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Mercury Exposure in Terrestrial Insectivorous Birds

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Abstract

From 1929-1950, the South River in the Shenandoah Valley of Virginia was polluted with mercury by an industrial source. Mercury can have adverse effects on wildlife and is known to bioaccumulate in aquatic invertebrates, fish, piscivorous wildlife, and aquatic-foraging insectivores. Only recently was it shown that terrestrial insectivores are also at risk of bioaccumulating mercury. To determine if terrestrial insectivores were accumulating mercury from the contaminated South River, I compared the blood / feather mercury levels of Carolina wrens, *Thryothorus ludovicianus*, and house wrens, *Troglodytes aedon*, caught within 50 m of the contaminated South River to a reference population caught within 50 m of the upper (unpolluted) South River, the Middle River, or the North River. I found that Carolina and house wrens from the polluted portion of the South River had significantly elevated blood and feather mercury levels compared to the reference population.

Mercury is accumulated by vertebrates via their prey, with fish and aquatic invertebrates being the assumed route of exposure for predatory vertebrates. Finding that terrestrial insectivores were also accumulating mercury was novel and warranted the question: through which prey items were terrestrial insectivores accumulating mercury? To determine this, I used Carolina wrens, house wrens, and eastern bluebirds, *Sialia sialis*, nesting in man-made nest-boxes along South River and at the reference sites. Avian diets are known to vary geographically and seasonally; therefore, it was necessary to determine the diets of terrestrial insectivores in the Shenandoah Valley. To ascertain their diet I used the ligature method to collect prey items gathered by adults and delivered to their nestlings.

By collecting the actual prey items birds were consuming, I avoided the questionable assumption that potential prey items collected by researchers from the bird's habitat are similar to those birds are actually eating. I successfully collected prey items from all three species, from both the contaminated and reference sites. The diets of all three species consisted primarily of Aranea, Lepidoptera, and Orthoptera, with eastern bluebirds also consuming a high proportion of Coleoptera. Prey items from the contaminated sites had total mercury levels that were significantly elevated over those from the reference sites. Of the major prey groups collected from the contaminated sites (Aranea, Lepidoptera, Orthoptera, and Coleoptera), Coleoptera had the highest mercury levels, followed by Aranea. Lepidoptera and Orthoptera from the contaminated sites had elevated mercury levels compared to a reference population but had mercury levels approximately one third of that found in Aranea and one fourteenth of that found in Coleoptera.

To determine if prey mercury levels can explain avian mercury exposure, I used a novel approach by developing a simulation that employed both bootstrapping and Monte Carlo techniques. The simulation correctly predicted the relative rank order of mercury exposure for the three species of terrestrial insectivores. Lastly, I compared the mercury levels found in the prey items of terrestrial insectivores to that of aquatic-foraging insectivores and fish-eating species. I plotted the distribution of prey mercury levels for all three foraging guilds and found a high degree of overlap, suggesting that mercury exposure for terrestrial insectivores is equivalent to that of aquatic-foraging insectivores and fish-eating species.

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Introduction

1. Humans and mercury

Mercury (Hg) was one of the first metals used by humans (Grigal, 2003; Hylander and Meili, 2003), and is now a global pollutant, posing a risk to both humans and wildlife (Hylander and Meili, 2003; Mergler et al., 2007; Scheuhammer et al., 2007; Thompson, 1996; Wiener et al., 2003). Its symbol on the periodic table, Hg, comes from the Greek word *hydrargyrum*, meaning liquid silver, and it is often referred to as quicksilver because it is a liquid at room temperature. In the past, prior to the industrial revolution, mercury was used for medicinal purposes, preservation, and as a dye. While excavating ancient Egyptian sites dated to the 2nd millennium BC, archeologists found evidence of mercury use (Hylander and Meili, 2003; Sznopek and Goonan, 2000). Mercury is now used most commonly in household devices (e.g., thermostats), and to enhance the recovery of precious metals in the mining process.

2. Sources of mercury

Mercury can be released into the environment through both natural and anthropogenic processes, and is found naturally in the earth's crust at a concentration of 0.09 ppm, in soil at 0.03-0.16 ppm, in streams at 0.00007 ppm, and in ground water at 0.0005-0.001 ppm (Clesceri LS et al., 1998).

Natural deposits of mercury are mostly in the form of cinnabar (HgS) and can be released by volcanic activity, weathering of rocks, and sea floor venting (Nriagu and Pacyna, 1988; Thompson, 1996; United States Department of the Interior, 1998; Wiener et al., 2003). While natural releases of mercury have occurred regularly across geologic time scales, anthropogenic sources of mercury have been increasing since the Industrial Revolution (Schwarzbach, 1998; Swain et al., 2007; Wiener et al., 2003), and now make up 50 to 75% of atmospheric emissions (Monteiro and Furness, 1997).

2.1. Atmospheric versus aquatic emissions

Atmospheric mercury comes from mercury released in the vapor state or adhered to airborne particles, which is then mobilized by the Earth's atmosphere and transported great distances (non-point source). Aquatic contamination in fresh water habitats is often the result of point source releases. Aquatic point source contamination impacts the habitat immediately surrounding a specific source (e.g. a factory or mine). In cases of aquatic contamination, mercury is often released in the liquid form directly into a nearby river, lake, or harbor. Because mercury is 13.5 times heavier than water it can find its way into small crevices on river and lake bottoms (Carter, 1977). Once sequestered, the mercury can later be remobilized when changes in topography occur (e.g. flooding, landslides, land development). The three

most important sources of anthropogenic mercury are fuel combustion, mining, and industrial waste (Monteiro and Furness, 1997; Swain et al., 2007).

2.1.1. Fuel Combustion

Mercury exists in trace amounts in fossil fuels, but when large quantities are burned the amount of mercury released is substantial. Since the industrial revolution, the main source of anthropogenic mercury has been the combustion of fossil fuels (Hylander, 2001; Sznopek and Goonan, 2000). The current global demand for energy has resulted in the continued and growing combustion of coal. In 2006, the combustion of coal was responsible for the majority of anthropogenic emissions (Swain et al., 2007). The increase in fuel combustion since the industrial revolution has resulted in a 50-300% increase in mercury deposition around the world (Swain et al., 2007).

2.1.2. Mining

Mercury's chemical affinity for precious metals has been exploited throughout history and is a major source of local mercury pollution. Gold and silver miners use mercury to enhance recovery (Alpers et al., 2005; Hylander, 2001). The Chinese were the first to use mercury in the mining process. Following the Chinese, Spaniards used mercury to mine silver in South America from the 16th to 19th century (Hylander, 2001). Mercury's use in gold mining continues in the 21st century. As occurred in 1849, during the gold rush

of the American West, wherever gold is discovered, fortune seekers follow, and so does the legacy of point source mercury pollution.

To enhance recovery of gold and silver, mercury is mixed with crushed rock and soil. The mercury then binds to the precious metal and the excess rock, soil, and mercury are washed away. The gold or silver-bound mercury is left behind due to its greater weight. The gold is then removed from the mercury by heating to evaporate the mercury and leave concentrated gold or silver behind. The vaporized mercury is deposited nearby on land while the liquid mercury ends up in nearby bodies of water (Hylander, 2001). Although the use of mercury in the mining process has been stopped in most of Europe and North America, it continues on a large scale among artisinal miners of South America, Asia and Africa.

2.1.3. Industrial Sources

Mercury is used in many industrial chemical processes, resulting in both atmospheric and aquatic pollution. At the start of the 20th century the use of mercury in industrial processes increased dramatically (Hylander and Meili, 2003; United States Department of the Interior, 1998). Some of the more common uses include the production of firecrackers, military weapons, paper, and synthetic fibers, as well as waste incineration, felting and chlor-alkali plants (Clesceri LS et al., 1998).

The largest industrial use of mercury during the 20th century was in chlor-alkali and synthetic fiber plants. During the decomposition process of chloride compounds, small amounts of mercury are lost to the environment. In 1996, it was estimated that chlor-alkali plants were responsible for 37 percent of all mercury consumed in the United States. The majority of the mercury used in chlor-alkali plants goes unaccounted for and is presumed lost to the environment (Sznopek and Goonan, 2000). Like the chlor-alkali process, the production of many synthetic fibers requires the use of mercury in the form of mercuric sulfate as a catalyst (Carter, 1977; Newman and Unger, 2003). Similar to the chlor-alkali process, during the production of synthetic fibers, mercury is often accidentally lost to the environment.

3. Mercury's Chemical Form

Due to the many sources and chemical forms of mercury, its fate, transfer, and distribution is poorly understood. Depending on the medium in which it is deposited, mercury can undergo numerous chemical transformations and be remobilized at varying rates (United States Environmental Protection Agency, 1997a). As with many other contaminants, the degree of mercury toxicity is highly dependent on its chemical form (Compeau and Bartha, 1985; Harris et al., 2003).

Generally, anthropogenic inputs of mercury are in the inorganic phase as Hg^0 or Hg (II) (United Nations Environment Programme, 2003; Wiener et al.,

2003). However, to humans and wildlife Hg^0 and Hg (II) are not the most toxic forms (Celo et al., 2006). The more toxic form of mercury is methylmercury (Celo et al., 2006).

4. Methylmercury

Methylmercury is of concern because compared to other forms of mercury it readily enters the food web, biomagnifies and bioaccumulates (United States Environmental Protection Agency, 1997b). Compared to inorganic mercury, which is not readily absorbed via the intestine in vertebrates, intestinal absorption of methylmercury can reach 100% (Scheuhammer, 1987). Once absorbed by the intestine, methylmercury easily passes the placental or blood-brain barriers, and can be a potent neurotoxin. In the food web, methylmercury bioaccumulates within individuals, and biomagnifies with increasing trophic position. The conversion process of elemental mercury to methylmercury is known as methylation.

4.1. Methylation

The methylation process, the addition of a methyl group (CH_3), is the most important transformation of elemental mercury (Wiener et al., 2003). The formation of methylmercury can occur via biotic and abiotic mechanisms, with the biotic pathway, via sulfate-reducing bacteria, considered to be dominant (Compeau and Bartha, 1985; Wiener et al., 2003). However, abiotic processes

are likely more important than once thought (Celo et al., 2006). The methylation process is not fully understood. It appears that to be methylated by sulfate-reducing bacteria a neutral dissolved mercury complex must cross the cell membrane of a bacteria (Benoit et al., 1999a; Benoit et al., 1999b). In addition to sulfate reducing bacteria, iron-reducing bacteria were recently shown to methylate mercury (Fleming et al., 2006).

4.2. Rates of Methylation

Rates of methylation can vary greatly depending on a host of environmental factors. Most methylation occurs in anaerobic sediments and wetlands (Compeau and Bartha, 1985; Schwarzbach, 1998; United Nations Environment Programme, 2003). The highest rate of methylation occurs in aquatic environments, under anaerobic conditions, high temperatures, and low pH (Celo et al., 2006; Wiener et al., 2003). In riverine environments the rates of methylation, and in turn the bioavailability to wildlife, can vary greatly with changing stream flow patterns. During periods of low stream flow, methylation rates can increase because dissolved oxygen decreases creating an anaerobic environment. In sum, the process of methylation is essential for mercury to become toxic, bioaccumulate and bioconcentrate (Harris et al., 2003; Thompson, 1996; Wiener et al., 2003).

4.3. Bioaccumulation and Biomagnification

One of the most important factors in understanding the fate and toxicity of methylmercury is the fact that it regularly bioaccumulates and biomagnifies (Celo et al., 2006; United Nations Environment Programme, 2003). Bioaccumulation refers to the net accumulation of a contaminant within an individual from all sources and occurs when the rate of intake is greater than the rate of elimination. Biomagnification refers to the increase in concentration of a contaminant from one trophic level to the next due to contamination of food (Newman and Unger, 2003). Because mercury continuously bioaccumulates over an individual's lifetime and biomagnifies in the food web, species that are long-lived and feed at high trophic levels are at the greatest risk of mercury poisoning. The presence of inorganic mercury in tissues is not uncommon but only methylmercury is highly bioavailable (Newman and Unger, 2003).

How methylmercury enters the base of the food web and transfers up the lower levels of the food web is poorly understood (Wiener et al., 2003). On the other hand, our understanding of mercury accumulation higher on the food web is better and is believed to be similar in all aquatic systems (marine, river, lake etc.), with top predators having a higher exposure than herbivores (Wiener et al., 2003). Differences in trophic position, diet, age, size, metabolic rate, fractionation, and life history can often explain differences in mercury levels, both within and between species (Wiener et al., 2003).

5. Human Epidemics

Although humans and wildlife have long been exposed to low concentrations of mercury, it was not until mercury was used in industrial processes that its toxic nature was recognized. From 1932 to 1968, the Chisso Corporation in Minimata, Japan, a manufacturer of chemicals (e.g. acetaldehyde), used mercuric sulfate as a catalyst. Beginning in the mid-1950s, the citizens and cats of Minimata began showing symptoms that indicated a disease of the central nervous system (Saito, 2004) and it was eventually concluded that the cause of the disease was methylmercury obtained via seafood consumption (Harris et al., 2003; Saito, 2004). This was the first time mercury was identified as the cause of an epidemic and ever since mercury has been suspected in many human and wildlife ailments. Traditional societies consuming a diet high in seafood, such as Native Alaskans and residents of the Seychelles Islands, are believed to be at high risk to mercury exposure (Mergler et al., 2007; Pirrone and Mahaffey, 2005). In addition to adversely affecting humans, methylmercury has neurological and reproductive effects on wildlife.

6. Wildlife Exposure

As with humans, it is commonly believed that fish-eating wildlife are most at risk to mercury exposure (Scheuhammer et al., 2007). Species such as the northern pike (*Esox lucius*), otter (*Lutra* spp.), mink (*Mulesta spp*),

osprey (*Pandion haliaetus*), and kingfisher (*Alcedo* spp.) have long been thought to be most at risk of mercury bioaccumulation (Scheuhammer et al., 2007). Considerable effort has been expended in studying piscivorous wildlife to determine the level of contamination, and risk, faced by species living in mercury polluted waterways. Since 2000, over 250 publications have used the key words "mercury" and "piscivorous" or "fish-eating".

7. Aquatic Food Webs

The majority of our knowledge on the exposure and bioaccumulation of mercury comes from studies of aquatic species and aquatic food webs for the simple reason that seafood consumption is the main exposure route to humans. Additionally, most point source pollution involves aquatic habitats and the methylation process is most rapid in aquatic environments (Grigal, 2003; Harris et al., 2003; Scheuhammer et al., 2007; Schwarzbach, 1998; Thompson, 1996; Wiener et al., 2003). Because methylation is greatest in aquatic environments and fish is the main route of exposure for humans, combined, the U.S. Environmental Protection Agency, U.S. Fish and Wildlife, Canadian Wildlife Service, and the BioDiversity Research Institute have over 4,700 records reporting a mercury concentration in some avian tissue from the northeastern United States and southeastern Canada (Evers et al., 2005).

7.1. Aquatic invertebrates

Invertebrates represent the base of the food chain and are exposed to both inorganic and methylmercury. The ratio of methylmercury to total mercury varies across habitats, season, and species (Boening, 2000; Defreitas et al., 1981; Riisgard et al., 1985; Watras et al., 1998; Wiener et al., 2003). Although the percent of mercury present as methylmercury can vary greatly, methylmercury comprises a higher percentage of the total mercury present in predatory invertebrates than in non-predatory invertebrates. When benthic invertebrates are classified by diet, percent methylmercury increases from detritivores to grazers to omnivores to predators, reaching 95% in predatory dragonfly larvae (Tremblay et al., 1996). In two similar studies in Maryland and Virginia, the percent of methylmercury increased from periphyton to filter feeders, to scrapers, to shredders, to predators (Mason et al., 2000; Murphy, 2004). In predatory insects methylmercury, as a percent of total mercury, approaches 100% (Mason et al., 2000).

7.2. Fish

Similar to predatory insects, the percent of methylmercury in fish tissue approaches 100% (Kannan et al., 1998; Wagemann et al., 1997; Westoo, 1973; Wiener et al., 2003). Much of our knowledge about mercury's distribution in different habitats comes from the thousands of studies on fish because this is the main exposure route for humans. Many of the most

desirable fish species for human consumption are also at risk to mercury exposure due to their predatory habits. For example, in saltwater the long lived and top predatory tuna and billfish species are known to have high mercury concentrations and children and women of reproductive age are advised against consuming them. In freshwater, bass, walleye, and pike, all predatory species, are often the targets of fish consumption warnings. As of 2007, there are 2500 fish consumption advisories in the United States, with 12 states having statewide advisories for all freshwater systems (http://www.epa.gov /waterscience /fish/advisories/index.html, updated January 29, 2007).

7.3. Fish-eating predators

Many terrestrial species living along contaminated waterways feed on aquatic prey and thus are exposed to mercury. Otter (*Lutra spp.*) and mink (*Mustela spp.*) are two groups of fish-eating mammals for which the most mercury exposure information is available (Scheuhammer et al., 2007; Wiener et al., 2003). Mercury levels in the brains of wild otters and mink ranged from 0.1 to 1.0 ppm wet weight (ww) , with some individuals having concentrations exceeding 5.0 ppm ww (Wiener et al., 2003). Mink consuming a diet with a concentration of 1.0 ppm ww methylmercury or higher have been shown to suffer adverse neurological effects (Dansereau et al., 1999; Wobester et al., 1976; Wren et al., 1987). Higher levels of mercury in the brain (>5.0 ppm ww)

are believed to cause mercury poisoning in mink (Wobester et al., 1976) and otters.

Mink and otter may be at high risk to mercury exposure, but they do not make easy study organisms or good biomonitors. They are hard to catch, do not persist in disturbed habitats, cannot be found in high densities, and are difficult to sample non-lethally. In contrast, many bird species that are at risk of mercury accumulation persist in disturbed habitats, occur at high densities and are easy to sample non-lethally (Brasso, 2007). In addition, birds are familiar and of interest to the general public. As with mammals, fish-eating birds have traditionally been thought to be the species most at risk and have therefore become favorite organisms for biomonitoring (Scheuhammer et al., 2007; Wiener et al., 2003).

8. Mercury in Birds

Researchers are not only interested in birds because they are effective biomonitors, but also because they warrant conservation concern. All native avian species in North America are protected at the federal level under the Migratory Bird Treaty Act of 1918. Decreased reproductive success as a result of exposure to mercury could cause population declines or changes in source-sink dynamics. As a result, numerous studies have measured mercury concentrations in free-living birds. This is especially true for fish-eating birds, in both marine and freshwater environments.

Although considerable attention has been focused on freshwater avian communities, until recently, mercury contamination was not considered a threat to terrestrial species (Scheuhammer et al., 2007). It has recently been established that in some cases terrestrial species are at equal if not greater risk than fish-eating and aquatic insectivorous birds (Cristol et al., in prep). Here, I will focus on terrestrial birds and only address studies of fish-eating and aquatic birds as a baseline for comparison.

8.1. Laboratory and field studies of birds

Despite the fact that many studies have used birds as biomonitors, in field studies it is often difficult to isolate the biological effects of a contaminant because correlations do not imply causation. Furthermore, monitoring reproductive success in free-living birds can be labor intensive and impractical. To detect small differences in reproductive success in free-living birds requires large sample sizes that are often unattainable even for those species that nest colonially (Wiener et al., 2003).

8.2. Avian tissue interpretation

Prior to designing any study using birds, the tissue being studied must be chosen. Four tissues are commonly used: blood, feathers, liver, and eggs. In all but the liver, methylmercury as a percent of total mercury approaches 100%, but total mercury concentrations differ greatly between tissues and have

different turnover rates (Evers et al., 2005). Some tissues represent an endpoint (e.g., liver and feathers) where mercury cannot be remobilized, while other tissues are not endpoints and thus may reflect more recent exposure (e.g., blood and muscle tissue).

The two tissues most commonly sampled non-lethally are blood and feathers. Blood mercury levels reflects short-term dietary uptake of about two weeks but turnover rates in blood vary from species to species and by molting stage (Evers et al., 2005). Mercury in the blood is mostly in the methylated form (Rimmer et al., 2005). The half-life of mercury in the blood ranged from three days in loon chicks actively growing feathers (Fournier et al., 2002) to 84 days for non-molting male mallard ducks, *Anas platyrhynchos* (Stickel et al., 1977).

As in other tissues, mercury in feathers is found as methylmercury. Feather mercury reflects blood and muscle mercury levels at the time of molt (Bearhop et al., 2000b; Evers et al., 2005). Feather mercury can therefore reflect both site specific (incorporation from blood) and long-term body burdens (remobilization and incorporation from muscle tissues; Evers et al., 2005). Once incorporated into the feathers mercury is stable (Appelquist et al., 1985) and provides a window into an individual's long-term mercury exposure, even for preserved museum specimens. In common loons, the ratio of mercury concentration in blood : feather was 1 : 6, a ratio that held true for adult bald eagle and tree swallow (Brasso and Cristol, 2007).

8.3. Laboratory studies

Mercury in the diets of captive birds has been shown to cause mortality and at low levels is associated with adverse reproductive effects (Schwarzbach, 1998; Thompson, 1996; Wiener et al., 2003). Chickens fed a diet of wheat dressed with methylmercury were sacrificed and fed to northern goshawks, *Accipter gentilis*. All goshawks died within 39 days (Borg et al., 1970).

In a dosing study of four species (n = 14 of each: Starlings, *Sturnus vulgaris*, common grackles, *Quiscalus quiscula*, red-winged blackbirds, *Agelaius phoeniceus*, and brown-headed cowbirds, *Molothrus ater*) adults were fed a diet containing 40 ppm methymercury. After five of the 14 individuals died, five survivors were sacrificed and mercury concentrations in tissues were measured in both dead and sacrificed individuals. No differences in mercury concentrations in specific organs between dead birds and sacrificed birds were found, suggesting that sensitivity to mercury toxicity can vary within a species (Finley et al., 1979).

Zebra finches, *Poephila guttata*, fed a diet containing 1.0 and 2.5 ppm methylmercury showed no signs of intoxication. However, zebra finches fed a diet containing 5.0 ppm methylmercury showed symptoms consistent with mercury poisoning and 25% of the high-dose group died. Surviving individuals

were lethargic, had fluffed feathers and difficulty balancing (Scheuhammer, 1988), consistent with methylmercury toxicity in wildlife.

Heinz (1979) described the effects of methylmercury on three generations of mallards, dosed with 0.5 ppm mercury via their food. The first generation was dosed starting when the breeders were adults. The second and third generations were dosed starting at nine days of age. This allowed Heinz (1979) to determine if continued exposure to mercury over multiple generations had cumulative effects on duckling behavior and reproductive behavior of adults. Female mallards laid fewer eggs and produced fewer ducklings than control birds. Exposed ducklings had decreased responsiveness to parental calls and hyper-responsiveness to a frightening stimulus. Though the effects tended to become progressively more severe over the three generations there was no statistical evidence for this (Heinz, 1979).

In a dosing study on great egrets, *Ardea albus*, there was no difference between experimental and control individuals in the time required for individuals to capture live prey. However, experimental individuals showed lower activity levels and were less likely to forage for fish (Bouton et al., 1999). In sum, dosing studies have shown biological effects but relating these levels to free living birds is difficult because few field studies have determined the mercury levels of prey and thus choosing relevant dosing levels is difficult.

8.4. Field studies of insectivores

Until recently, non-aquatic birds were not believed to be at risk of mercury exposure and little is known about the availability or toxicity of mercury in terrestrial insectivorous birds (Adair et al., 2003; Rimmer et al., 2005; Thompson, 1996; Wiener et al., 2003). Of terrestrial species, insectivores are believed to be most at risk of mercury exposure (Rimmer et al., 2005), but studies to date have reported levels that appear to be far below lowest observed adverse effects levels (LOAEL) from the literature.

8.4.1. Terrestrial insectivores and atmospheric mercury

In pied flycatchers (*Ficedula hypoleuca*) nesting in Northern Sweden, mercury concentration decreased with increasing distance from a sulphide ore smelter (Nyholm, 1995). Clutch size increased, and the frequency of eggshell defects decreased, with increasing distance from the metal source. Nestling liver concentrations were reported to be 0.25 ppm ww. However, mercury's role is unknown because many other metals were present.

Rimmer et al. (2005) investigated mercury levels in montane forest breeding adult birds and found that Bicknell's thrush, *Catharus bicknelli*, yellow-rumped warblers, *Dendroica coronata*, blackpoll warblers, *Dendroica striata*, and white-throated sparrows, *Zontrichia albicollis*, accumulated mercury. Blood mercury levels for these three species ranged from 0.03 to 0.42 ppm ww. This was the first study to quantify the extent of mercury exposure in

montane forests, and also underscored the ability of mercury to accumulate in wilderness areas remote from any point source of mercury.

8.4.2. Terrestrial insectivores and riverine mercury pollution

In southern Alabama a chlor-alkali facility released mercury into the flood plain of the Tombigbee River (Adair et al., 2003). Compared to those from reference sites, prothonotary warbler chicks (*Protonotaria critrea*) had elevated mercury levels in their tissues (Adair et al., 2003; Reynolds et al., 2001). Adult kidney mercury levels on the two contaminated sites average 0.93 ppm ww.

The Sudbury and Charles Rivers in Massachusetts were polluted with mercury from an industrial source. In a study of 11 songbird species nesting on or near the two rivers, blood mercury levels were found to be elevated (Evers et al., 2005). Insectivorous songbirds had significantly higher blood mercury levels compared to granivorous songbirds (Evers et al., 2005). The terrestrial insectivore with the highest blood mercury level was the song sparrow (*Melospiza melodia*) at 0.2 ppm ww, and the insectivore with the overall highest mercury levels was the northern waterthrush (*Seiurus noveboracensis*) at 0.6 ppm ww.

In Nevada, the Carson River drainage was polluted with mercury as a result of mining practices during the 1800s. In the mining-impacted areas most sampled organisms accumulated mercury (Custer et al., 2007). Compared to

birds from a reference site, house wrens (*Troglodytes aedon*), a terrestrial insectivore, had elevated mercury levels in their eggs and in the livers of nestlings. Mercury levels were significantly lower in wren (2.72 ppm, n = 11) than in tree swallow (7.34 ppm dw, n = 9) eggs from the same study site, but there was no difference in liver mercury concentrations (3.79 ppm dw, n = 10 and 2.87 ppm dw, n = 8 respectively). These levels were considerably higher than those detected in house wren eggs (0.1 – 0.2 ppm dw) and livers (0.1 ppm dw) from mine affected areas in South Dakota and Wyoming (Custer et al., 2002).

In the most comprehensive study to date, over a period of two years, 11 of 12 terrestrial songbirds nesting within 50 meters of the contaminated South River in Virginia were found to have elevated blood mercury levels compared to reference birds (Cristol et al., in prep). The South River was contaminated with industrial mercury prior to 1950 (Carter, 1977). Five of the 11 terrestrial songbirds sampled by Cristol et al. (2007) had blood mercury levels comparable to or higher than the fish-eating kingfisher, three aquatic insectivores (tree swallow, rough-winged swallow, *Stelgidopteryx serripennis*, and eastern phoebe, *Sayornis phoebe*), and one duck (mallard*).*

Cristol et al. (2007) found that blood mercury levels in terrestrial songbirds ranged from 0.45 ppm ww in Carolina chickadees (*Poecile carolinensis*; n = 7) to 6.72 ppm ww in red-eyed vireos (*Vireo olivaceus, n* = 6). The next highest terrestrial insectivore, the Carolina wren, *Thryothorus*

ludovicianus, had a blood mercury level of 4.49 ppm ww. The fish-eating belted kingfisher had a blood mercury level of 3.35 ppm ww (n = 21) and the tree swallow, an aquatic insectivore, had a blood mercury level of 3.66 ppm ww (n = 78). In summary, terrestrial insectivorous songbirds are at risk of accumulating potentially harmful levels of mercury even if the original source of contamination was aquatic in nature.

9. Mercury exposure in terrestrial insectivorous birds

The recent scientific documentation that terrestrial insectivores, including shrews and bats, can accumulate mercury at levels comparable to aquatic species has highlighted a gap in our knowledge regarding mercury pollution and its effects on wildlife. Accurately quantifying a species' exposure and having the ability to predict differences in exposure between species is important in identifying the species most at risk of mercury poisoning. Further, determining through which prey items terrestrial insectivores are accumulating mercury can serve to identify: (i) the route of mercury exposure and (ii) other avian species with similar diets that could also be at risk. Food chain length, diet, metabolic processes, and migratory status all have the potential to explain differences in mercury exposure between avian terrestrial insectivores.

9.1. Food chain length

Accurately describing a species' diet is important to many ecological

studies (Rosenberg and Cooper, 1990). Bill size, body size, habitat, feeding ecology, fecal samples, gut content, prey collection, and other methods have all been used to predict or describe avian diets (Bearhop et al., 2004; Rosenberg and Cooper, 1990). The emerging field of stable isotope analysis offers a potentially powerful method of measuring both food chain length and trophic niche width (Bearhop et al., 2004).

9.1.1. Stable isotopes

The field of stable isotope analysis deals with the assimilation of heavy versus light stable isotopes of nitrogen, carbon and other elements. The ratio of heavy to light isotopes in predators reflects the ratio in their prey (Hobson, 1999; Hobson and Clark, 1992a). Stable isotope analysis has become increasingly popular among ecologists to untangle complex food webs (Bearhop et al., 2004). Both carbon and nitrogen have been used for this purpose. Carbon is used to determine the source of a consumer's diet, and nitrogen to determine food chain length. The ratio of ^{15}N to ^{14}N (expressed as $\delta^{15}N$) has become a standard metric for ecotoxicologists when assigning risk of bioaccumulating a contaminant. Consumers tend to have $\delta^{15}N$ levels 2.5‰ to 5‰ higher than the organisms in their diets (Hobson and Clark, 1992b). Contaminants that bioaccumulate, such as mercury, are positively correlated with $\delta^{15}N$ both within and between species (Bearhop et al., 2000b; Newman and Unger, 2003).

9.2. Diet

Inter-specific differences in mercury levels are often attributed to differences in diet. Many researchers have attempted to show this relationship by classifying species according to their assumed diets, for example "herbivores" versus "primary consumers" versus "top predators". Fewer researchers have actually collected prey items and analyzed them for mercury (Cabana and Rasmussen, 1994; Longcore et al., 2007; Monteiro et al., 1998; Nisbet et al., 2002; Stewart et al., 1997). Compared to studies describing mercury exposure in birds, those describing mercury exposure in actual prey items are rare. If mercury in prey items is investigated, the putative prey items are often not the actual prey items eaten but hypothesized prey items collected by researchers using nets, traps or other sampling methods. Collecting actual prey items is difficult and in some cases impossible.

9.2.1. Mercury concentration in actual prey items of fish-eating birds

To my knowledge, only five studies have collected actual prey items from fish-eating birds. Collection of prey items from fish-eating birds can be accomplished during banding because when handled by researchers both nestlings and adults will often regurgitate their stomach contents. When stomach samples are not regurgitated voluntarily, regurgitation can be induced (Monteiro et al., 1998). These regurgitated prey items have provided a window

into the route of mercury exposure in piscivores. Comparisons between studies is difficult because only two of these studies collected avian tissue samples for comparison to prey mercury levels and one of the five studies did not report actual prey mercury levels.

In the Azores, feathers and dietary samples of six seabirds were collected and analyzed for mercury. Mean body feather mercury in the six species ranged from 2.1 to 22.3 ppm fresh weight (fw). Mercury concentrations in their prey ranged from 0.05 to 0.43 ppm dry weight (dw). There was a highly significant and positive correlation between mercury in the food and mercury in the feathers (Monteiro et al., 1998).

In the North Atlantic, feather, blood, and prey samples from adult great skuas, *Catharacta skua,* were collected and analyzed for mercury. Mean blood mercury ranged from 3.5 ppm dw to 6.7 ppm dw, and mean body feather mercury ranged from 4.7 to 6.2 ppm dw. In regurgitated prey samples mercury concentrations ranged from 0.04 ppm dw in sand eels to 0.89 ppm dw in auk muscle (Bearhop et al., 2000a). Sample sizes were low ($n \leq 4$), therefore, statistical comparisons between prey groups were not possible.

Nesting great skuas are also known to prey upon other fish-eating birds. Stewart et al. (1997) used regurgitated pellets (indigestible portion of food) to describe the diets at individual nests and found that mercury concentration in the feathers of adults, chicks, and chick down of skuas was positively correlated with the proportion of bird remains in their pellets. Mercury levels of

actual dietary items were not available since the pellets did not represent what was eaten but what was not digested. What this study did show is that mercury levels can vary as a result of different feeding strategies.

Wading birds also commonly regurgitate prey items when handled. While banding 20-40 day old nestling wood storks (*Mycteria american*), Gariboldi et al. (1998) collected 200 prey items. The collected prey items were identified and analyzed for total mercury. Mean mercury concentrations in prey ranged from below the detection limit to 2.36 ppm dw. Overall, freshwater fish had higher mercury concentration than saltwater fish (Gariboldi et al., 1998). Using several assumptions, the authors calculated an average daily dose for nestling wood storks of 0.02 - 0.13 ug Hg/Kg body weight/day (Gariboldi et al., 1998). No mercury levels were reported for blood or feathers from nestlings or adults.

Prey items regurgitated by great egret nestlings from the Everglades were collected, identified, and analyzed for mercury. Over a four year period fish comprised 95% of their diet, and mercury concentration in the fish ranged from 0.04 -1.4 ppm ww (Frederick et al., 1999). The mean mercury concentration across all years and all prey items was estimated to be 0.4 ppm ww and over the 80-day nestling period it was estimated that nestlings ingested on average 4.2 mg of mercury (Frederick et al., 1999). Again, feather or blood mercury levels were not reported making comparisons difficult.

9.2.2. Mercury in Prey of insectivores

Even fewer studies exist that examine mercury concentrations in the actual prey items of insectivores. Until recently it was not technically possible to determine mercury concentrations in small invertebrates due to low sample mass. Furthermore, terrestrial species have been traditionally of little interest to researchers studying mercury bioaccumulation.

Prey items collected from nestling prothonotary warblers consisted of both terrestrial (Lepidoptera and Aranea) and aquatic (Odonata) invertebrates. There was no relationship between mercury levels in an individual's food and its kidney (Adair et al., 2003). However, prey items collected from contaminated sites were significantly elevated compared to those from reference sites (Adair et al., 2003). Spiders, a predatory invertebrate, were significantly elevated compared to all other prey items combined (Adair et al., 2003). Mean adult kidney mercury levels from three contaminated sites ranged from 0.3 to 1.6 ppm ww. In nestlings, kidney mercury levels ranged from 0.03 to 0.19 ppm ww, with means ranging from 0.05 to 0.17 ppm ww. Mean prey mercury levels ranged from 0.03 to 0.07 ppm ww.

The liver samples and stomach contents, not individual prey items, of three insectivorous species (one largely aquatic, tree swallow; and two presumably terrestrial, house wren and western bluebird) were collected from nestlings reared on sites contaminated by precious metals mining (Custer et al., 2007). Mean liver samples for tree swallows, house wrens and bluebirds

were 3.8, 2.9, and 1.3 ppm dw respectively. Mercury concentrations in food averaged 1.2 ppm dw for tree swallows (n = 5 items), 1.7 ppm dw for house wrens (n = 3), and 1.8 ppm dw for western bluebirds (n = 2), but statistical comparisons were not possible because sample sizes were low.

That tree swallows would feed on contaminated prey in a river valley is not surprising, because they are known to feed over water, collecting emerging aquatic insects (Robertson et al., 1992). More surprising is the fact that house wrens and bluebirds were also feeding on mercury contaminated prey items. Furthermore, the range of mercury concentrations reported (0.7-3.1 ppm dw) is similar to that of many fish-eating birds (Frederick et al., 1999). However, stomach contents collected from the birds were never identified, and sample sizes were miniscule, so identifying through which prey items house wrens and western bluebirds accumulated mercury was not possible.

Another recent study examined eggs, feathers, and prey from tree swallows nesting in New England. Mean total mercury concentrations in eggs ranged from approximately 0.25-0.6 ppm ww, in feathers from 1.5-3.5 ppm ww, and in food from 0.1-0.3 ppm ww (Longcore et al., 2007). Comparing these results to other reports of feather mercury levels is difficult because feather mercury levels were not separated by feather type and included all feathers from de-feathered nestling carcasses that were 14 days of age or greater (Longcore et al., 2007). Further, egg mercury levels are difficult to compare because in some cases the third egg of each clutch was collected and in others

the first three eggs were collected for a composite sample. This causes a problem because egg mercury levels differed by as much as 50% between eggs from the same clutch (Longcore et al., 2007).

9.2.3. Predatory invertebrates

The results of Adair et al. (2003) suggest that predatory invertebrates (i.e., spiders) could be a major potential exposure route of mercury for terrestrial birds. Many terrestrial insectivores consume spiders, predatory beetles, and Odonates, hence increasing food chain length and in turn increasing the potential for bioaccumulation of contaminants. A diet high in predatory invertebrates has the potential to increase the bioaccumulation of mercury. Furthermore, when I examined published diet reports of the species of songbirds occurring along the South River, predatory invertebrates (e.g. spiders) comprised a high percentage of many of the species' diets (Gowaty and Plissner, 1988; Grubb and Pravosudov, 1994; Haggerty and Morton, 1995; Johnson, 1998; Mostrom et al., 2002).

9.3. Metabolic processes

Metabolic processes, including assimilation and fractionation, potentially affect how mercury moves within the body of an individual. Smaller species generally have higher metabolic rates, consume more food, and associated mercury for their size. Assimilation of methylmercury in the digestive tract is

similar across all species and nears 100% (Fournier et al., 2002; Scheuhammer, 1987). Fractionation refers to the transfer and incorporation of mercury in specific tissues within an individual's body. Fractionation can vary greatly from one species to another and can be related to metabolism. That is, once mercury has been assimilated via the digestive tract and incorporated into the blood, the latency with which mercury becomes incorporated into the liver, kidneys, brain, and other tissues is variable, as well as the proportion of body burden found in each tissue. A major factor affecting fractionation of mercury in birds is molt and feather growth. Determining differences in metabolism, assimilation, and fractionation in birds requires dosing studies where birds are regularly sacrificed. None of these were experimentally addressed in the field study presented here, and each may have additional explanatory power for differences observed between the study species.

9.4. Migratory Status

When characterizing mercury exposure in birds on a contaminated site it is essential to determine which species are migrants and which are year-round residents. Migrants leave the contaminated site after breeding and are presumably only exposed to mercury during the 3-5 months of the breeding season. Resident birds remain on the contaminated site and although they may change their diet with the changing season they are potentially exposed to mercury year-round. This suggests that when sampling a tissue (see section:

avian tissue interpretation) that reflects long-term exposure (feathers), migratory species could have lower mercury levels than non-migratory species. In addition, migration behavior is closely related to molt schedule (i.e., migrants often molt before or after migration whereas residents can molt more gradually), so differences may arise from this biological constraint as well. To my knowledge, only one study has addressed the relationship between migration and mercury level in songbirds. In a study that included pied flycatchers, collared flycatchers (*Ficedula albicollis*), nuthatches *(Sitta europa*) and coal tits, *Parus ater*, nesting near a mercury production plant in Slovakia, a zinc smelter in Norway, or a reference site, it was concluded that mercury levels in eggs were lower in migrants (Rosten et al., 1998).

10. Objectives

10.1. Accumulation

Question: Are terrestrial insectivores accumulating mercury from the contaminated South River?

Approach: To rule out the possibility that terrestrial insectivores were accumulating mercury due to atmospheric deposition, I compared blood and feather mercury levels from Carolina and house wrens captured within 50 m of the South River to those of a nearby reference population sharing the same depositional environment. To accomplish this, adult Carolina and house wrens

were captured at their nest boxes or using mist nets and audio lures along the contaminated South River and three reference sites in 2006.

10.2. Exposure

Question A: What prey types make up the majority of the diet of terrestrial insectivores and what are the mercury levels of these prey items?

Approach: To determine the extent of mercury exposure in the prey items of terrestrial insectivores, the ligature technique (Mellott and Woods, 1993; Orians and Horn, 1969) was used to collect prey items from Carolina wrens, house wrens, and eastern bluebirds in 2006 and 2007. The diets of the three avian species were compared as a percentage of total biomass on a fresh weight basis. Mercury levels were compared between prey items collected from the three avian species and between years.

Question B: Do prey items collected from birds nesting within 50 m of the contaminated South River have elevated mercury levels compared to prey items collected from birds nesting on reference sites?

Approach: To rule out the possibility that prey items of terrestrial insectivores were accumulating mercury due to atmospheric deposition I compared the mercury levels of the major prey groups collected from contaminated sites to the mercury levels of the same prey groups collected from reference sites.

Question C: Does mercury accumulation differ by prey type?

Approach: To determine from which prey items terrestrial insectivores were accumulating mercury, I compared the mercury levels of the prey groups making up the major portion of each species' diet to each other (e.g. spider mercury compared to caterpillar mercury).

10.3. Modeling exposure

Question: Can prey mercury levels explain differences in bird mercury levels?

Approach: To determine if prey mercury levels can explain avian mercury exposure, I used the total mercury values of prey items along with life history characteristics (avian size and daily food consumption) in a Monte Carlo simulation designed to estimate the likelihood of particular exposures. To interpret how diet and prey mercury levels determine mercury exposure in adult birds, I generated a distribution of daily mercury exposure per gram of bird for each of the three terrestrial species. These were compared to one another.

10.4. Comparisons to aquatic and piscivorous birds

Question: How does daily mercury exposure and the mercury level in the prey items of terrestrial insectivores compare to the mercury level in the prey items of an aquatic insectivore (tree swallow) and a fish-eating species (belted kingfisher)?

Approach: To accomplish this, I analyzed for total mercury, food boluses collected from adult tree swallows during the summer of 2006, and fish collected from belted kingfishers during the summers of 2005, 2006, and 2007. I then examined the distributions of prey mercury levels of the three feeding strategies (terrestrial insectivore, aquatic insectivore, and fish-eating) for degree of overlap.

Methods

1. Study site

In Waynesboro, Virginia, from 1929-1950, mercuric sulfate was used as a catalyst in the manufacturing of acetate fiber by E.I DuPont de Nemours and Company (Carter, 1977). In 1977, DuPont took responsibility for discharging unknown quantities of mercury into the South River. Sediment testing downstream of their factory revealed heavy mercury contamination (Carter, 1977). Mercury levels in fish have been deemed unsafe for human consumption and there is a consumption warning from the foot bridge at the old plant in Waynesboro to Front Royal, Virginia, on the South Fork of the Shenandoah River, comprising approximately 167 km of river (Murphy, 2004).

Mercury contamination was predicted to decline over time, but it has not (Don Kain, South River Science Team. pers. comm.). The South River Science Team (SRST) was formed in 2000 as a joint effort between Dupont and the Virginia Department of Environmental Quality (DEQ) to assess the damage done by mercury to the fish and wildlife living in and around the contaminated river. From 2000 to 2004, attention was focused on water quality monitoring and contamination of aquatic organisms (i.e. fish and their aquatic invertebrate prey).

In 2005, the first study to focus on any wildlife other than fish was started by D. Cristol. The focus of the study was the aquatic-foraging

insectivorous tree swallow, the fish-eating belted kingfisher and the eastern screech-owl, a predator primarily on small birds and mammals (see Brasso, 2007; White, 2007). All three bird species had elevated blood and feather mercury levels compared to a reference population. Blood mercury levels varied along the South River, peaking near Grottoes, Virginia, approximately 40 km downstream of the original contamination source.

In addition to focusing on these three species, many other birds were sampled within 50 m of the South River and on reference sites. The species with the highest mercury level in 2005, even higher than the fish-eating belted kingfisher, was the Carolina Wren. The one other terrestrial insectivore sampled in sufficient numbers in 2005 –the eastern bluebird– was also found to have elevated blood mercury levels.

In all species sampled, blood mercury levels dropped significantly downstream of Port Republic, Virginia where the South River and North River join to form the South Fork of the Shenandoah. The study presented herein focuses on the contaminated section of the South River from Waynesboro to Port Republic, and three reference sites: upstream of the contamination site on the South River and the entire Middle and North Rivers. For a detailed description of the study site see Brasso (2007).

1.1. Choice of individual study sites

Study sites were chosen based on the presence or absence of suitable habitat for the target species. Suitable habitat was identified by using habitat descriptions found in the literature (Haggerty and Morton, 1995; Johnson, 1998) and consulting with experts (Pers. Comm. T.M. Haggerty). Permission to use all study sites was granted by the appropriate land owner or jurisdictional agency. Many locations were selected because they had been used in 2005 and thus access was simple. If suitable habitat existed on these properties for wrens and bluebirds, they were incorporated into the present study. Eastern bluebirds regularly used the nest boxes erected for tree swallows, and the same type of box could be used for both wrens, albeit in different habitat (see "Box placement" below). For a detailed description of individual study sites see Brasso (2007) and White (2007).

2. Nest boxes

Nest boxes were erected on all accessible contaminated sites with suitable wren habitat. Two types of nest boxes were used. For Carolina wrens, house wrens, and eastern bluebirds a standard eastern/western bluebird box, as described by the North American Bluebird Society (www.nabluebirdsociety.org) was used. On the poles of these nest boxes a stovepipe-style predator guard warded off raccoons, domestic cats, and snakes.

2.1. Tubes

I developed a second type of nest box, made out of plastic drainpipe, specifically for Carolina wrens (herein after referred to as 'tubes'). The design consists of a black plastic garden drain pipe cut to approximately 45.5 centimeters in length and 10.16 centimeters in diameter. At each end a plastic flower pot was inserted, bottom inward, and glued. In one of the flower pots a 3.8 centimeter entrance hole was drilled. On one side of the tube a 25.4x10.16 centimeter rectangular access hole was cut out. This access hole could be sealed with the cut-out piece that was held in place with a loop of monofilament. The tube was then attached with two screws to the side of a tree, 1-2 m off the ground.

2.2. Box Placement

For Carolina and house wrens, 3-5 nest boxes or tubes were clustered in what could become a single territory. The nest boxes were placed as close as 10 m apart. This was done because both species often build multiple dummy nests that are never used. By placing several boxes on a single territory each pair of wrens was given the opportunity to build dummy nests (T.M. Haggerty, pers. comm.). For Carolina wrens, boxes were placed in forest openings lacking brush in the immediate surroundings (2 – 5 m). The nest box holes were oriented so the entrance hole faced the nearest bush, fallen tree, or

brush pile. For house wrens, nest boxes were placed on the edge of forested habitat with the entrance hole oriented towards the forest. All boxes were checked weekly (as per Brasso 2007) to determine the ideal time to capture adults and ligature nestlings (see below).

3. Study species

Mercury exposure in Carolina and house wrens was characterized during the summer of 2006. In 2006, prey items from Carolina wrens, house wrens and eastern bluebirds were sampled and in 2007 additional prey items from eastern bluebirds were sampled. The three species differed in their choice of habitat, migratory status, nesting behavior, and foraging strategy. Thus, each species faces different potential risks of mercury exposure.

3.1. Carolina wren

Carolina wrens are small songbirds found throughout the southeastern United States and into northern Mexico. They occupy a wide range of forested habitats but dense shrubs or brushy cover are a unifying component (Haggerty and Morton, 1995). They are non-migratory, maintaining territories throughout the year. In the southern end of the range, breeding starts as early as the last week of March and continues through August. Clutch size is typically four eggs (Haggerty and Morton, 1995). Nest site characteristics vary greatly from tree cavities and upturned roots to old shoes and flower pots (the inspiration for the

tube design). Using a gleaning technique, their main prey items consist of insects and other invertebrates, which are found primarily on or near the ground. Their large beak (11 – 12 mm) is often used to turn over leaves and dismember large prey items (Haggerty and Morton, 1995).

3.2. House wren

House wrens are smaller than Carolina wrens and breed throughout the central and northern latitudes of the United States and southern Canada. They occupy edge habitats between forested areas and open fields, and they avoid habitats that are heavily vegetated. These wrens are frequently found near areas of human disturbance (Johnson, 1998). Most individuals migrate to the southern United States or Mexico (Johnson, 1998). Breeding starts in mid-May and clutch size ranges from 4-7 eggs (Johnson, 1998). House wrens use natural cavities and old woodpecker holes as nesting sites but readily use nest boxes (Johnson, 1998). Using a gleaning technique in the sub-canopy, house wrens acquire small invertebrates using their smaller beak.

3.3. Eastern bluebird

Eastern bluebirds are small thrushes found throughout the eastern United States and southern Canada. They nest and forage in open habitats. Migratory status varies greatly among and within populations. Some individuals migrate, some wander, and some remain on the breeding grounds

all year. No systematic study has addressed what causes some individuals to migrate while others do not (Gowaty and Plissner, 1988). Breeding starts in April and clutch size is usually 4-5 eggs. Eastern bluebirds use natural cavities but are found most commonly in nest boxes. Hunting prey visually from perches, their main prey items consist of insects, spiders, and small fruits which are found primarily in open habitats with sparse ground cover. All prey item data associated with eastern bluebirds were collected for my study, whereas blood mercury levels from adults and nestlings were collected by A. Condon and graciously provided for comparisons to the two species of wrens.

4. Capture method

Both nestlings and adults were sampled to characterize mercury exposure. All nestlings were sampled at their nest boxes 3-5 days before the predicted fledge date. Many field studies require adult birds to be captured at their nest boxes and several techniques have been devised. Adults of all three species were captured in one of three ways (see below). Capture method varied by species, sex, number of previous captures (i.e. wariness), and microhabitat characteristics.

4.1. Brooding females

Since each nest box was checked on a regular basis it was often possible to predict within 3-4 days when a clutch would hatch. Hatch date was

predicted based on incubation periods, 15 days for Carolina wrens and 13 days for house wrens (Gowaty and Plissner, 1988; Haggerty and Morton, 1995; Johnson, 1998). At the end of the incubation period and the beginning of the nestling period adult females could often be found incubating eggs or brooding nestlings. This was especially true during the first hours of daylight. If the nest box was approached quietly and the entrance hole quickly covered I could often trap the female inside the box. If I was not successful in capturing females this way, they were captured, along with all males sampled, using one of the following two methods.

4.2. Nest box traps

Several nest box trap designs have been described in the literature (Cohen and Hayes, 1984; Litovich et al., 1983; Mock et al., 1999; Rendell et al., 1989; Stutchbury and Robertson, 1986). All but the 'basket trap' described by Rendell et al. (1989) rely on some variation of a trap door. These trap door designs range from the simple to the complex and from the inexpensive to the expensive. The simplest design, described by Stutchbury and Robertson (1986) relies on a square plate propped up by a stick or a piece of stiff grass. The most complex design relies on a radio-controlled release of a trap door (Litovich et al., 1983; Mock et al., 1999).

All of the traps cited above work well when first tried, but once an individual has been trapped or managed to escape they can become extremely

wary at the sight of the trap door (pers. observation). This is often the case when the same individual needs to be caught for a second, third or fourth time, often at a precise time (e.g. 24 hours after treatment; Mock et al., 1999). In such cases it is often necessary to catch a specific member of a pair (male or female) without catching the mate and creating additional disturbance.

I would only use a trap door during the nestling stage so as to take advantage of the frequent feeding trips made by adults. When first attempting to catch an individual I would use a trap door propped up by a stick (Stutchbury and Robertson, 1986). However, this was often unsuccessful because the males were extremely wary if the female had already been caught, or the male successfully avoided the falling trap door. Males would often land in the entrance hole to feed their young with a prey item visible in their beak but not enter the box. Females would also exhibit this behavior if they had been caught previously. Believing that it was likely the adult birds were able to see the stick and trap door, I devised an alternative trap.

The same size trap door as described in Stuchbury and Roberston (1986) was taped above the hole using duct tape. The trap door was colored black with a marker to blend in with the roof of the box. Instead of propping the door open with a stick with one end balanced on the nest itself, a drinking straw was placed in the ventilation gap between the side of the box and the roof. The trap door was then pushed all the way to the ceiling of the box and the straw was used to hold it in place. The straw was colored black with a

permanent marker and cut so that it would not stick out beyond the edge of the roof. If placed properly, the straw and trap door were nearly invisible.

Attached to the straw with a small piece of tape was a length of 4-6 pound test green or clear monofilament fishing line. The monofilament was strung down the back of the box and along the pole to the ground. The researcher then walked 30-50m away and watched for the adult bird to enter the box. Since the trap door and straw were nearly invisible, even wary birds readily entered the box. In 2006 and 2007, this method was successfully used to trap four species of insectivorous birds including Carolina and house wrens. Also in 2006 and 2007, as part of a larger study by D. Cristol, it was necessary to recapture tree swallows 24 hours after having injected them with phytohemagglutinin as part of an immune system study. After being captured more than once, and being injected with phytohemagglutinin after the most recent capture, the tree swallows became extremely wary of entering the box. This method had a big advantage over the prop-trap method in that the researcher could allow an unwanted member of a pair to come and go without triggering the trap, until the targeted member of the pair entered the nest box.

4.3. Mist Net

In some cases, both species of wrens would build nests that were unsuitable for a trap door due to the excessive amount of nesting material in the nest box. In these cases it was necessary to place a mist net directly in

front of the box. Time needed to capture individuals varied greatly from box to box and it was often not possible to capture the more wary males. Mist nets were also used after many failed attempts with a trap door and considerable disturbance. Therefore, it often took several visits from the male and female until I was successful in capturing the bird. The male and female could usually see the mist net and easily avoided it by flying around it and approaching the box from behind. Eventually, these birds would be captured by the mist net upon leaving the box.

In addition to capturing Carolina and house wrens at their nest boxes, I also used audio lures (Shy and Morton, 1986) to capture them in areas where they were using natural nest. Mist nets were placed in areas where Carolina wrens had been previously heard singing. Once the mist net was erected, a recording of a male wren or an eastern screech-owl was played. This would elicit an aggressive response with the birds often caught in the mist net within an hour.

5. Tissue sampling

Blood samples were taken to determine short term exposure to mercury (Evers et al., 2005). Blood was taken from adults and nestlings of all three species and followed the procedures described in Brasso (2007). Approximately 50 µL of blood was collected. Both heparinized and non-heparinized 75 µL capillary tubes were used for each bird. Heparin is used as

an anti-clotting agent and non-heparinzed tubes were used because heparin contains nitrogen and therefore any blood collected in heparinized tubes would be unsuitable for possible future stable isotope analysis. Feathers were sampled to determine long-term exposure to mercury. In 2006, approximately ten back and body feathers were collected from adult wrens. Effort was made to pull ten feathers from different parts of the body to avoid sampling feathers that grew in simultaneously. In 2007, when a wren banded in 2006 was re-captured, the tenth primary feather was collected. All samples were frozen within 12 hours.

6. Prey item sampling

Prey items were sampled from Carolina wrens, house wrens, and eastern bluebirds using the ligature method (Mellott and Woods, 1993). Prey items from tree swallows and belted kingfishers were collected opportunistically as part of a larger study on mercury exposure and reproductive success in the two species (see Brasso, 2007; White, 2007). Prey items from tree swallows and belted kingfishers were collected by removing prey from the beaks of recently captured adults.

6.1. Prey item collection

To determine diets, I used the adult birds as "bug collectors" to ascertain what the species as a whole was eating. An assumption of the study was that

adult and nestling diets are closely related. Prey items fed to nestlings may not exactly mimic what adults are consuming, but when adult and nestling diet studies form the literature were compared, there is evidence that the prey groups that make up the major proportion of a species' diet are similar between adults and nestlings (Beal et al., 1916; Chapman HH, 1947; Gowaty and Plissner, 1988; Johnson, 1998; Laskey, 1948; Pinkowski, 1978; Pitts, 1978). Further evidence that nestling and adult diets are similar is found in their similar stable nitrogen isotopic signatures and the correlation of isotopes between nestling and parents across nest sites (Cristol et al., in prep).

6.1.1. Ligatures

In the ligature method, a constrictive ligature is placed around a nestling's neck, preventing it from swallowing food items while not inhibiting breathing. In the past, several different materials have been used as constrictors with varying degrees of success, including copper wire, plastic-coated wire, pipe cleaners and thread (Johnson et al., 1980; Rosenberg and Cooper, 1990). Recently, plastic cable ties have gained popularity due to their ease of use and low nestling mortality rate (Mellott and Woods, 1993). Regardless of the material used, care must be taken not to fasten the ligature too tight or on nestlings that are too young. In both cases, the result is a high mortality rate (Orians, 1966). When done properly, mortality through

strangulation can be reduced to less than one percent (Mellott and Woods, 1993), and, in the case of my study, to zero.

An advantage of the ligature method is that multiple prey items can be collected in a single day (Johnson et al., 1980). Either the researcher can wait for the parent to deliver several food items or remove food items after each delivery. However, there are drawbacks to both methods.

If the parents are allowed to deliver several food items before the researcher collects them, the potential for the removal and consumption of food by the adults increases (Johnson et al., 1980). Also, the longer the researcher waits to remove a food item the greater the chance of the food item slipping past the ligature (Johnson et al., 1980). Alternatively, when food items are removed after each delivery the adults' behavior may be affected by the disturbance. This can result in altered food delivery rates (Johnson et al., 1980). Both cases can result in a bias in prey size and abundance. Small prey may slip past ligatures and large prey items may be removed by adults if not swallowed by nestlings (Johnson et al., 1980; Orians, 1966). However, overall diet composition was shown not to be affected by ligatures (Johnson et al., 1980).

Initially, I experimented with many variations on the ligature technique but settled on using four-inch cable ties as described by Mellot and Woods (1993). Cable ties were chosen for their ease of application and removal, associated low mortality rate, and low cost. Cable ties could not be reused like

wire or pipe cleaners but they are relatively inexpensive and can be found at any hardware store.

6.1.2. Ligature application

Ligatures were only applied to nestlings after their wing feathers erupted but before their tail feathers were unsheathed. This time period was chosen for two reasons. First, using any time period standardized the collection of prey across species with slightly different developmental rates. Second, this time period avoided many of the risks associated with ligature method. When the nestlings are very young it is necessary to tighten the cable ties completely, increasing the potential of strangulation. Once the tail feathers have completely grown in, the risk of nestlings fledging prematurely with a ligature still on increases. (This occurred once during my study, when a house wren nestling jumped out of the nest with the ligature still on and could not be recaptured.)

To apply the ligatures all nestling were removed from the nest and placed in a cloth bag. One by one, each nestling was removed from the bag, a ligature applied, and the nestling returned to the nest. All nestlings in a brood were ligatured simultaneously for a period of approximately one hour. At the end of an hour tweezers were used to remove un-swallowed prey items from the crop. Each nestling was then placed back in the cloth bag. Again, one by one, each nestling was removed from the bag, the cable tie was removed using

wire cutters (Mellott and Woods, 1993), and the nestling was returned to the nest. Placing the nestlings in a bag and applying/removing nestlings one-by-one assured that a ligature was never left on by accident. This process was repeated 3-4 times during the 10 days that nestlings were of the right age.

7. Collection/Handling of prey items

Prey items were collected in clean glass jars (1-2 dram shell vials) and stored on ice. Within 12 hours, all prey items were individually weighed, placed in a glass jar, sealed in a Ziploc © bag and frozen at –30° C. In 2006, prey items were identified to order after the completion of the field season. In 2007, all prey items were identified to order at the time of weighing. To obtain a dry weight and solid fraction each sample was individually freeze dried using a Labconco © Benchtop Freeze Dry System. Once each sample was freeze dried it was weighed again and the solid fraction was calculated as total dry weight divided by total wet weight.

8. Mercury Analysis

Analysis for total mercury was completed using a Direct Mercury Analyzer (DMA-80 Milestone, Inc.) at three laboratories (Trace Elemental Research Laboratory (TERL) at Texas A&M University in College Station, TX, US EPA Region One Laboratory (EPA) in North Chelmsford, MA, and the College of William and Mary (W&M) in Williamsburg, VA). The Milestone DMA-

80 uses cold vapor atomic absorbance spectroscopy, the preferred method for mercury analysis (Clesceri LS et al., 1998), and detailed methodology can be found in the owners manual (DMA Manual, Milestone Inc.). The factory calculated instrument detection limit (IDL) is 0.005 ng Hg. Every 20 samples consisted of a combination of two of three standards reference materials (SRM: DORM-2, DORM-3, or DOLT-3), a methods blank, and a sample blank. Mean percent recoveries for THg of standard reference materials was 97.995% ± 0.637 (DORM-2; n = 13), 97.831% ± 0.426 (DORM-3; n = 31), and 96.553 ± 0.512 (DOLT-3; n = 50).

8.1. Minimum detection limit

The minimum detection limit (MDL) was calculated by running seven aliquots of a sample with a low mercury concentration. The standard deviation of the seven concentrations was then calculated. Then the standard deviation was multiplied by the appropriate t-statistic for seven replicates and six degrees of freedom (Helsel, 2005b). The MDL was calculated twice at W&M and both times it was 0.0055 ppm. EPA and TERL calculated their own MDL. The MDL of 0.0055 was the highest MDL for the three labs, though only slightly (e.g. TERL MDL = 0.0051) and 0.0055 was used for all samples. Avian blood samples were run at W&M (27%) and TERL (77%). All of the feather samples were done at W&M. All of the 2006 prey items were done at EPA and all of the 2007 prey items were done at W&M.

8.2. Duplicate samples

All samples reported herein were analyzed as part of a larger study on mercury exposure in avian species by D. Cristol. As part of the larger study, over 2,500 blood, feather, and prey samples were analyzed for total mercury with a DMA-80 at one of the three laboratories listed above. Duplicates were done when possible but were often not possible because many prey items and feather samples were run whole due to their small size and to avoid homogenization problems. Furthermore, blood was often not collected in a sufficient amount to allow for duplicates. Inter-laboratory duplicates were done when possible and exist for W&M-TERL and W&M-EPA. However, due to time and cost constraints, duplicates between TERL-EPA were not possible.

8.2.1. Duplicate methods

There were three methods for duplicate samples. Duplicates were done by (1) crushing and homogenizing large prey items, (2) splitting the total number of back and body feathers in half, and (3) analyzing blood from the same bird but collected in two different tubes. It should be noted that all four of the methods mentioned above were not duplicates of the exact same material, for example different drops of blood may contain different amounts of mercury, or different aliquots of a homogenized insect may vary in mercury load. Achieving a perfectly homogenized insect sample was not possible due to the

presence of indestructible parts such as wings. Although every effort was made to mix feathers thoroughly this was often difficult because it is hard to cut small enough pieces. The third method, two tubes of blood from the same bird, was also not a perfect duplicate because in some cases one tube of blood was collected from the right wing and the other from the left wing. One last caution must be given when interpreting duplicate samples and that is that the interval between inter-laboratory duplicates ranged from six months to two years, so there could have been effects of storage time.

8.2.2. Duplicate mercury values

The difference between duplicate samples is reported as the relative percent difference (RPD) between the first sample and second sample. For samples below the MDL (n = 13), 1/2MDL was substituted. The mean RPD was then calculated separately for all samples with a mean concentration less than two times the MDL (n = 8), between two and ten times the MDL (n = 26) and all samples with a mean concentration greater than ten times the MDL (n =192). The mean RPD for all samples with an average concentration less than two times the MDL was 48.3 ± 67.0%, for samples between two and ten times the MDL the mean RPD was 54.50 ± 65.3%, and for those samples with a mean concentration greater than ten times the MDL the RPD was 15.73 ± 27.53%.

In sum, the MDL was 0.0055 ppm and recovery for all SRMs was greater than 95%. For all duplicate samples greater than ten times the MDL the RPD was less than the accepted 20 percent when inter-laboratory duplicates are included. Therefore, all values were considered highly comparable (Minnesota Clean Water Partnership Program, 2000). The high RPD values for duplicates with less than 10 times the MDL, while discouraging, represent a small number of samples (<20%) and only those with biologically unimportant mercury levels (< 0.05 ppm), and thus should not affect any of my conclusions.

9. Values below the detection limit

In many ecological and epidemiological studies some values fall below the MDL (commonly called 'non-detects'). The proportion of values falling below the MDL varies greatly from study to study and often determines what is to be done with these values. How non-detects are handled statistically can have consequences for the study's conclusions and ultimately determine policy decisions. Several methods, each with their own biases, have been suggested for dealing with values below the detection limit (Helsel, 1990; Helsel, 2005a).

9.1. Deleting values

Deleting all values below the MDL is used when a biased answer is considered better than no answer. This method can be considered

conservative if a contaminated site is simply being compared to a reference site and all values below the detection level are from the reference site. However, this has the potential of eliminating a whole class of data and in policy situations is unacceptable and a waste of money and time (Helsel, 2005a).

9.2. The substitution method

The substitution method (0, 1/2MDL, or the MDL) is probably the most common method because it is easy and allows for statistical comparisons. However, it has fallen out of favor because variation is eliminated and there is no basis for selecting a particular substitution value (Helsel, 1990). When less than then 10% of the samples fall below the MDL (as is the case in this study) it has arbitrarily been deemed acceptable to use the substitution method (Lubin et al., 2004).

9.3. The fill in method

When 10-30% of the data are below the detection limit the "fill in" method has been shown to produce unbiased parameters (Helsel, 1990; Lubin et al., 2004). In this method, the data are determined to fit a specific distribution and maximum likelihood estimates (MLE) are used to produce summary statistics. Then, values from below the detection limit are randomly sampled and used as replacement values for the all values below the MDL

(Helsel, 1990; Lubin et al., 2004). This method works poorly if the distribution of the data is unknown, the sample size is low, or greater than 30% of the data is below the MDL (Helsel, 1990; Lubin et al., 2004). Another consideration is that the fill in method performs very well when estimating the median and percentiles but less well when estimating the mean and standard deviation (Helsel, 1990).

9.4. Using the actual readings

Reporting the machine readings is another method used to deal with values below the detection limit (Helsel, 2005a). This method preserves the variation needed for statistical comparisons but does not allow the researcher to determine if values differ from zero or each other. In some senses, the machine is being treated as a random number generator. For instance, if the MDL is ten, one can not claim that a sample with value of four is more than one of two because no confidence can be instilled in the magnitude of results below the MDL. Additionally, the variation generated by this method can also be biased in a random direction effecting conclusions and decision making.

9.5. Qualitative comparisons

A final method is qualitative instead of quantitative. When two data sets are being compared and a high proportion of the values from one data set fall below the detection limit some argument can be made that statistical

comparisons are not necessary to tell that these two data sets are different. This argument is flawed because it is often necessary to tell the magnitude of difference between two data sets or use the data set with values below the MDL as a baseline in a future study.

9.6. Statistical treatment of non-detects in this study

In all, 502 prey items were analyzed for total mercury with 44 (8.8%) falling below the detection limit. No avian tissue samples fell below the detection limit. Although the substitution method is acceptable in this case, variation is still eliminated and therefore specific comparisons were not possible. The fill in method was used to replace the values below the MDL. First, the data was determined to conform to a lognormal distribution. Then using maximum likelihood estimates, the mean and standard deviation were determined. Using the software package Crystal Ball © a distribution with these parameters was created. Next, using Monte Carlo simulation the distribution was randomly sampled, with replacement, between zero and the detection limit. These values were then used to replace the values falling below the MDL in the original data set.

10. Statistics

When comparing contaminated populations to reference populations nonparametric Mann-Whitney *U* tests were used because of non-normal

distributions. I also used Mann-Whitney *U* tests when comparing sexes and ages within a species. The three avian species and prey groups within the contaminated site were compared with Analysis of Variance (ANOVA). When the ANOVA identified a significant difference I used post-hoc Tukey's test determine which groups were significantly different from one another. To determine the individual effects of multiple parameters I used a general linear model (GLM) to run an ANOVA. For all statistical comparisons of prey groups, years were combined. However, in the Monte Carlo/bootstrapping simulation, prey items from 2006 were used for Carolina and house wrens, but for eastern bluebirds, I used prey items from 2007 (see below for detailed explanation).

10.1. Migration and feather mercury

To test the hypothesis that a year-round resident (Carolina wren) was at greater risk to mercury exposure than a migratory species (house wren) I looked at the ratio between feather mercury and blood mercury. If year-round residents were exposed to more mercury during the course of the year than migratory birds, the ratio between feather and blood mercury levels would have been greater for year-round residents.

10.2. Daily mercury exposure

To determine if prey mercury levels could explain avian mercury accumulation I modeled exposure in the three species of terrestrial

insectivores. I used a novel approach that corrected for many of the assumptions made in other bioaccumulation/exposure models (Newman and Unger, 2003). To my knowledge this is the first time such an approach has been used and was only possible because I collected a sufficient number of the actual prey items each species was consuming.

The actual prey item weights and mercury levels were used in the simulation. For each species, a daily intake was determined from the literature for house wrens and scaled for the other two species (for which no comparable estimates were available). Also, for each bird species an average mass, standard deviation of the mass, minimum mass, and maximum mass were calculated from the actual weights collected from our field site in 2006 and 2007. These intake and mass values were then used to create distributions for a Monte Carlo simulation. Ten thousand daily intake values and ten thousand weights were randomly selected, with replacement, for each species. These intake and weight values were then correlated using a rank correlation coefficient of 0.75 for all three species. That is, larger individuals of each species had a larger daily intake compared to their smaller counterparts. This resulted in ten thousand simulated individuals of each of the three species. Each individual had a body weight (g) and consumed a given amount of food (g dw) per day.

10.3. The 'pool of prey items'

The actual dry weights and mercury values of the entire sample of prey items collected from each bird species was used as the 'pool of prey items' from which each simulated bird of that species could 'forage.' It is this aspect of the model that makes it unique and more informative than any previous models used for bioaccumulation of contaminants. This approach requires that the prey items in the model's 'pool of prey items' exist in the same proportions that they are found in avian species diet. This was only possible because a large number of prey items were collected from each avian species.

10.3.1. The source for the 'pool of prey items'

For wrens I used prey items collected in 2006 and for eastern bluebirds I used prey items collected in 2007. This was necessary because mercury values and dry weights for nearly half of the prey items collected from eastern bluebirds in 2006 were never obtained due to the failure of laboratory equipment. Therefore, the remaining biased sample of 2006 prey items was not used in the simulation where the nature of the entire pool of prey items. For all other statistical comparisons the prey items from 2006 and 2007 were combined because overall distribution of prey item types was not relevant (see above).

10.4. Values below the detection limit in the simulation

A total of 325 prey items were used in the analysis, of which only 15 (4.6%) had mercury values below the detection limit. In this case the percentage of values below the minimum detection limit was considerably < 10%, therefore the effects on the overall variation of replacing these unknown values with an estimate were negligible. I assigned half of the minimum detection limit to each (Lubin et al., 2004). Additionally, I was not using these values in a statistical test that was sensitive to the overall variation.

10.5. Individual simulated birds

Each of the ten thousand birds from the simulation randomly 'foraged' by choosing a single prey item, with replacement, from the given avian species' 'pool of prey items' until the individuals' daily intake was reached. Each prey item selected had a given amount of mercury (ng Hg) associated with it. These mercury values were summed for the day and divided by the bird's mass to generate a daily intake rate. The ten thousand daily intake values were then used to determine the distribution of exposure for each of the three species. The distributions of mercury values for prey items of terrestrial insectivores, aquatic insectivores, and piscivores were not normal. The values were therefore first log transformed to approximate a normal distribution. Then the log transformed percent distributions were plotted against each other to examine to what degree they overlapped.

It is possible that increasing or decreasing the proportion of each major prey group could alter a simulated birds' daily mercury exposure (ng Hg / day / gram of bird). By including individual values for each prey item and using the method I did, each simulated individual had a different diet. This allowed me to rigorously examine the relationships between the proportion of each major prey group in a simulated birds' diet and that birds' daily mercury exposure.

To determine the effect, the proportion of each prey group had on the daily mercury exposure for each species I examined the relationship between the proportion of each major prey group and the daily mercury exposure for 1000 simulated birds. First, from the simulation I calculated the proportion each of the major prey groups made up in the diet of 1000 individuals for each species. I then plotted the proportion of each of the major prey groups against daily mercury exposure. This resulted in three plots for Carolina and house wrens (Aranea, Lepidoptera, and Orthoptera) and four for eastern bluebirds (Aranea, Lepidoptera, Orthoptera, and Coleoptera).

10.6. Comparison of terrestrial prey items to aquatic prey items

To compare prey items collected from terrestrial species to those collected from aquatic species I combined all terrestrial prey items and log transformed the distribution of mercury levels. I then did the same for the prey items collected from the aquatic-foraging tree swallow and fish-eating belted kingfisher. This resulted in three log transformed distributions. I plotted the

distributions against one another to determine the degree of overlap. I did not included the aquatic and piscivorous birds in the intake simulation because the samples of actual prey items was not large enough to generate robusts 'pools of prey items' for the simulation.

Results

1. Nest box occupancy

Carolina wrens only used the nest boxes erected in habitats that specifically targeted them (small clearings under forest canopy). House wrens used boxes that were placed in open field habitats targeting tree swallows or eastern bluebirds. It is therefore with caution that I report nest box occupancy rates for house wrens because they used many nest boxes targeted for other species and thus the true number of "available" nest boxes is difficult to estimate.

Carolina wrens used both the plastic tube boxes and the wooden boxes. In both types of boxes Carolina wrens sometimes built partial nests that were never completed. In some cases a complete nest was built but never used. House wrens used only the wooden boxes, and like Carolina wrens, built many partial and complete nests that were never used.

The Carolina wren occupancy rate in plastic tubes along the South River was 10.6%. Downstream of the contamination source, there were a total of 94 plastic tube boxes erected at 11 sites that targeted Carolina wrens (Table 1). Of these, 12 received at least some nesting material characteristic of Carolina wrens, but only 10 clutches were initiated (Table 1).

In the wooden nest boxes specifically erected targeting wrens, the Carolina wren occupancy rate in wooden boxes along the South River was 16.3% and the house wren occupancy rate in wooden boxes along the South

River was 15.3%. In 2006, downstream of the contamination source, there were a total of 98 wooden boxes erected at 11 sites that targeted Carolina and house wrens (Table 1). Of these, 23 received at least some nesting material characteristic of Carolina wrens and 31 received at least some nesting material characteristic of house wrens. Clutches were initiated in 16 Carolina wren nests and 15 house wren nests (Table 1). In addition to initiating clutches in the wooden boxes targeting wrens, 11 house wren clutches were initiated in wooden nest boxes erected for tree swallows (for tree swallow nest box distribution see Brasso 2007), but these have not been included in the occupancy statistic.

2. Number of birds sampled

During the spring and summer of 2006, a total of 48 adult Carolina wrens were caught. Of these, 10 were caught on reference sites and 38 were caught on the contaminated sites (Table 2). Also during the summer of 2006, a total of 34 adult house wrens were caught. Of these birds, eight were caught on reference sites and 26 were caught on contaminated sites (Table 3). During the summer of 2006, a total of 33 nestling Carolina wrens were sampled from nine broods (Table 5). A total of 88 nestling house wrens were sampled from 17 broods (Table 6). Wren nestlings were not sampled on reference sites.

Table1

Number of boxes per site and number of clutches initiated per site in 2006

Site	Plastic Tube Boxes	Wooden Boxes	Carolina Wrens	House Wrens
Basic Park	12	0	0	0
Hopeman Parkway	9	5	4	2
Genicom	0	0	0	1
Dooms	4	5	2	4
Wertman	15	12	2	0
Dubai	4	16	4	0
Harris	0	4	0	2
Wampler	4	7	1	3
Boes	5	4	0	3
Harriston Crossing	9	10	3	0
Renkin	6	3	1	0
Grand Caverns	13	10	2	8
Grottoes City Park	13	8	5	4
Bradburn Park	0	14	2	0
Total	94	98	26	27

Table 2

Number of adult Carolina wrens sampled in 2006

Site	River	Hg Status	Males	Females	Unknown Sex	Total
Water Treatment Plant	South	C	0	0	1	1
Basic Park	South	C	0	0	0	0
Hopeman Parkway	South	C	1	1	0	2
Genicom	South	C	0	0	0	0
Dooms	South	C	1	1	0	2
Wertman	South	C	0	1	0	1
Augusta Forestry Center	South	C	0	1	2	3
Dubai	South	C	1	4	0	5
Harris	South	C	1	0	0	1
Wampler	South	C	1	0	0	1
Boe	South	C	2	0	0	2
Harriston Crossing	South	C	3	3	0	6
Renkin	South	C	3	0	0	3
Grand Caverns	South	C	0	1	0	1
Grottoes City Park	South	C	3	2	0	5
Bradburn Park	South	C	3	2	0	5
Contaminated Subtotal			**19**	**16**	**3**	**38**
P. Buckley Moss Barn	South	R	1	0	0	1
Ridgeview Park	South	R	3	0	0	3
Dories' Property	Middle	R	1	1	0	2
Fort River Road	Middle	R	2	0	0	2
Auckerman's Property	North	R	1	0	0	1
Wildwood Park	North	R	1	0	0	1
Reference Subtotal			**9**	**1**	**0**	**10**
Total			**28**	**17**	**3**	**48**

Table 3

Number of adult house wrens sampled in 2006

Site	River	Hg Status	Males	Females	Unknown Sex	Total
Water Treatment Plant	South	C	0	0	0	0
Basic Park	South	C	0	0	0	0
Hopeman Parkway	South	C	0	1	0	0
Genicom	South	C	0	0	0	1
Dooms	South	C	3	2	0	5
Wertman	South	C	0	0	0	0
Augusta Forestry Center	South	C	0	0	0	0
Dubai	South	C	0	0	0	0
Harris	South	C	1	1	0	2
Wampler	South	C	1	1	0	2
Boe	South	C	3	1	0	4
Harriston Crossing	South	C	0	0	0	0
Rankin	South	C	0	0	0	0
Grand Caverns	South	C	4	4	0	8
Grottoes City Park	South	C	1	3	0	4
Bradburn Park	South	C	0	0	0	0
Contaminated Subtotal			**13**	**13**	**0**	**26**
P. Buckley Moss Barn	South	R	0	0	1	1
Ridgeview Park	South	R	3	0	0	3
Dories' Property	Middle	R	2	1	1	4
Fort River Road	Middle	R	0	0	0	0
Auckerman's Property	North	R	0	0	0	0
Wildwood Park	North	R	0	0	0	0
Reference Subtotal			**5**	**1**	**2**	**8**
Total			**18**	**14**	**2**	**34**

Table 4

Number of wren nestlings (broods in parentheses) sampled along the contaminated portion of the South River in 2006

Site	Carolina Wren	House Wren
Water Treatment Plant	0	0
Basic Park	0	0
Hopeman Parkway	8 (2)	0
Genicom	0	0
Dooms	4 (1)	22 (4)
Wertman	0	0
Augusta Forestry Center	0	0
Dubai	9 (3)	0
Harris	0	5 (1)
Wampler	2 (1)	11 (2)
Boe	0	11 (2)
Harriston Crossing	5 (1)	0
Renkin	0	0
Grand Caverns	0	29 (5)
Grottoes City Park	0	10 (3)
Bradburn Park	5(1)	0
Total	**35 (9)**	**88 (17)**

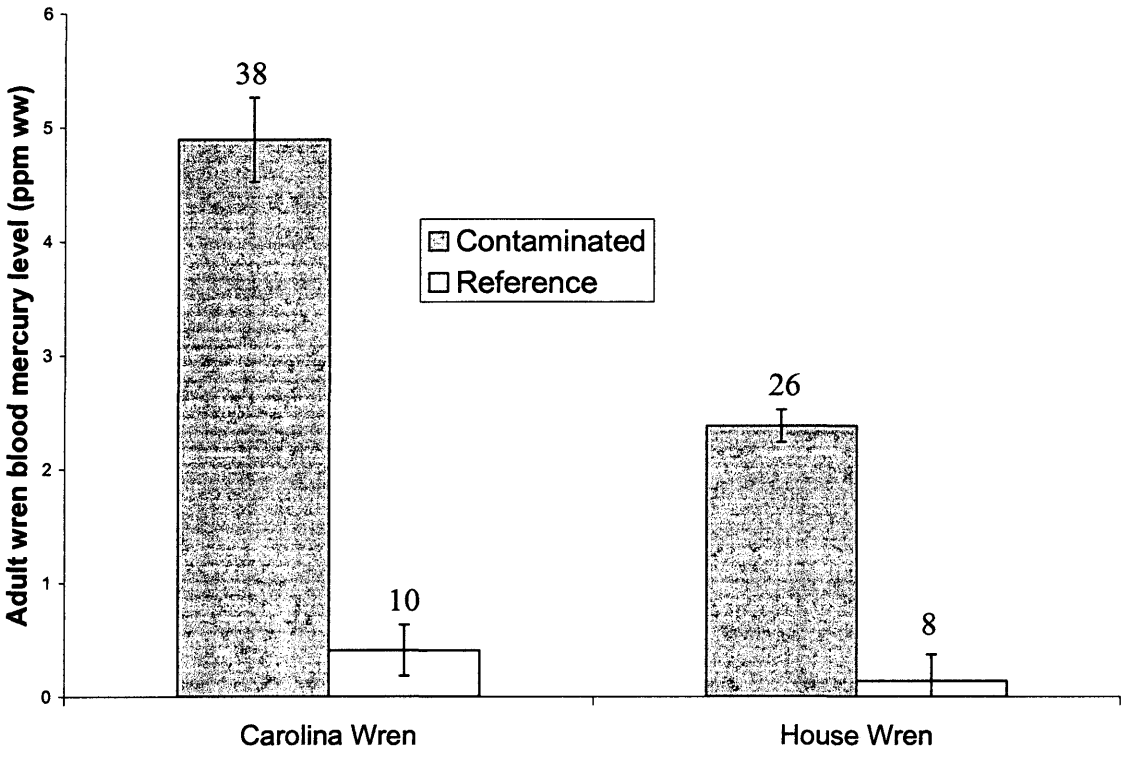
3. Mercury levels

In 2006, adult Carolina wrens nesting within 50 m of the South River had elevated blood mercury levels compared to the reference population (Fig.; $w = 1118.0$, $p < 0.001$). Likewise, in 2006, adult house wrens nesting within 50 m of the South River had elevated blood mercury levels compared to the reference population (Fig. 1; $w = 559.0$, $p < 0.001$). In 2006, on the contaminated site, adult blood mercury levels were significantly different among the two species of wrens and bluebirds (Fig. 2; $F_{2,102} = 53.35$, $p < 0.001$). Post hoc comparisons showed that significant differences existed for all comparisons (Carolina wren>house wren, Carolina wren>eastern bluebird: $p < 0.0001$; house wren>eastern bluebird: $p = 0.01$).

Compared to nestlings, adult Carolina and house wrens had significantly elevated blood mercury levels (Fig. 3; Carolina: $w = 1992.0$, $p < 0.001$; house: $w = 2627.0$, $p < 0.001$). In 2006, nestling Carolina and house wrens were only sampled on contaminated sites. Therefore, no comparisons between contaminated and reference nestlings was possible. When I compared nestling Carolina and house wrens and eastern bluebird nestlings there was a significant difference in blood mercury levels (Fig. 4; $F_{2,183} = 80.08$, $p < 0.001$). Post hoc comparisons showed that significant difference existed for all comparisons (Carolina wren>house wren, Carolina wren>eastern bluebird, and house wren>eastern bluebird: $p = 0.0001$).

Figure 1

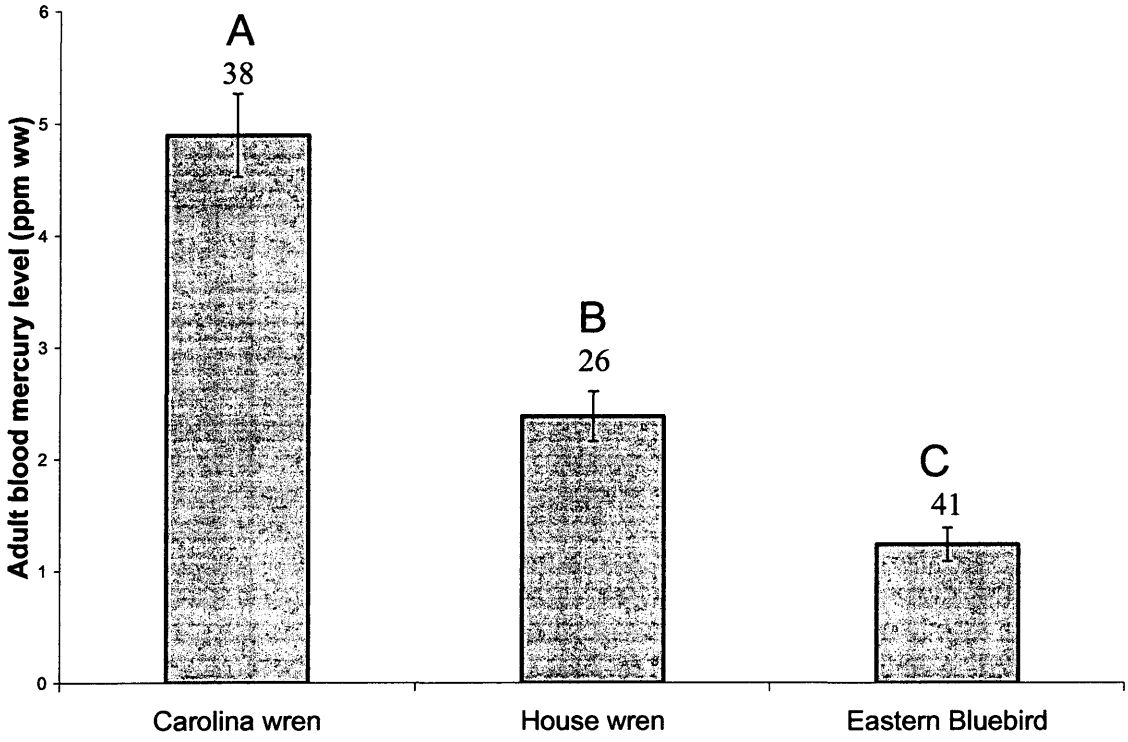
Comparison of contaminated and reference Carolina wren adult blood mercury levels in 2006



Error bars represent standard error of the mean. Samples sizes are shown above error bars.

Figure 2

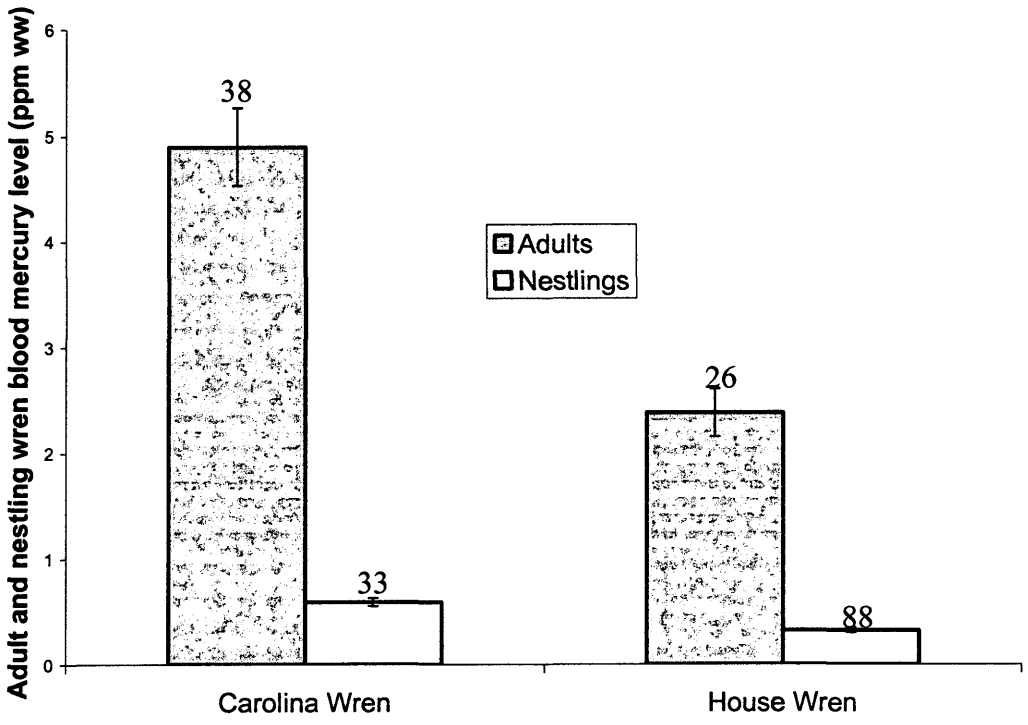
Comparison of contaminated Carolina wren, house wren, and eastern bluebird adult blood mercury levels in 2006



Error bars represent standard error of the mean. Samples sizes are shown above error bars.

Figure 3

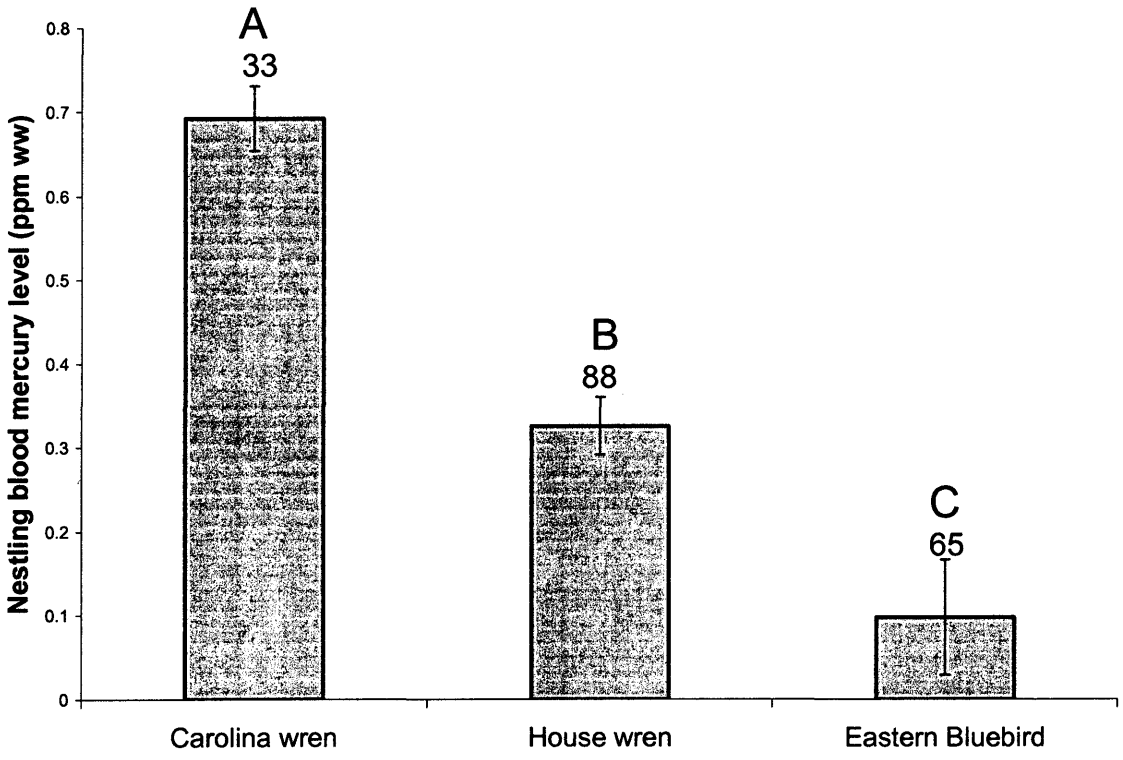
Comparison of adult wren blood mercury level to nestling blood mercury levels in 2006



Error bars represent standard error of the mean. Samples sizes are shown above error bars.

Figure 4

Comparison of contaminated Carolina wren, house wren, and eastern bluebird nestling blood mercury levels in 2006



Error bars represent standard error of the mean. Samples sizes are shown above error bars.

3.1. Variables that could affect mercury exposure in adults

3.1.1. Sex

The fact that female Carolina wrens are smaller than males (Haggerty and Morton, 1995) and in both species of wrens, females can eliminate mercury via egg production (Evers et al., 2005), suggest that females might have lower mercury body burdens. I tested this hypothesis by comparing male and female blood mercury levels on the contaminated site in both species. There was no difference in blood mercury levels between adult male and female Carolina Wrens (Fig. 5; $w = 301.0$, $p = 0.69$) or adult male and female house wrens (Fig. 5; $w = 185.0$, $p = 0.64$).

3.1.2. Spatial and temporal variation

Adult blood mercury varied considerably from kilometer zero in Waynesboro to kilometer 38.3 in Port Republic. Mercury sometimes varies with date because it can become more available for bioaccumulation during the warmer months due to increased methylation rates. To rigorously determine the independence of river kilometer or date on blood mercury levels, it would have been necessary to collect large samples from each study site across a period of time. This was not possible because species density was not sufficient and birds nest synchronously within sites. However, in an attempt to detect dramatic effects of river kilometer or date on blood mercury level I grouped collection dates by 14-day periods and used river kilometer and the

grouped date intervals as factors in an ANOVA. For Carolina wrens I found no significant effect of either river kilometer (Fig. 6, $F_{6,37} = 1.07$, $p = 0.41$) or date (Fig. 6, $F_{13,37} = 0.54$, $p = 0.87$). Likewise, for house wrens there was no effect of either river kilometer (Fig 7, $F_{4,25} = 0.7$, $p = 0.60$) or date (Fig. 7, $F_{6,25} = 1.05$, $p = 0.431$).

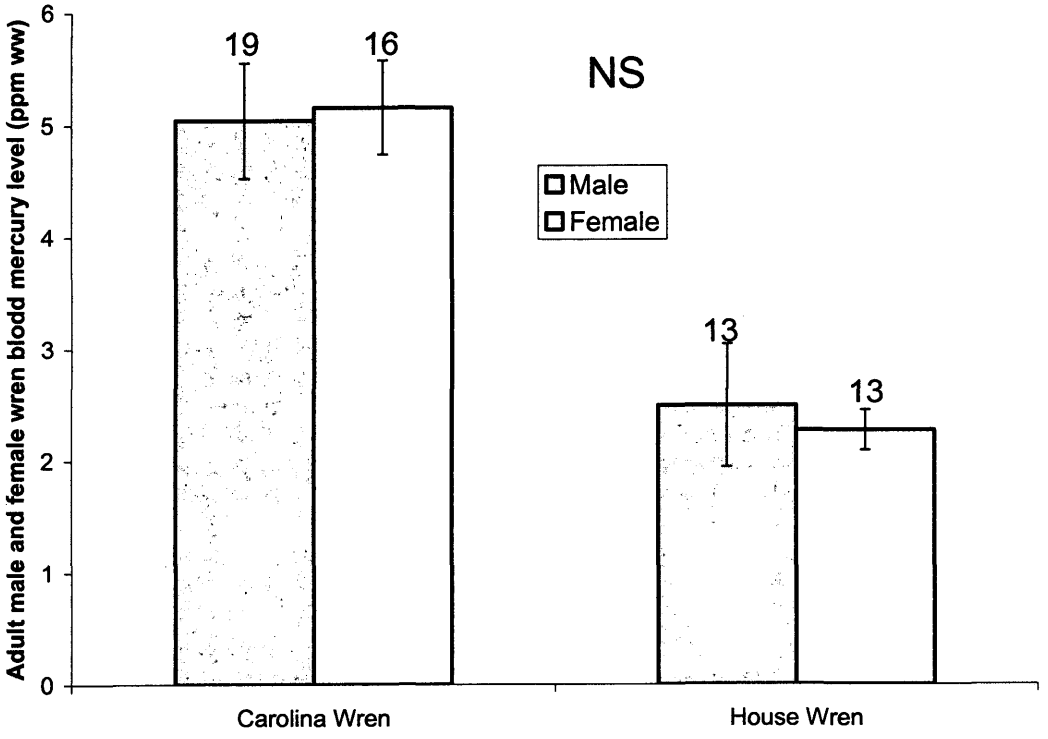
4. Feather mercury

From the contaminated site, 35 adult Carolina wrens and 26 adult house wrens were sampled for back and body feather mercury. From the reference site, nine Carolina wrens and seven house wrens were sampled for back and body feather mercury. Both contaminated Carolina ($w = 945.0$, $p < 0.001$) and house wren ($w = 496.0$, $p = 0.02$) body feather mercury were significantly elevated over the reference site (Fig. 8).

Carolina wrens are year-round residents, while house wrens migrate, only spending four months on the contaminated site. To test the hypothesis that duration of exposure would affect feather mercury levels, I compared the ratio of feather mercury to blood mercury levels between the two species. The mean feather to blood ratio for Carolina wrens was higher (2.442 ± 0.226 SE) than for house wrens (0.5849 ± 0.0829 SE; $w = 1490.0$, $p < 0.0001$).

Figure 5

Comparison of contaminated adult male and female Carolina wren blood mercury levels in 2006



Error bars represent standard error of the mean. Samples sizes are shown above error bars.

Figure 6

Carolina wren blood mercury levels with collection date grouped by river Kilometer (larger symbols indicate greater distance from the source of mercury)

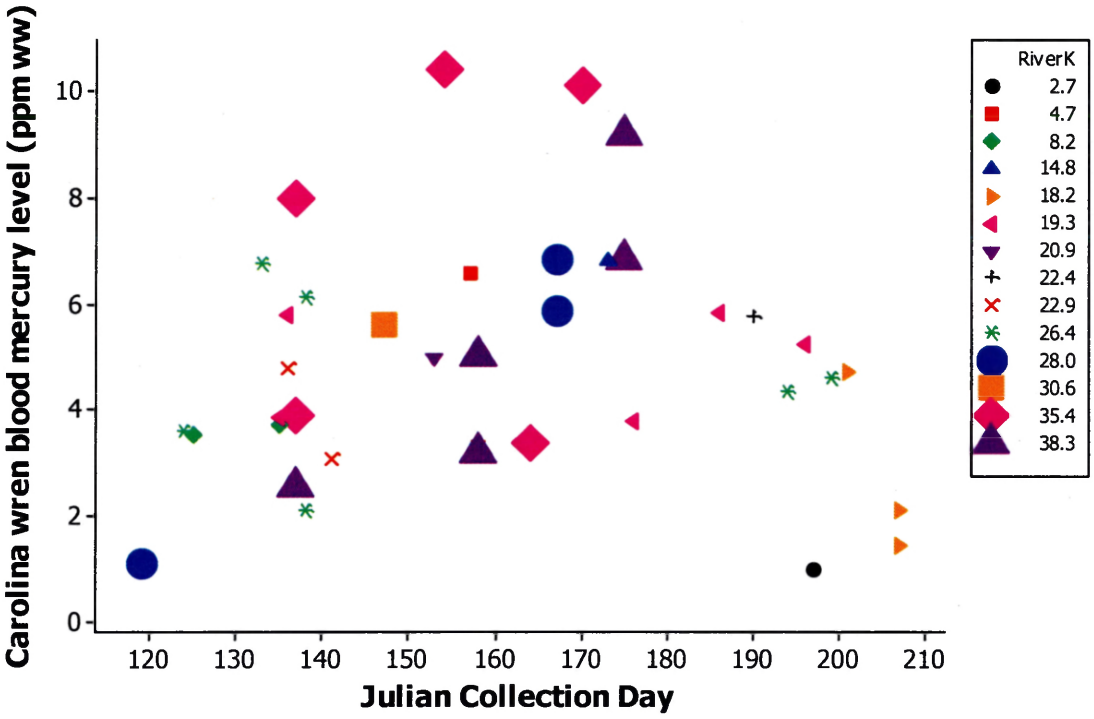


Figure 7

House wren blood mercury levels with collection date grouped by river Kilometer (larger symbols indicate greater distance from the source of mercury)

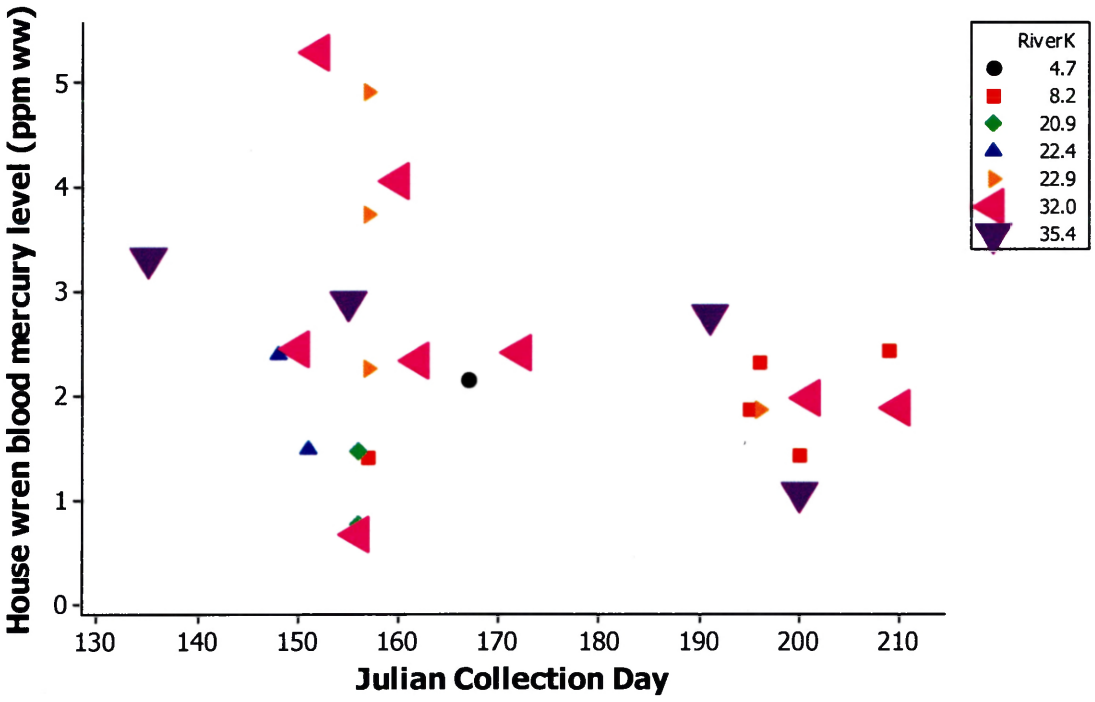
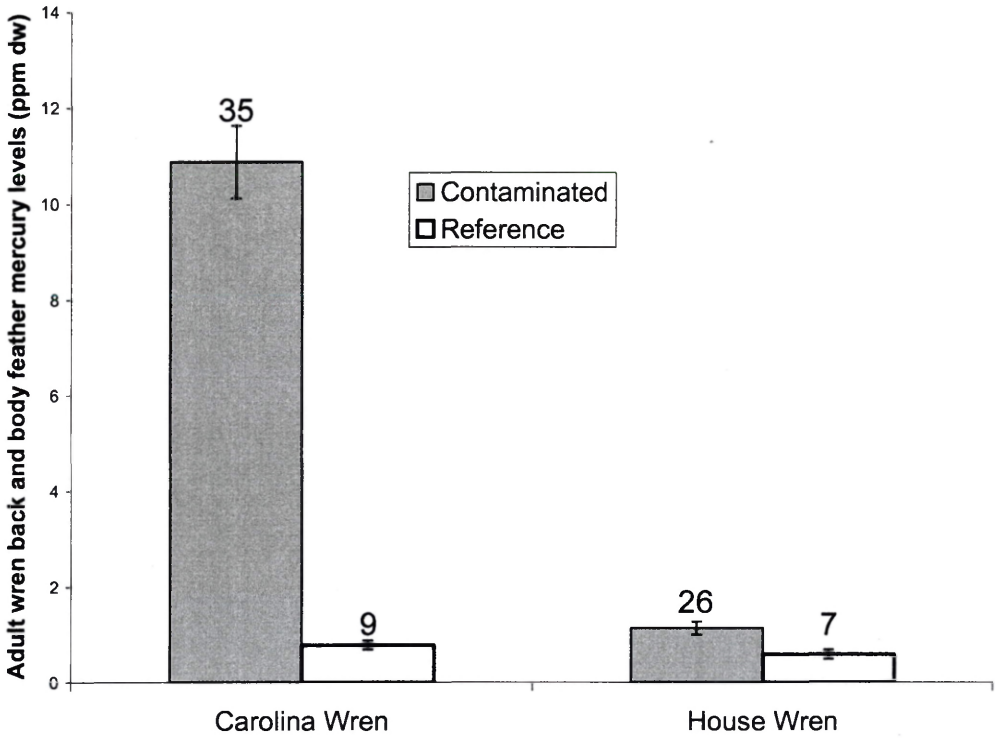


Figure 8

Comparison of contaminated adult wren back and body feather mercury levels



Error bars represent standard error of the mean. Samples sizes are shown above error bars.

5. Sample sizes of prey

Prey items were successfully collected from all three species of terrestrial insectivores. During the summer of 2006 a total of 363 prey items were collected from three species of terrestrial insectivores: 72 from Carolina wrens, 139 from house wrens, and 152 from eastern bluebirds. Of the 72 prey items collected from Carolina wrens 70 were identifiable to order. Of the 139 prey items collected from house wrens 126 were identifiable to order. Of the 152 prey items collected from eastern bluebirds during the summer of 2006 147 were identifiable to order (Table 5). During the mercury analysis process in 2006, eastern bluebird prey items were destroyed, due to equipment failure; thus mercury values were not obtained for 68% of the Aranea, 33% of the Coleoptera, 68% of the Lepidoptera, and 33% of the Orthoptera. This made it impossible to include the 2006 eastern bluebird prey items in the simulation because prey groups were no longer represented in the same proportions in the 'prey population' as they were found in the diet. However, these mercury values were used when comparing contaminated sites to reference sites and mercury levels between prey groups, because the lost samples were not biased with respect to mercury values.

At the end of 2006 it became clear that there were insufficient samples of prey from eastern bluebirds. In addition, I realized that obtaining reference prey items would be beneficial in terms of demonstrating that prey was a route

of exposure for birds at contaminated sites. In 2007, 149 prey items were collected from bluebirds on the contaminated site and 92 from the reference site. Because reference prey were unlikely to have much mercury I decided it was not necessary to collect from additional bird species.. Of the 241 prey items collected from eastern bluebirds during the summer of 2007, 229 (95.0%) were identified to order and analyzed for total mercury.

Since I collected prey items that the birds were actually eating, rather than sampling from traps or nets, I avoided relying on the dubious assumption that prey collected by humans is similar to that eaten by the birds. I did make the assumption that each prey item collected was an independent sample from the contaminated site, even though this may not be the case, because items collected from the same bird or nest could be considered pseudo-replication. As previously mentioned, I also assumed that prey brought back for nestlings was the same as that eaten by adults.

6. Diet description

In 2006 fresh weights were obtained for all but two items from Carolina wrens and three from house wrens. From eastern bluebirds, fresh weights were obtained for all but two items collected on the contaminated site in 2006 and 2007 (100% from reference samples). On a fresh weight basis, Fig. 9 shows that all three species consumed a diet consisting of mainly Aranea (spiders), Lepidoptera (moths, butterflies, and their larvae), Coleoptera

(beetles), and Orthoptera (crickets and grasshoppers). On a fresh weight basis, these four prey groups made up more than 70% of all three species' diets (Fig.10; unknown included in other). The prey group making up the next highest component of any species' diet was opiliones (daddy longlegs) at 8.65% from house wrens. For Carolina wrens and house wrens the same relationships held true when the diets were examined on a dry weight basis (Fig. 11). It was not possible to examine the diet of eastern bluebirds from 2006 on a dry weight basis because, due to the mishap in the laboratory, dry weights were obtained for less than 60% of prey items collected from eastern bluebirds and the relationship between fresh and dry weight across prey groups was not clear. For eastern bluebirds, the only species for which prey items were collected across years and on the reference sites, diets were qualitatively similar between the contaminated and reference sites in 2007 and between 2006 and 2007 (Fig. 12). In sum, Aranea, Lepidoptera, and Orthoptera made up the majority of the diet of Carolina and house wren with eastern bluebirds also consuming Coleoptera in substantial amounts.

Table 5

Number of prey items collected from the three terrestrial insectivores in 2006

	Carolina Wren		House Wren		Eastern Bluebird		Total	
Prey Group	N	N for THg	N	N for THg	N	N for THg	Total	Total for THg
Aranea	16	16	35	34	28	9	79	59
Plant Matter	0	0	0	0	6	6	6	6
Coleoptera	0	0	3	3	34	22	37	25
Dermaptera	0	0	9	9	1	1	10	10
Dictoptera	0	0	1	1	1	1	2	2
Diptera	0	0	10	10	1	1	11	11
Hemiptera	1	1	4	3	1	1	6	5
Hymenoptera	1	1	0	0	1	1	2	2
Isopod	4	4	0	0	0	0	4	4
Lepidoptera Adult	11	10	19	19	9	0	39	29
Lepidoptera Larvae	22	22	17	17	24	11	63	50
Lepidoptera Pupae	7	7	2	2	2	0	11	9
Lepidoptera Total	40	39	38	38	35	11	113	88
Myriapoda	0	0	0	0	1	1	1	1
Odonata	0	0	0	0	1	0	1	0
Opilione	3	3	14	14	1	0	18	17
Opisthopora	0	0	0	0	5	5	5	5
Orthoptera	3	3	12	11	30	20	45	34
Mollusc Shell	2	1	0	0	1	0	3	1
Unknown	2	0	13	0	5	0	20	0
Total	72	68	139	123	152	79	363	270

Figure 9

Diet comparison for the three species of terrestrial insectivores on a fresh weight basis from 2006 (Carolina wren, house wren, and eastern bluebird) and 2007 (eastern bluebird only)

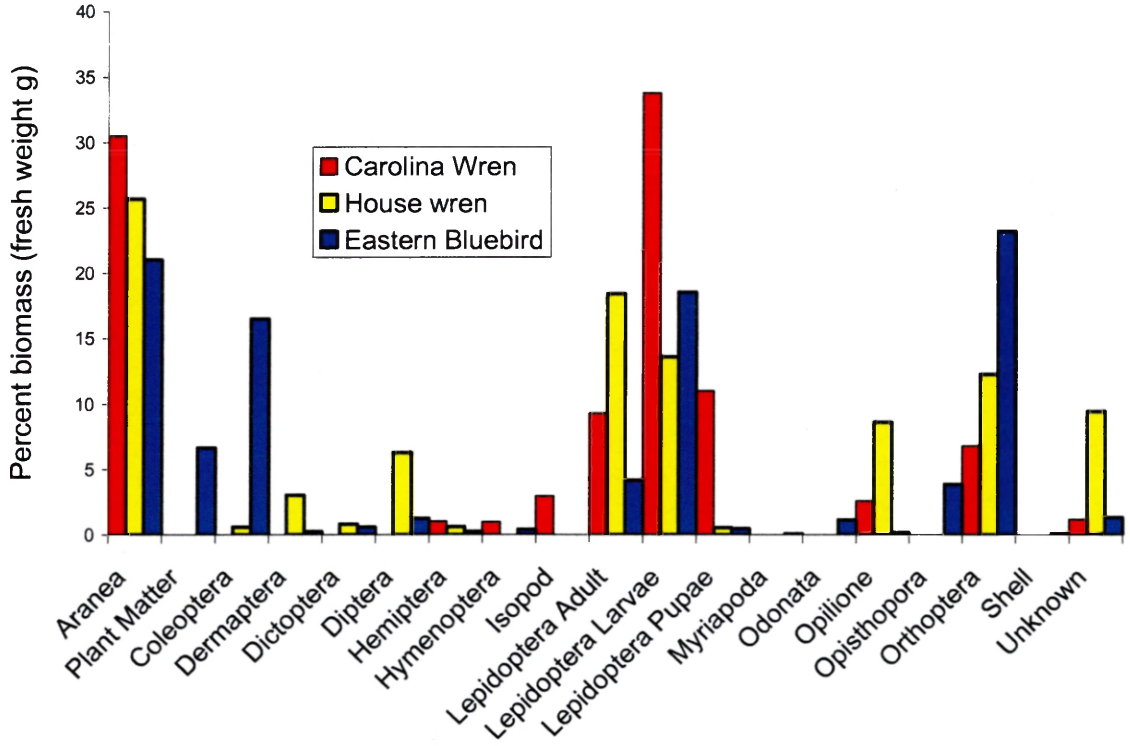


Figure 10

Diet comparison by major prey groups for the three species of terrestrial insectivores on a fresh weight basis from 2006 (Carolina wren, house wren, and eastern bluebird) and 2007 (eastern bluebird only)

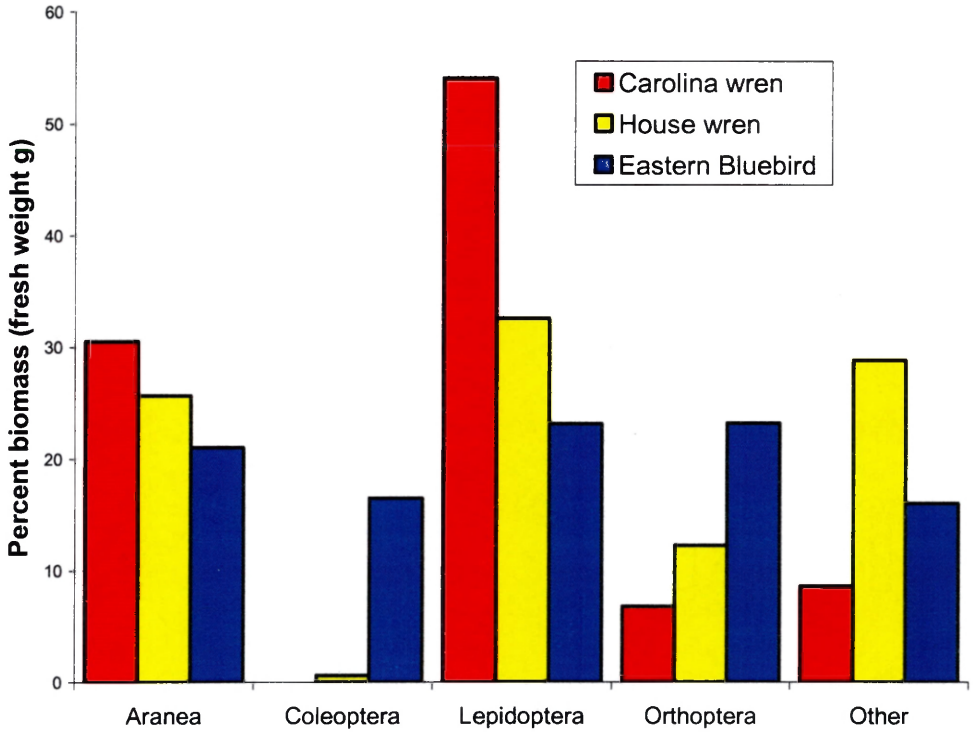


Figure 11

Diet comparison by major prey groups for Carolina and house wrens on a dry weight basis from 2006

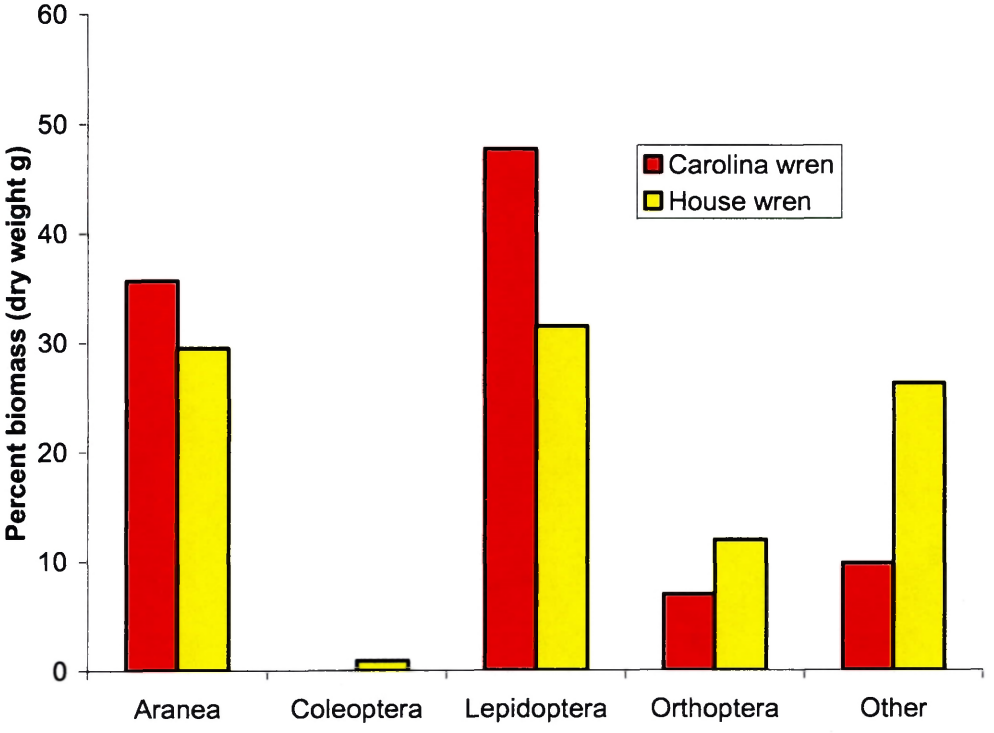
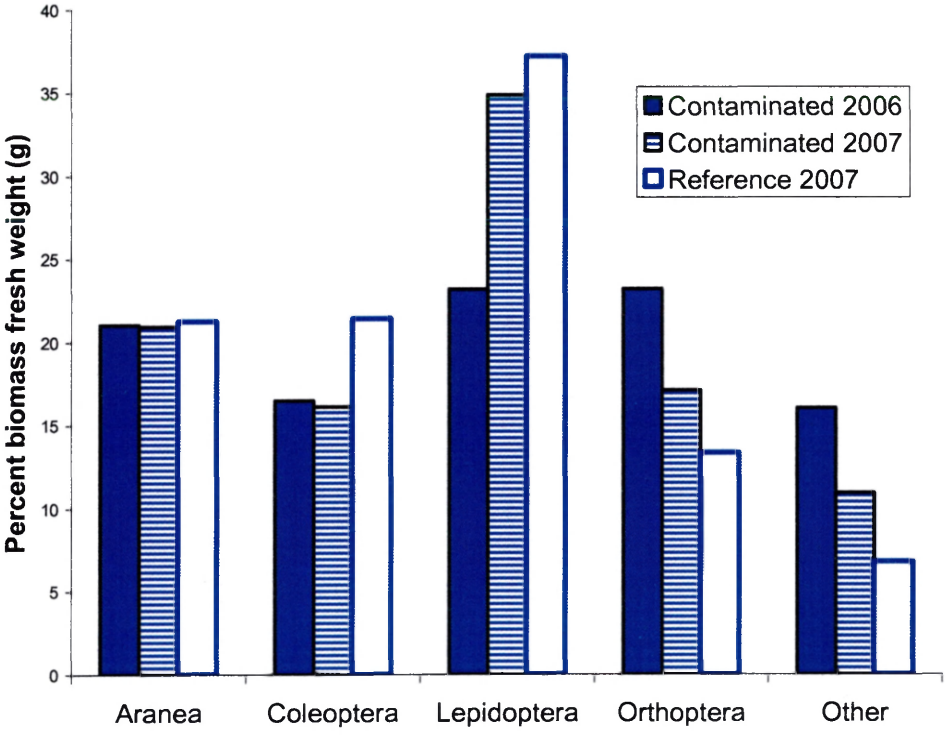


Figure 12

Diet comparison by major prey groups for eastern bluebirds on a fresh weight basis from the contaminated and reference sites in 2006 and 2007



7. Prey mercury analysis

Of the prey items collected from Carolina wrens, 68 (94%) were individually analyzed for total mercury. From house wrens, 123 of 139 (88%) prey items were individually analyzed for total mercury and from eastern bluebirds in 2006 79 of 152 (52%) prey items were analyzed for total mercury (Table 7). Only 52% of the prey items from eastern bluebirds during 2006 were analyzed for total mercury due to mechanical complications with the mercury analysis process.

7.1. Can prey groups be combined across avian species?

The two main goals of this study were to determine through which prey items terrestrial insectivores as a whole were accumulating mercury and whether prey from contaminated sites had elevated mercury levels compared to prey from reference sites. Therefore, I combined prey groups across avian species, for example combining all contaminated spiders regardless of which avian species collected them. This gave me the ability to compare the major prey groups (Aranea, Coleoptera, Lepidoptera, and Orthoptera) consumed by terrestrial insectivores.

Statistical support exists for combining prey groups across avian species. To determine if the avian species from which a prey item was collected had a significant effect on that prey item's mercury level I used a GLM to run an ANOVA with prey group, avian species, and river kilometer as

factors. I found a significant effect of prey group but not for avian species or river kilometer (Table 6). Therefore, for all analyses to follow, prey groups were combined across avian species, making for greater and more robust comparisons.

8. Can mercury levels be combined across years?

To further increase sample sizes I wished to combine bluebird prey collected on contaminated sites in 2006 and 2007. To determine if mercury levels differed between 2006 and 2007 I compared both adult blood mercury levels and prey items from the two years. Adult eastern bluebird blood mercury levels between the two years did not differ significantly (Fig. 13; $w = 1965.0$, $p = 0.35$). This suggests that mercury exposure was similar across the two years. Comparing prey items from 2006 to those from 2007 was not straightforward because not all prey groups were collected in the same numbers from the same river kilometers in the two years. To examine the effect year had on prey mercury levels I used a GLM to run an ANOVA with prey group, avian species, and river kilometer as factors. There was a highly significant effect of prey group and marginally significant effect of river kilometer (Table 7). Combined with the fact that adult blood mercury levels did not differ, for all analyses to follow except the Monte Carlo/bootstrapping simulation, prey items from 2006 and 2007 were combined.

9. Were prey items from the contaminated sites elevated compared to prey items from the reference sites?

To determine if prey items from the contaminated sites had elevated mercury levels relative to those from the reference sites I compared average mercury levels of the major prey groups (Aranea, Coleoptera, Lepidoptera, and Orthoptera). All major prey groups collected from terrestrial insectivores nesting within 50 meters of the contaminated South River had significantly elevated mercury levels compared to those collected from a reference population of terrestrial insectivores (Table 8; all $P < 0.0001$).

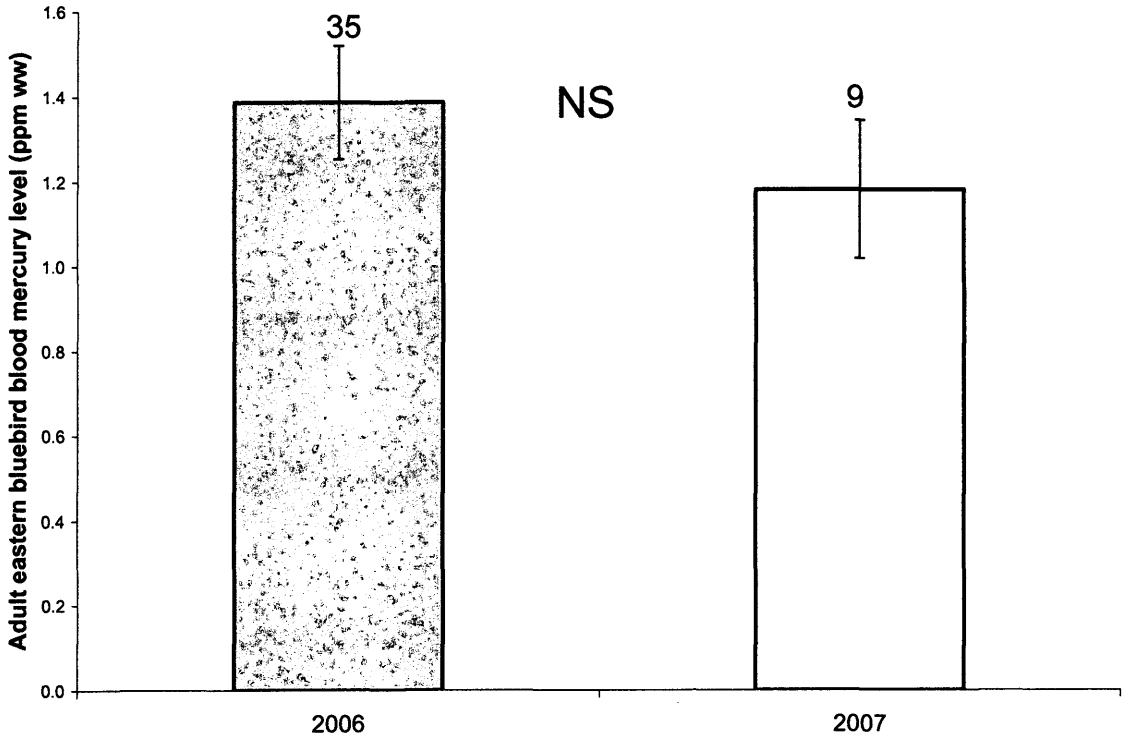
Table 6

The individual effects of Prey Group, River Kilometer, and Avian Species on prey mercury levels (ppm dw)

Factor	Degrees of Freedom	F	P-value
Prey Group	4	7.59	0.001
Avian Species	2	0.58	0.559
River Kilometer	15	1.58	0.080

Figure 13

Comparison of adult eastern bluebird blood mercury levels between 2006 and 2007



Error bars represent standard error of the mean. Samples sizes are shown above error bars.

Table 7

The individual effects of Prey Group, River Kilometer, Avian Species, and Collection Year on prey mercury levels (ppm dw)

Factor	Degrees of Freedom	F	P-value
Prey Group	4	6.20	0.001
Avian Species	2	0.17	0.840
River Kilometer	16	1.79	0.030
Year	1	0.73	0.394

Table 8

Contaminated prey group mercury levels compared to reference prey group mercury levels (ppm dw)

	Contaminated		Reference	
Prey Group	Mean (SE)	n (<DL)*	Mean (SE)	n (<DL)*
Aranea	1.242 (0.145)	101 (0)	0.0500 (0.006)	25 (0)
Coleoptera	5.550 (2.370)	48 (0)	0.1397 (0.311)	27 (1)
Lepidoptera	0.382 (0.178)	137 (13)	0.0221 (0.133)	23 (20)
Orthoptera	0.307 (0.173)	50 (3)	0.0020 (0.001)	6 (6)

*Number of samples below the detection limit

10. Did mercury accumulation differ by prey type?

To determine through which prey items terrestrial insectivores were accumulating mercury I compared the mercury levels between the prey groups that together represented the major portion (>70%) of each avian species' diet. For Carolina and house wrens, whose diets were similar, I compared the mercury levels of Aranea, Lepidoptera, and Orthoptera. Neither Carolina nor house wrens consumed Coleoptera and together, Aranea, Lepidoptera, and Orthoptera made up 91.4% and 70.5%, respectively, of their diets. There was a significant difference between the mercury levels of the three prey groups (Fig. 14; $F_{2,285} = 8.26$, $p = 0.001$) and post hoc comparisons showed that the differences existed between Aranea and the other two groups (Aranea>Lepidoptera: $p = 0.001$ and Aranea>Orthoptera: $p = 006$), but not between Lepidoptera and Orthoptera ($p = 0.963$).

For eastern bluebirds, I compared the mercury levels of Aranea, Coleoptera, Lepidoptera, and Orthoptera, which comprised 83.6% of their diet. There was a significant difference between the mercury levels of the four prey groups (Fig. 15; $F_{3,335} = 8.42$, $p < 0.001$) and post hoc comparisons showed that Coleoptera contained more mercury than the other three groups ($p < 0.001$) but there were no significant differences between Aranea, Lepidoptera, and Orthoptera ($p > 0.05$).

11. Did prey mercury levels explain avian mercury exposure?

To determine if prey mercury levels could explain avian mercury exposure I modeled exposure in the three species of terrestrial insectivores. The daily intakes determined from the literature (Johnson, 1998) and the average mass, standard deviation of the mass, minimum mass, and maximum mass are shown in Table 9. Figures 16 and 17 show the distributions created for the Monte Carlo simulation. Figure 18 shows the relationship between mass and intake when a correlation coefficient of 0.75 is assumed.

The Monte Carlo simulation, randomly sampled weights and intakes from the distributions, resulted in ten thousand daily mercury exposure values (ng Hg/day/gram of bird) for each species. On average, simulated Carolina wrens were exposed to more mercury on a daily basis than house wrens, which were exposed to more mercury than eastern bluebirds (Fig. 19). To test if these mean values were statistically different (Carolina wren>house wren>eastern bluebird), I used an ANOVA on the exposure values generated from the simulation. I found a statistical difference ($F_{2,2997} = 7718.86$, $p < 0.0001$) and post hoc comparisons showed that statistical differences existed for all comparisons (Fig. 19; $p < 0.001$ for all comparisons). There was considerable overlap in the natural log transformed percent distributions of daily mercury exposures for the three species, demonstrating that although, on average, they were exposed to different amounts of mercury, a portion of each species populations are experiencing the same exposure (Fig. 20).

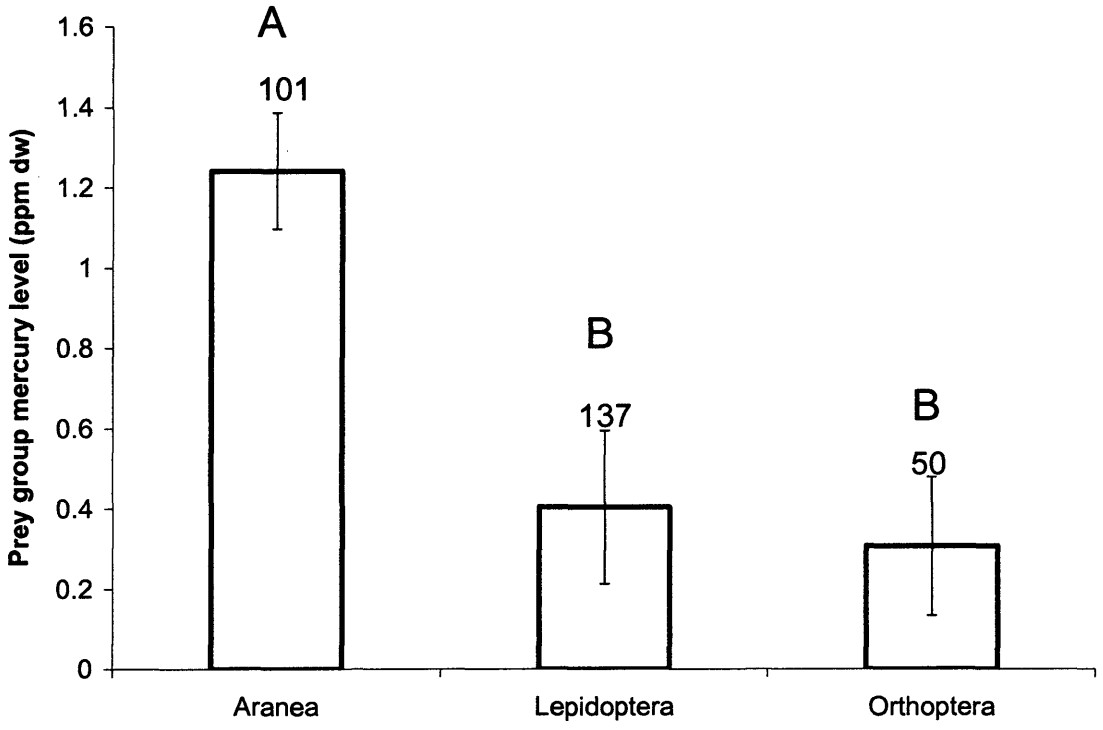
For Carolina and house wrens there was a positive, significant correlation (Table 10) between the proportion of Aranea in the diet and daily mercury exposure (Figs. 21 and 22, panel 1). The opposite was true for Lepidoptera and Orthoptera; there was a significant (Table 10), negative correlation between the proportion of the diet consisting of Lepidoptera or Orthoptera and daily mercury exposure (Figs. 21 and 22, panels 2 and 3). For eastern bluebirds there were no trends or significant relationships (Table 10) between the proportion of major prey groups in the diet and daily mercury exposure (Fig. 23, panels 1, 2, 3, and 4).

12. Comparison to aquatic insectivores and piscivores

Boluses of flying insects and whole fish were collected from breeding adult tree swallows and from belted kingfishers respectively (see Brasso and Cristol in press; White 2007 for methodology). The swallow boluses which contained primarily Diptera and Ephemeroptera, had mean total mercury concentrations of 0.974 (± 0.207; n = 29). The fish, which were of dozens of species had a range of sizes, had a mean total mercury concentration of 1.292 (± 0.384; n = 21). The overall mean total mercury for all invertebrate prey items collected from terrestrial species was 1.326 (± 0.297; n = 412). The natural log transformed percent distributions of total mercury concentrations, for the three groups of prey items showed a high degree of overlap (Fig. 24).

Figure 14

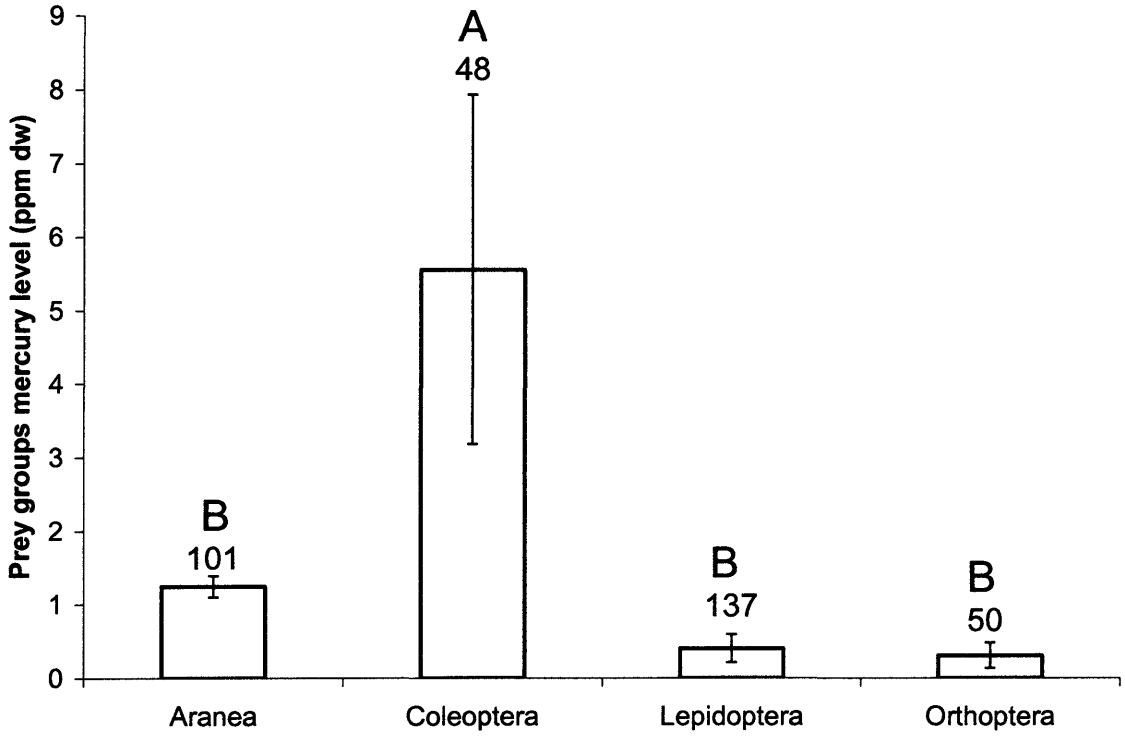
Comparison between major prey groups consumed by Carolina and house wrens



Error bars represent standard error of the mean. Samples sizes are shown above error bars.

Figure 15

Comparison between major prey groups consumed by eastern bluebirds (Note change in y-axis)



Error bars represent standard error of the mean. Samples sizes are shown above error bars.

Table 9

Daily intake (±SD) estimated from the literature, and average mass (±SD), maximum and minimum mass values from field data for birds used in the simulation

Species	Daily intake	Mass	Minimum mass	Maximum Mass
Carolina Wren	4.77(± 0.38)	19.48 (±1.79)	16	23
House Wren	2.42 (± 0.19)	10.66 (±0.78)	9	12
Eastern Bluebird	6.41 (± 0.64)	28.90 (±2.91)	22	37
Tree Swallow	4.80 (± 0.38)	21.20 (±1.55)	18	23
Belted Kingfisher	1/2 Body Weight	151.6 (±20.80)	125	215

Figure 16

Frequency distributions of daily food intake of Carolina wrens (gray bars), house wrens (black bars), and eastern bluebirds (open bars) for Monte Carlo simulation of mercury exposure in terrestrial insectivores

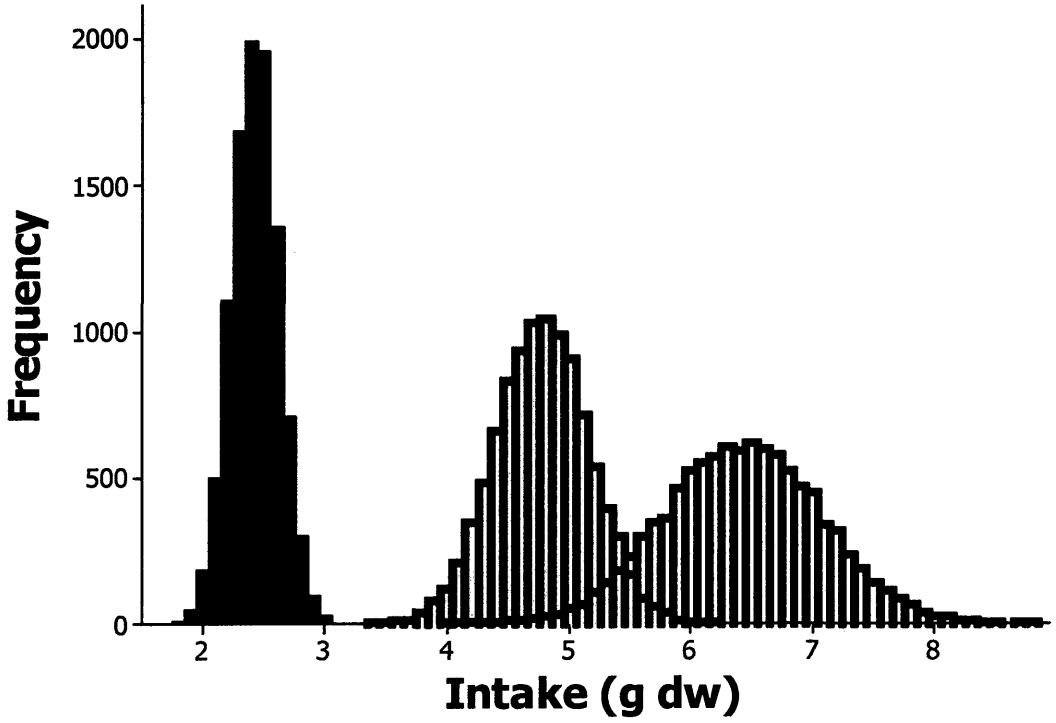


Figure 17

Frequency distributions of mass of Carolina wrens (gray bars), house wrens (black bars), and eastern bluebirds (open bars) for Monte Carlo simulation of mercury exposure in terrestrial insectivores

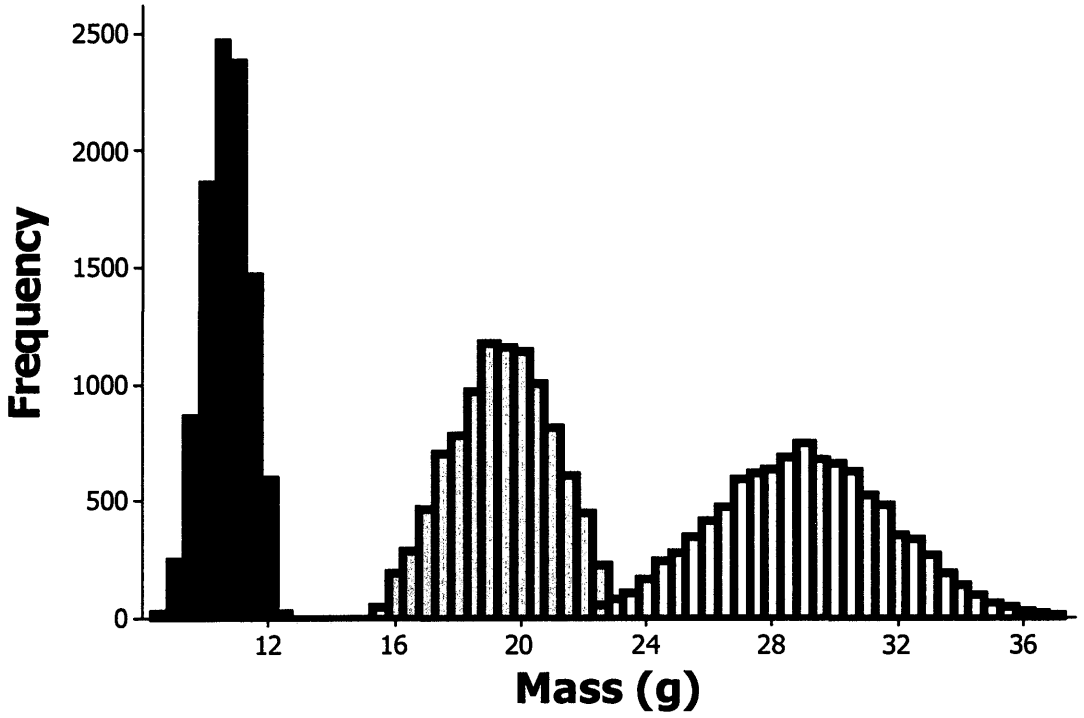


Figure 18

Correlation of mass and daily intake for Carolina wrens (closed circles), house wrens (open circles), and eastern bluebirds (open squares) for Monte Carlo simulation of mercury exposure in terrestrial insectivores

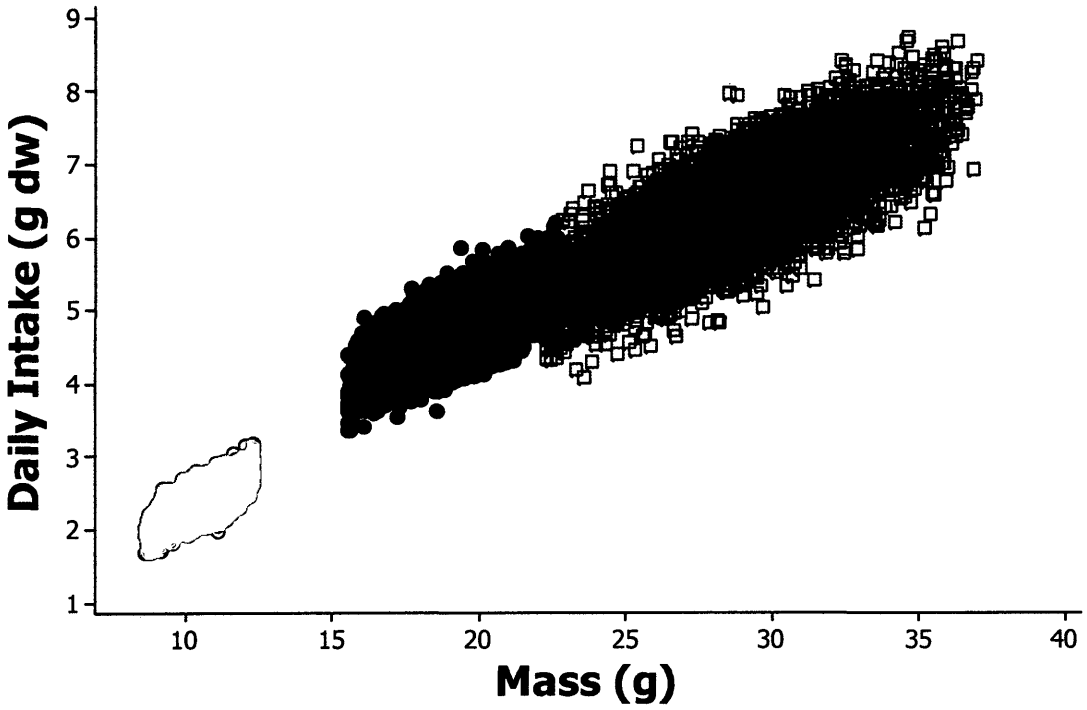


Figure 19

Comparison of average daily mercury exposure values generated from the Monte Carlo simulation (n = 1000 for all categories) for Carolina wrens, house wrens, and eastern bluebird (bars not sharing a common letter are significantly different)

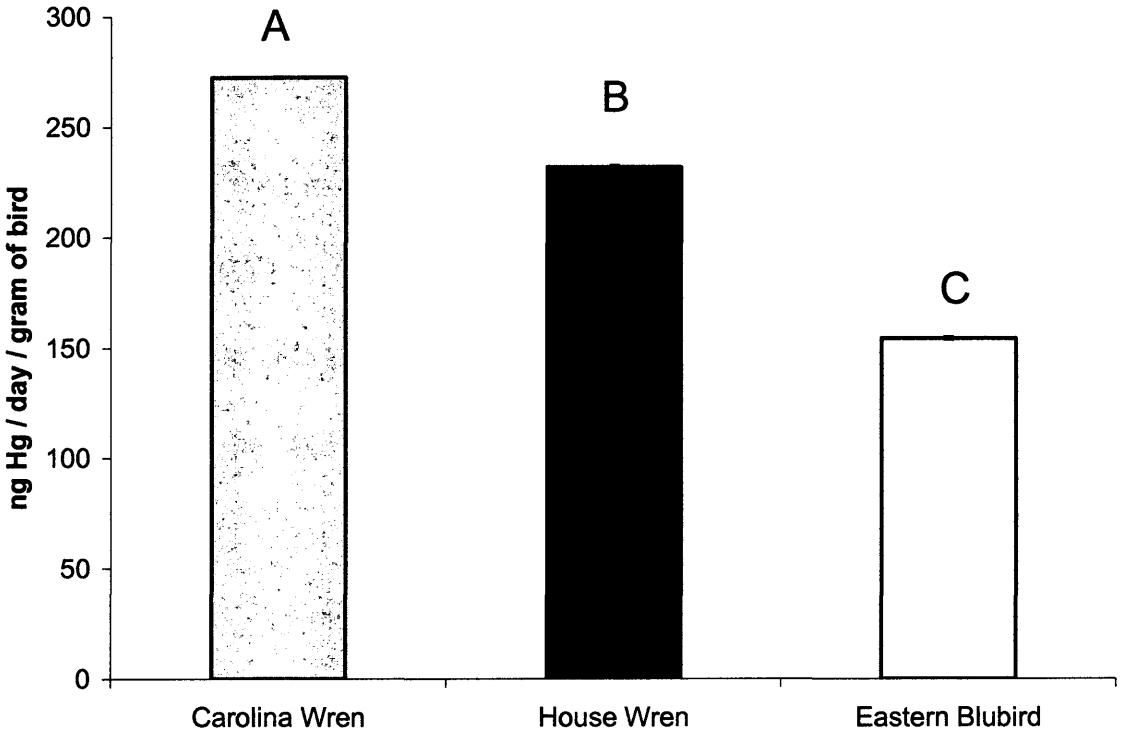


Figure 20

Comparison of frequency distributions of daily mercury exposure values generated from the Monte Carlo simulation for Carolina wrens, house wrens, and eastern bluebird

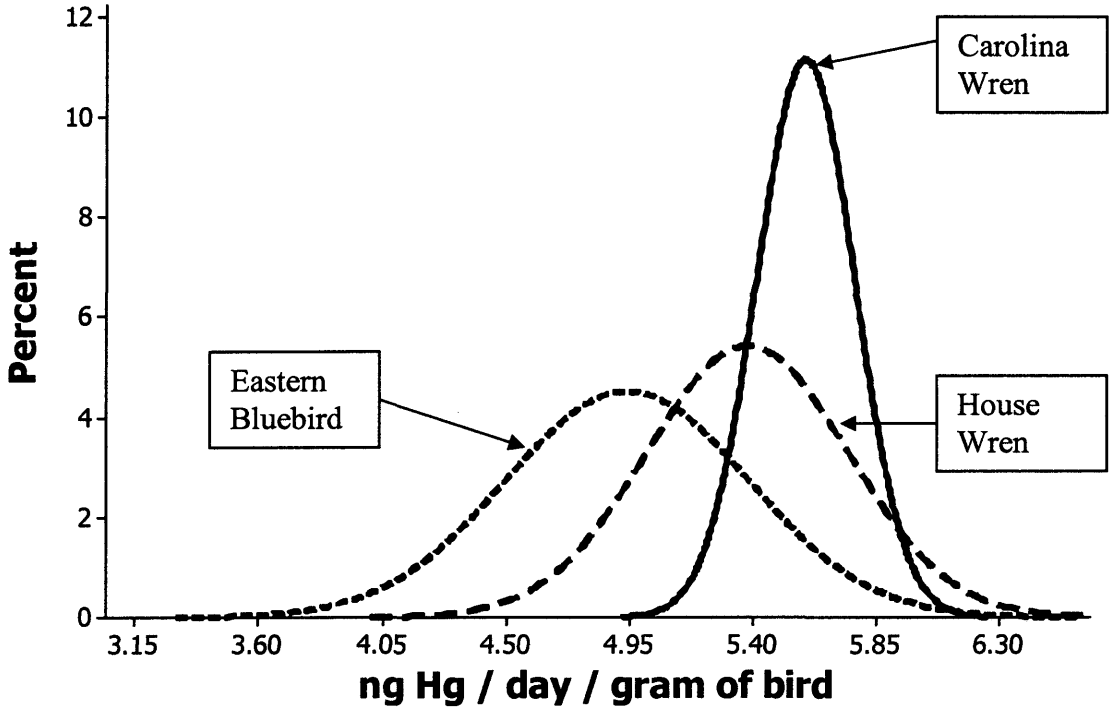


Table 10

Statistics of the relationship between the proportion of each major prey group in the diets' of each species and daily mercury exposure

	Aranea			Lepidotpera		
Species	Pearson Corr. Coef	p-value	R^2 of the fitted line	Pearson Corr. Coef	p-value	R^2
Carolina Wren	0.734	<0.001	0.53	-0.526	<0.001	0.28
House Wren	0.602	<0.001	0.67	-0.365	<0.001	0.13
Eastern Bluebird	0.033	0.301	0.01	-0.014	0.648	<0.01

	Orthoptera			Coleoptera		
Species	Pearson Corr. Coef	p-value	R^2 of the fitted line	Pearson Corr. Coef	p-value	R^2
Carolina Wren	0.287	<0.001	0.08	NA	NA	NA
House Wren	-0.145	<0.001	0.02	NA	NA	NA
Eastern Bluebird	-0.029	0.36	<0.01	-0.001	0.982	<0.01

Figure 21

Relationship between the proportion of daily mercury exposure (ng Hg / d / g of bird) and Aranea, Lepidoptera, and Orthoptera in Carolina wrens

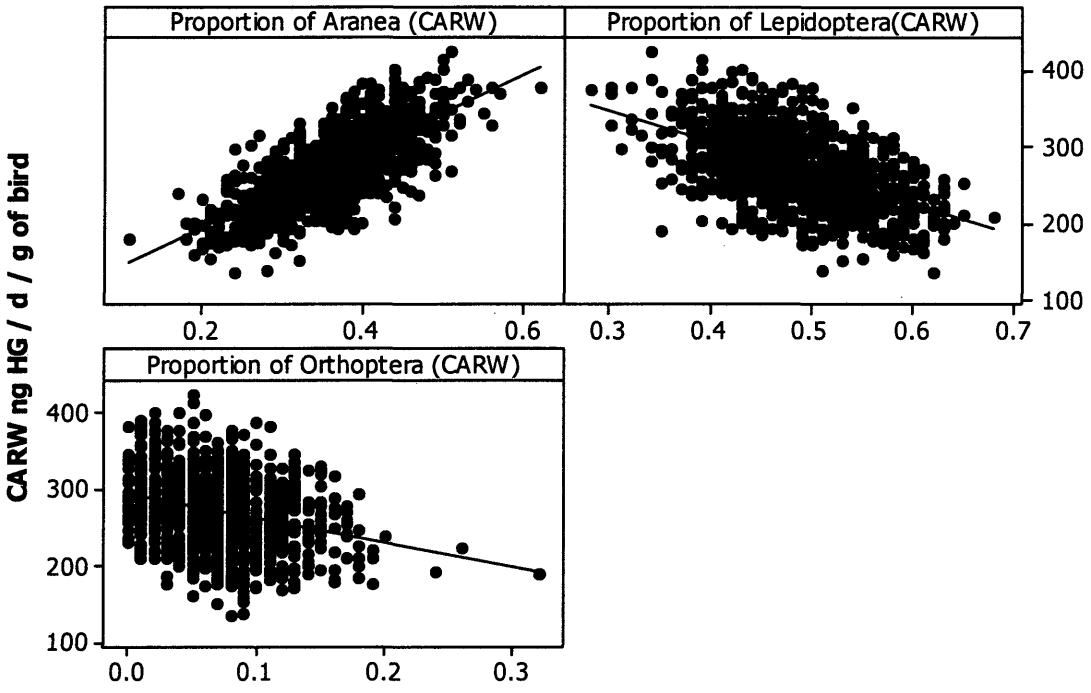


Figure 22

Relationship between the proportion of daily mercury exposure (ng Hg / d / g of bird) and Aranea, Lepidoptera, and Orthoptera in house wrens

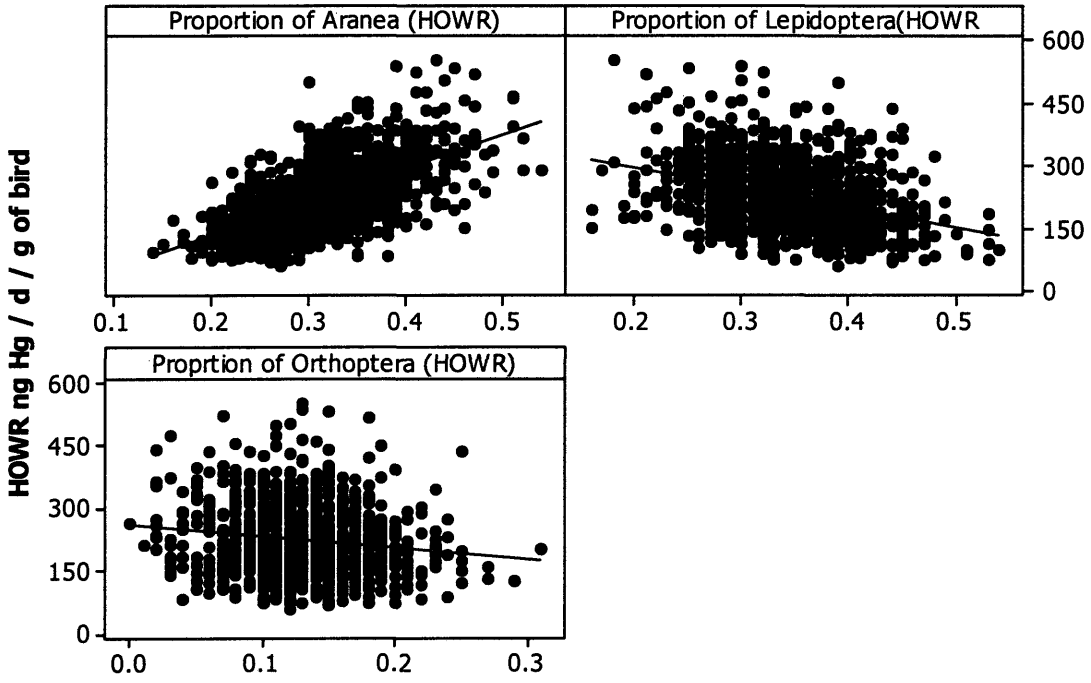


Figure 23

Relationship between the proportion of daily mercury exposure (ng Hg / d / g of bird) and Aranea, Lepidoptera, Orthoptera, and Coleoptera in eastern bluebirds

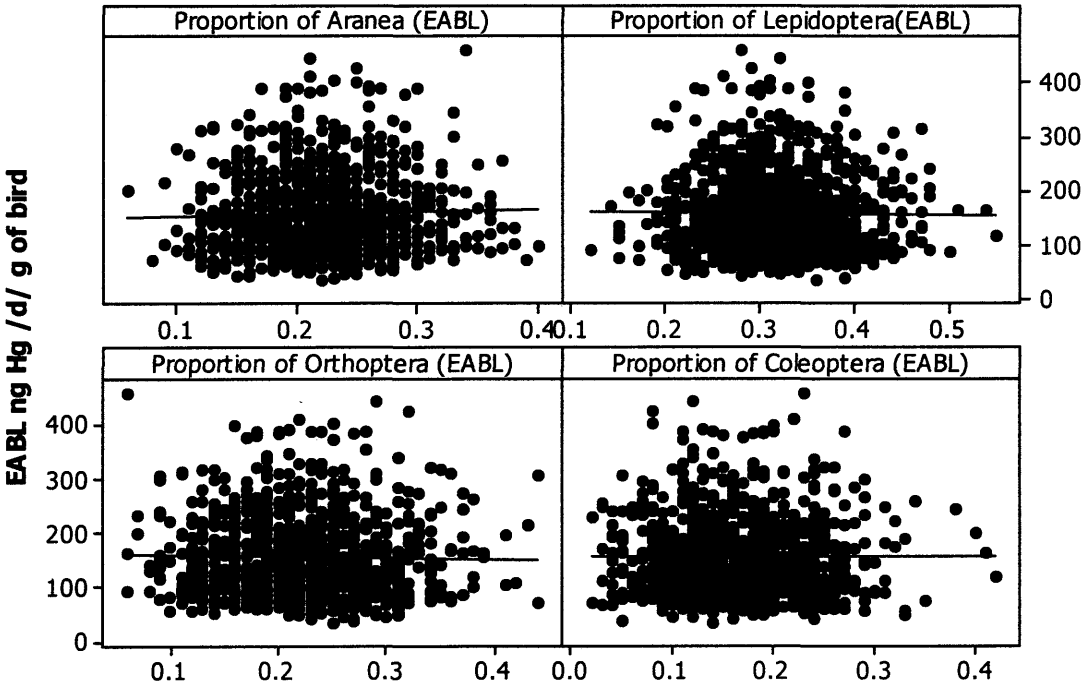
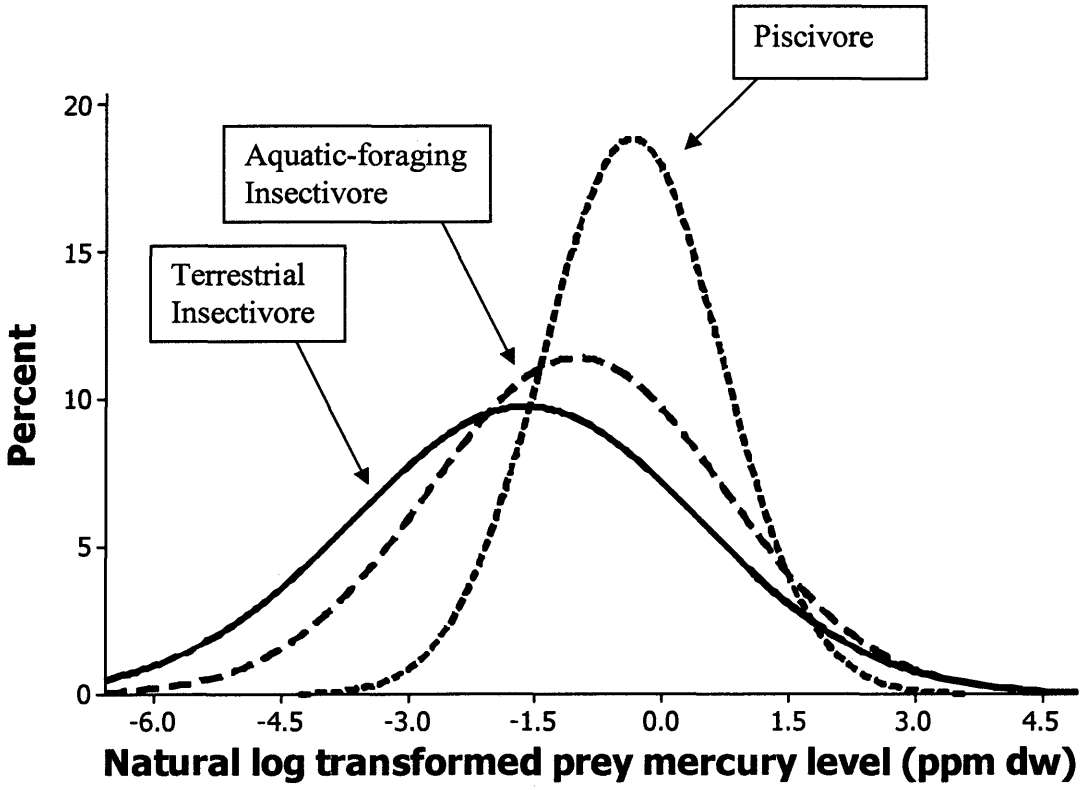


Figure 24

Comparison of percent distributions of the log transformed total mercury concentrations values in individual prey items collected from terrestrial insectivores, aquatic insectivores, and piscivores



Discussion

1. Mercury levels of birds

1.1. Blood mercury levels

Both Carolina and house wrens nesting within 50 m of the contaminated South River had significantly elevated blood and feather mercury levels compared to birds from a reference population. The two species' average contaminated blood mercury levels were more than ten times those of the average reference blood mercury levels. This confirms that insectivorous species nesting along the contaminated portion of the South River are accumulating mercury from the river and not from atmospheric sources. If contamination was solely from atmospheric sources, one would expect blood mercury levels to be similar across the relatively homogeneous Shenandoah Valley.

1.1.1. Sex

In neither species of wren was there a difference in blood mercury level between the sexes. The majority of females were caught 14-21 days after completion of egg laying; therefore, mercury excretion via the deposition in egg would not be reflected in these blood measurements because the half life of mercury in blood is on the order of a few weeks (Evers et al., 2005). Blood represents short term mercury exposure (Evers et al., 2005). Carolina wrens are sexually size dimorphic (Haggerty and Morton, 1995) and it is possible that

they have different diets which could result in different mercury levels, as occurs in common loons. Male common loons had higher mercury levels than females and they also are known to eat larger fish (Evers et al., 2005). House wrens are not sexually size dimorphic and not surprisingly the sexes did not differ in their blood mercury levels. This finding agrees with the findings of Brasso (2007) likely the only other study to address blood mercury levels and sex differences in a passerine. In that study no sex difference was detected in a large sample of tree swallows.

1.1.2. Spatial and temporal variation

Brasso (2007) reported that tree swallow blood mercury levels peaked at the Augusta Forestry Center site (relative river kilometer 18.2) and that a similar pattern existed for fish and sediment (South River Science Team, pers. Comm.). In 2005 and 2006 there also appeared to be a trend of decreasing blood mercury levels throughout the summer (Brasso, 2007). However, this relationship was difficult to untangle from site differences and would require samples to be collected from a single site across a range of dates, or better yet, from the same individuals across time. Unlike tree swallows, which nested in high densities, it was not possible to rigorously untangle spatial and temporal variation for either species of wren due to low nesting densities. Using an imperfect analyses, I detected no effect of date or location, but this result must be interpreted with caution due to lack of the ideal experimental design.

1.2. Are terrestrial insectivores accumulating mercury at a rate similar to aquatic species?

Adults: Two aquatic-foraging species nesting along the contaminated portion of the South River were intensively sampled during the summers of 2005 and 2006. The tree swallow, an aquatic-foraging insectivore, had an average blood mercury level of 3.66 (±2.42 SD) ppm ww and the belted kingfisher, a piscivore, had an average blood mercury level of 3.35 (±2.67 SD) ppm ww (Brasso, 2007; White, 2007). Carolina wren blood mercury levels were higher than both tree swallows and belted kingfishers. House wren blood mercury levels averaged below that of both the tree swallow and belted kingfishers. Among the 12 other insectivorous avian species sampled along the South River, the only species with a higher blood mercury level was the red-eyed vireo, *Vireo olivaceus*, average blood mercury level of 6.72 (± 4.60 SD; n = 5). Thus Carolina wrens are at greater risk of mercury exposure than aquatic-foraging insectivorous and piscivorous species.

Nestlings: In both species of wrens, adult blood mercury levels were elevated compared to nestling blood mercury levels. This agrees with what is already known about avian blood mercury levels. Nestling blood mercury levels are believed to be lower than that of adults because they are eliminating mercury into their newly growing feathers (Evers et al., 2005). This finding was consistent with not only previous studies across the United States and Canada

but also three other concurrent avian mercury exposure studies on the South River. Adult tree swallows, belted kingfishers, and eastern bluebirds all had significantly elevated blood mercury levels compared to nestlings (Brasso, 2007; White, 2007; A. Condon, pers. comm.). The nestling blood mercury levels reported here for Carolina wrens (0.69 ± 0.0385 SE) and house wrens (0.3257 ± 0.0224 SE) were the highest nestling blood mercury levels reported for any species nesting along the contaminated portion of the South River. The next highest nestling blood mercury level was reported for belted kingfishers at 0.26 (± 0.16 SD) ppm (White, 2007) followed by tree swallow nestlings (0.23 ± 0.17 SD) ppm and lastly, nestling eastern bluebirds had a blood mercury level of 0.0975 (± 0.07 SE).

When adult blood mercury levels are compared Carolina wren > belted kingfisher > tree swallow > house wren > eastern bluebird. However, when nestling blood mercury levels are compared the house wren increases in rank relative to the other species and the order changes to Carolina wren > house wren > belted kingfisher > tree swallow > eastern bluebird. This discrepancy, between the relative rank order of adult and nestling house wrens, cannot be explained with any data I or any of my colleagues collected. All nestlings were sampled just prior to fledging. In this case, fractionation, the movement of mercury within a bird's body, has the most explanatory potential. It is possible that nestling feather growth patterns, overall growth rates, and incorporation of mercury into feathers differs between species. Another, simpler explanation

would be if house wren nestlings grow relatively few feathers before leaving the nest. To determine if this is the case, a dosing study would be required; to my knowledge no appropriate dosing study has been performed on any songbird.

1.3. Feather mercury

Both species of adult wrens from the contaminated site had back mercury levels in their body feathers that were significantly elevated over those from the reference sites (nestling back and body feathers were not sampled). Feather mercury values must be interpreted with caution because an individual's residency on a contaminated site, the location where molt occurs, and feather type sampled all can affect feather mercury levels. An individual's length of exposure should be the first thing considered when sampling feather mercury. Ideally all individuals sampled should have spent the previous breeding season on the site that the researcher wishes the feathers to represent. This was not possible in my study as all feathers sampled in 2006 come from unbanded birds with unknown age and breeding history. A large sample size should correct for this factor because, although some individuals may have moved in recently from an uncontaminated site the likelihood of a wren having spent the previous breeding season on the contaminated sampling site is much higher for birds sampled from contaminated sites. This was probably the case for both species of wrens, the migrant (house wren) and the year round resident (Carolina wren) because both showed a high degree of

variation in feather mercury levels but nonetheless were significantly elevated over the reference site.

1.4. Comparisons to other studies on wrens

Only recently have researchers employed non-lethal sampling methods (blood and feathers), rendering it difficult to compare with studies employing lethal methods (liver, kidney, whole body, etc.). Evers et al. (2005) suggested a ratio for converting tissues based on common loon tissue mercury levels. This ratio was used with limited success by Brasso (2007) to compare blood mercury levels of tree swallows from the South River to studies that reported concentrations in other tree swallow tissues. The ratio of blood : feathers for Carolina and house wrens reported here does not follow the 1:6 ratio reported by Evers et al. (2005). The reasons for this could be many. One possibility is that the feathers reported in this study were back and body feathers and not wing feathers. It is also possible the individuals sampled in this study are less faithful to previous breeding sites than loons or swallows.

However, the problem inherent in comparing tissues is made infinitely less difficult by the lack of other studies on mercury exposure in wrens. To my knowledge this is the first study to report mercury levels for any tissue for Carolina wrens and the second for house wrens. Nestling house wrens from a mine impacted area, assuming 84% moisture, had mercury concentrations in the egg on a wet weight basis of 0.44 ppm. Making the additional assumption

that Evers et al. 2005 ratio of 0.4 : 1.2 for egg : blood is correct the females that laid the eggs would have had a blood mercury level of about 1.3 ppm ww, well below the blood mercury levels reported here for house wrens nesting within 50 m of the South River.

1.5. Comparisons to studies on terrestrial insectivores in other geographic locations

Only recently have insectivorous birds come to the attention of ecotoxicologists studying mercury. Therefore, the number of studies on insectivorous birds and mammals is small but growing. Blood mercury levels of female great tits (*Parus major*) nesting in Belgium, Europe in an area impacted by industrial practices, assuming 75% moisture, had blood mercury levels ranging from 0.02-0.07 ppm ww (Dauwe et al., 2005). This is below the levels reported here for Carolina and house wrens.

Assuming that mercury concentrations are always highest in the liver (Evers et al., 2005) Carolina and house wren nestlings from the South River are exposed to higher mercury concentrations than nestling pied flycatchers collected near a sulphide ore smelter in Sweden, Europe (Nyholm, 1995). In the case of the Carolina wren, nestling blood levels (0.69 ppm ww) were more than twice as high as nestling pied flycatcher liver mercury levels. In the case of the house wren, nestling blood levels (0.3257 ppm ww) were almost 1.5 times higher then nestling pied flycatcher liver levels. This clearly shows that

Carolina and house wren nestlings from within 50 m of the South River were accumulating more mercury than pied flycatcher nestlings near a Swedish smelter.

Four species of insectivorous birds accumulating mercury from atmospheric deposition in montane habitats had blood mercury levels (Rimmer et al., 2005) that were below that of the levels reported here for Carolina and house wrens. The highest adult mercury level reported in the montane songbird study was 0.42 ppm ww, which is only a fraction of the level reported here for adults and resembles the mercury level of nestling wrens from the South River, and. This further demonstrates that insectivorous species nesting within 50 m of the South River are exposed to high levels of mercury relative to that reported elsewhere.

As of yet there is no conversion factor for kidney mercury levels but kidney and liver mercury levels are similar (Evers et al., 2005). Adult prothonotary warblers nesting near a chlor-alkali plant had an average kidney mercury level of 0.93 ppm ww. Brasso (2007) used this relationship to compare the percentage of adult tree swallows with blood mercury levels lower than the average kidney mercury level reported for prothonotary warblers. Whereas, only 11% of the tree swallows nesting along the South River had blood mercury levels lower than the average kidney mercury level reported for prothonotary warblers, none of the Carolina or house wrens on the South River had lower mercury levels in their blood than prothonotary warblers had in their

kidney. Assuming that kidney and liver mercury levels are always highest, this is further evidence that wrens nesting along the South River are exposed to some of the highest concentrations of mercury ever reported.

On the Sudbury River, in Massachusetts, a contaminated superfund site the terrestrial insectivore with the highest blood mercury level was the song sparrow and the species with the overall highest blood mercury level was the northern waterthrush at 0.6 ppm (Evers et al., 2005). Again, these adult mercury levels from other sites more closely resemble the mercury levels of nestling Carolina wrens from the South River, providing more evidence that terrestrial insectivores nesting along the South River are at a higher risk to mercury exposure than at other study sites.

2. Prey mercury levels

When the diets of three terrestrial insectivores was compared Aranea, Lepidoptera, and Orthoptera made up the majority of all three species' diets with Coleoptera also being consumed by eastern bluebirds. This agrees with published diet reports of the three species (Gowaty and Plissner, 1988; Haggerty and Morton, 1995; Johnson, 1998). Aranea comprised between 20 and 30% of each species' diet and differences in proportions of the diet consisting of Aranea were small. The avian species and year from which a prey item was collected had little effect on the prey items' mercury level,

therefore prey items were grouped across avian species to test specific hypotheses.

2.1. Do prey items collected on the contaminated site have elevated mercury levels?

All prey groups collected on the contaminated site had significantly higher mercury levels than their counterparts collected from the reference sites. It could be argued that terrestrial songbirds are actually feeding on emerging aquatic insects or possibly drinking contaminated water directly. By demonstrating that none of the terrestrial species consumed emerging aquatic insects in any great numbers and that the prey groups that made up the majority of their diet had elevated mercury levels, I have clearly shown that the most likely route of mercury for terrestrial species is their terrestrial prey, particularly spiders.

The fact that terrestrial herbivores (e.g., Orthoptera), and not just terrestrial predators, also have elevated mercury levels shows that mercury has entered the terrestrial environment and is accumulating in the base of the food chain. If only spiders had had elevated mercury levels one could argue that these predators were accumulating mercury by preying on emerging aquatic invertebrates. It may still be the case that this is how terrestrial predators are accumulating mercury, but obviously this is not the case for

terrestrial herbivores. Further study is needed to determine through which plants herbivores are accumulating mercury.

2.2. Prey mercury levels compared to other studies

Coleoptera (beetles) were not eaten by either wren, but comprised a major portion of the eastern bluebird's diet. Mercury levels were highest in Coleoptera, followed by Aranea. This could be consistent with the idea that predatory invertebrates are at a higher risk of bioaccumulation of contaminants. However, not all Coleoptera are predatory, and although not all individual prey items were identified to species, many of the Coleoptera collected were not predatory (e.g. Japanese beetles; pers. observation.). The high mercury levels observed in non-predatory Coleoptera may be misleading because only a small percentage may be in the most toxic form of methylmercury. Whereas, in Aranea, a predatory invertebrate, the majority of total mercury is more than likely in the most toxic form of methylmercury (Rimmer et al., 2005). Due to the high cost of methylmercury analysis ($280/sample), at the present time no samples in this study have been analyzed for methylmercury.

2.2.1. Mercury in Coleoptera

Although very few studies have addressed mercury accumulation in terrestrial invertebrates there is some evidence in the literature that a very low percentage of total mercury in Coleoptera is in the form of methylmercury.

Murphy (2004) collected Coleoptera from two locations along the South River and found total mercury levels to be greater than 14.5 ppm ww. This is even higher than reported in this study or a study that sampled Coleoptera larvae in the flood of the South River (3.27 ppm dw; Cocking et al., 1991) . Only one study has addressed the percentage of methylmercury in Coleoptera and found that only 5.2% of total mercury was in the methylated form (Murphy, 2004). Therefore, the high total mercury concentrations reported for Coleoptera in this study may be misleading with regard to the availability of mercury from Coleoptera to the avian species consuming them. This may be an explanation for why eastern bluebirds, the only avian species consuming Coleoptera in high numbers, had the lowest mercury levels of the three avian species reported here. However, non-predatory beetles are known to accumulate organic contaminants, such as chlordane, to levels high enough to poison insectivorous predators such as bats and birds (Stansley et al., 2001). The role Coleoptera play in the bioaccumulation and biomagnification of mercury in food chains needs further study.

2.2.2. Mercury in Aranea

Aranea had the second highest mercury levels after Coleoptera and levels significantly higher than that of terrestrial herbivores such as caterpillars and grasshoppers. This is consistent with studies on aquatic invertebrates that found predatory groups to have higher mercury levels than omnivorous and

herbivorous groups (Mason et al., 2000; Murphy, 2004; Tremblay et al., 1996). Similar to Coleoptera, few studies have examined mercury accumulation in Aranea. Aranea collected from nestling prothonotary warblers in Alabama had an average mercury concentration of 0.1211 ppm ww (n = 17) well below the value reported here for Aranea (1.242 ppm dw). The mercury concentrations reported here for Aranea are more than twice as high as reported in an early study done on the South River that found mercury concentration to be 0.4 ppm dw for composite samples Aranea (Cocking et al., 1991). Assuming bioaccumulation in terrestrial food webs is similar to aquatic food webs, mercury in Aranea, because they are predatory, is most likely in the toxic form of methylmercury and therefore readily available to insectivorous birds (Murphy, 2004; Wiener et al., 2003).

2.2.3. Mercury in Lepidoptera and Orthoptera

Mercury in Lepidoptera and Orthoptera was similar and the lowest of all the major prey groups. Both were elevated over their counterparts collected from the reference area but well below that of Aranea and Coleoptera. This is contrary to what Cocking et al. (1991) found for Orthoptera of the family *Gryllidae*. Composite samples had a mean mercury concentration of 0.8 ppm dw. For all other non-predatory invertebrates mercury was not detected (Cocking et al., 1991). Invertebrates are not popular biomonitors and therefore

to my knowledge there are no additional studies that have reported mercury levels for free living Lepidoptera or Orthoptera.

2.2.4. Mercury accumulation by prey type for the three avian species

I found that Carolina and house wrens were exposed to mercury mainly through their Aranean prey and eastern bluebirds were exposed to mercury through Coleoptera and Aranea. Finding that mercury concentration differs by prey types is not surprising and agrees with other studies. In a study on the diets of six species of seabirds from the Azores, mercury levels differed by prey types and varied from 0.05 to 0.43 ppm dw (Monteiro et al., 1998). Likewise, prey samples collected from great skuas in the North Atlantic had a similar range of mercury values (Bearhop et al., 2000a). This is within the range of mercury levels reported here for prey items of terrestrial insectivores, but terrestrial insectivores were consuming prey items with a wider range of mercury levels. Prey items collected from wood storks in Georgia, USA also showed high variation and were more similar to the range reported here for prey items from terrestrial insectivores (Gariboldi et al., 1998).

Similar to this study, Aranea collected from prothonotary warblers nestling near a chlor-alkali plant had the highest mercury levels (Adair et al., 2003). The mean mercury levels for all prey collected from prothonotary warblers were reported on a wet weight basis (Adair et al., 2003). Assuming an average solid fraction of 0.25, the range of prey mercury levels on a dry

weight basis was 0.12 to 0.28 ppm in that study, well within the range of mercury levels reported here. Aranea from that study had a mercury level of 0.48 ppm dw (assuming a solid fraction of 0.25) which is less than half the level reported for Aranea in this study.

In a study similar to the one presented here, a small sample of prey items collected from the stomachs of two species of terrestrial insectivores near a mine impacted area had an average mercury level of 1.49 ppm dw (Custer et al., 2007). The ranges reported in that study are within the range of mercury levels reported here, but sample size was small (n = 5) and samples represented pooled stomach contents as opposed to the mercury level of each individual prey item. Furthermore, an average of 1.5 ppm dw is very similar to the average mercury value reported for Aranea here and therefore one would expect avian tissue mercury levels to be much higher if the entire diet had an average mercury level that high. To the contrary, avian tissue mercury levels reported by Custer et al. (2007) are lower than the ones reported here. By mixing prey groups and collecting partially digested prey samples a great deal of information was lost.

In sum, few studies have attempted to determine the mercury levels of prey items and those that have found a wide range of mercury levels. The mercury levels reported here for the prey items of three terrestrial insectivores represent some of the highest values ever reported. By identifying prey items to order, collecting a large enough sample size to represent the species' diet,

and individually analyzing each prey item, I was able to identify Aranea as having the highest mercury levels in the diets of terrestrial insectivores. To my knowledge this is the most comprehensive study of its kind.

3. Simulation

By collecting a large enough number of prey items from each of three species to accurately represent their diet, and analyzing each prey item individually I was able to develop a novel technique to predict mercury exposure. This technique allowed me to generate a distribution of potential mercury exposures which can be used to design future dosing experiments and make restoration/remediation decisions. To my knowledge, no study has attempted to explain bioaccumulation of a contaminant in any terrestrial insectivorous bird.

Other exposure models assume a constant mercury concentration or a distribution of mercury values based on the literature, or a small sample size of potential prey items collected from the environment. Furthermore, the proportion of each prey item in a species' diet is assumed from the literature. An additional problem with the traditional exposure models is that rare prey items are often not accounted for. That is not the case with the model I developed. Diets can vary greatly from one location to another. By collecting actual prey items I have circumvented the shaky assumption that diets are similar across geographic regions, seasons, and habitats. The model correctly

predicted the relative degree of mercury exposure (Carolina wren>house wren>eastern bluebird) for the three species of avian terrestrial insectivores studied here. This simulation also allowed me to examine the effect the proportion each major prey group had on daily mercury exposure. For both species of wren the simulation identified spiders as being the source of mercury. This suggests that a diet high in predatory invertebrates increases an individual's risk of bioaccumulating mercury. However, for bluebirds there was no relationship. This could be due to the fact that bluebirds had the lowest blood mercury levels and no one prey group influences daily mercury exposure compared to other prey groups.

Collecting actual prey items is time consuming, but this model may have applications to other avian species and potentially any species at risk to exposure of any contaminant. This model was designed with the software package Crystal Ball © which is a plug-in for Microsoft Excel and is user friendly. The model can be easily adapted to other locations, using a different 'pool of prey items' or target species. Furthermore, this model can now serve as the basis for a future dosing study which, combined with the model, can serve to assist managers in making decisions regarding restoration/remediation.

3.1. Future dosing study

The next step and broader use of this approach would be to design a dosing study in order to determine the effect this level of mercury exposure will have on terrestrial insectivores. The model shows that the range of daily mercury exposures is 22 to 707 ng hg / d / g of bird. This should be the basis for determining the LOAEL and NOAEL for terrestrial insectivorous birds, which has yet to be determined. A series of dosing studies can be performed based on this range, to determine what percentage of the population is at risk. If, for example, the LOAEL is 707, 0% of the population is affected. However, if the LOAEL is 22, 100% of the population is affected. This information can then be used to determine the effect mercury is having on the populations of Carolina wrens, house wrens, and eastern bluebirds nesting within 50m of the South River and appropriate action can be taken.

4. Comparisons to aquatic species' prey

When the distributions of prey mercury levels collected from the aquatic-foraging tree swallow and fish-eating belted kingfisher were compared to the distribution of mercury levels from terrestrial prey items, the distributions had a high degree of overlap. This clearly demonstrates that terrestrial insectivores are exposed to a similar amount of mercury as aquatic-foraging species nesting along the South River. This is the first study that has compared mercury levels in the prey items of both terrestrial and aquatic species from the

same location. The results of this study are contrary to the dogma that mercury is an aquatic problem for birds (Wiener et al., 2003) and future studies determining the impact of riverine mercury pollution should consider terrestrial species as well as aquatic-foraging species.

5. Conclusion

Both Carolina and house wrens were accumulating mercury from the contaminated South River. Likewise, the prey items of all three avian terrestrial insectivores sampled here were accumulating mercury from the contaminated South River. When the collected prey items from the three species are used as 'pool of prey items' in a simulation designed to predict mercury exposure, the three avian species' mercury exposure predicted by the model corresponded to their relative blood mercury levels. When the mercury levels in the prey of terrestrial-foraging species was compared to that of aquatic-foraging species there was a high degree of overlap, demonstrating that mercury exposure is similar. All future studies investigating the impact of mercury on avian communities should include terrestrial species and not just aquatic species as was common in the past.

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