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Mercury Levels in Newly Independent Songbirds

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ABSTRACT PAGE

The South River, in the Shenandoah Valley of Virginia, was contaminated with mercury between 1929-1950 from an industrial source, and mercury is still present in sediment and aquatic biota. Adult songbirds breeding along the contaminated river have elevated blood mercury levels compared with reference sites. However, nestling blood mercury levels are an order of magnitude lower than adult levels on the contaminated site. I hypothesized that the low levels of mercury in nestling blood were a result of the buffering effect of growing feathers, which extract mercury from the blood. In 2006 and 2007, I found an increase in blood mercury levels in eastern bluebirds (Sialia sialis) during the three months following fledging, when feathers have finished growing and can no longer serve as a harmless reservoir for mercury. On the contaminated sites, mean blood mercury was 0.09 ± 0.06 ppm (mean ± standard deviation; n=156) in nestling bluebirds with growing feathers, as compared to 1.21 ± 0.57 ppm (n=86) in adults.

In 2006, bluebirds from 13 families were radio-tagged and trapped at intervals after leaving the nest, between May and August. Blood samples were taken from 46 fledgling bluebirds of different ages, with up to four repeated measures per individual, out to 92 days post fledging. In 2007, telemetry was not used, but an additional 12 individuals were caught. Mean blood mercury increased to 0.52 ± 0.36 ppm (n=44) when there was no discernible feather growth. When the first prebasic molt began, and feathers were growing again in numbers, mean blood mercury levels dropped to 0.20 ± 0.09 ppm (n=11) in fledglings. Fledglings originating from the second clutch began molting within a shorter time frame than birds from the first clutch; thus they were not exposed to mercury accumulation as long as birds originating from the first clutch. Stable isotopes of nitrogen were anlayzed to ensure that the change in blood mercury was due to feather growth, rather than a dietary shift. Nitrogen values increased with age, showing a different and unrelated pattern than the blood mercury levels.

Most studies of mercury contamination in young birds have focused on the nestling stage, when birds are buffered from mercury toxicity by growing feathers and have low levels of mercury in the blood. Nestling blood mercury levels were not indicative of the contamination at this site. Risks to young birds and possible effects on populations may become apparent in the vulnerable period after fledging, when birds learn to forage on their own. These findings suggest that further research is needed examining the effects on juvenile survival. Understanding what happens to mercury levels in young birds after they leave the nest is of importance for determining whether mercury is affecting reproductive fitness.

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Introduction

1.0 Mercury and human history

Mercury has been of use to humans for mining, industry, and agriculture for thousands of years. Since 1500 B.C., civilizations including the Egyptians, Greek, and Romans, have used this heavy metal as a preservative, in ointments, and as an amalgam with other metals. Cinnabar (HgS, mercuric sulfide), a naturally occurring ore of mercury, was first mined for use as a red pigment, and later in order to extract liquid mercury (Wiener et al. 2003). Mercury has been used in mining processes, primarily in the extraction of gold and silver. Mercury has also been used in the manufacturing process of mirrors and hats, in dental amalgams, electronics, and a variety of medical treatments. Isolated cases of mercury poisoning have been linked to these applications throughout history (*e.g.*, the "mad hatters" of the mid 18^{th} to 19^{th} century hat-making industry).

1.1 Human exposure events

The intentional use of mercury for industrial and agricultural purposes has also resulted in large-scale mercury poisoning events. In 1971-1972, thousands of Iraqis were poisoned with mercury through consumption of bread made with wheat seeds treated with a methyl mercury fungicide. Other cases of mercury poisoning via contaminated grains occurred in Ghana, Guatemala, and Pakistan (Elhassani 1983). However, the most well-known event was associated with contaminated fish in Minamata Bay, Japan, and human risk today is most related to fish consumption.

In the 1950s, Minamata Bay was contaminated with mercury from an industrial source. Hundreds of people were exposed to mercury through contaminated fish, and suffered from 'Minamata disease' for years following (Khera 1979; Clarkson 1987; Eisler 1987). By 1974 there had been more than 700 cases of methyl mercury poisoning in Minamata (NAS 1978). Those inflicted with this disease showed low IQ, muscular incoordination, difficulty walking, loss of hearing, difficulty speaking and constriction of vision (Khera 1979). All symptoms arose as a result of damage to the central nervous system, which is characteristic of mercury poisoning.

1.2 Human health advisories in the US

By 2001, 9% of river miles and 23% of lake acreage in the United States had been subjected to some level of fish advisory (Jakus et al. 2002). As of 2004, there were mercury-related fish consumption advisories on water bodies in 44 states (USEPA 2005). The United States Environmental Protection Agency (USEPA; section 304(a) of the Clean Water Act) has designated a criterion of 0.3 μg/g for methyl mercury in fish (Driscoll et al. 2007). Mercury levels are reported in different ways in the literature; currently, μg/g, mg/kg, parts per billion (ppb) and parts per million (ppm) are most often used and indicate the same concentration (see Appendix A). In the following reviews of other research, I will convert concentrations to parts per million (ppm). Concentrations may also be reported as wet weight (ww) or fresh weight (fw), when water is present in the sample, or as dry weight (dw), where no

water is present. Most mercury concentrations are reported as ww, and I will note if otherwise.

The Food and Drug Administration (FDA) action level for mercury in fish is 1 ppm, although several state governments use the standard of 0.5 ppm (USDI 1998). For reference, the median mercury concentration in fish caught in Minamata Bay was 11 ppm (NAS 1978).

2.0 Anthropogenic releases of mercury

Coal-fired power plants are responsible for approximately 50% of anthropogenic mercury emissions (USEPA 1997; Hylander 2001). Mining processes (for cinnabar ore or gold) can release mercury into the air, or directly into the environment as a waste product. The smelting of lead, copper, and zinc also release airborne mercury as a byproduct. Chlor-alkali plants, electric utilities, and wastewater treatment plants release mercury through improper disposal practices. Paint and wood pulping processes, fungicides, and seed dressings also are historic sources of mercury. All of these sources have contributed to increased airborne and waterborne emissions of mercury (Boening 2000; Evers et al. 2005; Driscoll et al. 2007). Even though industrial emissions have decreased in recent years in developed countries, mercury continues to be released into the environment in large quantities (Hylander 2001). In developing countries, mercury emissions have actually increased in the past 25 years, due to industrialization (Hylander 2001).

2.1 Anthropogenic increases in atmospheric mercury

Atmospheric mercury has increased by a factor of 2-5 since the beginning of the industrial period (Boening 2000; Driscoll et al. 2007). Sediment cores from remote lakes in Minnesota showed an increase of mercury about three times the preindustrial atmospheric deposition of 140 years ago (Swain et al. 1992). Mercury emissions directly from anthropogenic sources constitute 33-36% of the total tonnage of mercury emitted into the air annually (Driscoll et al. 2007). Airborne mercury has a variable residence time of up to 2 years, and may be transported up to thousands of kilometers (km; Boening 2000; Driscoll et al. 2007). As a result, mercury may be transported from industrial sources and deposited in land or water in remote and distant areas, contributing to global pollution.

Mercury 'hotspots' have been identified within the United States. In 1973, states with a high number of chlor-alkali plants or copper smelting facilities such as Arizona, Montana, Nevada, New Mexico, Delaware, Kentucky, Louisiana, and West Virginia were labeled 'hotspots' (NAS 1978). In 2007, 'hotspots' from atmospheric deposition were identified in northeastern North America, where levels of mercury deposition are up to six times higher than levels from 1900 (Evers et al. 2007). These areas are associated with landscape qualities that are favorable for mercury methylation and accumulation (*e.g.*, reservoirs with fluctuating water levels, forests, and presence of wetlands; see below 3.2 Methylation), or are in close proximity to a local emissions source (Evers et al. 2007). As early as 1978, NAS called for the

identification of areas that may be affected by direct mercury spills, where mercury concentrations in organisms may be well above background levels.

2.2 Point-source contamination

While sources of airborne emissions can be difficult to identify, waterborne or terrestrial emissions are often linked to a specific source. In cases of point-source pollution, mercury is released on a localized scale, often as an industrial spill or leak, contaminating the adjacent area to a degree that greatly exceeds natural background levels. Often, concentrations of mercury are highest in organisms and sediments closest to the source of contamination (Eisler 1987). Past studies have shown that mercury concentrations in biota in areas not directly contaminated by mercury were <1 ppm fw, whereas concentrations in biota near chlor-alkali plants were >1 ppm fw (Eisler 1987). However, given time and the right environmental conditions, contamination may move from the original source, as in the contamination of the South River, VA, where fish consumption advisories are in place for 100 miles downstream of the source (Carter 1977; http://www.deq.virginia.gov/fishtissue/pdf/mercurytext.pdf).

2.2.1 Classic cases of point-source contamination

In the United States, there are numerous sites of point-source mercury contamination associated with mining sites or industry. Some major case studies are described below. Clear Lake, in California, was contaminated with 100 tons of mercury from a mining operation and is now considered a superfund site (USDI 1998). At Clear Lake, mercury levels in brain, muscle and kidney in western grebes

(*Aechmorphorus occidentalis*) were twice those found in reference lakes (Elbert and Anderson 1998). In sediment, mercury concentrations ranged from 0.27 to 183 ppm dw (USDI 1998). The Carson River in Nevada was contaminated with mercury from approximately 75 gold and silver mines (Henny et al. 2002; Custer et al. 2007). Mean mercury concentrations in livers of tree swallows (*Tachycinta bicolor*) and house wrens (*Troglodytes aedon*) were 3.79 and 2.87 ppm dw respectively (Custer et al. 2007). The Willamette River Basin in Oregon was also contaminated with historic mine sites; American dippers (*Cinclus mexicanus*) had a mean total mercury of 0.0385 ppm in eggs and 1.158 ppm in nestling feathers at the most highly contaminated study site (Henny et al. 2005).

The Savannah River Site in South Carolina and the Holston River in southwestern Virginia were contaminated with mercury waste from chlor-alkali plants (Powell 1983; Kennamer et al. 2005). At the Holston River, five species of passerine had mean mercury levels between 0.41 and 2.4 ppm, significantly elevated over reference species (Powell 1983). Wood duck (*Aix sponsa*) albumen (where the majority of mercury was found) averaged 0.22 ppm at the Savannah River Site (Kennamer et al. 2005). The South River in Virginia, my study site, was contaminated with mercury from an industrial source approximately 80 years ago (see below Methods, 1.0 History of Study Area; Carter 1977). Adult female tree swallows had mean blood mercury levels of 3.56 ppm on the South River, compared to 0.17 ppm on reference sites (Brasso 2007). Adult belted kingfishers (*Ceryle alcyon*) had mean blood mercury of 3.35 ppm on the South River, significantly elevated over

reference birds (White 2007). Carolina wrens (*Thryothorus ludovicianus*) also had elevated mercury levels, up to 5 ppm, significantly higher than wrens on reference sites (Friedman 2007). Three species of turtles (*Chelydra serpentine*, *Sternotherus odoratus*, *Chrysemys picta*) had blood mercury levels that were 20 to 108 times higher than turtles on reference sites, up to 3.60 ppm (Bergeron et al. 2007).

3.0 Transport and cycling of mercury

Mercury is a naturally occurring element, typically released into the environment by the degassing of the earth's crust and ocean (*i.e.*, through volcanic eruptions, rock and soil volatilization), or through evaporation of the ocean's surface waters (Eisler 1987; USEPA 1997; Boening 2000). Mean background concentrations of mercury in surficial materials have been reported as 0.065 ppm dw (USDI 1998). Mercury may be emitted as a gas, in particulate form, or in solution, from natural and anthropogenic sources (see above 2.0 Anthropogenic releases of mercury).

Once released into the atmosphere, mercury may be transported great distances, in the form of mercury vapor, usually elemental mercury, Hg^0 (Eisler 1987; NAS 1987; Boening 2000). Mercury may then be deposited on land (60%) or water (40%) by wet precipitation of dissolved mercury, or dry deposition through adsorption to particles (such as aerosols; Eisler 1987; Morel et al. 1998). Amount of deposition varies depending on distance from the source, surface composition and climate (Rimmer et al. 2005). Residence time of mercury has been estimated at 11 days in the atmosphere (on the low end), 1000 years in soils and 2.5×10^8 years in sediments (NAS 1978). Once deposited, mercury may be reemitted to the

atmosphere, sequestered in the soil, or transported to a water body (Morel et al. 1998; Driscoll et al. 2007). Mercury does not dissolve easily in water and water bodies may become saturated with aqueous mercury, which may be re-released into the atmosphere or converted to another form of mercury (Morel et al. 1998; Driscoll et al. 2007).

3.1 Forms of mercury

Mercury exists in many chemical forms, inorganic and organic, but only the organic forms are thought to be toxic to organisms (NAS 1978). Mercury has three stable valence states (USDI 1998). Elemental (metallic) mercury (Hg^0) occurs commonly as a gas in the environment or dissolved in waters (Morel et al. 1998). The other two states are the mercuric ion (Hg(II) or Hg^{2+}, monovalent mercury), and the mercurous ion (Hg(I) or Hg_2^{2+}, divalent mercury) (Eisler 1987; Boening 2000). Mercuric forms are more common in the environment, occurring as dissolved mercury and bound to sulfides or dissolved organic carbon (DOC; Morel et al. 1998). Other forms commonly found in the environment include inorganic mercury salts (mercuric sulfide, HgS), mercuric oxide (HgO) and mercuric chloride ($HgCl_2$) (USEPA 1997). Organic forms of mercury are available to organisms in the form of a methyl mercury complex with chloride (CH_3HgCl), or as dissolved methyl or dimethyl mercury (Morel et al 1998).

Alkylmercury salts, such as methyl mercury (MeHg, CH_3Hg^+), are the most dangerous of organomercurials, mercury with one covalent link to a carbon atom (Elhassani 1982). The mercuric ion, Hg^{2+}, is the most toxic inorganic form.

However, all inorganic forms (*e.g.*, metallic mercury and mercury sulfide) are subject to easy conversion to the toxic methyl mercury or other organic forms in the environment or in the body, and thus are also an indirect hazard (NAS 1978). Throughout its cycle, mercury may be transformed several times, so availability of any type of mercury is important to consider in terms of possible risk.

3.2 Methylation

The process of methylation was discovered in the 1960s, and occurs when a methyl group is transferred from an organic compound to the metal ion, *i.e.*, when Hg^{2+} becomes CH_3Hg^+ (Jensen and Jernelov 1969; Carty and Malone 1979; Morel et al. 1998). All forms of mercury may be converted to methyl mercury through both biotic and abiotic processes. The conversion process is complex, influenced by many environmental factors, including the amount of mercury in the system. Low pH enhances methylation, so certain wetlands and areas subject to acid precipitation are sensitive to increased methylation (Zillioux et al. 1993; Wiener et al. 2003). Methylation also increases with temperature, water hardness and water flow (Boening 2000; Driscoll et al. 2007). Low phosphorous, high sulfides, high dissolved organic carbon, and low acid neutralizing capacity also increase methyl mercury formation in a system (Driscoll et al. 2007).

Furthermore, land use and type also may play a role; methylation is typically highest in watersheds with mixed agriculture and forest land cover and lower in upland soils (Driscoll et al. 2007). The anoxic waters and sediments of wetlands provide optimal conditions for methylation, and specifically seem to act as sources of

methyl mercury. Wetlands usually have higher levels of mercury compared with background levels (Morel et al. 1998; Driscoll et al. 2007). However, terrestrial soils also contain a considerable amount of mercury in various forms, which may be transported to surface waters (Gabriel and Williamson 2004).

Mercury methylation may occur abiotically in soils, perhaps in a broader range of ecological settings, *e.g.* a wider range of pH, than biological methylation (Celo et al. 2006). Photochemical processes may contribute to the formation of methyl mercury (Morel et al. 1998). Methylation may occur in a variety of soil types, and is influenced by the quantities of the methylating substance in the fulvic acid of the soil (Rogers 1977). Rates of methylation have been associated with soil organic material (Rogers 1977). Methylation decreased with a pH above 4.5 in three soil types: clay, loam and sand (Rogers 1977).

Some bacteria are able to convert inorganic mercury species to methyl mercury under both anaerobic and aerobic conditions in soil and waters (Eisler 1987). Sulfate-reducing bacteria (*e.g.*, *Desulfovibrio desulfiricans*) are the primary methylators in freshwater anoxic sediments, and their presence will increase methylation in water or sediment (Celo et al. 2006; Driscoll et al. 2007). Methylation by sulfate-reducing bacteria occurs in lakes with pH of 4 to 6, and with sulfate concentrations lower than 5mM (ocean water is 28mM) (Celo et al. 2006). Iron-reducing bacteria have also been shown to methylate mercury (Fleming et al. 2006). The methylation process (possibly a specific enzyme-mediated process) occurs

internally, after mercuric ions pass through bacterial lipid membranes either by way of diffusion, or via transporters designed for other metals (Morel et al. 1998).

4.0 Methyl mercury and trophic transfer

Methyl mercury enters consumer organisms almost entirely through the gastrointestinal tract. It is readily absorbed by the intestinal wall into the bloodstream and distributed to organs and tissues (Morel et al 1998). The inorganic mercuric complex, $HgCl_2$, is lipid soluble and may also pass through cellular membranes, but will not biomagnify through the food chain (Eisler 1987; Morel et al. 1998). Once absorbed, inorganic mercury may be bound to cellular materials and excreted. Methyl mercury is assimilated four times as efficiently as inorganic forms of mercury (USDI 1998; Morel et al. 1998; Bouton et al. 1999; Driscoll et al. 2007). Intestinal absorption of inorganic mercury is between 1 and 3%, compared with nearly 100% absorption of methyl mercury (Elhassani 1982; Scheuhammer 1987; USDI 1998).

In food webs, methyl mercury passes through lipid membranes of unicellular organisms and primary producers, then bioaccumulates within individual organisms and biomagnifies through the upper levels of the food chain (Morel et al. 1998). The proportion of methyl mercury to total mercury increases from 10% in water column, to 15% in phytoplankton to 30% in zooplankton to 95% in fish (Driscoll et al 2007). This increase in mercury accumulation with trophic level is seen in a variety of organisms, including birds (Zillioux et al 1993). In dosed zebra finches (*Poephila guttata*) mercury in the kidney and liver showed a 30-fold increase from the dietary mercury, and in the brain a 13-fold increase (Scheuhammer 1988).

4.1 Trophic level described by stable isotope analysis

Since the 1970s, stable isotopes have been used in ecology studies on food web structure, migration, food stress, and identification of food sources (Hobson 1987; Peterson and Fry 1987; Hobson et al. 1993; Hobson 1999; Podlesak et al. 2005). Isotopes are also increasingly being used as an analytical tool in ecotoxicology studies of contaminants that biomagnify through the food chain (Elbert and Anderson 1998; Thompson et al. 1998; Morrissey et al. 2004; Shaw-Allen et al. 2005). Fractionation (Δ 13C or 15N), as defined by Hobson and Clark (1992b), is the change in isotopic signature between diet and tissues of the consumer. Fractionation of carbon and nitrogen isotopes are used to gather information about the trophic status of an organism (Bearhop et al. 2002). See Methods, 9.0 Isotope analysis, for calculation and explanation of notation (δ).

4.1.1 Carbon isotopes

Carbon isotopes ($^{13}C/^{12}C$, or $\delta^{13}C$) reflect the contribution to the diet of plant energy produced by two distinct photosynthetic pathways, and are used to determine if a prey source is from a certain biome, *i.e.*, to distinguish marine or terrestrial sources, or areas with C_3, C_4, or CAM plants as the dominant vegetation type (Smith and Epstein 1971; DeNiro and Epstein 1978; Hobson 1987; Kelly 2000). The typical range of carbon isotopes for C_3 plants is -20 to -35‰, C_4 plants -7 to -15‰, and CAM -10 to -22‰ (Ehleringer 1989). Carbon isotopes are not as readily used to interpret trophic level due to the relatively small trophic enrichment of carbon in consumers of 0.8‰ (range -1.2-6‰) and also diet may not fall into a distinct $\delta^{13}C$-

defined biome (DeNiro and Epstein 1978; Hobson and Clark 1992; Kelly 2000; Pearson et al. 2003).

4.1.2 Nitrogen isotopes

Nitrogen isotopes ($^{15}N/^{14}N$, or $\delta^{14}N$) have been used more reliably to show food web position. Stable isotope composition of tissues (proteins) of an organism predictably reflects the isotope composition of proteins in their diet. The heavier isotope,^{15}N, is preferentially retained in tissues, while ^{14}N is excreted, so each trophic level accumulates more ^{15}N. Typically a 3.0‰ increase in $\delta^{15}N$ per trophic level is seen in a variety of bird species, with a range of 1-5‰ (DeNiro and Epstein 1981; Minagawa and Wada 1984; Hobson and Clark 1992; Pearson et al. 2003). There is variation between species, but generally herbivores have a lower $\delta^{15}N$ value than carnivores (Kelly 2000). Typically the ^{15}N value will show a positive relationship with mercury concentration, because mercury also bioaccumulates up the food web (Bearhop et al. 2000a; Bergeron et al. 2007), but there have been studies that have detected no relationship (Thompson et al. 1998).

4.1.3 Tissues and isotope analysis

Different tissues will provide different information on trophic level. Tissues with rapid isotopic turnover will indicate recent dietary uptake, and those with a longer turnover will indicate a long-term diet (Tieszen et al. 1983; Hobson and Clark 1992a). Whole blood provides information on recent or short-term diet from 20 - 30 days prior to sampling (Hobson and Clark 1992a; Bearhop et al. 2002). The half-life in blood is 10 - 16 days for ^{13}C and 9 - 15 for ^{15}N (Hobson and Clark 1992a; Bearhop

et al. 2000; Bearhop et al. 2002). Pearson et al. (2003) found that yellow-rumped warblers (*Dendroica coronata*) had a slightly shorter half-life of 3.9 - 6.1 days for ^{13}C. Variation in metabolic rate or stage of development may influence turnover rate in tissues (Hobson and Clark 1992a). Isotopic turnover (the ratio of ^{14}N:^{15}N retained) is faster during high metabolic activities, such as growth or molt, however it is unlikely that this increase would affect the overall signature of the tissue (Hobson and Clark 1992a; Bearhop et al. 2002).

4.1.4 Variation in isotopic signatures

However useful this technique has become, caution is still required when using isotope information in bird studies. Differences in $\delta^{15}N$ and ^{13}C are seen in individuals of the same species eating the same diet (DeNiro and Epstein 1978; DeNiro and Epstein 1981). There is some evidence that $\delta^{15}N$ values may become more enriched with age, despite feeding at the same trophic level, *e.g*, in walleye (*Stizostedion vitreum*), $\delta^{15}N$ increased with age (in years) of the fish (Overman and Parrish 2001). Other studies have shown no relationship with age and $\delta^{15}N$, but rather that size had a significant effect regardless of age, although often associated with age (Jardine and Curry 2006). Growth processes may also have an effect on $\delta^{15}N$; lower values were seen as nestling snowy egrets (*Egretta thula*) were growing, possibly due to the retention of protein in order to gain weight rapidly (Shaw-Allen et al. 2005).

Differences may be explained by location and time period sampled; there can be variation in isotopic signature over large and small spatial scales, *e.g.*, bird feather

^{15}N ratios were higher from agricultural land compared with boreal forest (DeNiro and Epstein 1981; Hobson 1999). Soil ^{15}N content may influence variation in plants at the base of the food chain, and thus organisms at upper levels. Disturbed soils, such as agricultural soils, have a high level of biological activity and nitrogen content, and may be enriched by nitrogen fertilizers as well (Alexander et al. 1996; Hobson 1999). The use of fertilizers (nitrate or ammonia), and sewage inputs may limit the interpretation of $\delta^{15}N$ values (DeNiro and Epstein 1981). Baseline isotopic signatures may be quite variable between ecosystems, and must be known in order to standardize $\delta^{15}N$ of organisms in a particular place (Cabana and Rasmussen 1996; Post 2002)

Nutritional stress or starvation have been shown to elevate ^{15}N ratios in birds and spiders (Hobson et al. 1993; Oelbermann and Scheu 2002). Although other studies on mysids (small crustaceans) and song sparrows (*Melospiza melodia*) did not confirm this (Gorokhova and Hansson 1999; Kempster et al. 2007). Feeding rate or quality of food may also affect the isotopic signature. Increased feeding rate on a constant diet caused decreased $\delta^{15}N$ in tilapia fishes (Gaye-Siessegger et al. 2003). High quality food caused increased $\delta^{15}N$ in spiders, however, low quality food caused increased $\delta^{15}N$ in tilapia (Oelbermann and Scheu 2002; Gaye-Siessegger et al. 2003).

As in mercury studies, most isotope studies with birds and contaminants have been conducted on large-bodied birds (*e.g.*, seabirds) or in the laboratory and few have been on passerines (but see Kelly 2000 and Pearson et al. 2003). Clearly, there is

much to be learned about interpretation of isotopic signatures, and they will be used with caution in this thesis.

5.0 Mercury contamination and birds—aquatic and terrestrial

Birds have long been used as biomonitors (Burger 1993). In Sweden in the 1950s and 1960s, birds were poisoned en masse through ingestion of seeds treated with a mercurial fungicide (Berg et al. 1966; NAS 1978). Mercury levels up to 270 ppm in livers and kidneys were found in the dead birds and 48% had liver mercury concentrations greater than 2 ppm (NAS 1978; Fimreite 1979). This was the first major ecological problem related to mercury poisoning, and led to a ban on mercurial fungicide use in 1966 (Burger 1993). Except in these cases of direct application, mercury has historically not been considered a problem in terrestrial systems. Most studies since this terrestrial-based catastrophe have focused on aquatic bird species. These large-bodied, fish-eating study species inhabiting marine or wetland ecosystems seem to be most at risk for accumulating mercury to dangerous levels (see above 3.2 Methylation, 4.0 Methyl mercury and trophic transfer), and have qualities that make them effective biomonitors (*e.g.*, long-lived, common, top-predators, charismatic; see Burger 1993). Extensive research has been conducted on the common loon (*Gavia immer*), wading birds, piscivorous raptors, and other long-lived species feeding at high trophic levels.

There is increasing evidence that methyl mercury accumulation is occurring in insectivorous passerines (Evers et al. 2005; Rimmer et al. 2005; Brasso and Cristol 2007; Driscoll et al. 2007; Cristol unpublished data). Elevated mercury levels have

been reported for northern waterthrush (*Sieurus noveboracensis*) and red-winged blackbird (*Agelaius phoeniceus*) and attributed to atmospheric deposition and point-source contamination (Powell 1983; Evers et al. 2005). Two species of sharp-tailed sparrows (*Ammodramus* spp.) in coastal salt-marshes had mean blood mercury ranging from 0.256 to 0.868 ppm (Shriver et al. 2006). Prothonotary warbler (*Protonotaria citrea*) nestlings accumulated significantly more mercury in their kidneys (mean=0.1688 ppm) than reference nestlings (0.0259 ppm) (Adair et al. 2003). Tree swallows (*Tachycineta bicolor*) and other species of terrestrial songbirds had elevated levels of mercury on the South River in Virginia (Brasso and Cristol 2007; Cristol unpubl. data). Terrestrial insectivorous species on this site had the highest mercury levels, and Friedman (2007) showed that these passerines were receiving mercury through their terrestrial prey items. Levels in these birds far exceeded other reported levels of terrestrial passerines, *e.g,*. the Bicknell's thrush (*Catharus bicknelli*) in high elevation habitat in northeastern North America (Rimmer et al. 2005).

6.0 Mercury toxicity in birds

The presence of mercury in birds is of concern because mercury has no known biological purpose in living organisms, and instead may cause neurotoxic, teratogenic, and carcinogenic effects. Toxicity of methyl mercury has been investigated in several bird species, and is associated with hematological and immune system damage, and may affect adult survival, reproductive success and behavior (Heinz 1976, 1979; Eisler 1987; Scheuhammer 1987; Thompson 1996; Hughes et al.

1997; Wolfe et al. 1998; Bouton et al. 1999; Sepulveda et al. 1999). Methyl mercury is characterized by high stability, lipid solubility, and the ability to penetrate membranes (*e.g.*, blood-brain and placental; Eisler 1987). It has a high affinity for, and forms stable complexes with, sulfhydryl groups (-SH) which are found in most proteins, and may inhibit any SH-containing enzymes and damage structural proteins (Carty and Malone 1979; Elhassani 1983; Clarkson 1987; Eisler 1987). Cell division may also be inhibited, and cellular swelling may result from the disrupted cell membrane sodium and potassium (Wolfe et al. 1998). Methyl mercury may destroy neurons and damage the central nervous system, possibly causing brain damage (Fimreite 1979; Eisler 1987; Evers et al. 2005).

These effects are often not evident immediately after exposure, there may be a considerable period of latency. Therefore, determining the concentration at which sublethal effects occur has been difficult (NAS 1978; Eisler 1987). How quickly effects become apparent may differ between species. Uptake and accumulation of mercury varies between species so generalizing trends is difficult (Eisler 1987). For example, similar effects of mercury may occur at different levels in species due to body size, diet, migratory patterns, and physiology (Scheuhammer 1988).

6.1 Distribution among tissues

In birds, as in other organisms, mercury is ingested and absorbed into the bloodstream, where it is distributed between red blood cells and plasma (mostly found in red blood cells; Elhassani 1982). Mercury is then distributed among other tissue compartments. The highest mercury levels are typically found in the feathers,

liver and kidney (Honda et al. 1986b; Boening 2000). Percentage of the body burden was highest in feathers compared with brain, muscle, blood, liver and kidney in osprey (*Pandion haliaetus*) chicks: 86.2% in feathers, 6.4% in muscles, 2.6% in liver, 3.8% in blood, 0.8% in kidneys and 0.07% in brain (DesGranges et al. 1998). Mercury concentrations decreased in the following order of tissues in great egrets (*Ardea alba*): growing scapular feathers, powder down, mature scapular feathers, liver, kidney, blood, muscle, pancreas, brain, bile, fat, and eye (Spalding et al. 2000b). Feather mercury concentrations were higher on average than blood in both adult and nestling common loons (*Gavia immer*) (Scheuhammer et al. 1998). Common loon tissues had relative mercury concentrations in egg:blood:muscle:feather:liver of 0.4:1:2:6:15 (Evers et al 2005).

6.2 Tissues used in sampling

Historically, mercury studies have used birds found dead or birds killed for the purposes of the study, and analyzed multiple internal tissues and organs for mercury (Stewart et al. 1994; Stewart et al. 1997b). More recently, blood and feathers are often used as non-destructive monitoring tissues. Eggs have been used to indicate the contamination ingested by the female just before the egg was laid (Lewis et al. 1993). Feathers indicate the total mercury burden of the bird (see below 9.2 Excretion via feathers). Blood is used to indicate current exposure through dietary uptake, generally thought to span 2 weeks (Kahle and Becker 1999). In humans, peak blood mercury concentrations were achieved within 4-14 hours of ingestion, and half-life of blood mercury was 52 days (Kershaw et al. 1980). In birds, peak blood

mercury concentrations occur between 4-8 hours after ingestion, and half life is from 30 to 90 days (Scheuhammer 1987; Monteiro and Furness 2001b; Monteiro and Furness 2001a; Fournier et al. 2002).

7.0 Sublethal effects and associated levels

Sublethal effects include effects on reproduction, growth, development, behavior, vision, hearing, immune function, and metabolism (Eisler 1987; Wolfe et al. 1998). Adverse effects in birds have been associated with concentrations of 5.0 ppm fw in feathers, 0.90 ppm fw in eggs, 0.05-0.10 ppm in diet (Eisler 1987). A level of 1 ppm in the blood is sometimes used as a reference for possible concern (D. Evers pers. comm.). However, there is currently no lowest observed adverse effects level (LOAEL) established for bird blood mercury concentrations. The level of 0.80 ppm in mallard eggs has been associated with sublethal effects, and has been extrapolated to other studies (Heinz 1979; Henny et al. 2002; Henny et al. 2005). Studies on common loons have resulted in a criterion level for high risk of 3 ppm for blood mercury (Driscoll et al. 2007). These levels should be used only as a general guide, as there may be species-specific differences in LOAELs (Henny et al. 2002).

7.1 Sublethal reproductive effects

Reproduction may be affected at chronic low levels of mercury, 1/5 the levels that cause overt toxicity in adult birds (USDI 1998). Reproductive effects include reduced hatchability (due to increased embryo mortality), reduced clutch size, some eggshell thinning, eggs laid outside the nest, and aberrant behavior of juveniles (USDI 1998).

In dosing studies with mallards (*Anas platyrhynchos*), fewer eggs were laid, and fewer young were produced in dosed birds, and young birds were less likely to survive to one week (Heinz 1976, 1979). Levels associated with reproductive and behavioral abnormalities in mallards were 9-11 ppm in feathers, and greater than 2 ppm in other tissues (Eisler 1987). Adult loons with mercury levels as low as 2 ppm in the brain had lowered incubation success and higher rates of territory abandonment (Barr 1986). In another study, no reproductive effects were seen in loons when adult males and females had mean blood mercury levels of 1.77 or 2.55 ppm (Champoux et al. 2006).

In general, mercury concentrations in eggs of 0.5 ppm (~2.5 ppm dw) have not been indicative of reproductive failures, but levels between 0.5 and 2 ppm are linked to some level of impairment (Thompson 1991). Several studies found that elevated egg mercury levels did not lead to reproductive failures. Levels ranging from 2.3 to 15.8 ppm in the first laid egg showed no subsequent effect on hatching success in herring gulls (*Larus argentatus*) (Vermeer et al. 1973). However, mercury concentrations greater than 3 ppm dw in merlin (*Falco columbarius*) eggs were associated with a reduction in brood size (Newton and Haas 1988).

No effect on reproductive parameters or survival was found at concentrations in body feathers of 1.2 to 33.4 ppm fw in great skuas (*Catharacta skua*) (Thompson et al. 1991). When interpreting feather mercury levels, both diet and age of the bird need to be considered, as well as life history traits (*e.g.*, where and when they grew their feathers).

7.2 Sublethal behavioral effects

Signs of acute or chronic mercury toxicity include ataxia, slowness, fluffed feathers, calmness, withdrawal, drooping eyelids, hypoactivity; reduced food intake resulting in weight loss, weakness in wings and legs, and general difficulty standing, flying or walking (Fimreite 1979; Eisler 1987; USDI 1998). Great egret nestlings dosed with 4-5 ppm of methylmercury chloride exhibited weakness of the legs and could no longer stand after 8-10 weeks of the dosing program (Hoffman et al. 2005). Other studies on great egret juveniles showed decreased appetite in all dose groups, including the low-dose group which had blood mercury levels of 11.9 ppm (Spalding et al. 2000a). In egrets dosed in the wild, declines in appetite were seen at even lower doses, suggesting that mercury may have similar effects at lower levels in wild populations (Spalding et al. 2000a). Mercury-dosed juvenile great egrets also spent significantly more time in the shade (Bouton et al. 1999).

In dosed zebra finches (*Poephila guttata*), behavioral signs of mercury intoxication—lethargy, difficulty balancing—were seen in the group dosed with 5.0 ppm dw mercury, and 25% of the group died before the end of the experiment. Birds that developed neurological impairment had at least 15 ppm in the brain and 30-40 ppm ww in the kidney, and were ingesting dietary levels of 5 ppm (Scheuhammer 1988). Similar concentrations fed to larger birds (mallards and black ducks) did not have a visible neurological effect comparable to that of zebra finches (Scheuhammer 1988).

7.3 Other sublethal effects (histologic, biochemical)

There was a significant correlation between blood mercury concentration and packed cell volume in free-ranging great egrets (*Ardea albus*), but no other health effects were observed (Sepulveda et al. 1999). In another study, dosed egrets showed significantly lower packed cell volume, *i.e.* anemia, in dosed groups than controls; blood mercury in dose groups ranged from 1.1 ppm in the low dose group to 19.2 ppm in the high dose group (Spalding et al. 2000a). Activities of 3 plasma enzymes, as well as liver and brain enzymes, were significantly different between dose groups of great egret nestlings, and oxidative stress was apparent in brain tissue of the high-dose group (Hoffman et al. 2005). Oxidative stress was also seen in young double-crested cormorants that averaged 5.42 ppm in the blood (Henny et al. 2002). Brain lesions were seen in a high-dose group of great egrets (Spalding et al. 2000a). Juvenile wading birds and cormorants showed peripheral nerve damage on a contaminated site, with blood mercury levels from 2.67 to 5.42 ppm (Henny et al. 2002). Growth of organs was affected in young wading birds; mercury contaminated juvenile snowy egrets (*Egretta thula*) had mean blood mercury levels of 2.67 ppm, and showed enlarged livers and kidneys, and decreased brain size compared to reference birds (Henny et al. 2002). Double-crested cormorant (*Phalacrocorax auritus*) juveniles, with mean blood mercury levels of 5.42 ppm on the contaminated site, had enlarged spleens compared with reference juveniles (Henny et al. 2002).

8.0 Lethal effects and associated levels

Residues of mercury in poisoned red-winged blackbirds, European starlings (*Sturnis vulgaris*), brown-headed cowbirds (*Molothrus ater*) and common grackle (*Quiscalus quiscula*) were greater than 20 ppm (fw) (Finley et al. 1979; Eisler 1987). These four species had liver concentrations of 54.5 to 126.5 ppm, brain concentrations of 21 to 45 ppm, kidney concentrations of 40.4 to 86.4 ppm, and muscle concentrations of 30 to 57.1 ppm at death (Finley et al. 1979). A diet containing 40 ppm of mercury (methylmercury dicyandiamide) was lethal to these species (Finley 1979). In general, laboratory studies have showed that death occurred in birds (passerines, raptors and pheasants) with liver concentrations of 30 ppm and above, or dietary mercury of 10 ppm (Thompson 1996). However, mercury-related effects found in lab experiments may not occur similarly in the natural environment.

9.0 Mitigation of toxicity

Even when ingesting high quantities of mercury, birds may be able to avoid effects. Simply migrating from a contaminated site would cause detoxification. Osprey (*Pandion haliaetus*) feathers grown in a contaminated part of Sweden had mercury concentrations of 20 ppm, and those grown after migrating had concentrations of 5.0 to 6.3 ppm (NAS 1987). However, there is the possibility that migratory birds may be exposed to mercury on the migratory pathway, as well as on both breeding and wintering grounds.

Before methyl mercury is even ingested, certain circumstances may reduce exposure. In some environments, methyl mercury may be degraded by bacteria or

light (Morel et al. 1998). Microbes in water, soils and the guts of animals are capable of demethylating mercury (Eisler 1987). Demethylation may occur in the kidney, liver or spleen of some seabirds and wading birds (Kim et al. 1996; Spalding et al. 2000a; Henny et al. 2002). However, all things being equal, demethylation occurs at a slower rate than methylation (NAS 1978). Selenium may also protect against mercury toxicity in fish, mammals, aquatic invertebrates and birds by releasing mercury from its bonds with proteins (Eisler 1987; Henny et al. 2002).

9.1 Elimination of mercury from body

Mercury may also be eliminated through egg laying, defecation and feather growth. Methyl mercury accumulates in the white of the egg, while inorganic mercury accumulates in the yolk (Boening 2000; Kennamer et al. 2005). Mercury may also accumulate in small amounts in the eggshell (Burger 1994). Egg laying is only a route of excretion for breeding females; female herring gulls may excrete an estimated 20% more mercury than males via eggs (Lewis et al. 1993). Female Cory's shearwaters (*Calonectris diomedea)* excreted an average of 14% of the dose ingested 3-4 weeks prior to laying into eggs (Monteiro and Furness 2001). However, mercury content of eggs is usually low compared with female burdens, and different species have varying capacity to eliminate mercury into the egg (Stewart et al. 1994).

Between 11 and 15% of mercury administered to dosed great egrets (in the low-dose group) was recovered in feces, although this estimate may be slightly inflated due to remnants of feather sheaths that were mixed in with feces (Spalding et

al. 2000b). In laboratory-reared black-headed gull (*Larus ridibundus*) chicks, over 20% of the dose was recovered in feces (Lewis and Furness 1991).

9.2 Elimination via feathers

Feather growth is thought to be the primary means of elimination of mercury in birds. Feathers are made of about 90% protein, mostly beta-keratin (Stettenheim 2000). Keratin is characterized by high-sulfur content, and is rich in disulfide bonds (Crewther et al. 1965). Feathers emerge from a follicle in the skin, and as they grow, are connected to the body by a blood vessel running from the follicle up the shaft (Stettenheim 2000). The tips of feathers form first, and new keratin is added at the base until the entire feather is grown (Burger 1993; Burger and Gochfeld 1997). The blood vessel atrophies from the distal portion of the feather to the proximal end as keratin is laid down, disappearing completely when the feather is fully formed (Stettenheim 2000). Typical feather growth lasts one to three weeks, and when growth is complete, feathers are physiologically separate from the body (Burger and Gochfeld 1997).

Mercury is transported into feathers along with nutrients in the blood, and binds to disulfide bonds of keratin molecules during formation (Crewther et al 1965; Goede and de Bruin 1984; Furness et al. 1986; Fournier et al. 2002). Methyl mercury has a high affinity for the free thiol groups (-SH) in amino acids, e.g. cysteine and glutathione, that are present in keratin (Crewther et al. 1965; Amirbahman et al. 2002). Therefore, much of the mercury circulating in the bloodstream at the time of growth becomes incorporated into feather keratin. When the feather is no longer

supplied with blood, mercury remains physically and chemically stable within the feather, resistant to leaching (Appelquist et al. 1984; Goede and De Bruin 1984; Burger 1993; Thompson et al. 1998; Stettenheim 2000).

10.0 Past studies on the role of plumage

It is widely stated in the literature that birds are able to excrete large amounts (50-93% of the body burden) of mercury into growing feathers (Honda et al. 1986a; Braune 1987; Braune and Gaskin 1987; Lewis and Furness 1991; Bryan et al. 2001; Agusa et al. 2005). However, lower amounts have been reported as well, indicating some variation between species or perhaps a dose-dependant elimination strategy (Kim et al. 1996; Monteiro and Furness 2001a). All mercury excreted into plumage is methyl mercury, so it is likely that feather growth serves as a buffer from toxic effects (Thompson and Furness 1989; Spalding et al. 2000b).

Past studies have established a strong foundation of evidence that mercury in the body is preferentially bound into feathers as they grow. The bulk of these studies have used seabirds as study species, and the remaining studies have used large-bodied aquatic species. To my knowledge, this relationship has not been examined in passerines. The following review of the past work is divided into studies on collected specimens, dosing studies, and non-destructive field studies. These studies examined the dynamics of mercury in molting adults and juveniles. Most of these studies sampled feathers or tissues at different stages of molt to compare the changing distribution and body burden of mercury. Dosing studies have measured elimination rates of mercury in the body at different stages of feather growth.

10.1 Collected specimens

Age-related accumulation of metals was examined in adult and juvenile eastern great white egrets (*Egretta alba modesta*; Honda 1986a). A high percentage of the mercury concentration in the whole body was present in feathers, suggesting that mercury is preferentially shunted into the feathers (Honda et al 1986a). In chicks, whole body mercury levels increased with age until fledging, when a decrease in concentration was observed in a few individuals (Honda 1986a). This same age-related increase in mercury concentration was seen in the livers of seven species of juvenile wading birds (Ciconiiformes) (Sundlof et al. 1994). Mercury levels in down of common tern (*Sterna hirundo*) chicks (up to 10 days old) was significantly and positively correlated with age, but liver and whole body concentrations showed no relationship with age (Becker et al. 1993). Down contained 38% of mercury in the body, and may have had an important effect in eliminating mercury from the body (Becker et al. 1993).

Other studies related mercury accumulation directly with stages of feather growth or molt, rather than age. In a well-known study (cited over 140 times), eight species of breeding adult seabirds were collected and feathers analyzed for mercury. In every species there was a decrease in mercury with primary molt sequence; *i.e.*, feathers grown last had the least mercury (Furness et al. 1986). This suggested that much of the body burden, as well as ingested mercury, was eliminated into the first growing feathers, and was depleted by the end of the molt sequence (see also Braune 1987). Small numbers of Bonaparte's gulls (*Larus philadelphia*) were collected near

New Brunswick, Canada, in different stages of molt (Braune and Gaskin 1987). Primary feathers showed a decrease in concentration with growth sequence in adults, indicating a decrease in the body burden of mercury, as seen in Furness et al. (1986; Braune and Gaskin 1987). Mercury in the feathers represented 88-93% of total body mercury (Braune and Gaskin 1987). Tissues (muscle, kidney, brain and liver) showed a non-significant decreasing trend in mercury concentration during post-breeding molt followed by an increase once molt had stopped (Braune and Gaskin 1987). Another study found a temporal decrease in mercury concentrations in tissues of adult and juvenile (year-old) guillemots (*Uria aalge*) collected in April, June and November, which was attributed to the complete post-nuptial molt that occurs in July (Stewart et al. 1994). Mercury concentrations in tissues of adult black-eared kites (*Milvus migrans lineatus*) differed between pre-molt, molt and post-molt classifications, and were lowest during the molt period (Honda et al. 1986b). Feathers contained 70% of the mercury burden (Honda et al. 1986b). Again, these studies suggest that a high proportion of mercury in the body is shunted into the growing feathers.

However, similar studies on other species did not detect age-related trends in down, feather or body mercury. There was no relationship between down or feather mercury concentration and age in arctic skua (*Stercorarius parasiticus*) or arctic tern (*Sterna paradisaea*) chicks, and there was a negative relationship in guillemots and kittiwake (*Rissa tridactyla*) (Stewart et al. 1997b). Known-age adult black-tailed gulls (*Larus crassirostris*) showed a decrease in mercury levels in the kidney with age

(Agusa et al. 2005). Similarly, adult age was negatively correlated with liver mercury in great skuas, and not significantly correlated with feather, kidney or muscle mercury (Thompson et al. 1991). These different relationships may be a result of lower levels of exposure to mercury, when intake was not constant, allowing a lessening of the mercury load in the body (Agusa 2005). The decline of mercury levels in the down of chicks was suggested to be due to growth dilution, *i.e.*, mercury concentrations become diluted as muscles grow during high rates of protein synthesis (Stewart et al. 1997b). However, it is not completely understood why levels in certain tissues decrease with age in some species and increase in others, and likely has to do with diet, metabolism or capacity for demethylation.

10.2 Dosing studies

Lab studies have corroborated the finding that a high percentage of blood mercury ends up in plumage. Black-headed gull (*Larus ribidundus*) chicks (n=15) raised in the laboratory were assigned one of 4 dose groups (0, 20, 100 or 200 μg total dose of mercury). An average of 71% of the dose administered was excreted—22% into feces and 49% into plumage (Lewis and Furness 1991). A total of 65% of the body burden of mercury was found in plumage, and feathers grown last had lower levels of mercury than primaries and body feathers grown first, consistent with some of the studies described above (see above 10.1 Collected specimens). Body feathers and coverts accumulated 19-20% of mercury intake. In this study, there was no relationship between dose level and rate of mercury excretion (Lewis and Furness 1991).

Recent dosing studies provided more information on the dynamics of mercury in the blood and feathers. Bearhop et al. (2000b) dosed 9 individual juvenile great skuas (*Catharacta skua*) for 21 weeks, and used blood mercury from post-dosing profiles to fit a pharmacokinetic model of mercury. Each individual represented a different dose 'group' fed between 300 and 1300 μg/kg body weight per day. Mercury levels increased throughout the dosing period in blood and feathers, as birds were completing molt, suggesting a rapid transfer of mercury to feathers. Feather growth occurred in all birds, although it was variable among birds, and this variability was not accounted for in the model. The estimated half-life of blood during the "slow terminal phase" was over 30 days even while feathers were growing (Bearhop et al. 2000b).

A more comprehensive study examined excretion of mercury in both adults and young of a different species of seabird. Free-living adult Cory's shearwaters (*Calonectris diomedea*) were dosed with 250, 1000 or 2000 μg mercury; peak blood concentrations occurred 24-48 hours after a single dose and 33% of intake was excreted into the plumage (Monteiro and Furness 2001a). In a related study, free-living pre-fledging Cory's shearwater chicks dosed with mercury experienced shorter half-lives in the blood, ranging from 5.5 to 6.3 days, than adults, which ranged from 40 to 65 days (Monteiro and Furness 2001b; Monteiro and Furness 2001a). Molting adults exhibited shorter mercury half-lives than non-molting adults. In chicks, 42-60% of the dose was excreted into feathers over the experiment. Both of these studies relied on the assumption that a single dose of mercury will be handled in the same

way as constant exposure to mercury (Monteiro and Furness 2001a; Monteiro and Furness 2001b).

A similar study on young common loons examined the difference in excretion of two different stages of development. Fournier et al. (2002) dosed eight loon chicks either orally or intravenously, with single doses administered during and after feather growth. During feather growth, blood mercury rose to a peak level 2 – 8 hours after administration, then decreased to background levels within 4 – 8 weeks after administration, with a half-life of 3 days (Fournier et al. 2002). When flight feather growth was complete, at 11 weeks, elimination was 25 times slower than elimination during maximum feather growth (Fournier et al. 2002). The pharmacokinetic model for the older birds included a rapid initial phase, where mercury was distributed to tissues (half-life of 0.9 days), and a slow terminal phase where mercury was lost to feces (half-life of 116 days; Fournier et al. 2002),

Dosing studies have also been used to determine effects in juvenile birds. Kenow et al. (2002) collected common loon eggs from lakes with known elevated mercury levels. These chicks were raised in the laboratory from hatching, dosed with CH_3HgCl, and their blood sampled weekly. An increase in blood mercury concentration was seen in all dose groups, including after the point when all feather growth was complete (Kenow et al. 2002). Feathers contained a mean of 26% of total mercury body burden in chicks at 105 days, after they were fully feathered, a lower value than observed in other studies (see above Dosing studies and 10.1 Collected specimens; Kenow et al. 2002). The control group showed a contrasting decrease in

mercury (from the egg) until feather growth was complete, then a rise in mercury concentration (Kenow et al. 2002). The decrease in mercury of control birds likely represented the elimination of egg mercury residues, since eggs were originally collected from lakes of known mercury contamination. The increase of mercury was then due to consumption of low levels of mercury in food, and the cessation of feather growth, allowing mercury to accumulate (Kenow et al. 2002). This study found no effects on growth or survival of chicks, even in the high dose group, suggesting that the feather growth buffered the chicks from toxicity. High dose birds had mean blood levels of 3.33 μg/g at five weeks, which may not have been enough to cause adverse effects.

Another series of related studies on great egrets also examined dose response and behavioral effects. Great egret juveniles were raised in captivity and dosed with mercury, either 0, 0.5 or 5 mg/kg/day (Spalding et al. 2000a; Spalding et al. 2000b; Hoffman et al. 2005). Concentrations of mercury in the blood were found to be higher in all dose groups after feathers stopped growing between weeks 9 and 11 (Spalding et al. 2000b). A companion study found that birds were experiencing decreased appetite and growth at this time as well, suggested that this time of increased accumulation corresponded with effects (Spalding et al 2000a).

10.3 Field studies

There have been relatively few field studies looking at the relationship of feather growth and body mercury levels. A study on ospreys (*Pandion haliaetus*) that examined mercury levels and biomagnification near hydroelectric reservoirs in

Quebec also provided some evidence that the same phenomenon is seen in the wild (DesGranges et al. 1998). Osprey chicks had higher mercury levels in the feathers (mean of 37.3 ppm) than blood (mean of 1.9 ppm; DesGranges et al. 1998). The increased ratio of feather to blood mercury corresponded generally to the start of feather growth. Presumably there would be less mercury circulating in the blood during this time, as it would be deposited in feathers. The ratio then decreased after growth was complete, and more mercury was again circulating in the blood (DesGranges et al 1998).

In 2005, black-footed albatross chicks (*Phoebastria nigripes*) in Japan were sampled for blood mercury at the nest, and one of three growth stages were assigned to generally describe the loss of down (Ikemoto et al. 2005). Blood mercury increased in the later growth stages, with completed plumage growth, suggesting that excretion of mercury into feathers had kept blood mercury levels down. Mean blood mercury levels increased from 0.52 to 0.70 to 1.1 ppm in the three growth stages. Common loon chicks showed a 0.025 μg/g increase in blood mercury between 2 and 5 weeks of age (Fevold et al. 2003). In contrast, double-crested cormorant nestlings sampled twice over time (within 10-12 days) showed no change in blood mercury levels (Caldwell et al. 1999).

11.0 Age class of biomonitors—nestlings, adults or fledglings

In mercury studies, both adults and nestlings have been used as monitors, but rarely fledglings. Nestlings have been suggested to be important monitors because they indicate mercury exposure in a very specific area and time period (Janssens et al.

2003). Although they are receiving some mercury from the egg, most mercury accumulated will be via prey items during the period between hatching and fledging. They are also relatively easy to sample compared with mobile adults and fledglings. However, nestling tissue mercury levels have typically been lower than adults from the same region; mercury concentrations are found to be 2-10 times higher in adults than their young (Evers et al 2005). Mercury in blood of common loon chicks was an order of magnitude lower than their parents, while still being significantly correlated with parent levels (Scheuhammer et al. 1998). In contrast, feather levels of loon chicks were comparable to those of adults (Scheuhammer et al 1998). Prefledging juvenile mercury levels were 80-94% of adult levels in guillemots in liver, kidney, feathers and muscle (Stewart et al. 1994). Levels in kidney and liver were significantly higher in adults, while feathers and muscle did not show a significant difference between adults and juveniles (Stewart et al. 1994). Tree swallow blood mercury levels were higher in adults than nestlings (Evers et al. 2005; Brasso 2007). However, adult and fledged young blood mercury levels were not significantly different in song sparrows sampled in northeastern North America (Evers 2005).

Early developmental stages, *i.e.*, young birds, are thought to be the most susceptible to toxic effects of mercury (Eisler 1987; Scheuhammer 1988). However, accumulation of mercury, and subsequent toxicity may actually be lessened in young birds. Some mercury may be diminished by growth dilution, but elimination due to feather growth may be the most important factor (March et al. 1983). Many birds do not fully complete feather growth until after they have already left the nest. Thus,

nestlings may be more at risk during the post fledging period, when both of these protective mechanisms have ended.

12.0 Post-fledging period

Few studies have looked at metal concentrations in fledglings, especially in free-living birds. Stewart et al. (1997a) examined cadmium in fledgling Cory's shearwaters in the Azores. Dead fledglings were collected and kidney and livers were examined for heavy metals. Cadmium was shown to have accumulated during the nestling period, but was still lower than adult values (Stewart et al. 1997a). Other studies related mercury levels as nestlings to juvenile survival, but did not measure the mercury of the fledglings. Great egret juveniles that were dosed with mercury for 15 days as nestlings (when feathers are still growing) showed no effect on probability of survival as fledglings (Sepulveda et al. 1999). Post-fledging survival of wood storks was also not affected by nestling mercury levels (Hylton et al. 2006).

Few studies have measured both mercury levels and effects in fledglings. One study on wading birds showed pronounced effects on fledgling birds compared to adults; fledgling wading birds and double-crested cormorants who had completed feather growth were found to experience neurological and histological damage (see above 7.3 Other sublethal effects; Henny et al. 2002). These cormorants had elevated blood, feather, kidney, liver and brain mercury concentrations over reference birds of the same age (Henny 2002). Other than these studies, there is little information on the effects and levels of mercury during the vulnerable post-fledging period.

The post-fledging period in migratory birds is considered the time between independence from the parents until departure from the natal site (Vega Rivera et al. 1998). Little is known about this period since fledglings are difficult to observe and study during this time. It is a period of high-risk in which young birds are learning to forage and avoid predators, and is characterized by high-mortality (Baker 1993). Overall juvenile survival estimates range from 0.321 to 0.423 for long and short distance migrants (Sullivan 1989; Elbert and Anderson 1998).

Predation is a major cause of mortality in fledglings (Sullivan 1989). In some passerine species, *e.g.*, yellow-eyed juncos, mortality is high immediately after fledging when flight ability is poor, then drops when young birds are able to fly well, and rises again when parental feeding stops (Sullivan 1989). Other studies did not find the second increase in mortality upon independence, though the first few weeks post-fledging still produced the highest mortality (Kershner et al. 2004). Overall probability of predation in wood thrushes (*Hylocichla mustelina*) post-fledging (week 1-8) was 0.506, but during week 4-8, probability was 0 (Elbert and Anderson 1998).

13.0 Research question

The present study examined total (*i.e.*, methylated plus inorganic) blood mercury levels in post-fledging, free-living songbirds in a natural setting. I asked whether mercury levels rise in the body after feather growth is complete. I was testing the common assumption that growing feathers serve as an elimination route for ingested mercury, and that blood mercury levels rise after feather growth ceases. To do this, fledglings were sampled for blood mercury repeatedly after leaving the

nest, and age and feather growth were recorded. I predicted that blood mercury levels would be related to the intensity of feather growth across individuals, and would rise to adult levels over the course of the first summer.

13.1 Why more research on plumage?

Most previous studies on feathers and mercury have recommended a need for more research during the post-fledging time period. Though there are multiple studies examining the relationship of feathers to body burden of mercury (see above 10.0 Past studies on the role of plumage), most have been indirect measures of the phenomenon. Almost all of the studies using collected specimens were conducted on un-marked individuals and considered mercury acquired over a large and ill-defined spatial area. Movement and changing diet of the bird were not always considered in analysis. All studies used large-bodied, fish-eating birds as study species, which may have different pharmacokinetic patterns of mercury excretion than smaller bodied passerines.

Many of the studies examined the relationship of feather mercury to mercury in tissues and organs, but not in the blood—a more relevant tissue for non-lethal sampling. Lab studies have shown convincing and detailed models of the fate of ingested mercury in the body, using blood as a sampling tissue. However, lab studies are confounded by small sample sizes in some cases, and an unnatural diet and activity regimen; mercury doses are often not comparable in amounts or frequency of exposure to what birds may receive in the wild (see Bearhop et al. 2000b). The fate

and toxicity of mercury may show considerable variation between species, sex, form of mercury, route of administration, and age (Fimreite 1979).

No other study has examined what happens to mercury levels in the blood of wild fledgling birds living in a known contaminated environment, which is in a sense a continuous dosing study under natural conditions. In the present study, birds were exposed to mercury continuously through diet, and mercury was measured directly in the blood and feathers before, during and after feather growth was completed.

13.2 Implications of research

Juvenile survival may be impacted by mercury contamination. Little is known about population level risks and effects of mercury contamination on insectivorous passerines (Rimmer et al. 2005). Many birds complete feather growth after they have left the nest, and when they are still foraging on contaminated prey items. Experiencing an increase in mercury at this time may have implications for juvenile survival of birds in contaminated landscapes. This study attempted to define a period of high risk for juvenile songbirds related to feather growth.

It has been suggested that mercury detected in nestlings is a good indicator of what is present in the local environment (Janssens et al. 2003). Nestlings may be buffered from any toxicity of mercury because most of the mercury circulating in the body could be eliminated into growing feathers. Thus, although they are commonly used as biomonitors, not all nestling tissue may give an accurate picture of the level of contamination in an area, and interpretation of adult and nestling levels differ.

This study may provide guidance for the selection and interpretation of the monitoring tissue.

14.0 Predictions

1. Mercury levels will rise in the blood of independent fledgling birds as feather growth terminates. I used radio-telemetry to monitor eastern bluebirds (*Sialia sialis*) after they left the nest box, and throughout the post-fledging period. Individual birds were re-trapped repeatedly and blood and feather mercury was measured with each capture.

2. If an increase in mercury levels is seen, it will correlate better with feather growth than with a shift in diet leading to more mercury exposure. Trophic level was determined by stable isotope analysis of nitrogen in blood. I looked at nitrogen isotope signature because it has been shown to correlate with increasing mercury levels and thus must be addressed as a potential explanatory variable for temporal changes in blood mercury (see above 4.1.2. Nitrogen isotopes).

Materials and Methods

1.0 History of Study Area

Between 1929 and 1950, mercury escaped into the South River from an industrial plant in Waynesboro, VA (Carter 1977). Mercuric sulfate was used as a catalyst in the production of synthetic (acetate) fibers at the E.I du Pont de Nemours and Company (hereafter "DuPont") plant, and undetermined quantities leaked into the river and soils while the plant was operating (Carter 1977; Murphy 2004). After the contamination was discovered in 1976, sediment downstream from the plant was determined to have mercury concentrations of 240 ppm, compared to less than 1 ppm upstream (Carter 1977; Murphy 2004). Fish tissue samples downstream from the plant contained mercury concentrations above the Food and Drug Administration's then 'action level' of 0.5 ppm, even up to 77 miles from the contamination source (Carter 1977). Operations using mercury as a catalyst ceased in 1950, and since then presumably no new mercury has been added to the river (Carter 1977). Yet the river remains contaminated up to 130 miles downstream from the site of the original leak more than a half-century later (Carter 1977).

Today, mercury is present in sediment, fish, and other aquatic and terrestrial biota in elevated levels compared to natural background levels (Carter 1977; Cocking et al. 1991; Murphy 2004; Brasso 2007; White 2007). In the 1980s, the Virginia Department of Environmental Quality (VDEQ) issued a health advisory (no consumption) on fish consumption for all species on the South River downstream from the plant in Waynesboro to the confluence with the North River at Port Republic

(VDEQ 2000; Murphy 2004; Brasso 2007). The river is stocked with hatchery-raised trout, which are exempt from the advisory because trout from the hatchery had average mercury levels of 0.1 ppm even after six months of exposure to the South River (VADEQ 2005). The advisory continues on the South Fork Shenandoah River, from Port Republic all the way to the confluence with the North Fork Shenandoah River at Front Royal (Murphy 2004).

To monitor the mercury in the river, a trust fund was created in a settlement between DuPont and the Virginia State Water Control Board (predecessor of VDEQ) in the early 1980s (Murphy 2004). For a 100-year period, the fund would support projects that monitor mercury in water, sediments and fish throughout the Shenandoah River basin, including the South River, South Fork Shenandoah River and Shenandoah River (VDEQ 2000). In 2000, VDEQ and DuPont formed the South River Science Team (SRST), a coalition of stakeholders including state and federal agencies, citizen and industry groups, and academics (VDEQ 2000; Murphy 2004).

SRST research has primarily focused on locating potential sources of mercury near the former DuPont plant and in the South River, and monitoring water, sediment, and fish of the South River, *i.e.*, aquatic components (Murphy 2004). Cocking et al. (1991) did an exploratory study of the distribution of mercury in different terrestrial compartments, such as, soils, vegetation, small mammals, and insects. However, the bulk of ongoing research continues to focus on the mercury within the river. The presence of mercury contamination in the surrounding terrestrial environment was examined in a comprehensive study starting in 2005 using

the avian community as biomonitors (Brasso 2007; Friedman 2007; White 2007). My study was part of that larger research project. The three tributaries of the South Fork Shenandoah, the South, Middle and North Rivers, used in the project were located in Augusta and Rockingham counties in the Shenandoah Valley of Virginia, west of the Blue Ridge Mountains and east of the Appalachian and Allegheny Plateaus.

1.1 The South River

The contaminated study area encompassed a 38.6-km portion of the South River (Figure 1) downstream from the source of mercury in Waynesboro to the confluence with the North River (the river flows in a northeasterly direction). With headwaters in the Blue Ridge Mountains, the South River joins the North River in Port Republic to form the South Fork Shenandoah River which ultimately drains into the Potomac River and Chesapeake Bay.

When referring to sites along the South River, "river mile" is used by researchers of the SRST. Here, "river mile" has been converted to "river kilometer (km)", to be consistent with scientific literature. River km 0 was designated as the footbridge at the former DuPont plant. The South River study sites started at river km 1.7 and extend to km 38.3. Within this stretch of river, approximately 20 sites were used—public parks and private property. The surrounding land was primarily agriculture (hay fields and livestock use, with one large tree seedling plantation) and suburban residential (*e.g.*, houses and city parks). The river was patchily buffered by riparian woods of varying thickness.

A portion of the South River upstream from the former DuPont plant was also used as a reference site (river km 0 to -22.5). Four sites were located in this stretch of river. For details on sites, see Brasso (2007).

1.2 The North River—reference site in bird study

The North River, a tributary to the South Fork Shenandoah River that has not been contaminated with mercury, was one of the three reference rivers. The headwaters of the North River are in the Allegheny Mountains, and it merges with the Middle River near Grottoes, then becomes the South Fork Shenandoah River when it meets the South River in Port Republic (Figure 1). The surrounding land is both agricultural and suburban residential, with some riparian buffer. The five specific study sites on the North River were mostly on developed land, either private yards or public parks. See Brasso (2007) for a detailed explanation of reference sites.

1.3 The Middle River—reference site in bird study

The second reference river was the Middle River. With headwaters in the Great Valley southwest of Staunton, it becomes the North River west of Grottoes, and then joins the South River to form the South Fork Shenandoah River (Figure 1). The surrounding land is primarily agriculture and forest. Nest boxes on the Middle River were placed in agriculture fields, used either for livestock or hay, at eight sites.

1.4 Nest box trail

In 2005 and 2006, elevated mercury levels were documented in both piscivorous (belted kingfisher, *Ceryle alcyon*) and insectivorous bird species nesting along the South River, compared with the reference sites (Brasso 2007; White 2007;

Cristol unpubl.). Kingfishers nested in cavities in the banks adjacent to the river, while tree swallows and other species used man-made nest boxes placed within 50 meters (m) of the river. An extensive, though not continuous, nest box trail was established starting in 2005 along the South, North and Middle Rivers (initially 102 boxes at contaminated sites, 89 at reference sites; see Brasso 2007).

The trail was established for the purpose of attracting tree swallows (*Tachycineta bicolor*), so most sites shared open habitat with little or no riparian buffer (Brasso 2007). In 2005, the nest boxes were occupied in lesser numbers by eastern bluebirds (*Sialia sialis*), Carolina chickadees (*Poecile carolinensis*), Carolina wrens (*Thryothorus ludovicianus*), and house wrens (*Troglodytes aedon*). In 2006 and 2007, boxes were added to existing sites, as well as a few new sites, in order to increase the numbers of these other species. At the start of the 2006 season, there were 221 nest boxes on the contaminated river, and 183 on the reference sites.

At the start of the 2007 field season, there were 255 boxes on the contaminated South River within 50 m of the river. An additional 93 boxes were added at various distances from the river up to 400 m. There were 172 boxes on reference rivers in 2007, all within 50 m of the shoreline (Table 1). Boxes were placed both in open and wooded habitat, many in microhabitats selected for wrens, chickadees and bluebirds.

Sites were defined as groups of nest boxes on the same property, with a common access point (see Brasso 2007). Sites were of varying size and could hold between 2-50 nest boxes. Several sites were large and close enough to each other that

some within-site boxes were actually closer to boxes on the neighboring site. When referring to the river km or latitude/longitude of a site, an estimated midpoint or central point was used. For a detailed description of all sites used in 2005 and 2006, see Brasso (2007). Here, I will provide additional information only on the specific sites used in the fledgling study in 2006 and 2007. Although eastern bluebird nest boxes were monitored on all rivers, the fledgling study was conducted only along the contaminated South River and only at five sites.

1.5 Nest boxes and placement

Bluebird nest boxes measured 16 x 16 x 23.8 cm, with a 3.8 cm diameter entrance. The design was borrowed from the standard bluebird box design of the North American Bluebird Society (see assembly instructions Eastern/Western bluebird house http://www.nabluebirdsociety. org/eastwestbox.htm). Boxes were mounted on the top of a hollow metal pole, approximately 1.5 m in length. A smaller diameter metal pole, approximately 1 m in length, was driven into the ground, serving as an anchor. The nest box apparatus was placed over the supporting pole, so the nest box stood 1.5 m above ground. A predator collar (a cylindrical stovepipe-style metal baffle; Erva Tool, Illinois, USA, "raccoon guard") was placed around the pole to discourage predators.

Boxes were placed within approximately 50 m (a few were up to 65 m away) of the river, initially to attract tree swallows—which prefer large open areas in which to feed in close proximity to the nest site. In 2007, some bluebirds nested in the newly placed boxes 50-400 m from the river, but it is not currently known if birds at

this distance are still exposed to mercury, so I was still primarily concerned with birds nesting within 50 m.

Boxes were placed on edges of sparsely forested areas and in clearings, with suitable perches in close proximity (*e.g.*, fences, wires, trees). Spacing between boxes at my study sites was 20-100 m. At a few sites there was only one nesting pair, because of space limitations within a site. Bluebirds prefer a distance of between 36 and 100 m to the nearest neighbor, so often more than one pair could not "fit" in a particular area (Gowaty and Plissner 1998).

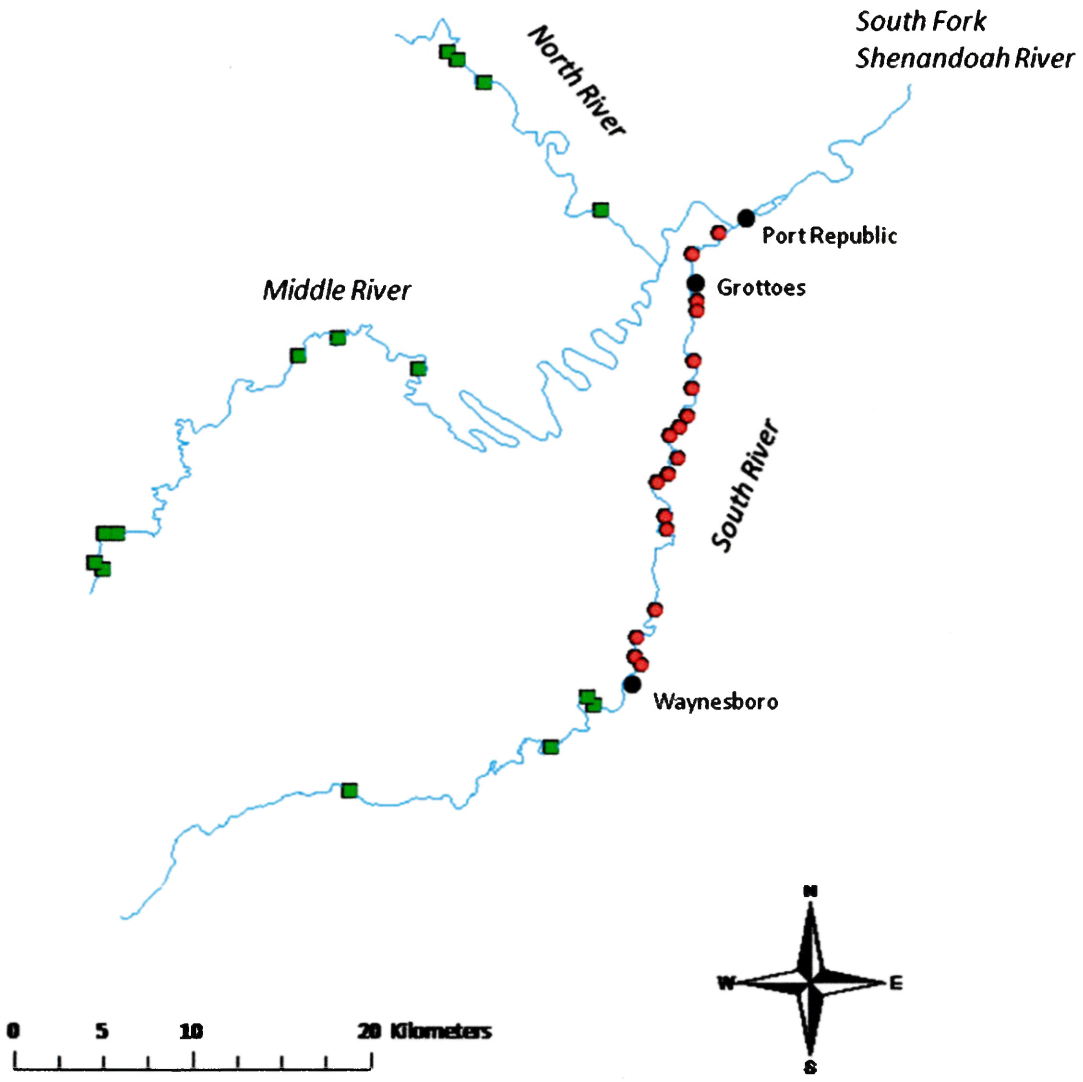

Figure 1. Map of contaminated and reference study sites in 2006-2007. The South River flows north. Red circles indicate contaminated sites, and green squares indicate reference sites. Three landmark towns are indicated with black circles.

1.6 Sites for monitoring fledglings

Sites for monitoring fledglings were chosen based on accessibility for radio-tracking and mist-netting, and the suitability of adjacent properties. Successful trapping sites had a mixture of low and high perches that the birds would regularly use, and where mist nets could easily be set. In 2006, although bluebirds nested at 12 sites on the South River, only five sites were used to follow fledglings: 1) Water Treatment Plant (WH20), 2) Basic Park (BAPA), 3) Genicom (GENI), 4) Augusta Forestry Center (AUFC), and 5) Grottoes City Park (GRCP; Figure 2). In 2007, bluebirds nested at 17 sites. Because fewer bluebirds were needed in the second field season, only AUFC was used as a site for trapping fledglings in 2007.

Sites not used were small, isolated properties that were difficult to access, were surrounded by equally inaccessible land, or had few or no successful eastern bluebird nests. Sites that were used are described below (river km locations indicate a mid-point of the site). All sites were located between river km 2 and 35. Sites were located on the east and west side of the river. Sampling effort attempted to represent the river spatially (*i.e.*, birds were sampled at intervals along the length of the contaminated river), however effort was necessarily concentrated in the areas with dense concentrations of bluebird nests.

1.6.1 Waynesboro Water Treatment Plant (WH20)

The Waynesboro Water Treatment Plant was located at river km 2.7, closest to the plant. In 2006, there were 10 boxes placed along the river with a riparian buffer between them and the open gravel lot of the water treatment works. The

property encompassed approximately 12 hectares (ha), and included a large gravel lot and several buildings, discarded heavy equipment and vehicles. The lot acted as a storage place for old supplies, equipment and temporary structures, many of which were used as perches by bluebirds. This site was accessible every day at all hours, yet was unvisited by the public, so disturbance to nets was very low.

Bordering the plant was a city park just upstream and across the road (North Park), and private property. I was able to obtain access to adjoining private property just west of the plant. Across the river was a dirt bike track and Basic Park (see below 1.6.2 Basic Park). Although there was only one successful bluebird nest at WH20 in 2006, it seemed possible that the fledglings there would join those from Basic Park because of the close proximity of the two sites. Fledglings were not trapped at WH20 in 2007.

1.6.2 Basic Park (BAPA)

Basic Park was at river km 3.2, and had nine boxes in the open field (within 50 m of the river) in 2006 and 2007. This site was approximately 7 ha of open mowed lawn and athletic field. There was a medium to thick riparian buffer bordering the field. Trees, fences, and light posts were used as perches by the bluebirds. This site was accessible at all times, and used sporadically by the public. Here, accessibility was occasionally restricted when there were athletic events or large crowds.

The park was adjacent to tracts of woods both upstream and downstream, and industrial buildings to the east. The WH20 was upstream and on the opposite bank,

and the dirt bike track was adjacent on the upstream (south) end of the site. The opposite bank at BAPA was steep and wooded, with private property on the other side of the woods. In 2006, there were two bluebird nests here, only one of which produced young (box 101). Again, because of the accessibility and extremely close neighboring sites, BAPA was used to track fledglings. Fledglings were not trapped at BAPA in 2007.

1.6.3 Genicom (GENI)

Genicom was at river km 4.7, and had 13 boxes in a hayfield with a continuous riparian buffer obstructing access to the river. The site was approximately 16 ha of open hay field with various sized bordering woodlots. Aside from the tall perches offered by trees, there were no other on-site perches used by the bluebirds. Accessibility to this area was unlimited, and again disturbance was low because of the locked gate.

The buildings of the former Genicom company were to the east of this site. The approximate 1.5 km to Basic Park upstream was accessible on foot, but intervening habitat was densely wooded. Opposite the river were private residences and farms. This site proved to be a relatively poor choice, because the birds moved across the river and I was unable to obtain permission from all of the property owners on the other side of the river. Trapping attempts were not continued after one month in 2006. There were two successful bluebird families at this site. Fledglings were not trapped at GENI in 2007.

Table 1. Total boxes available on South, Middle and North Rivers in 2006 and 2007. Status "C" = contaminated sites, "R" = reference sites.

						2006		2007		
Site Name	Site Code	River Km	Status	Latitude (N)	Longitude (W)	< 50 m	< 50 m wooded	< 50 m	< 50 m wooded	> 50 m
Water Treatment Plant	WH20	2.7	C	38° 04' 55"	78° 52' 21"	10	0	10	0	0
Basic Park	BAPA	3.2	C	38° 05' 07"	78° 52' 36"	9	5	9	5	8
Genicom	GENI	4.7	C	38° 05' 22"	78° 52' 34"	13	1	14	3	0
Dooms Crossing	DOOM	8.2	C	38° 06' 31"	78° 51' 43"	9	5	7	3	0
Wertman property	WERT	14.8	C	38° 08' 50"	78° 51' 28"	0	12	0	12	10
Wertman North	WERN	15.3	C	38° 09' 11"	78° 51' 25"	0	0	1	0	9
Crimora Crossing	CRIM	15.9	C	38° 09' 58"	78° 51' 14"	3	0	5	0	5
Augusta Forestry Center	AUFC	18.2	C	38° 10' 13"	78° 51' 43"	31	0	34	0	16
Wolf property	DUBA	19.3	C	38° 10' 34"	78° 51' 11"	0	16	0	16	12
Harris property	HARI	22.2	C	38° 11' 01"	78° 50' 46"	0	4	0	4	0
Wampler property	WAMP	22.4	C	38° 11' 40"	78° 51' 02"	6	7	6	7	0
Boe property	BOES	22.9	C	38° 11' 56"	78° 50' 44"	6	4	6	4	10
Desportes property	DESP	24.0	C	38° 12' 16"	78° 50' 28"	0	0	0	0	10
Harriston Crossing	HARR	26.4	C	38° 13' 02"	78° 13' 02"	3	10	3	10	0
Rankin property	RANK	28.0	C	38° 13' 49"	78° 50' 08"	8	3	4	8	3
Cosby Mill Road	COMI	31.5	C	38° 15' 20"	78° 49' 57"	0	0	3	1	4
Grand Caverns	GRCA	32.0	C	38° 15' 34"	78° 49' 35"	7	10	7	13	14
Grottoes City Park	GRCP	35.4	C	38° 17' 02"	78° 50' 04"	16	8	16	12	17
Bradburn Park	BRAD	38.3	C	38° 17' 40"	78° 48' 41"	4	14	0	14	0
Total boxes						125	99	125	112	118

Table 1 continued.

Site Name	Site Code	River Km	Status	Latitude (N)	Longitude (W)	2006		2007		
						< 50 m	< 50 m wooded	< 50 m	< 50 m wooded	> 50 m
Cowbane Preserve	SCOW	-22.5	R	38° 01' 19"	79° 03' 37"	16	0	16	0	0
P. Buckley Moss	PBUC	-8.0	R	38° 02' 29"	78° 55' 56"	11	0	13	0	0
Locust Street	SLOC	-2.4	R	38° 03' 42"	78° 54' 11"	7	0	5	0	0
Ridgeview Park	SRDG	-2.4	R	38° 03' 59"	78° 54' 28"	11	9	8	9	0
Whitescarver Farm	MWHI	NA	R	38° 08' 06"	79° 12' 59"	20	0	20	0	0
Opposite Whitescarver	MOPW	NA	R	38° 08' 16"	79° 13' 12"	17	0	17	0	0
Godfrey Farm	MGOD	NA	R	38° 09' 05"	79° 12' 20"	14	0	13	0	0
Smith's Pond	MSMP	NA	R	38° 09' 05"	79° 12' 50"	17	0	17	0	0
Fort River Road	MFOR	NA	R	38° 13' 45"	79° 00' 39"	4	0	4	0	0
Dories property	MDOR	NA	R	38° 14' 12"	78° 05' 17"	14	3	8	3	0
Concrete Bridge	MRBR	NA	R	38° 13' 57"	79° 05' 34"	8	0	8	0	0
Shapcot property	MSHA	NA	R	38° 14' 44"	79° 03' 42"	5	0	5	0	0
Crawford property	NCRA	NA	R	38° 22' 51"	78° 58' 58"	11	0	10	0	0
Auckerman property	NAUC	NA	R	38° 22' 54"	78° 58' 56"	3	0	3	0	0
Sandy Bottom Park	NSBP	NA	R	38° 22' 11"	78° 57' 55"	4	0	2	0	0
Wildwood Park	NWWP	NA	R	38° 23' 05"	78° 59' 17"	6	0	6	0	0
Rt. 276 river crossing	276B	NA	R	38° 18' 20"	78° 53' 33"	7	0	7	0	0
Total boxes						175	12	162	12	0

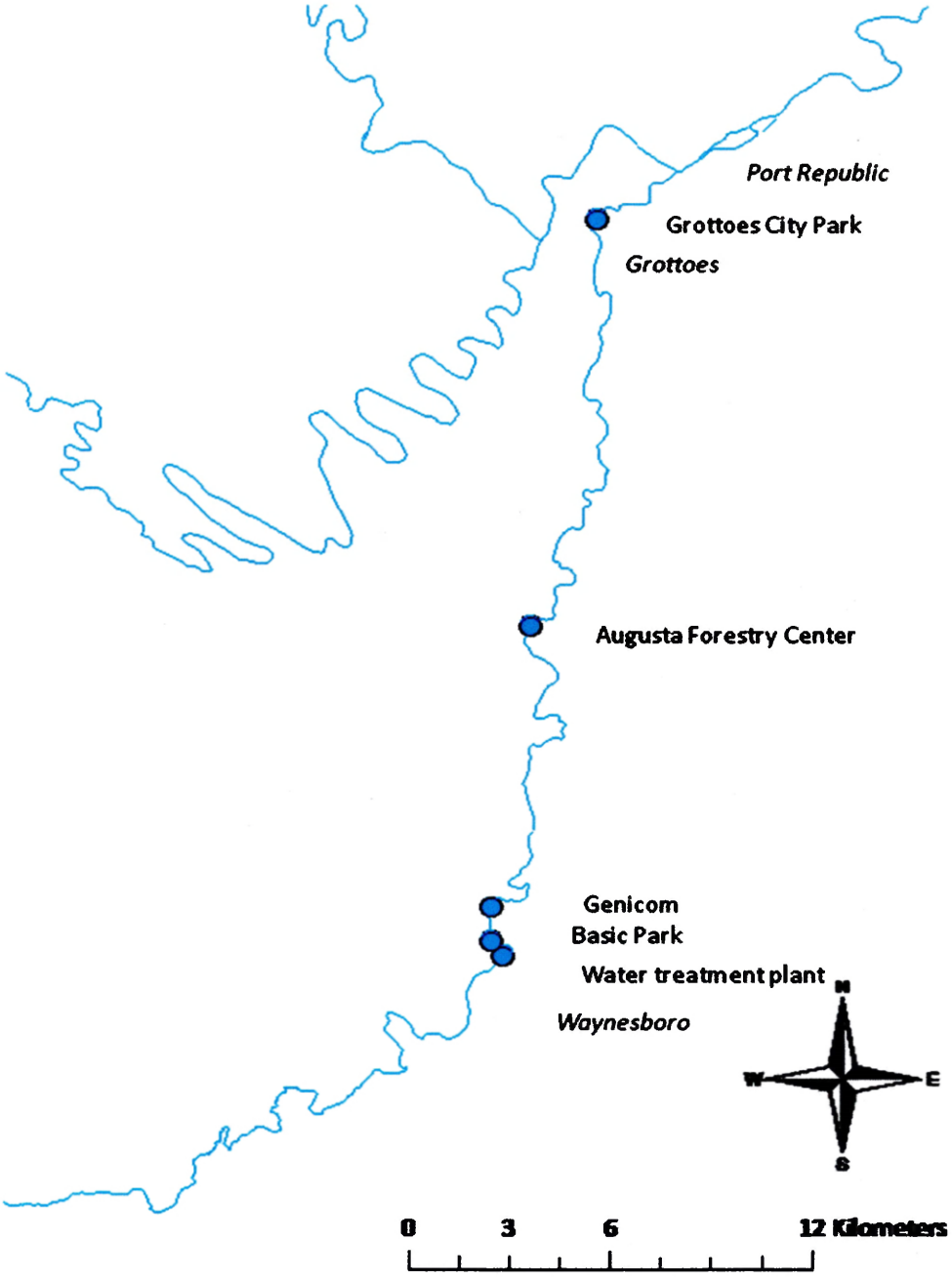

Figure 2. Map of sites used in fledgling study. Sites along the South River are indicated by blue circles.

1.6.4 Augusta Forestry Center (AUFC)

The Augusta Forestry Center (AUFC) was at river km 18.2. This was the largest site on the South River, spanning approximately 3 km, with a minimum of 31 nest boxes on site. The AUFC encompassed 114 ha, approximately 40 of which were tree seedling beds. The site was characterized by open fields and large tracts of growing plants with a thin buffer of trees along the river. Sprinkler heads in the growing fields, as well as a long stretch of barbed wire fencing, provided often-used perches for bluebirds. This site was isolated and secure (with a locked gate), enabling me to leave net poles erected undisturbed.

The upstream and downstream borders of the AUFC were cow pasture. These fields were also used as net sites only when cows were absent. Across the river were more open cow fields, and private residences. Permission to access property was received from almost all of the residents across the river. There were 10 bluebird families at AUFC in 2006, eight of which produced young. There were eight families in 2007, plus an additional seven in boxes >50 m from the river. In 2007, there were the 31 boxes present since 2005, plus an additional three erected in 2007, located within 50 m of the river. An additional 16 boxes were added from 50-400m away from the river as part of another study. The large numbers of bluebirds at this site made it an ideal trapping site, and in 2007 this was the only site where trap attempts were made.

1.6.5 Grottoes City Park (GRCP)

Grottoes City Park (GRCP) was the northernmost site used, at river km 35.4, a few km upstream from the confluence with the North and Middle River at Port Republic.

Sixteen boxes were set up at the park—in the open field, on a gravel bar in the river, and along the path that follows the river. The park was a large open space of approximately 27 ha, with a riparian buffer of varying thickness. Fences, trees, sign posts, telephone wires, and park structures (e.g. basketball backboards and playground equipment) were used as perches. The park was accessible, even at early hours of the morning. However, the park was heavily used by the public which could sometimes delay or hinder trapping operations (*i.e.*, net poles could not be left overnight, or permanently set up, and some areas became too crowded to set up nets at certain times).

Downstream from the park was an agricultural field and forested area, and upstream a gravel company operation and more woods. The opposite side of the river was a steep wooded bank, with private homes at the crest of the hillside. There were three bluebird families at the park in 2006, two of which produced young. The site was not used for trapping in 2007.

2.0 Study species: Eastern bluebird, *Sialia sialis*

Eastern bluebirds are small passerines in the thrush family (*Turdidae*), weighing approximately 28-32 g (Gowaty and Plissner 1998). They are an ideal general study species because they are secondary cavity nesters that readily use nest boxes, and are easily monitored and trapped at the box. In the preliminary study of 2005, bluebirds were found to have elevated levels of mercury; mean adult blood mercury was 1.99 ppm (n=10) and nestling blood was 0.08 ppm (n=36) in the contaminated section of the South River (Cristol 2005, unpubl. data).

Bluebirds typically begin nesting in April, and the breeding season may last up to 201 days (Gowaty and Plissner 1998). Clutch size is typically 4 eggs, and up to 3 clutches may be completed in a breeding season (Gowaty and Plissner 1998). Overall nest success is 55-84 % across the breeding range (Gowaty and Plissner 1998).

Bluebirds are "partial migrants", *i.e.*, after breeding, some birds within a population may migrate short distances while others remain on site throughout the winter (Gowaty and Plissner 1998). In Michigan, approximately 30% were non-migrants and 70% were migrants (Pinkowski 1977). Whether or not a bird migrates may be determined by the winter weather at the breeding site; birds may be more likely to migrate in harsh winters (Gowaty and Plissner 1998). In the Shenandoah Valley, bluebirds occur frequently in summer months and during spring and fall migration, but are uncommon in the winter (http://www.audubon-nsvas.org/birdlist.htm). It is not known how many of the breeding birds in my study site depart in winter.

Bluebirds are terrestrial insectivores (and frugivores in autumn and winter). The main foods taken during the breeding season are ground-dwelling arthropods, including butterfly and moth larvae, adult grasshoppers, crickets, spiders and beetles, but they will also eat fruits to some extent (Pinkowski 1978; Friedman 2007). They use sit-and-wait perch hunting as the primary means of catching prey (Gowaty and Plissner 1998). During the breeding season, foraging is most active during the mornings and evenings, and is slow during the middle of the day (Gowaty and Plissner 1998).

2.1 Eastern bluebird fledglings

For the first 7-10 days after leaving the nest, fledglings tend to remain near cover (Gowaty and Plisser 1998), and often perch high in well-foliated trees. They progressively become more mobile with time. Fledglings can feed independently two weeks (25 - 34 days old) after departing the nest, but may still be fed by an adult for an additional 10 days (35 - 47 days old) (Pinkowski 1975; Gowaty and Plissner 1998). Food fed to fledglings may be different from nestlings, and has been found to be smaller sized and from a more localized source (within a few meters of the begging fledgling) than that fed to nestlings (Pinkowski 1978).

Family groups often remain together throughout the summer and into the fall (Pinkowski 1975, 1977; Zeleny 1976). Young of the second brood are more likely to remain associated with their parents near the nest site throughout the winter; earlier broods may leave parental territories upon independence, but may rejoin flocks later in the breeding season (Gowaty and Plissner 1998). The exact 'home range' of fledgling birds is unknown, but they may make flights up to several km from their natal site (Gowaty and Plissner 1998). Home range of adults during the breeding season averaged about 2.1 ha in New York and South Carolina, but may range from 1.1-8.4 ha (Sloan and Carlson 1980; Gowaty and Plissner 1998). After breeding, territory sizes are unknown, but may be up to 120 ha in the winter (Saverno 1991). Fidelity of fledglings to the natal site the following year is variable, only 11% of fledged young returned to breed on a study site in Minnesota (Fiedler 1974).

2.1.1 Feather growth of fledglings

Feather growth is rapid in the second week of development, and nestlings are almost completely feathered by day 12 (Pinkowski 1975). Birds fledge between 18 and 22 days, and feathers continue growing after fledging, when wing chord is 75% of adult wing chord (Pinkowski 1977; Gowaty and Plissner 1998). Young achieve full adult dimensions in wing chord and primary length at 35-40 days (Pinkowski 1975).

Juvenile bluebirds undergo a partial or incomplete first prebasic molt, replacing most body feathers, *i.e.*, all contour feathers including wing and tail coverts, as well as some flight feathers, *i.e.*, primaries, secondaries and tail. This molt may include 3-10 inner greater-coverts (forewing), 0-12 rectrices (tail), and 1-3 tertials or secondaries (inner wing) (Pinkowski 1975; Pyle et al. 1987; Gowaty and Plissner 1998). Spring brood juveniles molt when they are 2-3 months old (July to September), summer brood juveniles molt when they are less than two months old (August to October; Gowaty and Plissner 1998). The mean duration of the molt period is 49 days; young from early broods molt for approximately 10 weeks, and later broods molt for 6 weeks (Pinkowski 1975). Thus, sampling fledglings for a month after they leave the nest provided data on mercury levels during moderate feather growth, from 40-60 days provided data during a period of no feather growth, and beyond that feather growth (molt) may or may not have re-started.

3.0 Nest box monitoring and reproductive success

Nest boxes were monitored from April-August 2006 and 2007. With other students I collected reproductive data, including: clutch initiation, number of eggs,

number hatched, and number fledged on sites on the South, Middle and North Rivers. Weekly box checks began on 1 April in 2006 and 2007 to determine nesting status. Presence or absence of a nest was noted, and if appropriate, stage of nest building, species, and number of eggs. House sparrow (*Passer domesticus*) nests, with or without eggs, were removed. Eastern bluebirds began building nests in late March or early April. Nests were checked more frequently starting in May, to establish accurate clutch initiation, and to determine the total number of eggs laid. Bluebirds lay one egg a day, and incubation typically begins on the day the last egg was laid, lasting 14 days (range of 11-19 days; Gowaty and Plissner 1998). Eggs usually hatch within one day, sometimes two, of each other (Gowaty and Plissner 1998). Hatch dates were predicted using the typical clutch size of four eggs and incubation period of 14 days. Nests were visited on, or shortly after, the predicted hatch date to determine the actual hatch date based on nestling age. Nests were visited again to band nestlings at the appropriate age (see below 4.0 Banding and morphological measurements), and to determine fledging success 18 to 22 days after hatching. If eggs were cracked or missing, predation was assumed (*e.g.*, by house sparrows or snakes).

4.0 Banding and morphological measurements

At all sites in 2006, nestlings (hereafter, hatch-year birds, abbreviated as HY) were banded between nine and 17 days old with a US Geological Survey (USGS) leg band (size 1B) on the right leg and a unique combination of three plastic color bands (red, yellow, black, light green, lavender, pink, or white). When transmitters were attached, nestlings were banded closer to fledging age (15-17 days; see below 5.0 Telemetry).

Weight and morphological measurements, such as, wing chord and tail length were obtained for all HY bluebirds. When possible, nestling sex was determined based on amount of blue in primaries, secondaries, primary coverts and rectrices, and the extent of white edging on outer rectrices, as described by Pinkowski (1974). This method was most reliable after nestlings were 13 days old.

Adults (hereafter, after hatch-year, abbreviated as AHY) were captured at the nest box after the hatch date and banded in the same manner as nestlings. Adults were trapped at the nest box using a nest-box trap—a small piece of metal or plastic, approximately 7.5 x 7.5 cm, that was duct-taped on the inside of the box above the entrance hole and propped open with a thin stick (Stutchbury and Robertson 1986). When an adult entered the nest to feed young, the stick would be knocked out of place, allowing the door to close over the hole and trapping the adult inside the box. Attempts were made to trap all breeding adults. Traps would be set, and left or watched (a maximum of three traps at a time) for up to one hour. Weight and wing chord measurements were taken from all adults at the time of banding.

In 2007, nestlings and adults on reference sites were only banded with a USGS silver band on the left leg; nestlings and adults in 2007 on the contaminated site were still banded with color bands, and the USGS band was on the left leg. The same measurements were taken in 2007 as in 2006.

5.0 Telemetry

In 2006, transmitters (0.9g model BD-2 transmitters, Holohil Systems Ltd., Ottawa, Ontario, Canada) were attached to bluebird nestlings while they were still in the

nest box. In each brood, 2-5 young were outfitted with transmitters. At first, transmitters were attached to all nestlings of a brood. Some members of the brood are likely to die, and this method ensured the possibility of tracking any survivors of that brood. However, because of the limited number of transmitters, and in order to obtain data from more families, I changed techniques and put transmitters on only 1-3 of the heaviest nestlings. Heavier nestlings are more likely to bear the weight of a transmitter with no ill effects, and to survive to the next breeding season (Sullivan 1989; Magrath 1991).

The expected life of the transmitters was 50 days, and the signal could be detected up to 800 m away. Transmitters were removed from all birds that were captured after approximately 45 days had passed. In five cases a replacement transmitter was placed on a bird to allow continued monitoring. Transmitters were not retrieved from 23 birds by the end of 2006. Five of these birds were likely to have died early in the season. In 2007, telemetry was not used to monitor fledglings.

5.1 Attachment of transmitter

Transmitters were attached using a Rappole harness (Rappole and Tipton 1991) constructed of 1 mm elastic bead cord glued in a figure-8 shape to the transmitter with cyanoacrylate glue (Krazy glue®). The cotton sheath surrounding the cord was removed (unraveled) so only the elastic was used as a harness (Jewelry & Craft Essentials®, Hirschberg Schultz & Co. Inc.). Each loop measured approximately 27 mm from the edge of the transmitter to the end of the stretched loop. There was slight variation in the loop size, some were larger than others. Transmitters were prepared ahead of time, and several transmitters were brought to each outfitting of a brood. The best-fitting harness

was used depending on an individual's body size. The figure-8 harness was supplemented with two more loops of equivalent size of 0.5 mm elastic threaded through the hollow tubing at the front and back end of the transmitter (Holohil Systems Ltd.; Figure 3). The weight of the transmitter plus harness was 1.1 g, which is between 3 and 5% of the bluebird's body weight (Caccamise and Hedin 1985). Weight of nestlings upon attachment ranged from 23.5 to 32.6 g (mean = 27.9, n = 41); thus the transmitters were between 3.4 and 4.7% of nestling mass. The effects of transmitters on flight ability may be more pronounced in young birds just learning to fly; however, adverse effects of transmitters have been studied in several species, and no effects have been detected in behavior or physical condition (Sanzenbacher et al. 2000; Naef-Daenzer et al. 2001; Bowman et al. 2002).

5.2 Tracking

Each transmitter had a frequency between 150.800 and 151.800 MHz. I used a hand-held receiver (model R-1000, 149-152 MHz, Communications Specialists Inc., Orange, CA) and a hand-held folding Yagi three-element directional antenna (model F 151-3FB, AF Antronics, Urbana, IL). I tracked birds primarily on foot at each site, although tracking by vehicle was also used. Telemetry is used as a means of tracking birds movements, in dispersal and migration studies, and has been used as a means of estimating post-fledging juvenile survival, and to study foraging behavior (Williams 1990; Elbert and Anderson 1998; Vega Rivera et al. 1998; Kershner et al. 2004; White 2007). In this study, telemetry was used only as a means for locating individuals or groups of juveniles in order to trap them repeatedly over time.

Individuals were located by telemetry, homing in until visual identification of individuals could be made via leg bands. Locations were recorded by global positioning system (GPS) coordinates (Garmin® etrex data logger). Because all nestlings were color banded, individuals without transmitters in the group were identified as well. Fledglings were observed until a typical pattern of movement was established, and frequently used perches or flight paths or often visited areas were identified (typically 1-3 hours of observation). Mortality of fledglings is high at this time, when flight ability is poor (Sullivan 1989; Kershner et al. 2004). If possible, transmitters were recovered from dead birds and reused on other birds. Any dead fledglings found were stored for possible mercury analysis of tissues. Absent signals were due to transmitter failure, mortality, or movement of the bird out of range. I assumed a bird was dead if: 1) a signal was lost within the first two weeks of fledging, when it is unlikely that they moved off site and out of range, or 2) if the transmitter was found with bluebird remains such as feathers or leg bands.

6.0 Trapping

Spring broods typically fledge synchronously (Pinkowski 1977), and the first nests on my study sites fledged in early to mid-May. At sites with multiple boxes, as predicted, fledglings from different families congregated together. Juvenile bluebirds form cohesive flocks (see above 2.0 Study species), and family groups often remain together in the natal area throughout the summer and into the fall (Pinkowski 1977, 1975; Zeleny 1976; Gowaty and Plissner 1998). This social behavior of juveniles facilitated

trapping of fledglings and also made it likely that fledglings were still feeding in the contaminated area.

Fledglings (hereafter, independent hatch-year, abbreviated IHY) were tracked and observed daily for the first 2 weeks post-fledging and locations were recorded in ArcGIS® 9 (Environmental Systems Research Institute, Inc., Redlands, California). Starting approximately 2 weeks after fledging, when they became more mobile, trapping attempts began. Mist nets (4-shelf, 30 or 38 mm-mesh mist nets; 6, 9 and 12 m; Avinet, Inc., Dryden, New York) were set up in the area where fledglings were located, between 50 and 400 m from the river. Nets were set up before dawn and in late afternoon, when birds were most likely to be foraging, and left open for 4-10 hours. Fledglings were continuously tracked during this time and nets were moved as needed.

Audiotape lures and decoys were used in the early season without obvious benefit, so nets alone were used for the remaining attempts. Five pairs of extending painting poles were modified to use as telescoping mist net poles (Wooster Positive lock Sherlock®, 8' -16' and Mr. LongArm® 6'-12'). The attachment end of the pole was sawed off, leaving an opening to the hollow pole. Rebar (approximately 1 m in length) was pounded into the ground as an anchor for the poles. Nets were opened on the poles, and extended up to approximately 5 m. I also used four 8-10 foot galvanized conduit pipes as poles for additional nets. At each capture, the GPS (Global Positioning Systems) coordinate was taken of the net and time was noted. Placement of nets was based on careful observation of the behavior. Nets were placed either: 1) between or just in front of perches—*e.g.*, sprinkler heads, fences, bird boxes; 2) in a flight line—sometimes in the

middle of a field between perches; or 3) raised next to taller trees frequented by the birds. The layout of some sites enabled "chasing", when birds were herded along a row of trees into a net at the end of the line.

I attempted to locate birds with transmitters every 2-3 days, to check for mortality and to see if they remained in the same general area. I attempted to keep a regular schedule of trapping birds as well. However, because my primary objective was trapping as many birds as possible in different stages of feather growth, I was somewhat flexible in my methods. If a bird seemed particularly "trappable", or if I wanted to spend extra time on a bird that was difficult to trap, I would allow for extra time at a site. As a result, all data I collected on movements was anecdotal. Because I did not trap birds in the reference areas, I can not directly compare the survival data from the contaminated sites to local data.

In 2007, the trapping effort was entirely focused at AUFC because only a small sample was needed. The chance of catching multiple individuals was greatest at this location. Telemetry was no longer necessary in 2007, when my goal was to catch birds later in the season. Instead, individuals were located visually or aurally (by the *Tu-a-wee* call—often given by fledglings while foraging in flocks (Gowaty and Plissner 1998), and IHY birds were identified by color bands. Once located, nets were placed in the appropriate locations.

7.0 Data collection - morphology

At the time of capture, morphological measurements (weight, wing chord and tail length) were recorded. Individual growing feathers, both flight and body, were counted,

unless more than 100 were growing in a section (counted as >100). Bird age was determined by counting days since hatching (day 0).

Before analysis, I defined four feather growth categories, the first corresponding with the nestling period, and following three with the fledgling period. The categories were related to the amount of feathers growing, and were also chronological. Feather growth was classified as follows: "nestling", referring to the period when thousands of feathers were growing simultaneously in the nest, 10-17 days old; "waning", referring to the period when feather growth was decreasing after fledging, but >10 flight feathers and/or body feathers were still growing, 27-41 days old; "none", referring to the stage when feather growth had stopped entirely, or <10 body feathers and no flight feathers were growing, 32-80 days old; and "molt", referring to the stage when body feathers (>10) were growing to replace juvenile plumage during the first prebasic molt, 43-106 days old.

Unbanded fledglings from off-site were often caught with groups of banded fledglings. These birds were banded and blood and feathers were sampled for mercury. However, they were only used in some analyses because of their unknown mercury exposure and exact age.

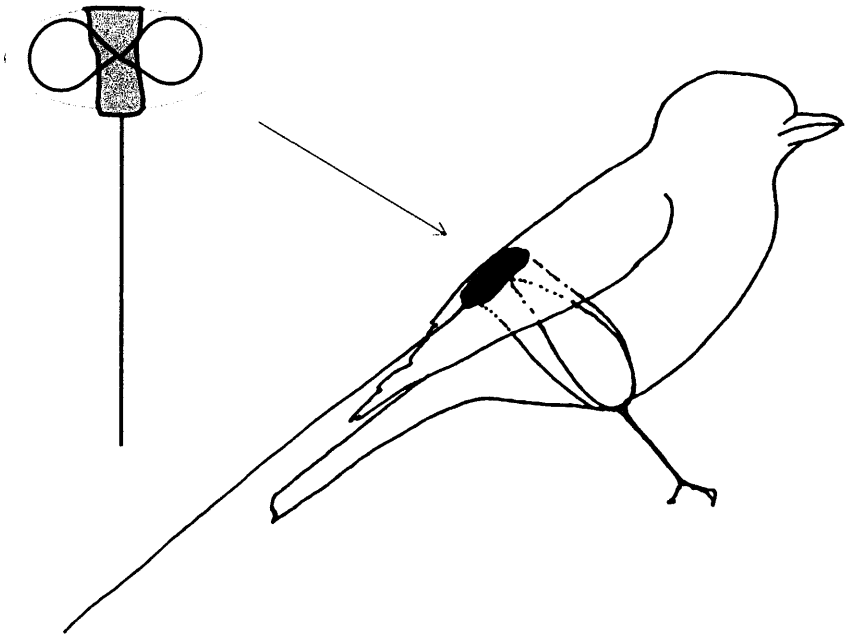

Figure 3. Diagram of transmitter apparatus. Two sets of elastic loops attached around the birds thigh, so the transmitter sat on the lower back with antenna extending beyond the tail.

8.0 Data collection - Mercury

Blood and feather samples were taken from all fledglings, nestlings and adults. The entire sample from one bird—feathers and blood—was placed in a 1-quart Ziploc® bag labeled with date, species, age, sex and site in permanent marker (Sharpie®) and stored in a freezer (-25°C) before analysis. In 2007, nestlings on the reference sites were not sampled for blood or feathers. All fledglings and adults captured at all sites were sampled in both years. Blood mercury is representative of short-term exposure, indicating recent dietary uptake (Kahle and Becker 1999). Feathers are indicative of mercury exposure at the time of growth, and indicate both dietary uptake and body burden (see above Introduction 9.2 Excretion via feathers).

A 26 G ½ gauge needle (Becton, Dickinson and Co.(BD), Franklin Lakes, New Jersey) was used to puncture the cutaneous ulnar vein (brachial vein), and between 50 and 225 µL (2-3 75 µL heparinized capillary tubes, partially to completely full) of blood was taken from each individual. The 2 or 3 capillary tubes were sealed with Crito-caps®, and stored in a 6 mL BD® vacutainer (13x100mm). Multiple capillary tubes were used in case of loss and as duplicates in analysis. Blood samples were placed on ice immediately, and then stored in a freezer (-25°C) until analysis. Vinyl gloves were worn while sampling blood.

In 2006, feathers were sampled from the belly (6-9) and back/rump (6-9) of all birds and stored in a Ziploc® bag, placed in a cooler. Back, belly, breast and rump feathers show the least variation in mercury levels (Furness et al. 1986; Lewis and Furness 1991). In 2007, only feathers from the back (9) were sampled from all ages. In

both years, additional flight feathers were taken from adults for related studies (see White 2007 and E. Langer unpubl. data). Nestling body feathers were analyzed whole, despite that they were partially growing and the total mercury may be underestimated because of the blood residue in the shaft (Burger 1993). The feathers collected from fledglings were always completely grown, even if the bird was molting. Thus, feathers collected from fledglings would reflect the mercury exposure from the nestling period and shortly thereafter (see above Introduction 9.2 Excretion via feathers), rather than more recent exposure. To remove any external contamination, feathers were washed with de-ionized water and dried in a coin envelope in a low-humidity container for at least 48 hours.

9.0 Isotope Analysis

Blood for isotope analysis was taken at the same time as for mercury analysis, but was stored in nonheparinized capillary tubes (Fisher Scientific®). This did not represent additional blood sampling beyond the 1-2 tubes described above in 8.0. To prepare for analysis, the blood was transferred from the capillary tube directly into a small centrifuge tube with a small hole in the lid. Samples were then freeze-dried using a Labconoco® Benchtop Freeze Dry System for 24 to 48 hours. The dry samples were placed in 8 x 5 mm (Costech Analytical Technologies Inc., Valencia, VA, USA) tin capsules and approximately 0.002 g weighed out using an analytical balance. Tins were compacted on a crimper plate, and placed in a 96-well microtiter plate, wrapped in parafilm, with the sample labeled as the corresponding row and column. Samples were shipped to the UC Davis Stable Isotope Facility (Davis, CA) for analysis. Ratios of stable isotopes of carbon and nitrogen were measured by continuous flow isotope ratio mass spectrometry

(20-20 mass spectrometer, Sercon, Crewe, UK) for high precision analysis of combusted solid samples. The samples were combusted to CO_2 and N_2 at 1000° C in an on-line elemental analyzer (PDZEuropa ANCA-GSL). Sample ratios were compared to those of pure cylinder gases, injected into the spectrometer before and after the sample peaks.

Stable isotope ratios are reported in parts per thousand (‰), in the standard delta (δ) notation, of the standard for C (Pee Dee Belemnite (PDB) limestone formation) and N (atmospheric nitrogen (AIR)). The equation:

$$\delta X=[(R_{sample}/R_{standard})-1]x1000$$

was used to calculate values: X is the heavier isotope, either ^{15}N or ^{13}C; R_{sample} is the isotopic ratio in the sample; and $R_{standard}$ is the ratio in the standard (Peterson and Fry 1987). Measurement errors averaged ± 0.1‰ for nitrogen and ±0.3‰ for carbon. Replicate standards were analyzed every 12 samples to ensure accuracy.

10.0 Mercury Analysis

In 2006, samples were analyzed for total mercury at the Trace Element Research Laboratory (TERL, Texas A&M University, College Station TX). In 2007, all samples were analyzed for mercury at the College of William & Mary, including a few remaining samples from 2006. Some adult bluebird blood from reference sites, blood from previously unbanded fledglings, and all fledgling feathers from 2006 were analyzed at this time. The amount of total mercury approximates the amount of methyl mercury in a sample, because 90-100% of mercury in avian blood and feathers is methyl mercury. In the Bicknell's thrush (also family Turdidae), the methyl mercury to total mercury ratio was 0.983 ± 0.254 (Rimmer et al. 2005). Therefore samples were analyzed for total

mercury, which was a more cost effective procedure (Evers et al 2005, Rimmer et al 2005).

At TERL and William & Mary, blood and feathers were analyzed with a Milestone DMA-80 direct mercury analyzer using cold vapor atomic absorption spectroscopy (CVAAS). Samples are analyzed directly, without first being digested. The samples were weighed in clean nickel boats before analysis, and placed in one of 40 positions in the carousel of the DMA. The carousel automatically moves positions allowing each boat to pass through the machine, initially dried by a flow of O_2 passing through a heated coil. CVAAS is a process that combusts the samples at 750 °C to release mercury (Hg^0), which collects on a gold trap and enters an atomic absorption cell. Light from a mercury vapor lamp is absorbed by the Hg ions, and absorption is compared with an external calibration standard to determine mercury in the sample. The instrument detection limit was 0.005 ng Hg. Minimum detection limit (MDL) at William and Mary was 0.0055 ppm, and at TERL was 0.0051 ppm (see Friedman 2007 for description).

A sample blank, methods blank and two of three standard reference materials (DORM-2, DORM-3 or DOLT-3) were run every 20 samples. Recovery of total Hg was above 96% for all three standards (see Friedman 2007). Duplicate samples were obtained by splitting the total number of feathers into two samples, or analyzing two capillary tubes of blood from the same collection of the same bird. Duplicates were run when possible (*i.e.*, when there was enough blood taken), every 20 samples. Inter-laboratory duplicates were also run, to ensure comparability between TERL and William and Mary. The relative percent difference (RPD) between duplicates was 15.73 ± 27.53

% for samples greater than 10 times the MDL, less than the generally accepted 20%. See Friedman 2007 for detailed description of calculations of RPD. Mercury levels are reported in parts per million (ppm) wet weight (ww) or fresh weight (fw; for feathers).

11.0 Statistical Analysis

Statistical tests were performed using Minitab 15 (Minitab version 15, Minitab Inc., State College, PA, USA) or R 2.5.1 (R Development Core Team (2005). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org). Non-normal data were log-transformed, or non-parametric tests were used as noted. A significance level of $\alpha < 0.05$ was used for all tests.

11.1 Nesting and reproductive data

The reproductive data: total clutch size, Julian date of clutch initiation, proportion hatched (total hatched/total eggs), proportion fledged (number fledged/number hatched), and total number of young produced were compared between treatment groups each year. Nests were not included in analyses if eggs were collected for other studies (2006 only), if monitoring late in the season was inconsistent, or the fate was unknown for any reason. In a few instances when the first clutch failed due to predation and the birds re-nested, the second clutch was not included in the second clutch analysis, since these nests may not have been equivalent to actual second clutches. In 2007, five nests were eliminated from analyses for these reasons. In 2006, when eggs were collected from eight nests for another study, 13 were eliminated from analysis.

Data were not normally distributed, and proportion and count data could not be transformed to a normal distribution in order to use an ANOVA. Instead, I compared medians of the treatment group populations for each parameter using Kruskall-Wallis tests adjusted for ties separately for each year and clutch. I also used the GLM function in ANOVA to compare each parameter with treatment group, year and treatment group*year interaction, even though data violated the assumption of normality.

11.2 Mercury levels of adults and nestlings

Blood mercury levels of birds that were caught more than once over the season, *i.e.*, during the first and second clutch, were averaged to create one value for that bird. For AHY birds on the contaminated site, I used an ANOVA using the GLM function with Type III sums of squares in Minitab, which allows for unbalanced design. I compared log-transformed mercury levels between years, date of capture, river km, sex, and interactions between year*time frame, year*river km, year*sex, time frame*sex, and river km*sex. The variable 'date of capture' described 14-day periods beginning with the first capture (Julian day of first capture = 119; day 119-132 = time period 1, 133-146=2, 147-160=3, 161-174=4, 189-212=7). River km and date were assigned as covariates. Non-significant interactions were removed for the final models. Polynomial and linear regression were used to analyze blood mercury along river km. Blood mercury levels from AHY birds on reference sites were compared using a GLM with years, time frame, sex and interactions between year*sex, and time frame*sex. There was no equivalent of 'river km' for reference sites.

Nestling mercury on the contaminated site was averaged per brood and log-transformed to fit a normal distribution. I tested for differences between years using a GLM with year, date and river km as factors. Not all nestlings could be reliably sexed, so sex was not used as a factor. Nestling feather mercury was analyzed using the same model. To relate nestling to adult blood mercury, a linear regression was used between the average blood mercury level per brood and the blood mercury level of the parent, which was an average of the male and female adults when both were sampled.

Comparisons between captures of the same AHY birds (*i.e.*, between clutches or years) were made with a paired t-test and log-transformed mercury level. Nestling mercury was compared between first and second clutches (that had one or both of the same parents) with a paired t-test using log-transformed blood mercury. Mann-Whitney U tests were used to compare mercury levels of age classes between treatment groups, and between age classes within contaminated or reference sites.

11.3 Mercury levels of IHY bluebirds

I used repeated measurements of the same individuals over time to directly monitor changes within an individual and between individuals. If an individual IHY was caught twice during the same feather growth stage, values were averaged (for mercury level, date, age and morphological measurements). Mercury levels of blood and feathers were log-transformed to fit a normal distribution, but $\delta^{15}N$ was normally distributed and no transformation was necessary. I used the lme function in the statistical program R 2.5.1.

I first compared blood mercury levels between feather growth categories, combining unknown and known individuals. I used a linear mixed-effects model to account for fixed and random effects, fit by maximum likelihood estimates. The model was simplified as non-significant interaction terms and factors (*e.g.*, sex) were eliminated. The final model was: log-transformed Hg ~ river km + feather growth category, incorporating the random effects of repeated captures of related individuals ("box/band", or individuals nested in a box). I used the same model to test for differences in feather mercury and $\delta^{15}N$, using just known-origin birds, as feathers of unknown origin birds may not have been grown in a comparable level of contamination, and $\delta^{15}N$ was not analyzed for unknown birds. Birds originating from the first and second clutch were analyzed both together and separately.

To show the relationship of mercury with age, rather than with growth stage, I also used a linear mixed effects model with a polynomial regression. The model was: log-transformed blood mercury ~ river km + poly (age, second order polynomial), with the random effect of related individuals. This model was used to analyze feather mercury and $\delta^{15}N$ as well. This analysis was another way of presenting the data, as feather growth categories are close proxies for age.

Results

1.0. Nests and reproduction

Bluebirds were present on sites in February and possibly were year-round residents (pers. obs.). Nesting in both years began in early April and went through August, in nest boxes intended for tree swallows, as well as some placed near the woods. Only nests within 50 m of the river are included in analyses of reproductive data.

In 2006, there were 37 total bluebird nests on the South River and 39 on reference rivers (Table 2). For a related study, eggs were collected from four reference nests, and four contaminated nests, these nests were not included in analyses. In 2007, there were 35 total bluebird nests on the South River and 41 on reference rivers in 2007 (Table 3). No eggs were collected in 2007. Third clutches were not monitored in either year, because they were so few (< 3 each year) and late in the season.

In 2006, five nests on the contaminated river, and two on the reference sites were depredated while on eggs, or on hatch day. Two other nests on reference areas failed while on eggs due to nest box takeover by tree swallows or Carolina chickadees (*Poecile carolinensis*). One nest on the reference site never hatched, and was considered to be abandoned. Four other nests on the contaminated site and six on reference sites failed as nestlings, due to predation or apparent starvation. Overall success rate (including both clutches, of at least one fledged young) was 0.76 (28/37) on contaminated sites, and 0.72 (28/39) on reference sites. Because nests were monitored frequently there was no uncertainty as to fate, and thus no benefit of using the traditional Mayfield Method of calculating survivorship based on days of exposure (Mayfield 1961, 1975).

For nests in the first clutch in 2006, there was no difference between contaminated and reference sites in total clutch size (H=0.26, df=1, p=0.611), proportion hatched (H=0.22, df=1, p=0.637), proportion fledged (H=0.20, df=1, p=0.656), total number fledged (H=0.10, p=0.75), or Julian date of clutch initiation (H=0.01, df=1, p=0.932; Table 4). Nests on reference sites appeared to have smaller second clutches than those on contaminated sites (H=35.44, df=1, p=0.020). Second clutches were initiated earlier on contaminated sites (H=6.11, df=1, p=0.013; Table 4). Among second clutches, there was no significant difference between treatment groups in proportion hatched (H=2.81, df=1, p=0.094), proportion fledged (H=1.33, df=1, p=0.248), or total number fledged (H=3.18, df=1, p=0.075).

In 2007, three nests on contaminated sites, and four on reference sites were depredated, and one nest on the reference site was taken over by a house sparrow while on eggs. Three nests on the contaminated and two on the reference sites failed while on nestlings. One of the three failures on contaminated sites was due to human vandalism. Overall success rate was 0.80 (28/35) on contaminated sites, and 0.85 (35/41) on reference sites. For both years combined, success rates were similar across treatment groups, 0.76 on contaminated sites and 0.79 on reference sites.

For nests in the first clutch in 2007, there was no difference in total clutch size (H=1.90, df=1, p=0.168), proportion hatched (H=0.72, df=1, p=0.395), proportion fledged (H=0.05, df=1, 0.823), total number fledged (H=0.03, df=1, p=0.872), or Julian date of clutch initiation (H=1.12, df=1, p=0.289; Table 4). For the second clutch, a higher proportion of nestlings may have fledged on the contaminated site (H=4.99, df=1,

p=0.025—not significant if not adjusted for ties). Second clutches were also initiated earlier on contaminated sites (H=4.42, df=1, p=0.036). There was no difference in total clutch size (H=3.43, df=1, p=0.064), proportion hatched (H=0.75, df=1, p=0.385), or total number of fledglings produced (H=0.98, df=1, p=0.322; Table 4).

Using a GLM with treatment group, year and treatment group*year interaction, I performed an alternative analysis of reproductive success. For total eggs laid in the first clutch, there was no significant effect of treatment group ($F_{1,93}$=1.87, p=0.174), year ($F_{1,93}$=0.36, p=0.543) or the interaction term ($F_{1,93}$=1.00, p=0.319). For Julian date of clutch initiation, there was no significant effect of treatment group ($F_{1,93}$=1.32, p=0.253), but there was an effect of year ($F_{1,93}$=24.01, p<0.001). There was no effect on this variable of the interaction between year and treatment group ($F_{1,93}$=0.63, p=0.431). Post-hoc tests showed that clutches were initiated significantly later in 2007. This difference between years, and those mentioned below, were of course not tested in the separate analyses of years presented above. For proportion hatched, there was no significant effect of treatment group ($F_{1,93}$=0.54, p=0.464), or interaction term ($F_{1,93}$=0.15, p=0.703), and year was marginally significant ($F_{1,93}$=3.74, p=0.056). Post-hoc tests showed a slightly lower hatching success in 2007. For proportion fledged, there was no significant effect of treatment group ($F_{1,84}$=0.01, p=0.909), year ($F_{1,84}$=0.80, p=0.372) or the interaction term ($F_{1,84}$=0.00, p=0.984). For total birds fledged, there was a significant effect of year ($F_{1,94}$=4.44, p=0.038), and post-hoc tests showed that more fledglings were produced per nest in 2006. There was no significant effect of treatment group

($F_{1,91}$=0.01, p=0.943) or the interaction term ($F_{1,91}$=0.01, p=0.913) on total fledglings produced.

For the second clutch, the same effects were used in the GLM. For total clutch size, there was a significant effect of treatment group ($F_{1,46}$=9.72, p=0.003); reference total clutch size was smaller, as detected in the 2006 analysis above. Year ($F_{1,46}$=0.16, p=0.689) and the interaction term ($F_{1,46}$=0.00, p=0.988) had no significant effect on total clutch size. For Julian date of clutch initiation, there was a significant effect of treatment group ($F_{1,46}$=16.97, p<0.001), and post-hoc comparisons showed that contaminated birds initiated nests earlier than reference birds, as found in the analysis of each year separately. Year ($F_{1,46}$=0.01, p=0.926) and the interaction term ($F_{1,46}$=0.31, p=0.581) had no significant effect on clutch initiation date. For the proportion of eggs that hatched, there was no significant effect of treatment group ($F_{1,46}$=2.36, p=0.131), year ($F_{1,46}$=1.03, p=0.315) or interaction term ($F_{1,46}$=0.00, p=0.997). For the proportion of nestlings that fledged, there was a significant effect of year ($F_{1,43}$=5.90, p=0.019), with a greater proportion fledging in 2007. Treatment group ($F_{1,43}$=0.11, p=0.741) and the interaction term ($F_{1,43}$=3.13, p=0.084) had no significant effect on proportion fledged. For the total number of birds that fledged, there was a marginally significant effect of treatment group ($F_{1,46}$=3.85, p=0.056) and a significant effect of year ($F_{1,46}$=4.61, p=0.037), but no effect of interaction term ($F_{1,46}$=0.40, p=0.532). Post-hoc comparisons showed that fewer birds fledged on reference sites, as in the initial analysis of 2007, and more fledged overall in 2007. Thus the findings of the alternative analysis were consistent with the findings of the initial analysis by year, except differences between 2006 and 2007 were revealed.

Table 2. Bluebird nests on contaminated and reference sites in 2006. Total number of bluebird nests and nests that fledged at least one young, per site. Status "C" refers to contaminated sites, "R" refers to reference sites. A "*" next to a site name indicates that eggs were collected from one nest.

Site	Status	Nests		Nests fledged	
		Clutch 1	Clutch 2	Clutch 1	Clutch 2
Water Treatment Plant	C	1	1	1	1
Basic Park*	C	1	1	1	1
Genicom	C	2	2	2	2
Dooms Crossing	C	2	2	0	2
Wertman property	C	1	0	0	0
Crimora Crossing	C	1	0	1	0
August Forestry Center	C	9	5	8	3
Wampler property*	C	1	1	1	0
Harriston Crossing*	C	0	0	0	0
Rankin property	C	0	1	0	1
Grottoes City Park	C	3	2	2	2
Bradburn Park*	C	1	0	0	0
Total Contaminated		22	15	16	12
276 Bridge crossing*	R	1	1	1	0
Auckerman property	R	1	1	1	1
Wildwood Park	R	1	1	1	1
Crawford property	R	1	0	0	0
Fort River Road	R	1	0	0	0
Concrete Bridge	R	1	1	1	1
Dorries property*	R	0	0	0	0
Shapcot property	R	1	1	0	1
Whitescarver farm	R	2	2	2	1
Smith's Pond	R	1	1	1	0
Opposite Whitescarver	R	3	3	2	2
Godfrey property	R	1	1	1	1
Ridgeview Park*	R	4	3	3	1
P. Buckley Moss property*	R	1	1	1	1
Cowbane nature preserve	R	4	0	4	0
Total Reference		23	16	18	10

Table 3. Bluebird nests on contaminated and reference sites in 2007. Total number of bluebird nests and nests that fledged at least one young, per site. Status "C" refers to contaminated sites and "R" refers to reference sites.

Site	Status	Nests		Nests fledged	
		Clutch 1	Clutch 2	Clutch 1	Clutch 2
Water Treatment Plant	C	1	0	1	0
Basic Park	C	2	1	0	1
Genicom	C	1	1	1	1
Dooms Crossing	C	2	0	2	0
Wertman property	C	3	0	2	0
Wertman North	C	1	0	1	0
Crimora Crossing	C	1	1	1	1
August Forestry Center	C	8	1	7	1
Wolf property	C	0	1	0	1
Wampler property	C	2	1	1	1
Boe property	C	1	0	1	0
Harriston Crossing	C	1	0	1	0
Rankin property	C	1	0	1	0
Grand Caverns	C	1	0	0	0
Grottoes City Park	C	2	2	1	2
Total Contaminated		27	8	20	8

Table 3 continued.

Site	Status	Nests		Nests fledged	
		Clutch 1	Clutch 2	Clutch 1	Clutch 2
276 Bridge crossing	R	1	1	1	1
Auckerman property	R	1	0	1	0
Wildwood Park	R	1	1	1	1
Crawford property	R	0	0	0	0
Fort River Road	R	2	1	1	1
Concrete Bridge	R	2	2	2	2
Dorries property	R	2	1	2	1
Shapcot property	R	1	1	1	0
Whitescarvers farm	R	2	1	1	1
Smith's Pond	R	3	0	3	0
Opposite Whitescarver	R	4	1	3	1
Godfrey property	R	1	1	1	1
Ridgeview Park	R	3	2	2	2
P. Buckley Moss property	R	2	1	2	1
Locust Street	R	1	0	1	0
Cowbane nature preserve	R	2	0	1	0
Total Reference		28	13	23	12

Table 4. Reproductive parameters, mean± standard deviation, for contaminated and reference sites, 2006 and 2007 both clutches. Sample size (n) is in parentheses.

Clutch 1	Year	Total clutch	Proportion hatched	Total fledged	Proportion fledged	Clutch initiation
Contaminated	2006	4.6 ± 0.5 (n=22)	0.8 ± 0.3 (n=22)	2.8 ± 1.9 (n=22)	0.8 ± 0.4 (n=19)	109 ± 13 (n=22)
Reference	2006	4.6 ± 0.6 (n=25)	0.7 ± 0.4 (n=25)	2.8 ± 2.1 (n=25)	0.8 ± 0.4 (n=21)	108 ± 12 (n=25)
Contaminated	2007	4.4 ± 0.8 (n=24)	0.9 ± 0.2 (n=24)	3.6 ± 1.5 (n=22)	0.9 ± 0.3 (n=22)	126 ± 18 (n=21)
Reference	2007	4.7 ± 0.7 (n=26)	0.9 ± 0.2 (n=26)	3.6 ± 1.6 (n=26)	0.9 ± 0.3 (n=26)	120 ± 14 (n=26)
Clutch 2						
Contaminated	2006	4.6 ± 0.5 (n=14)	0.8 ± 0.3 (n=14)	3.0 ± 1.5 (n=14)	0.8 ± 0.3 (n=13)	157 ± 4 (n=14)
Reference	2006	4.0 ± 0.7 (n=15)	0.7 ± 0.3 (n=15)	1.9 ± 1.6 (n=15)	0.6 ± 0.4 (n=14)	168 ± 13 (n=15)
Contaminated	2007	4.5 ± 0.5 (n=8)	0.9 ± 0.1 (n=8)	3.6 ± 1.2 (n=8)	0.9 ± 0.2 (n=8)	155 ± 17 (n=8)
Reference	2007	3.9 ± 0.8 (n=13)	0.8 ± 0.3 (n=13)	3.1 ± 1.2 (n=13)	1.0 ± 0 (n=12)	170 ± 10 (n=13)

2.0 Blood mercury levels 2006-2007

Because of the small sample size of HY blood samples on reference sites in 2007 (n=3), they were not included in any analyses. Only birds within 50 m of the river were included in these analyses. Mercury levels may show natural variation with time (both between years and within a year), due to changes in environmental conditions, as with temperature, that may affect rates of methylation and mercury availability. Mercury levels may also vary with location, *i.e.*, lower concentrations of mercury may be expected further from a source of contamination. Mercury levels may also vary with sex, due to the female's ability to eliminate mercury into the egg (Evers et al. 2005). Whenever possible or appropriate, all of these factors were included in comparisons of blood mercury levels between years.

2.1 Blood mercury levels of AHY birds 2006-2007

On the contaminated site, AHY bluebirds had significantly elevated blood mercury levels compared with the reference AHY population ($w=9674$, $p<0.001$; Figure 4). Feather mercury of AHY bluebirds will be presented in a related study (L.Langer in prep.).

There was a significant effect of river km, but not of date of capture, year, or sex on AHY blood mercury on the contaminated site (Table 5). No interactions were significant and thus all were removed from the model. To further explore the relationship of blood mercury and distance from the source, mercury levels were plotted by river km. River km had a significant, but weak linear and quadratic effect

on log-transformed blood mercury (linear: F=9.79, p=0.002, quadratic: F=7.22, p=0.009; R^2=0.176, R^2(adj)=0.156; Figure 5).

Blood mercury of AHY birds on reference sites did not have a significant relationship with year, date of capture or sex (Table 6, 7). Average blood mercury levels for adults of individual contaminated and reference sites are presented in Table 8.

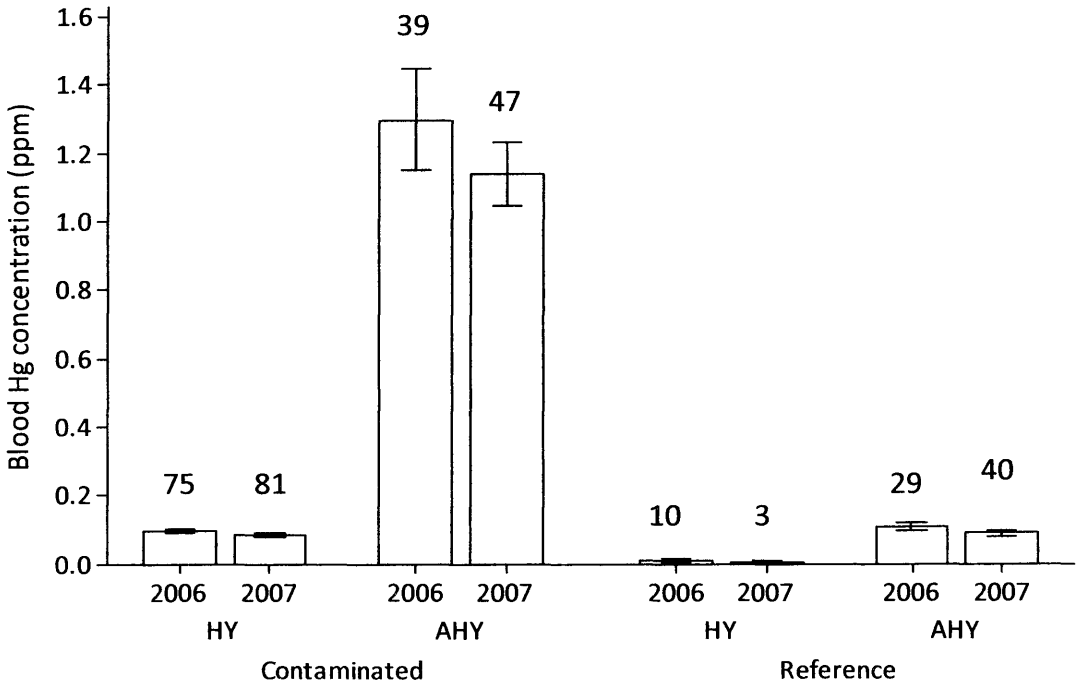

Figure 4. Blood mercury levels of AHY and HY bluebirds at contaminated (grey bars) and reference (white bars) sites in 2006 and 2007. Error bars represent one standard error of the mean. Sample sizes are indicated above the bars.

Table 5. ANOVA table for AHY bluebird blood mercury level on the South River.

Factors	DF	F	P
Year	1	0.24	0.626
Date of capture	1	2.00	0.161
River km	1	8.63	0.004
Sex	1	2.12	0.149
Error	80		

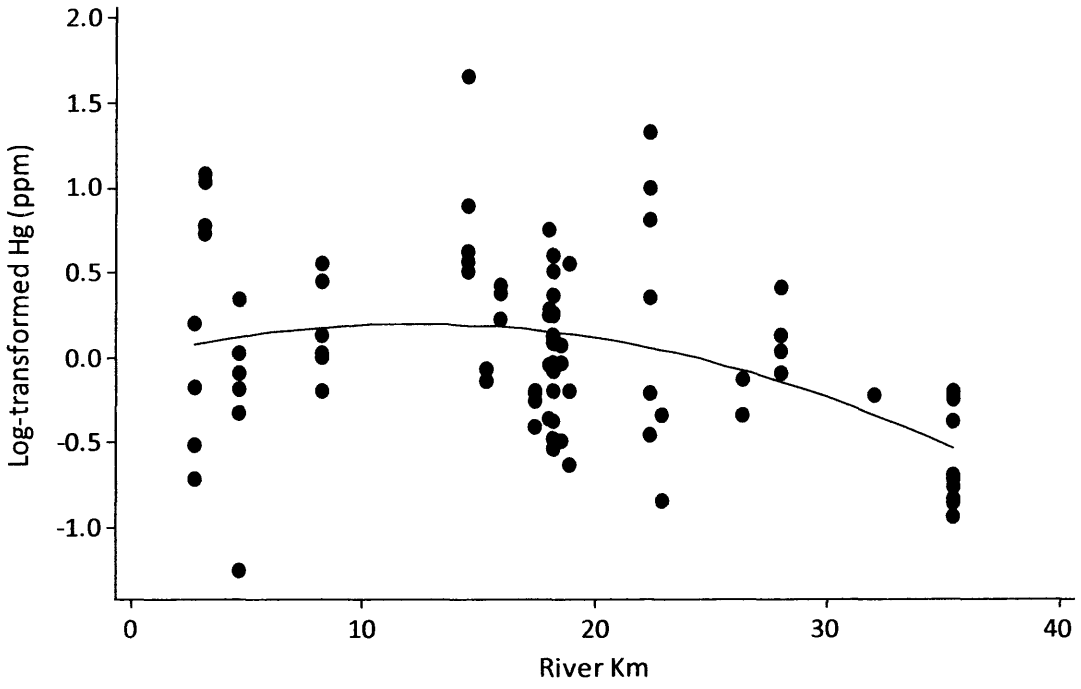

Figure 5. Log-transformed blood mercury of AHY bluebirds on the South River with distance from source in river km (Log Hg= 0.0028 + 0.03262 river km– 0.001344 river km **2).

Table 6. ANOVA table for AHY bluebird blood mercury level on reference rivers.

Factors	DF	F	P
Year	1	0.04	0.835
Date of capture	4	1.65	0.174
Sex	1	0.05	0.831
Year*sex	1	0.37	0.547
Date of capture*sex	4	0.49	0.739
Error	57		

Table 7. Mean blood mercury levels of female and male AHY birds.

	Status	N	Mean	St Dev	Range
Female	Contaminated	48	1.125	0.804	0.287 - 5.310
Male	Contaminated	38	1.321	0.755	0.426 - 3.810
Female	Reference	40	0.102	0.056	0.031 - 0.276
Male	Reference	29	0.097	0.054	0.036 - 0.291

Table 8. Mean blood mercury levels ± standard deviation for individual sites on contaminated and reference rivers, 2006 and 2007.

			2006		2007	
Site	River	River km	N	Blood Hg (ppm ± STDV)	N	Blood Hg (ppm ± STDV)
WH20	South	3	2	1.10 ± 0.86	2	0.73 ± 0.17
BAPA	South	3	2	2.85 ± 0.57	2	2.15 ± 0.08
GENI	South	5	4	0.92 ± 0.47	2	0.79 ± 0.08
DOOM	South	8	4	1.04 ± 0.10	4	1.33 ± 0.42
WERT	South	14	2	3.6 ± 2.43	3	1.98 ± 0.43
WERN	South	15	0	NA	2	0.91 ± 0.04
CRIM	South	16	1	1.27	2	1.47 ± 0.45
AUFC	South	18	13	1.12 ± 0.52	16	1.13 ± 0.44
WAMP	South	22	2	2.52 ± 0.34	4	1.68 ± 1.46
BOES	South	23	0	NA	2	0.58 ± 0.2
HARR	South	26	1	0.89	1	0.72
RENK	South	28	2	1.34 ± 0.27	2	0.98 ± 0.08
GRCA	South	32	0	0	1	0.81
GRCP	South	35	5	0.55 ± 0.23	4	0.49 ± 0.13
Mean South River			39	1.32 ± 0.96	47	1.16 ± 0.65
276B	North		1	0.13	2	0.11 ± 0.0001
NAUC	North		1	0.08	1	0.07
NWWP	North		2	0.13 ± 0.002	2	0.11 ± 0.01
MGOD	North		1	0.06	2	0.03 ± 0.004
MOPW	Middle		6	0.06 ± 0.02	5	0.08 ± 0.03
MSMP	Middle		2	0.06 ± 0.005	4	0.07 ± 0.03
MWHI	Middle		2	0.14 ± 0.10	1	0.12
MSHA	Middle		1	0.06	1	0.05
MDOR	Middle		0	NA	2	0.10 ± 0.01
MFOR	Middle		0	NA	3	0.05 ± 0.002
MRBR	Middle		0	NA	4	0.10 ± 0.02
PBUC	South		2	0.13 ± 0.04	3	0.08 ± 0.01
SRDG	South		6	0.17 ± 0.08	5	0.11 ± 0.07
SLOC	South		0	NA	2	0.24 ± 0.05
SCOW	South		5	0.11 ± 0.03	2	0.10 ± 0.02
Mean Reference Rivers			29	0.11 ± 0.04	40	0.09 ± 0.05

2.1.1. Returning AHY birds

Fifteen breeding adults from 2006 returned to breed in 2007. Ten of 47 adults (21%; 5 male, 5 female) on the contaminated site, and five of 40 adults (13%; 1 male, 4 females) on the reference site were returning breeders in 2007 (Table 9). Three nestlings from 2006 returned to breed in 2007 on the contaminated site only (Table 9, 10). In addition, four adult birds breeding in 2007 were first banded as fledglings in 2006 on the contaminated site (Table 10).

There was no significant difference in blood mercury levels in these individuals from one year to the next (paired t-test: $t=-1.88$, $p=0.083$). When comparing mercury levels across years for treatment groups separately, there was again no difference between years among birds on the contaminated sites ($t=-2.09$, $p=0.066$), or on the reference site ($t=2.37$, $p=0.099$). However, mercury levels of most individuals increased between 2006 and 2007 among contaminated birds and decreased among reference birds, albeit slightly.

Nine breeding adults from 2005 (the preliminary study) returned to breed in 2006. Five of 39 adults (13%; 1 male, 4 female) on the contaminated site, and four of 29 adults (14%; 1 male, 3 female) on the reference site were returning breeders in 2006 (Table 11). Four nestlings (2 male, 2 female) returned to breed in 2006 on the contaminated site, and one nestling (female) on reference sites (Table 11). There was no difference in mercury levels of individual AHY birds from one year to the next when contaminated and reference samples were combined ($t=1.97$, $p=0.097$), as well

as on the contaminated site alone (t=2.05, p=0.109). Birds on the contaminated site showed no general trend of increase or decrease in mercury levels from 2005 to 2006. There were only two AHY birds from the reference site that had available mercury values for both years, so no statistical analysis was performed, but both birds' mercury levels declined in 2006. Two AHY birds bred in the contaminated sites in all three years, and no AHY birds in the reference sites bred in all three years.

2.2 Blood and feather mercury levels of HY birds 2006-2007

Only nestlings from the first brood were used in these analyses (but see below 2.3 Mercury differences between clutches). Individual values were averaged within a family. Sex was not used as a factor. On the contaminated site, mean blood mercury levels of a brood showed no effect of year, river km or date of capture (Table 12). Feather mercury of a brood showed no effect of river km or time frame, but there was a significant effect of river km (Table 13). Linear regression showed a significant, though weak, negative relationship with river km and HY feather mercury ($F=4.16$, $df=1$, $p=0.052$; $R^2=0.14$, $R^2(adj)=0.11$; Figure 6).

On the contaminated site, feather and blood mercury of a brood were positively correlated ($F=54.49$, $df=1$, $p<0.001$, $R^2=0.70$, $R^2(adj)=0.69$; Figure 7), and mean feather mercury (mean=2.96 ± 1.18 ppm, n=27) was significantly elevated over mean blood mercury (mean=0.0984 ± 0.06 ppm, n=33; paired t-test; $t=-11.91$, $p<0.001$; Figure 8).

Table 9. AHY bluebirds recaptured from 2006 to 2007. A '*' indicates no mercury level for that individual. Status "C" refers to contaminated sites, "R" to reference sites. Mean for C and R is presented ± standard deviation

Band	Sex	Status	River km	Site 2006	Box 2006	Blood Hg 2006	Site 2007	Box 2007	Blood Hg 2007
225170376	F	C	3	WH20	35	0.491	WH20	36	0.605
225170413	F	C	5	GENI	166	0.287	GENI	990	0.731
196141369	M	C	5	GENI	166	1.420	GENI	990	0.839
225170363	M	C	8	DOOM	239	1.040	DOOM	170	1.576
225170437	M	C	8	DOOM	187	*(HY)	DOOM	238	1.762
225170345	F	C	16	CRIM	345	1.270	CRIM	133	1.784
196141095	F	C	18	AUFC	191	1.024	AUFC	164	1.144
196141604	M	C	18	AUFC	228	0.673	AUFC	199	1.298
225170346	M	C	18	AUFC	0	0.781	AUFC	164	1.140
196141335	M	C	22	WAMP	342	2.760	WAMP	341	3.810
225170370	F	C	26	HARR	357	0.887	HARR	358	0.715
Mean ± standard deviation						1.063 ± 0.687			1.400 ± 0.901
196141318	F	R	-27	SCOW	172	0.112	SCOW	176	0.085
225170407	F	R	-2	SRDG	251	0.153	SRDG	251	0.082
225170408	M	R	-2	SRDG	251	0.111	SRDG	251	0.086
225170313	F	R	NA	MOPW	291	*	MOPW	291	0.068
225170427	F	R	NA	NAUC	97	0.075	NAUC	97	0.068
Mean ± standard deviation						0.112 ± 0.032			0.078 ±0.009

Table 10. Adult birds breeding in 2007 on contaminated sites, caught as fledglings in 2006 on contaminated sites. Mean ± standard deviation is only for IHY values of 2006.

Site	Box	Band	Sex	Blood Hg 2007	Blood Hg 2006	Status 2006
AUFC	230	196141720	M	1.49	0.28	IHY-unknown
AUFC	M076	196141737	M	1.29	0.16	IHY-unknown
AUFC	M078	225170454	F	0.23	0.04, 0.25	HY, IHY- known
AUFC	M087	225170484	M	0.55	0.38	IHY-unknown
DESP	M046	225170482	M	0.93	0.62	IHY-unknown
GRCP	9	225170456	M	0.47	0.03, 0.13	HY, IHY- known
Mean ± standard deviation				0.827 ± 0.495	0.303 ± 0.179	

Table 11. AHY bluebirds recaptured from 2005 to 2006. A '*' indicates no mercury level for that individual. Status of "C" refers to contaminated sites, "R" to reference sites. Mean for C and R is presented ± standard deviation, and is only for AHY mercury levels.

Band	Sex	Status	River km	Site 2005	Box 2005	Blood Hg 2005	Site 2006	Box 2006	Blood Hg 2006
196141009	F	C	3	BAPA	100	2.74	BAPA	101	2.98
196141041	M	C	5	GENI	168	2.39	GENI	168	1.03
196141057	F	C	18	AUFC	159	*(HY)	AUFC	225	0.911
196141072	F	C	18	AUFC	194	2.84	AUFC	195	1.82
196141095	F	C	18	AUFC	195	1.3	AUFC	191	1.28
196141604	M	C	18	AUFC	192	0.0501 (HY)	AUFC	228	0.673
196141602	F	C	18	AUFC	192	0.0501 (HY)	AUFC	UNK	0.133
196141080	F	C	35	GRCP	9	1.97	GRCP	12	0.821
196141090	M	C	35	GRCP	9	0.032 (HY)	GRCP	9	0.439
Mean ± standard deviation						2.248 ± 0.630			1.121 ± 0.848
196141002	F	R	-2	SRDG	23	*	SRDG	251	0.235
196141081	M	R	-2	SRDG	22	0.142	SRDG	27	0.075
196141086	F	R	-2	SRDG	22	0.232 (HY)	SRDG	27	0.068
196141042	F	R	-27	SCOW	175	*	SCOW	245	0.104
196141087	F	R	NA	MSWO	82	0.115	MSWO	83	0.064
Mean ± standard deviation						0.129 ± 0.019			0.109 ± 0.072

Between sites, feather mercury was significantly elevated on the contaminated site compared to the reference site (Mann-Whit U: w=880.0, p<0.001; Figure 9). Contaminated HY bluebirds also had significantly higher blood mercury levels than reference HY birds (w=14202.0, p<0.001; see above Figure 4).

Adult birds in both contaminated and reference sites had blood mercury levels significantly elevated over respective nestling levels (contaminated site: w=17157.0, p<0.001; reference site: w=3285.0, p<0.001; see above Figure 4). Adult blood mercury (the average of both parents when possible, otherwise just one parent) on the contaminated site, was significantly correlated with both nestling blood (F=18.12, df=1, p<0.001, R^2=0.39, R^2adj=0.37; Figure 10) and feather mercury (F=23.53, df=1, p<0.001, R^2=0.52, R^2(adj)=0.50; Figure 11).

2.3 Mercury differences between clutches

Nineteen AHY birds were sampled twice during the breeding season (Table 13). There was no significant difference between paired values (t= -0.46, p=0.648). There were only 4 AHY from reference sites that were sampled twice in the season (Table 14). On the contaminated site only, HY blood mercury from clutch 1 did not differ significantly from clutch 2 (paired-t test: t=0.27, p=0.797; Table 15). Feather mercury levels from clutch 1 did not differ significantly from clutch 2 (t=-1.39, p=0.213). However, it should be noted that sample size was small at 7 families, and some families only contained one individual.

Table 12. ANOVA table for HY bluebird blood and feather mercury levels (first clutch only).

	Blood Mercury			Feather mercury		
Factors	DF	F	P	DF	F	p
Year	1	3.32	0.079	1	1.54	0.227
Date of capture	1	0.40	0.532	4	1.94	0.117
River km	1	1.58	0.219	4	4.62	0.042
Error	29			17		

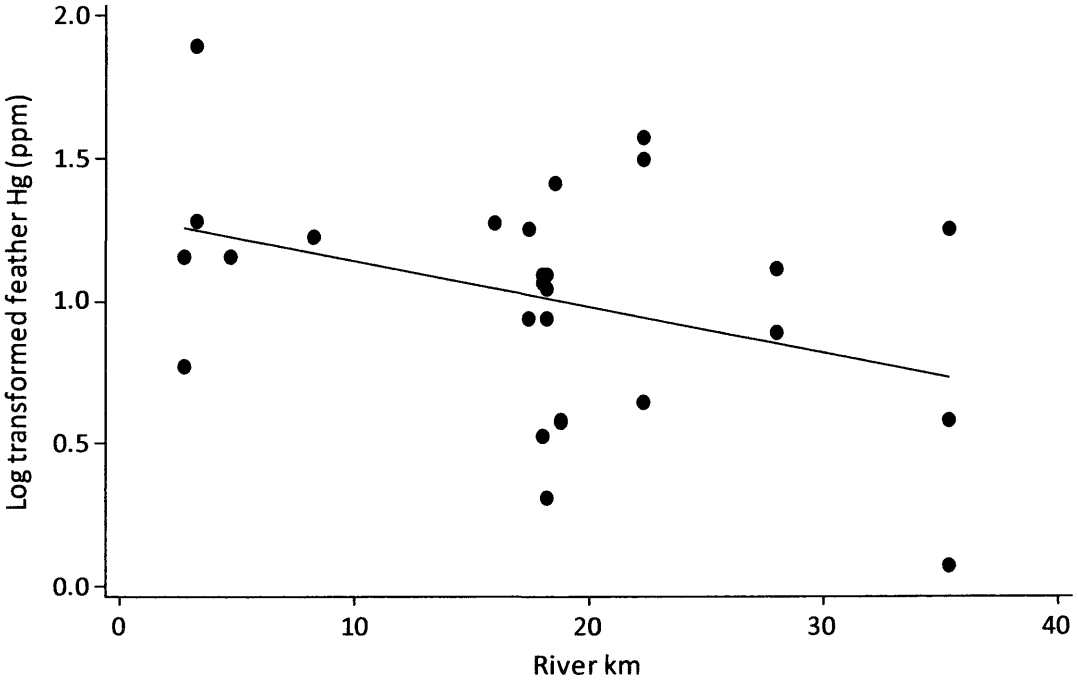

Figure 6. Log-transformed feather mercury level and river km of HY birds 2006-2007 on the contaminated site only (Log HY feather Hg= 1.3-0.01605 river km).

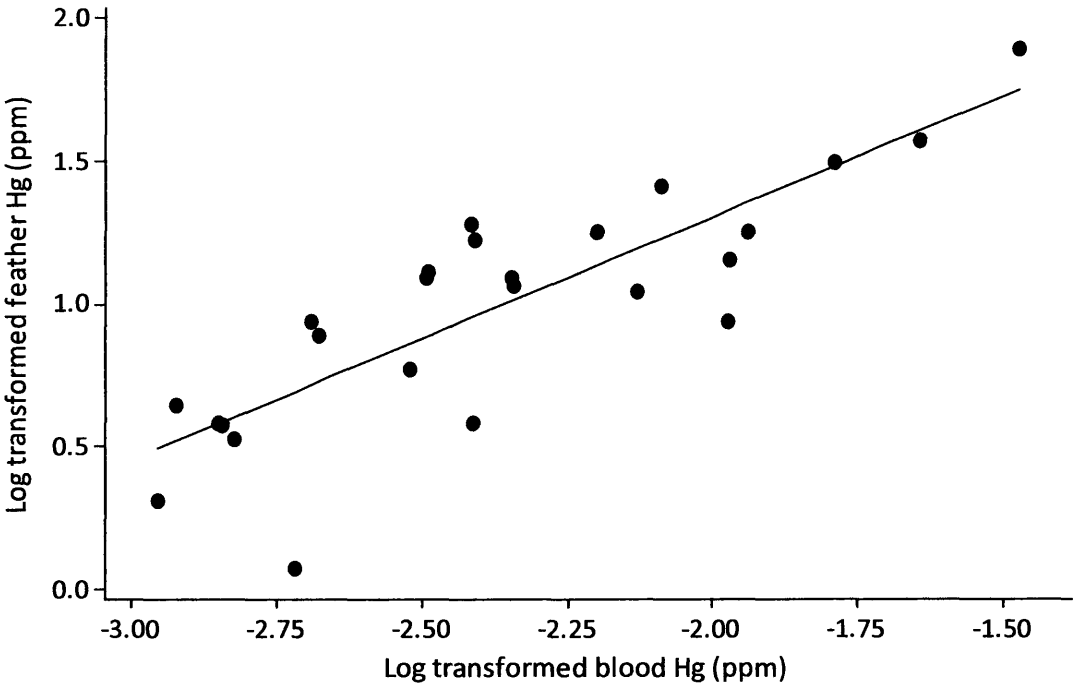

Figure 7. Log-transformed HY blood and feather mercury correlation on the contaminated site only 2006-2007 (log HY feather Hg=3.001+0.8489 log HY blood Hg).

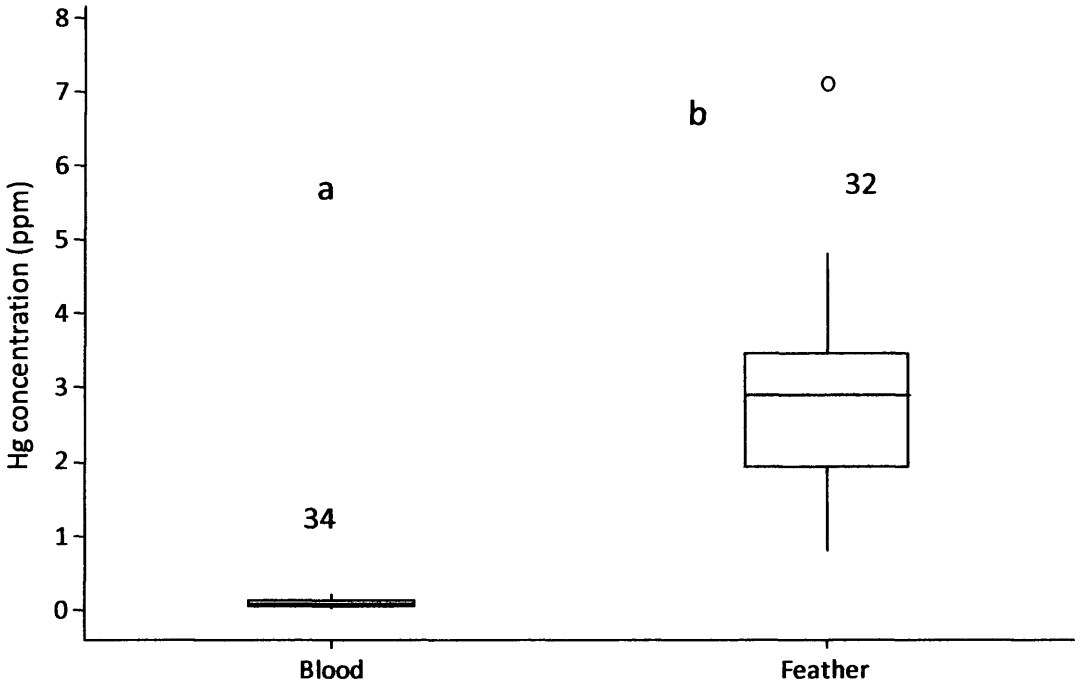

Figure 8. Blood and feather mercury levels (ppm) of HY bluebirds within the contaminated site 2006-2007. Sample sizes above the bars. Different letters indicate a significant difference.

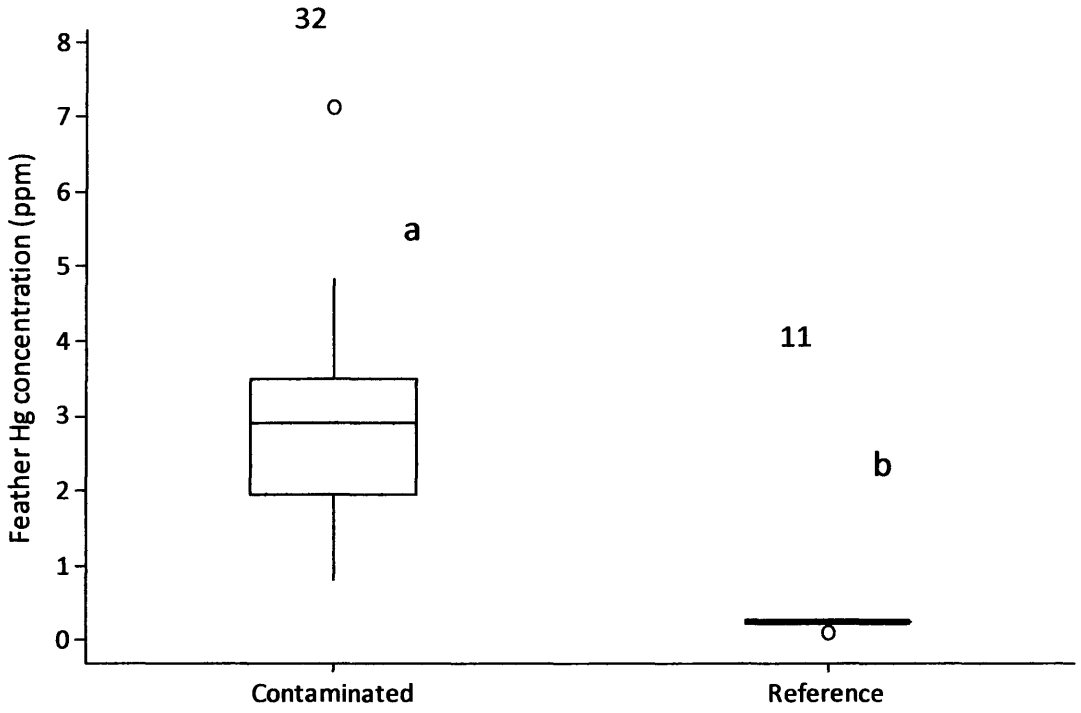

Figure 9. Feather mercury levels (ppm) of HY bluebirds 2006-2007 combined, on contaminated and reference sites. Sample size above the bars. Different letters indicate significant differences.

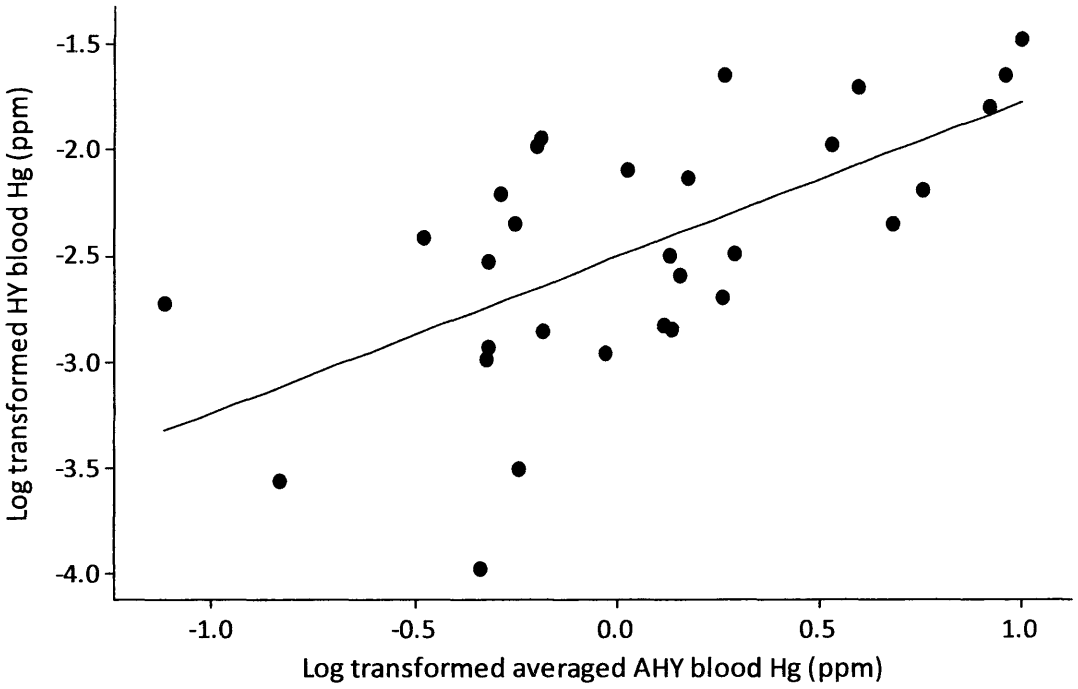

Figure 10. Average AHY blood mercury and brood average blood mercury (log-transformed) on the contaminated site only. AHY blood mercury represents an average of both adults when possible, otherwise just one of the parents (Log HY blood Hg = -2.505+0.7350 AHY average blood Hg).

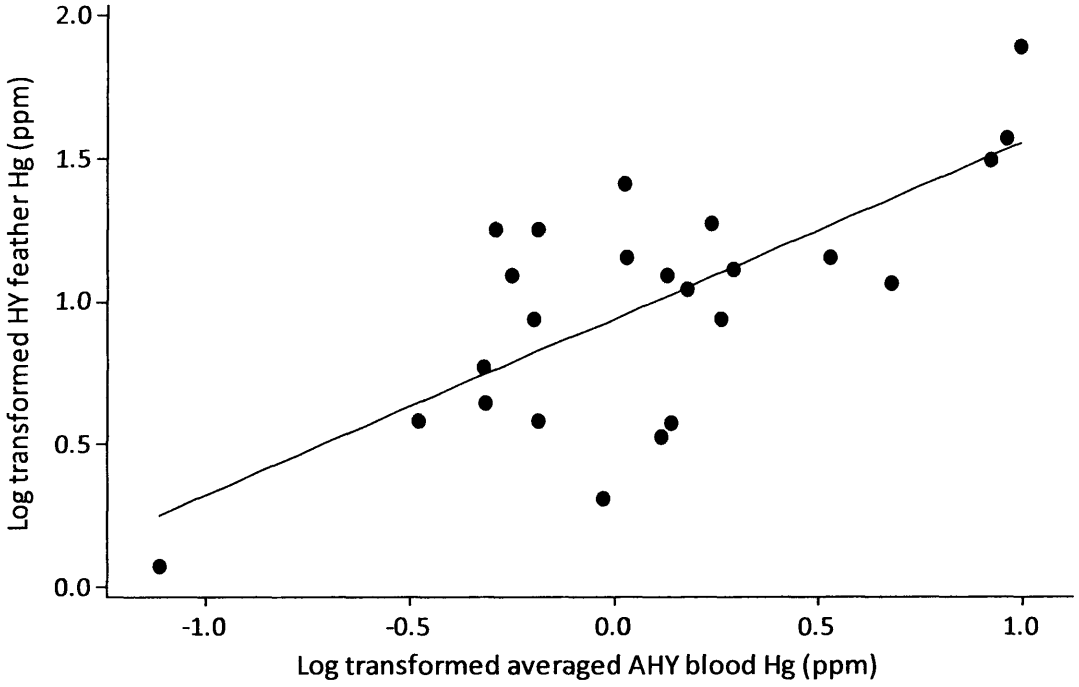

Figure 11. Average AHY blood mercury (average of both parents when possible, otherwise just one of the parents) with HY brood average feather mercury on the contaminated site only (Log HY feather mercury = 0.9387 + 0.6175 AHY average blood Hg).

Table 13. Mercury levels of AHY bluebirds caught twice in a breeding season on the contaminated site.

Year	Clutch	Site	Band	Sex	Clutch 1 Hg(ppm)	Clutch 2 Hg (ppm)
2006	2	AUFC	196141057	F	0.832	0.990
2006	2	AUFC	196141072	F	1.820	1.265
2006	2	AUFC	196141095	F	1.550	1.024
2006	2	AUFC	196141305	F	0.777	1.159
2006	2	BAPA	196141009	F	3.250	2.710
2006	2	DOOM	196141333	F	1.040	1.260
2006	2	DOOM	196141334	F	1.170	0.851
2006	2	GRCP	196141080	F	0.719	1.003
2006	2	GRCP	196141372	F	0.367	0.646
2007	2	GRCP	220185107	F	0.677	0.908
2007	2	AUFC	196141720	M	2.192	1.487
2006	2	BAPA	196141359	M	2.440	3.240
2007	2	CRIM	196141748	M	1.147	1.797
2006	2	DOOM	196141388	M	0.923	1.370
2006	2	GENI	196141041	M	1.030	1.042
2006	2	GRCP	196141090	M	0.289	0.588
2006	2	GRCP	196141398	M	0.522	0.867
2007	2	GRCP	225170456	M	0.477	0.470
2006	2	WH20	196141304	M	0.829	0.779
Mean					1.161	1.235

Table 14. Mercury levels of AHY bluebirds caught twice in a breeding season on reference sites

Year	Clutch	Site	Band	Sex	Clutch 1 Hg (ppm)	Clutch 2 Hg (ppm)
2007	2	SRDG	225170408	M	0.09	0.11
2006	2	MWHI	196141375	M	0.07	0.16
2007	2	276B	220185106	M	0.11	0.09
2006	2	MOPW	193194038	F	0.10	0.13
Mean					0.09	0.12

Table 15. Mean blood (above) and feather (below) mercury levels (ppm) of HY bluebirds from the first and second clutch on the contaminated site.

Year	Box	Site	n Clutch 1	Clutch 1 mean	n Clutch 2	Clutch 2 mean
2006	9	GRCP	4	0.660	3	0.280
2006	12	GRCP	4	0.058	3	0.070
2006	101	BAPA	4	0.230	1	0.104
2006	191	AUFC	5	0.059	2	0.340
2006	195	AUFC	3	0.096	2	0.114
2006	199	AUFC	5	0.096	4	0.048
2007	9	GRCP	2	0.029	2	0.019
Mean blood				0.175		0.139
2006	9	GRCP	3	1.074	3	1.451
2006	12	GRCP	1	1.470	2	2.251
2006	35	WH20	4	3.178	4	2.057
2006	101	BAPA	4	6.645	4	7.671
2006	191	AUFC	1	1.700	3	2.398
2006	195	AUFC	3	2.909	2	4.054
2006	199	AUFC	5	2.982	4	2.935
Mean feather				2.851		3.260

3.0 Telemetry

In 2006, transmitters were attached to 36 nestlings of 13 first broods between 5 May and 15 June. Five transmitters were attached to nestlings of two second broods from 3-7 July. Five transmitters were added onto captured fledglings that were previously banded as nestlings but not already fitted with transmitters. Five transmitters were used to replace transmitters with battery failure in order to extend tracking time. A total of 46 individuals had transmitters at some point during the season (see Appendix B). Birds were regularly located on the natal site. At times, fledgling were located as much as 800 m from the natal box.

3.1 Fledgling fate

Of birds initially fitted with transmitters, 15 were found dead (Table 16). Five (one brood) were dead pre-fledging in the nest box, apparently due to abandonment or possible death of the parents. Four were found after an unusually violent storm, under a tall tree that had snapped in half. The birds were observed perching in this cluster of trees often, and had probably been roosting during the storm. Six birds were predated; the transmitter and feathers (and legs in some instances) were found together on the ground. All of these birds originated from the first clutch.

An anecdotal estimate of mortality is 0.30 (14/46), including only birds that were fitted with transmitters. However, this is likely an underestimate. Four signals were lost just after the storm event, probably due to death in the storm, but possibly the result of battery failure or dispersal out of range. Six other signals were lost in late June. These birds may have died as well, but more likely they moved off-site

and/or the batteries of the transmitter died. Several of these late-disappearing birds were confirmed to be alive by re-sighting after signals ceased to be detected. Two transmitters died soon after the birds fledged, likely due to mortality or battery failure, as the birds would not have been able to move far enough away at that point. If all of the missing birds died, mortality was 0.57 (26/46).

The identified dead IHY birds had a mean of 0.120 ppm blood mercury as nestlings (Table 17). The IHY birds that presumably survived had a mean mercury level of 0.083 ppm as nestlings, ranging from 0.024 to 0.243. Five of the 15 dead birds had been caught previously as IHYs, with mercury levels ranging from 0.3 to 1.18 ppm (Table 17). The average parent mercury value for the birds that died was 1.101 ppm, and for the survivors was 1.096 ppm.

3.2 Trapping 2006

In 2006, 46 individuals were caught, and of these 20 did not have transmitters attached previously. Individuals were caught from the first (n=31) and second (n=15) nesting attempts, belonging to 12 families, including from 1- 4 members of each brood (Table 18). Once one fledgling was caught, others would often be attracted to its vocalization as it was being removed from the net, so multiple birds were caught at a time. Unbanded fledglings (n=45) were also caught in this way.

Of all 46 known birds caught at least one time, 24 individuals were caught at least twice, eight were caught at least three times, and only one individual was caught four times. Attempts at capturing individual fledglings were timed approximately every two weeks; however, due to difficulty in trapping, the time between captures

Table 16. IHY bluebirds found dead in 2006, with mercury levels as nestlings. "Age" refers to the age of the bird when found. A "*" means that bird didn't have a transmitter, but was identified via remains of leg bands.

Date Banded	Site	Box	Band	Age	Date of death	Hg (ppm) as HY	Cause of death
23-Apr	GRCP	14	1961-41313	19	12-May	0.144	Unknown (HY)
23-Apr	GRCP	14	1961-41314	19	12-May	0.131	Unknown (HY)
23-Apr	GRCP	14	1961-41315	19	12-May	0.125	Unknown (HY)
23-Apr	GRCP	14	1961-41316	19	12-May	0.116	Unknown (HY)
23-Apr	GRCP	14	1961-41317	19	12-May	0.204	Unknown (HY)
29-Apr	AUFC	191	1961-41336	59	27-Jun	0.059	Storm
29-Apr	AUFC	195	1961-41342	59	27-Jun	0.109	Storm
27-Apr	AUFC	199	1961-41347	61	27-Jun	0.108	Storm*
27-Apr	AUFC	199	1961-41348	61	27-Jun	0.089	Storm
29-Apr	AUFC	225	1961-41351	23	22-May	0.071	Predator
3-May	BAPA	101	1961-41380	45	17-Jun	0.236	Predator
1-May	GENI	168	1961-41385	20	21-May	NA	Predator
1-May	GENI	168	1961-41387	30	31-May	NA	Predator
7-May	AUFC	226	2251-70338	51	27-Jun	0.048	Predator
30-May	AUFC	228	2251-70352	32	1-Jul	0.113	Storm

Table 17. Mercury levels (ppm) of five IHY birds that were found dead, as nestlings and from a previous capture as an IHY. "Age" is the age at which the bird was captured live as an IHY. "Feather growth" corresponds to the stage of feather growth at the live IHY capture. A '*' indicates an average of 2 captures.

Site	Box	Band	Age at death	Hg as HY	Hg as IHY	Age	Feather growth	Mean Hg of parent(s)
AUFC	191	1961-41336	59	0.059	0.300*	36	Waning	0.994
AUFC	195	1961-41342	59	0.109	0.702	38	Waning	1.738
AUFC	199	1961-41347	61	0.108	0.697	55	None	0.968
AUFC	199	1961-41348	61	0.089	0.532	42	Waning	0.968
BAPA	101	1961-41380	45	0.236	1.180	33	None	2.910
Mean				0.120	0.682	40.8		1.653

varied. The time between fledging and first capture ranged from 9-57 days, between 1st and 2nd capture ranged from 6-32 days, between 2nd and 3rd capture ranged from 8-22 days, and from 3rd to 4th capture was 15 days.

There were a total of 79 captures, including recaptures of the same individual. Birds were caught in various locations, sometimes in the same net several weeks later. The furthest site of capture was just over 450 m from the edge of the river; 41 captures were within 100 m of the river, 72 were within 200 m (see Appendix C for maps of capture locations). Distance to the river was not included in any further analysis, because, with birds making frequent long flights, exact site of capture did not indicate the proximity of feeding areas to the river (pers. obs.).

Table 18. Number of IHY individuals caught per site in 2006

Site	River Km	Number IHY	Number families
WH20	2.7	3	1
BAPA	3.2	4	1
GENI	4.7	1	1
AUFC	18	25	7
GRCP	35.4	13	2

3.3 Trapping 2007

In 2007, when my goal was to catch birds later in the season, 12 individuals were caught and no transmitters were used. Nine individuals originated from the first

brood, and three from the second. Individuals belonged to only six families. Five unbanded birds were caught in addition.

Four birds were caught twice after fledging. Time between fledging and the first capture ranged from 16-55 days, and between the 1st and 2nd capture ranged from 15-31 days. In general, time between captures ranged from 15-55 days. There were a total of 17 captures, 16 within 100 m, and all within 200 m from the river. All birds were captured at AUFC. There was no difference in HY or AHY mercury between years, so I combined IHY data from both years for all analyses.

4.0 Feather growth categories

Of birds with known nest sites, a total of 58 birds were sampled during the "nestling" period, between the ages of 10 and 17 days. A total of 28 birds were re-caught in the "waning" period, between the ages of 27 and 41 days; one bird was caught twice in this phase. A total of 44 individuals were re-caught in the "none" period, between 32 and 80 days; nine birds were caught twice in this phase. A total of 15 birds were caught during the "molt" period between the ages of 43 to 106 days; four birds were caught twice during this period. There was some overlap in the ages because birds from the second brood started molting at a younger age than first brood birds. Of birds with an unknown origin, two were caught in the "waning" period, 31 during the "none" period, and 14 during the "molt" period.

4.1 Blood mercury levels with feather growth category

For birds banded as nestlings, individuals caught twice within a growth category were averaged (mercury levels, age, dates). IHY birds that had not been

banded as nestlings were included in the analysis of mercury level and feather growth stage because whether birds came from the first or second clutch and actual age of the bird were not factors in this analysis. Average mercury levels rose until the "none" stage, and then dropped back down during "molt" (Table 19). For unknown origin fledglings, blood mercury levels decreased with growth stage (Table 19). However, the mean blood mercury levels were within the same range as known fledglings.

To analyze mercury levels with feather stage, I used a linear mixed effects model with log-transformed blood mercury as the response variable and feather growth stage and river km as explanatory terms. There was no effect of river km (-0.021, $p=0.190$). There was a significant linear and quadratic effect of feather growth category, increasing until the no growth stage and then decreasing (lin:0.615, $p<0.001$; quad:-1.060, $p<0.001$; Figure 12).

Although the unbanded IHY birds were feeding and flocking together with birds that were banded as nestlings on-site, they may have started with lower mercury levels (as nestlings). They also may have been spending time on their own natal territory, which was likely outside of 50 m from the river, possibly lessening their overall exposure to mercury. To ensure that the pattern seen in blood mercury was not a result of possible lower mercury levels of unknown birds, this same analysis was done with only the IHY birds that were banded as nestlings on the study site. There was no effect of river km (-0.026, $p=0.064$), although this factor was closer to significance than the previous analysis. There was a significant linear, quadratic, and

cubic effect of feather growth category; increasing until the no growth stage, then decreasing (lin:0.590, $p<0.001$; quad:-1.12, $p<0.001$; cub:-0.178, $p=0.02$; Figure 13).

In a previous model, distance of capture from the river was included as an explanatory term along with river km and feather growth category. This analysis was for known-origin IHY birds only. Distance from the river did not have a significant effect on blood mercury levels (-0.001, $p=0.085$; Figure 14), so was eliminated for the final model (see above). It is presented here to illustrate that mercury levels were not decreasing in the later growth stages as a function of the birds moving from the river. However, in this model, river km did have a significant effect on blood mercury (-0.026, $p=0.042$). Feather growth category also had a significant effect on blood mercury (lin:0.623, $p<0.001$; quad:-1.18, $p<0.001$; cub:-0.202, $p=0.01$).

Table 19. Mean blood mercury level (ppm) for the four feather growth stages for known and unknown IHY bluebirds.

	Known				Unknown			
	N	Mean	St Dev	Range	N	Mean	St Dev	Range
Nestling	56	0.08	0.05	0.02 - 0.24	NA	NA	NA	NA
Waning	28	0.28	0.16	0.05 - 0.70	1	0.31	NA	NA
None	44	0.52	0.36	0.12 - 1.92	32	0.29	0.27	0.06 - 1.57
Molt	11	0.20	0.09	0.07 - 0.31	14	0.16	0.10	0.02 - 0.42

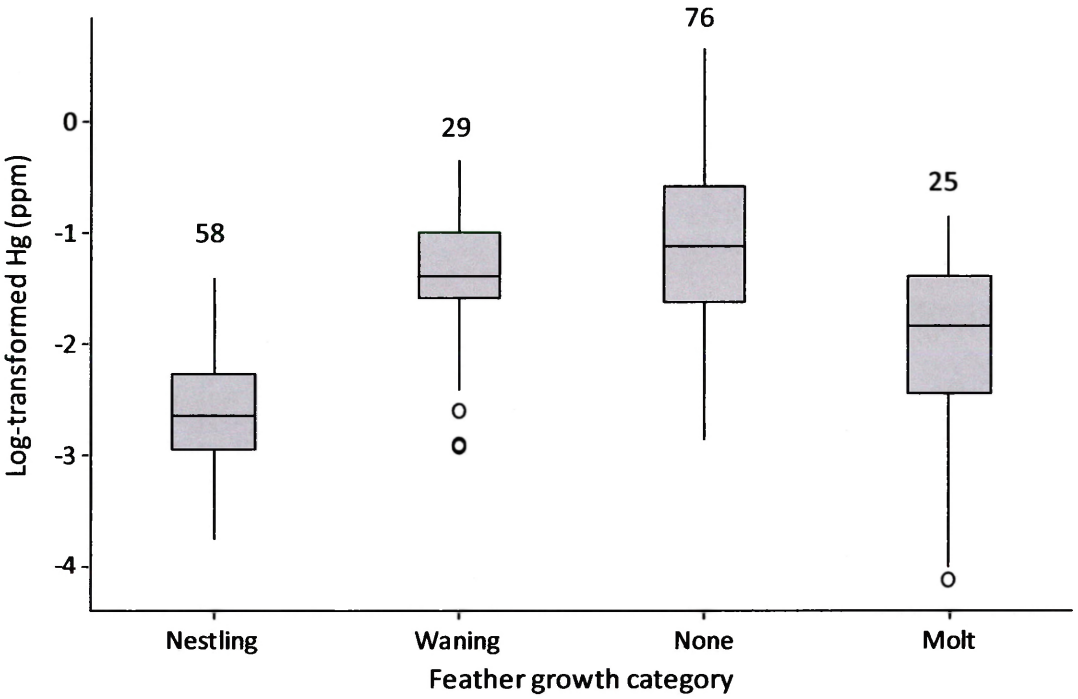

Figure 12. Log-transformed IHY bluebird blood mercury (ppm) in the four feather growth stages, including known and unknown origin birds. Samples sizes are above the bars.

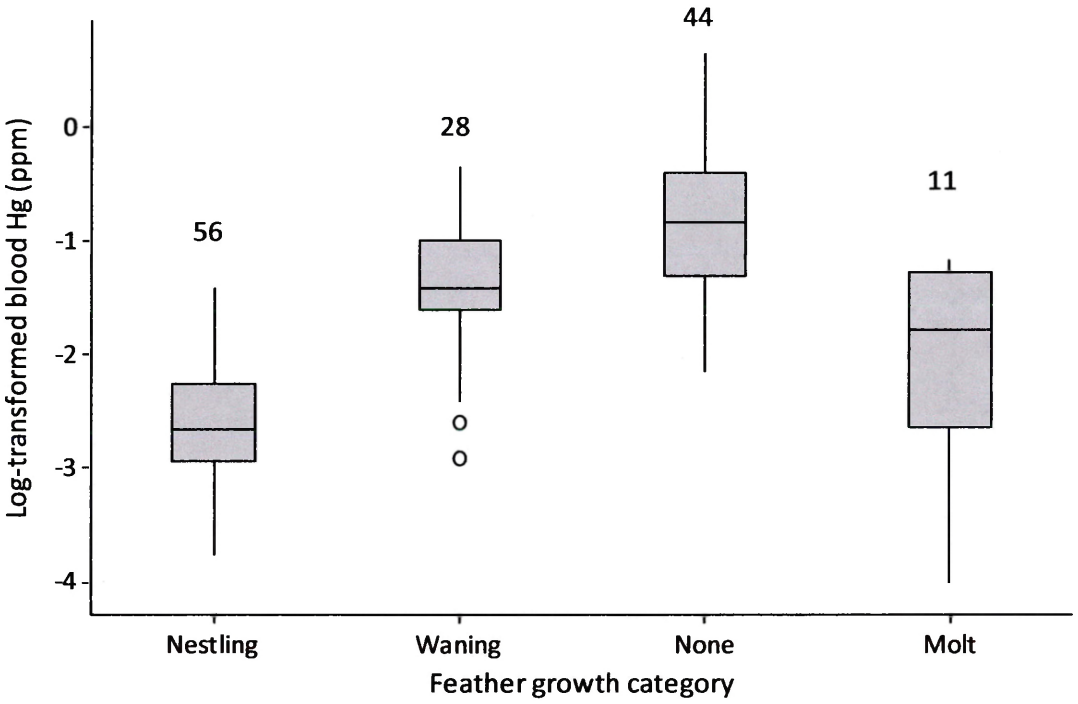

Figure 13. Log-transformed IHY bluebird blood mercury (ppm) in the four feather growth stages, only known origin birds. Samples sizes are above the bars

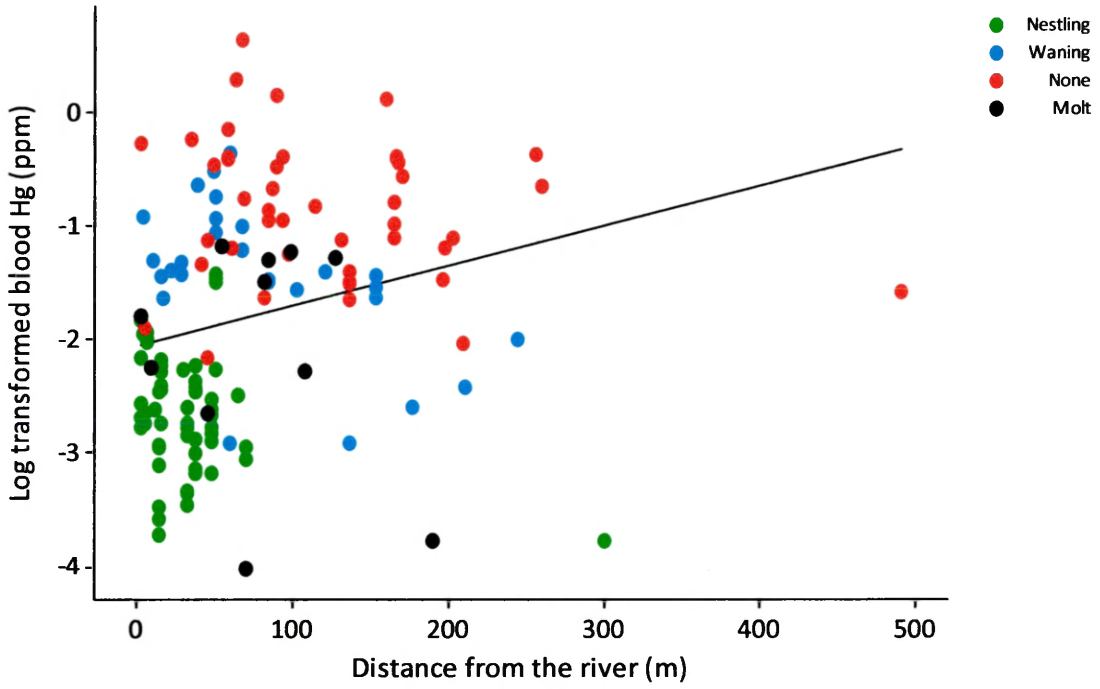

Figure 14. Log-transformed IHY bluebird blood mercury levels at varying distances from the river. Both clutches are represented. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

4.2 Feather mercury levels with feather growth category

Feathers of fledgling birds reflect mercury exposure as nestlings. Seven of the unknown fledglings had feather mercury levels above 0.99 ppm, which would be comparable to the birds nesting within 50 m of the river (Table 20). These birds could have nested in a natural cavity on site, or in an unmonitored box on private property near the site. Mean feather mercury level of HY birds on reference sites was 0.234 ± 0.048 ppm (range 0.107-0.278). The unknown IHY birds had generally higher feather mercury levels (mean and range) than reference nestlings (Table 20). There were no nestling mercury levels for unknown birds, and only two birds were sampled during the waning period (Table 20). I compared the feather mercury of reference nestlings to that of unknown IHY birds during feather stages of "molt" and "none" to compare exposure levels between the two groups. Feather mercury of unknown IHY birds was significantly elevated over reference nestlings (molt: $w=89.0$, $p=0.0089$; none: $w=101.0$, $p=0.0001$). I also compared the feather mercury levels between known and unknown IHY birds during the feather growth stages of "none" and "molt". Known IHY birds had significantly elevated feather mercury in both the "none" stage ($w=589.0$, $p<0.001$) and the "molt" stage ($w=81.0$, $p=0.0003$). Because the feather mercury of unknown IHY birds was lower than known contaminated birds, unknown birds were not included in feather mercury analysis.

The same terms were used in analyzing feather mercury as with blood mercury (river km and feather growth stage). Feather rank had a weak positive effect

on feather mercury (lin:0.175, p=0.0017; Figure 15). River km had a negative effect on feather mercury (-0.316, p=0.0022; Figure 16).

Table 20. Mean feather mercury levels (ppm) of four feather growth stages for both known and unknown IHY bluebirds. No unknown birds were sampled as nestlings.

	Known				Unknown			
	N	Mean	St Dev	Range	N	Mean	St Dev	Range
Nestling	45	2.93	1.53	0.95 - 7.72	NA	NA	NA	NA
Waning	26	2.86	1.16	1.23 - 5.61	2	0.29	0.02	0.28 - 0.31
None	42	3.52	2.28	1.17 - 10.39	31	0.80	0.87	0.20 - 4.51
Molt	9	3.31	1.76	1.21 - 6.08	12	0.56	0.47	0.21 - 1.95

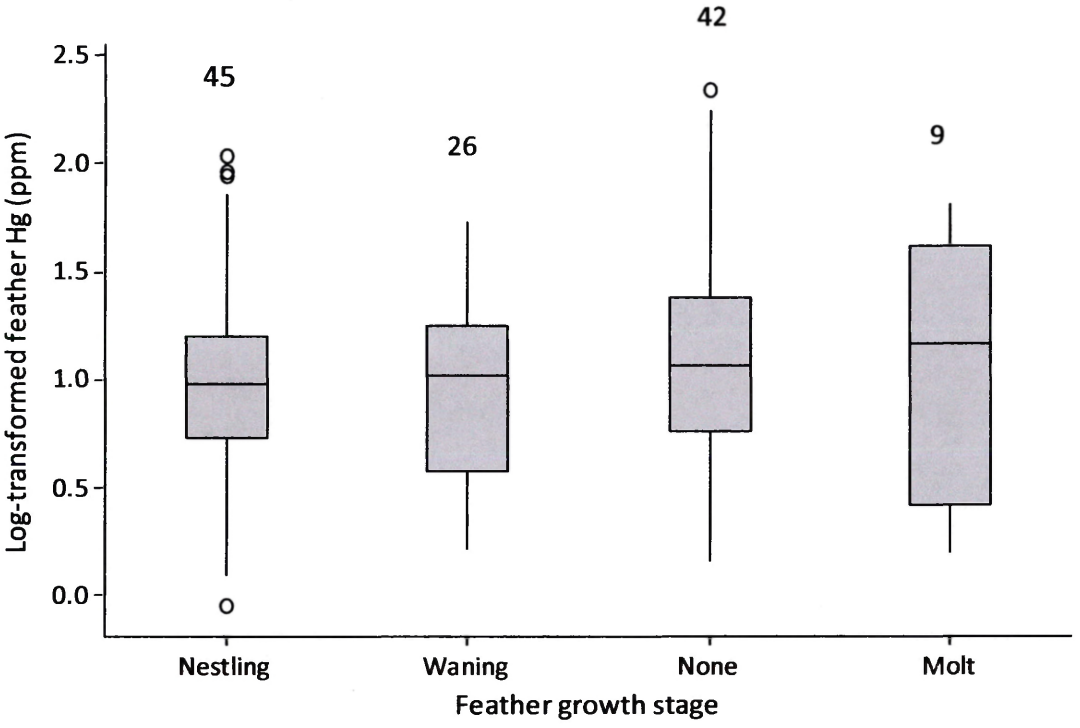

Figure 15. Log-transformed feather mercury (ppm) of IHY bluebirds at each growth stage. Sample sizes above bars.

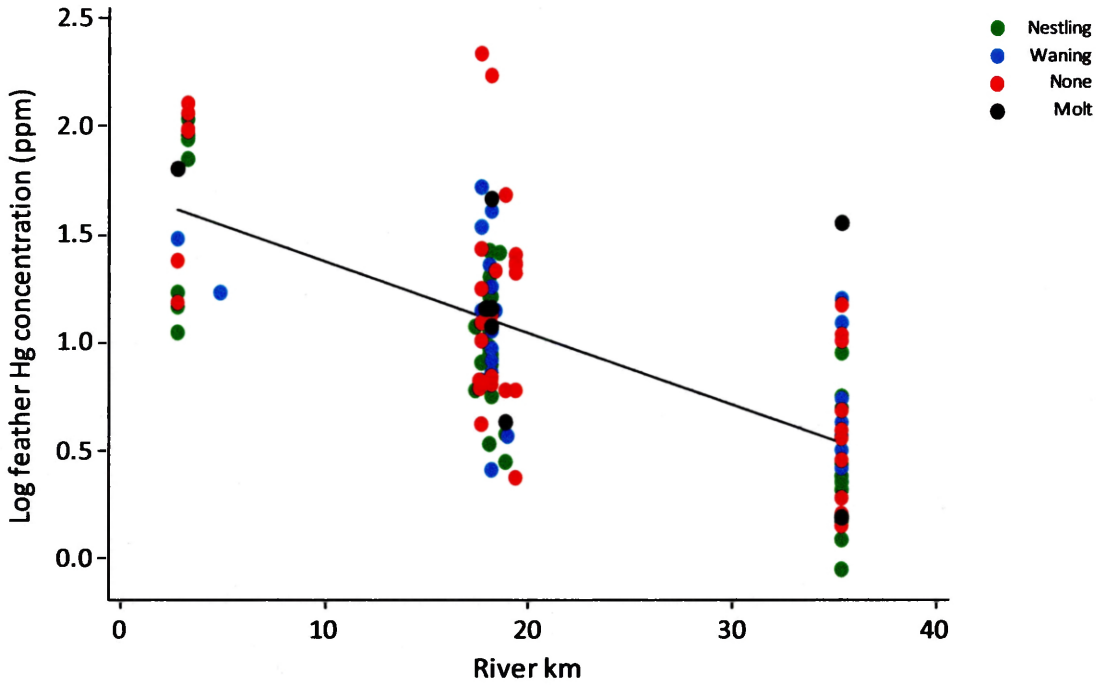

Figure 16. Log-transformed IHY feather mercury levels along river km. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

4.3 Stable isotope values with feather growth category

A subset of IHY birds of a known origin were analyzed for isotope ratio. Isotopic ratios of nitrogen had a significant positive linear relationship with feather growth category (lin:1.54, $p<0.001$; Figure 17). River km had a negative linear effect on $\delta^{15}N$ values (lin:-0.06, $p=0.046$; Figure 18).

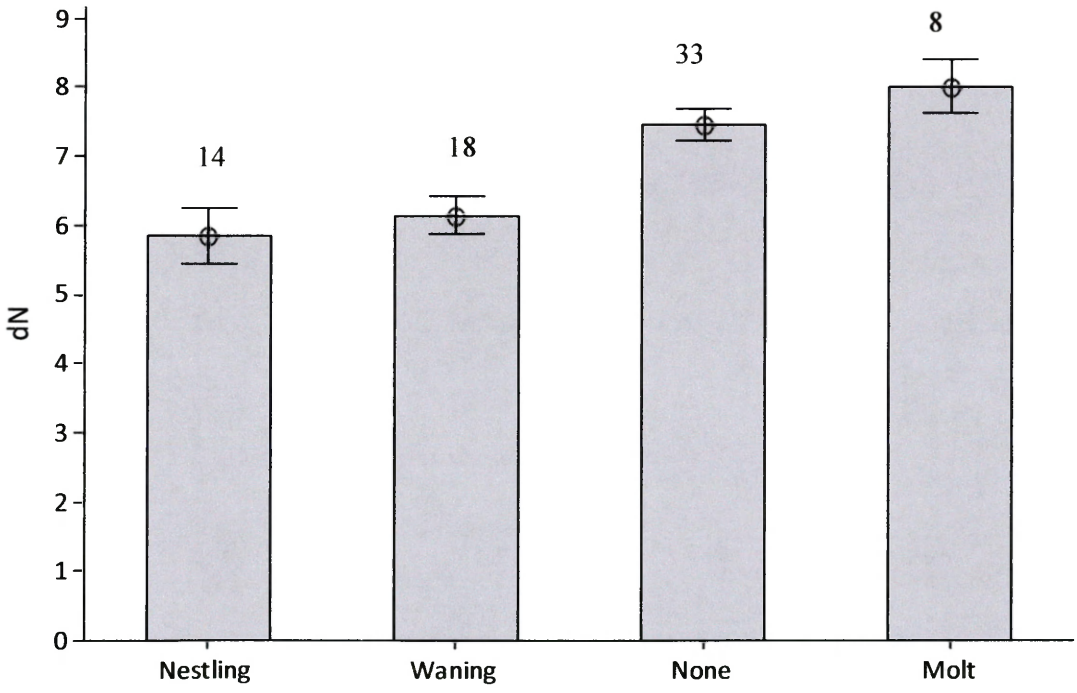

Figure 17. $\delta^{15}N$ (or dN) of IHY bluebirds of different feather growth categories. Sample sizes are above the bars.

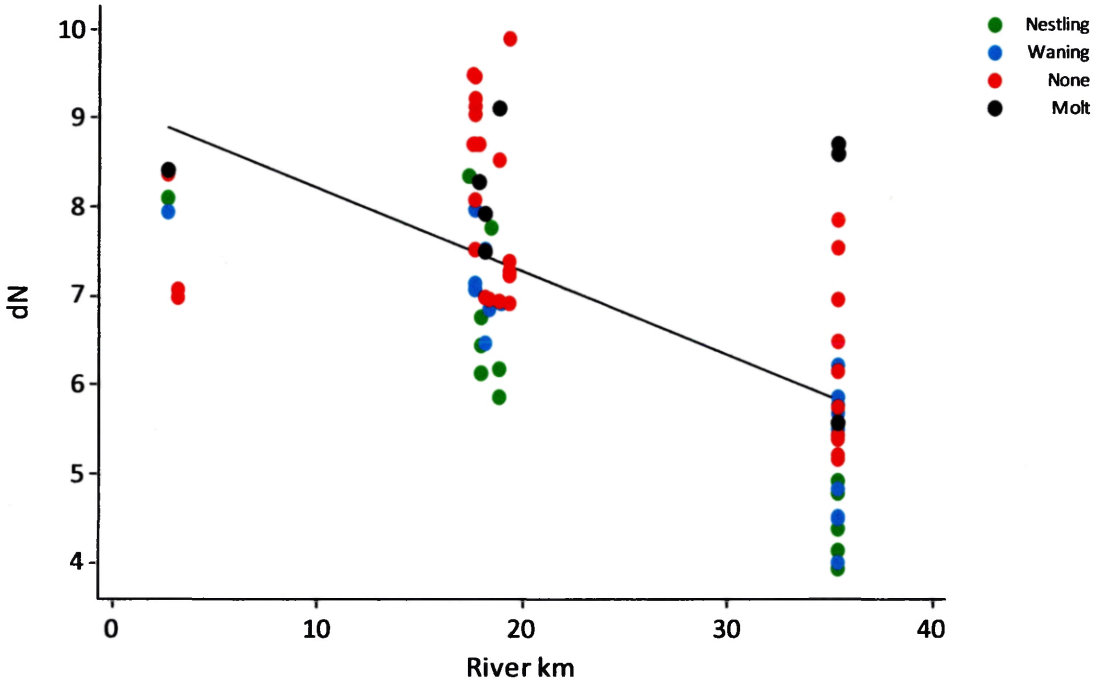

Figure 18. $\delta^{15}N$ (or dN) of IHY bluebirds at different distances from the source. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

5.0 Comparisons with age

I also used age as an explanatory term in order to see how feather growth stage related to the actual age of the bird. In this analysis, only birds that had been monitored as nestlings were included, because exact age was crucial to analysis. All individuals were from nest boxes within 50 m except one, which nested in a box at approximately 300 m from the river. Clutches were analyzed both combined and separately for blood mercury, feather mercury and $\delta^{15}N$.

5.1 Blood mercury levels with age

Log-transformed blood mercury of the combined first and second brood showed a significant quadratic relationship with age (lin:6.908, $p<0.001$, quad:-5.235, $p<0.001$; Figure 19), and no significant relationship with river km (lin:-0.026, $p=0.104$; Figure 20).

Second-clutch birds start molting at a younger age than first-clutch birds, which would explain why some molting birds (black circles) were less than 60 days of age (Gowaty and Plissner 1998). To examine this difference in timing of molt, each clutch was also examined separately for blood mercury levels. Birds from the first clutch showed a significant relationship with age (lin:5.79, $p<0.001$, quad:-5.95, $p=0.0017$; Figure 21) and with river km (lin:-0.024, $p=0.0188$; Figure 22). Birds from the second clutch also had a significant relationship with age (lin: 2.927, $p<0.001$; quad:-1.68, $p=0.0017$; Figure 23), but their blood mercury did not vary significantly with river km (lin:-0.005, $p=0.8290$; Figure 24).

Because in some analyses (see above) river km had a significant effect on blood mercury levels, blood mercury within each feather growth stage was correlated with river km. Doing the analysis within each growth stage allowed the effect of river km to be analyzed without the effect of different feather growth stages. Within each feather growth stage, except during molt, blood mercury levels show a general decrease with distance, just as in adult mercury levels. "Nestling" and "none" were significantly negatively related to river km, while "waning" and "molt" had no relationship (Nestling: R^2=0.32, p<0.001; Waning: R^2=0.08, p=0.128; None: R^2=0.17, p=0.005; Molt: R^2=0.07, p=0.429; Figure 25).

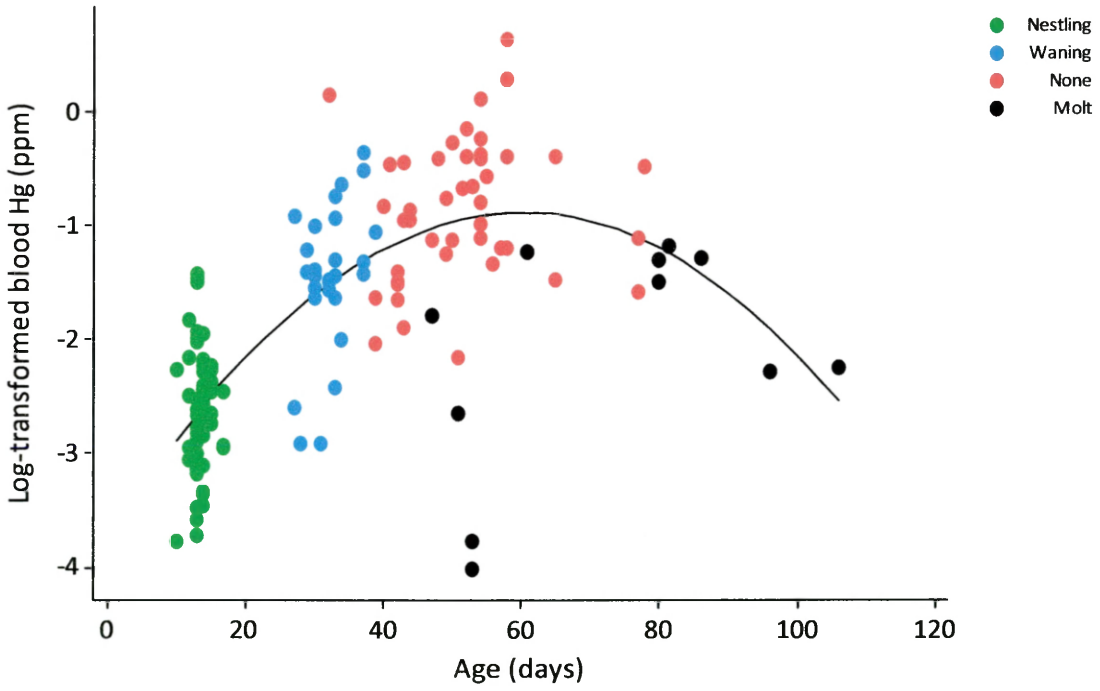

Figure 19. Log-transformed IHY blood mercury plotted against age (days), includes both clutches. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

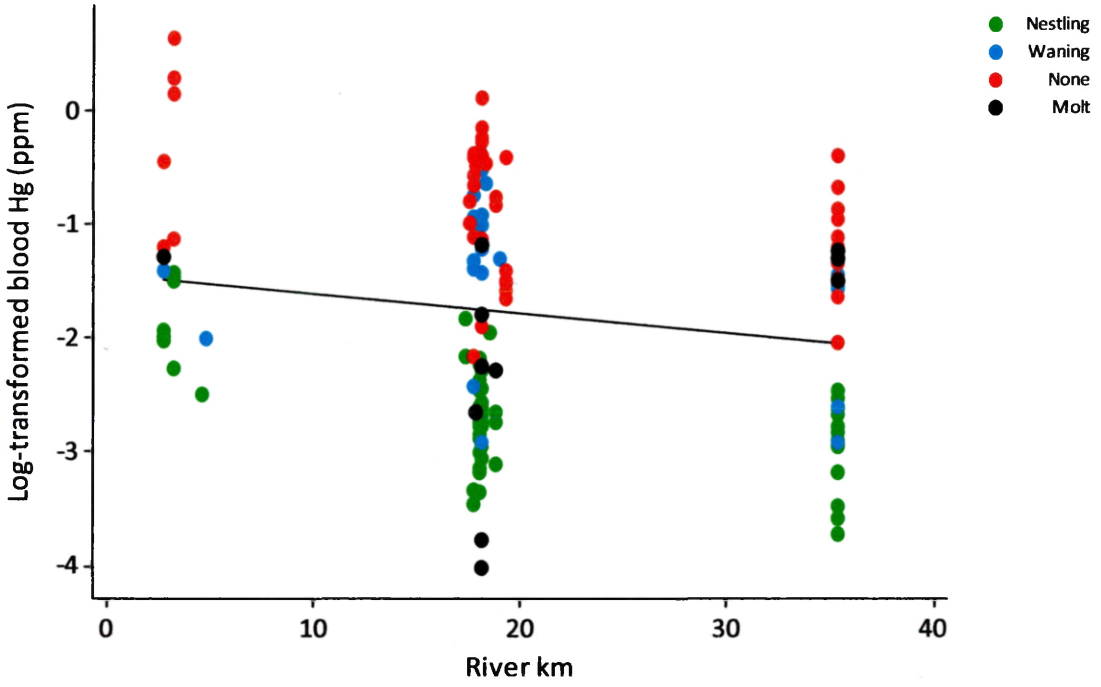

Figure 20. Log-transformed IHY blood mercury plotted against river km, includes both clutches. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

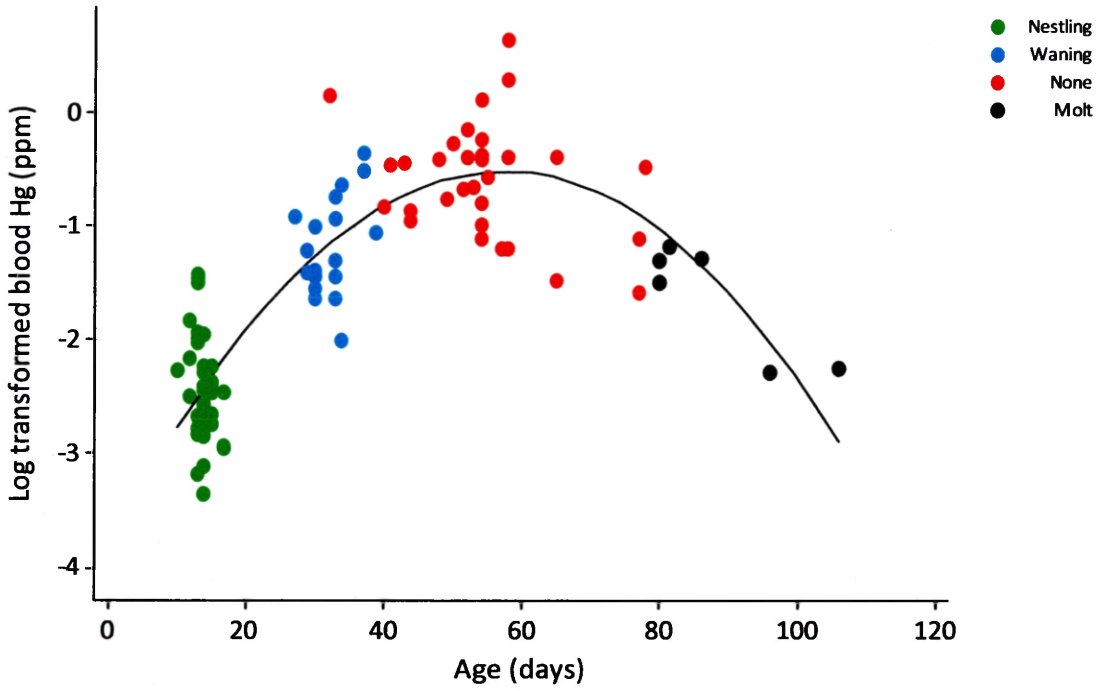

Figure 21. Log-transformed IHY blood mercury plotted against age, only birds that originated from the first clutch. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

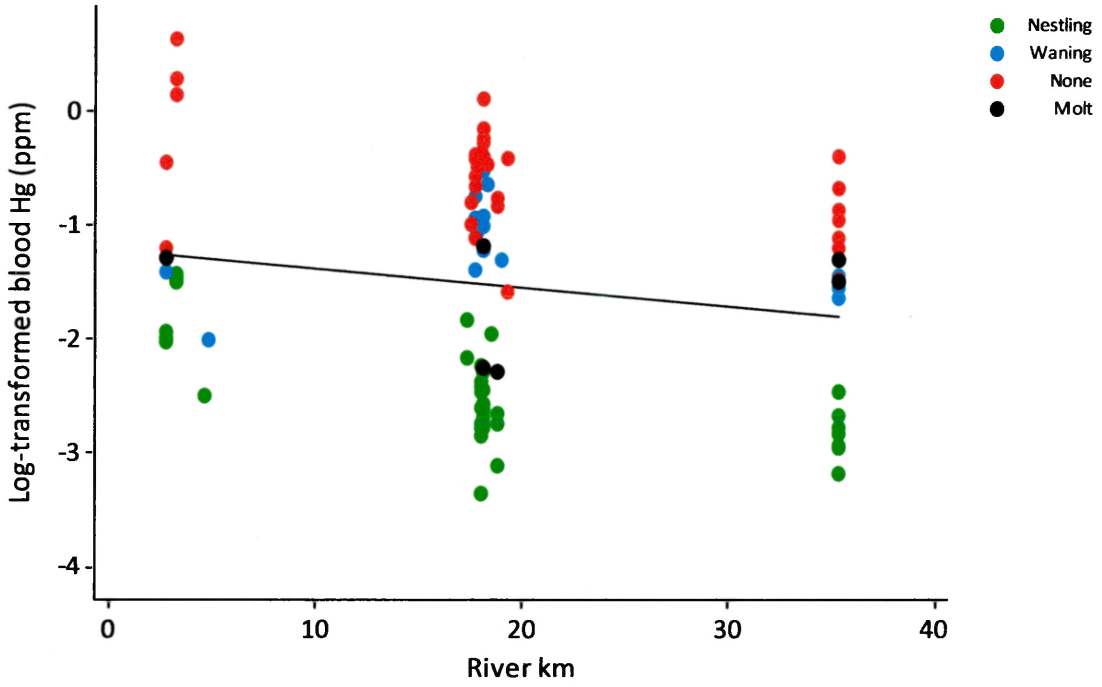

Figure 22. Log-transformed IHY blood mercury plotted against river km, only birds that originated from the first clutch. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

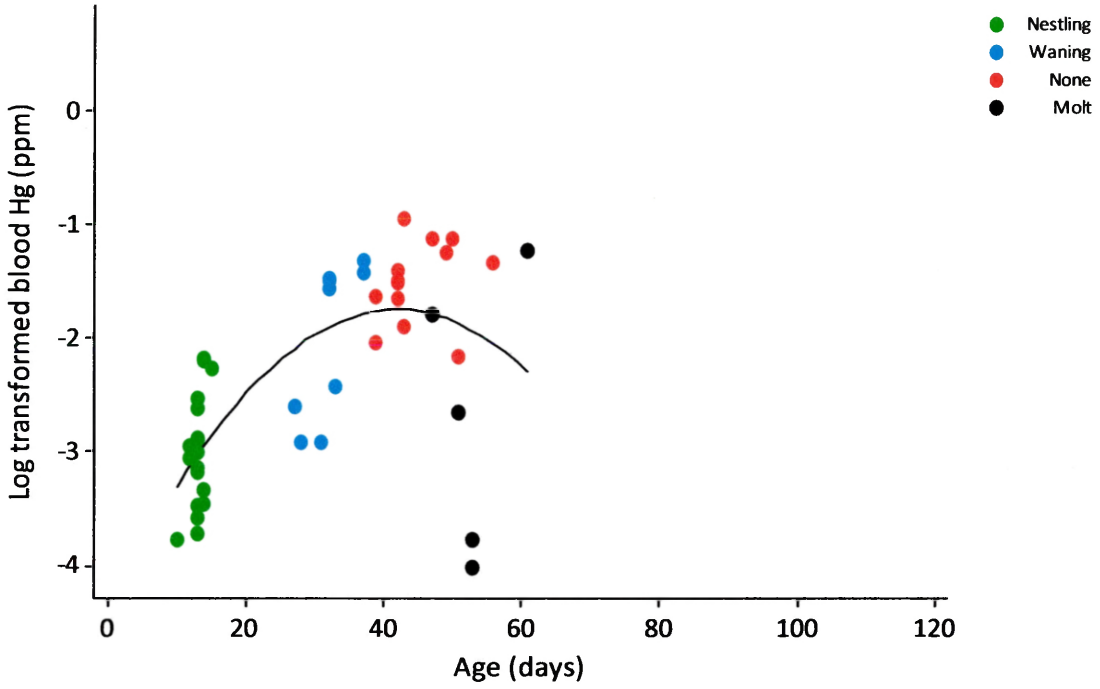

Figure 23. Log-transformed IHY blood mercury plotted against age, only birds that originated from the second clutch. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

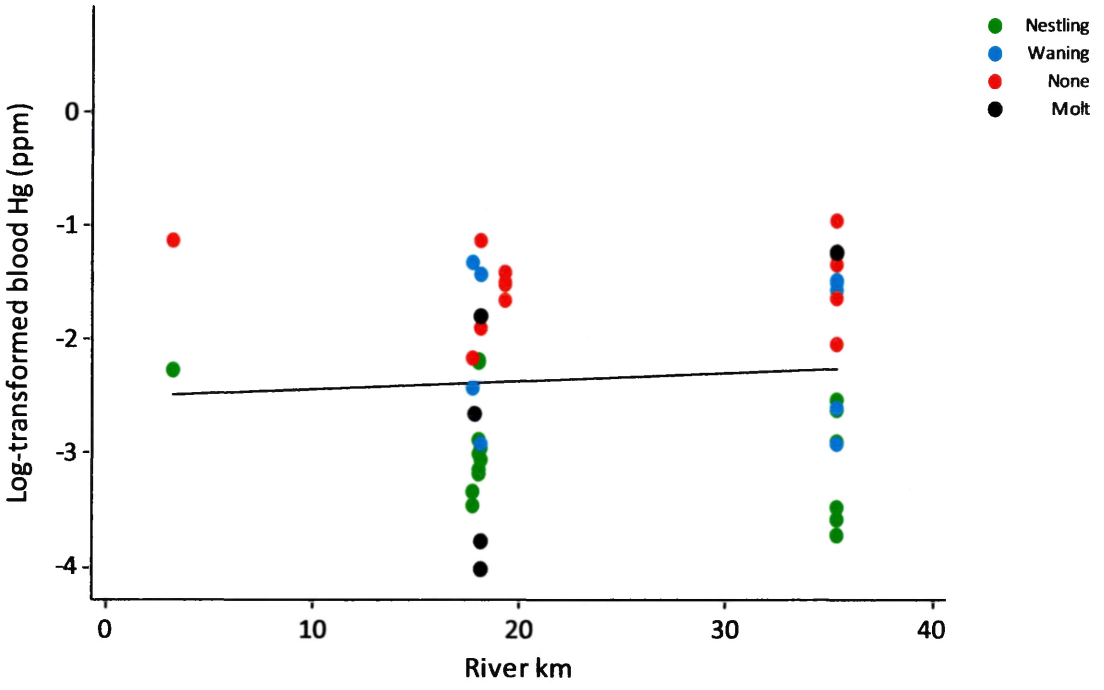

Figure 24. Log-transformed IHY blood mercury plotted against river km, only birds that originated from the second clutch. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

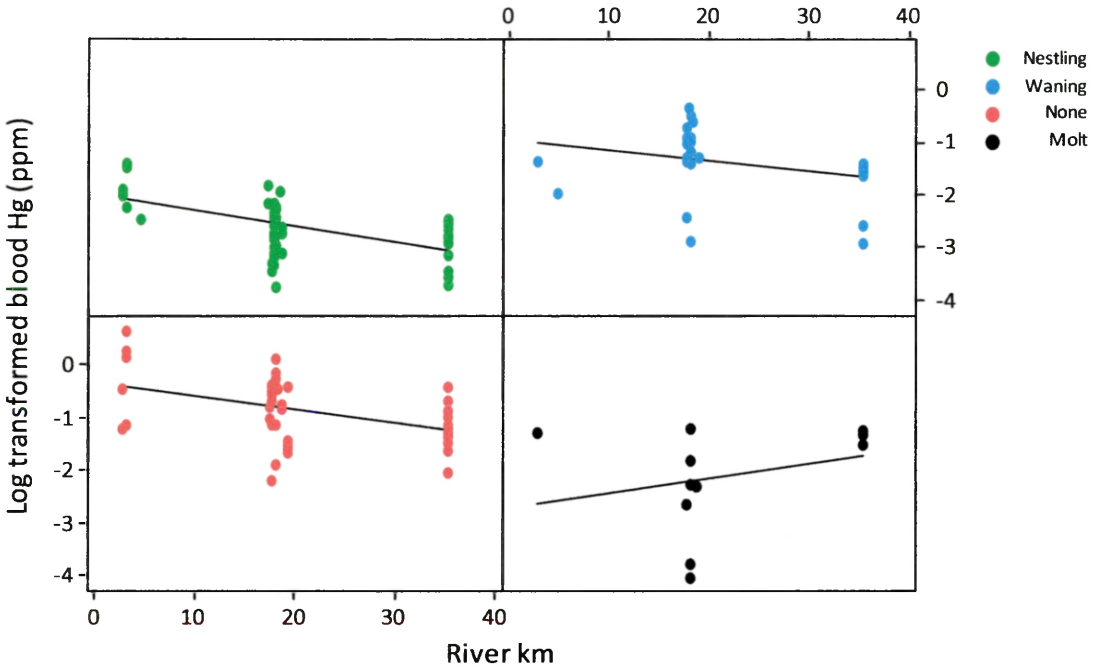

Figure 25. Log-transformed IHY blood mercury and river km within each growth category, includes birds from both clutches. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

5.2 Feather mercury levels with age

Clutches were combined for the following analyses. Log-transformed feather mercury had a significant weak positive relationship with age (lin:0.756, p=0.001, quad:-0.750, p=0.002; Figure 26) and negative relationship with river km (lin:-0.032, p=0.0015; see above Figure 16). Each clutch was also analyzed separately. For the first clutch only, there was a significant effect of age (lin:0.003, p=0.011; Figure 27) and river km (lin:-0.039, p=0.0001; Figure 28). For the second clutch only, there was

a significant effect of age (lin:0.008, p<0.001; Figure 29) but not of river km (lin: -0.028, p=0.095; Figure 30). For clutches combined, the ratio of feather to blood mercury changed depending on the stage of feather growth, and was lowest during the stage of no growth (Figure 31).

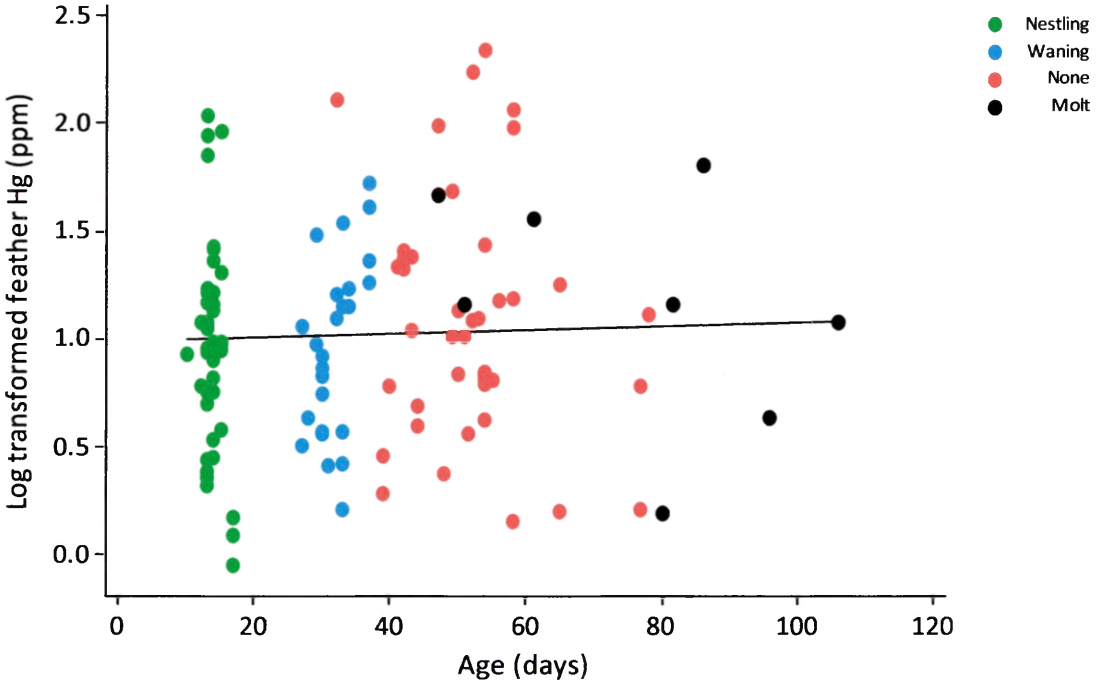

Figure 26. Log-transformed feather mercury with age, both clutches, both years. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

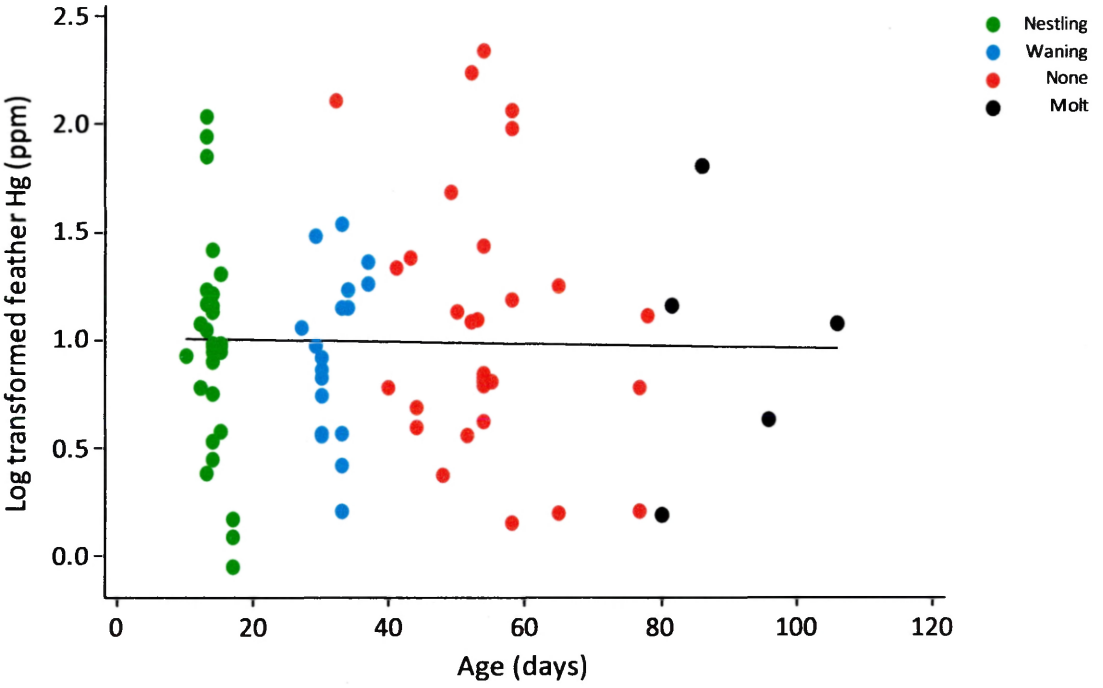

Figure 27. Log transformed feather mercury with age, first clutch only. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

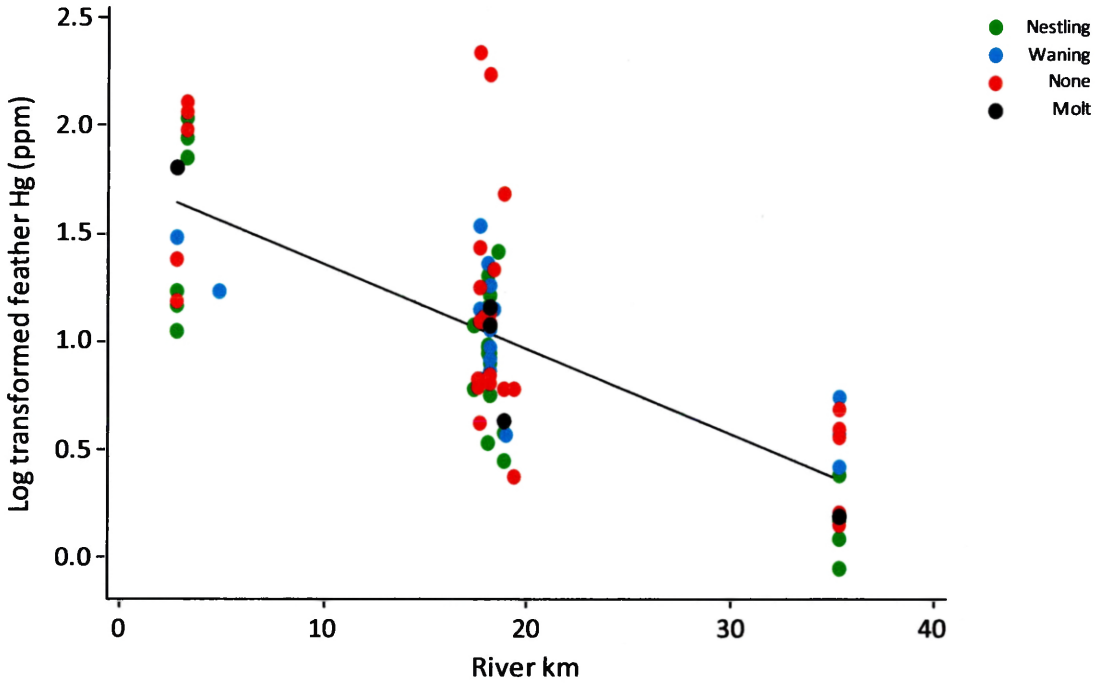

Figure 28. Log-transformed feather mercury along river km, first clutch only. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

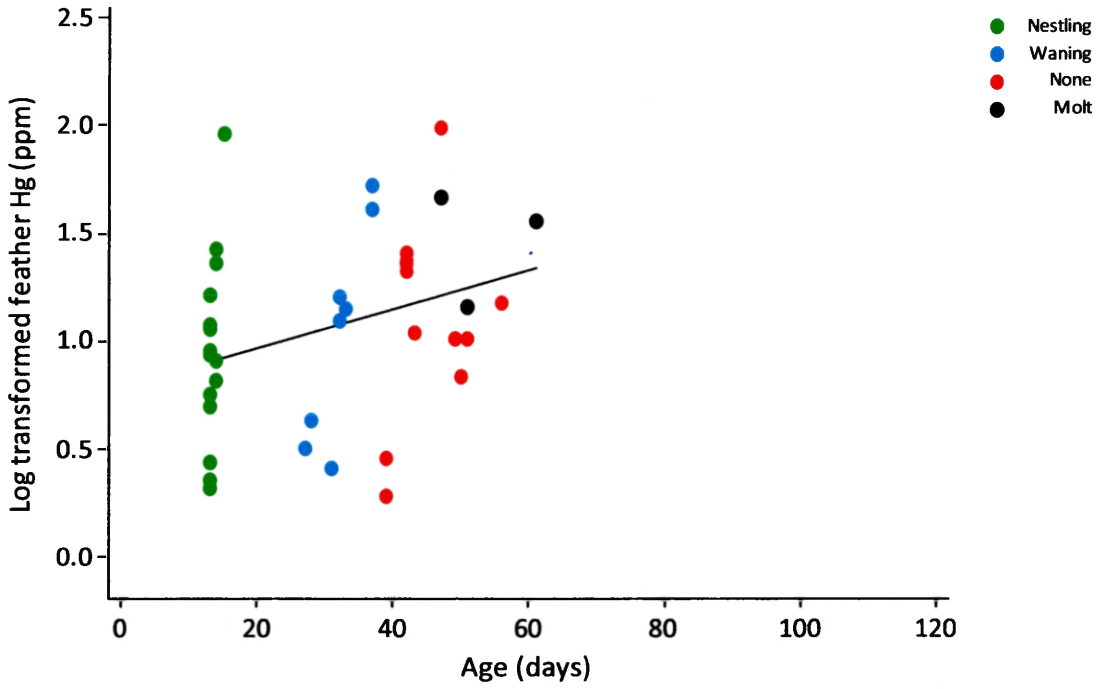

Figure 29. Log-transformed feather mercury with age, second clutch only. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

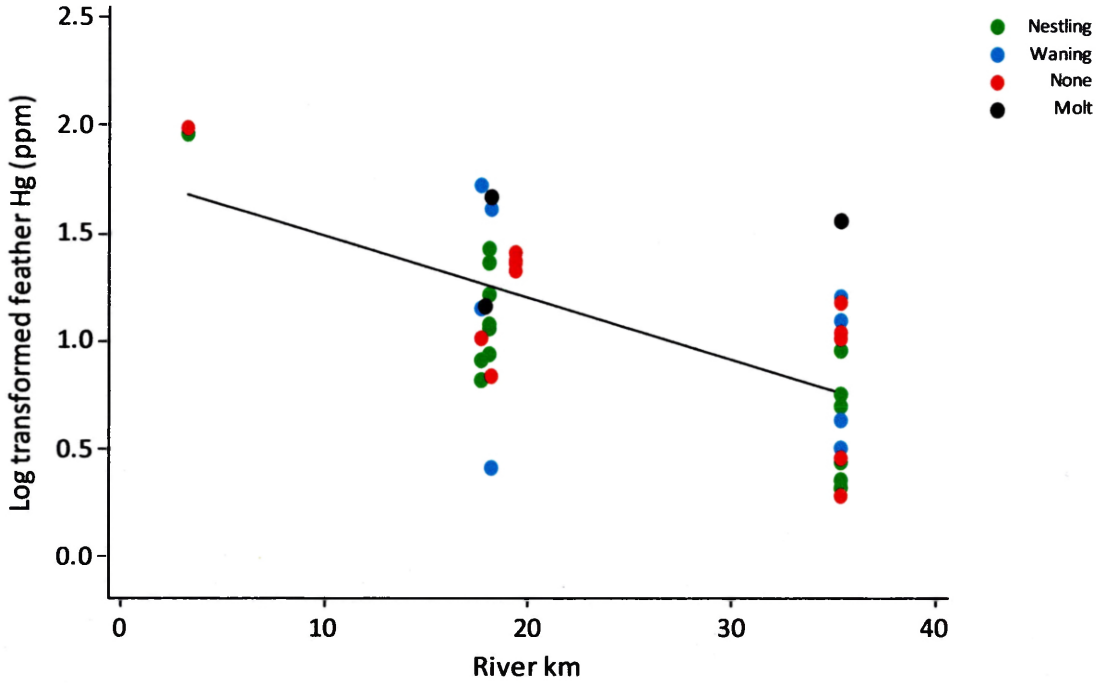

Figure 30. Log-transformed feather mercury along river km, second clutch only. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

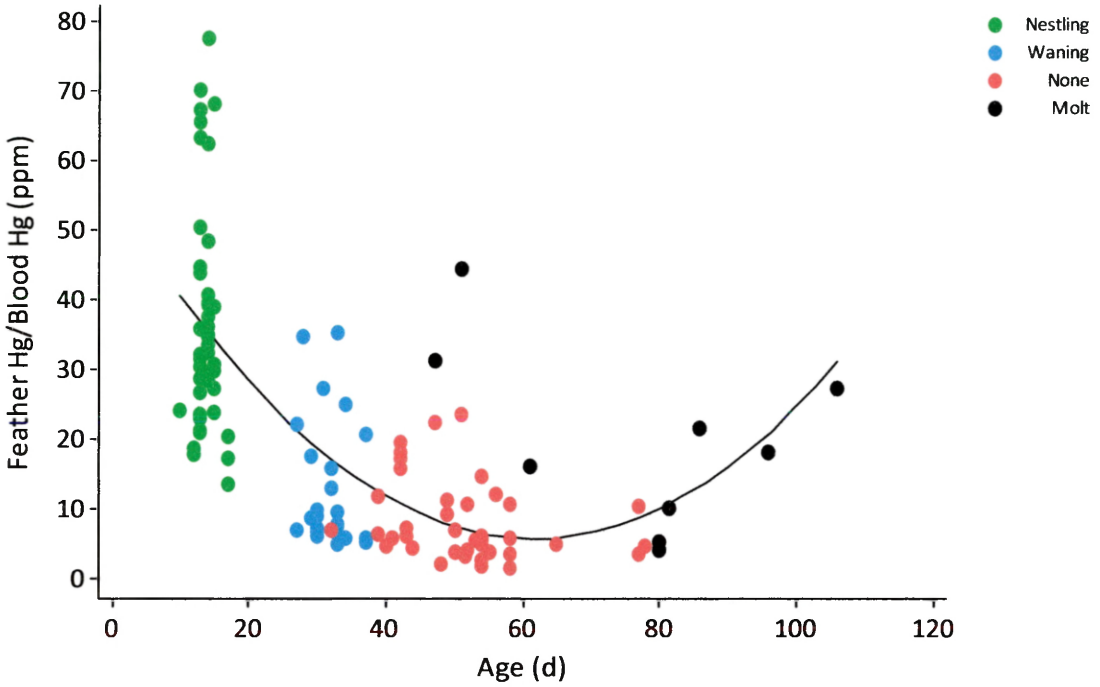

Figure 31. Feather to blood mercury ratio of different aged fledglings of the four feather growth stages. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

5.3 Stable isotope values with age

The $\delta^{15}N$ value was positively correlated with age (lin: 6.497, $p<0.001$; Figure 32), and marginally correlated with river km (-0.57, $p=0.0564$; see above Figure 18). The $\delta^{15}N$ value of the first clutch only was positively correlated with age (lin: 6.440, $p<0.001$; Figure 33), and not correlated with river km (-0.062603, $p=0.0604$; Figure 34). The $\delta^{15}N$ value of the second clutch only was not correlated with age (0.4355, $p=0.3349$; Figure 35) or river km (-0.0526, $p=0.0889$; Figure 36).

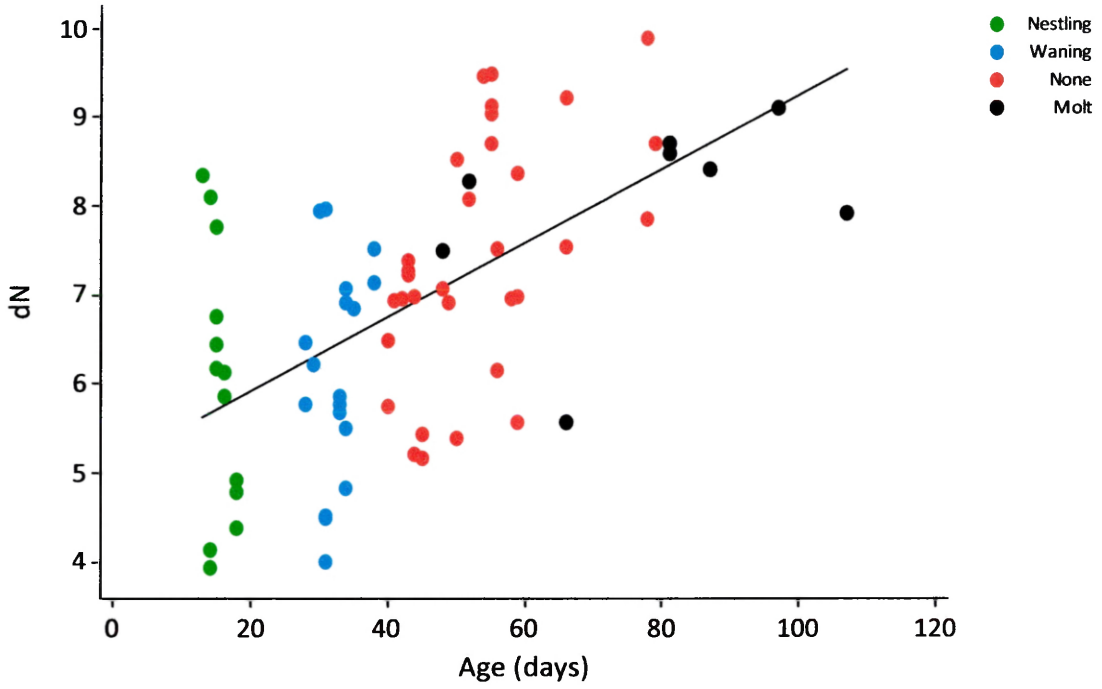

Figure 32. IHY $\delta^{15}N$ (dN) with age (days), both clutches. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

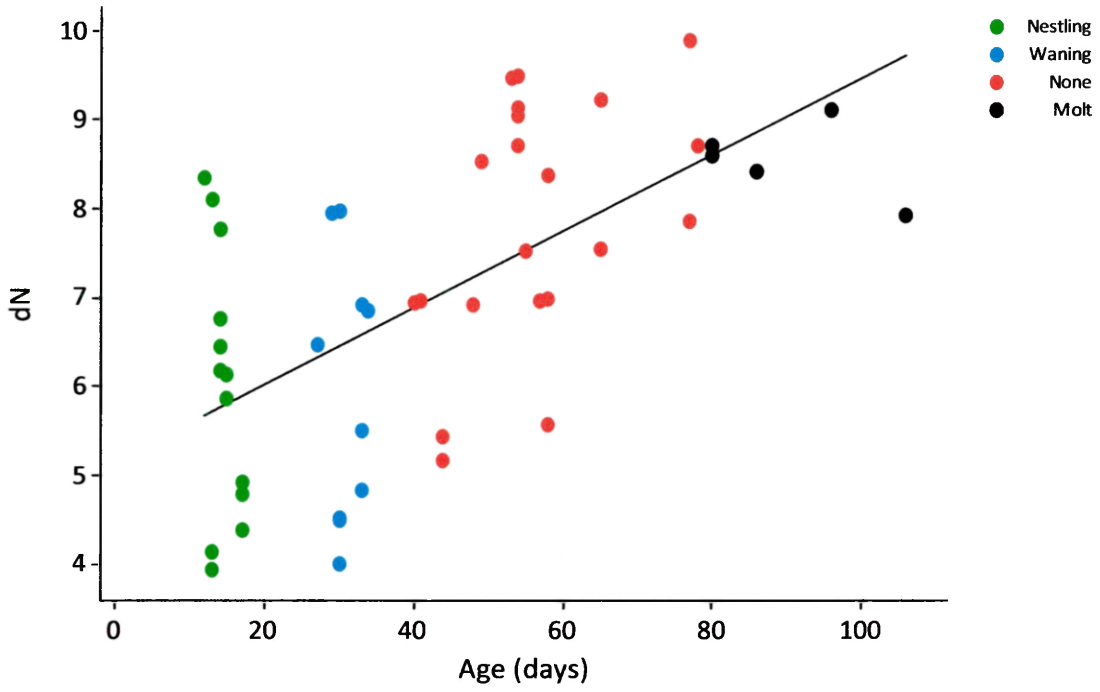

Figure 33. IHY $\delta^{15}N$ (dN) with age (days), first clutch only. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

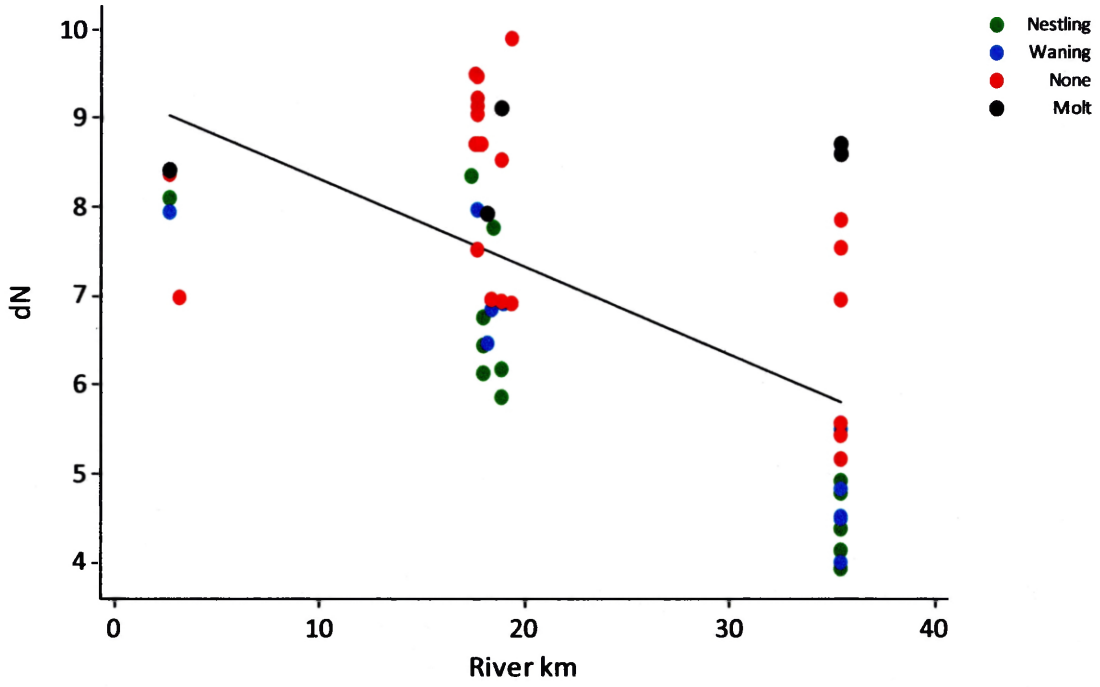

Figure 34. IHY $\delta^{15}N$ (dN) along river km, first clutch only. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

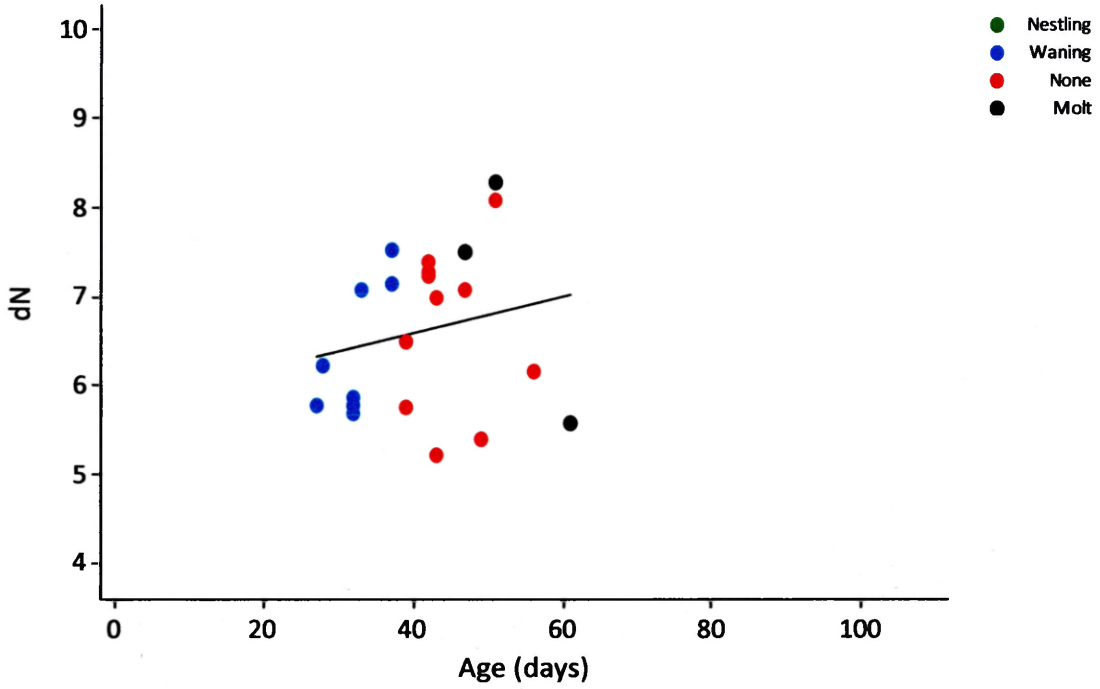

Figure 35. IHY $\delta^{15}N$ (dN) with age (days), second clutch only. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

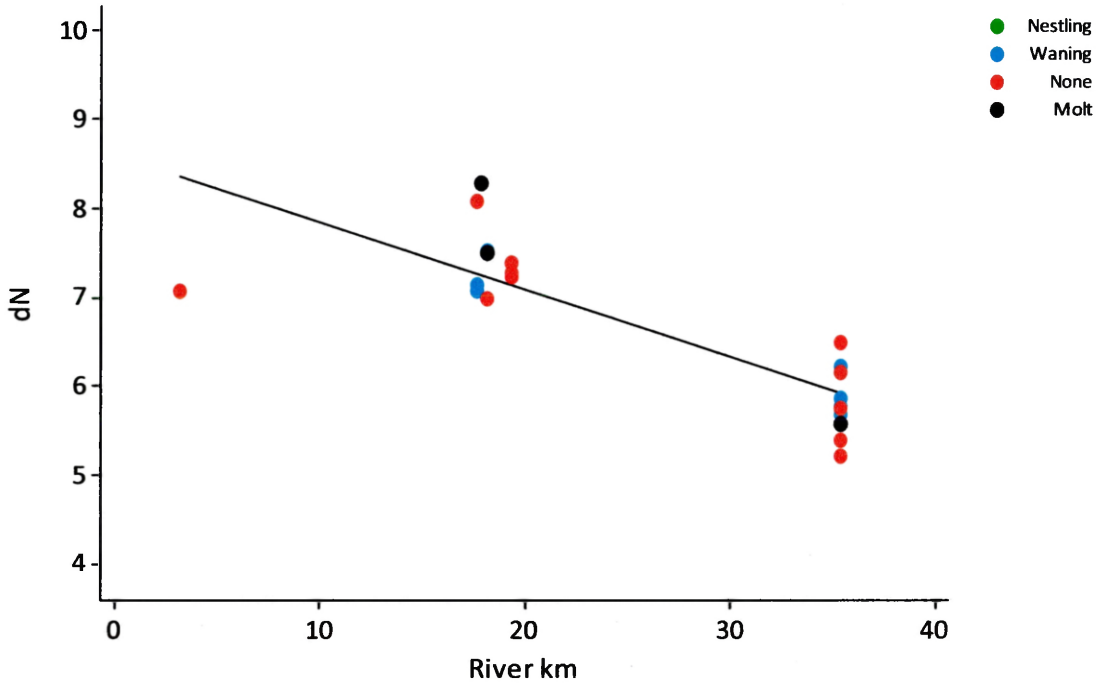

Figure 36. IHY $\delta^{15}N$ (dN) along river km, second clutch only. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

6.0 Comparison of mercury levels and $\delta^{15}N$

Blood mercury levels and $\delta^{15}N$ were analyzed separately because they each have a separate relationship with age and feather growth, and were both repeated measures of the same individuals. Within each feather growth category (so no individuals were repeated) $\delta^{15}N$ did not correlate with blood mercury except for during the nestling stage ($F_{1,12}$=6.36, p=0.027, R^2=0.35, R^2(adj)=0.29; Figure 37).

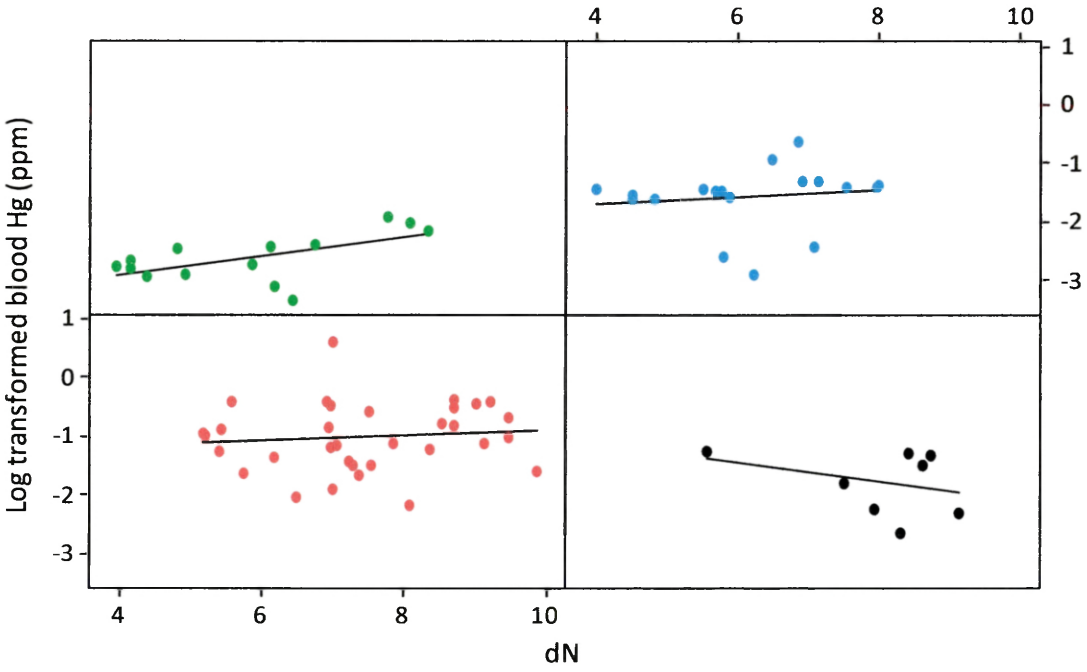

Figure 37. $\delta^{15}N$ (dN) and log-transformed blood mercury within each feather stage. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt. The relationship was non significant for waning ($F_{1,16}$=0.26, p=0.63, R^2=0.01), none ($F_{1,31}$=0.39, p=0.539, R^2=0.012), and molt ($F_{1,6}$=0.72, p=0.430, R^2=0.1).

Discussion

1.0 Fledgling blood mercury levels

As predicted, blood mercury levels showed an increase as feather growth halted, followed by a subsequent decrease in birds that began their first prebasic molt. A window of massive mercury elimination was provided when young nestlings grew thousands of feathers in a short period of time (~20 days), but shortly after fledging this route of elimination was no longer available and blood mercury levels rose, approaching adult levels. However, once molt began, the elimination route opened again and blood mercury levels fell. This is direct evidence that blood mercury levels fluctuate as the result of feather growth. Blood mercury levels showed the same pattern when compared with age, as opposed to feather growth category, but this was because feather growth category corresponded closely with age. The drop in blood mercury during molt is not explained well as a response to age because age continued to increase.

Despite differences in methods, the existing research on mercury levels and plumage overwhelmingly support my finding that mercury is eliminated into growing feathers, though often indirectly. Mercury is known to have a high affinity for feather keratin, especially sulf-hydryl amino acids (Crewther et al. 1966). It seems likely that most of the mercury circulating in the body during feather growth would be incorporated into feather keratin. Pied flycatcher (*Ficedula hypoleuca*) nestlings (n=2) sampled at sites of varying mercury contamination had liver mercury levels that increased with the age of nestlings at the site of highest contamination (Rosten et al.

1998). Past studies on seabirds, piscivores and wading birds have shown that 50-93% of body mercury burden and 42-60% of the mercury ingested is found in the feathers (Honda et al. 1986a; Lewis and Furness 1991; Monteiro and Furness 2001a; Monteiro and Furness 2001b; Kenow et al. 2002; Fournier et al. 2002; Agusa 2005). Studies on collected seabird specimens found that feather mercury levels decreased with molt sequence, suggesting a reduction of body burden (Furness et al. 1986; Braune and Gaskin 19897). Mercury levels in internal tissues of seabirds and wading birds decreased in molting birds, and increased during the inter-molt period (Honda et al. 1986; Braune and Gaskin 1987). However, these studies did not account for wide feeding ranges and differences in mercury intake among species sampled. They also did not track the change within individuals, or examine the relationship between blood and feathers throughout the sensitive post-fledging period. Instead, mercury levels were indirectly associated with relative stage of feather growth, or time period of molt, usually in adults.

Dosing studies have effectively showed that elimination rates of mercury during periods of high feather growth are more rapid than during periods of no feather growth, and have tracked the pattern within individuals (Bearhop et al. 2000b; Monteiro and Furness 2001a; Monteiro and Furness 2001b; Fournier et al. 2002). However, sample sizes were small in some cases (*e.g.*, nine great skuas, Bearhop et al. 2000). Also, these studies relied on the assumption that the kinetics of a single large dose would be the same as constant intake of mercury that would probably occur in a natural situation (Monteiro and Furness 2001a; Monteiro and Furness

2001b; Fournier et al. 2002). Bluebirds, and several other species on the South River, remain on or near the natal site throughout the late summer and fall. They are likely receiving doses of mercury on a regular basis rather than one large dose, though the level may vary slightly depending on the prey items. Although my study provided no information on excretion rate or half-life of mercury in the blood, as in the dosing studies, blood mercury levels decreased during periods of maximum feather growth. It is important to relate lab dosing studies to field studies to ensure that the phenomenon documented under artificial circumstances is occurring in a similar way in wild populations.

In this study, blood mercury levels were measured over time within individuals, assuming a relatively constant mercury intake. Mercury levels were low in nestlings and approached adult levels as feather growth ended. Some individuals exceeded the mean mercury concentration of adults (see Figure 27, see also Appendix D of mercury within individuals). My findings support the hypothesis that feathers serve as the primary elimination route in for mercury in nestling birds, and that once feather growth ceases, the mercury will accumulate in the body. The afore-mentioned previous research examined the phenomenon in large-bodied fish eating birds that may be up to 100 times the mass of a passerine. These larger birds have different molting patterns, metabolism, and thus kinetics of mercury in the body. They may also have been under selective pressure for millennia to eliminate dietary mercury, which has always been present at some level in marine ecosystems. This is the first demonstration that this oft-cited phenomenon of elimination into plumage occurs in

small birds, in terrestrial birds, or in free-living birds that were followed as individuals.

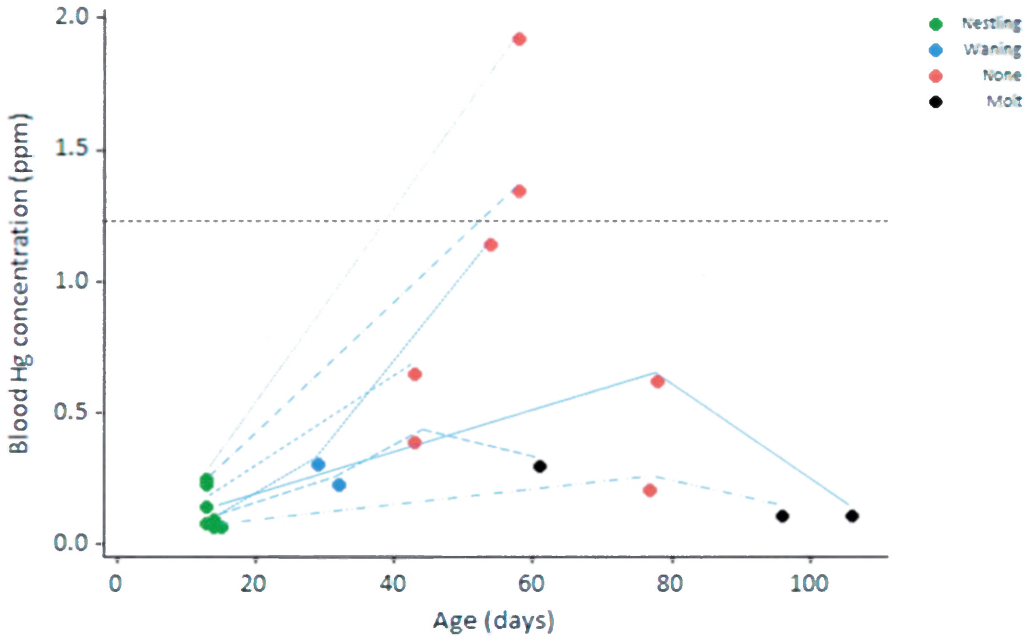

Figure 27. Example of pattern of mercury within seven individuals sampled over time. Dotted line represents mean AHY blood mercury level for bluebirds in 2006 and 2007. Colored circles represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt. Birds are: 196141331, 196141343, 196141381, 196141382, 220185162, 225170340, 225170419.

1.1 Other possible effects on mercury levels

There are other possible influences on mercury accumulation or exposure that deserve consideration. River km was sometimes a significant effect in analyses of blood mercury levels with feather growth stage or with age. However, differences

caused by river km reflect the relative amount of mercury available spatially. Some individuals had generally less blood mercury than others because they lived at sites (*i.e.,* river km) with lower mercury levels in the food web, but this is not relevant to the question of whether feathers are routes for mercury elimination.

There may also be variation between individual kinetics of mercury in the body that are unrelated to river km or feather growth (Bearhop et al. 2000b). Certain individual great skuas showed lower concentrations of mercury in the blood than was expected from dose level, suggesting variable excretion abilities (Bearhop et al. 2000b). Some of these differences in accumulation and excretion were sex-related, *i.e.*, Monteiro and Furness (2001a) found that females accumulated more mercury at a lower dose than males. However, sex was not a factor in the variation of mercury levels of fledgling bluebirds, and individual familial differences were accounted for in analysis (by the random term, individual, within a family).

Mercury may also be excreted into feathers on a dose-dependent basis, possibly accounting for some individual differences in feather mercury, but likely not the overall pattern of mercury levels between growth stages, which included birds of all exposure levels. In great skuas, individuals within the lower dose group exhibited longer mercury half-lives in blood, indicating some dose dependency (Bearhop et al. 2000b). However, there was no relationship between dose administered and amount of excretion evident in black-headed gulls or Cory's shearwaters (Lewis and Furness 1991; Monteiro and Furness 2001a). There may be a threshold above which all doses

are eliminated in the same manner. This was beyond the scope of my research, but may also explain some of the individual variation in mercury levels.

Another possible mechanism of reducing mercury in the body is through growth and protein turnover; as muscles grow, mercury concentrations are diluted (March et al. 1983). Growth dilution is partly responsible for keeping nestling blood mercury levels low. However, this does not diminish the importance of feather growth in mercury elimination because molting adults experience a similar reduction in mercury during molts (Monteiro and Furness 2001b). In this study, mercury levels were low as nestlings and again as molting fledglings, two separate phases in terms of body growth. I found no evidence of an important role for growth dilution.

In this study, I assumed that mercury intake was relatively constant. Friedman (2007) showed that bluebird prey was contaminated with mercury within 50 m of the river, and although there was variation in the mercury of prey, it was significantly elevated over reference prey (Friedman 2007). However, some of the captures of fledglings were greater than 50 m from the river, and it is currently not known how far the contamination extends in the floodplain. In adult bluebird blood mercury, preliminary data showed no decrease with distance from the river up to 400 m (M. Howie unpubl. data). Distance of capture from the river did not have a significant effect on blood mercury levels in a preliminary analysis. Despite movements up to 400 km from the river, I observed IHY birds along the river more often than away from the river (pers. obs.), and because blood is representative of two weeks' dietary intake, it is likely that a relatively constant 'dose' was ingested over time.

Another possibility is that I was preferentially capturing fledglings with low blood mercury later in the season because those with higher mercury had already died. This would potentially negate the role of feather growth in affecting blood mercury levels. However, a similar pattern was observed in birds with varying starting mercury levels. The direction of change in mercury level between feather growth stages within individuals was as I predicted 97% of the time (76/78), evidence that blood mercury levels were related to feather growth. Between the stages of "nestling" and "waning", I expected there to be an increase in mercury level; an increase was observed in 28 out of 28 cases where birds were caught in both stages. Between the stages of "waning" and "none", I expected there to be an increase in mercury level; an increase was observed in 16 out of 17 cases where birds were caught in both stages. Between the stages of "none" and "molt", I expected there to be a decrease in mercury level; a decrease was observed in five of six cases where birds were caught in both stages. Between the stages of "nestling" and "none", I expected there to be an increase in mercury level; an increase was observed in 27 out of 27 cases where birds were caught in both stages. In five other cases, birds were caught between "waning" and "molt" (mercury levels decreased in two cases), or between "nestling" and "molt" (mercury levels increased in one case and decreased in two cases).

2.0 Fledgling feather mercury levels

There was a statistically significant increase in feather mercury with both feather growth stage and age. This was not expected, as mercury in feathers is stable

after growth is complete, and only full grown feathers were sampled from IHY birds (Appelquist et al. 1984). The slight increase is likely due to the fact that feathers were still growing as nestlings when they were first sampled, and additional mercury may have been deposited until growth was complete. There also may have been natural variation in amount of mercury in different feathers. Some studies have found an increase in feather mercury levels with the age of the nestlings, while several studies on seabirds and wading birds have failed to see a relationship (Thompson et al. 1991; Stewart et al. 1997; Bearhop et al. 2000b; Goutner et al. 2001). Another possibility for the increase is that mercury is added to the outsides of feathers via the preen gland oil, as birds age (Goede and De Bruin 1984). The pattern of feather mercury varied between individuals (see Appendix E for individual plots of feather mercury).

3.0 Fledgling $\delta^{15}N$

The reason for the increase in $\delta^{15}N$ with age is unclear, and is likely not a factor in the pattern seen in blood mercury. If the increase in $\delta^{15}N$ reflected a change towards feeding higher in the food web as summer progressed, this might explain some or all of the change in blood mercury level that I have attributed to feather growth stage. Bluebird prey (fed to nestlings) on the South River consisted primarily (>75% of biomass) of Aranea (spiders), Lepidoptera (moths and butterflies), Orthoptera (crickets and grasshoppers) and Coleoptera (beetles; Friedman 2007). There are no data on bluebird diets as fledglings, but it is likely they are eating similar prey items. However, the availability of certain insects may change throughout the

summer (see M. Howie unpubl. data). Based on anecdotal observation, there were temporary increases of abundance of June bugs (*Phyllophaga* spp.) and crickets (family Gryllidae) at different periods over the season. Bluebirds may shift their diets depending on what is available.

There are other reasons that $\delta^{15}N$ may increase besides a change in trophic level. Stable isotope ratios may change with age or growth of an organism. There is conflicting evidence both for and against the claim that isotopic ratios will increase with age. With age, development and body weight, $\delta^{15}N$ values increased in the spider *Pardosa lugubris* (Oelbermann and Scheu 2002). In two species of mysids, the nitrogen ratio increased with increasing body size (which is often related to age; Gorokhova and Hansson 1999). However, Hobson and Clark (1992b) found that nitrogen fractionation from diet to blood did not changed with age in adult peregrine falcons (*Falco peregrinus*). Nitrogen isotopic ratios also did not differ in age of marine mussels (from 0 to 8 years of age; Migawa and Wada 1984). The weight of fledglings used in my study showed a positive, though weak, linear increase with age ($F_{1,134}$=5.13, p=0.025, R^2=0.04). Weight may have played a small role in influencing the isotopic ratio, however, it would require a controlled laboratory study monitoring $\delta^{15}N$ over time in birds on a known diet to be able to untangle the relationship of age and growth to isotopic signature.

Baseline nitrogen levels may vary across sites, so birds at different sites may have started with different $\delta^{15}N$. Disturbed soils, such as agricultural land, tend to be more enriched in $\delta^{15}N$ than other soils due to increased biological activity (Hobson

1999). Inputs of nitrogen from fertilizer application and sewage treatment effluent may increase nitrogen and change isotope ratios at a site. The Waynesboro Water Treatment plant (WH20) at river km 2 was within 5 km upstream of BAPA and GENI, three sites where fledglings had relatively high $\delta^{15}N$ (see Results). Fledglings from AUFC, around river km 18, which also had high $\delta^{15}N$ were often observed or trapped on cow pastures adjacent to the forestry center. Birds at GRCP, at river km 35, had the lowest $\delta^{15}N$. These fledglings were trapped most often on-site, which was a public park, and were observed on adjacent agricultural land infrequently. Site may have been a factor in creating different isotopic signatures over time. Possible excessive nitrogen was available due to the wastewater treatment facility near WH20 and BAPA, or from the disturbed agricultural fields near the AUFC. However, relatively few individuals were collected from the sites near the water treatment facility (see Appendix F for individual plots). Only one individual at the WH20 was measured multiple times, and it is interesting to note that the nestling $\delta^{15}N$ value was high, and remained relatively stable over time up to 85 days. Whereas at the AUFC and GRCP, values started lower as nestlings, and increased.

Other possibilities of increasing $\delta^{15}N$ include nutritional stress or time of sampling. Enrichment of $\delta^{15}N$ has also been seen in birds experiencing nutritional stress (Hobson and Clark 1992b). However, there were no significant differences in $\delta^{15}N$ in food-stressed song sparrows (Kempster et al. 2006). Isotope enrichment may indicate an extreme of starvation, rather than just poor nutrition (Kempster et al. 2006). This seems an unlikely reason for the increase in $\delta^{15}N$ in birds on the South

River, based on anecdotal observation and the weight increase that was seen with age. There also may be variation in isotopic signatures depending on the last meal eaten, and how quickly after it the blood was sampled—dietary lipid circulating in the blood during digestion may influence isotopic signatures (Bearhop et al. 2002). However, these factors are difficult to describe or account for and their role has not been investigated thoroughly in the literature.

3.1 Were mercury and $\delta^{15}N$ related?

Studies using isotopes have shown an increasing contaminant load with increasing food chain length using isotopes (Morrissey et al. 2004). However, Thompson et al. (1998) found no effect of trophic level on mercury levels, using stable isotopes. In this study, it is possible that the increase in $\delta^{15}N$ indicates a true diet shift, but if so, the diet shift was not closely tied to blood mercury levels. Individual diets, as indicated by isotopic signatures within stages of feather development, were not related to individual mercury levels. Thus, individuals with the highest mercury were not necessarily those with the highest $\delta^{15}N$. The pattern of change in $\delta^{15}N$ with age and feather growth was different altogether from that of blood mercury. For example, as blood mercury levels fell during the molting period, $\delta^{15}N$ increased. Feather growth, rather than a putative shift in diet reflected by isotopic signature, was closely tied to the change in mercury level. Even if there was a dietary shift towards prey items higher in the food chain, and thus presumably with more methyl mercury content, birds were still able to reduce these mercury levels through growing feathers.

4.0 Possible implications and effects

Although young birds may be more sensitive to contaminants, *i.e.*, intestinal absorption of heavy metals is enhanced in very young organisms, it is likely that young birds are buffered from toxicity by growing feathers (Jugo 1979). Only after feather is growth completed, often during the post-fledging period, may they be susceptible to accumulating high concentrations of mercury. That feather growth predicts blood mercury level can be usefully applied in a risk analysis for any species for which the molt schedule is known.

The primary objective of this study did not include quantifying effects of mercury on fledglings, however some anecdotal survival estimates were made. In general, bluebird fledgling survival estimates are high, 81.5%, from fledging to independence (Pinkowski 1977). My anecdotal estimates are slightly lower, which may be accounted for by the unusual and highly localized storm event (tornado) where 4-9 birds died. Estimates of survival in this study ranged from 41 to 67%, and include birds that died after independence (after approximately 30 days post-hatch). Thus, although I did not determine survivorship on reference sites, bluebird fledgling survivorship on contaminated sites may have been lower than expected for this species. This would also relate to the reproductive finding suggested below, of contaminated birds initiating second clutches earlier; *i.e.*, if it is true that fledglings from the first brood on contaminated sites experienced high mortality, then adults would be able to start a second nest earlier.

It is likely that bluebirds on my study site are at least short-distance migrants, although some may remain on site year-round. Other general estimates for juvenile survival in long and short-distance migrants range from 37 to 42% for up to eight weeks after fledging (Sullivan 1989; Anders et al. 1997). My anecdotal estimate of survival of bluebirds does fall within this range, and these estimates allow for birds that died after independence. However, without reference data survival estimates are strictly anecdotal, because even the effects of transmitters cannot be evaluated. The highest levels of fledgling bluebird blood mercury were similar to that of bluebird adults, and were lower than adults of many other species on the South River. Thus, species that are accumulating more mercury than bluebirds may be at risk during the post-fledging period, especially when feather growth stops. In addition, if birds do not begin a molt within two months of fledging, as bluebirds do, they may extend the time period in which they are accumulating mercury in the body, with no elimination route.

Risk of predation in some species has been found to be high during the first week of fledging, and again when juveniles are no longer attended by the parents (Sullivan 1989; Anders et al. 1997). This may correspond with increases in mercury levels in species as feather growth is ending, but birds are still foraging on contaminated food items. Accumulation of mercury in internal tissues then may have neurological and behavioral effects, possibly making birds more susceptible to predation. If juvenile survival is negatively impacted, there may be population-wide

repercussions. Juvenile survival is one of the main factors considered in population demographic studies (Pulliam et al. 1992; Fair et al. 2003).

With the bluebirds from this study, the period of risk would be greater for birds originating from the first clutch. Second clutch fledglings begin molting at an earlier age (<60 days) than first clutch fledglings (>60 days; Figure 38). Fledglings from the first clutch are exposed to mercury accumulation during the stage of no feather growth for approximately 20 more days than fledglings from the second clutch (see Figure 38). Fledgling birds from the second clutch stop growing juvenile feathers on a similar time scale than birds from the first clutch (Figure 39). However, within a short period of time, birds from the second clutch will begin to grow feathers again during the first prebasic molt compared to birds from the first clutch (Figure 39). Because birds from the second clutch fledge later in the season, the molt process is accelerated in order to "catch up" to the first clutch (Figure 40). Fledglings from the first clutch may experience higher mortality than birds from the second clutch due to this temporal difference. Exploring a difference in mortality between the first and second clutch fledglings was beyond the scope of this thesis. All of the identifiable dead fledglings in this study originated from the first clutch. However, there may be differential survival between clutches.

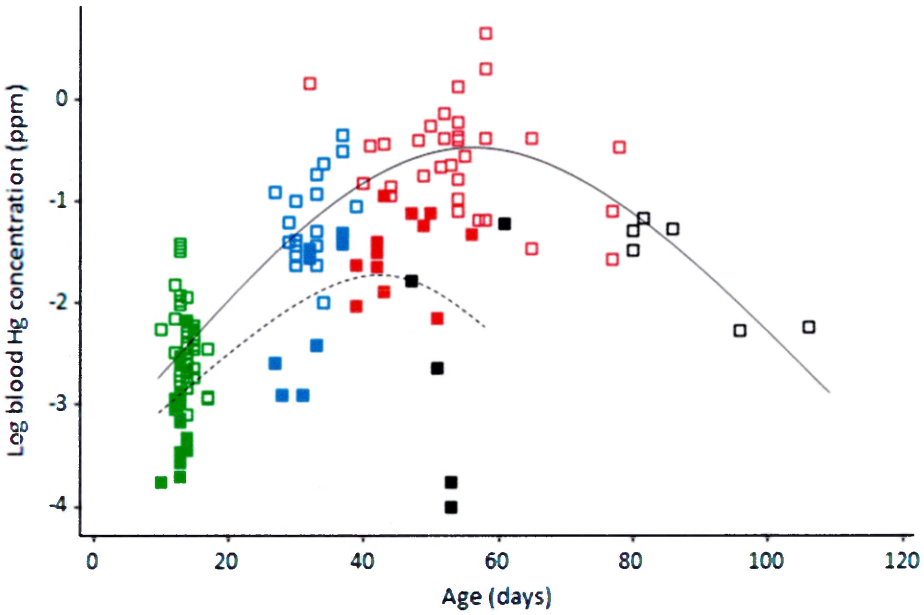

Figure 38. Log-transformed IHY blood mercury with age, both clutches. First clutch birds are represented by open squares and second clutch birds are represented by closed squares. Colors represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

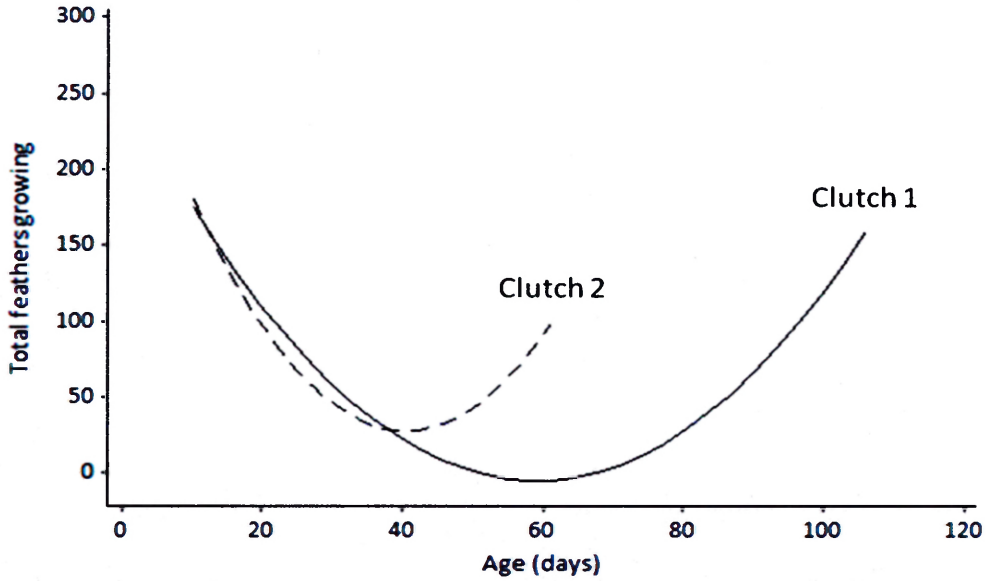

Figure 39. Number of growing feathers over age (days) of IHY bluebirds from the first clutch and from the second clutch. Solid line represents the feather growth of first clutch birds, dotted line represents the feather growth of second clutch birds.

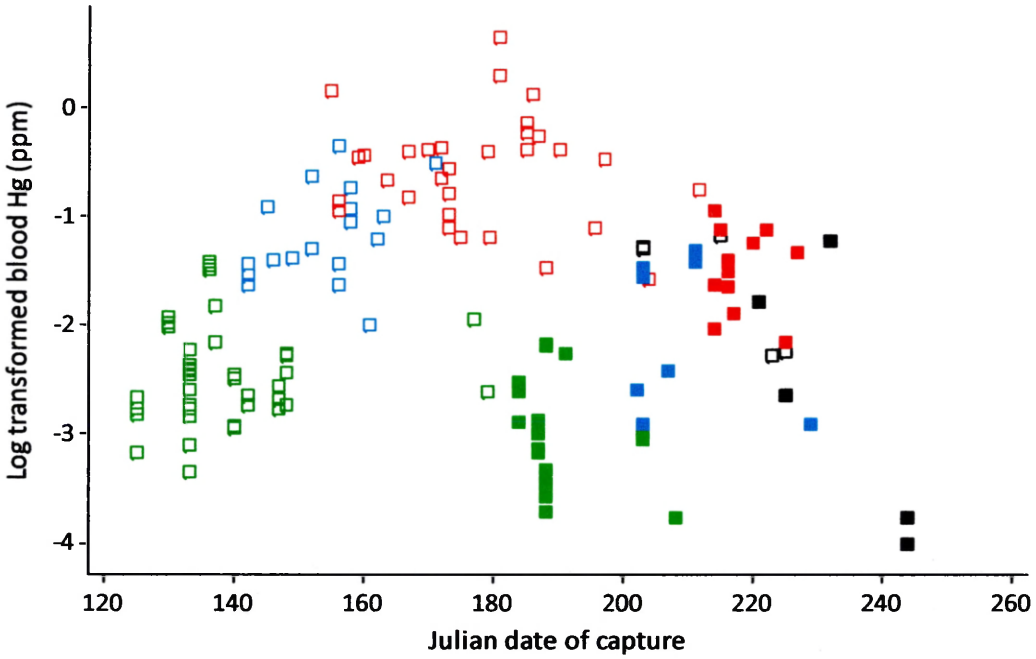

Figure 40. Log-transformed IHY blood mercury and Julian capture date, both years combined. First clutch birds are represented by open squares and second clutch birds are represented by closed squares. Colors represent different stages of feather growth: green=nestling, blue=waning, red=none, black=molt.

Other toxicological studies on fledgling survival have mostly related it to the levels of the contaminants in nestlings, rather than as fledglings. European dipper (*Cinclus cinclus*) juvenile survival was not effected by elevated levels of PCBs (Ormerod et al. 2000). There was no effect on survival of great egrets that were dosed with mercury as nestlings, with the average nestling blood mercury level of 0.7 and 1.2 ppm per each year of the study (Sepulveda et al. 1999). My data on fledglings that died or survived is not illuminating: fledglings found dead on the South River had blood mercury levels of 0.04 – 0.24 ppm as nestlings, while birds that presumably survived had blood mercury levels of 0.02 – 0.24 ppm. No studies, to my knowledge, have related survival to mercury levels within fledglings. It is important to continue monitoring these birds during the post-fledgling period to comprehensively assess the risks to survival.

5.0 Future work

Using telemetry is recommended to rigorously compare juvenile survival between contaminated and reference sites. Although bluebirds make good study species, they may not be appropriate for a survival study, due to their relatively low levels of mercury. Carolina wrens, which are year-round residents on site, have small territory sizes along the river, and are accumulating high levels of mercury, would be an ideal study species for this research (see Friedman 2007). Analysis of tissues and organs (especially, liver, kidney and brain) of fledglings found dead would provide some data on how the blood levels relate to accumulation in tissues.

A dosing study of passerines would be required to determine rate of uptake, half-life and whether passerines also fit a two-compartment model as seen in Cory's shearwater and common loon, of rapid initial uptake by target organs, then a slow terminal phase (Monteiro and Furness 2001a; Monteiro and Furness 2001b; Fournier et al. 2002). This study would provide additional information on periods of high risk for passerines by determining the length of time of mercury elimination. Bluebirds are accumulating mercury between 40 and 80 days of age, during no feather growth. They are then eliminating mercury during molt, which may last from 35 - 96 days. A dosing study would reveal how much mercury they are able to eliminate in this time frame compared to a constant intake.

Lastly, the relationship of $\delta^{15}N$ should be examined more closely in relation to mercury levels, and in relation to growth and age of passerines. In my study, it was not clear if the increase in $\delta^{15}N$ was due to age and growth, or environmental factors such as fertilizer input. A lab study where birds are fed a constant diet from hatching through fledging would provide information on $\delta^{15}N$ as birds grow. To better understand spatial and temporal differences between sites on the South River in $\delta^{15}N$, insects feeding low on the food chain could be collected at different times throughout the summer to detect site and seasonal differences in $\delta^{15}N$.

Discussion of other results

6.0 Adult blood levels

AHY bluebird levels did not vary between years. Mercury availability in the environment may vary between years due to environmental factors, and this variation is often reflected in bird blood. Tree swallow mercury levels were approximately twice as high in 2006 as in than 2005 on the South River, likely due to decreased stream flow and higher water temperature in 2006, which may have affected rates of methylation (Brasso 2007). Tree swallows may be highly sensitive to weather changes affecting the aquatic environment, since they forage on emerging aquatic insects (Robertson et al. 1992). However, differences in stream flow and water temperature probably do not quickly affect mercury levels in the terrestrial prey items of the bluebird.

Bluebirds may be able to naturally keep their mercury levels down through their varied diet and large foraging range, regardless of amount of mercury present in the aquatic environment. Foraging range of adults during the nestling period ranges widely from 4.5 to 38.9 ha (Pinkowski 1977). The foraging range for breeding adults on the South River is unknown other than anecdotal observations, but mercury has been found in food items of nestling bluebirds, which may be indicative of adult diet as well (Friedman 2007). Mercury levels in bluebird prey items varied from approximately 0.5 to 5.5 ppm (Friedman 2007). Mercury is present in variable quantities in the prey of bluebirds, and is likely the driving factor behind the variation

of mercury levels. However, the environmental factors determining how mercury levels vary within terrestrial prey items is currently unknown.

River km had a significant effect on bluebird blood (both AHY and IHY), with the furthest site from the contamination source showing lowest mercury levels. This pattern of decreasing mercury with distance suggests that mercury is less abundant further from the source of the original leak. Adult tree swallow blood mercury peaked around river km 18, the same general area of highest levels for bluebirds (Brasso 2007). No relationship was seen with river km in house wren (*Troglodytes aedon*) or Carolina wren blood mercury, however the sample size was smaller (Friedman 2007). The relationship with blood mercury and river km may be more complex than simply distance from the historic contamination source. Within and between sites, there may be differences in mercury availability due to microhabitat; Cocking et al. (1991) found variation of 11 to 84 μg/g of Hg in soil at one site on the South River using 100 m^2 quadrats.

Neither date of capture nor sex had effects on blood mercury levels in AHY bluebirds. Although females may deposit mercury into eggs, lessening their body burden, this may only be apparent shortly after egg-laying, which is before I collected samples. All females in this study were caught after eggs had hatched, and nestlings were 1 to 10 days old. Other studies have also shown no effect of sex on mercury levels (Furness et al. 1990; Evers et al. 2005; Brasso 2007; Friedman 2007).

AHY blood mercury on contaminated sites was elevated over reference sites by at least an order of magnitude. This same pattern has been seen in other

insectivorous passerines, as well as piscivores and owls on this site (see Brasso 2007; Friedman 2007; White 2007). This confirms in another species that the South River is significantly contaminated above background atmospheric deposition (see also Brasso 2007; Friedman 2007; White 2007). The level of contamination on the South River roughly equates to that in some dosing studies; *i.e.*, egret nestlings were administered doses that effectively tripled the natural levels of mercury at a study site in south Florida, causing a mean brood mercury concentration of between 0.7 and 1.2 ppm (Sepulveda et al. 1999). Bluebird nestlings on my site had a mean brood average of 0.913 ppm, comparable to that of the egrets. It is important to note that the doses of mercury received through prey items on the South River were similar to those seen in some lab studies.

6.1 Returning AHY

All of the AHY birds that returned from 2006 nested in the same sites. Of adults caught in 2006, 26% returned (10/39) on the contaminated site, compared with 17% (5/29) on reference sites. Only 3% (3/112) of nestlings from 2006 returned to breed as adults on the contaminated river. The literature estimates that between 11 and 13% of banded and fledged individual bluebirds return to natal sites to breed (Gowaty and Plissner 1998). Only 9% of total birds banded in 2006 returned on the contaminated site (13/152) and 5% on the reference site (6/125). The reason for low return rates compared to range-wide averages for the species is unknown. One possibility for the low return rates is displacement by the enormous population of tree swallows attracted to the site beginning in 2005. Tree swallows had high levels of

mercury on the contaminated site in 2006 (see Brasso 2007), possibly increasing mortality. If fewer tree swallows nested on the contaminated site, more nest boxes might have been available for the bluebirds, thus more returning bluebirds were found on contaminated sites. While on reference sites, healthier tree swallows would be able to out-compete bluebirds for nest box occupancy. Both areas have other nesting options available besides the study nest box trail, whether it be natural cavities, or other nest boxes on nearby private property. Another possibility is that birds from nearby locations may move to boxes on site, *e.g.*, four bluebirds were caught as fledglings of unknown origin in 2006 and nested on site in 2007. Likewise, some birds that nested on-site one year may move to private property to breed in the next year, and thus go undetected.

Even fewer AHY birds from 2005 returned in 2006, although the proportion returning was higher. Bluebirds were only banded opportunistically in 2005, and it is not known how many unbanded birds nested in the study area. Of adults caught in 2005, 33% (5/15) banded on the contaminated site returned to breed in 2006, compared with 36% (4/11) on reference sites. Only 7% (4/55) of nestlings returned to breed on contaminated sites in 2006, and 4% (1/23) on reference sites. Of total birds banded in 2005, 13% (9/70) on the contaminated site, and 15% (5/34) on reference sites returned in 2006. This estimate is within the upper range suggested by the literature, but may be inaccurate due to lower sampling effort in 2005.

7.0 HY blood and feather levels

Blood and feather mercury of nestling bluebirds from the South River was significantly elevated over reference nestlings, even at extremely low concentrations of mercury. Both feather and blood mercury captured the differences between contaminated and reference sites, but the levels in blood were so low that with a smaller sample size, the difference between contaminated and reference might not have been detectable above natural variation. Nestlings make good biomonitors because they are easy to sample and are ingesting mercury from a defined area, however caution is urged when interpreting HY blood results.

Nestling blood levels were highly correlated with their parents, as is seen in other species (Fevold et al. 2003; Evers et al. 2005; Brasso 2007; Friedman 2007; White 2007). However, HY blood levels were at least an order of magnitude lower than their parents' blood levels, while HY feather levels were similar to their parents' blood levels. The values for HY bluebird feather mercury on the South River ranged from 0.805 to 6.865 ppm. Mercury in the blood of adults on the South River ranged from 0.286 to 5.02 ppm. Feather mercury levels of nestlings and blood mercury of adults fall may be used interchangeably to detect contamination. Feather mercury levels of HY birds were higher than their own blood levels, and there is a strong correlation between the two, indicating that most of the mercury ingested is being shunted into the growing feathers. Contaminated HY blood levels were similar to reference AHY blood levels (nestling mean = 0.098 ± 0.06, reference adult mean = 0.102 ± 0.05), while contaminated HY feather mercury was elevated over reference

AHY blood mercury levels, as expected if nestlings were eliminating their blood mercury into feathers.

The level of contamination on the South River might be falsely described by using nestling blood as a monitoring tissue. Nestling feather mercury provides a more accurate description of the contamination, similar to that of adult blood levels, but with more ease of sampling. However, the level of contamination is different between species. Tree swallow, Carolina wren and belted kingfisher blood and feather mercury levels were elevated over bluebird levels (Brasso 2007; Friedman 2007; White 2007). For comparison, mercury levels in feathers of birds collected between 1955-1980 in Minamata Bay ranged from 4.6 to 13.4 ppm (fw), higher than the South River bluebird population, indicating relatively less contamination (Eisler 1987). However, belted kingfisher nestling feather mercury averaged 9.9 ppm, and resident Carolina wren adult feather mercury was close to 11 ppm, both being closer to the level of contamination at Minamata than bluebird feather mercury (Friedman 2007; White 2007). When sampling feathers, and including other species, the contamination in birds on the South River appears to be similar to that at the most infamous industrial mercury contamination event in history. Had a naïve researcher examined only nestling bluebird blood, a far different picture would have emerged.

8.0 Nesting data

Bluebirds showed few significant differences in reproductive parameters between contaminated and reference sites. With non-parametric tests, differences were only detected in the second clutch, where smaller sample sizes made the

analysis less reliable. There was a smaller mean clutch size and a lower proportion of fledglings on reference sites in 2006. These results are contrary to the prediction that nests on contaminated sites would show reproductive effects.

Although there may be a general decline in clutch size and number of fledglings produced between the first and second clutch of bluebirds, this does not explain the differences seen between treatment groups within the second clutch (Pinkowski 1977). A smaller clutch size may be due to physiological demands on a female who had raised a successful first brood, irrespective of contaminant levels (Pinkowski 1977). Of the second clutches on the reference sites, the preceding first clutch was successful in 12 instances, and failed in one. Similarly, on the contaminated sites, the preceding first clutch was successful in 11 instances and failed in two. It seems unlikely that the smaller second clutch size on reference sites was a result of reference birds raising more successful first broods than contaminated birds.

Instead, there may have been site effects that contributed to the reproductive success of later clutches, *i.e.*, food availability or level of predation. There are no data on food availability differences over the season for either site; this would require consistent insect sampling (*e.g*, pitfalls and sweep netting) throughout the season. Still, a smaller clutch size may indicate less available food on site later in the season (Lack 1948). There may have been fewer resources available on the reference sites, so fewer eggs were laid.

Increased predation of nestlings or eggs may have contributed to fewer fledglings produced on reference sites. The two most likely predators within both

sites were snakes and house sparrows. House sparrow competition seemed to be greater on reference sites (pers. obs.), although it showed no evidence of increasing later in the summer, during second clutches. At one reference site of 20 boxes, house sparrow nests were regularly removed from 13-15 boxes from early May all the way through late July. One nest on the reference sites failed due to a confirmed house sparrow take-over, and two additional nests failed during the nestling stage, possibly due to house sparrows. One more nest failed due to abandonment of eggs, making a total of four nest failures on the reference sites. On the contaminated sites, only two of the second clutches failed (one as eggs, one as nestlings). Therefore, it seems likely that fewer fledglings were produced on reference sites due to more predation of eggs and nestlings compared with contaminated sites, something that is unrelated to mercury levels.

The clutch initiation date was significantly earlier in contaminated second clutches in both years. Earlier second clutches may be due to failed first clutches, but this is not the case here, since second clutches that followed failed first clutches were excluded from analysis for this reason. This difference may be due to slight weather differences between the South, North and Middle Rivers. Double-clutching also involves a certain partitioning of resources of adults between the fledged young and the new brood. It is possible that fledglings from the first clutch on contaminated sites experienced higher mortality, thus "freeing up" the parents to initiate a second clutch earlier than otherwise would have been possible energetically. Data are not available for post-fledging survival (see below 6.0 Possible implications and effects).

However, it is not immediately clear why there are differences between treatment groups of the second clutch; these differences may simply be a result of small sample size and sampling error.

Analysis using a GLM mirrored the analysis by Kruskal-Wallis tests done separately by year: reference nests had a smaller clutch size and produced fewer fledglings in the second clutch. The GLM analysis also included a comparison between years. In 2006, more fledglings were produced than in 2007 in the first clutch. In 2007, there was an overall later clutch initiation date and a slightly lower proportion of eggs that hatched in the first clutch. In 2007, there was an overall larger proportion of fledged young and more fledglings produced in the second clutch. These differences may be driven by a poor performance in nesting birds on contaminated sites if one year had higher mercury levels than the other. However, these differences are not readily explained by mercury differences between years; adults and nestlings showed no difference in mean blood mercury between 2006 and 2007. More likely these differences are due to slight changes in weather, food availability or predation between years.

Literature estimates for nesting success are: 83% of eggs hatch, 75-90% of hatched chicks fledge, and 55-84% of clutches produce some fledglings (Gowaty and Plissner 1998). For both years combined, 85% of eggs hatched on the contaminated site, and 79% on reference sites. Reference sites had slightly lower estimates than the literature. For both years combined, 84% of hatched chicks fledged on the contaminated site, and 83% on reference sites, both within the range estimated by the

literature. Overall nesting success was 82% on the contaminated sites, and 80% on reference sites for both years combined. Again, these fall within the range provided by literature. Overall, bluebirds nesting in the Shenandoah Valley show similar productivity to that reported in other studies.

The tree swallow is the only other species for which reproductive data are available on the South, Middle and North Rivers (for 2005 and 2006; see Brasso 2007). Second-year females were found to produce one less young on the contaminated site than on reference sites (Brasso 2007). Tree swallows nested in greater numbers than bluebirds, which would allow for greater statistical power to detect differences. In 2006, adult tree swallows had 2-3 times the mean blood mercury levels of adult bluebirds. It is probable that bluebird mercury levels are low enough that there are no reproductive effects; mean adult bluebird blood mercury for both years combined was 1.21 ± 0.78 (n=86), barely above the generally-utilized level of concern of 1 ppm. Almost all reproductive failures in bluebirds were caused by predation, rather than decreased hatchability or nestling mortality. It is possible that mercury-laden birds are less successful at defending their territories from predators, but not likely in this study since predation occurred on both contaminated and reference sites.

9.0 Conclusions

This is the first study, to my knowledge, that has specifically examined the relationship of blood mercury with plumage in a free-living passerine. All other research on the role of plumage as an excretory route for mercury has been on large-

bodied seabirds, loons or wading birds. This phenomenon had not yet been directly studied in passerine bird species. Currently there is increasing concern about mercury accumulation in terrestrial songbirds, and the risk may be increased during the post-fledging period (Rimmer et al. 2005; Cristol unpubl. data; Friedman 2007). My results indicate that fledgling blood mercury level is predicted by feather growth. Thus, fledgling birds face increasing mercury loads in their internal tissues at the most sensitive life stage. However, I also found that fledgling blood mercury levels did not reach typical adult levels before they were "rescued" via the first pre-basic molt. The pattern of blood mercury is not related to a dietary shift, since $\delta^{15}N$ showed a different pattern over time than blood mercury. The relationship that I found by following young songbirds throughout the fledgling period is similar to that gleaned from numerous direct and indirect studies in the seabird and wading literature.

APPENDICES

Appendix A. Different units of mercury concentration, equivalents of ppm and ppb.

1 ppm=1000 ppb
1 ppb = 0.001 ppm

ppm	ppb
mg/kg	µg/kg
µg/g	ng/g
ng/mg	
ug/g	

Appendix B. List of all IHY birds with transmitters throughout the season.

Transmitter Id	Frequency	Box	Site	Date	Band
1	150.997	14	GRCP	5-May	1961-41314
1	150.997	225	AUFC	13-May	1961-41350
2	151.272	14	GRCP	5-May	1961-41313
2	151.272	191	AUFC	13-May	1961-41340
3	151.372	12	GCP	5-May	1961-41309
4	151.480	12	GCP	5-May	1961-41311
5	151.609	14	GRCP	5-May	1961-41316
5	151.609	199	AUFC	13-May	1961-41345
6	151.781	36	WH20	11-May	1961-41331
7	151.109	12	GRCP	5-May	1961-41312
8	151.519	14	GRCP	5-May	1961-41317
8	151.519	9	GRCP	20-May	2251-70332
9	151.201	12	GRCP	5-May	1961-41310
10	151.701	14	GRCP	5-May	1961-41315
10	151.701	233	AUFC	17-May	2251-70303
11	151.761	199	AFC	13-May	1961-41348
12	151.242	36	WTP	11-May	1961-41330
13	151.500	36	WTP	11-May	1961-41329
14	151.562	168	GENI	16-May	1961-41387
14	151.562	228	AUFC	13-Jun	2251-70352
15	151.021	195	AFC	13-May	1961-41341
16	151.312	195	AFC	13-May	1961-41342
17	151.742	191	AFC	12-May	1961-41336
18	151.460	168	GENI	16-May	1961-41385
18	151.460	195	AUFC	5-Jun	1961-41342
19	151.039	225	AUFC	13-May	1961-41351
19	151.039	9	GRCP	5-Jun	2251-70333
20	151.289	9	GCP	5/20/06	2251-70331
21	151.080	233	AFC	5/17/06	2251-70304
22	151.389	9	GCP	5/20/06	2251-70330
23	151.680	226	AFC	5/22/06	2251-70339
24	151.330	101	BAPA	5/16/06	1961-41382
25	151.130	101	BAPA	5/16/06	1961-41380
26	151.220	166	GENI	5/20/06	2251-70327
27	151.358	199	AFC	6/8/06	1961-41344
28	151.430	226	AFC	5/22/06	2251-70338

Appendix B
continued.

Transmitter Id	Frequency	Box	Site	Date	Band
29	151.640	226	AFC	5/22/06	2251-70340
30	151.541	166	GENI	5/20/06	2251-70326
31	150.821	191	AFC	7/7/06	2251-70460
32	150.828	161	AFC	6/30/06	2251-70371
33	150.841	9	GCP	7/21/06	2251-70458
34	150.864	226	AFC	7/23/06	2251-70340
35	150.881	36	WTP	7/16/06	1961-41329
37	150.898	195	AFC	7/16/06	1961-41343
38	150.910	195	AFC	7/30/06	2251-70463
39	150.921	191	AFC	7/7/06	2251-70461
40	150.940	191	AFC	7/7/06	2251-70459
41	150.953	9	GCP	7/7/06	2251-70330
42	150.960	12	GCP	7/3/06	2251-70418
43	150.968	101	BAPA	6/30/06	1961-41382
44	150.980	12	GCP	7/3/06	2251-70419
45	150.990	233	AFC	6/28/07	2251-70303

Appendix C. Five sites where IHY bluebirds were trapped.

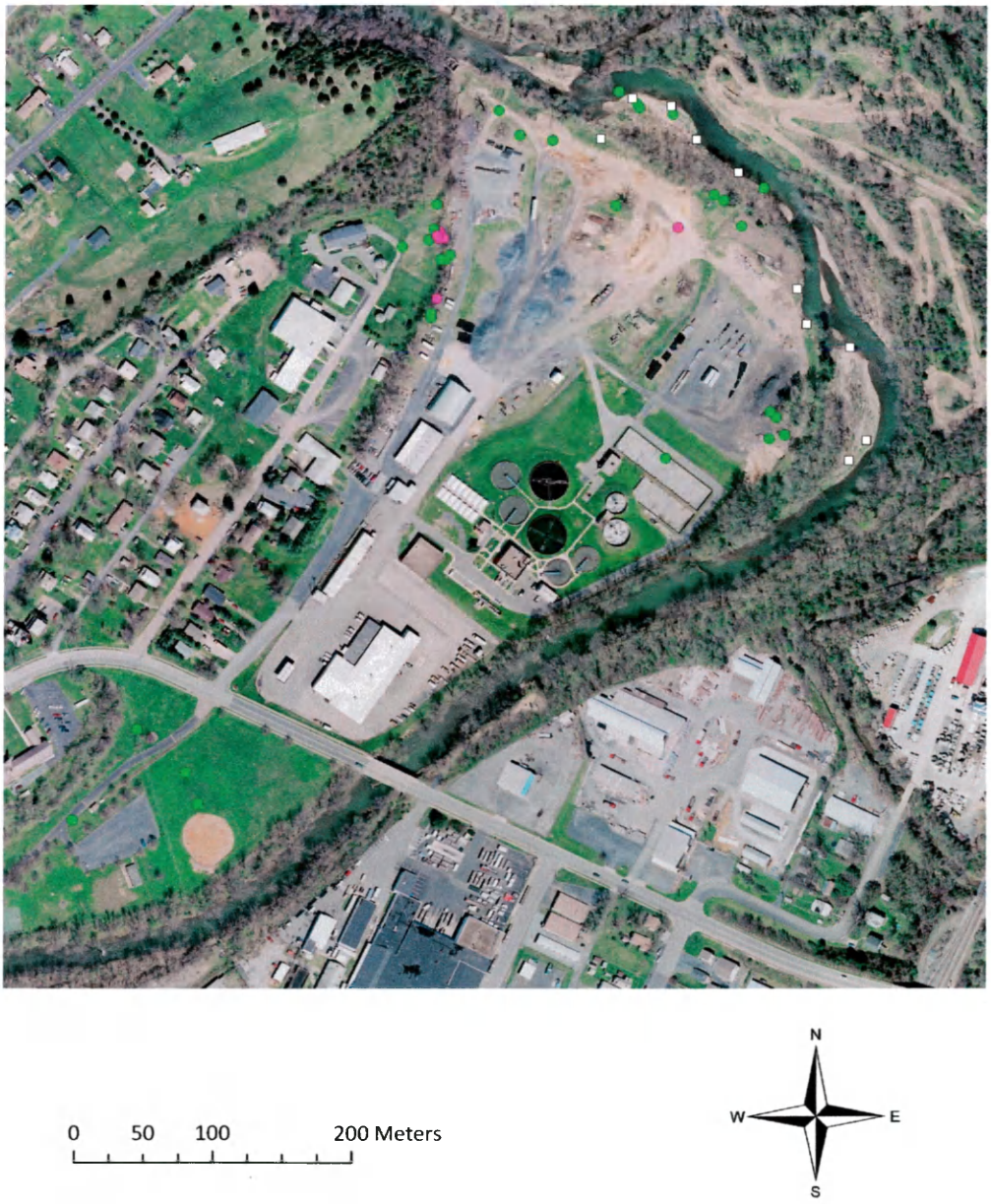

Figure C-1. Waynesboro Water Treatment Plant. Pink circles indicate net sites of capture. Red circles indicate where an IHY was found dead. White boxes are nest boxes on site

Figure C-2. Basic Park. Green circles indicate IHY locations. Pink circles indicate net sites of capture. Red circles indicate where an IHY was found dead. White boxes are nest boxes on site

Figure C-3. Genicom. Green circles indicate IHY locations. Pink circles indicate net sites of capture. Red circles indicate where an IHY was found dead. White boxes are nest boxes on site

Figure C-4. Augusta Forestry Center. Green circles indicate IHY locations. Pink circles indicate net sites of capture. Red circles indicate where an IHY was found dead. White boxes are nest boxes on site

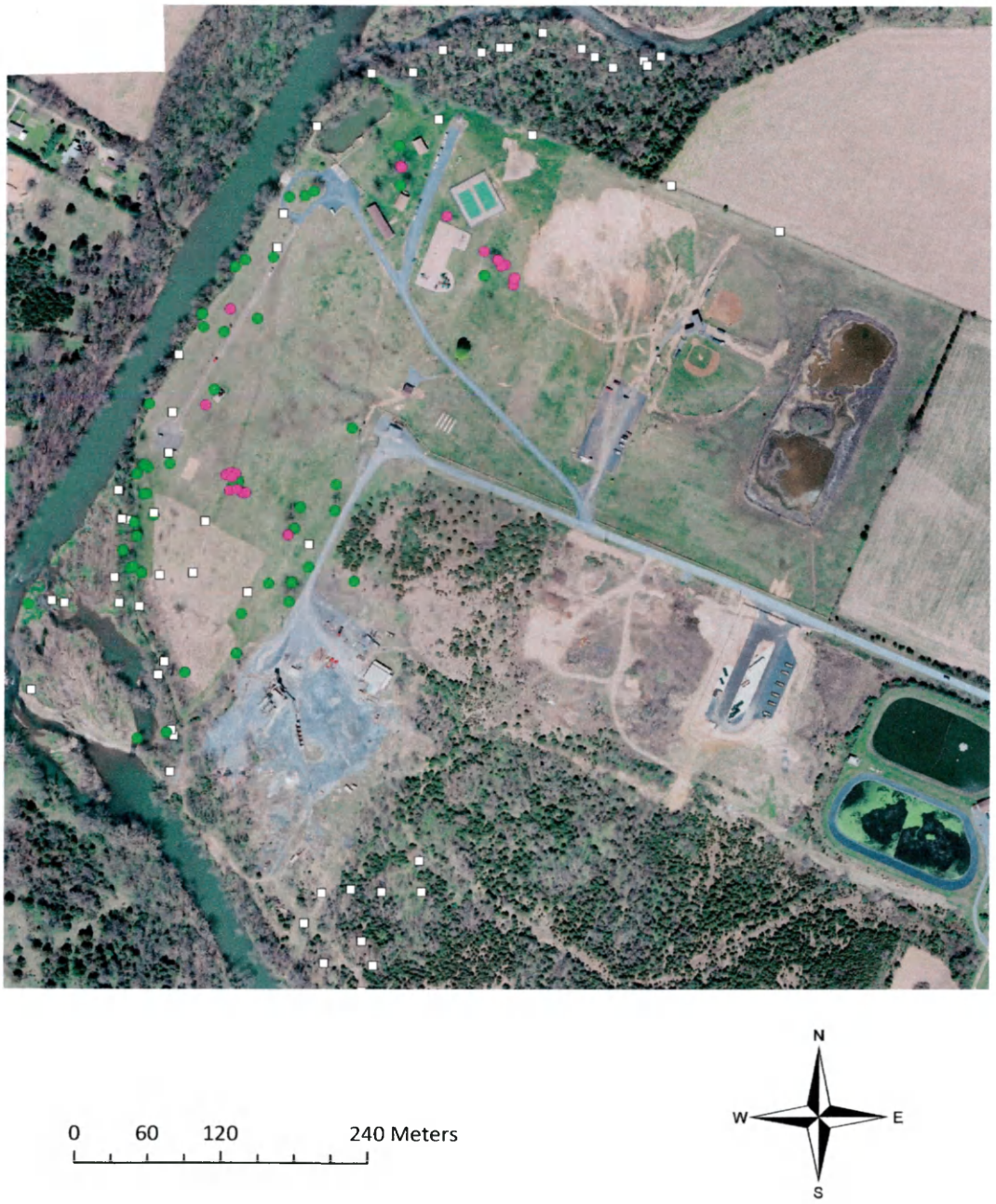

Figure C-5. Grottoes City Park. Green circles indicate IHY locations. Pink circles indicate net sites of capture. Red circles indicate where an IHY was found dead. White boxes are nest boxes on site.

Appendix D. Blood mercury (ppm) of IHY individuals over time by site, box and year. Only boxes that had individuals with at least 2 captures are presented.

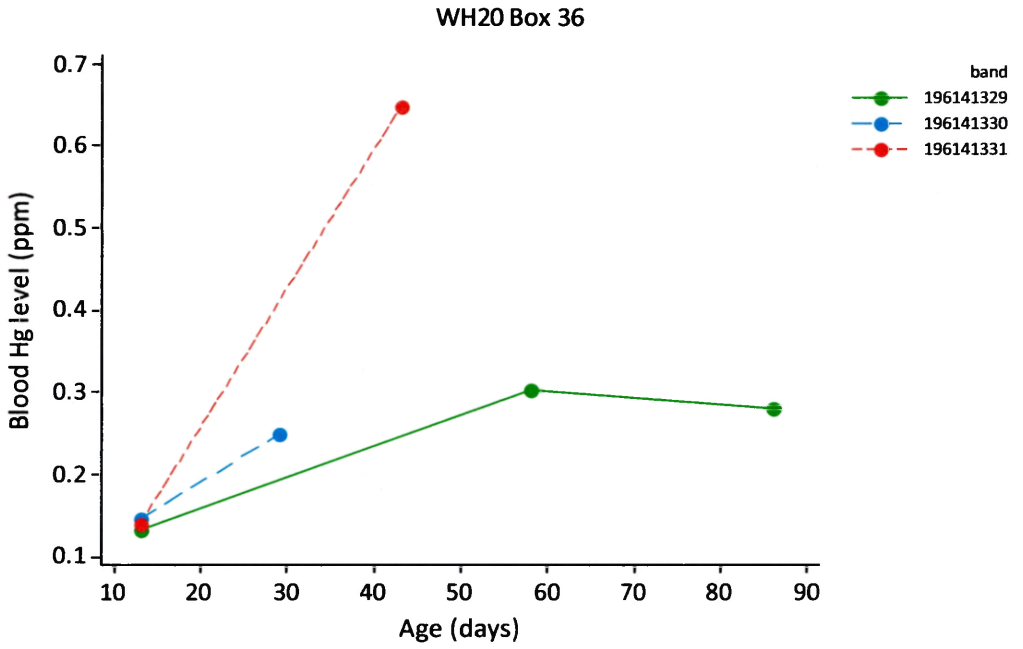

Figure D-1. Blood mercury of IHY individuals from Box 36, Water treatment plant, 2006, first clutch.

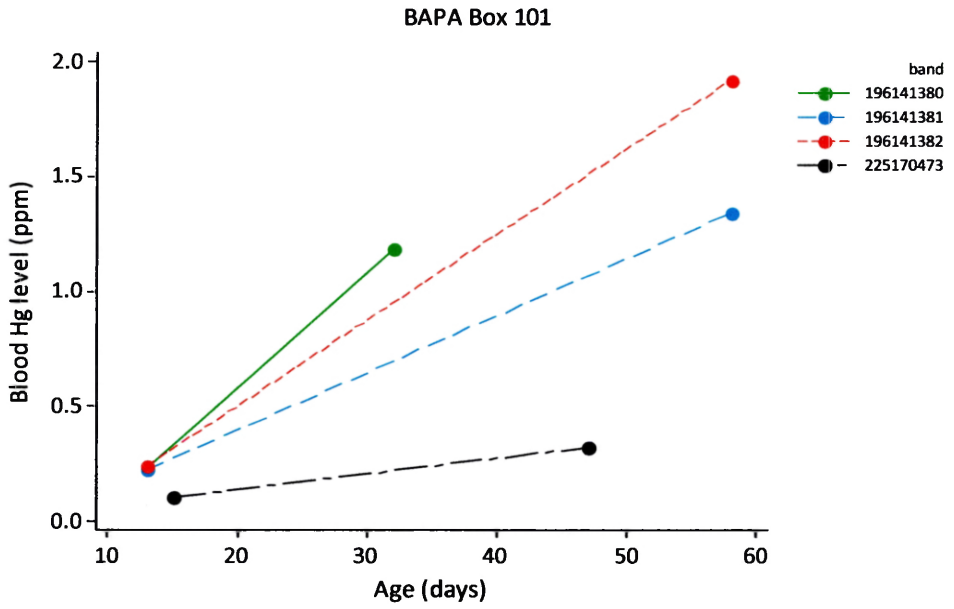

Figure D-2. Blood mercury of IHY individuals from Box 101, Basic Park, 2006, first and second clutch.

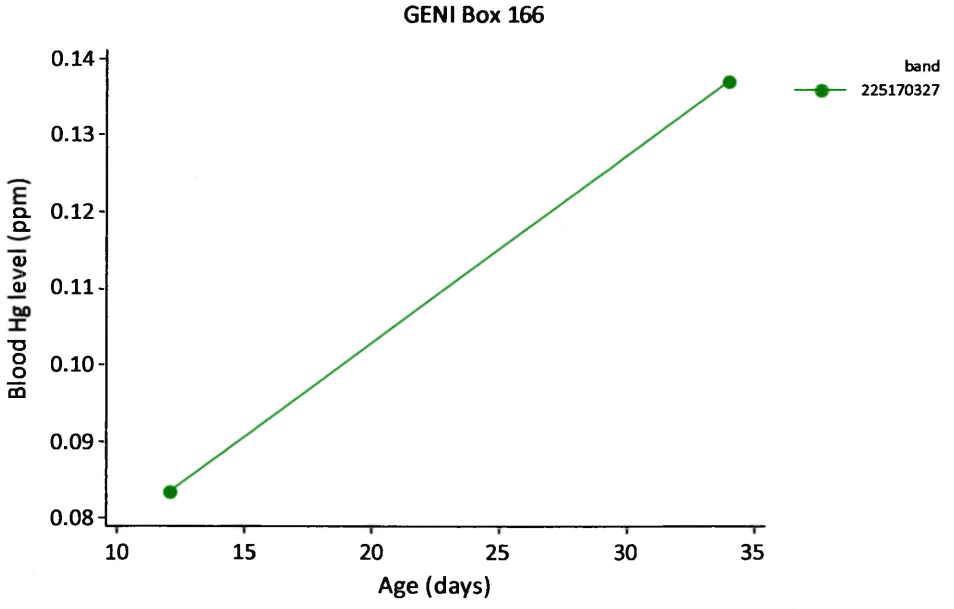

Figure D-3. Blood mercury of IHY individual from Box 166, Genicom, 2006, first clutch.

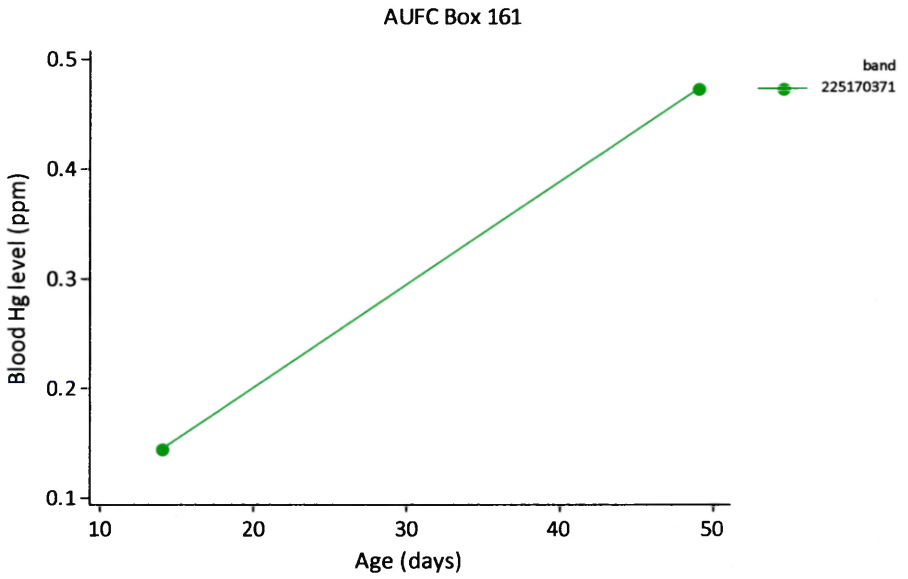

Figure D-4. Blood mercury of IHY individual from Box 161, Augusta Forestry Center, 2006, second clutch.

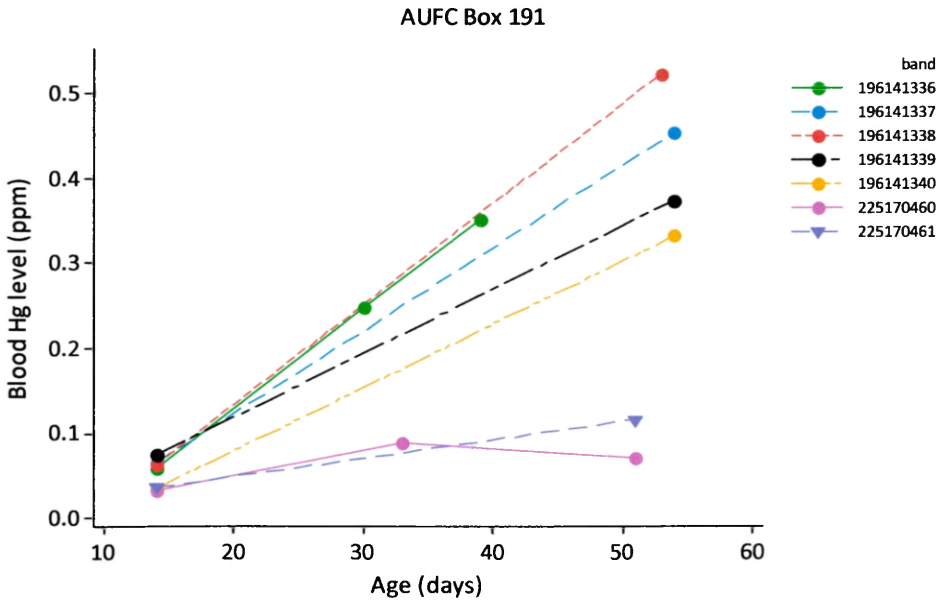

Figure D-5. Blood mercury of IHY individuals from Box 191, Augusta Forestry Center, 2006, first and second clutch.

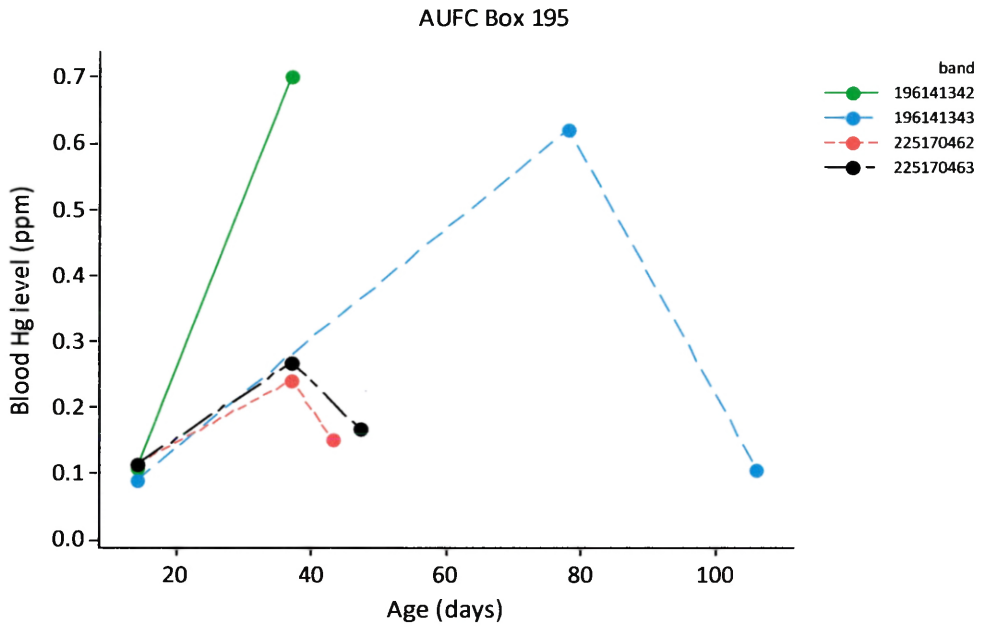

Figure D-6. Blood mercury of IHY individuals from Box 195, Augusta Forestry Center, 2006, first and second clutch

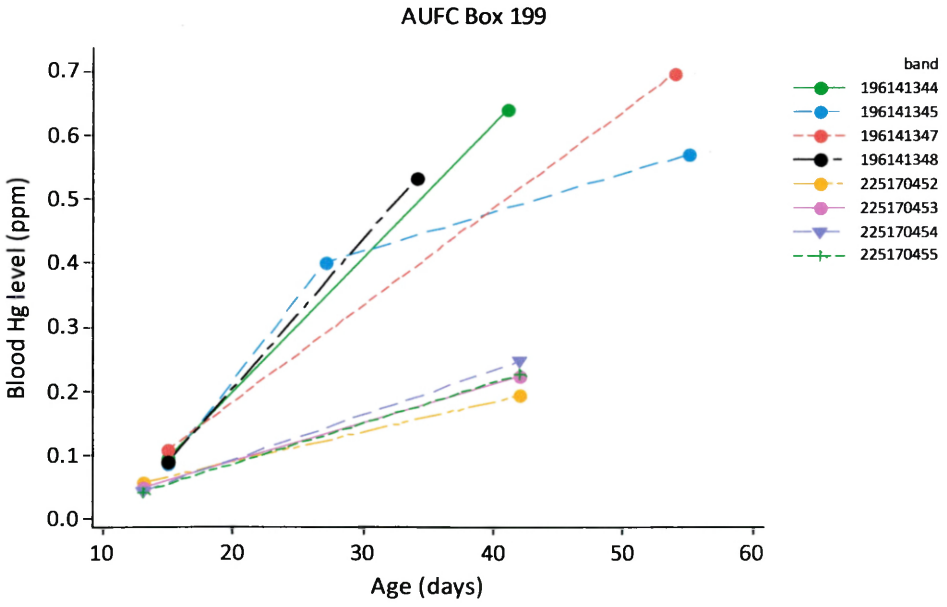

Figure D-7. Blood mercury of IHY individuals from Box 199, Augusta Forestry Center, 2006, first and second clutch.

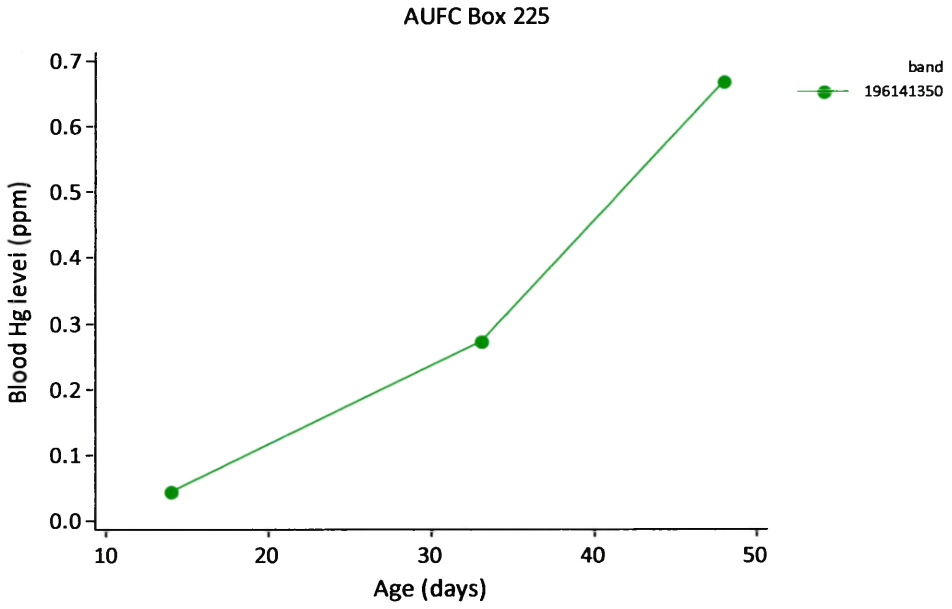

Figure D-8. Blood mercury of IHY individuals from Box 225, Augusta Forestry Center, 2006, first clutch.

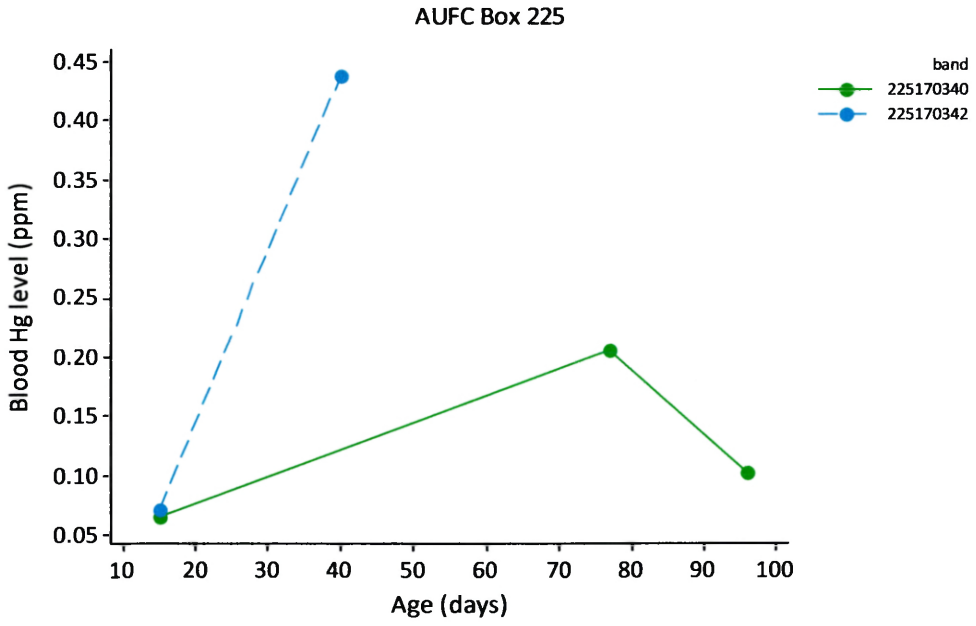

Figure D-9. Blood mercury of IHY individuals from Box 226, Augusta Forestry Center, 2006, first clutch.

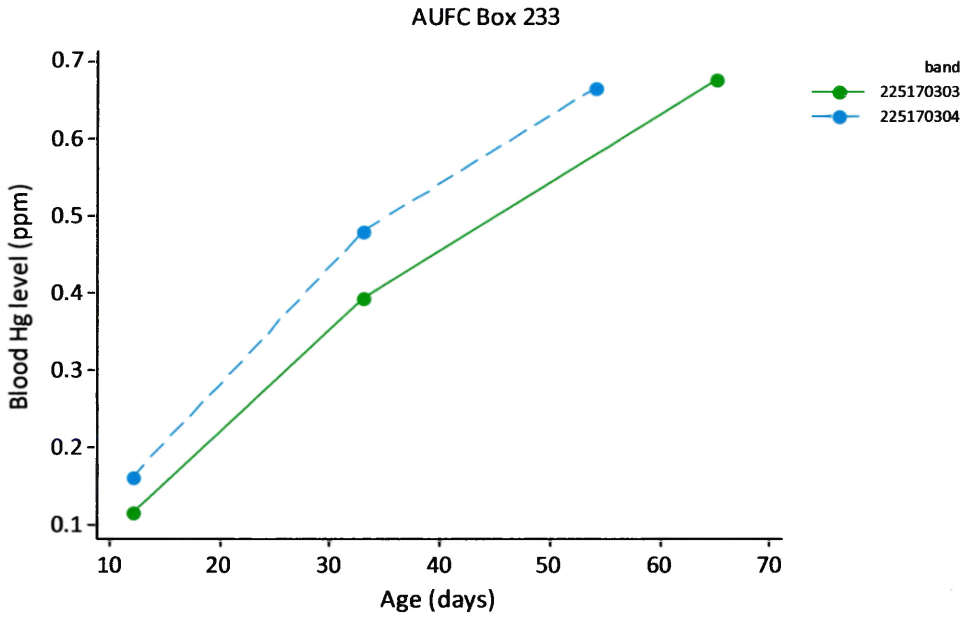

Figure D-10. Blood mercury of IHY individuals from Box 233, Augusta Forestry Center, 2006, first clutch.

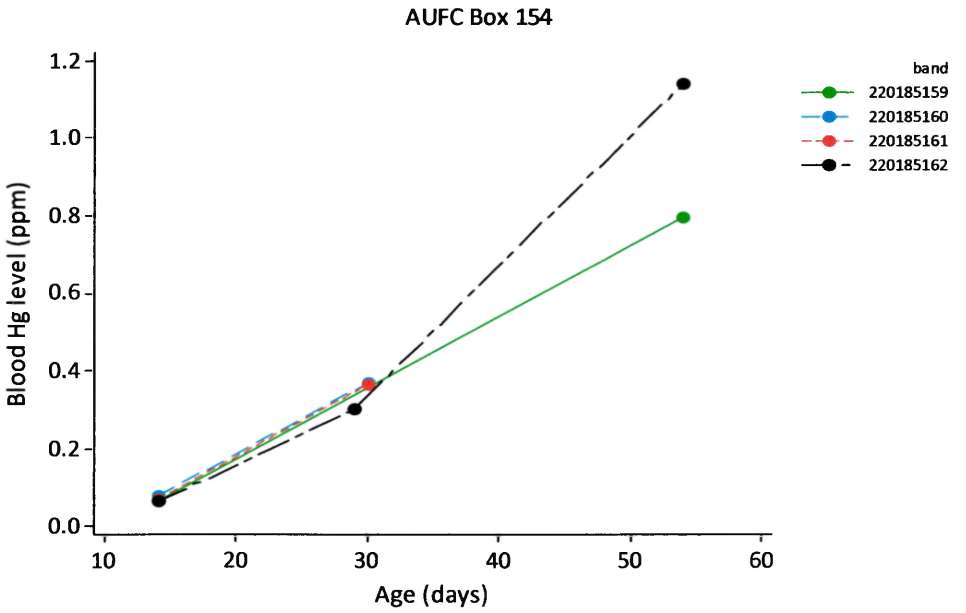

Figure D-11. Blood mercury of IHY individuals from Box 154, Augusta Forestry Center, 2007, first clutch.

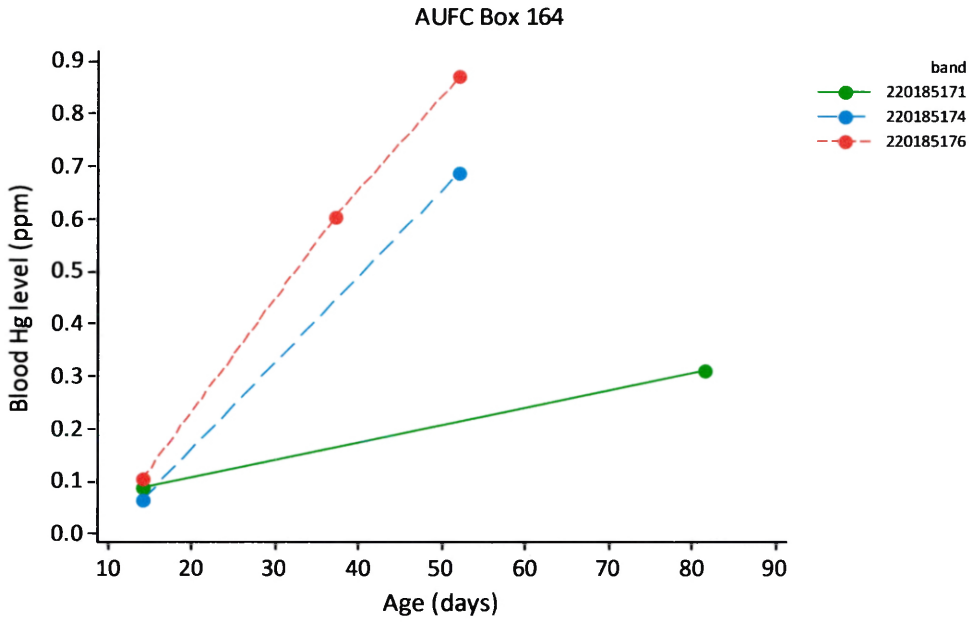

Figure D-12. Blood mercury of IHY individuals from Box 164, Augusta Forestry Center, 2007, first clutch.

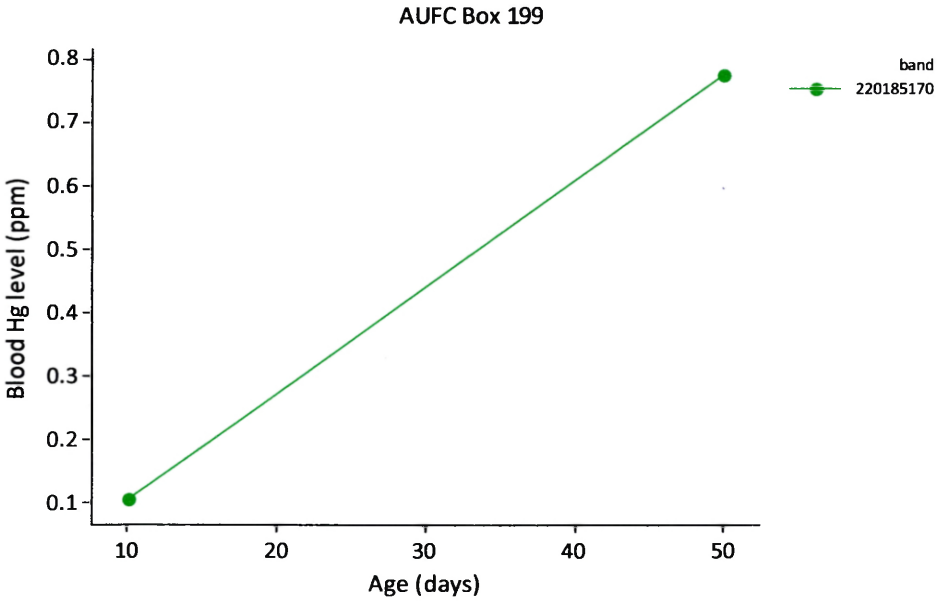

Figure D-13. Blood mercury of IHY individuals from Box 199, Augusta Forestry Center, 2007, first clutch.

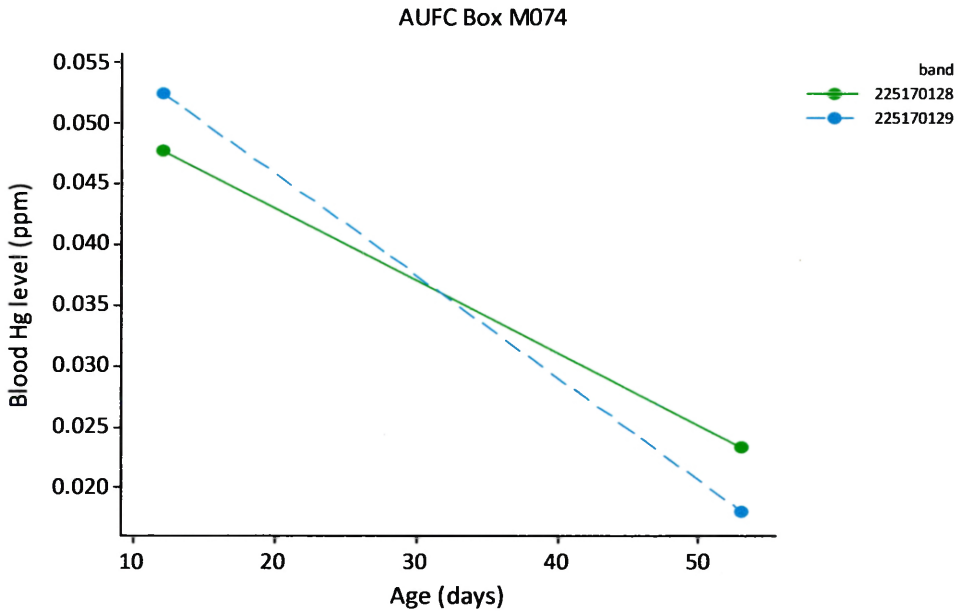

Figure D-14. Blood mercury of IHY individuals from Box M074, Augusta Forestry Center, 2007, second clutch.

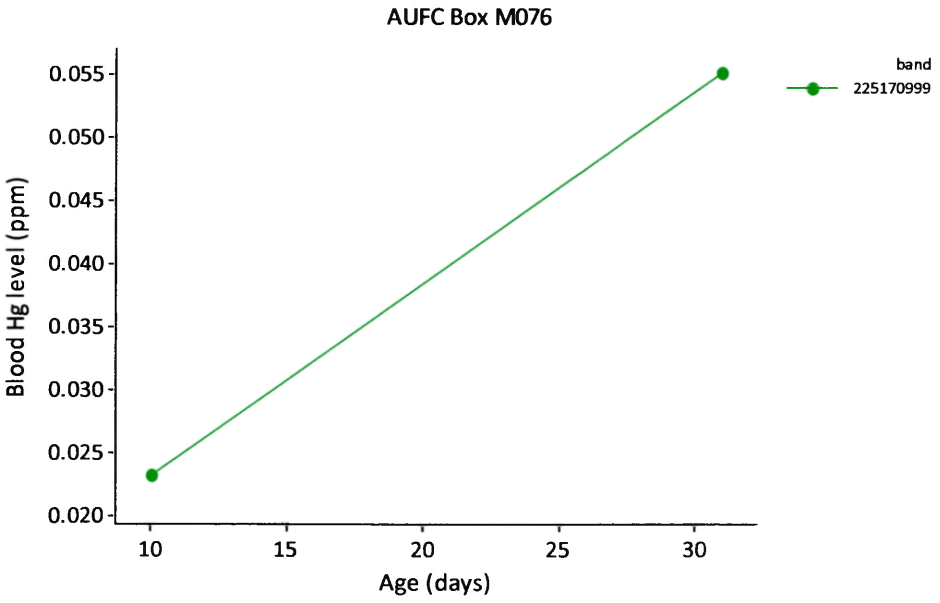

Figure D-15. Blood mercury of IHY individuals from Box M076, Augusta Forestry Center, 2007, second clutch.

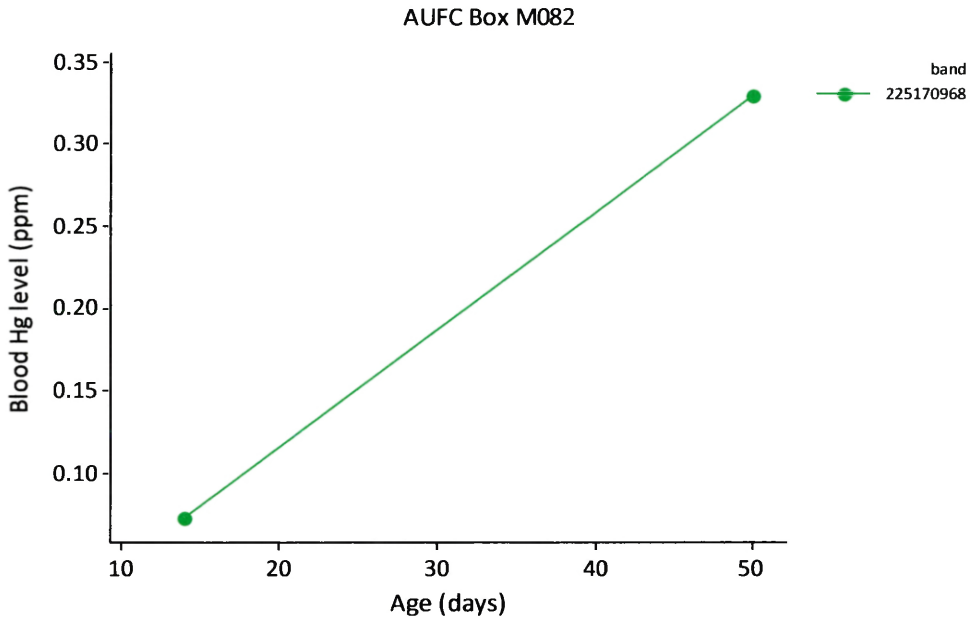

Figure D-16. Blood mercury of IHY individuals from Box M082, Augusta Forestry Center, 2007, second clutch.

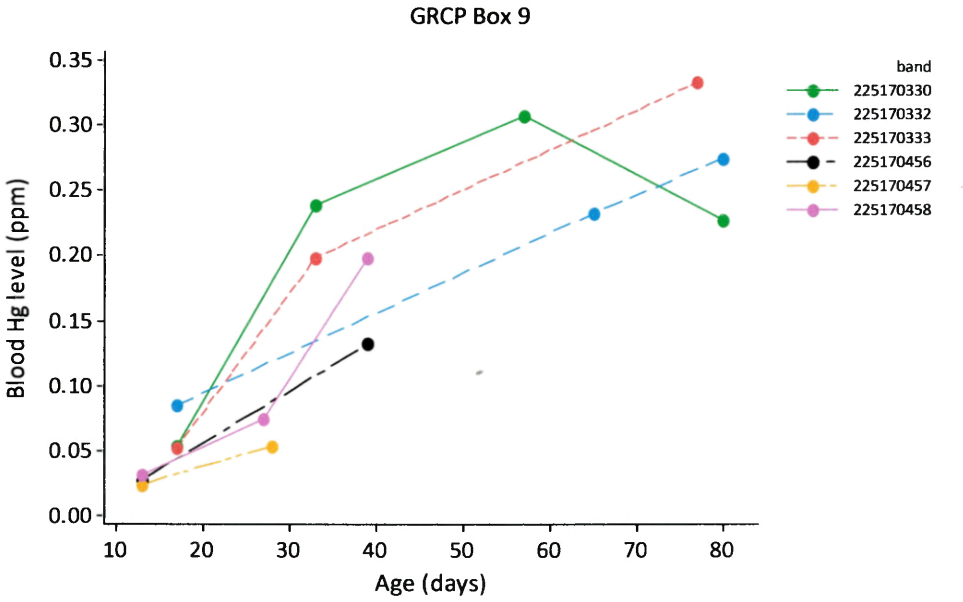

Figure D-17. Blood mercury of IHY individuals from Box 9, GRCP, 2006, first and second clutch.

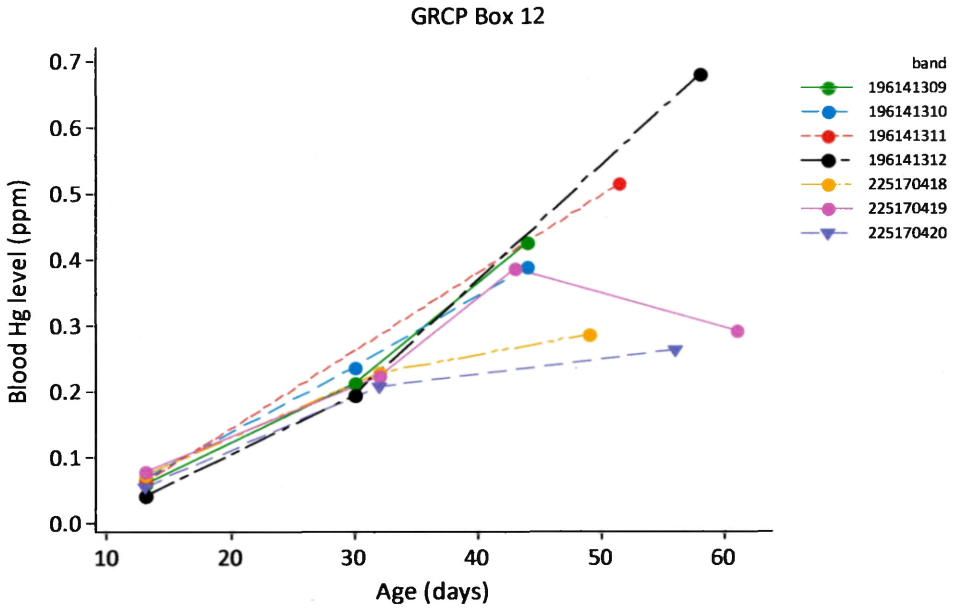

Figure D-18. Blood mercury of IHY individuals from Box 12, GRCP, 2006, first and second clutch.

Appendix E. Feather mercury (ppm) of IHY individuals over time by box, site and year. Only boxes that had individuals with at least 2 captures are presented.

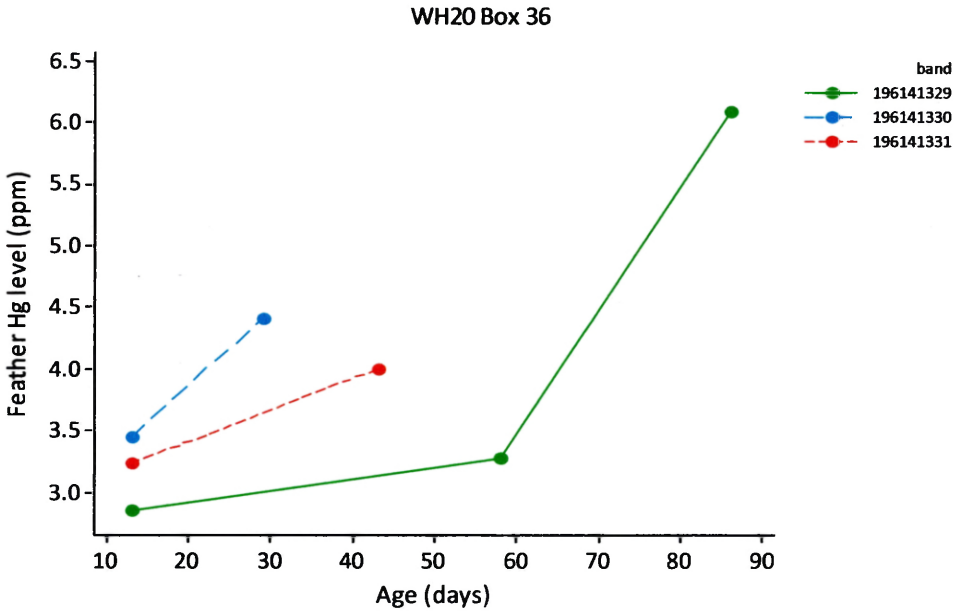

Figure E-1. Feather mercury of IHY individuals from Box 36, WH20, 2006, first clutch.

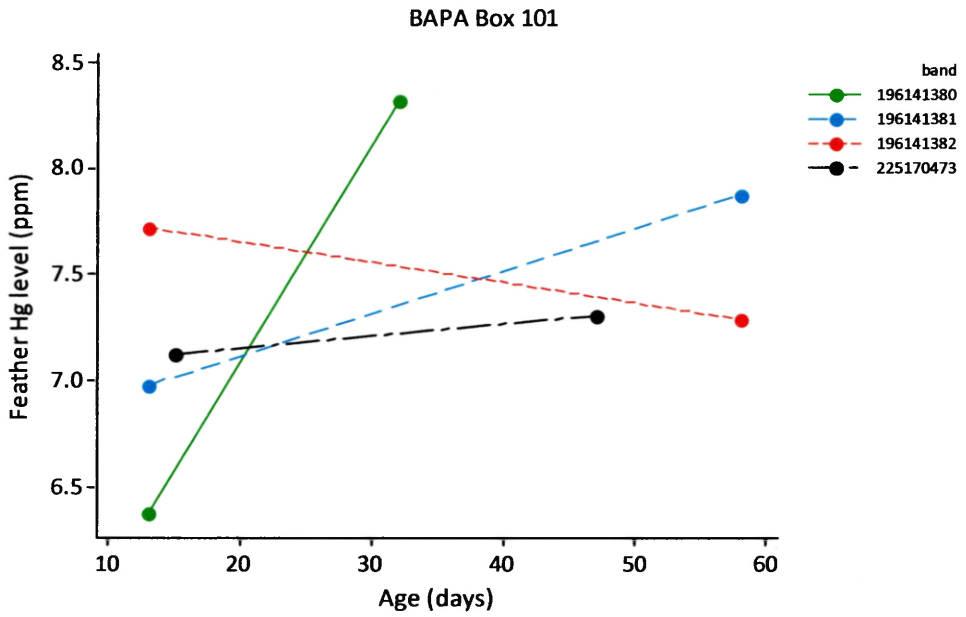

Figure E-2. Feather mercury of IHY individuals from Box 101, BAPA, 2006, first and second clutch.

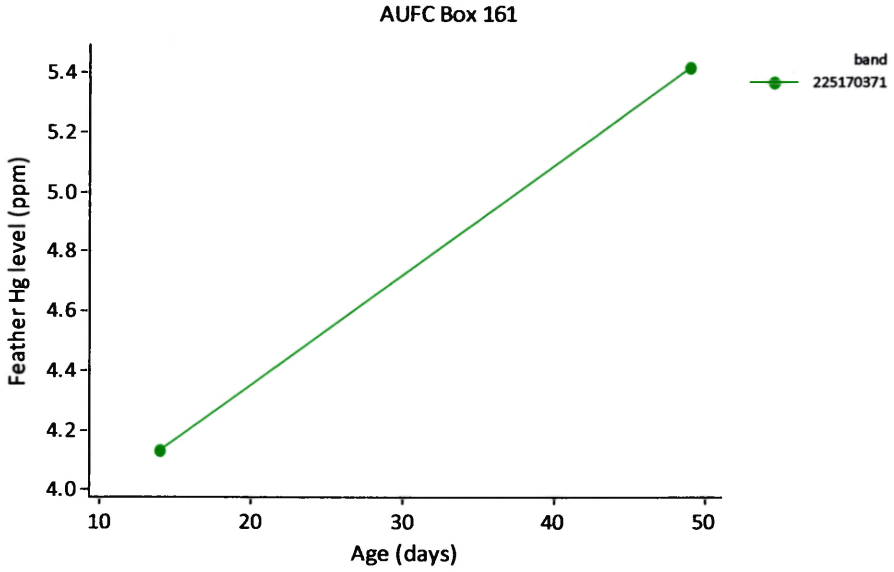

Figure E-3. Feather mercury of IHY individuals from Box 161, AUFC, 2006, second clutch.

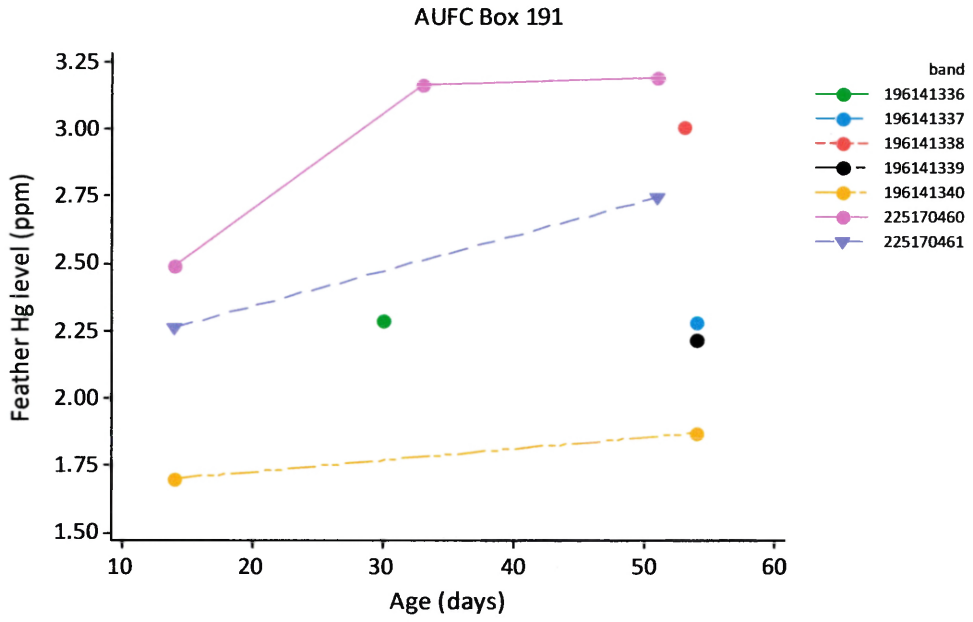

Figure E-4. Feather mercury of IHY individuals from Box 191, AUFC, 2006, first and second clutch.

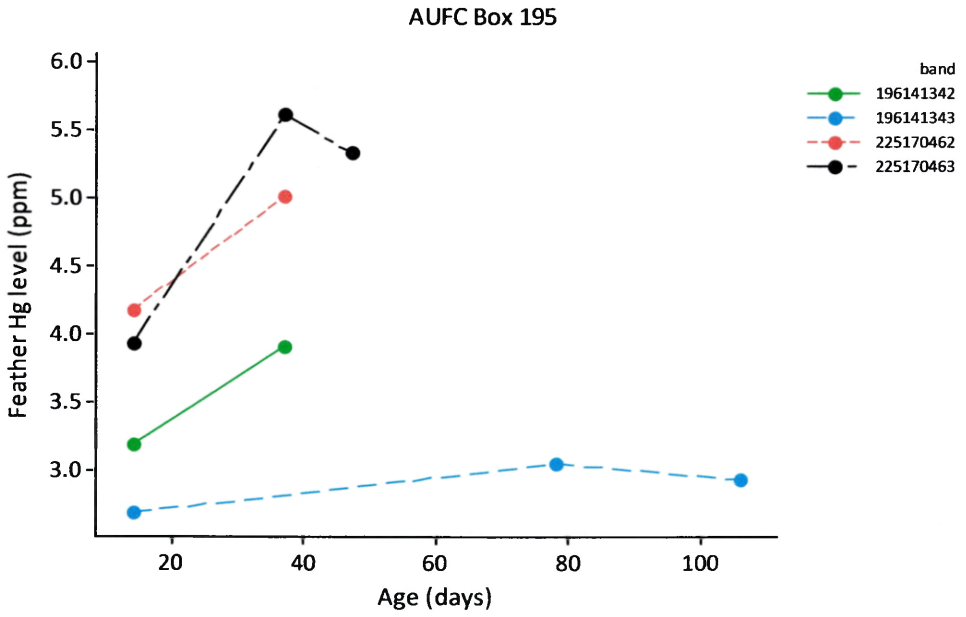

Figure E-5. Feather mercury of IHY individuals from Box 195, AUFC, 2006, first and second clutch.

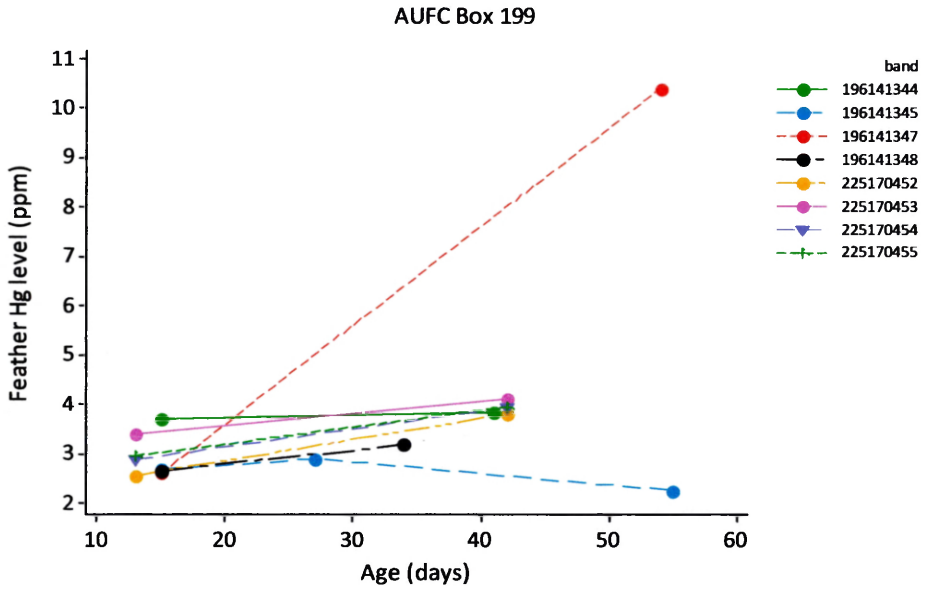

Figure E-6. Feather mercury of IHY individuals from Box 199, AUFC, 2006, first and second clutch.

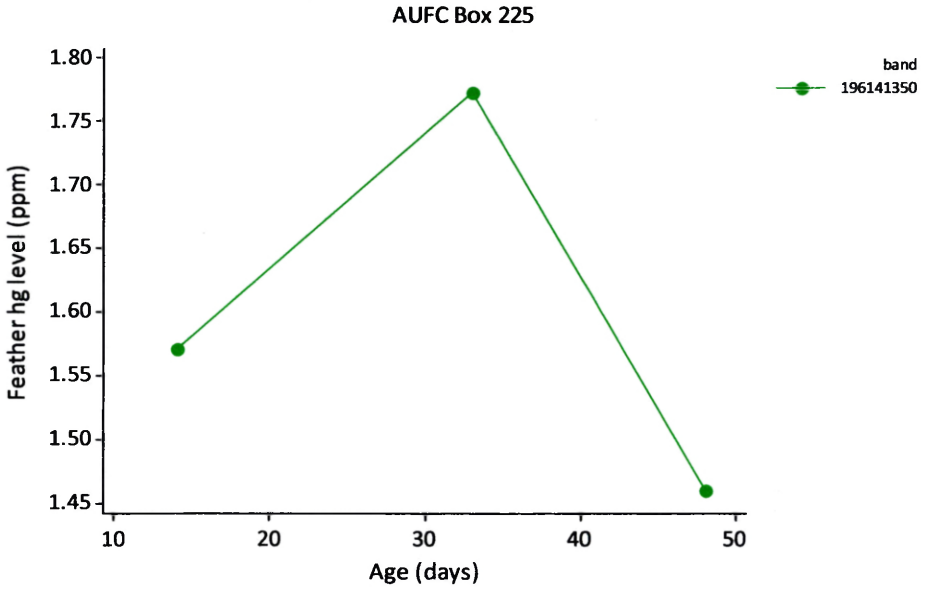

Figure E-7. Feather mercury of IHY individuals from Box 225, AUFC, 2006, first clutch.

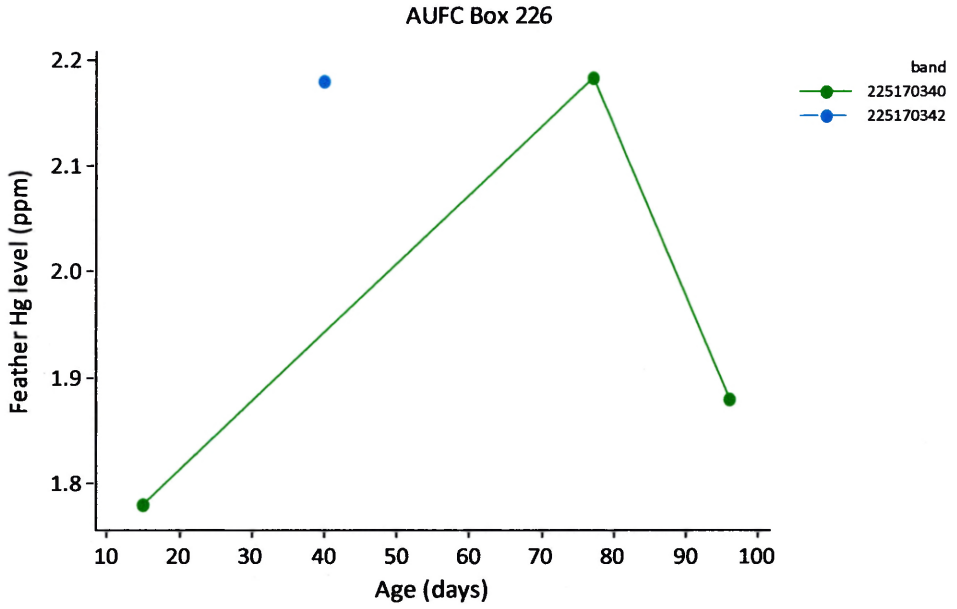

Figure E-8. Feather mercury of IHY individuals from Box 226, AUFC, 2006, first clutch.

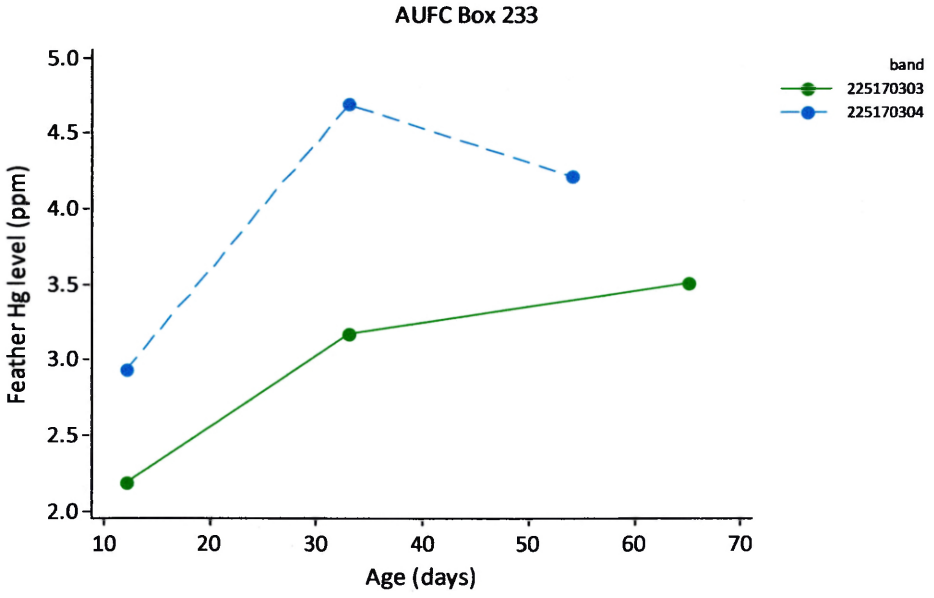

Figure E-9. Feather mercury of IHY individuals from Box 233, AUFC, 2006, first clutch.

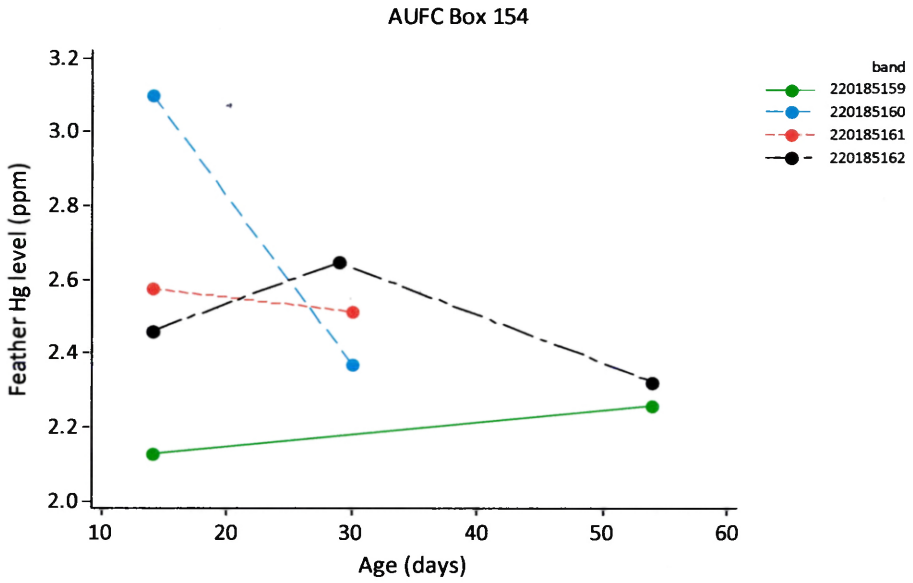

Figure E-10. Feather mercury of IHY individuals from Box 154, AUFC, 2007, first and second clutch.

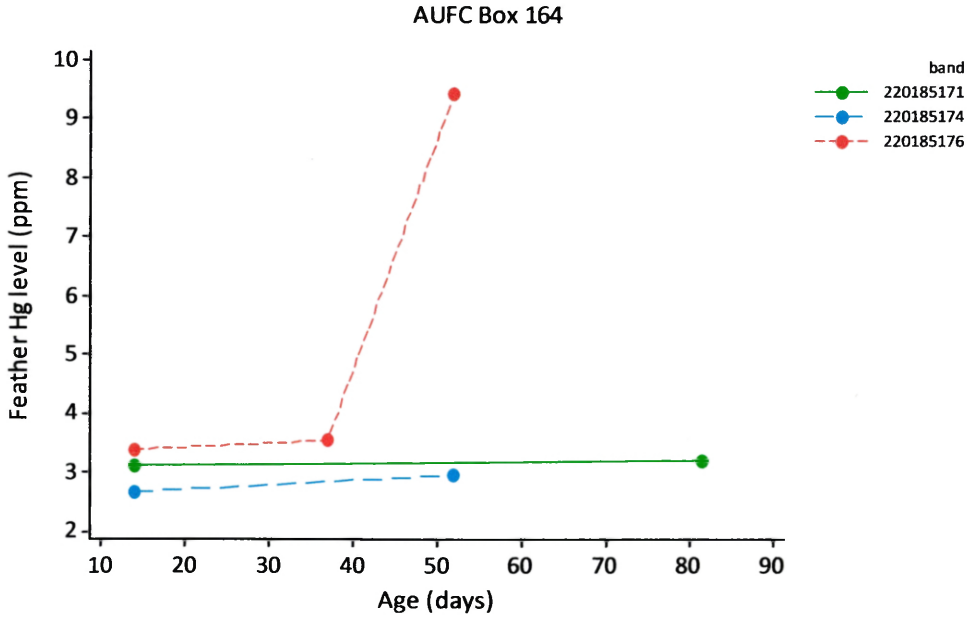

Figure E-11. Feather mercury of IHY individuals from Box 164, AUFC, 2007, first clutch.

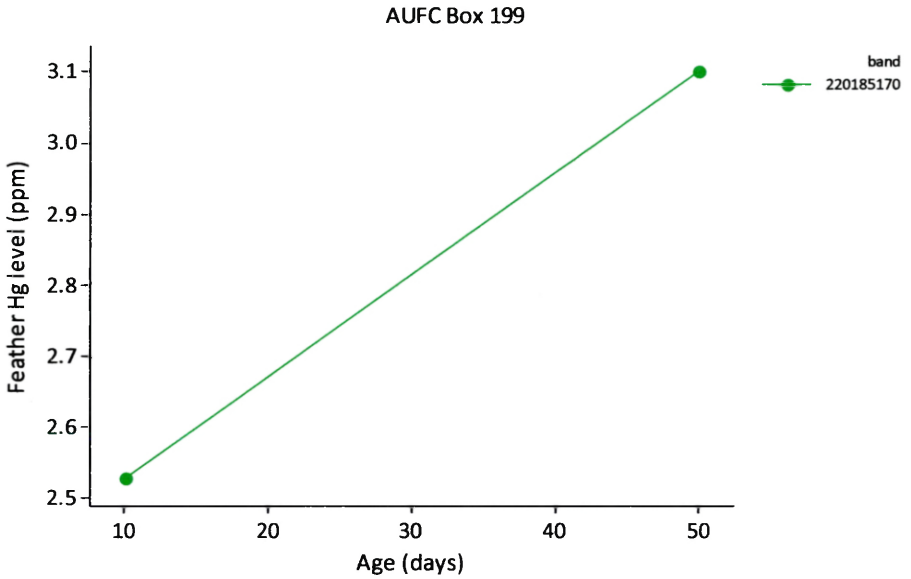

Figure E-12. Feather mercury of IHY individuals from Box 199, AUFC, 2007, first clutch.

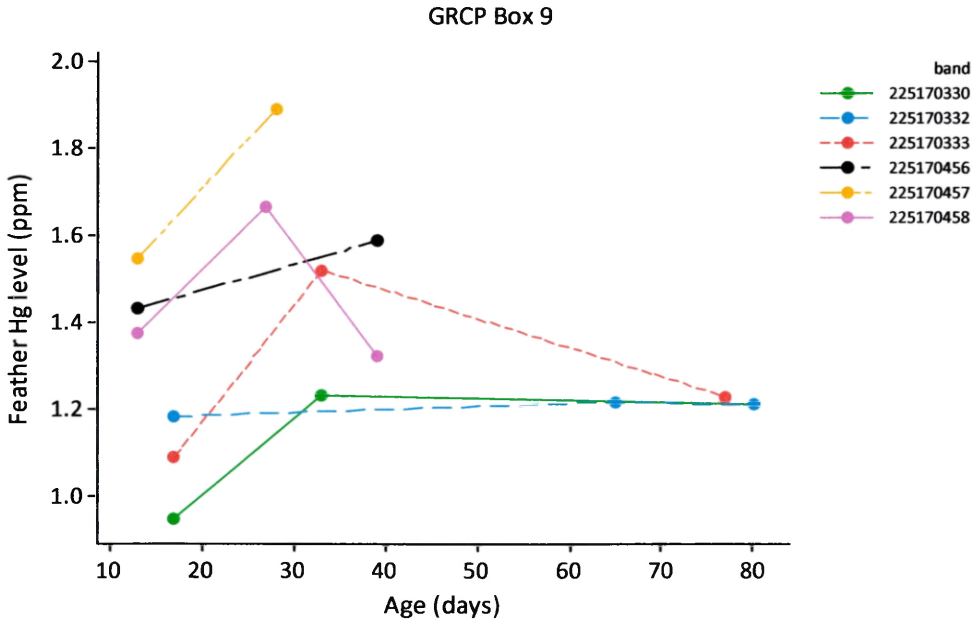

Figure E-13. Feather mercury of IHY individuals from Box 9, GRCP, 2006, first and second clutch.

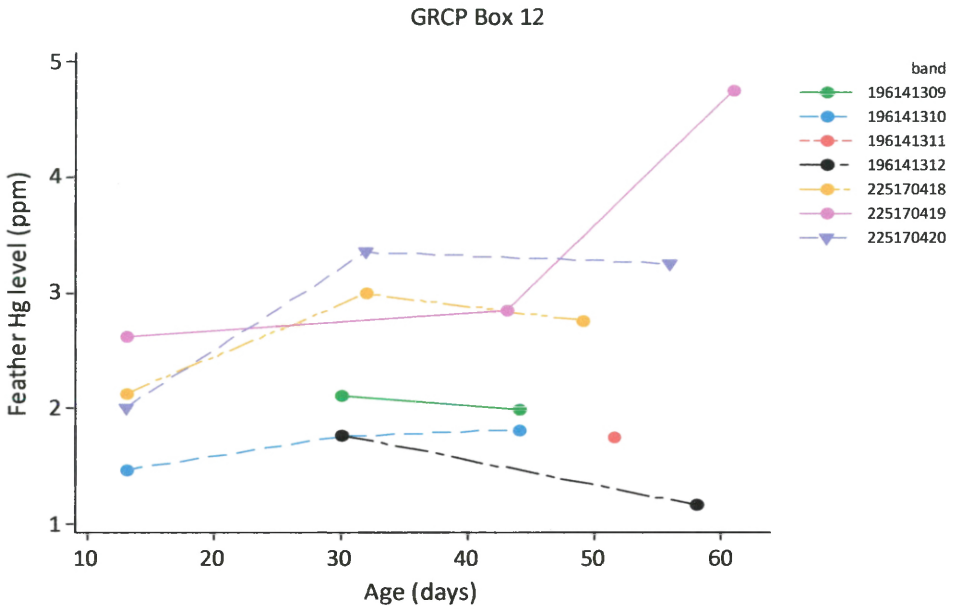

Figure E-14. Feather mercury of IHY individuals from Box 12, GRCP, 2006, first and second clutch.

Appendix F. $\delta^{15}N$ of IHY individuals over time by box, site and year. Only boxes that had individuals with at least 2 captures are presented.

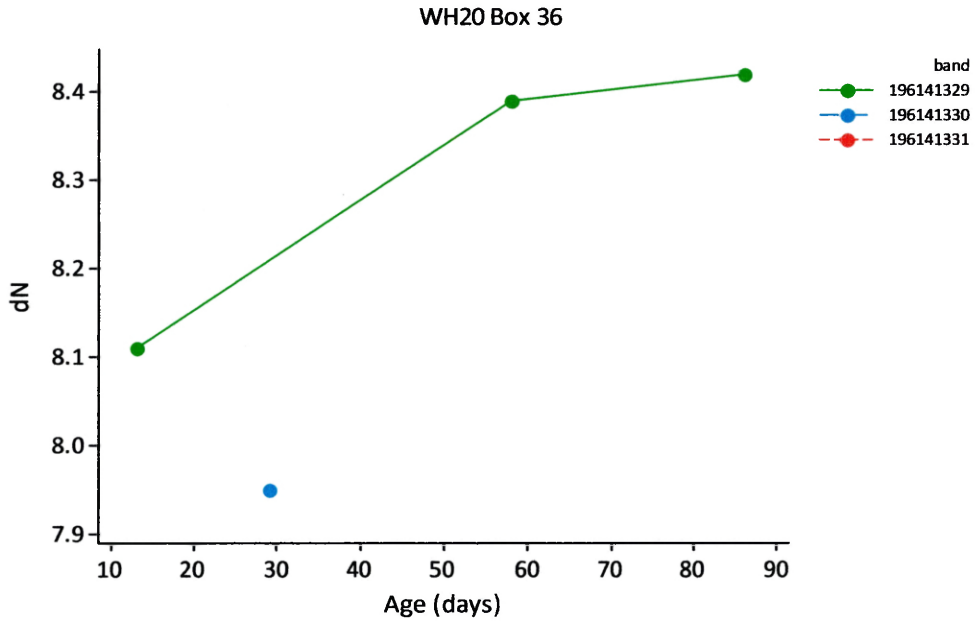

Figure F-1. IHY individuals and $\delta^{15}N$ over time from Box 36, WH20, 2006 first clutch.

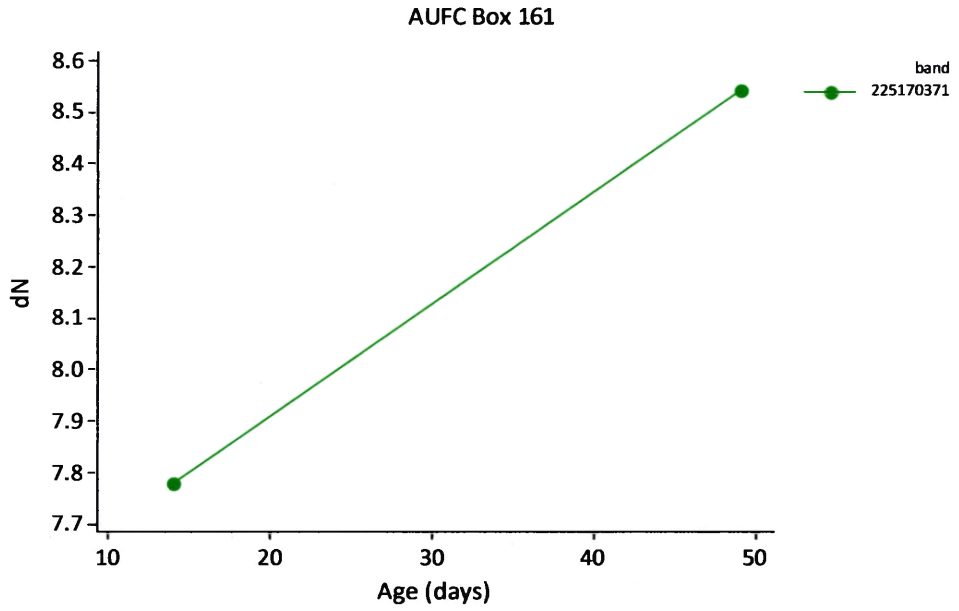

Figure F-2. IHY individuals and $\delta^{15}N$ over time from Box 161, AUFC, 2006 second clutch.

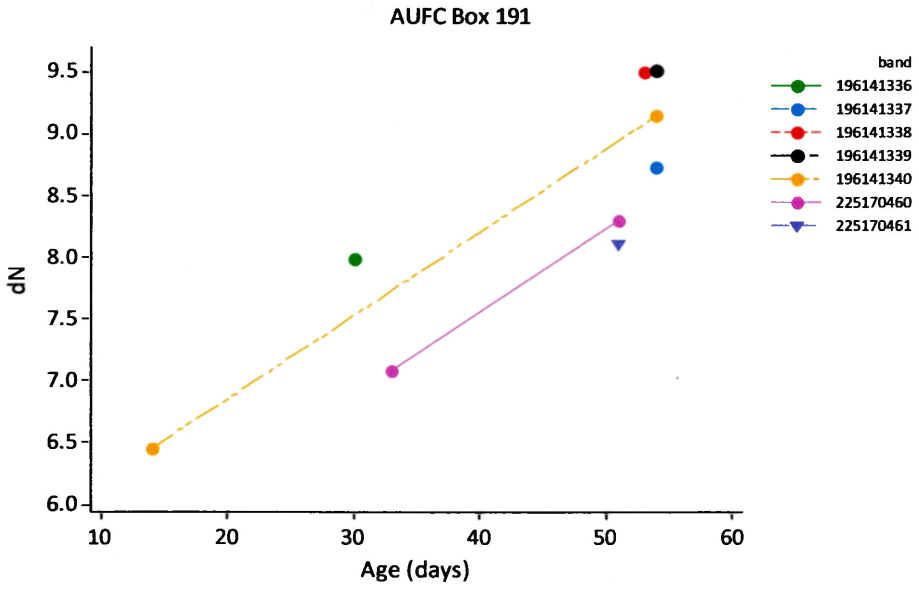

Figure F-3. IHY individuals and $\delta^{15}N$ over time from Box 191, AUFC, 2006 first and second clutch.

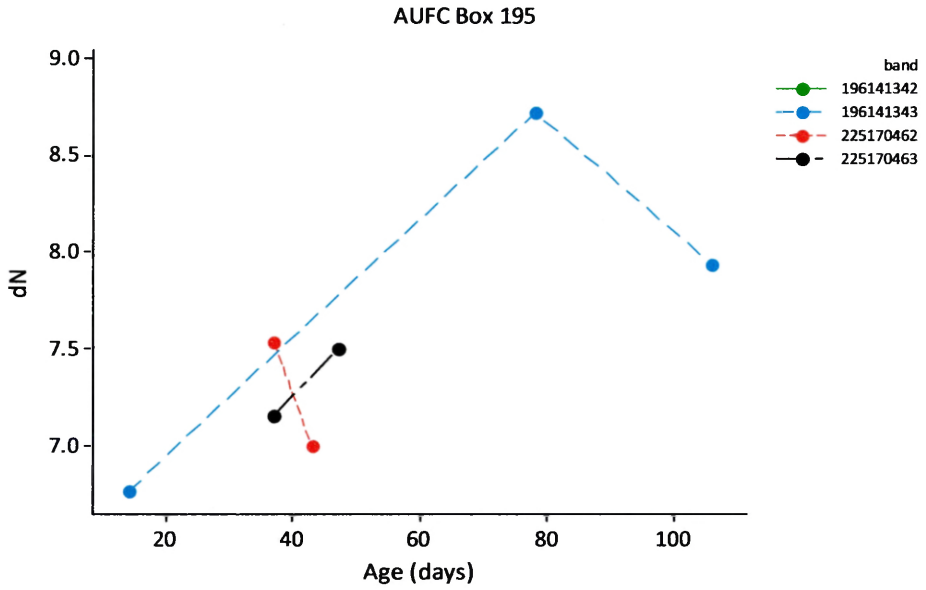

Figure F-4. IHY individuals and $\delta^{15}N$ over time from Box 195, AUFC, 2006 first and second clutch.

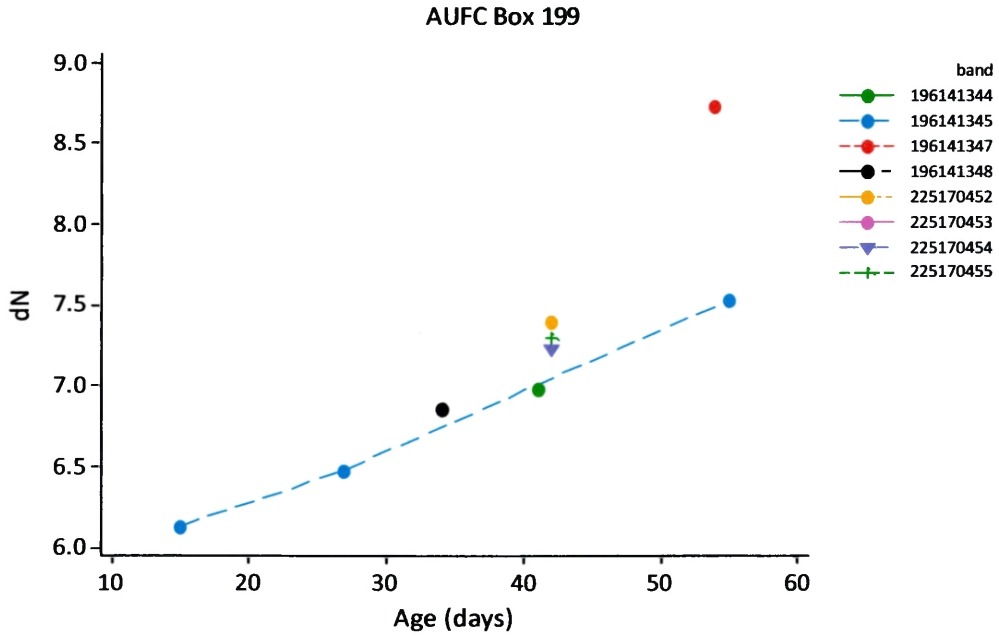

Figure F-5. IHY individuals and $\delta^{15}N$ over time from Box 199, AUFC, 2006 first and second clutch.

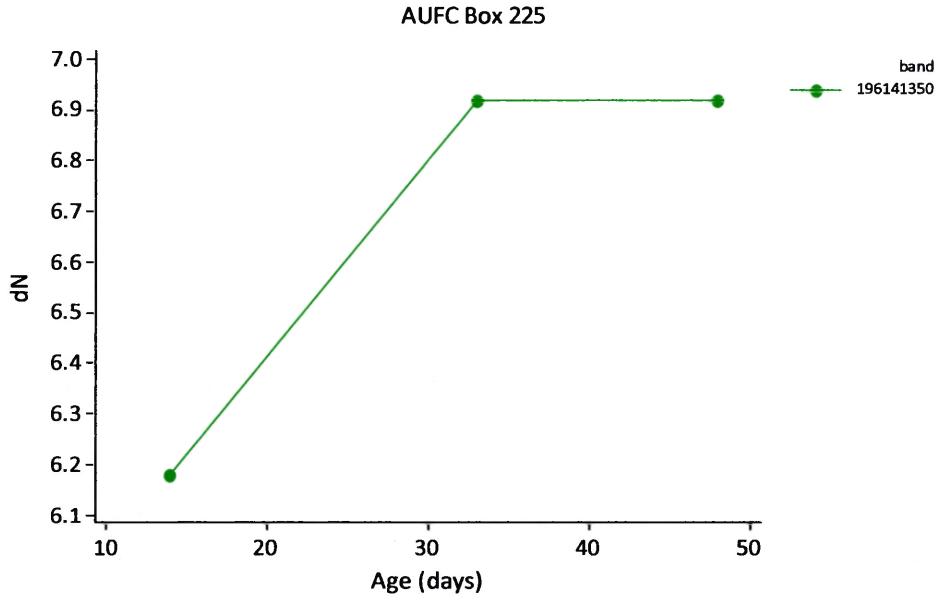

Figure F-6. IHY individuals and $\delta^{15}N$ over time from Box 225, AUFC, 2006 first clutch.

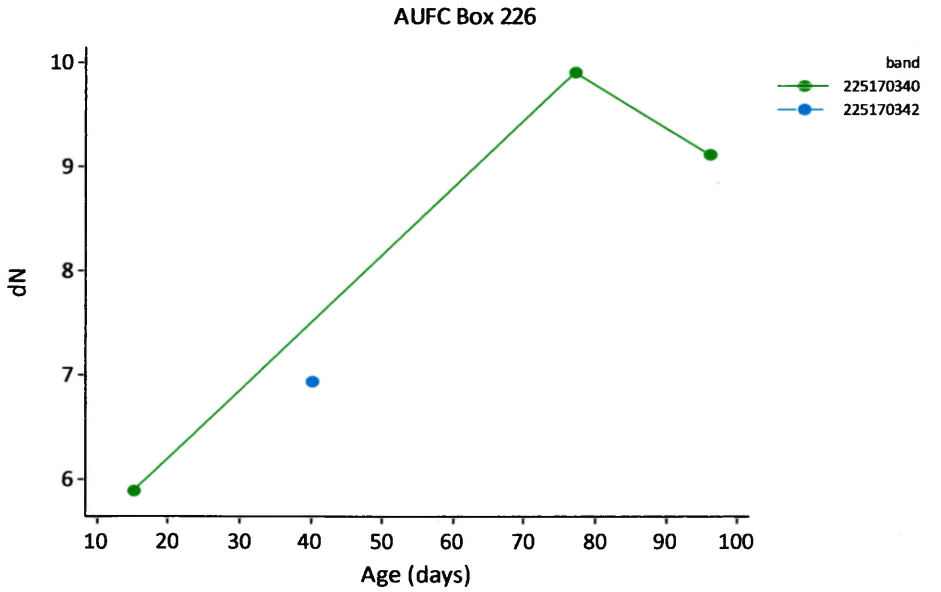

Figure F-7. IHY individuals and $\delta^{15}N$ over time from Box 226, AUFC, 2006 first clutch.

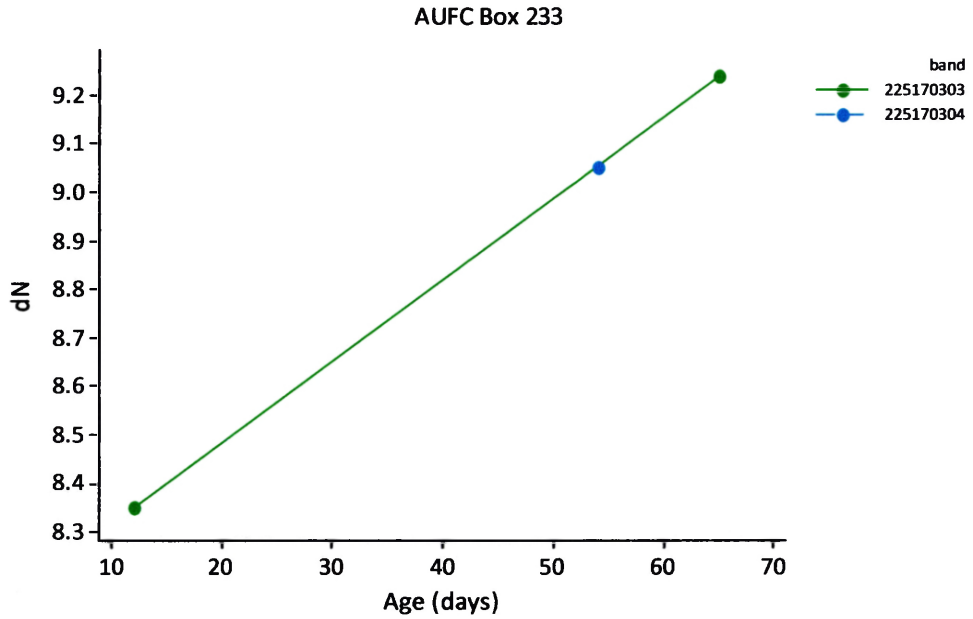

Figure F-8. IHY individuals and $\delta^{15}N$ over time from Box 233, AUFC, 2006 first clutch.

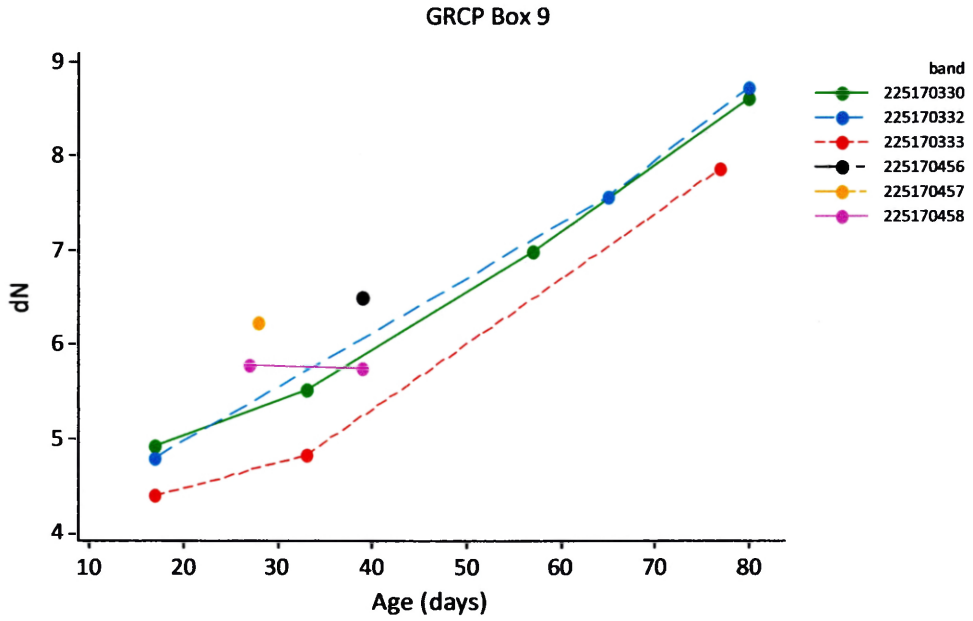

Figure F-9. IHY individuals and $\delta^{15}N$ over time from Box 9, GRCP, 2006 first and second clutch.

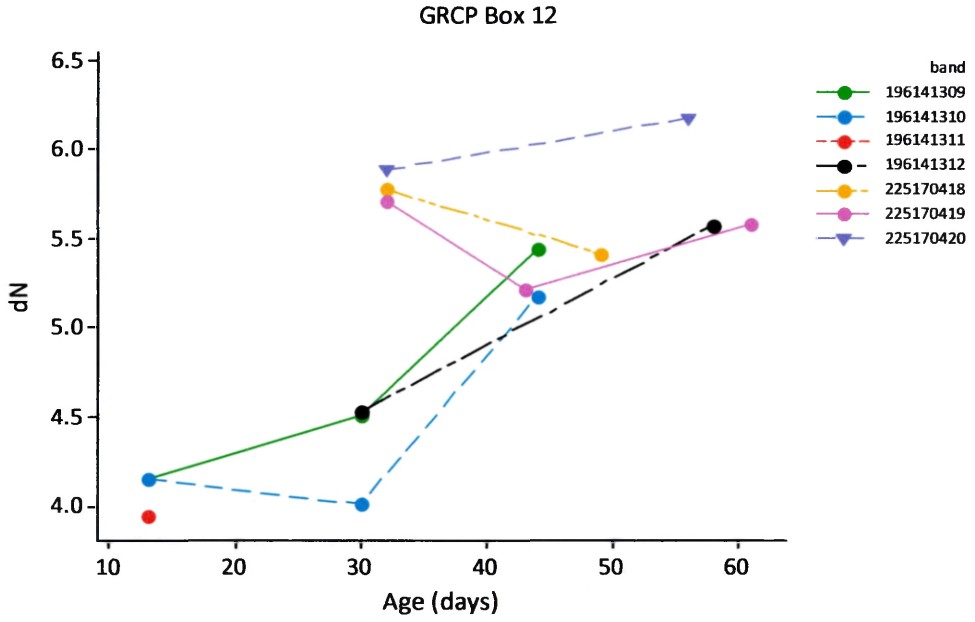

Figure F-10. IHY individuals and $\delta^{15}N$ over time from Box 12, GRCP, 2006 first and second clutch.

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