Pathways and Consequences of Contaminant Flux to Acadian Flycatchers (*Empidonax virescens*) in Urbanizing Landscapes of Ohio, USA

THESIS

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Abstract

Widespread increases in urbanization with a growing global population can result in a diverse assortment of threats to wildlife, including exposure to harmful chemical contaminants. Among environmental contaminants hazardous to humans and wildlife, mercury is of special concern due to its prevalence, mobility in aquatic systems, and persistence in sediments. Aquatic systems, in particular, can have high amounts of mercury in its bioavailable form (methylmercury), which can then bioaccumulate in insects and be transported to terrestrial food webs (i.e., to aerial insectivores). Such contaminant flux from aquatic to terrestrial systems is expected to disproportionately affect species reliant upon aquatic emergent insects, such as with aerial insectivorous birds. To understand the pathways of contaminant flux and the role that contaminants play in reproductive success, I addressed two broad questions: (1) Is avian exposure to metal contaminants influenced by territory placement at local and landscape scales? and (2) Do metal contaminant loads negatively impact individual body condition and reproductive output? From April-August 2011-2012 I tracked reproductive success of Acadian flycatchers (*Empidonax virescens*) in 19 mature riparian forest fragments located across an urban to rural land-use gradient in central Ohio, USA. I collected blood samples from adult ($n = 76$) and nestling ($n = 17$) Acadian flycatchers and collected samples from riparian systems (sediment, aquatic emergent insect, and water) to test for contaminants and examine relationships between landscape factors and contaminant concentrations. I

used reproductive data and flycatcher contaminant loads to evaluate the impact of mercury on reproductive success and body condition of adult flycatchers.

Factors that were most responsible for contaminant transfer to flycatchers remain unclear. Landscape factors, including proximity of flycatcher territories to rivers (i.e., access to aquatic emergent insects) and amount of urbanization surrounding forest sites, were not related to mercury levels in flycatchers, sediment, water, or insects. However, when separately analyzed, I detected a positive relationship between mercury in flycatchers and sediments in urban landscapes, and the opposite, a negative relationship, in rural landscapes. Unlike previous research, mercury concentrations in aquatic insects were not predicted by concentrations in sediments. The overall lack of concordance among mercury levels in Acadian flycatchers, aquatic insects, and sediments raises the possibility that flycatchers consumed less aquatic prey than expected or perhaps that the common aquatic insects were not the most important vectors of aquatic-to-terrestrial contaminant flux.

Although levels of mercury in the blood of Acadian flycatchers in this study were relatively low and appeared not to affect body condition, reproductive success declined with increasing mercury loads. My results are consistent with other studies that have documented reduced reproductive success in contaminated areas, though mercury loads were at trace levels in the flycatchers that I studied. Reduced reproductive success in my study system could possibly be explained by changes in adult behavior or by egg hatching success, driven by mercury loads in flycatchers; these factors need to be further examined as sublethal effects of mercury contamination in birds.

As a whole, my research contributes to a growing body of knowledge regarding pathways for contaminant flux from aquatic to terrestrial systems, and also to our understanding of mercury contamination effects on free-living songbirds. My research suggests that pathways for contaminant flux from aquatic to terrestrial systems are complex and do not always follow predictable routes. Moreover, that reduced reproductive success was associated with trace levels of mercury in a free-living songbird is a cautionary note that wildlife managers should not dismiss the impact of trace levels of contaminants that may biomagnify.

To my family, who encouraged me
to spend my childhood in nature, and whose
love and support I cherish.

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Chapter 1. Thesis Introduction and Literature Review

Introduction

As the global human population approaches the projected 9.3 billion by the year 2050 (United Nations 2012), anthropogenic stressors should reach unprecedented levels. Industrialization continues to increase worldwide, especially in developing countries. Increases in urbanization and industrialization may lead to increased emissions of contaminants, which may threaten wildlife at both the individual and population levels. For example, over the past 100 to 150 years, anthropogenic emissions of mercury to the atmosphere have increased globally and reached even remote areas of the globe (Fitzgerald and Engstrom 1998).

Environmental contaminants have played a central role in development of the modern conservation movement in the U.S. As Rachel Carson highlighted in “Silent Spring” (1962), contaminants can have major and unforeseen harmful effects to wildlife. Carson’s call to action helped spur public interest and legislative response to the issue of chemical pollutants in the environment. Many of the greatest victories of conservation have resulted from our ability to respond to and ameliorate some of the negative effects of contaminants on wildlife (e.g., DDT contamination in predators/scavengers such as

bald eagles (*Haliaeetus leucocephalus*), osprey (*Pandion haliaetus*), peregrine falcons (*Falco peregrinus*), and brown pelicans (*Pelecanus occidentalis*). However, historically polluted areas as well as contemporary contaminant sources continue to pose hazards to humans and wildlife.

Although more stringent regulations of chemical pollutant releases by industries have been in effect in recent years (e.g., US Environmental Protection Agency's Clean Air Act, Clean Water Act, and Safe Drinking Water Act), historical sources of chemicals continue to persist in the environment. Contaminants can persist for thousands of years in environmental compartments including sediments, soils, water, and the atmosphere (Swartzendruber and Jaffe 2012). Wildlife may be exposed to chemicals at locations far from point sources, or in areas where remediation efforts may not have removed all traces of pollutants (Fitzgerald and Engstrom 1998, Jackson et al. 2011b).

Understanding the threats that wildlife face is challenging because the amount of a contaminant in the environment may not indicate the likelihood or intensity of exposure. The complexity stems in part from the fact that contaminants move through systems by way of both biotic and abiotic processes. Mercury (Hg), for example, is primarily transported and deposited from the atmosphere into aquatic and terrestrial systems (Swain et al. 1992), and abiotic factors like weather, salinity, and pH can affect contaminant bioavailability (Noyes et al. 2009). At the same time, contaminants can biomagnify as they increasingly accumulate in consumer tissue with each trophic step in a food web. Movement through food webs also provides a route for contaminant flux between separate systems (e.g., from aquatic to terrestrial systems; Sullivan and

Rodewald 2012, Walters et al. 2008, 2010). Understanding contaminant flux pathways is key to predicting and ameliorating routes of exposure to wildlife in different systems.

Objectives

I examined contaminant flux pathways between aquatic and terrestrial systems, and the consequences of metal contaminants to riparian bird reproductive output and condition. To do this, I studied metal contamination in one bird species, the Acadian flycatcher (*Empidonax virescens*), within mature riparian forest fragments located across an urban to rural land-use gradient, in central Ohio, USA between 2011-2012. In my thesis, I specifically addressed the following questions:

1) Is avian exposure to metal contaminants influenced by territory placement at local and landscape scales?

My testable predictions were that:

- a) Individual birds with territories closer to a river will have higher contaminant levels in blood than individuals with territories farther from a river, because bioavailability of metals is frequently linked to aquatic systems and waterways, and aquatic invertebrates represent an important vector of exposure for aerial-foraging birds. Exposure is expected to increase with proportion of aquatic prey in diet, which should be related to distance from river.
- b) Territory position within the watershed and along the urbanization gradient is expected to be associated with an individual bird's contaminant load, because pollutants may accumulate over a greater area within the watershed. This

possibility may be especially likely for mercury, which primarily moves into watersheds through atmospheric deposition.

2) *Does exposure to metal contaminants negatively impact individual body condition and reproductive output?*

My testable predictions were that:

- a) Body condition will be negatively related to contaminant concentration in blood, because sub-lethal effects of metal pollutants include diminished body condition, lessened immune function, and altered behavior.
- b) Reproductive output will be negatively impacted by higher levels of contaminants in blood, because effects of metal pollutants on avian species include reduced reproductive productivity through lowered hatching success, embryo deformities, smaller egg sizes, altered behavior in adults or nestlings, and reduced fledging success.

Thesis Format

In this chapter I briefly review metal pollutants in the environment, with an emphasis on sources of contaminants and their effects on birds. Subsequent chapters represent separate manuscripts for submission to peer-reviewed journals. In Chapter 2, I examine how pathways for metal contaminant flux from aquatic to terrestrial systems are mediated by landscape factors and ultimately the extent to which aquatic system factors (namely sediment, water, and aquatic emergent insects) predict contaminant concentrations in

Acadian flycatchers. In Chapter 3, I evaluate the impact of mercury on reproductive success of Acadian flycatchers and body condition of adults.

Background

Emissions of metals, including mercury, lead, cadmium, and selenium, into the environment have increased with widespread industrialization (Chang and Cockerham 1994). Mercury is released into the environment from coal burning power plants, paper industry processes, and household materials such as batteries, fluorescent bulbs, and paints (Chang and Cockerham 1994, Pacyna et al. 2010). Lead is a persistent problem, especially in urban environments, as a result of past usage of lead-based paints and leaded gasoline (both of which were banned in the U.S., in 1978 and 1986, respectively). Cadmium is a toxicant released from industrial processes, and is found in paints, plastics, and batteries; it is also used as an anticorrosive for steel, iron, copper, and brass (Chang and Cockerham 1994). Selenium naturally occurs at low levels in the environment, but is also released into the environment primarily through coal combustion (Risher and McDonald 2003).

Urbanized areas have among the highest emissions of these metals from processes including fossil fuel combustion from industrial sites and engines, metal manufacturing plants, mines and smelters, and cement production sites (Chang and Cockerham 1994). Metals are concentrated at greater levels in the atmosphere in industrialized urban areas than at remote or rural sites (Van Metre 2012), and rivers moving through urban areas

also tend to have contaminants at greater than background levels, particularly for toxic metals including cadmium, mercury, and lead (Chang and Cockerham 1994).

Contamination of rivers and streams can occur through either point source or non-point source pollution. Point source pollution occurs when contaminants are released directly into the environment, such as a sewage treatment effluent or industrial wastewater effluent. Non-point source pollution includes runoff (i.e., from roads and agricultural fields) and atmospheric deposition (Ohio Environmental Protection Agency 2012). Contamination of sediments in rivers is widespread; each state in the U.S. had one or more locations with high enough levels of contaminated sediment to adversely affect aquatic organisms (U.S. Environmental Protection Agency 2004).

Exposure to contaminants in aquatic and terrestrial systems can have wide-ranging individual and population-level consequences for wildlife. Wildlife exposure to contaminants can occur through direct ingestion of contaminants (i.e., ingestion of lead shot) or indirectly through bioaccumulation. Biomagnification of metals up trophic levels in food webs has been documented in several studies (Cristol et al. 2008, Kleinow and Goodrich 1994), often leading to severe effects in fish and wildlife species. Effects of exposure can be complex; species exhibit differences in sensitivities to particular contaminants, due to differing physiological responses to contaminants (i.e., differences in organs, contaminant absorption, or detoxification mechanisms) (Chang and Cockerham 1994). Variation in factors such as geographic range, habitat requirements, and foraging preferences can affect exposure to pollutants. Negative effects of environmental contaminants on avian species include both mortality and sub-lethal

effects such as altered behavior, depressed immune function, reduced reproductive productivity, and diminished body condition (Hargreaves et al. 2010). Contaminants can also have population-level effects including sex-ratio shifts of offspring (Bouland et al. 2012), and shifts in the population age structure (Evers et al. 2008). Effects of three common metal pollutants are summarized in the following section.

Common metal contaminants in the environment

Mercury

Mercury is widespread and can persist for thousands of years in the atmosphere, aquatic, and terrestrial systems (Evers 2005, Swartzendruber and Jaffe 2012). Mercury can be present at high concentrations in urban and industrial areas, and poses serious health risks to both wildlife and people (Seewagen, 2010). Mercury has a high potential for flux from aquatic to terrestrial systems, thus increasing likelihood of exposure and adverse effects. Mercury can be transferred from water or sediment into aquatic insects through food intake or passive facilitated diffusion from water (Rainbow 1996). As these insects emerge from rivers as adults, they may transport mercury to terrestrial insectivores (Cristol et al. 2008, Menzie 1980, Sullivan and Rodewald 2012). In addition to trophic transfer of mercury from aquatic to terrestrial systems, mercury can be deposited onto floodplains with sediments. Mercury bioavailability may increase with flood events, due to increased microbial methylation of inorganic forms of mercury present in flooded soils (Wiener and Spry 1996).

The health consequences of mercury can be wide-ranging. For one, mercury is a highly potent neurotoxin (Seewagen 2009) and symptoms exhibited from high exposures

include motor and behavioral impairment (Wolfe et al. 1998). Recent research documented a shift in offspring sex ratios in three species of birds exposed to mercury in contaminated sites (ratio shifted toward female-biased clutches; mean proportion of male nestlings was 0.39 ± 0.029 in broods at mercury contaminated sites and was 0.50 ± 0.035 at reference sites; Bouland et al. 2012). This sex-ratio shift suggests there may be serious population-level effects on fitness resulting from mercury exposure, with a reduced effective population size resulting in reduced genetic diversity and/or evolutionary potential (Bouland et al. 2012). Additionally, population age structure may be shifted to younger individuals, as a result of mercury's toxic effects on common loon (*Gavia immer*) behavior, physiology, and reproductive success, and may thus result in lowered breeding performance and lower quality parents (Evers et al. 2008). Other studies, however, have not documented severe negative effects on the study population. For example, despite elevated blood-mercury levels in osprey (*Pandion haliaetus*), there was insufficient evidence for any deleterious effects in the resident population (Lounsbury-Billie et al. 2008). In several Arctic-breeding shorebird species, contaminant concentrations in the body were not related to reproductive success with the exception of mercury and lead, although those relationships were not strong (Hargreaves et al. 2010). Chronic exposure to low levels of mercury can lead to impaired reproductive success in birds, including thinned eggshells, embryo deformations, and behavioral changes in fledged young, leading to higher mortality rates (Wolfe, Schwarzbach, & Sulaiman, 1998).

Background levels of mercury range across species and tissue types. In herring gull eggs, the background level is 0.2 mg/L (wet weight), but is higher (2 mg/L, wet weight) in Forster's tern (*Sterna forsteri*) eggs (Burger and Gochfeld 1997). However, mercury levels as low as 0.5 to 6 mg/L (wet weight) in herring gull and Forster's tern eggs caused embryo malformations, reduced hatching success, and decreased chick growth and survival (Burger and Gochfeld 1997). Feather mercury levels in herring gulls and Forster's terns associated with negative effects range from 5 to 40 mg/kg (dry weight, Burger and Gochfeld 1997). Recent research indicated that mercury concentrations less than or equal to 0.7 mg/kg (wet weight) in blood results in less than 10% reduction in nest success (Evers et al. 2012, Jackson et al. 2011a).

Mechanisms exist that allow birds to rid themselves of mercury or store it in tissues where it may have no toxic effects. Birds can deposit mercury in their feathers, a protective mechanism especially in young birds with thousands of growing feathers (Condon and Cristol 2009, Evers et al. 1998, Spalding et al. 2000). Mercury can also be demethylated and stored in the liver and kidneys of birds (Boening 2000, Evers et al. 1998). Furthermore, antagonistic interactions between selenium and methylmercury may lessen toxic effects of mercury (Cuvín-Aralar and Furness 1991, García-Barrera et al. 2012). However, interactions between selenium and mercury may be additive or synergistic (increasing toxicity), depending on the chemical form of selenium. In studies reviewed by García-Barrera et al. (2012), antagonistic interactions occurred between inorganic mercury and the selenite form of selenium, while a synergistic interaction occurred between methylmercury and selenium. Another study determined that toxic

effects of dietary selenium and methylmercury (fed to the adult) on developing mallard (*Anas platyrhynchos*) embryos were greater than embryo effects resulting from a maternal diet with only added methylmercury (Heinz and Hoffman 1998). Additionally, in aquatic systems, selenium may decrease bioavailability of mercury and thereby the potential for trophic transfer (García-Barrera et al. 2012). In a study of mercury and selenium in American dippers (*Cinclus mexicanus*) and spotted sandpipers (*Actitis macularia*), neither species exhibited severe reproductive effects from selenium exposure, and mercury was only detected at trace concentrations in the eggs of these two species (Harding et al. 2005). The low levels of mercury in this study suggest that some other mechanism was influencing the marginal toxic effects of selenium; the fast-flowing water of the lotic system could reduce the likelihood of selenium transformation into bioavailable forms (Harding et al. 2005). In contrast, in the lentic (standing water) systems in the highly contaminated Kesterson Reservoir area in California, several bird species exhibited severe reproductive effects due to selenosis (Ohlendorf et al. 1988).

Selenium

Selenium naturally occurs at low levels in the environment and is essential for life, yet at elevated concentrations becomes highly toxic (Lenz and Lens 2009, Ratti et al. 2006). Excessive amounts of selenium are produced with large-scale coal burning, in the resultant fly ash. Although air quality regulations have reduced fly ash emissions into the air, disposal of seleniferous fly ash in landfills will result in some contaminated leachate (Lemly 2004). Selenium can reach toxic levels when leached from agricultural soils into drainwater (Heinz et al. 1989). In areas with a set of biogeochemical conditions known as

the “Kesterson Effect”, selenium build-up in irrigation drainage water can have significant negative effects on wildlife (Lemly 2004). In the western United States, conditions that lead to seleniferous drainage water include: 1) naturally seleniferous soils, 2) excessive irrigation in arid regions with alkaline, oxidized soils (promoting the formation of water-soluble forms of selenium), and 3) subsurface clay and drainage systems that prevent downward movement of water (Lemly 2004).

Accumulation of selenium in wildlife occurs through both biomagnification (dietary ingestion) and bioconcentration (from water; Barceloux 1999). Effects of exposure to selenium at high concentrations include mortality, reduced fertility, and teratogenicity (Heinz and Fitzgerald 1993, Heinz et al. 1989). Mortality of adults and reduced hatching success of eggs were observed in the field at a site of high selenium contamination (Ohlendorf et al. 1987). Laboratory experiments performed on mallards indicated that avian embryos were more sensitive to toxic levels of selenium than adult birds were (Heinz and Fitzgerald 1993).

Selenium toxicity thresholds have been determined primarily from laboratory experiments with aquatic birds (Albers et al. 1996); most studies examined concentrations in tissues including liver, brain, heart, eggs, and feathers. The threshold for negative impacts to birds in field conditions is disputed, and varies among species (Ratti et al. 2006). For eggs, the selenium threshold has been estimated at 6-7 mg/kg by the US Fish and Wildlife Service. More recently, a threshold was estimated at 12-14 mg/kg, which is more appropriate for waterfowl (i.e., mallards), but may not necessarily be applicable for other species (Adams et al. 2003, Harding et al. 2005). For whole blood,

a background concentration of selenium ranges from 0.1 to 0.4 mg/kg (dry weight) (Ohlendorf 2002). Other studies, however, have established a background concentration at 2 mg/kg (dry weight), and indicated that further study is needed for concentrations over 5 mg/kg (Conover and Vest 2009, Santolo and Yamamoto 1999). Only when selenium is concentrated in the environment (for example, in areas with agricultural runoff and evaporated surface waters) or when continued dietary intake exceeds approximately 15 mg/kg would incur toxic effects (Spallholz and Hoffman 2002).

Lead

Hazardous levels of lead in urban and suburban environments pose health risks to both people and wildlife. Lead toxicosis in wild birds from lead shotgun pellets has been widely recognized as a serious problem (Cockerham, 1994), and lead shot was banned for use in waterfowl hunting in 1991. Lead can be transferred through the food web, to top predators such as piscivorous birds, as well as scavenging birds. Wildlife can be exposed to lead at shooting ranges (Lewis et al. 2001); birds of prey ingesting game birds wounded with lead shot was a common problem prior to policy changes (Kendall et al. 1996). Exposure to lead can result in mortality, neurological dysfunction, immune suppression, and reproductive impairment (Kendall et al. 1996). Lead in maternal blood (maximum concentration = 47 ng/mL) was associated with reduced egg hatching and nest success in black-bellied plovers (*Pluvialis squatarola*) and ruddy turnstones (*Arenaria interpres*; Hargreaves et al. 2010). Birds can be exposed to lead in soils through their diet, and also through incidentally ingesting soil with their prey (Roux and Marra 2007, Scheifler et al. 2006). Roux and Marra (2007) observed decreased body condition with

increased lead levels in nestling gray catbirds (*Dumatella carolinensis*), but not for adult resident passerines, and found a significant correlation between blood and soil lead in five passerine species.

Background levels of lead in avian passerine species have been reported at various levels. In rural reference sites, blood lead concentrations in different passerine species varied from 0.011 ± 0.001 mg/kg to 0.079 ± 0.009 mg/kg (Roux and Marra 2007). Other studies reported different reference values, such as those reported for common blackbirds (*Turdus merula*): 0.05 ± 0.01 mg/kg in rural sites (Scheifler et al. 2006). As reviewed by Tsipoura et al. (2008), birds with blood concentration levels above 0.4 mg/kg typically exhibit toxic effects. Variation in lead toxic effects thresholds in blood vary across orders, from greater than 1 mg/kg in Falconiformes and greater than 2 mg/kg in Columbiformes, to greater than 5 mg/kg in Galliformes (Franson, 1996). Chronic lead exposure can result in anemia and a reduced overall blood hemoglobin concentration (Pattee & Pain, 2002).

Remediation

Although remediation and clean-up efforts can reduce the load of many contaminants in urban areas, certain metals can persist over much longer periods. Lead is a pervasive problem, especially in urban environments, where leaded gasoline residues and remains of lead-based paints are prevalent in soils, although toxicity is low. Selenium persists in the environment, and without treatment can result in dramatic biomagnification through food webs. Treatments for selenium contaminated water include various filtration, adsorption, or reductive processes (Lenz and Lens 2009).

Selenium produced in coal fly ash is most frequently buried in landfills, yet even the best-designed landfills can have leachate into groundwater (Lemly 2004). Better designs for landfills and cost-effective treatment options for water-borne selenium are needed. Mercury in particular is difficult to remove from the environment. It is a pollutant at a global scale, as it can be transported in the atmosphere far from point sources (Evers 2005). Efforts have been made to reduce mercury emissions worldwide and in North America (Evers 2005, Weiss 2005). However, despite mercury reaching peak deposition into lake sediments in the mid-1990s (Van Metre 2012), mercury from historical sources may continue to re-enter terrestrial systems in its bioavailable form (Jackson et al. 2011b). An extensive monitoring system to measure the magnitude and impacts of mercury pollution is needed for North America, and needs to begin with baseline data (Evers 2005, Mason et al. 2005). My research will contribute to a growing body of knowledge regarding contaminant flux between aquatic and terrestrial systems, and the health consequences of metal contaminants to terrestrial passerine birds.

Significance of research

With the steady increase of industrialization worldwide and historical stores of contaminants in the environment, it is important to understand how and where wildlife may be exposed to harmful pollutants. Through a comprehensive understanding of pathways by which contaminants flux between different environmental compartments such as aquatic and terrestrial systems, we can better understand potential transfers of contaminants. My research provides insight into pathways for contaminant flux from

aquatic to terrestrial systems. Additionally, my research adds to a growing body of knowledge regarding harmful effects of metal contaminants to passerine health and reproduction.

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Chapter 2. Pathways of aquatic-to-terrestrial contaminant flux in an aerial insectivore, the Acadian flycatcher (*Empidonax virescens*)

Abstract

Among environmental contaminants hazardous to humans and wildlife, mercury is of special concern due to its prevalence, persistence, and mobility. Aquatic systems, in particular, can have high amounts of mercury in its bioavailable form (methylmercury), which can then bioaccumulate in insects and be transported to terrestrial food webs (i.e., to aerial insectivores). Aerial insectivorous birds in riparian ecosystems should be especially vulnerable to aquatic-to-terrestrial contaminant fluxes. As such, I examined pathways of Acadian flycatcher (*Empidonax virescens*) exposure to mercury in 19 mature riparian forest sites in central Ohio, USA during 2011-2012. Specifically, I investigated the extent to which mercury exposure was predicted by local (distance from river) and landscape (urbanization in the surrounding landscape and watershed drainage area) factors. I collected samples of river water, sediments, and aquatic emergent insects, as well as blood samples from 76 Acadian flycatchers, all of which were tested for total mercury concentrations. Mercury concentrations in blood ranged from 47 to 584 $\mu\text{g/kg}$, which are levels considered to be of low risk of reproductive consequences in songbirds. Those factors that are most responsible for contaminant exposure in flycatchers remain

unclear. Mercury levels in flycatcher blood were not related to the proximity of flycatcher territories to the river (i.e., access to aquatic emergent insects), concentration of mercury in sediments, water, or aquatic emergent insects, nor to amount of urbanization surrounding forests. However, when rural and urban landscapes were considered separately, mercury in flycatchers was negatively related to mercury in sediments in rural landscapes but positively related in urban sites. Mercury levels in aquatic insects were not predicted by levels in sediments in neither rural nor urban landscapes. The overall lack of concordance among mercury levels in Acadian flycatcher, aquatic insects, and sediments raises the possibility that either flycatchers consumed less aquatic prey than expected, or that perhaps my results were an artifact of sampling or analytical approaches.

Introduction

Emissions of metals, including mercury, lead, cadmium, and selenium, into the environment have increased with widespread industrialization and the combustion of fossil fuels (Chang and Cockerham 1994). Urbanized areas have among the highest atmospheric concentrations of metals as a result of industrial activity; atmospheric mercury deposition was greater at sites sampled nearer to cities than at more remote sites (Van Metre, 2012). Rivers moving through urban areas also tend to have elevated contaminant levels compared to background levels, particularly for toxic metals including cadmium, mercury, and lead (Chang and Cockerham 1994). Moreover, sediments in rivers can function as a reservoir of concentrated contaminants, even after new inputs to aquatic systems have been stopped, because chemicals produced by industrial processes

are likely to bind with organic materials, especially in redox conditions. Contaminants can be transported with water flow, and elevated contaminant levels in sediments should be associated with larger watershed drainage areas, although this may be dependent on water flow rates (Kamman et al. 2004, Krabbenhoft and Babiarz 1992). Stream discharge was a significant positive predictor of both total mercury and methylmercury in streams in the Northeast U.S. (Shanley et al. 2005). Contamination of sediments is nearly ubiquitous, and each state in the U.S. has at least one location with high enough levels of contaminated sediment to adversely affect aquatic organisms (U.S. Environmental Protection Agency 2004). For example, in the upper Scioto River basin, Ohio, out of 50 total sediment sampling stations, there were 10 stations with samples classified as having probable adverse effects on aquatic life, and 32 stations with samples classified as having possible adverse effects, based on sediment chemistry, tissue residues, and/or toxicity to test organisms (U.S. Environmental Protection Agency 2004). Not only do contaminants concentrate in sediments, but they can also be transported downstream in water.

Contamination in sediments may include mercury, a particularly persistent and harmful pollutant. Mercury is released into the environment from coal burning power plants, paper industry processes, and household materials such as batteries, fluorescent bulbs, and paints (Chang and Cockerham 1994, Pacyna et al. 2010). At a global scale, mercury emissions have increased over the past 100 to 150 years, and atmospheric transport and deposition of mercury at locations far from point sources, even in “pristine” areas, necessitates regulation of releases at a large scale (Fitzgerald and Engstrom 1998, Weiss 2005). During the same time scale (in the past 140 years) within North America,

mercury emissions have increased by a factor of 3.4 (Swain et al. 1992). Mercury can persist for thousands of years in the atmosphere, aquatic systems, and terrestrial environments (Swartzendruber and Jaffe 2012). Deposition of mercury in lake sediments peaked in the mid-1990s (Van Metre, 2012), yet mercury from historical sources may continue to re-enter terrestrial systems in its bioavailable form (Jackson et al., 2011). Relative to shorter-lived contaminants such as organic chemicals, the persistence of mercury in the environment increases the likelihood for adverse health effects on humans and wildlife.

Mercury in the aquatic environment has high potential for transfer into terrestrial systems, posing hazards for terrestrial wildlife. Sediments containing mercury may be deposited on riparian floodplains with flooding events, redistributing mercury from aquatic systems into terrestrial systems (Jackson et al. 2011b). The bioavailability of mercury can increase with these flooding events, due to greater microbial methylation of inorganic forms of mercury present in terrestrial habitats (Wiener and Spry 1996). With the mobilization of contaminants through abiotic processes, and flux from aquatic to terrestrial systems through food webs, insectivorous wildlife in riparian habitats may have high potential for exposure to a toxic form of mercury.

Metal contaminants can be transferred from aquatic to terrestrial systems via aquatic emergent insects (insects that emerge from aquatic systems after metamorphosis into adult form; Menzie 1980). Aquatic insects can bioaccumulate mercury either through food intake or via passive facilitated diffusion from the water itself (Rainbow 1996). As these insects emerge as adults, they may then provide a vehicle for the transport of metals

from aquatic to terrestrial systems, as terrestrial insectivores consume them. Previous research shows that aquatic insects are an important food subsidy to terrestrial insectivores (Nakano and Murakami 2001). Aquatic emergent insects can provide up to 40% of the diet for insectivorous birds in the riparian zone (Baxter et al. 2005), and can export contaminants to riparian predators (Walters et al. 2008). In this way, food webs provide the pathway for mercury bioaccumulation across aquatic and terrestrial systems (Cristol et al. 2008, Menzie 1980, Sullivan and Rodewald 2012). Individuals with the greatest access to aquatic insects should be most vulnerable, and access should largely be a function of the distance between an individual's territory and the water source. Indeed, as distance from a stream or river edge increases, adult emergent insect abundance exponentially decreases, even at a scale of 10 to 25m (Baxter et al. 2005, Iwata et al. 2003). Accordingly, insectivorous birds with territories closer to rivers should consume greater quantities of aquatic emergent insects, thus increasing their likelihood of ingesting and amassing contaminants via bioaccumulation.

In this study, I evaluated pathways for aquatic-to-terrestrial contaminant flux in riparian forests using the Acadian flycatcher (*Empidonax virescens*) as a focal species given that it preferentially breeds in riparian forests (Bisson et al. 2000, Whitehead and Taylor 2002) and consumes a variety of insect prey, including aquatic insects (Whitehead and Taylor 2002). Aerial insectivores are thought to be threatened by widespread declines in insect populations as well as potential mismatches in reproductive timing with peak insect abundance resulting from climate change (Nebel et al. 2010, North American Bird Conservation Initiative, U.S. Committee 2010). As a member of the declining aerial

insectivore guild (Nebel et al. 2010), the flycatcher also is of conservation interest and is ranked as a species of “Medium” vulnerability in the 2010 State of the Birds Report (North American Bird Conservation Initiative, U.S. Committee 2010).

I hypothesized that exposure to mercury would be influenced by territory placement at local and landscape scales (Figure 1) and specifically predicted that (1) individuals with territories closer to a river, which presumably have a greater proportion of aquatic prey in diet, would have higher contaminant levels in blood than individuals with territories farther from a river, and (2) individuals with territories located in areas with a greater degree of surrounding urbanization or with greater watershed flow accumulation (a proxy for drainage area) would be exposed to more mercury, which primarily accumulates in sediments and water through atmospheric deposition, and can be higher than background levels in urban areas. In addition, I expected to find positive relationships between concentrations of metals in river sediment, aquatic emergent insects, and blood from the flycatchers (Figure 1).

Methods

Study system and sampling

Study sites ($n = 19$) were located within forested riparian floodplains along Alum Creek, Big Darby Creek, Big Walnut Creek, Blacklick Creek, the Olentangy River, and the Scioto River mainstem, in the upper Scioto River basin, Ohio, USA (Table 2.1,

Figure 2.2). River reaches adjacent to sites were approximately 250m in length. Dominant tree species at study sites were American elm (*Ulmus americana*), American

hackberry (*Celtis occidentalis*), black walnut (*Juglans nigra*), boxelder (*Acer negundo*), Eastern cottonwood (*Populus deltoides*), honey locust (*Gleditsia tricanthos*), silver maple (*Acer saccharinum*), sycamore (*Plantanus occidentalis*), sugar maple (*Acer saccharum*), and white ash (*Fraxinus americana*). Woody understory vegetation included honeysuckle (*Lonicera* spp.), multiflora rose (*Rosa multiflora*), common spicebush (*Lindera benzoin*), dogwood (*Cornus* spp.), hawthorn (*Crataegus* spp.), Ohio buckeye (*Aesculus octandra*), tall paw paw (*Asimina triloba*), and saplings of overstory trees (Borgmann and Rodewald 2004, Leston and Rodewald 2006).

Between 14 August 2012 and 6 October 2012, I collected river sediments at each study site following the protocol of (Walters et al. 2010). I collected samples at 5 locations evenly distributed along the river reach (approximately 200m along the study site), using a push corer and sampling 0-10cm in depth. Sediment samples were stored in plastic bags, and subsequently dried and homogenized. In addition to sediment, I collected samples of river water at each study site between 11 September and 8 October 2012. Although contaminant concentrations can vary with water depth and movement, water samples were collected from only one location per site. Water samples were stored in 250ml polyethylene bottles, and kept refrigerated until analysis. Sediment and water samples were analyzed for a panel of toxic elements (Table 2) at the Michigan State University Diagnostic Center for Animal and Population Health (DCPAH, methods described below). All contaminant concentrations in water samples were below detection limits, and thus were not included in analyses. The water quality criteria for the

protection of human health in non-drinking water is ≤ 0.012 $\mu\text{g/L}$ mercury (Ohio Environmental Protection Agency 2002).

Aquatic emergent insect samples were collected at each study site between 25 August 2012 and 9 October 2012, using floating, 1-meter square pyramidal emergent insect traps similar to the style of Aubin, Bourassa, & Pellissier (1973). Insects were preserved in 70% ethanol, and later identified to family in the laboratory with the use of Triplehorn & Johnson (2005). Dipteran families were among the most numerically-dominant families in the samples, with Chironomids dominating many samples. Insects were dried at 60°C for 48 hours, and homogenized for analysis at DCPAH. Analysis for a panel of toxic elements (Table 3) was conducted using inductively coupled plasma-atomic emission spectrometry (methods described below).

The watershed drainage area, represented by the amount of water flow within our system was measured by a derived watershed flow accumulation from DEM (digital elevation model) data (Table 2.1). The DEM data was processed in ArcGIS, in three computational steps following methods of Jenson (1991). First, raster cells in the DEM were “filled”, to raise elevations of depressions to their spill points; these depressions are typically products of the DEM creation process. Second, flow direction was computed. Finally, the flow accumulation value was calculated for each cell. This value was the count of upstream cells that contributed flow to the given cell, based on the direction of their flow. Larger values represented a cell, or location, that was farther downstream than smaller values, and thus a greater drainage area (Jenson 1991).

The degree of urbanization within 1-km of each forested site was previously quantified using a principle components analysis on five variables (percent cover of pavement, roads, buildings, agriculture, lawn, and number of buildings), with the resulting first component explaining >79% of variation among sites (eigenvalue = 3.99), and referred to as the “urban index” (Rodewald and Shustack 2008). This urban index represents a gradient of landscape composition change. Urban indices ≥ 0 represent more developed landscapes with greater numbers of buildings (>800) and higher percentages of road cover, pavement, and lawn, whereas indices < 0 are more rural with higher percentages of agriculture (Rodewald and Shustack 2008, Table 2.1).

I studied territorial Acadian flycatchers from May through August 2011 and 2012. As part of a companion study, I tracked settlement and reproductive activity of Acadian flycatchers by locating and monitoring their nests. Male flycatcher settlement in our study system typically occurred within the first two weeks in May, and females arrived roughly within a week thereafter. I recorded approximate settlement dates to ensure proper timing of our blood sampling (i.e., to reflect a bird’s diet while on the breeding grounds rather than from the migratory period); blood reflects the short term diet of birds (9-15 d; Bearhop et al. 2002, Hobson and Clark 1992). Finally, breeding territory distance to river was estimated as the average of nest distance from river edge across all nest attempts.

Adult territorial flycatchers were captured at each site using both passive and target mist-netting (i.e., using playback of conspecific vocalizations as a lure). Blood samples were collected between 14 June to 13 August 2011 and 29 May to 11 August

2012, on average 39 days after flycatcher settlement at breeding sites. This allowed for contaminant concentrations in blood to reach equilibrium (Evers et al. 2005), assuming that contaminants levels remained constant during the sampling period. Contaminant concentrations in blood reflect recent dietary exposure; half-life of methylmercury in non-molting adult shearwaters was between 44 and 65 days (Monteiro and Furness 2001), and after four weeks of continuous dietary exposure methylmercury, mercury in blood of American kestrels increased linearly (French et al. 2010).

Each individual flycatcher was banded with a unique combination of a numbered aluminum USGS size 0 band and three color bands. Blood was sampled immediately following banding and collecting morphological measurements (i.e., mass, tarsus length, fat). following procedures described by the Ornithological Council (Fair et al. 2010). Blood was sampled immediately following banding and collecting morphological measurements. Approximately 50 to 100 microliters of blood was collected (<1% of the individual's body mass) from the subcutaneous ulnar (brachial) vein punctured with a disposable 26G needle (BD PrecisionGlide Intradermal Bevel Needle). Blood samples were immediately transferred to Dried Blood Spot (DBS, PerkinElmer, Waltham, MA, USA) cards. DBS cards are useful for sampling blood from small-bodied birds (<20g; adult birds in our study had a mean weight of 12.8g, $SD = 0.7$ g, nestling mean weight = 9.7g, $SD = 1.4$ g) because small volume of blood is sufficient for analysis (Shlosberg et al. 2011, Stove et al. 2012). All flycatcher sampling was conducted in accordance with Institutional Animal Care and Use Committee protocol (2010A0003-R1), Ohio Division

of Wildlife Wild Animal Permit (11-68), and a US Department of the Interior, US Geological Survey Federal Banding Permit (22272).

All samples were analyzed at DCPAH for a panel of toxic elements, including mercury and selenium ($\mu\text{g/kg}$, dry weight). Instrument calibration [ICP-AES (Varian Vista, Agilent, Santa Clara, CA) and ICP-MS (Agilent 7500ce)] was performed with NIST-traceable stock solution standards for each element (GFS Chemicals, Inc., Cincinnati, OH). Lab reagent blanks [NIST (National Institute of Standards and Technology, Gaithersburg, MD)-traceable Multi-mix (Alfa Aesar Specpure, Ward Hill, MA)] and NIST Standard Reference Materials (SRM) digests (Mussel Tissue 2976, Montana II Soil 2711a, and/or Trace Elements in Water 1643e) were analyzed for quality assurance in each sequence. Cold vapor atomic absorption spectrometry (CETAC CVAA, Omaha, NE) was used for mercury analysis, with similar standards, instrument calibration, and quality control. Although total mercury was measured rather than methylmercury, concentration of mercury in blood of insectivorous birds is primarily methylmercury (>95%; Evers et al. 2005, Rimmer et al. 2005a). Despite Ohio being in a region of relatively low selenium concentrations, selenium was considered as a covariate in our models because selenium can act antagonistically with low levels of mercury (decreasing toxicity) in vertebrates (Civin-Aralar and Furness 1991, Scheuhammer et al. 2012, Spalding et al. 2000).

Statistical Methods

All statistical analyses were performed using R 3.0.0 software (R Core Team 2013) with the additional packages “Hmisc” (Harrell Jr and with contributions from

Charles Dupont and many others 2012), “lattice” (Sarkar 2008), “nlme” (Pinheiro et al. 2012), and “pastecs” (Ibanez et al. 2013). After checking normality, data on contaminant concentrations were $\log_e$ transformed to meet the assumption of normality prior to statistical analyses.

I used linear mixed-effects models fit by maximum likelihood estimates and weighted linear regressions to evaluate relationships between contaminants in the aquatic system and in flycatchers. In the mixed models, I treated study site as a random effect. Weighted linear regressions were used to account for differing numbers of flycatcher blood samples at each site, where site was the unit of measure (for comparison with sediment and insect samples). I assessed relationships between (1) flycatcher territory distance to river and blood contaminant concentrations, (2) watershed flow accumulation and contaminants in sediment, insects, and flycatcher blood, (3) urbanization and contaminants in sediment, insects, and flycatcher blood, (4) sediment and insect contaminant concentration, (5) insect and flycatcher blood contaminant concentrations, and (6) sediment and flycatcher blood contaminant concentration. Overall fit of the models was checked visually with plots of residuals and by examining parameter estimates. The alpha significance level was set at $P \leq 0.10$.

Results

Contaminant concentration ($\mu\text{g/kg}$, dry weight) in sediment was variable for mercury (range: 7 to 99 $\mu\text{g/kg}$, $\bar{x} = 26.33$, $SD = 22.73$) and selenium (range: 130 to 2440 $\mu\text{g/kg}$, $\bar{x} = 639.60$, $SD = 599.32$; Table 2.2). Likewise, concentrations in aquatic emergent insects were also variable (mercury range: 36 to 238 $\mu\text{g/kg}$, $\bar{x} = 95.25$, $SD = 58.37$;

selenium range: 1465 to 6332 $\mu\text{g/kg}$, $\bar{x} = 4267.25$, $SD = 1347.09$; Table 2.2). The majority of insects in the pooled samples were < 10 mm in length. Contaminant concentrations in water were below detection limits (i.e., mercury < 10 ng/kg, selenium < 0.02 mg/kg; Table 2.2). In Acadian flycatcher blood, mercury concentrations ranged from 47 to 584 $\mu\text{g/kg}$ ($\bar{x} = 211.76$, $SD = 95.47$), and selenium concentrations ranged from 788 to 15982 $\mu\text{g/kg}$ ($\bar{x} = 3823.77$, $SD = 2064.93$, Table 2.3).

The distance of an Acadian flycatcher's territory from the river was not related to blood mercury concentrations ($F_{1,42} = 0.084$, $P = 0.774$). Watershed flow accumulation was not related to either mercury in sediment ($F_{1,13} = 0.545$, $P = 0.472$, **Error! Reference source not found.**), or in insects ($F_{1,14} = 1.912$, $P = 0.188$). Likewise, watershed flow was not related to selenium in sediment ($F_{1,13} = 1.091$, $P = 0.315$, **Error! Reference source not found.**) or in insects ($F_{1,14} = 0.073$, $P = 0.791$). Additionally, watershed flow accumulation was not related to either flycatcher mercury ($F_{1,17} = 0.258$, $P = 0.618$) or selenium ($F_{1,17} = 2.313$, $P = 0.147$). Urbanization ("urban index") was not related to either mercury concentration in insects ($F_{1,14} = 1.722$, $P = 0.211$, **Error! Reference source not found.**) or sediments ($F_{1,13} = 0.3376$, $P = 0.571$) or to selenium in sediments ($F_{1,13} = 0.201$, $P = 0.661$) or insects ($F_{1,14} = 0.009$, $P = 0.924$). In Acadian flycatcher blood, neither mercury ($F_{1,17} = 0.002$, $P = 0.968$) nor selenium ($F_{1,17} = 0.190$, $P = 0.668$) concentrations were predicted by urbanization.

There was no significant relationship between sediment and insect mercury concentrations ($F_{1,8} = 2.988$, $P = 0.122$, Figure 2.6), nor between sediment and insect selenium ($F_{1,8} = 0.858$, $P = 0.382$). Mercury in insects did not predict mercury

concentration in flycatcher blood ($F_{1,60} = 2.145$, $P = 0.148$, Figure 2.7). There was, however, a significant positive relationship between insect selenium and flycatcher selenium concentration ($F_{1,60} = 3.74$, $P = 0.058$, Figure 2.8).

Mercury concentration in sediment was not significantly related to mercury in flycatchers ($F_{1,52} = 0.454$, $P = 0.504$, Figure 8). Similarly, selenium in sediment was not significantly related to selenium in flycatchers ($F_{1,52} = 0.009$, $P = 0.924$). Models including concentrations of both sediment mercury and selenium as predictors were not significant ($F_{2,51} = 0.245$, $P = 0.784$), nor was the interaction of sediment mercury and sediment selenium on the flycatcher mercury concentrations ($F_{3,50} = 0.203$, $P = 0.894$). Mercury and selenium were negatively correlated in flycatchers (Pearson's $r = 0.466$, $P < 0.001$, $n = 76$).

When urban and rural sites were analyzed in separate weighted linear regressions, a different pattern emerged. Although non-significant, there was a positive trend between sediment mercury and flycatcher blood mercury at urban sites ($F_{1,20} = 1.502$, $P = 0.235$, Figure 2.10). However, a significant negative relationship between sediment and flycatcher mercury was observed for rural sites ($F_{1,30} = 8.286$, $P = 0.007$, Figure 2.10).

Discussion

A growing body of literature suggests that terrestrial insectivorous birds in riparian habitats can be exposed to mercury and other contaminants via prey-mediated contaminant flux between aquatic and terrestrial systems (Cristol et al. 2008, Menzie 1980, Sullivan and Rodewald 2012, Walters et al. 2008). Given that the mercury concentration in sediment did not predict mercury in insects, and insect mercury did not

predict Acadian flycatcher blood mercury concentration, there may be a disconnect or missing link in the contaminant flux pathways I examined. Alberts et al. (2013) recently found a significant positive relationship between mercury in sediments and four swallow species in the same study system. However, the relationship between sediment and blood levels of mercury and selenium varied among landscapes. Despite similar levels of sediment contamination along the rural-urban gradient, both mercury and selenium concentrations in swallows were higher at rural than urban sites (Alberts et al. 2013). The different relationship between sediment and flycatcher mercury concentrations in my system (i.e., negative at rural sites, positive at urban sites) suggests there may be a change in biogeochemical processes or flycatcher behavior along the rural-urban gradient that mediates the relationship between sediment and flycatcher mercury concentrations. Indeed, among other predictor variables including stream discharge and annual precipitation, Shanley et al. (2005) found that percent urban land cover was an important predictor of total mercury in streams. Furthermore, Alberts et al. (2013) found that swallows at urban sites relied more heavily on aquatic insects than birds at rural sites, and foraged at a higher trophic level (i.e., larger-bodied insects) at urban sites than at rural sites. This behavioral shift was possibly due to denser forests along the riverbanks at urban sites, prompting swallows to spend more time foraging directly over water and consuming more aquatic emergent insects (Alberts et al. 2013).

Relationships between sediment and blood mercury levels also may be obscured by differences in the bioavailability of mercury in sediments, which can alter the amount of uptake by aquatic insects (Edmonds et al. 2012). It may be important to carefully

consider which aquatic insect taxa to include in analyses of contaminant flux pathways; insects that rely more on aquatic food resources than terrestrial inputs will generally have greater mercury concentrations due to differences in uptake (Jardine et al. 2012, Leady and Gottgens 2001). Because the amount of methylmercury (a bioavailable and toxic form of mercury) was not identified in laboratory analyses, but rather total mercury was measured, the proportion of mercury in sediment that was bioavailable for uptake by insects in this system is not known. Likewise, I lack data on the organic carbon content of sediments or water pH, which can influence bioavailability (Edmonds et al. 2010, Jardine et al. 2012). That said, several studies of trophic transfer pathways and biomagnification of contaminants have not measured or used organic carbon content in analyses, yet were able to detect significant relationships between aquatic prey and terrestrial predators in contaminant biomagnification (e.g., Walters et al. (2008, 2010)), including studies in the same study system (Alberts et al. 2013).

In our system, the lack of significant relationships that would confirm contaminant flux from sediment or aquatic insects to Acadian flycatchers suggests that (1) the diet of Acadian flycatchers may not be primarily composed of aquatic emergent insects, (2) that flycatchers are foraging at different locations where I did not sample (e.g., in upland habitat, farther than aquatic emergent insect dispersal distances), or (3) that the aquatic emergent insect taxa I sampled was not representative of taxa that relied more on aquatic food resources than on terrestrial inputs to aquatic systems, and thus had lower levels of mercury uptake than taxa that do rely more on aquatic resources.

However, there are a number of important caveats that must be considered. Importantly,

because insect samples were pooled and the composition of flycatcher diets remains unknown, I cannot omit the possibility that changes in insect communities and preferred prey species may have affected the proportion of aquatic prey in flycatcher diets. This possibility is of special concern given that insects and flycatchers were sampled at different times separated by 1 to 2 months. Further study is also needed in our system to determine if biogeochemical factors (e.g., water pH, dissolved organic matter) or forest structure along riverbanks are changing the pathways of contaminant flux to terrestrial insectivores along rural-urban gradients.

Our study contributes generally to the growing body of knowledge about pathways for contaminant flux from aquatic to terrestrial systems. In particular, I show that assumptions about pathways may not hold true given the complexity and variability of behavioral, biogeochemical, and landscape mediators of exposure. A predictor that may have provided better evidence of contaminant flux pathways might include ephemeral pools or flooded areas within the riparian floodplain. Proximity of wetlands to streams may be more important than wetland land cover in general and wetlands increase the potential for mercury methylation in redox conditions (Shanley et al. 2005). The different relationship I found between mercury in sediments and in flycatcher blood at urban and rural sites warrants further consideration, particularly to determine other landscape variables I did not examine such as dams or impoundments in relation to sediment mercury concentrations, or in relation to insect productivity. Because contamination of watersheds is widespread and persistent (U.S. Environmental Protection Agency 2004), wildlife managers should include an aquatic component to management of

terrestrial organisms, in order to be aware of contaminants that can biomagnify through food webs.

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Table 2.1. Study site location, watershed flow accumulation, and urban index for 19 riparian forests within the upper Scioto River basin, Ohio, USA. ¹Watershed flow accumulation. ²Urban index from Rodewald and Shustack (2008).

Study Site	Latitude	Longitude	River	County	Flow Acc ¹	Urban Index ²
Bexley	39.97297	-82.94560	Alum	Franklin	104055	1.23
Big Walnut	39.94430	-82.85602	Big Walnut	Franklin	220234	1.31
Camp Mary	40.12137	-83.03080	Olentangy	Franklin	6306879	0.21
Casto	40.08277	-82.92212	Alum	Franklin	4737080	1.25
Creeks	39.88168	-82.90453	Blacklick	Franklin	1128841	-0.71
Elk Run	39.89725	-82.89823	Big Walnut	Franklin	855349	-0.16
Galena	40.21565	-82.87890	Big Walnut	Delaware	3477786	-0.48
Gardner	39.89492	-83.21672	Big Darby	Franklin	676106	-0.87
Heisel	39.91025	-82.88600	Big Walnut	Franklin	462901	0.36
Kilbourne	40.32965	-82.95540	Alum	Delaware	2438689	-0.87
Lou	39.93638	-83.00118	Scioto	Franklin	500264	1.26
North Galena	40.35568	-82.92207	Alum	Delaware	2063592	-1.27
Prairie Oaks	39.98628	-83.24587	Big Darby	Franklin	84517	-1.12
Public Hunting	39.84723	-83.20280	Big Darby	Franklin	2504073	-1.15
Smith	39.90273	-82.91700	Alum	Franklin	628177	-0.28
Sunbury	40.23527	-82.83522	Big Walnut	Delaware	665729	-0.42
TNC	39.91700	-83.23150	Big Darby	Franklin	583058	-0.96
Tuttle	40.01200	-83.03100	Olentangy	Franklin	7402760	1.61
Woodside	40.04557	-82.88090	Big Walnut	Franklin	6389651	0.32

Table 2.2. Concentrations of mercury (Hg) and selenium (Se) in sediment, insect, and water samples collected at study sites in the upper Scioto River basin, Ohio, USA.

Study Site	Sediment		Insect		Water	
	Hg	Se	Hg	Se	Hg	Se
	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)	(ng/L)	(µg/L)
Bexley	10	130	.	.	<10	<20
Big Walnut	30	1269	65	3280	<10	<20
Camp Mary	29	551	66	2893	<10	<20
Casto	20	306	.	.	<10	<20
Creeks	43	317	41	3306	<10	<20
Elk Run	7	184	67	6257	<10	<20
Galena	16	566	58	4393	<10	<20
Gardner	.	.	124	4431	.	.
Heisel	8	187	126	5247	<10	132
Kilbourne	34	976	36	4093	<10	<20
Lou	99	2440	58	3853	.	.
North Galena	23	812	.	.	<10	<20
Prairie Oaks	.	.	104	4676	.	.
Public Hunting	.	.	112	5084	.	.
Smith	19	222	43	2877	<10	<20
Sunbury	9	234	91	6332	<10	<20
TNC	.	.	67	1465	.	.
Tuttle	31	700	58	4001	.	.
Woodside	17	699	214	6088	<10	<20

Table 2.3. Concentrations of mercury and selenium in blood of Acadian flycatcher adults from study sites in the upper Scioto River basin, Ohio, USA.

Study Site	Sample	Hg $\bar{x}$ (SD) ($\mu\text{g/kg}$)	Se $\bar{x}$ (SD) ($\mu\text{g/kg}$)
Bexley	Blood	236 (0)	2386 (0)
Big Walnut	Blood	218 (0)	5967 (0)
Camp Mary	Blood	290 (0)	3414 (0)
Casto	Blood	218 (189)	4216 (3862)
Creeks	Blood	236 (74)	4244 (1062)
Elk Run	Blood	291 (23)	4276 (770)
Galena	Blood	288 (115)	3256 (862)
Gardner	Blood	210 (62)	3192 (754)
Heisel	Blood	98 (40)	4707 (3519)
Kilbourne	Blood	212 (59)	3026 (1459)
Lou	Blood	232 (84)	3933 (1072)
North Galena	Blood	169 (63)	3201 (1122)
Prairie Oaks	Blood	183 (68)	4377 (2825)
Public Hunting	Blood	141 (64)	3323 (1467)
Smith	Blood	224 (28)	3883 (1230)
Sunbury	Blood	510 (105)	5578.5 (186)
TNC	Blood	158 (0)	1903 (0)
Tuttle	Blood	119 (31)	1722 (497)
Woodside	Blood	209 (84)	5586 (5879)

Figure 2.1. Potential pathways of mercury flux from an aquatic system to a terrestrial insectivore, and possible contributing landscape factors (simplified representation of food web and the pathways I examined). Positive relationships are represented by (+) and negative relationships are represented by (-).

Figure 2.2. Study site locations in the upper Scioto River basin, in Franklin and Delaware Counties, Ohio, USA

Figure 2.3. The relationship between watershed flow accumulation ($\log_e$) and mercury in sediment ($\mu\text{g/kg}$, $\log_e$) was not significant ($F_{1,13} = 0.545$, $P = 0.472$) at study sites in central Ohio, USA.

Figure 2.4. The relationship between watershed flow accumulation ($\log_e$) and sediment selenium concentration ($\mu\text{g/kg}$, $\log_e$) was not significant ($F_{1,13} = 1.091$, $P = 0.315$), at sites in central Ohio, USA.

Figure 2.5. The relationship between the urban index and insect mercury concentration ($\mu\text{g/kg}$, log_e) was not significant ($F_{1,14} = 1.722$, $P = 0.211$), at sites in central Ohio, USA.

Figure 2.6. Relationship between sediment mercury ($\mu\text{g/kg}$, $\log_e$) and insect mercury ($\mu\text{g/kg}$, $\log_e$) was not significant ($F_{1,8} = 2.988$, $P = 0.122$), at sites in central Ohio, USA.

Figure 2.7. The relationship between insect mercury ($\mu\text{g/kg}$, $\log_e$) and flycatcher blood mercury ($\mu\text{g/kg}$, $\log_e$) was not significant ($F_{1,60} = 2.145$, $P = 0.148$), at sites in central Ohio, USA.

Figure 2.8. The relationship between insect selenium ($\mu\text{g/kg}$) and flycatcher blood selenium ($\mu\text{g/kg}$, $\log_e$) was significant ($F_{1,60} = 3.74$, $P = 0.058$), at sites in central Ohio, USA.

Figure 2.9. The relationship between mercury concentrations ($\mu\text{g/kg}$, $\log_e$) in Acadian flycatcher blood and sediment mercury ($\mu\text{g/kg}$, $\log_e$) concentration was not significant ($F_{1,52} = 0.454$, $P = 0.504$), at sites in central Ohio, USA.

Figure 2.10. Weighted regression (negative) between sediment mercury in rural sites on blood mercury ($\mu\text{g/kg}$, $\log_e$) in Acadian flycatchers was significant ($F_{1,30} = 8.286$, $P = 0.007$, $R^2 = 0.216$). Weighted regression between sediment mercury ($\mu\text{g/kg}$, $\log_e$) in urban sites and Acadian flycatcher blood mercury ($\log_e$) was positive but not significant ($F_{1,20} = 1.502$, $P = 0.235$, $R^2 = 0.070$). Samples from study sites in central Ohio, USA.

Chapter 3. Reproductive consequences of trace mercury concentrations in Acadian flycatcher (*Empidonax virescens*) breeding in remnant riparian forests

Abstract

Environmental contaminants can impact the health of wildlife in a number of ways, including reduced reproductive productivity, diminished body condition, altered behavior, depressed immune function, and mortality. Predicting health outcomes can be especially difficult in cases where exposure is not tied to an animal's immediate environment, as when contaminants cross ecosystem boundaries through food web linkages. In riparian systems, consumers that rely on a combination of aquatic and terrestrial food resources can be exposed to both aquatic and terrestrial contaminant sources. Contaminant flux from aquatic to terrestrial systems is expected to disproportionately affect species reliant upon aquatic emergent insects, such as with aerial insectivorous birds. From 2011-2012, I used the aerial insectivore, Acadian flycatcher (*Empidonax virescens*), as a model to understand the reproductive consequences of mercury (Hg) along 6 rivers in central Ohio, USA. At 19 sites in riparian forest, I determined Hg concentration in the blood of 76 breeding flycatchers and the season-long reproductive success (as measured by total number of fledglings per territorial male) of tested and banded individuals. Hg concentrations in adult flycatcher

blood were relatively low (47 to 584 $\mu\text{g/kg}$, $\bar{x} = 211.76$, $SD = 95.47$) and were not related to adult body condition (i.e., size-adjusted mass). However, even at these low concentrations, Hg in blood was negatively related to reproductive success (number of fledglings declined by -0.83 with each $\mu\text{g/kg}$ ($\log_e$) increase of Hg in blood). Adult flycatchers had 11x greater concentrations of blood Hg than their offspring, and the youngest nestlings had the lowest concentrations of Hg in blood. That I observed reduced reproductive success at trace levels of mercury in a free-living songbird (lower than a previously described threshold for reduced nest success) is a cautionary note that wildlife managers should not overlook trace levels of contaminants that biomagnify.

Introduction

Although mortality is the most extreme consequence of chemical exposure, contaminants can elicit a wide range of sub-lethal effects, including altered behavior, depressed immune function, diminished reproductive productivity, and decreased body condition (Brasso and Cristol 2008, Cristol et al. 2008, Hargreaves et al. 2010, Jackson et al. 2011a). Among contaminants likely to adversely affect wildlife, mercury is of particular concern because it is geographically widespread due to atmospheric deposition (Blais 2005, Fitzgerald and Engstrom 1998, Pacyna et al. 2010, Swain et al. 1992), has the potential to remain in aquatic sediments over a long time period (> 50 years), and can be redistributed over large areas with flood events (Jackson et al. 2011b). Mercury can enter and biomagnify in aquatic systems through multiple pathways (Blais et al. 2007, Evers 2005, Leady and Gottgens 2001, Walters et al. 2008). Once in aquatic systems, Hg can bioaccumulate in aquatic emergent insect larvae from sediments or water, and can

subsequently be transferred into terrestrial food webs as adult insects emerge and are depredated by terrestrial consumers (Cristol et al. 2008, Menzie 1980, Sullivan and Rodewald 2012). Thus, insectivorous songbirds foraging in riparian habitats can accumulate contaminants at levels comparable to those detected in piscivorous bird species (Brasso and Cristol 2008, Cristol et al. 2008).

Previous studies have documented adverse reproductive effects of elevated mercury levels in insectivorous songbirds (Evers et al. 2012). Contaminants from adult female birds can be deposited in eggs, which may affect reproductive success through reduced hatching (Jackson et al. 2011a) or as embryonic deformities of the bill, eye, mandible, and foot (Hoffman et al., 1988). Due to this Hg depuration mechanism (into eggs), females may have lower blood Hg concentrations than adult males (Jackson et al. 2011a, Rimmer et al. 2005b). Body and blood Hg concentrations of nestling birds also may be unexpectedly low as they can deposit Hg into their growing feathers (Condon and Cristol 2009, Spalding et al. 2000). Mercury was an order of magnitude less in nestling swallows (multiple spp.) than adults (Alberts et al. 2013, Brasso and Cristol 2008). After fledging, however, Hg concentration in blood increased (post-feather growth) in great egrets (*Ardea alba*) (Spalding et al. 2000), which may lead to reduced fledgling survival. In an area with point-source contamination of Hg, tree swallows (*Tachycineta bicolor*) produced fewer fledglings than in an uncontaminated reference area, and second-year females produced smaller eggs than older females within the contaminated area (Brasso and Cristol 2008). Likewise, Hg was associated with reduced nest success in the Carolina wren (*Thryothorus ludovicianus*), a terrestrial insectivore (Jackson et al. 2011a).

In this study I examined the relationship between avian reproduction and blood concentrations of mercury in remnant riparian forests of central Ohio, where all major waterways contain Hg contamination (Ohio EPA 2013). I focused on the Acadian flycatcher (*Empidonax virescens*) a long-distance Neotropical migrant that breeds in mature deciduous and riparian forest. Acadian flycatchers consume a variety of insect prey from both aquatic and terrestrial systems, including beetles, moths, wasps, mosquitoes, crane flies, damselflies, dragonflies, deer and horse flies, and spiders (Whitehead and Taylor, 2002), thus allowing for pathways of exposure to metals from sediment and water as well as from terrestrial sources. I hypothesized that the presence of heavy metals in the blood of Acadian flycatchers would both reduce body condition and impair reproduction. As such, I predicted that the number of young fledged would be negatively related to metal concentration in blood. I also hypothesized that nestling flycatchers would have lower levels of Hg in blood than adults.

Methods

Study system and sampling

Our study system was located in the Upper Scioto River watershed in central Ohio, USA, within 19 mature riparian forest fragments (>100m wide and $\geq$ 250m long). Sites were located in the upper Scioto River basin, along Alum Creek, Big Darby Creek, Big Walnut Creek, Blacklick Creek, the Olentangy River, and the Scioto River mainstem (Figure 3.1, Table 3.1). Dominant tree species at study sites consisted of American elm (*Ulmus americana*), American hackberry (*Celtis occidentalis*), black walnut (*Juglans nigra*), boxelder (*Acer negundo*), Eastern cottonwood (*Populus deltoides*), honey locust

(*Gleditsia tricanthos*), silver maple (*Acer saccharinum*), sycamore (*Plantanus occidentalis*), sugar maple (*Acer saccharum*), and white ash (*Fraxinus americana*). Woody understory vegetation included honeysuckle (*Lonicera* spp.), multiflora rose (*Rosa multiflora*), common spicebush (*Lindera benzoin*), dogwood (*Cornus* spp.), hawthorn (*Crataegus* spp.), Ohio buckeye (*Aesculus octandra*), tall paw paw (*Asimina triloba*), and saplings of overstory trees (Borgmann and Rodewald 2004, Leston and Rodewald 2006).

I studied Acadian flycatchers from May through August 2011 and 2012. In our study system, male flycatchers typically settle at breeding sites within the first two weeks in May, and females arrive roughly within a week thereafter. Because blood reflects an organism's diet within the short term (9-15 d; Bearhop et al. 2002, Hobson and Clark 1992), I recorded approximate settlement dates in order to ensure that our blood collection activities reflected a bird's diet on the breeding habitat rather than from the migratory period.

Adult flycatchers were captured at each study site using passive and target mist-netting (i.e., using playback of conspecific vocalizations as a lure). Blood samples were collected from territorial flycatchers between 14 June 2011 to 13 August 2011 and 29 May 2012 to 11 August 2012, on average 39 days after flycatcher settlement at breeding sites. This post-settlement waiting period allowed for contaminant concentrations in blood to reach equilibrium (Evers et al. 2005), assuming that contaminants present at study sites remained constant during the sampling period. Half-life of methylmercury in non-molting adult shearwaters was between 44 and 65 days (Monteiro and Furness

2001), and after four weeks of continuous dietary exposure methylmercury, Hg in blood of American kestrels increased linearly (French et al. 2010). Blood samples were collected from nestling flycatchers when possible (i.e., when nest height was less than 3m) at 6 to 9 days after hatching to assess any age-related differences in contaminant concentrations.

Each individual flycatcher was banded with a numbered aluminum USGS size 0 band and a unique combination of three color bands. Blood was sampled immediately following banding and collecting morphological measurements (i.e., mass, tarsus length, fat), following procedures described by the Ornithological Council (Fair et al. 2010). Approximately 50 to 100 microliters of blood was collected (<1% of the individual's body mass) from the subcutaneous ulnar (brachial) vein punctured with a disposable 26G needle (BD PrecisionGlide Intradermal Bevel Needle). Blood samples were immediately transferred to Dried Blood Spot (DBS, PerkinElmer, Waltham, MA, USA) cards. DBS cards are useful for sampling blood from small-bodied birds (<20g; adult birds in our study had a mean weight of 12.78g, $SD = 0.71$ g, nestling mean weight = 9.67g, $SD = 1.36$ g), in that a small volume of blood is sufficient for analysis (Shlosberg et al. 2011, Stove et al. 2012). All flycatcher sampling was conducted in accordance with Institutional Animal Care and Use Committee protocol (2010A0003-R1), Ohio Division of Wildlife Wild Animal Permit (11-68), and a US Department of the Interior, US Geological Survey Federal Banding Permit (22272).

Blood samples were analyzed at the Diagnostic Center for Animal and Population Health, Toxicology Section, Michigan State University, for mercury and selenium (Se)

total concentrations ($\mu\text{g/kg}$, dry weight). Total mercury was measured rather than methylmercury, as mercury in blood is primarily methylmercury (>95%) in insectivorous birds (Evers et al. 2005, Rimmer et al. 2005a). Calibration of instruments [ICP-AES (Varian Vista, currently with Agilent, Santa Clara, CA) and ICP-MS (Agilent 7500ce)] was performed with NIST-traceable stock solution standards for each element (GFS Chemicals, Inc., Cincinnati, OH). Lab reagent blanks [NIST (National Institute of Standards and Technology, Gaithersburg, MD)-traceable Multi-mix (Alfa Aesar Specpure, Ward Hill, MA)] and NIST Standard Reference Materials (SRM) digests (Mussel Tissue 2976) were analyzed for quality assurance in each sequence. Cold vapor atomic absorption spectrometry (CETAC CVAA, Omaha, NE) was used for Hg analysis, with similar standards, instrument calibration, and quality control.

Reproductive monitoring

Territories of Acadian Flycatcher within study sites were intensively monitored at 2-5 day intervals, with shorter intervals as time of expected fledging approached, from May through August 2011 and 2012. Nest searching was conducted both through observing behavior and systematic searching of vegetation within territories. All nests were monitored until failure or fledging. I was able to track the total number of young produced by an individual for each year (i.e., across all nesting attempts) by monitoring color-banded individuals throughout the season.

Statistical methods

All statistical analyses were performed using R 3.0.0 software (R Core Team 2013) with the additional packages “Hmisc” (Harrell Jr and with contributions from Charles Dupont and many others 2012), “lattice” (Sarkar 2008), “nlme” (Pinheiro et al. 2012), and “pastecs” (Ibanez et al. 2013). After checking normality of the data, Hg concentrations were $\log_e$ transformed to meet the assumption of normality prior to statistical analyses.

I used a linear mixed-effects model fit by maximum likelihood estimates to evaluate the potential relationship between contaminant concentration in blood and productivity. Models included blood concentrations of mercury as a fixed effect and site as a random effect variable. Samples from both years were used in analyses because metal concentrations in blood were not significantly different between years ($F_{1,74} = 0.665$, $P = 0.418$). Because males and females did not differ significantly in blood concentration of Hg ($F_{2,73} = 1.045$, $P = 0.357$), I used only samples from males in the productivity models to avoid multiple samples for a given territory. Adult contaminant concentrations were compared to nestling concentrations using a paired t-test. Given our small sample size and the high variability among individuals, I used an alpha of 0.1 to indicate statistical significance. Overall fit of the models was checked by visually examining plots of residuals and parameter estimates.

The relationship between blood contaminant concentration and size-adjusted body condition in adult flycatchers was evaluated using linear mixed models with blood contaminant concentration as fixed effects and site as a random effect. The size-adjusted measure of body condition was calculated from a principal component analysis (PCA) on

wing, tarsus, and tail, resulting in a frame size PCA. Separately for each sex, I then ran a linear regression of the first principle component on mass (regression was significant for males: $F_{1,55} = 9.149$, $P = 0.004$, but not for females: $F_{1,15} = 0.012$, $P = 0.920$). I used the residuals from this regression (i.e., deviations of each bird from their expected mass given frame size) as my index of body condition for males (Appendix B).

Results

A total of 76 adult flycatchers were captured (57 males, 17 females, and 2 unknown sex; 16 birds in 2011, 60 in 2012, with 1-7 individuals per site). Metal concentrations in adult flycatcher blood ranged from 47 to 584 $\mu\text{g/kg}$ ($\bar{x} = 211.78$, $SD = 95.47$). Mercury in blood was not significantly related to male body condition ($F_{1,35} = 0.003$, $P = 0.955$). 17 nestling flycatchers were sampled in 2012; Hg concentration in nestlings ranged from below detection limits (i.e., $< 20 \mu\text{g/kg}$) to 38 $\mu\text{g/kg}$ ($\bar{x} = 19.06$, $SD = 13.52$). Mercury concentration in adult male flycatcher blood was negatively related to reproductive success as measured by number of fledglings over the entire season ($F_{1,26} = 3.021$, $P = 0.094$, Figure 3.2). Likewise, Hg concentration in adult female blood was negatively correlated with the number of fledglings ($r = -0.521$, $n = 16$). Adult male blood concentrations of Hg were 11x higher for adult males than for nestlings (paired t-test: $t = 4.595$, $df = 5$, $P = 0.006$) and were negatively correlated with nestling blood Hg concentration (Pearson's $r = -0.857$, $P = 0.029$, Table 3.2).

The youngest nestlings had the lowest concentrations of Hg in blood, but there was high variation among nestlings even within a given nest. In general, Hg

concentrations in nestlings tended to increase with nestling age (Figure 3.3), but this relationship was not significant ($F_{1,15} = 2.13$, $P = 0.165$).

Discussion

Although levels of mercury in the blood of Acadian flycatchers in our study were relatively low and appeared not to affect body condition, I found that reproductive success declined with increased Hg loads. Aquatically-derived food sources may be an important food source for flycatchers within riparian systems, yet I found that Hg concentrations from aquatic system samples (i.e., in sediments) were not related to blood concentrations of Hg in flycatchers (Rowse, unpublished data). Urbanization was likewise not related to Hg loads in flycatchers (Rowse, unpublished data), and thus was not a contributing factor to reproductive success in relation to Hg contamination. The reduced success due to Hg contamination that I observed could be explained by changes in adult behavior (e.g., nest abandonment, altered provisioning or incubation), or by egg hatching success (not documented in our study due to nest heights). Although brood parasitism and predation are common in our system and known to depress reproduction, the effect of Hg contamination on reproduction did not seem to be the consequence of those two processes. Even after excluding nests that were parasitized or depredated (partially or fully), the number of fledglings and blood Hg were still negatively correlated ($r = -0.44$, $n = 21$).

Reduced reproductive success due to Hg contamination has been documented in avian species (Evers et al. 2008; Wolfe et al. 1998), concordant with our results. Reduced reproductive success of Carolina wrens at Hg contaminated sites was attributed primarily

to nest abandonment (Jackson et al. 2011a). Likewise, Brasso and Cristol (2008) found that tree swallows produced fewer fledglings in a highly contaminated area compared to a reference area, but this pattern emerged only for second-year breeding females, and could be attributed to other factors besides Hg contamination. Longcore et al. (2007) found that fledging success for tree swallows was not affected by Hg loads in nestlings, thus it is more likely that adult Hg loads affect overall reproductive success rather than nestling Hg loads. To our knowledge, our study is the first to document negative reproductive consequences of trace levels of Hg in blood of a free-living songbird. In fact, birds in our study were on average 70% below the Hg concentrations expected to depress nest success by 10% in several passerine species in the northeast U.S. (Figure 3.4, Evers et al. 2012). Thus, our findings present a cautionary note given the prevalence and persistence of mercury in the environment (Evers 2005).

Relationships between adult and nestling mercury concentrations illustrate the difficulty of generalizing patterns of exposure among individuals even within the same species. For one, adult Hg was significantly higher than nestling Hg (on average, Hg concentration in adult blood was 11x greater than in nestling blood). The difference in blood Hg likely did not result from differing diets of the adult and nestling flycatchers, as nestlings are fed similar food items as the adults (Whitehead and Taylor 2002). However, I do not have evidence that adult and nestling flycatchers consumed similar sized prey items. Larger-bodied aquatic emergent insects may disperse farther from water, and can bioaccumulate more contaminants than insects placed lower in a food web; furthermore, larger-bodied insects are likely more energetically attractive to the flycatchers.

Provisioning effort by male and female flycatchers is approximately equal, so any differences in prey captured by males or females should not make a difference in nestling exposure to Hg through food items (Whitehead and Taylor 2002). In a similar study system to ours, Hg concentrations were greater in tree swallows at rural sites than urban sites, and appeared to be mediated by reliance on aquatic prey (Alberts et al. 2013). Alberts et al. (2013) also found that tree swallows both consumed a greater proportion of aquatic than terrestrial prey and fed at a higher trophic level in urban versus rural sites, and had elevated levels of Se but not Hg at urban sites. The lower Hg loads in nestlings versus adult flycatchers might result, in part, from differences in prey size or the amount of prey consumed, even if the diet was composed of similar food items. Mumford (1964) observed adults bringing large-bodied prey (e.g., damselflies and dragonflies) only to large nestlings (only after a few days post-hatching). Another probable cause of low Hg levels in nestling blood is the depuration of Hg into feathers during the period of rapid feather growth (Condon and Cristol 2009, Spalding et al. 2000). The low Hg levels in nestlings I found is consistent with companion studies in the same study system, where Alberts et al. (2013) observed that both Se and Hg concentrations in adult riparian swallows (multiple spp.) were significantly higher than those observed in nestlings. More surprising, I found that the concentration of Hg in adult blood was negatively related to that in the blood of nestlings, though the sample size of nestlings was small ($n = 17$).

I found no evidence that adult body condition was related to Hg load, thus suggesting that Hg did not affect reproduction by way of body condition. At the levels of Hg detected in this study, flycatchers may be able to partition Hg into tissues (such as the

liver or kidney) or excrete Hg to the extent that it is not toxic (Spalding et al. 2000). The process of Hg demethylation into its organic form reduces the toxic action of the element. There is, however, a limit to the amount of Hg birds can demethylate (due to cell damage or a lack of materials for detoxification); demethylation is a more efficient process at low levels of methylmercury in the blood (Spalding et al. 2000), as it is in the Acadian flycatchers. Selenium plays a role in demethylation of Hg; Se and methylmercury combine to form non-toxic protein complexes that can be stored in tissues (Civin-Aralar and Furness 1991, Scheuhammer et al. 2012, Spalding et al. 2000). In the adult flycatchers, however, Hg and Se were negatively correlated (Pearson's $r=0.466$, $P<0.001$, $n=76$, Appendix A).

Our observation of reduced reproductive output is likely a conservative estimate, as I did not account for potential sublethal effects of mercury such as egg hatching success (often not measurable due to nest heights) or altered behavior in adults provisioning nestlings or fledglings (Jackson et al. 2011a). However, trace level effects of mercury on reproduction in this system may suggest that higher levels of contaminants would have a greater negative effect. Future research may better explore other mechanisms by which mercury might affect reproduction and recruitment. This research adds to the growing body of knowledge regarding effects of mercury contamination on passerine reproductive success, and provides insight into effects of trace levels of mercury on a free-living terrestrial insectivore.

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Table 3.1. Study site locations for 19 riparian forests within the upper Scioto River basin, Ohio, USA.

Study Site	Latitude	Longitude	River	County
Bexley	39.97297	-82.94560	Alum	Franklin
Big Walnut	39.94430	-82.85602	Big Walnut	Franklin
Camp Mary	40.12137	-83.03080	Olentangy	Franklin
Casto	40.08277	-82.92212	Alum	Franklin
Creeks	39.88168	-82.90453	Blacklick	Franklin
Elk Run	39.89725	-82.89823	Big Walnut	Franklin
Galena	40.21565	-82.87890	Big Walnut	Delaware
Gardner	39.89492	-83.21672	Big Darby	Franklin
Heisel	39.91025	-82.88600	Big Walnut	Franklin
Kilbourne	40.32965	-82.95540	Alum	Delaware
Lou	39.93638	-83.00118	Scioto	Franklin
North Galena	40.35568	-82.92207	Alum	Delaware
Prairie Oaks	39.98628	-83.24587	Big Darby	Franklin
Public Hunting	39.84723	-83.20280	Big Darby	Franklin
Smith	39.90273	-82.91700	Alum	Franklin
Sunbury	40.23527	-82.83522	Big Walnut	Delaware
TNC	39.91700	-83.23150	Big Darby	Franklin
Tuttle	40.01200	-83.03100	Olentangy	Franklin
Woodside	40.04557	-82.88090	Big Walnut	Franklin

Table 3.2. Adult male and nestling Acadian flycatcher blood concentrations of mercury (Hg) and selenium (Se) in parts per billion (µg/kg), sampled at study sites in the upper Scioto River basin, Ohio, USA. Note: "Mean" is the mean concentration for all nestlings in one territory; "Individual" is the contaminant concentration for each nestling. Values below detection limit (e.g., <10) treated as 0 for mean and SD.

Site	Nest Number	Male Hg	Nestling Hg		Male Se	Nestling Se	
			Mean (SD)	Individual		Mean (SD)	Individual
Casto	12-05-101	351	6.7 (11.5)		6946	377 (98.85)	
				20			485
				<20			291
				<20			355
Kilbourne	12-14-140	191	NA		4074	NA	
				21			326
North Galena	12-11-005	115	25.3 (3.06)		3039	714 (161.58)	
				22			531
				26			774
				28			837
Public Hunting	* 12-11-006	106	13.2 (18.4)		2598	830.8 (421.19)	
				<20			462
				<20			535
				<20			581

Continued

Site	Nest Number	Male Hg	Nestling Hg		Male Se	Nestling Se	
			Mean (SD)	<i>Individual</i>		Mean (SD)	<i>Individual</i>
Public Hunting	12-11-004	87	25.3 (2.08)	38	2121	735.7 (10.69)	1341
				28			1235
	12-10-008			26			729
				23			748
				27			730
Tuttle	12-16-201	97	32.5 (6.4)	37	1370	1171 (12.73)	1162
				28			1180

*Two nests from the same male at Public Hunting were combined for the nestling averages.

Table 3.2 continued.

Figure 3.1. Study sites ($n = 19$) in the upper Scioto River basin, within Franklin and Delaware Counties, Ohio, USA.

Figure 3.2. Adult male Acadian flycatcher mercury in blood ($\log_e$, $\mu\text{g/kg}$) was negatively related to number of fledglings (corresponding to an individual male's territory, Pearson's $r = -0.857$, $P = 0.029$). Samples were collected in 2011-2012, in the Scioto River basin, Ohio, USA. *Note: Line shown is a graphical illustration, not the linear mixed model regression.

Figure 3.3. Acadian flycatcher nestling mercury concentration in blood ($\mu\text{g/kg}$) in relation to estimated nestling age (days). Samples were collected in 2012, in the Scioto River basin, Ohio, USA.

Figure 3.4. Blood mercury concentrations in songbird species associated with risk of reduced nest success (lines show 10% reduced success at 0.7 mg/kg, 20% at 1.2 mg/kg, and 30% at 1.7 mg/kg; Evers et al. 2008).

Appendix A. Body condition indices (the residuals from a regression of a frame size PCA on mass) for adult Acadian flycatchers sampled at study sites in the upper Scioto River basin, Ohio, USA. *Note:* ACFL = Acadian flycatcher; Condition = body condition index, Hg = mercury ($\mu\text{g/kg}$), Se = selenium ($\mu\text{g/kg}$).

Study Site	Species	Band Number	Sex	Condition	Hg	Se
Bexley	ACFL	231037369	M	-0.40	236	2386
Big Walnut	ACFL	245008696	M	-0.17	218	5967
Camp Mary	ACFL	231037355	M	0.40	290	3414
Casto	ACFL	249074166	M	0.33	351	6946
Casto	ACFL	231037387	F	-1.08	84	1485
Creeks	ACFL	231037349	M	-0.50	112	3330
Creeks	ACFL	240094058	M	-0.70	266	3838
Creeks	ACFL	240094058	M	-0.90	196	4671
Creeks	ACFL	249074133	M	0.50	151	2497
Creeks	ACFL	249074147	M	0.43	234	3724
Creeks	ACFL	249074147	M	0.60	298	5451
Creeks	ACFL	231037340	F	0.80	317	3876
Creeks	ACFL	231037348	F	-1.38	328	5737
Creeks	ACFL	231037350	F	-0.49	220	5074
Elk Run	ACFL	231037347	M	-1.38	273	3674
Elk Run	ACFL	240094054	M	0.18	316	5143
Elk Run	ACFL	240094054	M	0.15	283	4010
Galena	ACFL	231037346	M	0.01	413	3907
Galena	ACFL	231037358	M	0.37	162	2478
Galena	ACFL	249074157	M	0.04	225	2547
Galena	ACFL	231037382	F	-0.09	353	4090
Gardner	ACFL	231037337	M	-0.24	164	3500
Gardner	ACFL	231037339	M	-0.58	273	3115
Gardner	ACFL	231037342	M	-0.28	278	3166
Gardner	ACFL	231037343	M	0.74	186	2052
Gardner	ACFL	240094082	M	-0.98	147	4126
Heisel	ACFL	231037338	M	-0.26	70	2218
Heisel	ACFL	245009701	F	-0.35	126	7195
Kilbourne	ACFL	231037351	M	0.11	191	4074
Kilbourne	ACFL	231037371	M	0.04	166	1360
Kilbourne	ACFL	231037372	M	-0.21	278	3644
Lou	ACFL	231037357	M	0.89	265	3973
Lou	ACFL	231037378	M	-0.35	262	5617
Lou	ACFL	240094055	M	0.37	230	2907
Lou	ACFL	249074135	M	-0.05	106	2280
Lou	ACFL	249074135	M	0.17	327	4988
Lou	ACFL	231037377	F	0.04	186	3511
Lou	ACFL	231037379	F	0.03	140	3898
Lou	ACFL	249074132	F	0.90	342	4293

Study Site	Species	Band Number	Sex	Condition	Hg	Se
North Galena	ACFL	231037345	M	0.38	115	3039
North Galena	ACFL	231037370	M	0.42	177	4593
North Galena	ACFL	240094087	M	-0.39	198	3517
North Galena	ACFL	249074154	M	-0.22	294	4878
North Galena	ACFL	249074154	M	0.09	157	2027
North Galena	ACFL	249074156	M	0.52	76	1350
North Galena	ACFL	231037383	F	0.52	174	3244
North Galena	ACFL	249074134	F	-0.27	203	3508
North Galena	ACFL	249074134	F	1.02	125	2651
Prairie Oaks	ACFL	231037335	M	0.60	128	2829
Prairie Oaks	ACFL	240094049	M	0.20	285	9407
Prairie Oaks	ACFL	240094049	M	1.03	208	2859
Prairie Oaks	ACFL	249074153	M	0.59	177	3389
Prairie Oaks	ACFL	231037336	F	1.33	115	3400
Public Hunting	ACFL	231037363	M	-0.20	106	2598
Public Hunting	ACFL	231037368	M	-0.85	87	2121
Public Hunting	ACFL	249074150	M	0.01	139	5631
Public Hunting	ACFL	249074151	M	0.10	226	3133
Public Hunting	ACFL	249074151	M	0.66	242	3331
Public Hunting	ACFL	249074162	M	0.35	119	4049
Public Hunting	ACFL	249074162	M	0.58	177	5034
Public Hunting	ACFL	231037352	F	0.20	124	3222
Public Hunting	ACFL	231037367	F	-1.13	47	788
Smith	ACFL	245008698	M	0.26	243	4753
Smith	ACFL	231037344	F	0.95	204	3013
Sunbury	ACFL	231037356	M	-0.69	584	5710
Sunbury	ACFL	231037361	M	0.61	436	5447
TNC	ACFL	231037341	M	-0.83	158	1903
Tuttle	ACFL	231037354	M	-0.64	97	1370
Tuttle	ACFL	249074117	F	-1.00	141	2073
Woodside	ACFL	231037334	M	0.57	104	2784
Woodside	ACFL	231037359	M	-0.60	245	4225
Woodside	ACFL	231037360	M	-0.21	262	1748
Woodside	ACFL	249074128	M	-0.42	298	15982
Woodside	ACFL	249074128	M	-0.24	136	3191

Appendix B. Adult Acadian flycatcher total number of fledglings and concentration of mercury and selenium in adult blood at study sites in the upper Scioto River basin, Ohio, USA.

Study Site	Species	Date	Band Number	Sex	Fledglings	Mercury	Selenium
Bexley	ACFL	6/27/2012	231037369	M	0	236	2386
Big Walnut	ACFL	6/25/2012	245008696	M	1	218	5967
Camp Mary	ACFL	6/14/2012	231037355	M	2	290	3414
Casto	ACFL	6/29/2012	249074166	M	3	351	6946
Casto	ACFL	7/31/2012	231037387	F	3	84	1485
Creeks	ACFL	6/20/2012	240094058	M	1	266	3838
Creeks	ACFL	6/22/2012	231037340	F	0	317	3876
Creeks	ACFL	7/11/2012	231037348	F	2	328	5737
Elk Run	ACFL	7/2/2012	231037347	M	0	273	3674
Elk Run	ACFL	6/22/2012	240094054	M	0	316	5143
Galena	ACFL	6/28/2012	231037346	M	1	413	3907
Galena	ACFL	7/16/2012	231037382	F	1	353	4090
Gardner	ACFL	6/16/2012	231037337	M	1	164	3500
Gardner	ACFL	6/23/2012	231037342	M	0	278	3166
Gardner	ACFL	6/23/2012	240094082	M	5	147	4126
Heisel	ACFL	6/18/2012	231037338	M	2	70	2218
Heisel	ACFL	7/27/2012	245009701	F	2	126	7195
Kilbourne	ACFL	6/6/2012	231037351	M	1	191	4074
Lou	ACFL	6/16/2012	231037357	M	1	265	3973
Lou	ACFL	7/6/2012	231037378	M	0	262	5617
Lou	ACFL	6/21/2012	249074135	M	0	327	4988
Lou	ACFL	7/1/2011	249074132	F	0	342	4293
Lou	ACFL	7/7/2012	231037379	F	1	140	3898
Lou	ACFL	7/6/2012	231037377	F	0	186	3511
North Galena	ACFL	6/28/2012	231037345	M	5	115	3039
North Galena	ACFL	5/29/2012	240094087	M	2	198	3517
North Galena	ACFL	6/20/2012	249074154	M	0	294	4878
North Galena	ACFL	7/25/2012	231037383	F	3	174	3244
North Galena	ACFL	8/11/2012	249074134	F	2	203	3508
North Galena	ACFL	8/8/2011	249074134	F	2	125	2651
Prairie Oaks	ACFL	6/12/2012	231037335	M	0	128	2829
Prairie Oaks	ACFL	6/19/2012	240094049	M	5	285	9407
Prairie Oaks	ACFL	6/19/2012	249074153	M	3	177	3389
Prairie Oaks	ACFL	6/14/2012	231037336	F	3	115	3400
Public Hunting	ACFL	6/23/2012	231037363	M	0	106	2598
Public Hunting	ACFL	6/26/2012	231037368	M	3	87	2121
Public Hunting	ACFL	6/9/2012	249074150	M	0	139	5631
Public Hunting	ACFL	7/3/2012	249074151	M	0	226	3133
Public Hunting	ACFL	6/23/2012	249074162	M	4	119	4049

Study Site	Species	Date	Band Number	Sex	Fledglings	Mercury	Selenium
Public Hunting	ACFL	7/28/2012	231037352	F	0	124	3222
Public Hunting	ACFL	6/26/2012	231037367	F	3	47	788
Smith	ACFL	6/27/2012	245008698	M	0	243	4753
Smith	ACFL	6/25/2012	231037344	F	0	204	3013
Sunbury	ACFL	6/15/2012	231037356	M	1	584	5710
Sunbury	ACFL	6/22/2012	231037361	M	1	436	5447
TNC	ACFL	6/23/2012	231037341	M	4	158	1903
Tuttle	ACFL	6/14/2012	231037354	M	2	97	1370
Tuttle	ACFL	7/6/2012	249074117	F	2	141	2073
Woodside	ACFL	6/19/2012	231037359	M	0	245	4225
Woodside	ACFL	6/10/2012	249074128	M	0	298	15982

Appendix C. Contaminant concentrations for Acadian flycatchers at all sites in all years 2011-2012.

Study Site	Year	Band Number	Sex	Age	Contaminant Concentrations (all in µg/kg)					
					Arsenic	Cadmium	Lead	Mercury	Selenium	Thallium
Bexley	2012	2310-37369	Male	Adult	<10	<10	<10	236	2386	<10
Big Walnut	2012	2450-08696	Male	Adult	<10	<10	<10	218	5967	<10
Camp Mary	2012	2310-37355	Male	Adult	<10	<10	<10	290	3414	<10
Casto	2012	2310-37387	Female	Adult	<10	<10	<10	84	1485	<10
Casto	2012	2310-37388	Unknown	Nestling	<10	<10	<10	20	485	<10
Casto	2012	2310-37389	Unknown	Nestling	<10	<10	<10	<20	291	<10
Casto	2012	2310-37390	Unknown	Nestling	<10	<10	<10	<20	355	<10
Casto	2012	2490-74166	Male	Adult	<10	<10	<10	351	6946	<10
Creeks	2012	2310-37340	Female	Adult	<10	<10	<10	317	3876	<10
Creeks	2012	2310-37348	Female	Adult	<10	<10	<10	328	5737	<10
Creeks	2012	2310-37349	Male	Adult	<10	<10	<10	112	3330	<10
Creeks	2012	2310-37350	Female	Adult	<10	<10	<10	220	5074	<10
Creeks	2012	2400-94058	Male	Adult	<10	<10	<10	266	3838	<10
Creeks	2011	2400-94058	Male	Adult	<10	<10	<10	196	4671	<10
Creeks	2011	2490-74133	Male	Adult	<10	<10	<10	151	2497	<10
Creeks	2012	2490-74147	Male	Adult	<10	<10	<10	234	3724	<10
Creeks	2011	2490-74147	Male	Adult	<10	<10	<10	298	5451	<10
Elk Run	2012	2310-37347	Male	Adult	<10	<10	<10	273	3674	<10
Elk Run	2012	2400-94054	Male	Adult	<10	<10	<10	316	5143	<10
Elk Run	2011	2400-94054	Male	Adult	<10	<10	<10	283	4010	<10
Galena	2012	2310-37346	Male	Adult	<10	<10	<10	413	3907	<10
Galena	2012	2310-37358	Male	Adult	<10	<10	<10	162	2478	<10
Galena	2012	2310-37382	Female	Adult	<10	<10	<10	353	4090	<10
Galena	2011	2490-74157	Male	Adult	<10	<10	<10	225	2547	<10
Gardner	2012	2310-37337	Male	Adult	<10	<10	<10	164	3500	<10
Gardner	2012	2310-37339	Male	Adult	<10	<10	<10	273	3115	<10
Gardner	2012	2310-37342	Male	Adult	<10	<10	<10	278	3166	<10
Gardner	2012	2310-37343	Male	Adult	<10	<10	<10	186	2052	<10
Gardner	2012	2400-94082	Male	Adult	<10	<10	<10	147	4126	<10

Study Site	Year	Band Number	Sex	Age	Contaminant Concentrations (all in µg/kg)					
					Arsenic	Cadmium	Lead	Mercury	Selenium	Thallium
Heisel	2012	2310-37338	Male	Adult	<10	<10	<10	70	2218	<10
Heisel	2012	2450-09701	Female	Adult	<10	<10	<10	126	7195	<10
Kilbourne	2012	2310-37351	Male	Adult	<10	<10	<10	191	4074	<10
Kilbourne	2012	2310-37362	Unknown	Nestling	<10	<10	<10	21	326	<10
Kilbourne	2012	2310-37371	Male	Adult	<10	<10	<10	166	1360	<10
Kilbourne	2012	2310-37372	Male	Adult	<10	<10	10	278	3644	<10
Lou	2012	2310-37357	Male	Adult	<10	<10	<10	265	3973	<10
Lou	2012	2310-37377	Female	Adult	<10	<10	<10	186	3511	<10
Lou	2012	2310-37378	Male	Adult	<10	<10	<10	262	5617	<10
Lou	2012	2310-37379	Female	Adult	<10	<10	<10	140	3898	<10
Lou	2012	2310-37380	Unknown	Adult	<10	<10	<10	317	7292	<10
Lou	2011	2400-94055	Male	Adult	<10	<10	<10	230	2907	<10
Lou	2011	2490-74132	Female	Adult	<10	<10	<10	342	4293	<10
Lou	2011	2490-74135	Male	Adult	<10	<10	<10	106	2280	<10
Lou	2012	2490-74135	Male	Adult	<10	<10	<10	327	4988	<10
North Galena	2012	2310-37345	Male	Adult	<10	<10	<10	115	3039	<10
North Galena	2012	2310-37370	Male	Adult	<10	<10	<10	177	4593	<10
North Galena	2012	2310-37383	Female	Adult	<10	<10	<10	174	3244	<10
North Galena	2012	2310-37384	Unknown	Nestling	<10	<10	<10	22	531	<10
North Galena	2012	2310-37385	Unknown	Nestling	<10	<10	<10	26	774	<10
North Galena	2012	2310-37386	Unknown	Nestling	<10	<10	<10	28	837	<10
North Galena	2012	2400-94087	Male	Adult	<10	<10	<10	198	3517	<10
North Galena	2012	2490-74134	Female	Adult	<10	<10	<10	203	3508	<10
North Galena	2011	2490-74134	Female	Adult	<10	<10	<10	125	2651	<10
North Galena	2012	2490-74154	Male	Adult	<10	<10	<10	294	4878	<10
North Galena	2011	2490-74154	Male	Adult	<10	<10	<10	157	2027	<10
North Galena	2011	2490-74156	Male	Adult	<10	<10	<10	76	1350	<10
Prairie Oaks	2012	2310-37335	Male	Adult	<10	<10	<10	128	2829	<10
Prairie Oaks	2012	2310-37336	Female	Adult	<10	<10	<10	115	3400	<10

Study Site	Year	Band Number	Sex	Age	Contaminant Concentrations (all in µg/kg)					
					Arsenic	Cadmium	Lead	Mercury	Selenium	Thallium
Prairie Oaks	2012	2400-94049	Male	Adult	<10	<10	<10	285	9407	<10
Prairie Oaks	2011	2400-94049	Male	Adult	<10	<10	<10	208	2859	<10
Prairie Oaks	2012	2490-74153	Male	Adult	<10	<10	<10	177	3389	<10
Public Hunting	2012	2310-37352	Female	Adult	<10	<10	<10	124	3222	<10
Public Hunting	2012	2310-37353	Unknown	Adult	<10	<10	<10	172	3023	<10
Public Hunting	2012	2310-37363	Male	Adult	<10	<10	<10	106	2598	<10
Public Hunting	2012	2310-37364	Unknown	Nestling	<10	<10	<10	26	729	<10
Public Hunting	2012	2310-37365	Unknown	Nestling	<10	<10	<10	23	748	<10
Public Hunting	2012	2310-37366	Unknown	Nestling	<10	<10	<10	27	730	<10
Public Hunting	2012	2310-37367	Female	Adult	<10	<10	<10	47	788	<10
Public Hunting	2012	2310-37368	Male	Adult	<10	<10	<10	87	2121	<10
Public Hunting	2012	2310-37373	Unknown	Nestling	<10	<10	<10	38	1341	<10
Public Hunting	2012	2310-37374	Unknown	Nestling	<10	<10	<10	28	1235	<10
Public Hunting	2012	2310-37391	Unknown	Nestling	<10	<10	<10	<20	462	<10
Public Hunting	2012	2310-37392	Unknown	Nestling	<10	<10	<10	<20	535	<10
Public Hunting	2012	2310-37393	Unknown	Nestling	<10	<10	<10	<20	581	<10
Public Hunting	2012	2490-74150	Male	Adult	<10	<10	<10	139	5631	<10
Public Hunting	2012	2490-74151	Male	Adult	<10	<10	<10	226	3133	<10
Public Hunting	2011	2490-74151	Male	Adult	<10	<10	<10	242	3331	<10
Public Hunting	2012	2490-74162	Male	Adult	<10	11	<10	119	4049	<10
Public Hunting	2011	2490-74162	Male	Adult	<10	<10	<10	177	5034	<10
Smith	2012	2310-37344	Female	Adult	<10	<10	<10	204	3013	<10
Smith	2012	2450-08698	Male	Adult	<10	<10	<10	243	4753	<10
Sunbury	2012	2310-37356	Male	Adult	<10	<10	<10	584	5710	<10
Sunbury	2012	2310-37361	Male	Adult	<10	<10	<10	436	5447	<10
TNC	2012	2310-37341	Male	Adult	<10	<10	<10	158	1903	<10
Tuttle	2012	2310-37354	Male	Adult	<10	<10	<10	97	1370	<10
Tuttle	2012	2310-37375	Unknown	Nestling	<10	<10	<10	37	1162	<10
Tuttle	2012	2310-37376	Unknown	Nestling	<10	<10	<10	28	1180	<10

Study Site	Year	Band Number	Sex	Age	Contaminant Concentrations (all in µg/kg)					
					Arsenic	Cadmium	Lead	Mercury	Selenium	Thallium
Tuttle	2012	2490-74117	Female	Adult	<10	<10	<10	141	2073	<10
Woodside	2011	2310-37334	Male	Adult	<10	<10	<10	104	2784	<10
Woodside	2012	2310-37359	Male	Adult	<10	<10	<10	245	4225	<10
Woodside	2012	2310-37360	Male	Adult	<10	<10	<10	262	1748	<10
Woodside	2012	2490-74128	Male	Adult	<10	<10	<10	298	15982	<10
Woodside	2011	2490-74128	Male	Adult	<10	<10	<10	136	3191	<10

Appendix D. Insect contaminant concentrations ($\mu\text{g/kg}$), sampled in 2012 from sites in the upper Scioto River basin, Ohio, USA.

Study Site	Sample	Date	Arsenic	Cadmium	Lead	Mercury	Selenium	Thallium
Big Walnut	Insect	10/8/2012	212.26	7177.35	3830.43	64.96	3279.57	213.07
Camp Mary	Insect	9/10/2012	163.73	409.53	486.18	66.47	2892.76	87.91
Creeks	Insect	10/9/2012	120.44	3005.81	1245.04	41.44	3305.81	195.62
Elk Run	Insect	9/25/2012	379.79	856.38	828.01	66.56	6257.08	43.97
Galena	Insect	9/19/2012	326.87	2450.00	699.58	57.91	4393.35	30.76
Gardner	Insect	9/30/2012	257.73	838.84	1603.77	123.94	4431.02	101.49
Heisel	Insect	9/24/2012	760.91	1327.37	6485.65	126.10	5246.73	36.88
Kilbourne	Insect	10/6/2012	183.14	4840.06	1088.48	36.06	4092.56	360.24
Prairie Oaks	Insect	9/14/2012	336.88	1240.74	783.07	103.66	4675.66	171.06
Public Hunting	Insect	9/30/2012	712.29	2038.26	1840.61	112.23	5084.25	173.08
Smith	Insect	10/6/2012	169.34	1697.34	671.36	42.73	2877.10	52.80
Sunbury	Insect	9/19/2012	217.34	3954.29	1261.12	90.99	6332.42	12.04
TNC	Insect	9/14/2012	114.92	508.46	481.93	66.65	1464.87	172.70
Tuttle	Insect	8/25/2012	379.40	1552.60	3904.11	238.02	4001.10	184.52
Woodside	Insect	9/11/2012	710.49	4862.20	1857.14	214.39	6088.50	95.27

Appendix E. Sediment contaminant concentrations (Arsenic, Cadmium, Lead, Thallium in mg/kg; Mercury and Selenium in µg/kg), sampled in 2012 from sites in the upper Scioto River basin, Ohio, USA.

Study Site	Sample	Date Collected	Arsenic	Cadmium	Mercury	Lead	Selenium	Thallium
Bexley	Sediment	8/14/2012	<12.5	<1.25	9.65	20.85	130.48	<62.5
Big Walnut	Sediment	8/14/2012	17.78	<1.25	29.63	21.78	1268.75	<62.5
Camp Mary	Sediment	9/10/2012	<12.5	<1.25	29.29	22.84	550.75	<62.5
Casto	Sediment	9/11/2012	<12.5	<1.25	19.88	15.43	306.00	<62.5
Creeks	Sediment	8/14/2012	<12.5	<1.25	42.55	29.73	317.00	<62.5
Elk Run	Sediment	8/14/2012	<12.5	<1.25	7.37	35.34	183.53	<62.5
Galena	Sediment	9/19/2012	<12.5	<1.25	16.23	15.15	566.25	<62.5
Heisel	Sediment	8/14/2012	<12.5	<1.25	7.66	12.97	187.13	<62.5
Kilbourne	Sediment	10/6/2012	20.42	<1.25	33.85	21.43	975.75	<62.5
North Galena	Sediment	10/6/2012	22.74	<1.25	22.73	14.48	812.25	<62.5
Smith	Sediment	8/14/2012	<12.5	<1.25	18.93	19.16	221.58	<62.5
Sunbury	Sediment	9/19/2012	<12.5	<1.25	8.68	<12.5	234.13	<62.5
Woodside	Sediment	8/14/2012	14.79	<1.25	16.70	14.98	698.75	<62.5

Appendix F. Water contaminant concentrations (Arsenic, Cadmium, Lead, Selenium, and Thallium in mg/kg; Mercury in ng/L),
sampled in 2012 from sites in the upper Scioto River basin, Ohio, USA.

	Study Site	Sample	Date	Arsenic	Cadmium	Mercury	Lead	Selenium	Thallium
			Collected						
	Bexley	Water	10/8/2012	<0.010	<0.005	<10	<0.010	<0.020	<0.020
	Big Walnut	Water	10/8/2012	<0.010	<0.005	<10	<0.010	<0.020	<0.020
	Camp Mary	Water	8/31/2012	<0.010	<0.005	<10	<0.010	<0.020	<0.020
95	Casto	Water	9/11/2012	<0.010	<0.005	<10	<0.010	<0.020	<0.020
	Creeks	Water	10/9/2012	<0.010	<0.005	<10	<0.010	<0.020	<0.020
	Elk Run	Water	9/25/2012	<0.010	<0.005	<10	<0.010	<0.020	<0.020
	Galena	Water	9/19/2012	<0.010	<0.005	<10	<0.010	<0.020	<0.020
	Heisel	Water	9/24/2012	<0.010	<0.005	<10	<0.010	0.132	<0.020
	Kilbourne	Water	10/6/2012	<0.010	<0.005	<10	<0.010	<0.020	<0.020
	North Galena	Water	10/6/2012	<0.010	<0.005	<10	<0.010	<0.020	<0.020
	Smith	Water	10/6/2012	<0.010	<0.005	<10	<0.010	<0.020	<0.020
	Sunbury	Water	9/19/2012	<0.010	<0.005	<10	<0.010	<0.020	<0.020
	Woodside	Water	9/11/2012	<0.010	<0.005	<10	<0.010	<0.020	<0.020

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