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The Effects of Mercury on the Nesting Success and Return Rate of Tree Swallows (Tachycineta bicolor)

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THE EFFECTS OF MERCURY ON THE NESTING SUCCESS AND RETURN RATE OF TREE SWALLOWS (*TACHYCINETA BICOLOR*)

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ABSTRACT

Mercury released from an industrial source into the South River from 1929-1950 contaminated this tributary of the Shenandoah River. While piscivorous birds are frequently used as indicators of mercury contamination, insectivores have also been shown to accumulate metals at high levels. Tree swallows (*Tachycineta bicolor*) were used as biomonitors because they forage on flying insects with aquatic larval stages and so represent a likely non-piscivorous route for mercury entering the food chain. In total, 300 nest boxes were placed along the South, Middle, North, and South Fork Shenandoah Rivers. Half of the nest boxes were along contaminated portions of the watershed downstream from the mercury source; the other half were on uncontaminated reference tributaries or upstream from the source. Blood samples were taken from breeding adults and nestlings to determine mercury levels. To assess any impacts of mercury on nesting success, I compared first egg date, clutch size, proportion eggs hatched, and number of fledglings produced in contaminated and reference areas. Egg volume was measured in 2006. In 2005 and 2006, adults in contaminated areas had significantly higher mercury levels than adults in references areas. Despite elevated mercury levels in 2005, I did not detect any differences in nesting success between birds in the contaminated and reference areas. In 2006, mercury levels in the contaminated areas were twice as high as in 2005. Birds in contaminated areas produced significantly fewer fledglings than birds in reference areas in 2006. However, only first-time breeding females in contaminated areas experienced decreased nest success, perhaps due to production of small eggs. My findings suggest that mercury may most severely impact the success of less experienced breeders.

As part of the continuing study on the nesting success of tree swallows, I used the return rate of birds banded in 2005 as an estimate of short-term survivorship. I predicted that birds banded in contaminated areas would return at lower rates than birds banded in reference areas. Overall, 51% of the adult females banded in 2005 returned in 2006; similar proportions returned from contaminated and reference areas. Return rates were also similar to rates published in other studies.

THE EFFECTS OF MERCURY ON THE NESTING SUCCESS AND RETURN RATE OF TREE SWALLOWS (*TACHYCINETA BICOLOR*)

CHAPTER 1

THE EFFECTS OF MERCURY ON THE NESTING SUCCESS OF TREE SWALLOWS

Mercury

Mercury as an environmental stressor Late in the 15th century, anthropogenic mercury emissions began with the mining of precious metals, and since then the global cycle of mercury has been plagued by increased anthropogenic inputs (Monteiro and Furness 1997). Release of mercury from artificial sources is currently ten times higher than the amount released from natural processes (USDI 1998). Humans are responsible for approximately 50-75% of the total atmospheric mercury emissions, most coming from fossil fuel combustion (Monteiro and Furness 1997). There are currently over 3000 ways in which mercury is used around the world (Burger and Gochfield 1997b). Common anthropogenic sources of mercury into the environment include artisanal gold extraction (Burger and Gochfield 1997b), fungicides, paints, slimicides (USDI 1998), nonferrous waste production and incineration, chemical production processes, and sewage sludge dumping (Thompson 1996). Mercury contamination is becoming increasingly frequent in wetlands around the world due to transcontinental and global transport (Bouton et al. 1999, Chen et al. 2005). While approximately half of the mercury emissions from these sources ends up in the global atmospheric cycle, the remainder is absorbed into local and regional cycles severely impacting the immediate community (USEPA 1997). For example, wetlands are traps for mercury and methylation occurs rapidly due to high levels of dissolved organic compounds and anaerobic conditions (Zillioux et al. 1993). Upon disturbance, such as by fire, the mercury deposited in local wetlands is released and discharged into surrounding habitat (Zillioux et al. 1993).

Mercury emission into lakes and rivers has increased by a factor of 2-4 times since the beginning of the industrial age (USEPA 1997), as determined by lake sediments and peat bog cores (Monteiro and Furness 1997). Once mercury enters a riverine system it can remain in the water column, or be lost through revolatilization into the atmosphere, burial in the sediment, or absorption by aquatic biota (USEPA 1997). The transport of mercury from aquatic systems depends on the content of the organic matter (USDI 1998) and any disturbance of these sediments may increase the likelihood of mercury transport. Mercury transport is facilitated by variation in oxygen levels in the water column and sediment, resuspension of sediment-bound mercury in the water column, or lowering the pH (USDI 1998). However, the key step needed for mercury to be incorporated into the food chain is methylation (USEPA 1997).

Formation of methylmercury The formation of methylmercury (CH_3Hg^+) is significant as it is more toxic and more bioavailable than any other form of mercury (USDI 1998). The intestinal absorption of methylmercury can reach 100%, compared to absorption of only a few percent for inorganic mercury (Weech et al. 2006). Inorganic mercury decomposes quickly *in vivo* compared to organic forms such as methylmercury, which is one of the reasons methylmercury is so persistent in the body (Scheuhammer 1987). While the half-life of inorganic mercury may be as short as a week in a bird, the half-life of organomercurials (including methylmercury) in the blood may be as long as

2-3 months (Scheuhammer 1987). (see section *Elimination of mercury during molt* for details).

The methylation of mercury most commonly occurs through bacterial action in sediments or water (USDI 1998). Mercury in aquatic environments can be methylated by both biotic and abiotic processes and therefore can occur under either aerobic or anaerobic conditions (Celo et al. 2006). It is generally accepted that most environmental mercury methylation is microbially mediated and occurs in anoxic and reducing environments such as river bottom sediments. In anoxic marine sediments, over 95% of biotic mercury methylation was attributable to sulfate-reducing bacteria (Compeau and Bartha 1985). Isolates of sulfate-reducing bacteria have been observed to methylate mercury in pure culture and the bacterium *Desulfovibrio desulfuricans* has been used to examine the metabolic pathways involved in mercury methylation (Choi et al. 1994). Other studies have suggested that sulfate-reducing bacteria also dominate mercury methylation in freshwater sediments and that sulfate concentrations are a key controller of mercury methylation in anoxic environments (Gilmour and Henry 1991, Gilmour et al. 1992). More recently, an iron-reducing bacterium, *Geobacter* sp., has been shown to methylate mercury at rates similar to sulfate-reducing bacteria (Flemming et al. 2006). *Geobacter* sp. is the first iron-reducing bacterium found to methylate mercury at rates that are environmentally relevant suggesting that the process of mercury methylation is not necessarily dominated by the sulfate-reducing bacteria in freshwater systems (Flemming et al. 2006).

In summary, factors such as anaerobic conditions, high temperatures and low pH cause an increase in bacterial methylation rates, thus increasing the amount of methylmercury available for bioaccumulation (Andersson 1979, USEPA 1997). Therefore, characteristics such as slow moving water, acid rain, and the presence of wetlands and dams make a river more prone to methylation.

Mercury accumulation by birds

Physiological pathways Methylmercury enters the vertebrate digestive tract and becomes associated with free amino acids and other sulfhydryl-containing blood components allowing the metal to bind (USEPA 1997, Burger and Gochfield 1997). Thus, blood mercury concentrations in adult birds accurately reflect their recent dietary uptake (Evers et al. 2003). These newly formed complexes then move into tissues and membranes and can be transported across placental and blood-brain barriers (USEPA 1997). The lipophilicity and chemical stability of methylmercury allow it to penetrate cellular barriers. The inorganic forms cannot cross these barriers and are therefore not as toxic (USEPA 1997). Once mercury enters the blood, it is transported through the body and ultimately accumulates in the organs, such as liver, kidney, brain, spleen, and muscle (Bearhop et al. 2000, Evers et al. 2005).

Interpretation of tissues Until recently, comparative analysis of the data collected from various depuration and storage routes has been difficult. The tissue sampled has to be taken into careful consideration when interpreting the results of any study on mercury contamination. Commonly, the lowest mercury levels in the body are found in the blood as this tissue represents primarily recent dietary uptake, while the highest are found sequestered in the feathers and liver (Evers et al. 2005). Using blood from common loons (*Gavia immer*), Evers et al. (2005) developed an inter-tissue comparative ratio of 0.4:1:2:6:15 (egg:blood:muscle:feather:liver). This ratio allows for intra and inter-tissue comparison when samples are only available for one tissue type. These relationships among tissue types produced strong correlations between mercury levels in adult and juvenile blood, adult female blood and eggs, and juvenile feathers and blood (Evers et al. 2005). It is important to take care when selecting the tissue to match specific monitoring needs. Tissues such as blood will only provide information on short-term exposure while tissues that function as terminal endpoints (liver and feather) show elimination capabilities and overall body burdens.

Pathways of elimination in birds Once mercury has been deposited into the body tissue, it may be remobilized from certain tissues and eliminated through feathers, eggs, and excrement (Bearhop et al. 2000, Evers et al. 2005). Egg laying is a well known elimination route for female birds (Evers et al. 2003), while molt provides all birds at least an annual opportunity to transfer mercury from tissues into growing feathers, subsequently decreasing their body burden (Bearhop et al. 2000). However, once methylmercury reaches the liver, it is essentially unavailable for elimination (Evers et al. 2005).

Elimination of mercury during molt Feathers represent the major route for excretion of mercury in birds, accumulating 70%-93% of the body burden (Bearhop et al. 2000, Evers et al. 2005). Almost 100% of the mercury in a feather is methylmercury and due to its long-term stability in the feather, this tissue allows for retrospective analysis (Goutner and Furness 1997, Evers et al. 2005). Mercury is eliminated into feathers only at the time of molt, when feathers are growing and are still being supplied with blood (Wolfe et al. 1998). For this reason, feather mercury levels accurately reflect blood mercury levels at the time of molt (Evers et al. 2005). Mercury enters the growing feather through the blood stream and adheres to sulfide bonds within the feather keratin, after which it is no longer available for remobilization (Wolfe et al. 1998, Evers et al. 2005). Once molt is complete, body burdens will begin to increase as this route for elimination is closed (Bearhop et al. 2000). In non-molting adult mallards (*Anas platyrhynchos*) the half-life of methylmercury in the blood was estimated to be 74 days (Heinz and Hoffman 2004); in Cory's shearwater (*Calonectris diomedea)* the half-life was 40-60 days (Monteiro and Furness 2001). The half-life of methylmercury in the blood is highly correlated with the stage of molt at the time of sampling; the half-life of mercury in blood of great skuas (*Catharacta skua)* dosed with mercury during molt exceeded 30 days (Bearhop et al. 2000). During the intermolt period, the half-life would likely be longer and therefore it is important to consider the timing of cessation of the previous molt, or onset of the next molt, when monitoring birds using feathers and blood (Bearhop et al. 2000).

The amount of mercury eliminated into each feather as it grows is correlated with the pattern of molt of each bird species. This causes significant variation in the amount of mercury found in different feathers on an individual bird (Wolfe et al.1998). There is also individual variation in the amount of mercury an individual can excrete into the feathers (Bearhop et al. 2000). This can occur due to variation in the number of feathers growing at a time, because the half-life of mercury in blood will change depending on the number of feathers being grown (Bearhop et al. 2000). The first feathers grown in during molt have the highest mercury concentrations (Braune 1987, Goutner and Furness 1997, Wolfe et al. 1998). These levels reflect mercury accumulation during the intermolt period for adult birds (Bearhop et al. 2000). Feathers grown later in the molt cycle tend to reflect recent dietary uptake during the period immediately preceding feather growth (Bearhop et al. 2000). Higher levels of contamination in a given environment will lead to more variation in the amount of mercury present in the feathers on an individual bird (Wolfe et al. 1998). Species that have gradual molt cycles, such as seabirds of the order Procellariiformes, typically eliminate less mercury into feathers as fewer feathers are growing at any given time (Wolfe et al. 1998). Members of this order are known for their ability to decrease their overall body burden by demethylating mercury, perhaps as an adaptation to their gradual molt sequence (Wolfe et al. 1998, Bearhop et al. 2000).

Effects of mercury on birds

Effects of mercury on reproductive adults Despite the routes available for elimination, birds can still be heavily impacted by the toxic effects of mercury, even in small amounts. Reproduction has been identified as one of the most sensitive endpoints of mercury toxicity (Wolfe et al.1998). Mercury can affect avian reproduction through different pathways. Mercury in prey items consumed by a female bird can decrease reproductive success when it is eliminated directly into a female's eggs (Thompson 1996). Uptake of mercury by adult birds can also cause behavioral and physiological changes that could alter parental care; parents in poor condition may not be able to properly care for their young. Finally, impacts on nestling birds can occur via consumption of mercury-contaminated prey items delivered by the parents.

Decreases in reproductive success of 35-50% have been observed in birds with high methylmercury dietary uptake when there were no signs of impairment in the adults (USDI 1998). Female mallard ducks dosed with mercury laid fewer eggs, had thinner eggshells, and produced fewer ducklings (Heinz 1979). Although mercury is not commonly associated with eggshell thinning, the overall poor condition of the contaminated female may have led to decreased egg condition (Heinz 1979). Egg volumes in common loons decreased as mercury loading increased due to poor condition of the females (Evers et al. 2003).

Effects of mercury contamination in eggs Accumulation occurs in egg-white proteins (USDI 1998); the more mercury in a female's blood the more will be present in her eggs (Evers et al. 2003). Mercury level in the blood of adult female tree swallows (*Tachycineta bicolor*) was significantly correlated to the mercury present in her eggs (Evers et al. 2005). As mercury is deposited into the egg albumen and yolk (USDI 1998), it is directly absorbed by the growing chick inside. As a result, females may decrease the fitness and chance of survival of their offspring while lowering their own body burden of mercury (Heinz and Hoffman 2003).

Egg laying provides a route for elimination of mercury for female birds (Burger and Gochfield 1997b) and mercury can be differentially allocated to the eggs within the brood (Becker 1992). The first egg typically has the highest amount of mercury with significantly less in each successive egg, creating intraclutch variation (Becker 1992). In 3-egg clutches of herring gulls (*Larus argentatus*) and common terns (*Sterna hirundo*), the first laid eggs had mercury concentrations 39% and 37 % higher, respectively, than the last egg laid (Becker 1992). However, a recent study on mercury elimination into eggs in an insectivorous passerine, the great tit (*Parus major*), found that all eggs in the clutch had similar mercury levels, regardless of laying order (Dauwe et al. 2005). As gulls typically lay much smaller clutches compared to great tits which can lay up to 11 eggs per clutch, or tree swallows (which average five eggs per clutch, Robertson, et al.

1992), there may be differences in mercury elimination into eggs when more eggs are laid (Dauwe et al. 2005).

In general, effects such as decreased egg weight, hatchability, and chick survival are seen at egg mercury levels of 0.5-6.0 ppm ww in laboratory studies (Thompson 1996, Burger and Gochfield 1997b). Impairments and deformities seen in chicks include shortened legs, extra toes, deformed bills, and abnormal wings occurring at mercury concentrations as low as 1.00 ppm ww in the egg (Heinz and Hoffman 2003). Leg deformities have also been found in two songbirds species nesting in areas with heavy metal contamination. Great tit nestlings with an average of 11.0 ppm (dw) mercury in excrement had poorly developed legs; similar growth abnormalities have been reported for pied flycatchers (*Ficedula hypoleuca*) (Janssens et al. 2003). No growth deformities due to mercury contamination have been reported in tree swallows. Two studies have used tree swallow eggs as indicators of mercury availability, but apparently the levels were not sufficient to produce detectable effects on the embryos (Bishop et al. 1995, Custer et al. 2006).

Developmental and behavioral impacts of mercury In addition to an array of physiological effects, mercury contamination can cause significant behavioral problems in young birds. Mallard ducklings dosed with mercury at dietary concentrations as low as 0.5 ppm responded to maternal calls less often and were hyper-responsive in avoidance behaviors (Heinz 1976, Heinz 1979). Juvenile great egrets (*Ardea albus*) demonstrated an overall decrease in activity level and lack of motivation to eat when dosed with mercury (Bouton et al. 1999). Mercury dosed juveniles appeared to have a decreased tolerance to heat as they spent more time in the shade. They also took longer to catch food compared to control birds (Bouton et al. 1999). Mercury-dosed juvenile great egrets spent 30% less time standing and 40% more time sitting (Bouton et al. 1999). Control birds perched or sat on the pool edge more than dosed birds, which may have required more dexterity and motor control than was possible for the mercury-dosed birds (Bouton et al. 1999). This egret dosing study focused on the behavior of post-fledglings as the authors suggested that effects of methylmercury may be most apparent during this period. No other studies have examined the post-fledgling period.

Comparing laboratory and field results A large amount of literature exists on the effects of mercury contamination on birds in laboratory experiments, as well as in the field. However, few studies have looked at the relationship between the two (Burger and Gochfield 1997b). The lack of cross-validation has two causes: laboratory studies only report the effects of administered doses of methylmercury with no report of tissue levels, or field studies report tissue levels without effects and/or exposure levels (Burger and Gochfield 1997). When laboratory studies do report tissue levels, mercury levels in body tissues such as the liver, kidney, and muscle are reported while recent field studies have employed non-lethal sampling of blood and feathers (Burger and Gochfield 1997b). This creates difficulties in cross-validating, comparing results, or in determining what level of mercury in a wild population would produce the results observed in a lab dosing study (Burger and Gochfield 1997b).

Eggs are commonly sampled in both field and laboratory experiments; however, discrepancies exist when interpreting and comparing the results between the laboratory and the field even for this tissue. Laboratory studies contaminate eggs with mercury in one of two ways: indirectly through the female's diet or directly by injection. Heinz and

Hoffman (2003) suggest using caution when extrapolating the effects seen with external application of mercury to eggs in laboratory dosing experiments as they are not replicable in field situations. The mercury applied externally may have more significant and detrimental effects than mercury incorporated into the egg through the mother's diet because it is concentrated in one part of the egg. Burger and Gochfield (1997) suggest that to extrapolate laboratory results into field situations, careful studies need to be done to examine whether the same dose in the lab and field cause similar effects.

No studies have been published comparing environmentally relevant doses of mercury and its effects in free-living and/or captive insectivorous passerines. However, the use of free-living insectivores as biomonitors of heavy metal contamination is growing, especially using cavity nesting species. By establishing what levels of mercury are accumulated by free-living insectivores, dosing studies can be carried out based on realistic uptake. Sampling non-lethal tissues such as blood and feather allows individuals to be tracked across multiple years to gain perspective on ultimate reproductive endpoints of mercury contamination. As blood correlates well with recent dietary uptake (Evers et al. 2005), this tissue is easily sampled in a non-lethal manner to compare results from laboratory and field situations.

Biomonitoring

Birds as biomonitors of mercury contamination Birds are at high risk of contamination by mercury and other persistent pollutants for two primary reasons: 1) most birds are susceptible to biomagification because they eat at high trophic levels, and 2) they are relatively long-lived and are therefore prone to bioaccumulation (accumulation of a toxin over time). Birds are thus susceptible to contaminants that accumulate during their lifetime potentially reducing their reproductive fitness (Rosten et al. 1998). Due to these factors as well as the well-understood life history characteristics of many species, birds have become more commonly used as biomonitors. Birds also make good biomonitors because they are familiar and of interest to the general public (Eens et al. 1999, McCarty 2001). While piscivorous birds (e.g. loons, eagles, herons, kingfishers, cormorants) are frequently used as indicators of mercury contamination in freshwater ecosystems (Eens et al. 1999, Evers et al. 2003, 2005, Weech et al. 2006), insectivorous birds can also make effective bioindicators (Jones 2003, Evers et al. 2003, 2005). Historically overlooked, insectivorous passerines have recently been used as indicators of mercury contamination (Nyholm 1995, Eens et al. 1999, Dauwe et al. 2005, Custer et al. 2006).

Using nestling birds as biomonitors for local mercury availability is valuable as their body burdens closely reflect amounts of mercury in the food they are provided during development (Janssens et al. 2003). Chick feathers are commonly used as indicators of dietary mercury availability; any mercury accumulated in the growing feather of a chick could only have been ingested in the previous few weeks (Goutner and Furness 1997). Therefore nestling feathers represent mercury accumulated through the recent diet, not residues of previously accumulated body burdens (Goutner and Furness 1997). By studying nestlings, in contrast to adult birds, the entire lifetime exposure can be studied in detail and sampling methods can be standardized, as all individuals are of known age and origin (Janssens et al. 2003). Such studies have shown that metal levels in excrement from 15-day old great tits sampled along a contamination gradient surrounding a smelter in Antwerp, Belgium correlated with the distance the nest was from the pollution source (Janssens et al. 2003). Nestlings closer to the source had significantly higher levels of contamination than nestlings farther away. They also found that tit reproductive success decreased closer to the contamination source and nestling growth rate and fledging weight were sensitive indicators of contamination levels.

Study species

Tree swallow life history The tree swallow, an insectivorous passerine, is found across North and Central America and is a secondary cavity nester (Robertson et al. 1992). Tree swallows are migratory and begin to travel south in late August or early fall (Robertson et al. 1992). Eastern populations typically migrate to Florida and the Caribbean while populations from the American and Canadian Midwest follow the Mississippi Flyway to the southern Gulf Coast and Central America (Robertson et al. 1992). Males typically arrive on the breeding grounds first; older birds of both sexes arrive earlier than younger birds (Robertson et al. 1992). Tree swallows breed from the northern tree line through southern Tennessee and northern North Carolina in the eastern United States, with occasional records farther south (Wagner et al. 2002). Birds that arrive first on the breeding grounds quickly pair up and place small amounts of nesting materials, such as grass, in tree cavities or nest boxes; nest building begins in earnest at the end of April (Robertson et al. 1992).

Tree swallows rely on primary cavity nesters, such as woodpeckers, to excavate cavities in dead trees. They feed on flying insects and thus favor wetlands, especially those with high densities of dead trees, such as beaver ponds. Because nest cavities are a limiting resource, tree swallows are easily attracted to nest boxes erected in suitable habitat (Jones 2003, Mengelkoch et al. 2004) and will nest at high densities when nest boxes are provided near water or other sources of flying insects (McCarty 2001).

Tree swallows can be easily sexed during the breeding season by the presence of a brood patch on females or a cloacal protuberance in males (Pyle et al. 1987). Female tree swallows can also be aged by plumage patterns. Unlike most female birds, tree swallows maintain a distinct sub-adult plumage through their first breeding season (Robertson et al. 1992). These young females, in their first breeding season or second calendar year of life (hereafter SY females following the conventional abbreviation for “Second Year”), maintain the brown coloration of a juvenile bird. Sub-adult female tree swallows develop the blue-green iridescent coloration of adult males and females only when they molt at the end of their first breeding season. They would then be classified as “After-Second-Year” (hereafter ASY) females beginning in the following January, their third calendar year of life (McCarty and Secord 2000). Male tree swallows undergo a complete molt from juvenile to iridescent blue-green plumage with no intermediate plumage. Thus males cannot be aged and are classified as “After Hatching Year” (hereafter AHY) beginning in their first January of life (Robertson et al. 1992).

The delayed plumage maturation in females may function as a signal during mate choice, as a badge of age to reduce aggression from older birds or in other aspects of the tree swallow social system (Robertson et al.1992, McCarty and Secord 2000). SY females are generally smaller and have lower reproductive success than ASY females (McCarty and Secord 2000). However, another study found no difference between SY and ASY females in any component of parental care, including feeding rate, fecal sac removal, or aggression against intruders (Lombardo 1991). That study suggested the poor reproductive performance of SY females may be due in part to their attracting lower quality mates (also suggested by McCarty and Secord 1999, 2000).

Tree swallows as model organisms The most common method of exposure to toxins is through foraging, and for tree swallows foraging occurs almost exclusively on winged insects, including those emerging from an aquatic larval stage (McCarty 2001). The majority of the diet consists of Diptera (41%); however, other insects such as dragonflies and spiders are taken in small amounts (Robertson et al. 1992). In habitats with running water, such as the South River, VA, tree swallow diets typically include stoneflies (Plecoptera), caddisflies (Trichoptera) and mayflies (Ephemeroptera) (Robertson et al. 1992). Developing insect larvae accumulate toxins (such as mercury) from contaminated aquatic sediment as they grow (McCarty 2001, Mengelkock et al. 2004), transferring these to tree swallows after winged adults emerge. Adults swallows typically forage within a 400 m radius of their nest site (Mengelkoch et al. 2004, Stapleton and Robertson 2006); throughout the nestling period adults may remain within 100-250 m of the nest (Quinney and Ankney 1985, McCarty 2001). This suggests that most contaminants accumulated by nestling and adult swallows will have originated from a small area during the nesting period. A recent study using radio-telemetry to track home-range movements suggested that females may roost over 2000 m from their nest box during the pre-laying period (Stapleton and Robertson 2006).

Many of the life history characteristics of tree swallows make them one of the most highly recommended bioindicators for determining the effects of mercury on riverine systems (McCarty 2001, Evers et al. 2005). Female tree swallows are known to be highly philopatric once they begin breeding (Winkler et al. 2004), and thus can be monitored across multiple breeding cycles. Philopatry is an important trait to consider when choosing an avian biomonitor (Hollamby et al. 2006) as the effects of contaminants may be cumulative rather than immediately apparent. For example, reproductive success could decrease upon returning to a contaminated site year after year; however, few, if any, studies have addressed this issue. Other suggested traits of avian biomonitors possessed by tree swallows include: 1) characterization of the biology of the species to detect departures from normal; 2) a tissue type that can be easily sampled in an appropriate quality and stored until analysis (e.g. blood and feathers); 3) resistance to human disturbance (McCarty 2001), and 4) documented use of the species as a biomonitor of contamination with established sampling methods (Hollamby et al. 2006). Because they possess these characteristics the argument has been made that, in addition to being useful biomonitors, tree swallows deserve a place among classical model organisms such as the fruit fly (*Drosophilia melanogaster)* and nematode (*Caenorhabditis elegans*) (Jones 2003).

While there are a few disadvantages to using tree swallows in studies of environmental stress (e.g. migratory, not of conservation concern, difficult to rear in captivity), their natural abundance and cavity nesting habit more than compensate for this and make them ideal biomonitors. Environmental contaminants have been studied using other species in this guild, such as the barn swallow (*Hirundo rustica*), northern rough-winged swallow (*Stelgidopteryx serripennis*), and cliff swallow (*Petrochelidon pyrrhonota*) (McCarty 2001). The limited use of these species is mostly due to the fact that they are not cavity nesters and must use cliffs, banks, or other large, man-made structures to breed, limiting their availability. Hence, the tree swallow is a better choice logistically than other members of its guild based on its acceptance of artificial nest boxes.

Use of tree swallows in studies of environmental stress Tree swallows have been used in many studies of environmental contamination including mercury, PCBs (polychlorinated biphenyls), and pesticides. As of 2006, there were at least 44 completed or on-going studies in North America on the uptake of contaminants and the effects of anthropogenic stressors in tree swallows (McCarty 2001). Several examples follow.

Tree swallows have recently been employed to monitor increases and impacts of methylmercury after the creation of reservoirs (Gerrard and St Louis 2001). Reproductive success was measured and related to mercury levels from eggs and nestlings in two reference areas and a pre- and post-flood reservoir (Gerrard and St Louis 2001). As a rule, the rate of methylation of mercury increases with increased flooding due to decomposition of organic carbon in the flooded soil, causing potentially elevated rates of trophic transfer (Zillioux et al. 1993). Pre-flood, the mean methylmercury level in the bodies of the nestlings was 82.8±2.2 ppb dw compared to an average of 130±11 ppb dw post-flood, a highly significant increase in methylmercury availability (Gerrard and St Louis 2001). However, the increased accumulation of methylmercury after reservoir creation had no significant impact on reproductive parameters such as clutch size or hatching and fledging success. The unexpected lack of differences in reproductive success, despite an increase in dietary methylmercury, was interpreted as the result of increased food availability as dipteran insects emerged from the newly created reservoir (Gerrard and St Louis 2001). The link between food abundance and reproductive success is well established in tree swallows; food abundance has a large impact on the level of parental care provided during the nesting period, and in this case the benefit of extra food was not offset by the increased methylmercury (Quinney et al. 1986, Gerrard and St Louis 2001).

In a similar study in northwestern Minnesota, tree swallows were used to monitor mercury availability in re-flooded pools at the Agassiz National Wildlife Refuge (Custer et al. 2006). Because flooding of wetlands generally increases methylmercury availability to insectivores (Zillioux et al. 1993, Bishop et al. 1995), the re-flooding of a pool at this refuge provided the opportunity to test the effect on swallows. The mercury levels in tree swallow tissues, post-flood, averaged <0.25 ppm dw, similar to background levels (Custer et al. 2006). The authors suggest the lack of impacts on reproduction at this site was likely due to the low levels of mercury. The re-flooding of the pools on this refuge did not increase mercury availability as the pools likely remained wet during the original pool drawdown, and therefore re-flooding did not enhance methylation (Custer et al. 2006). In general, decomposition of organic material fuels methylation after flooding (Zillioux et al. 1993), and this may not have existed in the drawn-down pool. Tree swallows were also used to determine the transfer of mercury from wetland sediments in the Great Lakes Basin (Bishop et al. 1995). Mercury levels in tree swallow eggs ranged from 0.043 - 0.079 ppm; levels consistent with atmospheric mercury deposition rather than a point source (Bishop et al. 1995).

Contamination from PCBs, known endocrine disruptors, can cause a host of problems, including severe developmental abnormalities, decreased reproductive success, and physiological and metabolic changes (McCarty 2001). Tree swallows have been used as biomonitors of PCB contamination in Green Bay, WI (Custer et al. 1998) and along the Housatonic River in Massachusetts (Custer et al. 2003). Tree swallows nesting along the Housatonic River had the highest levels of PCB ever reported for this species, with levels higher than in piscivorous birds (Custer et al. 2003). Mean PCB values ranged from 31.5 – 100.9 ppm ww in 12-day old nestling carcasses. This study did report lower hatching success in contaminated areas but this varied with year and the correlation was weak. The study in Green Bay did not find any reproductive differences, perhaps due to lower PCB levels (Custer et al. 2003). Most interesting was that there was typically no difference in the level of PCBs in clutches in which all, none, or some of the eggs hatched, indicating extreme variation in how this contaminant impacts individuals (Custer et al. 1998).

As an endocrine disruptor, PCB can affect other aspects of reproduction by altering parental behaviors. The quality of the nests built in a contaminated site (Hudson River) was significantly lower, in terms of mass and number of feathers, than nests built on reference sites (McCarty and Secord 1999). A related study found that female tree swallows in the PCB-contaminated sites acquired ASY plumage early (McCarty and Secord 2000). This suggests that environmental endocrine disruptors, such as PCB's, may alter the expression of ornamental traits or androgen-stimulated behaviors.

Tree swallows have also been used to monitor the uptake of organochlorine pesticides. Exposure to these contaminants typically comes from consuming insects exposed to the pesticides or from pesticides being sprayed in close proximity to nesting sites (McCarty 2001). Tree swallows in a pesticide-sprayed apple orchard in southern Ontario did experience significant decreases in reproductive success with increased pesticide (organophosphorous) exposure (Bishop et al. 2000). Birds with higher toxicity scores experienced deceased egg fertility and chick survival (Bishop et al. 2000). Tree swallows have also been used to determine the trophic transfer of pulp and paper mill effluent from the aquatic to riparian ecosystems (McCarty 2001).

Though this list of the use of tree swallows in studies of environmental stress is not exhaustive, it highlights the most common uses of this species in determining the transfer of contaminants from aquatic ecosystems to birds. Typically used in studies of the environmental contaminants described above (metals, PCB, DDT), tree swallows have also been used to study radiation, acid deposition, and climate change (McCarty 2001). Despite the growing use of this species as a biomonitor, one thing many studies have in common is a lack of significant responses, in the form of decreased reproductive success or large scale mortality, to these environmental stressors (McCarty 2001). It has been suggested that the tolerance of tree swallows to contaminants may be higher than in other species (McCarty 2001). Though seemingly a negative trait, tolerance to a wide range of contaminants makes for a good biomonitor (McCarty 2001, Hollamby et al. 2006) as trace amounts do not cause large scale mortality that would hinder long-term research. Further, the lack of significant reproductive responses could also be due to the low, background levels of contaminants reported in many of these studies or the difficulty in detecting subtle effects in uncontrolled ecological studies.

Objectives

Is mercury accumulated by tree swallows, a non-piscivorous species, nesting along the South River?

To determine whether mercury was being accumulated by non-piscivorous species along the South River, I used the tree swallow, an aquatic insectivore, as a biomonitor. To assess the amount of mercury recently accumulated by adult and nestling tree swallows, blood samples were taken during the nestling period. Feather samples were also collected to determine long-term accumulation in adults and to reflect recent dietary uptake in nestlings. Mercury levels of birds nesting along the contaminated areas were compared to the levels of birds from reference areas nearby to eliminate the influence of airborne or background mercury contamination. Because mercury availability varied greatly along the contaminated stretch of the South River, direct comparisons between areas classified as reference and contaminated may not, in some cases, be as useful as comparisons between contaminated areas with higher and lower mercury availability.

Is mercury impacting the nesting success of tree swallows along the South River?

My second objective was to determine if mercury accumulation was impacting the nesting success of tree swallows along the South River. Nests in contaminated and reference areas were assessed for basic reproductive parameters including clutch initiation date, clutch size, hatching success, and number of fledglings produced. In 2006, egg volume was also measured. Chick growth and pre-fledging condition were also monitored to assess chick health during the nestling period as mercury could directly impact the chicks through developmental impairments.

METHODS

History of contaminated site

On April 14, 1977 E.I. DuPont de Nemours and Company (DuPont) claimed responsibility for discharging mercury into the South River (a tributary to the South Fork

Shenandoah River) in Waynesboro, VA. Released from 1929-1950, the source of the mercury was the mercuric sulfate used as a catalyst to produce acetate fibers in their Waynesboro, VA manufacturing plant (Carter 1977). Most of the spills of metallic mercury reportedly occurred in the 1930s and 1940s with unknown quantities released through discharges, runoff, or subsurface seepages (Carter 1977, Murphy 2004). Sediments tested in 1977 contained more than 240 parts per million (ppm) mercury downstream of the plant, compared to less than 1.0 ppm upstream of the source (Carter 1977).

In the mid-1980's, the Virginia State Water Control Board (now the Virginia Department of Environmental Quality, VDEQ) and DuPont created a trust fund for a 100-year monitoring plan for the South River to support mercury monitoring of the water, sediment, and fish (VDEQ 2000). The VDEQ established a health advisory in the 1980's warning against fish consumption in the South River downstream of the DuPont plant in Waynesboro to the Page/Warren County line on the South Fork Shenandoah River (VDEQ 2000). This advisory discourages the consumption of all fish except stocked trout that have been declared safe for human consumption (Murphy 2004). In 2000, DuPont and the VDEQ created the South River Science Team (SRST) to perform damage assessments in the South River. Progress has been made by the SRST in locating the source of continuing mercury inputs, monitoring mercury levels in the water and sediment, and determining the level of contamination in aquatic organisms such as clams and insects; many of these studies are ongoing. A comprehensive study on the contamination levels of fish and their prey in the South Fork of the Shenandoah River Basin was completed as a Master's thesis in 2004 (see Murphy 2004). Murphy (2004) identified baseline mercury levels and dietary uptake in selected fish species and their prey. While the levels of mercury in the aquatic ecosystem have been monitored since the establishment of the SRST, no studies had yet focused on determining whether the mercury was making its way into the terrestrial ecosystem and what potential impacts it may have on terrestrial or aquatic wildlife. Beginning in 2005, the present study is the first to examine the impact of mercury contamination on the terrestrial ecosystem of the South River by using non-piscivorous birds as biomonitors.

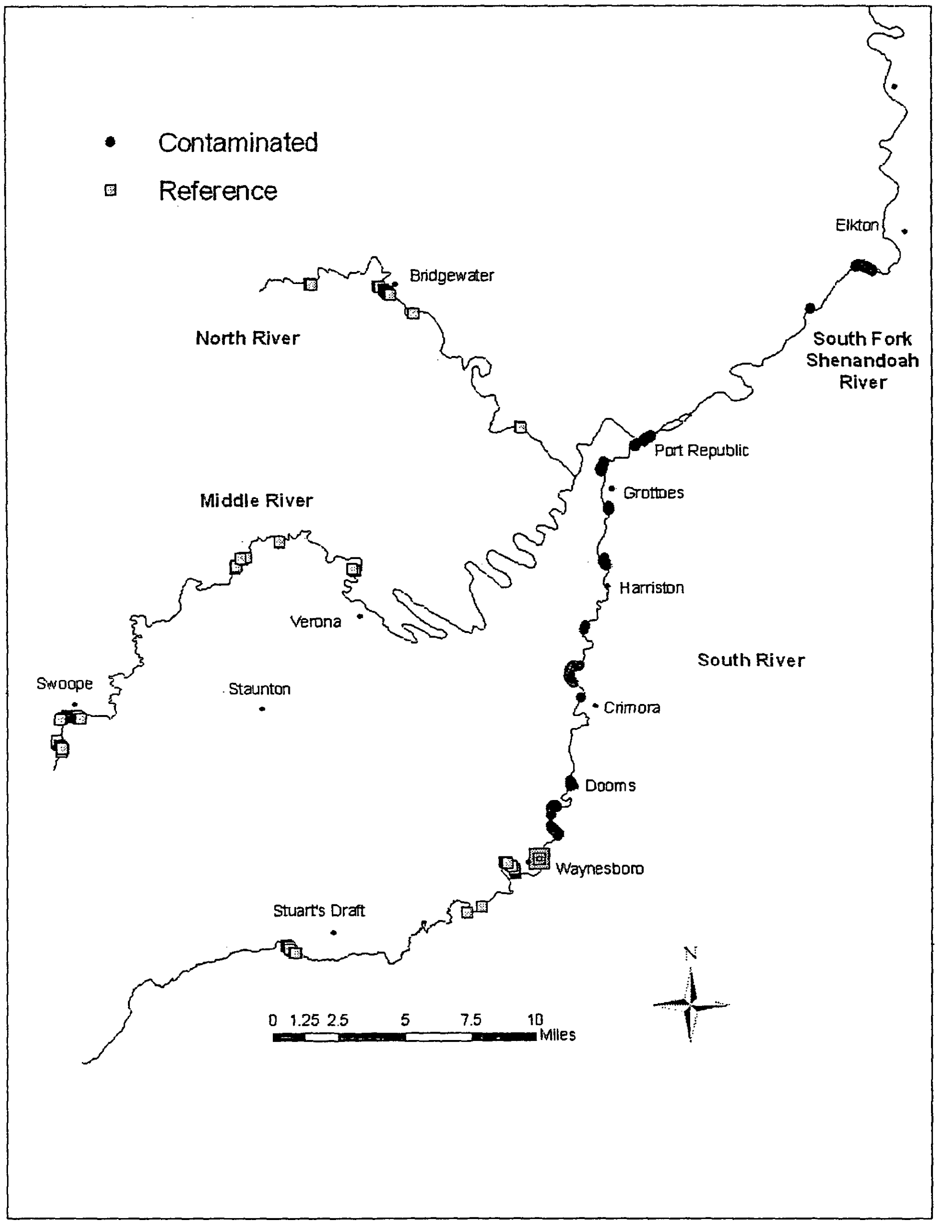
Study site

Description This study was carried out in the mercury-contaminated South River and South Fork Shenandoah River. The North and Middle Rivers, as well as uncontaminated sections of the South River upstream of Waynesboro, were used as uncontaminated reference sites. The South, Middle, and North Rivers make up the three tributaries of the South Fork of the Shenandoah River (Fig. 1). Stream order described here refers to the classification of a river based on the number of upstream tributaries that feed it (Strahler 1952). For example, a second order stream is formed downstream of the confluence of two first order, or headwater, streams; a third order stream is formed below the confluence of two second order streams (Strahler 1952).

South River The South River is the southernmost headwater of the Shenandoah River, beginning in the Blue Ridge Mountains south of Staunton, VA. The drainage basin of the South River, a fourth order stream, encompasses 373 km^2, discharging an average of 7.4 m^3/s (Murphy 2004). The river flows over substrate composed primarily of limestone and carbonate. The South River is characterized by a moderate gradient that increases in volume as it is fed by smaller streams and creeks north of Waynesboro. The

FIGURE 1

MAP OF NEST BOX SITES ALONG THE SOUTH, MIDDLE, NORTH, AND SOUTH FORK SHENANDOAH RIVERS



Black circles are nest boxes in contaminated areas; grey squares are nest boxes in reference areas. The source of contamination is labeled with a large square in Waynesboro.

surrounding landscape varies from urban to rural and is primarily used for growing hay and rearing livestock, but thin to moderate riparian zones exist throughout. There are two water treatment plants along the South River, located in Waynesboro and Grottoes. The South River flows north to Port Republic, VA where it meets with the larger North River to form the South Fork Shenandoah River.

South Fork Shenandoah River The South Fork Shenandoah River is a sixth order stream that flows for approximately 160 km, draining an area of 4,144 km^2 with an average annual discharge of 39 m^3/s (Murphy 2004). The substrate consists mostly of limestone ranging from cobble to boulders. The South Fork is a low gradient river, averaging 30.5 m wide, but producing a few class I and class II rapids. The shoreline of the South Fork consists of more vegetated patches than the South River, not being as severely impacted by agriculture (Murphy 2004). Like the South River, the surrounding landscape includes pasture, crop fields, and small patches of urbanization. This river meets with the North Fork Shenandoah in Front Royal, VA forming the Shenandoah River which ultimately runs into the Potomac River and Chesapeake Bay.

North River The North River, one of three reference tributaries in this study, begins in the Allegheny Mountains and flows over the carbonate rocks near the Natural Chimneys. From here, the substrate of the river becomes a deposit of alluvial sediment, decreasing the river gradient dramatically. The North River is a fifth order stream that has an annual discharge of 10.7 m^3/s and drains approximately 1140 km^2 (Murphy 2004). The landscape surrounding this river is similar to that of the South River with large expanses of urbanization mixed with agriculture as well as thin strips of forest. The

North River converges with the Middle River before joining the South River in Port Republic to create the South Fork Shenandoah.

Middle River The other reference tributary used in this study was the Middle River, which begins to the southwest of Staunton in the Great Valley. The Middle River is a fourth order stream that flows over a substrate of primarily shales and carbonates. It drains an area of approximately 971 km^2, with an average annual discharge of 8.6 m^3/s (Seagle 1980). Approximately 90% of the landscape surrounding the Middle River is forested or agricultural. Lewis Creek, a tributary to the Middle River, is contaminated with PCBs 12 miles upstream of its confluence with the Middle River south of Staunton. The Middle and North Rivers converge near Grottoes, after which the enlarged North River flows to meet with the South River in Port Republic, forming the South Fork Shenandoah. Although there is presently no warning of PCB contamination downstream of the confluence of Lewis Creek and the Middle River, as a precaution no study sites were selected downstream of Lewis Creek on the Middle and North Rivers.

Sites

Design and choice of sites In order to maximize use by tree swallows, I looked for properties with open fields along the river with little or no forested riparian strip (see section on *Microhabitat box placement* below). As few properties completely lacked a riparian zone, sites were then selected based on the expanse of open field available within 50 m of the river, and lack of canopy over the river. Nest boxes were erected on properties with thin riparian zones in areas where one or both banks had open habitat. This allowed swallows direct access to the river and access to emerging aquatic insects near the nest. Once a property was selected, nest boxes were erected after receiving verbal permission from all property owners.

The contamination source (DuPont/Invista plant) in Waynesboro was the point of reference for river mile along the South River; river mile zero was located at the footbridge in the plant. River miles were used rather than kilometers in order to be comparable with data from the other members of the South River Science Team. All boxes downstream of river mile zero were classified as mercury contaminated sites while those upstream, or on the other two tributaries, were classified as reference sites areas. The term "site" was used as an operational construct to describe clusters of nest boxes with common access points. Each nest box was considered an independent sample because nest boxes on one "site" were sometimes closer, as the swallow flies, to boxes on another "site" than to other boxes on the same "site". (For details of all properties used and locations see Appendix A1 and A2).

Description of sites

South River-Reference In 2005, boxes were set up along three properties upstream of the contamination source including Cowbane Natural Prairie Preserve in Stuart's Draft, Ridgeview Park in Waynesboro, and a residential neighborhood on Locust Street in Waynesboro. One additional property was added in 2006, between Cowbane and Ridgeview Park.

1) Cowbane Preserve is located approximately 14 river miles upstream of the contamination source in Augusta County and is maintained by the Virginia Department of Conservation and Recreation (formerly the South River Preserve maintained by The Nature Conservancy). This protected area consists of marsh and prairie habitats in the

floodplain with a few large sycamore trees on the riverbank. The property along the opposite riverbank is open cow pasture under conversion to residential housing. Twelve nest boxes were erected in open gaps along the riverbank or in the emergent wetland within 50 m of the river in 2005. In 2006, four additional boxes were added to this site.

2) Ridgeview Park is a public, city-owned park approximately 2 miles upstream of the contamination source. Nest boxes were placed along the open riverbank in areas that experienced frequent disturbance by fisherman and other park patrons. The opposite river bank has a riparian buffer several trees deep. Four of the 11 nest boxes at this site were placed in small gaps of a thin riparian buffer; the rest were placed on mowed lawn areas free of trees. This site had six nest boxes in 2005, and five were added in 2006 making a total of 11 nest boxes.

3) Locust Street consisted of four adjacent riverfront backyards. These properties are located approximately 1.5 miles upstream of the source. The upstream border of this site is adjacent to the downstream border of Ridgeview Park. Few trees line the riverbank on both sides of the river bank and the seven nest boxes at this site were placed on mowed lawns at the waters edge as far from trees as possible. No new nest boxes were added to this site in 2006 as it already held the maximum number of boxes space would allow.

4) P. Buckley Moss was a fourth property added in 2006, approximately 5 miles upstream of the contamination source at the exhibition barn of a local celebrity. This site encompassed seven adjacent properties and has a thick riparian buffer of trees and shrubs on both river banks; an ATV trail cut through the woods provided access to the river and gaps in which to place the nest boxes under forest canopy. Seven nest boxes were placed

in small gaps within the riparian buffer; the remaining four were located in large mowed clearings.

South River-Contaminated Downstream of the contamination source, along the South River in Augusta and Rockingham County, the eight properties used included public parks, cow pastures, and the county forestry nursery. In 2005, there was a gap from river mile 10 to 19 in which there were no nest boxes. In 2006, the nest boxes were removed from the South Fork Shenandoah River, providing additional nest boxes to fill this gap. Three new sites were established in 2006 along this previously unstudied section of river.

1) The Water Treatment Plant was the first site about 2 miles downstream of the plant in Waynesboro. The water treatment plant has a thick riparian buffer that leads to an unvegetated gravel bar. The opposite bank has a steep, dirt cliff in which a pair of belted kingfishers and northern rough-winged swallows nested, and a dirt bike course. Eight nest boxes were placed along the bar in open areas or in gaps within the riparian buffer in 2005. Two nest boxes were added to this site in 2006, for a total of 10 boxes.

2) Basic Park is also located 2 miles downstream of the source, opposite the Water Treatment Plant, and is a public park with athletic fields and a moderate to wide riparian buffer on both sides of the river. Five nest boxes were arranged in the mowed field within 50 m of the river in 2005; four new boxes were added in 2006 for a total to nine available boxes.

3) Genicom is approximately 2.5 miles downstream. Here, nest boxes were set up in a hayfield on the site of the former Genicom company (now lightly used as a storage depot by REO Distributing). Nest boxes at this site were placed in the field, rather than on the

shoreline, because both shores were forested and the riparian zone had no breaks. Eight nest boxes were erected at this site in 2005; six were added in 2006 bringing the total to 13 nest boxes.

4) Dooms Crossing is located five miles downstream in a cow pasture opposite the Holsinger dairy farm. Nest boxes were erected along open riverbank or placed in gaps within a moderate riparian buffer. The opposite shore also had a moderate riparian buffer. This is the "Dooms" study site frequently used by the South River Science Team. The nine nest boxes on this property were placed on the grazed and highly eroded river bank; five boxes were present in 2005 and four were added in 2006.

5) Crimora Crossing is 5 miles downstream from Dooms, at river mile 9.9, where a town park has been recently created on Rt. 612. This property is city-owned and was graded and reseeded with lawn between 2005 and 2006. This small park lacks a riparian buffer and the nest boxes were spaced in the open areas along the riverbank frequented by fishermen. The opposite bank has a few trees that increased in density downstream; however, my nest boxes did not stretch that far. In 2005, there were only two nest boxes at this site and one was added in 2006, making this one of the smallest sites.

6) The Augusta Forestry Center in Crimora, located from river miles 11-12 downstream of the source, was the largest property used along the South River. There is a single row of sycamore trees along this entire stretch of river, with few gaps, on both banks. Therefore a majority of the nest boxes at this site were put up in the open field between the riparian zone and the cultivated fields. The cultivated fields are primarily used to grow saplings and are irrigated with water pumped from the South River, which pools in one area creating a tiny wetland. The Augusta Forestry Center is also the location of

several ongoing studies by the South River Science Team. Twenty-one nest boxes were erected at this site in 2005; 10 were added in 2006 making this the largest site in the South River with 31 nest boxes.

7) The Wampler property is a dairy farm site that was established in 2006 at river mile 14 at the end of Rt. 616. This site has open pasture with a thin and patchy riparian buffer on both banks. The six nest boxes were placed between an electric fence and the highly eroded river bank.

8) Harriston Crossing is a small open hayfield on Patterson Mill Road 16 miles downstream of the contamination source. This site was used only in 2006 and was separated from the river by a thick riparian zone with three nest boxes in the hay field. This is the one site where the nest boxes were turned to face away from the river; instead, facing a road and large crop field.

9) The Rankin property was the final new site added in 2006 approximately 18 miles downstream. It is a working farm, owned by Ralph Rankin, with a small cornfield and moderate riparian buffer. Despite the nearly continuous riparian zone, nest boxes were erected in small gaps between the trees in the hopes of attracting tree swallows that would forage over this section of river. However, despite eight available nest boxes, this site attracted no swallows.

10) Grand Caverns, a public park and tourist attraction, is located approximately 20 miles downstream of the contamination source. Grand Caverns has a thick riparian buffer with a few large gaps along both riverbanks. This area was frequented by pedestrians, cyclists, and fisherman. All nest boxes were placed on the high western bank of the river under gaps in the canopy. This site had only two boxes in 2005, with five added in 2006.

11) Grottoes City Park, river mile 22, is just downstream of Grand Caverns across the Rockingham County line. This is a large town park with picnic pavilions, a boat launch, large playground, fishing pond, and a small golf driving range. The park property reaches to the river and has a thick riparian zone as well as open gravel bars and an oxbow lake. Three nest boxes were placed directly on the river on the shoreline gravel bars in 2005. Seven nest boxes were placed in the open lawn areas of the park away from frequent disturbance. Six additional boxes were added in this field and along the walking path in 2006. Altogether, 16 nest boxes were available at this park in 2006. In 2005, Grottoes City Park was the northernmost site upstream of the confluence in Port Republic.

12) In 2006, Bradburn Park was a new site just downstream of Grottoes City Park in Port Republic, approximately 24 miles downstream of the plant. Bradburn Park was the northernmost site on the South River in 2006. This park consists of a small grass field canopied by trees with a patchy riparian buffer on both banks. Four nest boxes were placed within small gaps along the riverbank. This site attracted no swallows.

South Fork Shenandoah River-Contaminated Downstream of the confluence, on the South Fork Shenandoah River, I had three sites in 2005. All nest boxes along the South Fork Shenandoah were removed in February 2006, before the swallows returned for the breeding season.

1) The first was a pair of residential properties in Port Republic immediately downstream of the confluence after river mile 24. Both properties had a thick riparian zone with several meters of open bank along the river. Nine nest boxes were erected along the riverbank.

2) At river mile 31, five nest boxes were erected along the river bank in a cow pasture owned by the Sheets family. The open cow pasture, lack of a riparian zone, and the wide river channel made this location ideal.

3) The northernmost site of this study in 2005 was located at the Merck Pharmaceutical Plant in Elkton. This site was the largest on the South Fork Shenandoah and was located 37 miles downstream of the contamination source. I had access to all of the riverfront property surrounding the Merck plant; large open fields extending to the river bank with a patchy riparian zone on both banks. Twenty-eight nest boxes were placed on the river bank in open gaps or in the adjacent un-mowed field when a thicker riparian zone was present.

Middle River-Reference There were a total of six properties used on the Middle River, also in Augusta County. The landscape surrounding the Middle River is more rural than that surrounding the South, North, and South Fork Shenandoah Rivers. Only cow pastures and residential backyards were used as nest box sites along the Middle River. The largest sites were located on three neighboring farms in Swoope.

1) The property furthest upstream was the Whitescarver farm. Tree swallows were probably nesting in this area before my study began as the property owner had erected a number of his own boxes along fence posts. This site is an open cow pasture with a few trees on either river bank. Nest boxes were erected on both sides of the river in CREP (Conservation Reserve Enhancement Program) habitat. CREP was created by the United States Department of Agriculture to promote sustainable land use practices by monetarily rewarding landowners for establishing practices such as creating filter strips and riparian buffers, or restoring wetlands. Fifteen nest boxes were erected along both sides of the

river bank in 2005. In 2006, 22 additional nest boxes were added to the contiguous cow pasture downstream.

2) The next site was created at Smith's Pond, an area well known to local birders. Smith's Pond was created by the damming of the Middle River at this location. The surrounding landscape is cow pasture and open, mowed fields. Ten nest boxes were spaced along the river bank upstream of the dam in 2005. In 2006, seven boxes were added downstream of the dam for a total of 17 boxes.

3) Immediately downstream is the Godfrey farm site. These boxes were in a CREP field adjacent to the cattle pasture. Eight nest boxes were placed along the open river bank or within the CREP field within 25 m of the river in 2005. Six nest boxes were added in 2006 for a total of 14 nest boxes on the Godfrey Farm.

4) The other three sites along the Middle River are residential properties. At all three sites, next boxes were placed on the river bank in open spaces, or in open gaps along a thin riparian zone. Two of the properties are located in Franks Mill and Spring Hill (respectively), near Staunton, along the riverfront property of the homeowners (Dories/Middle River Rd. and Shapcot properties). The Dories/Middle River Rd. property had six nest boxes along the river on the Dories family property in 2005. In 2006 this site was extended by adding eight nest boxes to the adjacent property upstream, for a total of 14 nest boxes. The Shapcot property had three nest boxes in 2005; two boxes were added in 2006. The third property was farther downstream at Fort River Road in Verona; there were four nest boxes in 2005 and 2006.

North River-Reference The North River had the fewest nest box sites of the three rivers used in this study. All of the sites were located in Bridgewater in Rockingham

County. In 2005, there were boxes on two residential properties. Two new sites were added in 2006.

1) The site farthest upstream in 2005 was at the Flora family property. This site had a thick riparian zone and therefore the nest boxes were placed as close to the river as possible to avoid dense groups of trees along both banks. This was a small property with four nest boxes, only one of which was used, and it was removed in 2006.

2) Downstream of the Flora property was the largest site on the North River, the Crawford Annex. The Crawford Annex consisted of three contiguous properties including two residential, the Crawford and Auckerman properties, and one public park, Wildwood Park. Nest boxes were only present at Wildwood Park in 2006. This riverfront drive (Crawford/Auckerman properties) lacks a riparian zone, with only a few large trees shading the river bank. The river is calm at this site due to a dam constructed immediately upstream. The landscape surrounding the nest boxes is suburban; however, each property has a large amount of open, mowed-grass suitable for tree swallow foraging and the river channel is very wide here. Wildwood Park is a public city property located upstream of the dam. Nest boxes were erected along sections of open river bank in areas that were frequented by fisherman. The Crawford/Auckerman properties held 14 nest boxes in 2006. The addition of Wildwood Park added six nest boxes for a total of 20 nest boxes along this stretch of river.

3) One of two new sites used in along the North River in 2006 was at Sandy Bottom Park, downstream of the Crawford annex. Sandy Bottom Park is a Par 3 golf course with moderate pedestrian traffic. Four nest boxes were placed along the river in open gaps within the thin riparian zone, away from frequent human disturbance.

4) The second new site added to the North River in 2006 was at the Rt. 276 river crossing in Weyers Cave. This site was located just upstream of the confluence of the Middle and North Rivers near Grottoes. This site is an undisturbed area with scattered trees, on both banks, which backs up to a cornfield. The seven nest boxes were erected next to the overpass along the river or in clearings within 50 m of the river.

Nest boxes

Construction The tree swallow boxes were constructed 23.8 cm deep from the floor to the top with a floor-hole height of 16.5 cm. The floor area was 16 cm^2 and the entrance hole was 3.8 cm in diameter. The nest box was attached to a 1.5 m metal pole. To erect the nest box, a 1.0 m long, 1.27 cm wide galvanized steel pipe was driven 0.5 m into the ground to provide stability for the nest box. The nest box pole then slid over this stabilizing pipe. Providing boxes with entrance holes no larger than 3.8 cm prevented usurpation or parasitism by European starlings (*Sturnus vulgaris)* and brown-headed cowbirds (*Molothrus ater*) (Gowaty and Plissner 1998). The nest box design used is the standard bluebird box of the North American Bluebird Society (see assembly instructions Eastern/Western bluebird house http://www.nabluebirdsociety.org/eastwestbox.htm). Predator guards were placed around the poles supporting the nest box in order to reduce predation (raccoon guard, Erva Tool, Chicago, Illinois). These metal "stovepipe" cylinders are commonly used to prevent predation by raccoons, domestic cats, and snakes (Gowaty and Plissner 1998).

Placement Beginning in February 2005, I erected 191 nest boxes along the South, Middle, North, and South Fork Shenandoah Rivers to establish a breeding population of tree swallows for spring 2005. There were 102 nest boxes at contaminated sites (South River and South Fork Shenandoah River), and 89 boxes at reference sites (Table 1).

In February 2006, all nest boxes along the South Fork Shenandoah River (n=42) were removed as my focus narrowed to birds nesting along contaminated portions of the South River and appropriate references. Adult female tree swallows are highly philopatric and will return to the same nesting site if they have a successful breeding season (Robertson et al. 1992). In order to accommodate the returning adults and recruit their young from 2005 back to their natal sites to breed, additional boxes were erected. Thus, new nest boxes were erected at many sites along the South, Middle, and North Rivers in order to create available nesting sites for returning juveniles from 2005 (Table 1). If no new boxes had been provided, the older birds, who return from migration earlier (Robertson et al. 1992), would have occupied a majority of the nest boxes before the young birds returned.

Where possible, the total number of available boxes per site was increased by approximately 60%. A few sites did not receive additional boxes or an increase of exactly 60% as suitable habitat and nest boxes were limited. Boxes were also erected at new sites in both the contaminated and reference areas. Twenty-two boxes were erected at three new reference sites along the South (upstream of the contamination), Middle, and North Rivers; 21 boxes were placed at four new sites along contaminated portions of the South River. Thus, at the beginning of the 2006 breeding season there were a total of 119 nest boxes in the contaminated areas and 167 boxes in reference areas; a total of 286 available boxes. Of the 119 nest boxes in the contaminated sites in 2006, 38 (63% increase) new boxes were erected at old sites and 21 (35% increase) were put up in new

TABLE 1

NUMBER OF NEST BOXES PER SITE IN 2005 AND 2006

Site	River	Hg Status	# boxes 2005	# boxes 2006
Basic Park	South	C	5	9
Water Treatment Plant	South	C	8	10
Genicom	South	C	8	13
Dooms river crossing	South	C	5	9
Crimora river crossing	South	C	2	3
Augusta Forestry Center	South	C	20	31
Wampler property	South	C	0	6
Harriston river crossing	South	C	0	3
Rankin property	South	C	0	8
Grand Caverns	South	C	2	7
Grottoes City Park	South	C	10	16
Bradburn Park	South	C	0	4
SUBTOTAL			**60**	**119**
Port Republic	South Fork	C	9	0
Sheets Farm	South Fork	C	5	0
Merck Plant	South Fork	C	28	0
SUBTOTAL			**42**	**0**
Cowbane Nature Preserve	South	R	12	16
P Buckley Moss Barn	South	R	0	11
Ridgeview Park	South	R	6	11
Locust Street	South	R	7	7
Whitescarver Farm	Middle	R	15	37
Godfrey Farm	Middle	R	8	14
Smith's Pond	Middle	R	10	17
Fort River Road	Middle	R	4	4
Dories /Middle River Rd	Middle	R	6	14
Shapcot property	Middle	R	3	5
Crawford annex	North	R	14	20
Flora property	North	R	4	0
Sandy Bottom Park	North	R	0	4
Rt. 276 river crossing	North	R	0	7
SUBTOTAL			**89**	**167**
TOTAL AVAILABLE BOXES			**191**	**286**

locations. In the reference areas, of the 167 nest boxes available in 2006, 60 (67% increase) were new nest boxes added to old sites and 22 (25% increase) were erected in new locations. Thus, the change in number of sites and number of nest boxes was similar for reference and contaminated treatment groups.

Microhabitat My goal was to place the nest boxes as close to the river as possible to ensure that the birds nesting there were feeding from the river rather than over the floodplain. When choosing a nest site, tree swallows avoid wooded pastures and more commonly nest in sites with tall grasses in extensive open areas to accommodate their aerial foraging (Willner et al. 1983, Munro and Rounds 1985, Lumsden 1989). Due to the presence of a thin riparian zone along the river bank at the majority of the study sites, some boxes were in close proximity to trees and shrubs.

The entrance hole of all boxes was oriented towards the river for uniformity with respect to microhabitat conditions. Rendell and Robertson (1994) reported that tree swallows prefer cavities with entrance holes facing S-SE; however, cavity orientation did not affect reproductive success in that study. Their study took place in Ontario, Canada, where increased thermoregulatory benefits to birds nesting in S-SE facing cavities may have been more important than in Virginia. As entrance hole orientation did not appear to affect reproductive success (Rendell and Robertson 1994), the orientation of my nest boxes remained facing the river, regardless of compass direction, to encourage foraging directly over the aquatic habitat and to provide an axis of uniformity across all sites.

The spacing of the nest boxes was taken into careful consideration based on the territoriality of the target species. Unlike the eastern bluebird (*Sialia sialis*), tree swallows do not defend feeding territories and will therefore nest in close proximity to each other, 10-15 m from the nearest conspecific (Muldal et al. 1985, Robertson et al. 1992). The boxes were spaced 20-40 m apart in hopes of reducing competition with bluebirds by providing multiple boxes within the same bluebird-sized territory (Gowaty and Plissner 1998).

Box checking Tree swallows arrive on the Virginia breeding grounds in March and lay their eggs in mid-May to early June (Robertson et al. 1992, Lane and Pearman 2003). Weekly nest checks began on April 2nd in 2005 and April 1st in 2006 ; each nest box was checked for the presence of a nest or a nest with eggs. The status of the nest box was noted, including the species, stage of the nest (either partial or complete), and number of eggs present, if applicable. All house sparrow nests were removed if found in any nest box, unless nestlings were present in which case they were left until the chicks fledged. After fledging, any new house sparrow nesting material was removed from the box to prevent a second clutch from being laid.

Nest boxes were checked with increasing frequency, every 3-4 days, in the first week of May in both 2005 and 2006, as tree swallows were predicted to begin laying eggs at that time. In 2006, this study site had the Virginia state record early egg date for tree swallows (4/18/2006, R. Clapp pers. comm.) In order to predict the hatch date of each nest, I needed to find the eggs before the clutch was complete, necessitating frequent visits. Once a tree swallow nest was found with eggs, it was checked one additional time to determine the complete clutch size and predict hatching date. All nests were removed from the boxes after the chicks had fledged as tree swallows prefer nest boxes free of old nesting material (Rendell and Verbeek 1996). Emptied nest boxes were checked on a weekly basis through the end of July to detect late-nesting or second-clutching birds.

Catching adult tree swallows

2005 During the incubation period, the female was not disturbed to prevent nest abandonment. Both SY and ASY female tree swallows are more prone to nest abandonment during the incubation period compared to the nestling period (Lombardo 1989). Once the chicks hatched, an attempt was made to catch the breeding female at all nest boxes. Adults were caught in one of two ways, either removed from the nest directly if found brooding the chicks, or by using simple nest box traps (Stutchbury and Robertson 1986). All adults were banded with USGS bands upon capture for later identification. Mass and wing cord of the right wing were recorded as well.

If neither adult was present in the nest box when opened, a trap was set and monitored from 25-30 m away. Nest box traps were frequently left unattended and checked within 30 minutes to see if the trap had been triggered with a bird inside. Nests were never disturbed for longer than one hour regardless of the success in catching either parent. If neither adult was caught, a second attempt was rarely made to catch them using the nest box traps due to time constraints.

Traps were left in for shorter periods of time when attempting to catch the male as I was focusing on catching as many of the females as possible and maximizing the number of nests visited per day. Stutchbury and Robertson (1986) noted a bias in catching more females than males, but were able to catch a small proportion of males by providing them with feathers during the nest building and incubation periods. As my nest boxes were not disturbed during incubation, that method of catching males was not an option.

2006 In 2006, all females were caught and greater emphasis was placed on catching the males as well. It was necessary to catch all females to determine return rates of female adults and nestlings banded in 2005. As a number of adult males were banded in 2005, including an unknown number of male nestlings, additional effort was put into determining male return rates. The nest box trap was modified in an attempt to catch wary males. Rather than having a stick prop the trap up inside the nest box, a stick was placed in the gap between the sides of the nest box and the roof (for design, see Appendix B). This removed the visual cue presented by the prop stick in the simple nest box trap. A piece of 4-6 lb blue or green fishing line was attached to the end of the stick and strung out 25-40m away where it was held taught by an observer. Once a bird entered the nest box, the fishing line was pulled to remove the stick and the trap door fell.

The first bird to enter the box was immediately caught and removed and the trap was reset. If unable to catch the female on the first day attempted, I returned to the box within a few days for a second attempt, never needing a third attempt. It was typically possible to determine whether or not the male was banded by looking for a band on the right leg using binoculars. If the male was unbanded, attempts to catch him were cut short on occasion due to time constraints. If the male was banded, every effort was made to catch him, including returning to the box on a different day if I was unable to catch him within an hour. If the adult captured was banded, the band number was recorded because it was likely to be a recapture from 2005. All unbanded adults caught in 2006 were banded with USGS bands. Mass and wing cord of the right wing were recorded for all birds regardless of band status. A photograph of the right tarsus of recaptured, banded birds from 2005 also taken to be used as a skeletal measurement in the index of body condition.

Life History/Reproductive Success Parameters

Hatching date Female tree swallows lay one egg per day and normally begin incubation on the day that the penultimate egg is laid (Robertson et al. 1992). The incubation period is most commonly 14-15 days; however, it has been recorded to go on for as long as 19 days (Robertson et al. 1992). By checking the nest boxes on a regular schedule, I was able to find most nests with eggs before the clutch was complete, allowing me to predict an accurate hatch date. Hatch date was calculated by adding 14 days to the date on which the penultimate egg was laid. Nest boxes were visited within 1-3 days of this predicted date to record the actual hatch date. If the eggs were unhatched upon the first visit after the predicted hatch date, the nest was revisited within three days.

In cases when the eggs were still not hatched more than five days after the predicted hatch date, I checked for the presence of the female and felt the eggs to see if they were still warm. The presence of the parents was determined by standing next to the box, in the pair's territory, for 10-15 minutes and visually scanning for either the male or female. If the nest was still active, either the female or male and female would quickly return to defend their territory from the intrusion. Nests were left intact if one or both parents were detected as incubation periods are variable. When eggs were cold to the touch more than five days after the predicted hatch date and no adults were present, the eggs were collected and the nest removed.

Nesting success Basic reproductive parameters were assessed at each nest including clutch initiation date, clutch size, proportion eggs hatched, proportion nestlings fledged, proportion eggs fledged, and number of fledglings produced. Clutch initiation date, the day on which the first egg was laid is reported as Julian date, where the date on which the first tree swallow egg was laid in this population was Julian Day 1. In this study, Julian day 1 corresponded with the calendar date of 4/29/2005 in year one and 4/18/2006 in year two. Proportion eggs hatched was defined as the number of eggs that hatched divided by the total clutch size. Proportion nestlings fledged was the number of chicks that fledged divided by the number of eggs that had hatched. Proportion eggs fledged was calculated as the number of chicks fledged divided by the number of eggs laid to provide an overall assessment of reproductive effort. Number of fledglings produced was the total number of nestlings fledged from the nest, regardless of clutch size.

Nest boxes were visited after the predicted hatching date to determine the number of eggs that hatched as well as at the end of the nestling period (20-22 days, Robertson et al. 1992) to remove the nest and any chicks that did not fledge. Because there was no uncertainty as to the fate of nestlings, as is typically the case with a study on birds nesting outside of artificial cavities, there was no need to adjust reproductive success parameters using the Mayfield Method (Mayfield 1961).

Egg dimensions In 2006 I measured 94 eggs from 16 nests on contaminated sites and 79 eggs from 14 nests on reference sites. Using calipers, the maximum length and breadth of each egg was measured to the nearest 0.1 mm. The mass (g) of each egg was determined using a digital balance and returned to the nest. Egg volume (V) was determined by the formula,

$$V=LB^2 * 0.51$$

where L= length (cm), B= breadth (cm), and 0.51 is the volume coefficient constant (Hoyt 1979). This formula estimates egg volume within 2% (Hoyt 1979).

Chicks

Chicks were weighed three times in order to determine whether there were differences in growth in chicks in the contaminated versus reference areas in 2005. Mass was recorded using a digital balance at days 4, 8, and 15 post-hatch, plus or minus one day. Not all broods were measured on all three of the target days and therefore some nests may only be represented by one or two data points in the growth curve. All nestlings were banded with USGS bands on day 15 for later identification.

Tree swallow chicks reach their peak weight between days 12-14 (De Steven 1980); therefore the final weight provided not only the completion of the growth curve, but also served as an estimate of the condition of the chicks at fledging (De Steven 1980). An attempt was made to record the growth trajectory for each individual chick by marking them with individually unique combinations of nail polish on the toenail of each chick. However, as I only visited the nests every 4-5 days, the polish typically came off before I returned to the nest making it impossible to follow the chicks individually before they were banded on day 15. The average of the brood was used to eliminate noise and gain a more representative value as I was interested in the success of the whole brood, not particular individual nestlings. Average brood mass was calculated at day 15 as a measure of pre-fledgling condition; brood mass was compared between nests contaminated and reference areas using the residuals of brood mass on age, to control to any differences in age. As the residuals were not normally distributed, I used a non-parametric, Mann-Whitney U test to compare pre-fledging condition.

2006 Only broods of chicks from returning, banded females were measured in 2006. Mass was determined on day 15, along with photographs of the right and left tarsi to provide an index of pre-fledging condition (see *Pre-fledging condition* for methods). Comparing pre-fledging condition of broods of returning females in contaminated and reference areas could determine whether parental care was impacted by breeding on a contaminated site two years in a row. However, as tarsus length did not correlate with body mass based on analyses from 2005, pre-fledging condition has not been analyzed for broods in 2006.

Pre-fledging condition (2005) On day 15 (±1-2 days) photographs of the right and left tarsi were taken in addition to mass. The photographs provided a skeletal measurement with which the mass at day 15 could be related to provide an overall pre-fledging condition. All tarsi were photographed while being held in a standardized position. Tarsi were measured using Adobe® Photoshop 5.0LE. The photograph of the right tarsus of each chick was measured three times. Using the line tool, the distance from the proximal end of the tarsus to the end of the second scale (moving proximal to distal) at the distal end of the tarsus was measured for each chick (LeClerc et al. 2005). Brood mass was regressed on tarsus length at day 15 to determine whether broods in contaminated areas and reference areas differed with respect to the residual of mass on age. However, when broods from contaminated and reference areas were combined, there was no significant linear relationship between brood mass and tarsus length ($F_{1,38}$=0.08, R^2=0.002, p=0.78, see Fig. 21). Further, when analyzed separately, there was no significant relationship between brood mass and tarsus length in contaminated ($F_{1,13}$=0.00, R^2=0.0, p=0.95) or reference ($F_{1,23}$=0.06, R^2=0.003, p=0.81) areas. The lack of a relationship between these two morphometrics precluded further comparison of pre-fledging condition in this manner and will not be discussed further.

Mercury sampling

Blood Blood samples provide the best gauge of short-term dietary uptake of mercury in insectivorous birds (Evers et al. 2005). The mercury present in the blood is greater than 95% methylmercury and accurately reflects the dietary availability of this contaminant to the bird (Evers et al. 2005). Blood samples were taken from all adult and nestling tree swallows in 2005. In 2006, blood was collected from all adults, but only from nestlings within targeted areas. Nestling blood and feather samples were taken from broods at Dooms, Augusta Forestry Center, and Grottoes City Park for a related isotope study. Blood samples were also taken from nestlings at new sites between Crimora and Grottoes to fill in gaps in the 2005 data set.

For the tree swallows, a small gauge (26G ½) needle was used to puncture the cutaneous ulnar, also known as the brachial, vein. Blood was collected into three, 75µL heparinized capillary tubes $^{2}/_{3}{}^{rds}$ full. The capillary tubes were then sealed with Crito-caps® and placed into a 10cc BD® vacutainer to prevent breakage. Protective latex gloves were worn at all times and field sharps containers were available for immediate disposal of all needles and blood-contaminated supplies. Blood samples were placed into labeled, Ziploc® bags and stored in a cooler with ice until I returned from the field site where they were transferred to a locked freezer (-25° Celsius).

Feathers Feather samples in this study were collected from adult birds to provide an index of long-term mercury accumulation and from chicks to assess mercury accumulated through the nestling diet. In 2005, the two second-to-outermost retrices as well as eight back feathers were collected from all adults. It was unknown where each bird grew in their feathers the previous year, but it almost certainly was not on my study site, as no nest boxes or appropriate habitat were available. Thus, these feathers provide a baseline mercury level with which to compare blood levels from 2005. Eight body feathers were collected from all nestlings at day 14 as well. Feathers were placed inside labeled Ziploc® bags and stored in a cooler with ice until I returned to the field house where they were transferred to a locked freezer (-25° C).

When the birds banded in 2005 returned to the breeding grounds in 2006, the innermost primary feather (P1) was collected from both wings in addition to nine back and nine chest feathers (to be used for another study on pigmentation). The flight feathers are the first to molt in tree swallows, beginning with P1 followed by the rest of the primaries, secondaries and retrices; body feathers are molted last (Stutchbury and Rohwer 1990). As flight feather molt typically begins in mid-July (Stutchbury and Rowher 1990), mercury accumulated on the breeding grounds in 2005 will occur in the highest amounts in this feather. P1 in returning birds represents not only dietary uptake, but also reflects the overall body burden of each bird during its time on the breeding grounds in 2005. P1 was also collected from all unbanded birds found using the nest boxes in 2006 in order to standardize any effects of removing these feathers on the reproductive success across all nests in the study.

Feathers from neither year have been analyzed at this time due to lack of funding. Therefore, no analysis will be presented on feather mercury values.

Laboratory analysis

All mercury analysis took place at the TERL laboratory at Texas A&M University. Mercury samples were analyzed using a Milestone DMA 80 direct mercury analyzer equipped with a 40 position autosampler and a dual cell detector. Mercury samples remained frozen until they were analyzed based on wet weight. As this machine analyzes total mercury, speciation was not a problem. Because approximately 95% of the mercury in blood is methylmercury (Evers et al. 2005), total mercury values produced will accurately reflect the amount of methylmercury present in the sample.

Mercury was analyzed using cold vapor atomic absorption spectroscopy (CVAA), a method pioneered by the work of Hatch and Ott (1968). This method allows mercury samples to be analyzed directly, without requiring sample digestion. After homogenization, this method combusts the samples to release the mercury onto a gold surface which traps the mercury as a concentrated slug ready for analysis via atomic absorption (AA). This methodology is particularly well suited for samples of small mass, limited to samples of 0.1-0.2 g at most (Hatch and Ott 1968).

In this study, samples were homogenized to allow a representative aliquot to be taken for analysis. Samples typically weighed less than or equal to 0.1 g. These samples were weighed on pre-combusted boats and placed on an autosampler carousel. The boats were moved into the sampler by a pneumatic arm and subjected to heating while under a constant flow of O_2. Combustion gasses were passed through a heated catalyst and then through a gold trap, releasing the mercury as a concentrated slug into the gas stream. The released mercury was then moved into a two-stage absorption cell where free mercury (Hg^0) atoms absorbed light from a mercury vapor lamp. This process resulted in two absorption peaks; a sensitive, long path length cell, and one from a less sensitive, short cell. The mercury concentrations in each sample were measured quantitatively by comparing peak absorption with that of known calibration standards.

QAQC sampling Measures of quality assurance/quality control are used to demonstrate the accuracy and precision in monitoring. Quality Assurance (QA) refers to maintaining quality in all aspects of a program while Quality Control (QC) consists of the steps taken to determine the validity of specific sampling and analytical procedures.

In the field, multiple blood samples were collected from each bird to be used in assessing the accuracy and precision of the laboratory analysis. For each sample collected, two duplicate samples were taken at the same time and same location for each bird. One duplicate was stored as a back-up in case of shipping or laboratory problems. The other was available for use as a blind field duplicate, which was included for one of every 20 samples, without the knowledge of the analytic laboratory. Duplicates of blood samples were not identified as method duplicates on the chain of custody form when shipped to the laboratory. Field blanks were not used.

Statistical analyses

Mercury levels The field sites were grouped as follows: the contaminated portion of the South River, the South Fork Shenandoah River (SFSR), and the reference rivers consisting of values from upstream of the contamination on the South River, and the Middle and North Rivers. The South River is referred to as the "contaminated area (or sites)" throughout the results section; the SFSR is treated as a separate entity because it receives all of the water from both the reference and South rivers. Statistical analyses were done using MINITAB 14.2 statistical software (Release version 14.2, LEAD

Technologies, State College, PA). Mercury levels were compared using the general linear model (GLM) to run an analysis of variance (ANOVA). Mercury samples from 2005 and 2006 were analyzed combined and separately as different sites and factors needed to be considered in 2005 and 2006 (see Appendix C1 and C2).

To determine the differences in mercury in 2005 only, I used a GLM to run an ANOVA using treatment group (contaminated, reference, or SFSR) and sex as factors; the interaction term used was treatment group*sex. To compare mercury levels in 2006 only, I used treatment group (contaminated or reference), sex, and recapture status (recapture from 2005 or new to the site) as factors. Interaction terms included treatment group*sex and treatment group*recapture status. To directly compare mercury levels in recaptured birds and birds new to the study site, only ASY birds were used to control for any effect of age. I used a GLM to run an ANOVA using treatment group, sex, and recapture status as factors and treatment group*sex, treatment group*recapture status, and sex*recapture status as interaction terms. This analysis was different from the previous one as recapture status in that case included birds of all ages.

Mercury levels were also compared between years. I combined all of the mercury samples from both years and used a GLM to run an ANOVA using year, treatment group, and sex as factors; interaction terms included year*treatment group, treatment group*sex, and year*sex. This analysis also determined the overall effect of sex on mercury level. To determine whether, overall, female age class had an effect on mercury levels, I used a GLM to run an ANOVA using treatment group, female age, and year as factors with treatment group*female age, treatment group*year, female age*year, and treatment group*female age*year as interaction terms. Because mercury levels appeared higher downstream of river mile 10, I used a GLM to run an ANOVA using levels from contaminated sites only to compare mercury levels in this "hot-zone" to levels upstream; zone, year, and sex were used as factors and zone*year and zone*sex were used as interaction terms. I have reported only those factors and interactions that were found to be significant ($p<0.05$) in the results section; any factor or interaction not mentioned was not found to have a significant effect ($p>0.05$). Tukey's post-hoc test was used for multiple comparisons; p-values are provided indicating significant differences ($p<0.05$).

In addition to comparing mercury levels between the "hot-zone" and areas upstream, I attempted to describe the spatial variation in mercury with river mile from the DuPont plant. I used a polynomial regression to look at the distribution of mercury along the South and South Fork Shenandoah Rivers; data were combined from 2005 and 2006 for this analysis as no samples were collected downstream of the confluence in 2006. In a second analysis, I used a linear regression to compare the spatial distribution of mercury with river along the South River only; data from 2005 and 2006 were analyzed separately in this case.

Nesting success

The tree swallow nesting season was divided up into two portions; the early nesting season and late nesting season. All analyses of nesting success presented represent data collected from the birds in the early breeding season. Small sample size precluded the use of the late nesting season data the analyses. Significant results ($p<0.05$) were reported for all analyses, non-significant results were reported only as necessary to provide clarity. In all analyses, data for the proportion of eggs hatched, nestlings fledged, and eggs fledged were arcsine square-root transformed in an attempt to normalize the data as these values were proportions. However, data are shown in all figures. Tukey's post-hoc test was used for multiple comparisons; p-values are provided for significant interactions ($p<0.05$).

Nesting success was compared between the contaminated and reference areas only (see Appendix D1 and D2 for averages). As the focus of this study was on the effects of mercury along the South River, the SFSR data were not included in these analyses. All analyses were performed using MINITAB 14.2 statistical software. The six nesting parameters were compared using a GLM run an ANOVA with treatment group, female age, and year as factors with treatment group*year, female age*year, treatment group*female age, and treatment group*female age*year as interaction terms. A one-way ANOVA was used to compare the nest success of recaptured ASY females and new ASY females to determine any impacts of mercury accumulation across two years. As each individual box was considered an independent sample, and "site" used only as an organizational construct, site was not included as a factor in the analyses. For nests in the contaminated areas, an additional factor of location was added to compare nests in the "hot zone" to those upstream.

It should be noted that 40 (20 contaminated, 20 reference) of the tree swallows nests from which reproductive data were collected in 2006 were also part of a related study on the effects of mercury on humoral and cell-mediated components of the avian immune system. Adult females were initially captured to determine whether or not they were a returning, banded bird from 2005. Once determined to be unbanded, the female was injected with PHA (phytohaemagglutinin) in the right patagium (wing web) approximately four days after her nestlings hatched. Patagial width was measured at the site of injection to 0.01 mm using a micrometer prior to and 48 hours after injection. Upon recapture for patagial measurements, birds were intra-abdominally injected with 5 x 10^7 sheep red blood cells (SRBCs) (MP Biomedicals, Irvine, CA, USA) suspended in 100 uL phosphate-buffered saline (PBS). A 50 uL blood sample from the brachial vein was collected prior to and eight days following injection. In total, female swallows used in the immune assay were caught at the nest box 3-4 times; compared to birds not used in this study that were only disturbed once. This protocol has been used extensively on tree swallow nestlings and adults and does not cause any detectable stress, abnormal development, or nest abandonment (Ardia 2005). To be sure the nests used in this study did not experience differential nesting success compared to those not in the study, I analyzed all reproductive data as described above except all nests from the immune study were removed. As the significance of the findings when all nests were used and when the immune nests were removed were the same, I have included the immune assay nests in my analyses.

RESULTS

Nest box use and population dynamics

2005 The early nesting season included all clutches initiated from 29 April to 3 June and the late nesting season included all clutches initiated 10 June to 26 June. No clutches were initiated between June 3-10, therefore this gap was used to divide the two nesting seasons. Two of the late nests were second clutches of females from the early nesting season. I documented only one pair from the early breeding season that failed on the first try and re-nested in the late nesting season. This is an underestimate of the true number of re-nesting attempts because adults were typically not banded until after their eggs hatched and would not have been recognized as re-nesters if they had not been banded.

The number of tree swallow nests in contaminated sites was similar to the number of nests in reference sites during the early nesting season (Table 2). At contaminated sites, 47% of the nest boxes erected were used by tree swallows, compared to 52% usage at reference sites (χ^2=0.12, DF=1, p=0.73). Data were collected from 95 nesting pairs of tree swallows in all. There were fewer late nests (n=22) than there were early nests. These 117 nests represent the entire breeding population of tree swallows using the nest boxes in 2005, as few, if any, nests were missed. There were no known nests outside of nest boxes either, and no suitable habitat was identified for natural nest sites.

Throughout the breeding season, 98 adult tree swallows were banded. Of these birds, 74 were females and 24 were males (Table 3). A larger proportion of female birds were banded as they were targeted in this study. Males were sampled opportunistically when they entered the nest box before females during trapping. A total of 506 nestling birds were banded across all of the study sites (Table 4). Among the adult females that were banded, a majority (n=52/74, 70%) were SY as opposed to ASY birds. In other words, most of the primary breeding population in 2005 was made up of females breeding for the first time.

2006 The nesting season in 2006 was also divided into two portions, an early nesting season (clutch initiation April 18–June 1) and late nesting season (clutch initiation June 5–21). Only four days separated the two portions of the season in 2006. In the late nesting population, nine of the 45 nests were second clutches of successful birds from the early nesting population. Four of the second clutches were in

TABLE 2

NUMBER OF NESTS IN THE EARLY AND LATE NESTING SEASONS 2005

Site	**River**	**Contamination status**	**# boxes erected**	**Early nesting**	**Late nesting**
Basic Park	South	Contaminated	5	2	0
Water Treatment Plant	South	Contaminated	8	3	0
Genicom	South	Contaminated	8	5	0
Dooms river crossing	South	Contaminated	5	3	1
Crimora river crossing	South	Contaminated	2	1	0
Augusta Forestry Center	South	Contaminated	20	10	1
Grand Caverns	South	Contaminated	2	1	0
Grottoes City Park	South	Contaminated	10	3	0
CONTAMINATED TOTAL			**60**	**28**	**2**
Port Republic	South Fork	Contaminated	9	4	0
Sheets family farm	South Fork	Contaminated	5	5	1
Merck Plant	South Fork	Contaminated	28	14	3
SFSR TOTAL			**42**	**23**	**4**
Cowbane Nature Preserve	South	Reference	12	8	4
Ridgeview Park	South	Reference	6	2	0
Locust Street	South	Reference	7	1	0
Whitescarver Farm	Middle	Reference	15	9	1
Godfrey Farm	Middle	Reference	8	4	5
Smith's Pond	Middle	Reference	10	6	3
Fort River Road	Middle	Reference	4	2	0
Dories/Middle River Rd	Middle	Reference	6	2	0
Shapcot property	Middle	Reference	3	2	1
Crawford annex	North	Reference	14	9	2
Flora property	North	Reference	4	1	0
REFERENCE TOTAL			**89**	**46**	**16**

TABLE 3

LOCATION AND NUMBER OF ADULT TREE SWALLOWS BANDED IN 2005

Site	River	Hg status	Males	Females	SY	ASY	Total banded
Basic Park	South	C	1	0	-	-	1
Water Treatment Plant	South	C	0	2	2	0	2
Genicom	South	C	2	4	2	2	6
Dooms river crossing	South	C	2	3	3	0	5
Crimora river crossing	South	C	0	1	0	1	1
Augusta Forestry Center	South	C	3	5	5	0	8
Grand Caverns	South	C	0	1	1	0	1
Grottoes City Park	South	C	1	2	2	0	3
TOTAL CONTAMINATED			**9**	**18**	**15**	**3**	**27**
Port Republic	SFSR	C	1	2	0	2	3
Sheets family farm	SFSR	C	2	2	2	0	4
Merck Plant	SFSR	C	1	14	11	3	15
TOTAL SFSR			**4**	**18**	**13**	**5**	**22**
Cowbane	South	R	2	8	2	6	10
Ridgeview Park	South	R	1	0	-	-	1
Locust Street	South	R	1	0	-	-	1
Whitescarver Farm	Middle	R	2	5	5	0	7
Godfrey Farm	Middle	R	4	5	3	2	9
Smith's Pond	Middle	R	0	6	4	2	6
Fort River Road	Middle	R	0	3	2*	0	3
Dories property	Middle	R	0	1	1	0	1
Shapcot property	Middle	R	0	3	2	1	3
Crawford annex	North	R	1	6	4	2	7
Flora property	North	R	0	1	1	0	1
TOTAL REFERENCE			**11**	**38**	**24**	**13**	**45**
TOTALS ALL SITES			**24**	**74**	**52**	**21**	**98**

* does not include 1 bird of unknown age

TABLE 4

LOCATION AND NUMBERS OF NESTLINGS AND BROODS BANDED IN 2005

Site	River	Hg status	Nestlings	Broods
Basic Park	South	Contaminated	8	2
Water Treatment Plant	South	Contaminated	11	2
Genicom	South	Contaminated	28	5
Dooms river crossing	South	Contaminated	18	4
Crimora river crossing	South	Contaminated	6	1
Augusta Forestry Center	South	Contaminated	40	8
Grand Caverns	South	Contaminated	5	1
Grottoes City Park	South	Contaminated	15	3
TOTAL CONTAMINATED			**131**	**26**
Port Republic	South Fork	Contaminated	14	3
Sheets family farm	South Fork	Contaminated	33	7
Merck Plant	South Fork	Contaminated	79	16
TOTAL SOUTH FORK			**126**	**26**
Cowbane Nature Preserve	South	Reference	50	10
Ridgeview Park	South	Reference	12	2
Locust Street	South	Reference	6	1
Whitescarver Farm	Middle	Reference	45	8
Godfrey Farm	Middle	Reference	35	8
Smith's Pond	Middle	Reference	33	6
Fort River Road	Middle	Reference	9	2
Dories property	Middle	Reference	9	2
Shapcot property	Middle	Reference	13	3
Crawford annex	North	Reference	37	8
Flora property	North	Reference	0	0
TOTAL REFERENCE			**249**	**50**
TOTALS FROM ALL SITES			**506**	**102**

contaminated areas, five were in reference areas. There were two documented re-nests in the primary breeding population, as well as one in the late nesting population that occurred as a result of a failed first nesting attempt, but again any unbanded birds failing before hatching would have escaped detection.

Although there were more nests in the reference areas than in the contaminated, there were also more boxes, so the proportion of available nest boxes that were used in the contaminated (56%) and reference (59%) areas did not differ (χ^2=0.07, DF=1, p=0.79). Altogether, data were collected from 165 nests in the early nesting season and 45 nests in the late nesting season (Table 5). With even more confidence than in 2005 I can say that, few, if any, tree swallow nests were missed in 2006.

Of the 245 adult tree swallows that were banded in 2006, 98 were male and 147 were female. A larger proportion of males were caught in 2006 as more emphasis was placed on determining the number of returning, banded birds from 2005. The numbers of SY and ASY females present in 2006 were almost equal at 68 and 72 birds, respectively (Table 6). In contrast to 2005, the breeding population consisted of almost equal numbers of birds nesting for the first time and older birds. In addition to the 245 birds banded in 2006, 49 birds first captured on the site in 2005 were recaptured, of which 30 were former breeding females, seven were former breeding males, and 12 were former nestlings (two males, 10 females) (see Chapter 2 for details on return rates). A total of 836 nestling birds were banded from 172 broods across all sites in 2006 (Table 7).

TABLE 5

NUMBER OF NESTS IN THE EARLY AND LATE NESTING SEASONS IN 2006

Site	River	Status	# boxes erected	Early nesting	Late nesting
Basic Park	South	C	9	8	3
Water Treatment Plant	South	C	10	7	0
Genicom	South	C	13	9	5
Dooms river crossing	South	C	9	6	0
Crimora river crossing	South	C	3	2	0
Augusta Forestry Center	South	C	31	17	4
Wampler property	South	C	6	5	0
Harriston	South	C	3	2	0
Rankin property	South	C	8	0	0
Grand Caverns	South	C	7	2	0
Grottoes City Park	South	C	16	9	1
Bradburn Park	South	C	4	0	0
TOTAL CONTAMINATED			**119**	**67**	**13**
Cowbane	South	R	16	13	3
P Buckley Moss Barn	South	R	11	4	0
Ridgeview Park	South	R	11	5	1
Locust Street	South	R	7	2	0
Whitescarver Farm	Middle	R	37	16	10
Godfrey Farm	Middle	R	14	13	6
Smith's Pond	Middle	R	17	15	3
Fort River Road	Middle	R	4	3	0
Dories/Middle River Rd	Middle	R	14	8	1
Shapcot property	Middle	R	5	3	1
Crawford annex	North	R	20	11	7
Rt. 276 river crossing	North	R	4	2	0
Sandy Bottom Park	North	R	7	3	0
TOTAL REFERENCE			**167**	**98**	**32**

TABLE 6

LOCATION AND NUMBER OF BANDED ADULT BIRDS 2006

Site	River	Hg status	Males	Females	SY	ASY	Total banded	Recaps
Basic Park	South	C	5	6	2	4	11	2
Water Treatment Plant	South	C	4	5	4	1	9	2
Genicom	South	C	8	10*	4	5	18	2
Dooms river crossing	South	C	2	5	3	2	7	1
Crimora river crossing	South	C	1	2	2	-	3	1
Augusta Forestry Center	South	C	12	13	7	6	25	6
Wampler property	South	C	3	3	2	1	6	1
Harriston river crossing	South	C	0	2	2	-	2	-
Rankin property	South	C	0	0	-	-	0	-
Grand Caverns	South	C	2	1	1	-	3	1
Grottoes City Park	South	C	5	10	4	6	15	1
Bradburn Park	South	C	0	0	-	-	0	-
TOTAL CONTAMINATED			**42**	**57**	**31**	**25**	**99**	**17**
Cowbane Nature Preserve	South	R	5	12	3	9	17	5
P Buckley Moss Barn	South	R	1	4**	1	1	5	-
Ridgeview Park	South	R	3	4*	2	1	7	-
Locust Street	South	R	2	1	-	1	3	1

(continued)

* 1 female of unknown age included in total.
** 2 females of unknown age included in total.

Table 6 (continued)

LOCATION AND NUMBER OF BANDED ADULT BIRDS 2006

Site	River	Hg status	Males	Females	SY	ASY	Total banded	Recaps
Whitescarver Farm	Middle	R	11	17	9	8	28	10
Godfray Farm	Middle	R	7	10	4	6	17	7
Smith's Pond	Middle	R	8	16**	7	7	24	3
Fort River Road	Middle	R	2	3	1	2	5	-
Dories/Middle River Rd	Middle	R	6	7*	3	3	13	1
Shapcot property	Middle	R	1	2	1	1	3	2
Crawford annex	North	R	7	11	3	8	18	2
Rt. 276 river crossing	North	R	1	2	2	-	3	-
Sandy Bottom Park	North	R	2	1	1	-	3	1
TOTAL REFERENCE			**56**	**90**	**37**	**47**	**146**	**32**
TOTAL ALL SITES			**98**	**147**	**68**	**72**	**245**	**49**

* 1 female of unknown age included in total.
** 2 females of unknown age included in total.

TABLE 7

NESTLINGS AND NUMBER OF BROODS BANDED IN 2006

Site	River	Hg status	Nestlings	Broods
Basic Park	South	Contaminated	28	7
Water Treatment Plant	South	Contaminated	28	6
Genicom	South	Contaminated	46	10
Dooms river crossing	South	Contaminated	20	4
Crimora river crossing	South	Contaminated	10	2
Augusta Forestry Center	South	Contaminated	75	16
Wampler property	South	Contaminated	12	3
Harriston river crossing	South	Contaminated	8	2
Rankin property	South	Contaminated	0	0
Grand Caverns	South	Contaminated	11	2
Grottoes City Park	South	Contaminated	50	10
Bradburn Park	South	Contaminated	0	0
TOTAL CONTAMINATED			**288**	**62**
Cowbane Nature Preserve	South	Reference	85	16
P Buckley Moss Barn	South	Reference	17	4
Ridgeview Park	South	Reference	22	4
Locust Street	South	Reference	13	2
Whitescarver Farm	Middle	Reference	105	24
Godfray Farm	Middle	Reference	72	12
Smith's Pond	Middle	Reference	82	15
Fort River Road	Middle	Reference	16	3
Dories property/Middle River Rd	Middle	Reference	28	7
Shapcot property	Middle	Reference	24	5
Crawford annex	North	Reference	66	14
Rt. 276 river crossing	North	Reference	9	2
Sandy Bottom Park	North	Reference	9	2
TOTAL REFERENCE			**548**	**110**
TOTAL ALL SITES			**836**	**172**

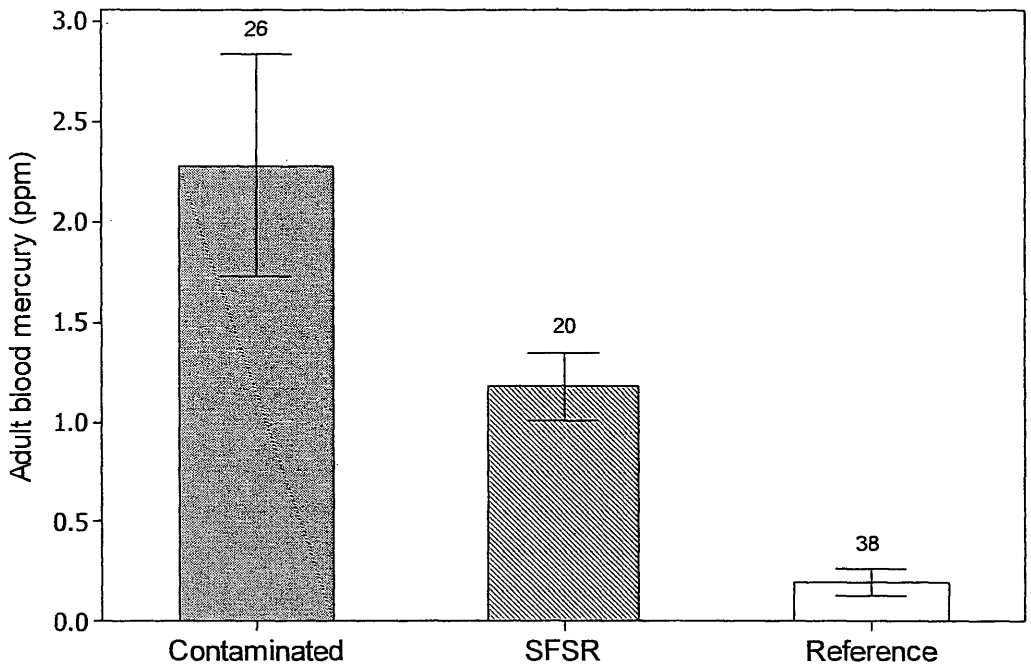
Objective 1: Was mercury accumulated by tree swallows, a non-piscivorous species, nesting along the South River?

Mercury levels 2005 I found a significant effect of treatment group on the mercury levels of adult birds in 2005 ($F_{2,77}=36.80$, $p<0.0001$). Post-hoc comparisons indicated blood mercury levels of adult birds nesting in contaminated areas to be approximately an order of magnitude higher than birds nesting in the reference areas (Fig. 2, $p<0.001$). I compared the mercury levels of birds nesting along the South River to those of the SFSR downstream of the confluence as well, using post-hoc comparisons. Birds nesting downstream of the confluence were predicted to have had lower mercury levels due to the influence of a large amount of clean water entering the South River at this point. Adult swallows nesting along the contaminated portions of the South River did have, on average, higher mercury levels than birds nesting downstream of the confluence on the South Fork Shenandoah River ($p=0.006$). Although adults from the SFSR had lower mercury levels than birds along the South River, post-hoc comparisons show they still had higher mercury than birds in reference areas ($p=0.02$).

There was also a significant effect of treatment group when brood mercury levels were compared ($F_{2,43}=16.55$, $p<0.0001$). Based on post-hoc comparisons, broods of nestling tree swallows in the contaminated areas had higher mercury levels than broods in the reference areas (Fig. 3, $p <0.0001$). Since adult mercury levels were significantly different above and below the confluence in Port Republic, I compared brood mercury in the same manner. Similar to the relationship found with adult birds, post-hoc comparisons indicated broods at sites along the South River had higher average mercury

FIGURE 2

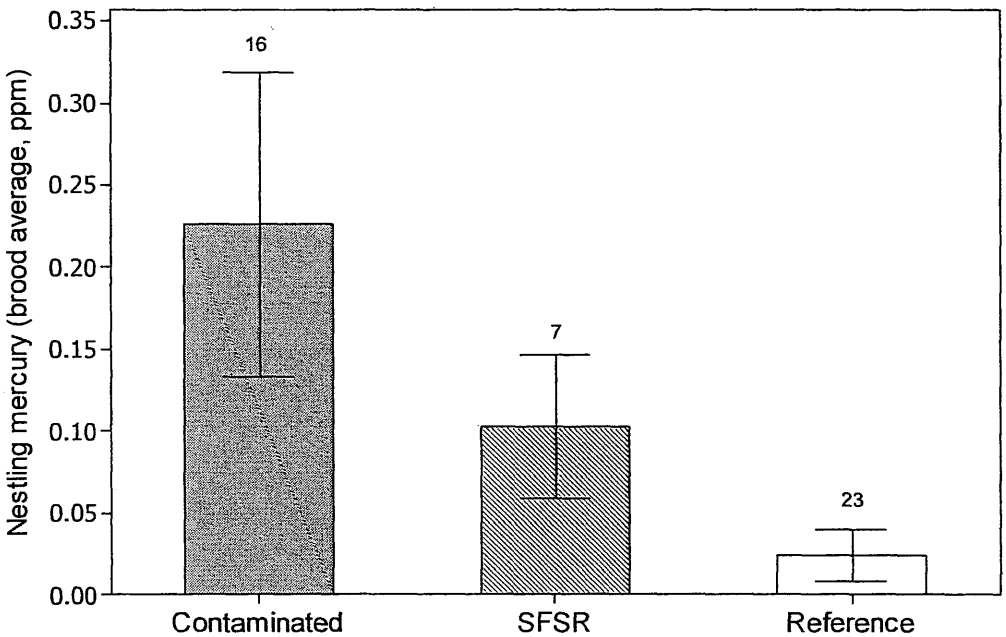
ADULT BLOOD MERCURY LEVELS 2005



Error bars represent 95% CI of the mean.

FIGURE 3

NESTLING BLOOD MERCURY LEVELS 2005



Nestling mercury levels displayed as brood averages. Sample sizes are number of broods sampled; error bars represent the 95% CI of the mean.

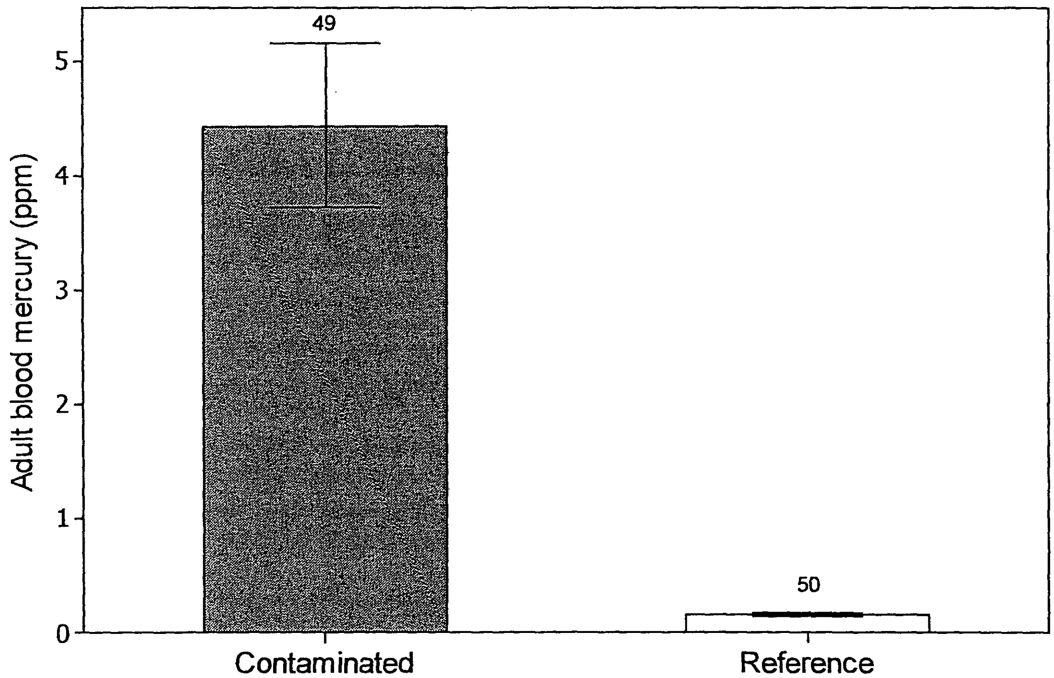
levels than broods on the SFSR, ($p=0.04$). While broods along the SFSR tended to have higher average mercury than broods in reference areas, the difference was not significant based on post-hoc comparison ($p=0.22$).

Mercury levels 2006 Similar to the findings in 2005, mercury in adult tree swallow blood in 2006 in contaminated areas was approximately an order of magnitude higher than birds nesting in reference areas (Fig. 4, $F_{1,92}=116.50$, $p<0.001$). There was no effect of recapture status; the blood mercury levels of recaptured ASY birds from 2005 were similar to ASY birds new to the site in 2006 in contaminated and reference areas (Fig. 5, $F_{1,62}=0.03$, $p=0.86$). This suggests that the increase in mercury from 2005 to 2006 may not have been due, in part or entirely, to the previous exposure of returning birds (unless ASY birds "new" to the study in 2006 were living on the contaminated sites as undetected floaters in 2005). Nestling blood mercury was not sampled at each site in 2006; samples that were collected have not yet been analyzed. No samples were collected along the SFSR in 2006.

Were mercury levels higher in 2006? I compared mercury levels in 2005 and 2006 and found significant effects of year ($F_{1,155}=19.96$, $p<0.0001$) and treatment group ($F_{1,155}=145.14$, $p<0.0001$), as well as a significant interaction between treatment group and year ($F_{1,155}=21.64$, $p<0.0001$). Post-hoc comparison indicated that adult tree swallow mercury levels were significantly higher in 2006 than in 2005 in contaminated areas (Fig. 6, $p<0.001$), and this difference was potentially biologically significant because it was an increase of approximately 100%. There was not a corresponding difference in the mercury levels of birds in reference areas between 2005 and 2006 ($p=0.97$). A number of

FIGURE 4

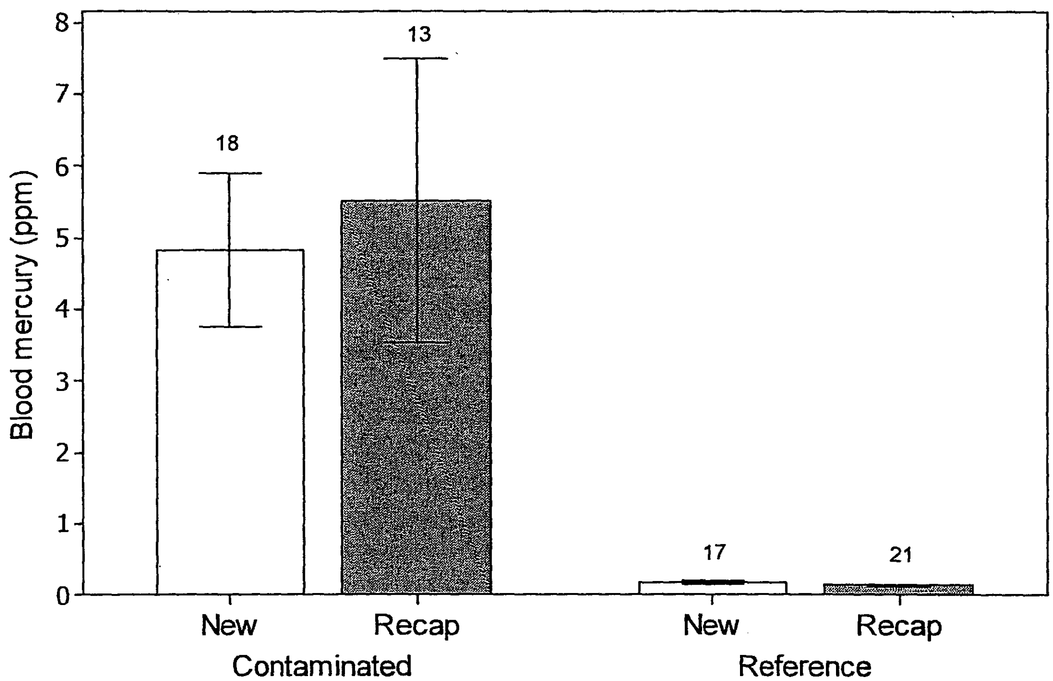
ADULT BLOOD MERCURY LEVELS 2006



Average adult blood mercury in contaminated areas was significantly higher than mercury levels in adult birds in reference areas in 2006. Error bars represent 95% CI of the mean.

FIGURE 5

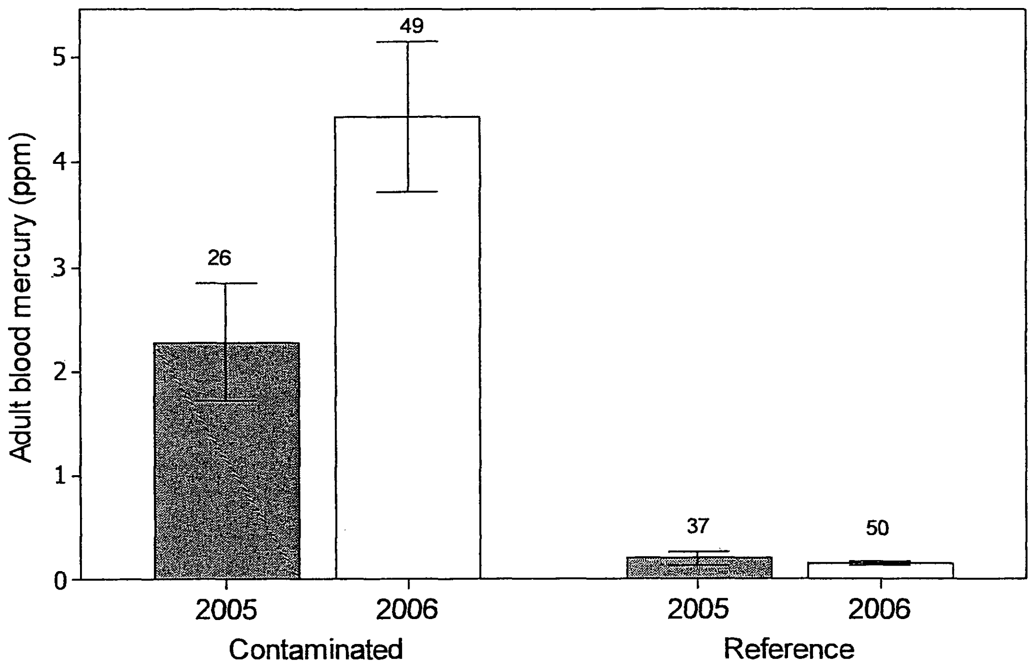
EFFECT OF RECAPTURE STATUS ON MERCURY LEVELS IN 2006



Mercury levels in recaptured ASY adults (male and female) in 2006 and ASY adults new to the study site in 2006 did not differ in the contaminated or reference areas. Error bars represent the 95% CI of the mean.

FIGURE 6

ADULT MERCURY LEVELS IN 2005 VS. 2006



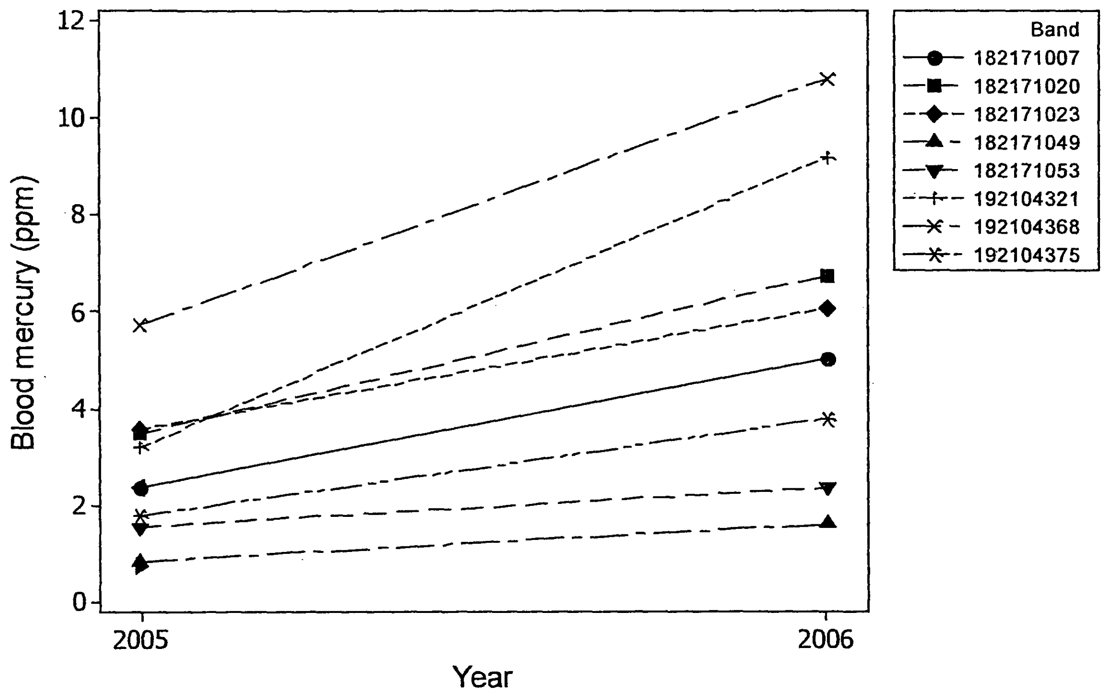
Adult blood mercury levels were significantly higher in 2006 than in 2005 in contaminated areas. There was no difference in mercury levels of adult birds in reference areas between 2005 and 2006. Error bars represent the 95% CI of the mean.

adult females were sampled in 2005 and again in 2006 upon returning to the study site. Using a repeated measures ANOVA, I compared the mercury levels of recaptured ASY females in 2006 to their mercury levels in 2005. This analysis included only banded, adult females that nested at the same sites along the South River in both 2005 and 2006 (n=8). The mercury levels of recaptured adult females in 2006 were significantly higher than in 2005 (Fig. 7, $F_{1,9}$=48.69, p=0.01), and like the ASY population as whole, increased over 100%. Because there was a difference in the age distributions between 2005 and 2006, it is relevant to compare mercury levels by age class. Although ASY mercury tended to be higher than SY in contaminated areas, the effect of female age on mercury level was not significant (F=$_{1,116}$ 3.27, p=0.07) nor was there a significant interaction of female age and treatment group ($F_{1,116}$=2.95, p=0.09) (see *Variables impacting mercury levels: age class* for figure).

In 2006, samples were collected from two new sites within the mercury "hot-zone" (see next section *Where was mercury the highest along the South River?*). As these two sites were located in areas of highest mercury (Wampler property, river mile 14.0, and Harriston, river mile 16.4) it was possible those values caused the average mercury in 2006 to appear higher than in 2005, when these sites were not sampled. To test if the addition of these new sites caused the increase in mercury from 2005 to 2006, I used a GLM to run an ANOVA with site status (new or old) and sex as factors and site status*sex as an interaction term. There was no significant difference in mercury level between the sites used in both 2005 and 2006 and the mercury level of adults at the two new sites (Fig. 8, $F_{1,45}$=0.62, p=0.44).

FIGURE 7

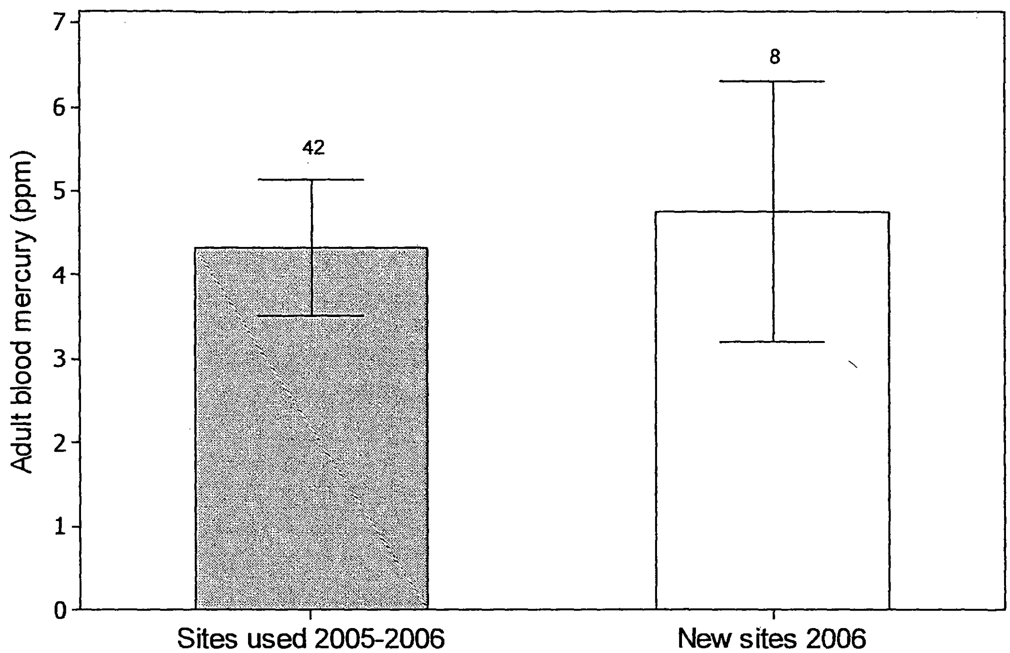
COMPARISON OF MERCURY LEVELS IN 2005 AND 2006 IN RECAPTURED FEMALES



Blood mercury levels of recaptured ASY females were significantly higher in 2006 than in 2005. Each line represents the mercury level of an individual in 2005 and 2006.

FIGURE 8

COMPARISON OF MERCURY LEVELS AT NEW SITES AND OLD SITES



The mercury levels of adult birds in the new sites (used only in 2006) did not differ significantly from the mercury levels of birds at sites used in both 2005 and 2006. Error bars indicate 95% CI of the mean, sample size is number of individuals sampled.

Where was mercury the highest along the South River? To determine the spatial distribution of mercury along the South River, including upstream of the contamination source and downstream on the SFSR, data were combined from 2005 and 2006 and plotted by river mile (Table 8). Reference sites began approximately 14 miles upstream of the contamination source; contaminated sites began one mile downstream of the source. Visual examination of the "Adult Hg" column in Table 8 clearly shows that mercury levels in birds upstream of the contamination source were approximately an order of magnitude lower than the mercury levels downstream of the source. Birds nesting closest to the contamination source had lower mercury than birds nesting further downstream, until the confluence in Port Republic, when mercury levels dropped off (Fig. 9).

Mercury levels increased with increasing river mile, but peaked around river mile 16 near Harriston, when sites downstream of the confluence were included. Mercury levels appeared to be highest between miles 10-22.3, and so this stretch of the South River was referred to as the "hot-zone". Mercury levels significantly declined downstream of river mile 24, at the confluence of the South and North Rivers in Port Republic. The mercury levels downstream of the confluence remained elevated compared to reference areas upstream of the contamination source. It should be noted that the range of mercury found in adult birds at a given site was highly variable. The largest range (0.53 - 11.90 ppm) was found at the largest site, the Augusta Forestry Center.

Some of this apparent variation may have been due to the difference in mercury between the two years; therefore I also plotted the mercury levels of birds from 2005 and

TABLE 8

AVERAGE ADULT MERCURY BY RIVER MILE FOR 2005 AND 2006

Site name	River mile	n_{adults}	Adult Hg	SD	Range	n_{broods}	Brood Hg (2005)	SD
Cowbane	-13.7	18	0.16	0.04	0.07 - 0.23	3	0.018	0.0009
Ridgeview Park	-1.7	2	0.28	0.17	0.17 - 0.40	1	0.0212	-
Locust Street	-1.5	3	0.34	0.09	0.27 - 0.44	1	0.0264	-
Contamination source	**0**							
Basic Park/ Water Treatment Plant	1.8	13	1.42	0.81	0.003 - 3.24	2	0.102	0.03
Genicom	2.0	11	2.52	1.11	1.45 - 5.02	4	0.102	0.08
Dooms river crossing	5.2	9	2.56	1.58	0.002 - 4.97	3	0.087	0.03
Crimora river crossing	9.9	3	4.63	0.43	4.22 - 4.58	1	0.562	-
Augusta Forestry Center	11.5	20	4.18	2.35	0.53 - 11.90	3	0.31	0.04
Wampler (2006 only)	14.0	5	5.68	1.60	4.02 - 7.71	-	-	-
Harriston (2006 only)	16.4	3	3.21	1.15	2.25 - 4.48	-	-	-
Grand Caverns	19.9	5	7.86	2.10	5.72 - 10.80	1	0.57	-
Grottoes City Park	22.3	7	4.98	2.34	2.72 - 6.20	2	0.345	0.03
Port Republic (2005 only)	24.1	3	1.39	0.08	1.34 - 1.48	-	-	-
Sheets Farm (2005 only)	31.0	3	0.63	0.58	0.008 - 1.16	-	-	-
Merck (2005 only)	37.0	14	1.24	0.22	0.99 - 1.73	-	-	-

FIGURE 9

ADULT BLOOD MERCURY WITH RIVER MILE IN 2005 AND 2006

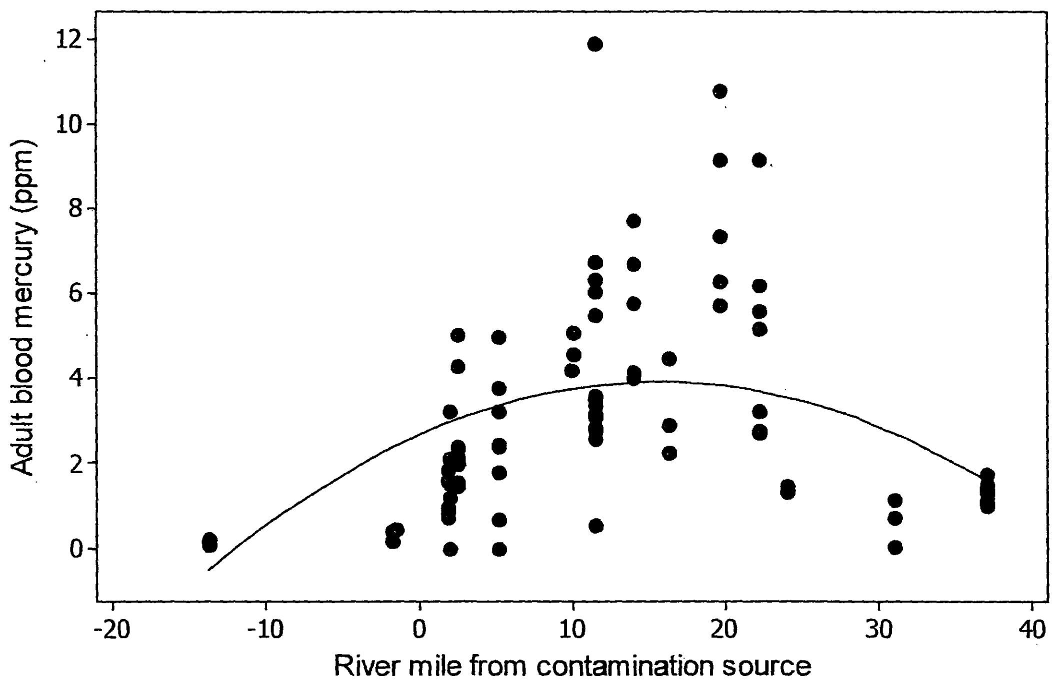
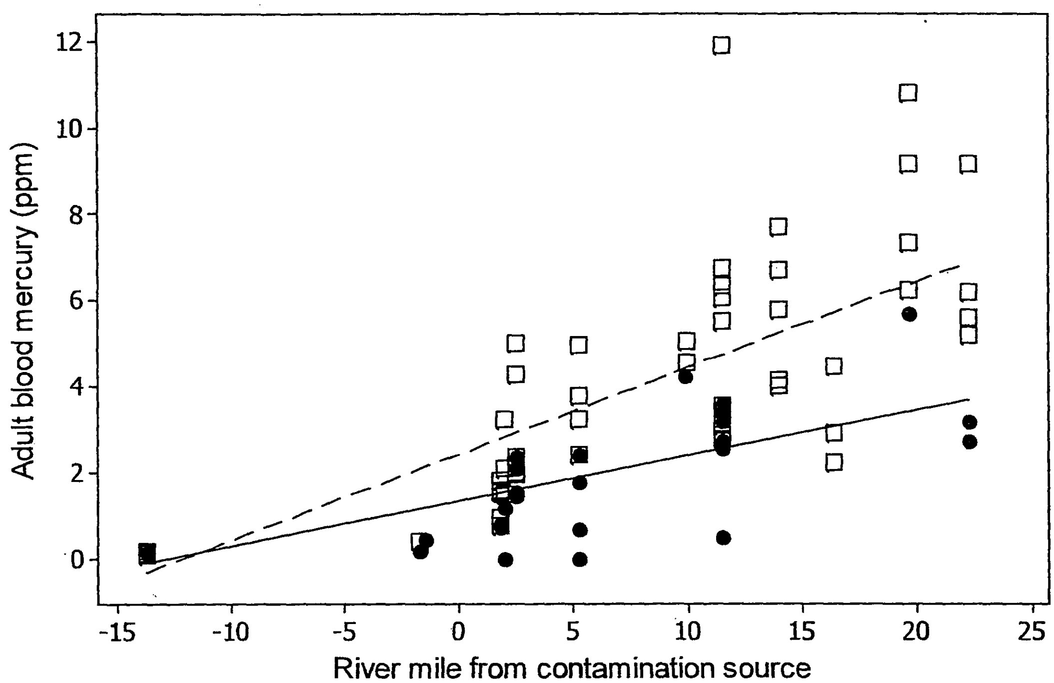


FIGURE 10

ADULT MERCURY WITH RIVER MILE ALONG THE SOUTH RIVER



There were positive, significant linear relationships between adult blood mercury level along the South River and river mile in 2005 (closed circles, solid line) and 2006 (open squares, broken line).

2006 separately. As no samples were collected downstream of the confluence in 2006, only individuals from the South River were plotted with river mile for this comparison (Fig. 10). When sites downstream of the confluence were removed, there were positive, significant linear relationships between mercury level and river mile in 2005 ($F_{1,33}$=46.68, R^2=0.59, p<0.0001) and in 2006 ($F_{1,59}$=97.09, R^2=0.62, p<0.0001).

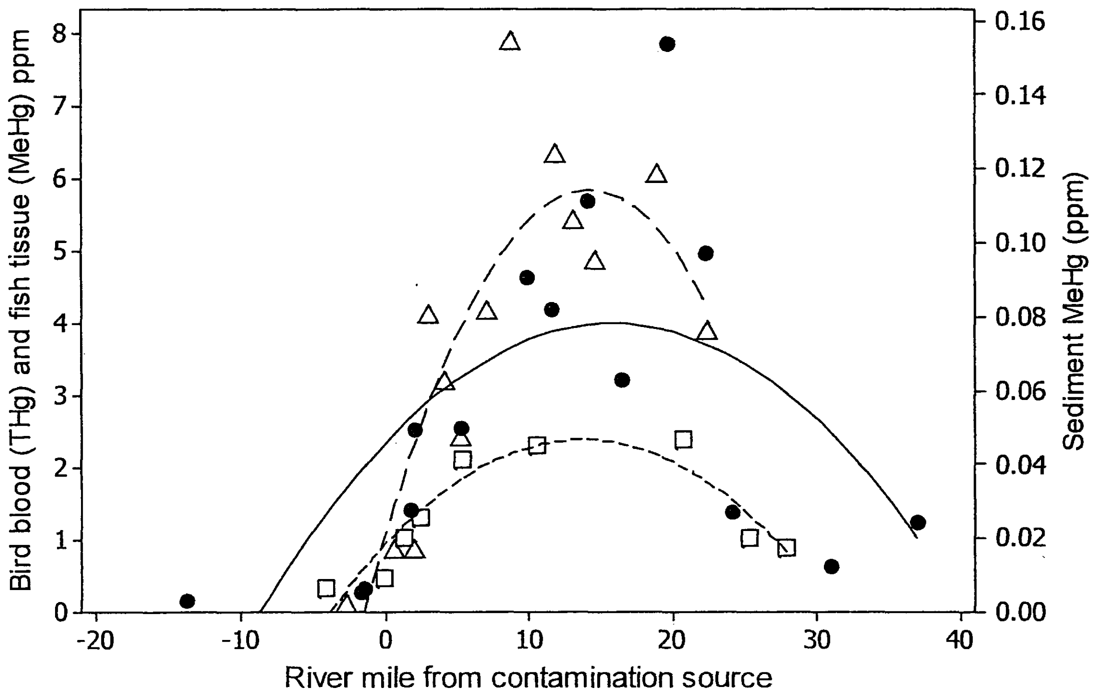
The methylmercury levels in fish and sediment followed similar spatial patterns to the birds (data provided by the SRST EcoStudy, Fig. 11). As almost all mercury in bird blood is methyl, I used the methylmercury values from sediment and fish collected along the South River to confirm that mercury availability peaked between river miles 10-22.3. Tree swallow mercury levels were averaged from 2005 and 2006 for this comparison. Fish data (small and largemouth bass) were collected in 2005 and sediment data were collected in 2006. By fitting a regression line to each set of data, I was able to compare where mercury levels were the highest along the South River by estimating the point at which the slope of the line changed from positive to negative. Sediment and fish methylmercury were highest just upstream of river mile 15. Tree swallow mercury reach the highest point at approximately river mile 16, near Harriston. Mercury levels in birds and fish decline downstream of river mile 24, downstream of the confluence. No sediment data were available downstream of the confluence. The pattern found in bird mercury with river mile is similar to the patterns found in fish and sediment indicating higher mercury bioavailability in certain areas along the South River.

Variables impacting mercury levels

Sex Male and female tree swallows are not size dimorphic and feed at the same trophic level (Robertson et al. 1992) and should therefore accumulate mercury at similar

FIGURE 11

MERCURY LEVELS WITH RIVER MILE FOR TREE SWALLOWS, FISH, AND SEDIMENT ALONG THE SOUTH RIVER



Closed circles, solid line = adult TRES (average 2005/2006); open squares, broken line = fish (2005); and open triangles, dotted line = sediment (2006)*.

*Fish and sediment data provided by the SRST EcoStudy.

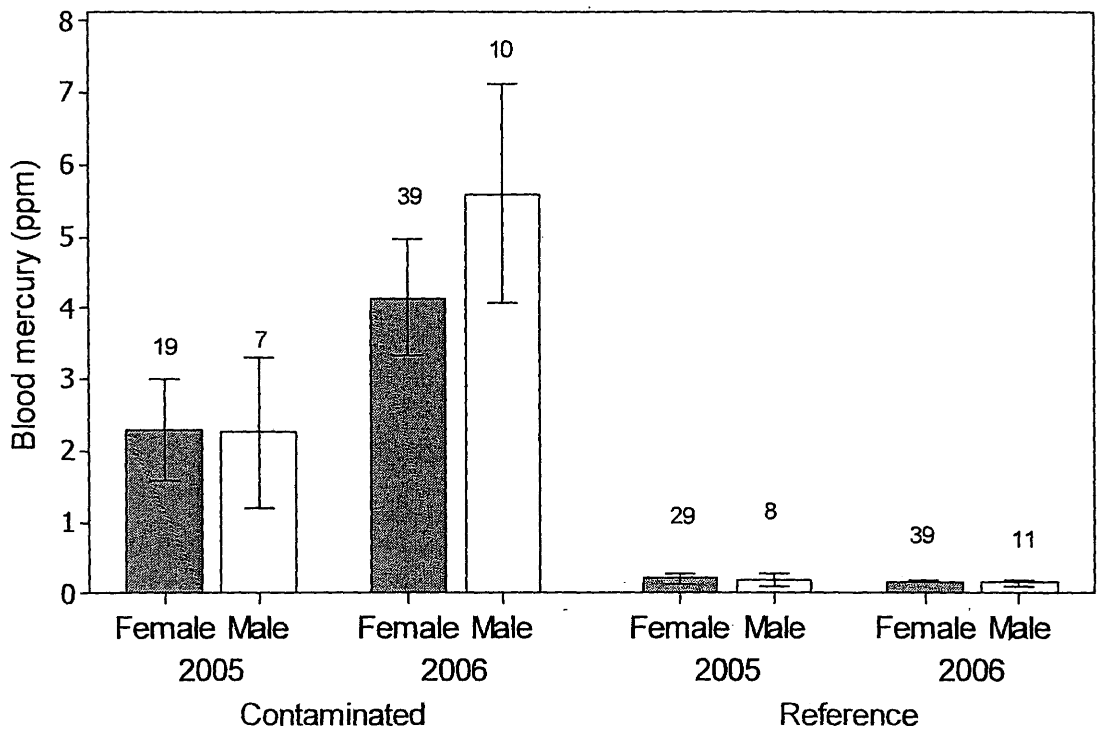
rates (Evers et al. 2005). The fact that females eliminate mercury into each egg would suggest that they would have lower body burdens, but blood sampling occurred >2 weeks after the last egg was laid, and thus might not be expected to reflect elimination into eggs. Overall, mercury levels in males and females in contaminated and reference areas did not differ across both years (Fig. 12, $F_{1,155}$=1.65, p=0.20). There was no interaction of sex and treatment group ($F_{1,155}$=2.36, p=0.13). No comparisons of nestling sex and mercury levels were made because nestling sex cannot be determined in the field for this species.

Age class Adult birds should have higher mercury levels than their young, as the main route of elimination, feather growth, is not available to adults until the prebasic molt that occurs after the cessation of breeding. Chicks should be able to eliminate dietary mercury rapidly as their feathers continue to grow throughout the nestling period. Using a one-way ANOVA, I compared mercury values for the female, or male if female mercury level was not available, to the average mercury level of their brood (Table 9). Adult mercury levels were significantly higher than their nestlings' levels in both the reference and contaminated sites. Adequate sample size was not available on SFSR.

Adult tree swallows forage, for themselves and their nestlings, within 400 m of their nest box during the nestling period (Mengelkoch et al. 2004). Therefore, adult mercury levels should correlate with the levels in their nestlings; as both represent recent dietary uptake from the same restricted area. Levels in adults and their nestlings in the contaminated areas were significantly, positively correlated (Fig. 13, $F_{1,11}$=25.53, R^2=0.72, p <0.0001). In other words, as parental mercury increased so did brood

FIGURE 12

COMPARISON BETWEEN MALE AND FEMALE MERCURY LEVELS



Error bars represent the 95% CI of the mean.

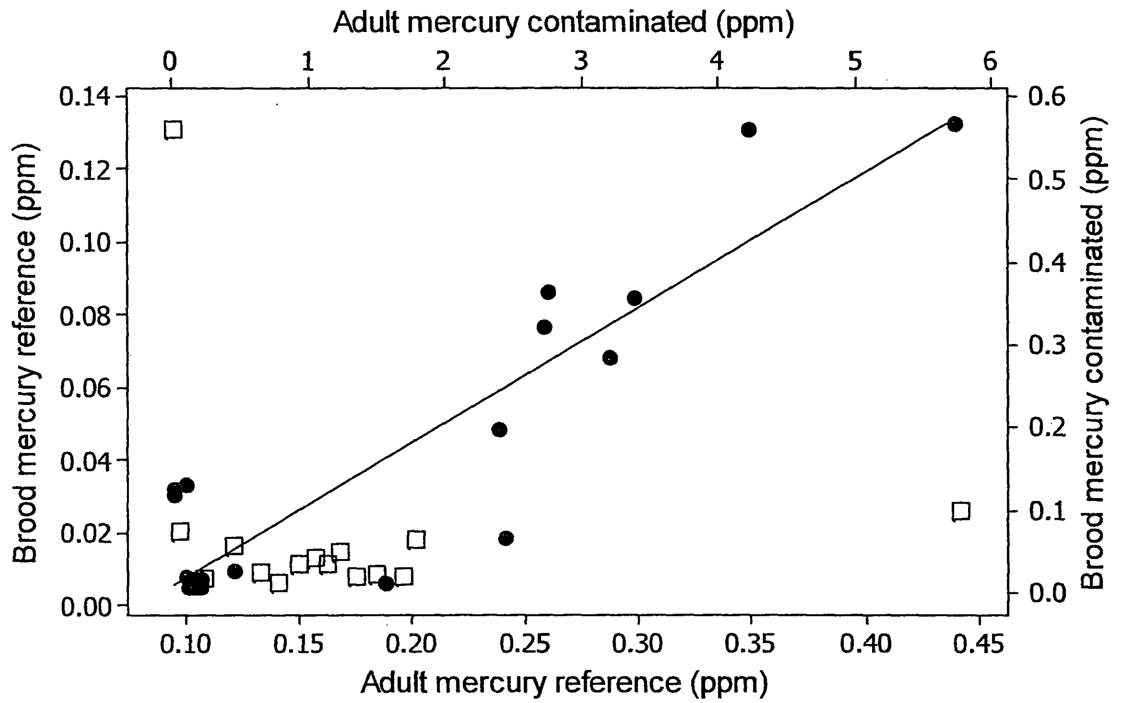
TABLE 9

COMPARISONS OF PARENTAL AND BROOD MERCURY LEVELS IN 2005

		N	mean	SE	SD	median	range	F	p
Contaminated	Adult	12	2.31	0.49	1.71	2.41	0.002 - 5.72	17.61	<0.001
	Brood	12	0.23	0.05	0.19	0.17	0.01 - 0.57		
Reference	Adult	18	0.16	0.02	0.08	0.16	0.09 - 0.44	56.51	<0.001
	Brood	18	0.02	0.007	0.03	0.012	0.007 - 0.13		

FIGURE 13

RELATIONSHIP BETWEEN PARENTAL AND BROOD MERCURY



There was a significant relationship between adult mercury levels and the mercury levels of their broods in contaminated areas (black circles, solid line). There was no corresponding relationship between parental and brood mercury in reference areas (open squares).

mercury. Adult mercury levels in birds in reference areas were not related to the mercury levels of their broods (Fig. 13, $F_{1,16}$=0.26, R^2=0.02, p=0.62).

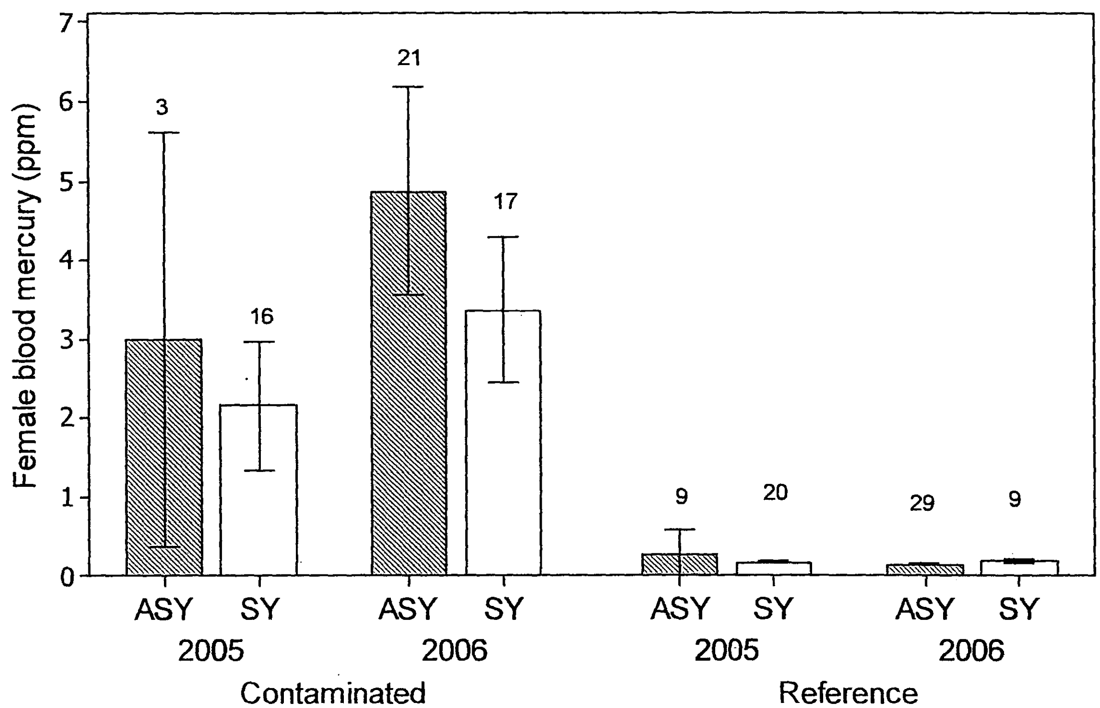
As SY and ASY females are identifiable based on plumage characteristics, I was able to compare mercury levels between these two adult age classes. Older birds may have higher body burdens of mercury due to bioaccumulation or different assimilation rates compared to younger birds. Across all sites, contaminated and reference, there was no effect of female age on mercury level at the time of sampling (Fig. 14, $F_{1,116}$=3.27, p=0.07). There was a significant effect of year ($F_{1,116}$=4.94, p=0.03) as well as a significant interaction of treatment group and year ($F_{1,116}$=5.72, p=0.02). This is not surprising as overall mercury levels were higher in 2006 than in 2005. There was no interaction between treatment group and female age ($F_{1,116}$=2.95, p=0.09); however, the difference in mercury level between ASY and SY females in contaminated areas was weakly significant as determined by post-hoc comparison (p=0.05). There was a tendency for ASY females to have higher mercury than SY females in 2006. The small sample size of ASY females in 2005 precluded a reasonable comparison; mercury levels were only available for three ASY females from 2005. Male age could not be determined and therefore no comparisons were made in this regard and there was not adequate sample size to include birds from the SFSR.

Date

As the rate of conversion of inorganic mercury to methylmercury can change due to changes in water temperature, pH, and/or oxygen availability, mercury availability could have changed due to variation in these (or other) factors over the course of the breeding season. Unfortunately, the effect of date was difficult to measure from my data

FIGURE 14

EFFECT OF FEMALE AGE ON BLOOD MERCURY LEVEL



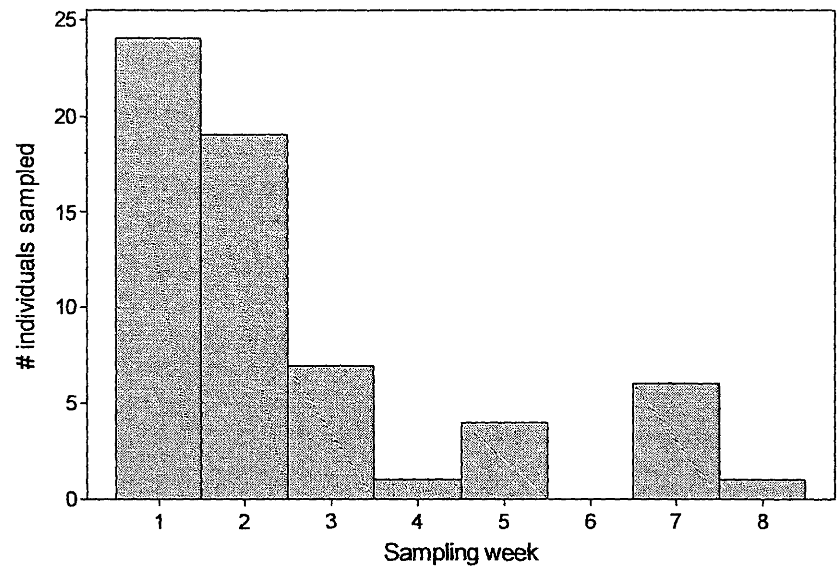
There was no effect of female age on mercury levels in contaminated or reference areas in 2005; however, only three ASY females were sampled in that year. In 2006, ASY females in contaminated areas had higher mercury levels than SY females. No differences were found in reference areas in either year. Error bars represent 95% CI.

as samples were not collected from each site each week. As previously discussed (see *Where was mercury highest along the South River?*), mercury availability changed depending on where an individual was sampled along the South River. I chose where to sample on a particular date based on the availability of birds; thus, it is difficult to separate out the temporal variation from the striking spatial variation in the data. In 2005, blood samples were collected from adult tree swallows beginning on 23 April and ending on 10 July. Samples were collected for a total of eight weeks representing individuals from the early and late nesting populations; however, a majority of the samples were collected during weeks 1-3 (Fig. 15a). Blood samples in 2006 were collected from adult tree swallows beginning on 12 May and ending on 21 June (Fig. 15b). A majority of the samples were collected during weeks two and three and all samples represent adults from the early nesting population in 2006. Samples were collected during weeks 8-10 in 2006, but have not yet been analyzed.

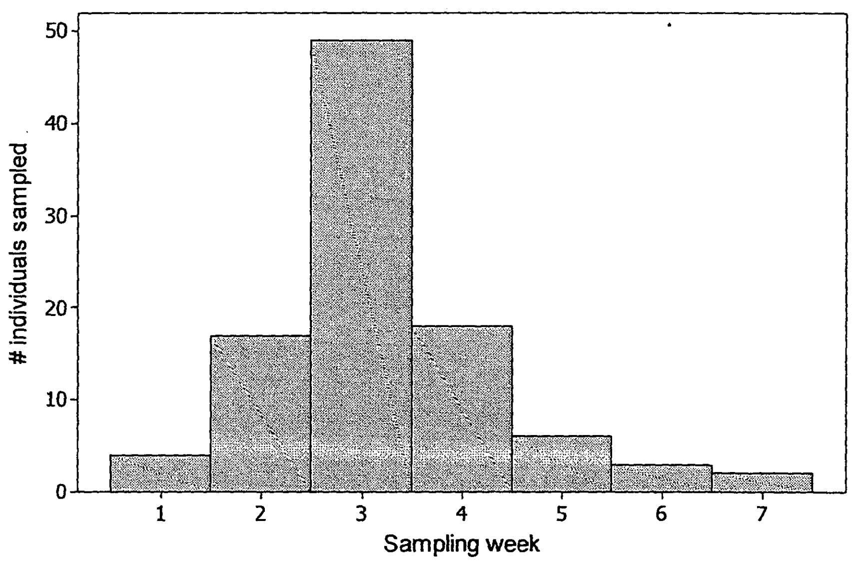
In order detect any effects of date, I would have needed to sample individuals from each site, each week throughout the season. As nesting appeared to be synchronized within sites, but not between sites, different sites were sampled at different times. Despite this shortcoming, I made an attempt to determine if there was a temporal trend in mercury level across all sites throughout the breeding season. To do so, I combined all mercury samples collected in 2005 and 2006. The calendar date, regardless of year, on which each sample was collected, was converted into a Julian day; for example, samples collected on 5/23/2005 and 5/23/2006 were both labeled as Julian day 12. These are different Julian dates than were used for analysis of reproductive success.

FIGURE 15

SAMPLES COLLECTED PER WEEK IN 2005 (A) AND 2006 (B)



a) Calendar dates associated with sampling week for 2005: week 1 = 5/22-5/28, week 2 = 5/29-6/4, week 3 = 6/5-6/11, week 4 = 6/12-6/18, week 5 = 6/19-6/25, week 6 = 6/26-7/2, week 7 = 7/3-7/9, week 8 = 7/10-7/16.



b) Calendar dates associated with sampling week in 2006 were as follows: week 1 = 5/7-5/13, week 2 = 5/14-5/20, week 3 = 5/21-5/27, week 4 = 5/28-6/3, week 5 = 6/4-6/10, week 6 = 6/11-6/17, and week 7 = 6/18-6/24.

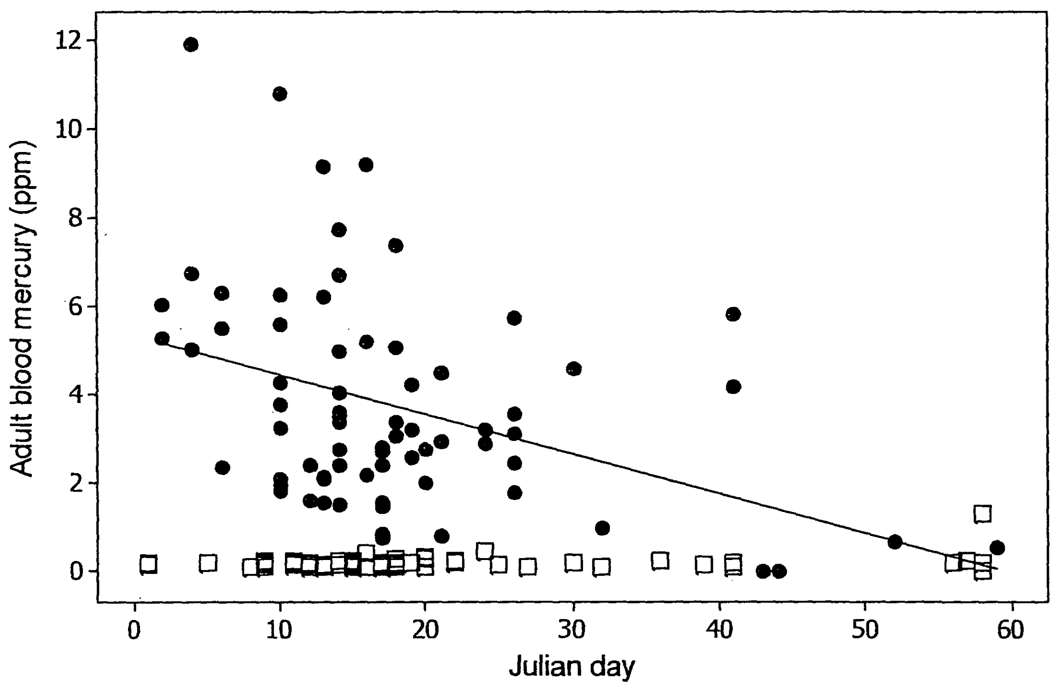
I used a linear regression to determine whether there was a relationship between mercury level and the day on which the sample was collected, separately for contaminated and reference areas (Fig. 16). In contaminated areas, there was a significant relationship of decreasing mercury levels as the sampling date increased; however, the relationship was weak ($F_{1,71}$=13.64, R^2=0.16, p<0.0001). The opposite relationship was found in the reference areas; mercury appeared to increase with Julian day ($F_{1,87}$=9.82, R^2=0.10, p=0.002), but this trend was likely driven by one outlier sampled during week seven (Julian day 58). The elevated mercury level (1.29 ppm) in this bird may have been due to its arrival on a reference site after spending time in a contaminated area earlier in the season. When this individual was removed from the analysis, there was no longer a relationship between mercury level and sampling day in the reference areas ($F_{1,86}$=0.04, R^2=0.00, p=0.85). As only seven individuals were sampled after Julian day 30 (10 June) in both years combined in contaminated areas, additional samples are needed late in the breeding season to determine whether this trend is real.

Objective 2: Is mercury impacting the nesting success of tree swallows along the South River?

Clutch initiation date and clutch size Despite elevated levels of mercury in adult and nestling birds along the South River in 2005 and even higher levels in adults in 2006, I detected no effect of treatment group (contaminated or reference) on clutch initiation date ($F_{1,178}$=0.15, p = 0.70) or clutch size ($F_{1,178}$=0.49, p=0.48). There was a significant effect of female age on clutch initiation date ($F_{1,178}$=15.35, p<0.0001), with ASY females nesting before SY females. There was also a significant effect of year on clutch initiation

FIGURE 16

ADULT MERCURY LEVELS WITH SAMPLING DATE



In contaminated areas (closed circles, solid line), mercury levels appeared to decline with increasing Julian day; no corresponding relationship was found in reference areas (open squares). However, too few samples were collected after Julian day 30 in contaminated areas to determine whether this is a real trend.

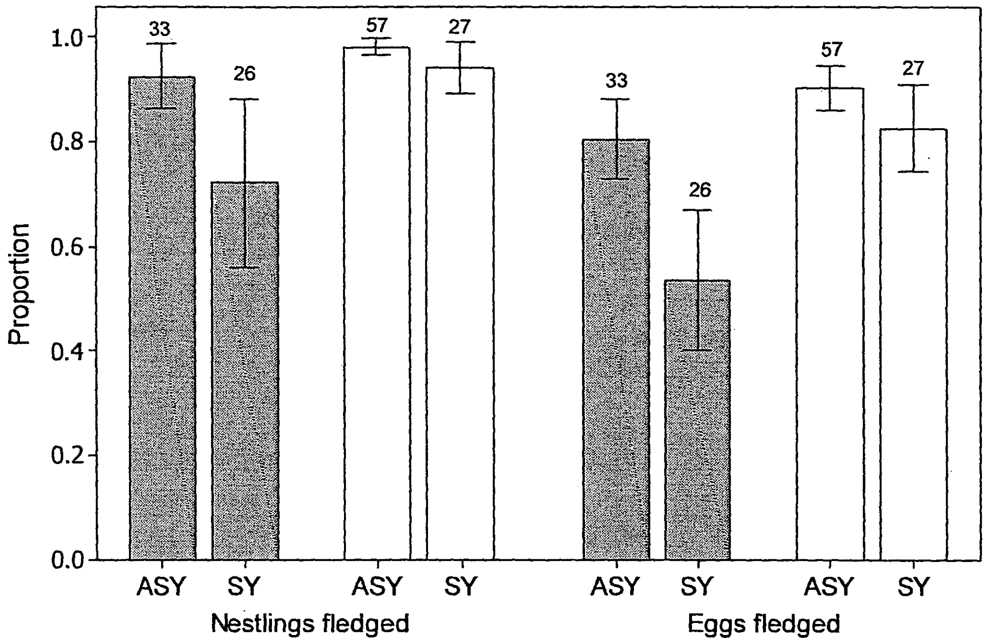
date ($F_{1,178}$=33.62, p<0.0001). The nesting season, based on clutch initiation date, began 5 days earlier in the contaminated and 7 days earlier in reference areas in 2006 than it did in 2005. ASY females also had larger clutches than SY females ($F_{1,178}$=7.29, p=0.008) across both years.

Proportion of eggs hatched There was no effect of treatment group ($F_{1,178}$=2.55, p=0.11) or female age ($F_{1,178}$=2.64, p=0.11) on the proportion of eggs that hatched across both years. However, there was a significant effect of year ($F_{1,178}$=5.48, p=0.02), overall the proportion of eggs hatched was lower in 2006 than in 2005. While there was a tendency for hatching success in contaminated areas in 2006 to be lower than in 2005, the difference was not significant based on post-hoc comparisons (p=0.23). Hatching success in reference areas did not differ between 2005 and 2006 (p=0.51).

Proportion of nestlings fledged There was a significant effect of treatment group ($F_{1,173}$= 4.22, p=0.04) and significant interaction of female age and year ($F_{1,173}$=4.73, p=0.03) on the proportion of nestlings fledged. Post-hoc comparisons indicated there was a significant difference between contaminated and reference areas in 2006 (p<0.01); no difference was detected in 2005 (p=0.92). Within the contaminated areas in 2006, the proportion of nestlings fledged by ASY females was significantly higher than that of SY females (Fig. 17, p=0.009). The proportion of nestlings fledged by SY females in contaminated areas in 2006 was also significantly lower than both ASY (p<0.0001) and SY (p=0.008) females in reference areas. The proportion of nestlings fledged from ASY females in contaminated areas was not significantly different from the proportion fledged by SY (p<0.99) or ASY (p=0.90) females in reference areas in 2006.

FIGURE 17

PROPORTION OF NESTLINGS AND EGGS FLEDGED IN 2006



SY females in the contaminated areas (grey bars) had a significantly smaller proportion of nestlings and eggs fledge compared to ASY females. In the reference areas (white bars), ASY and SY females had similar proportions of nestlings and eggs fledge. Error bars represent the 95% CI of the mean.

Proportion of eggs fledged Similar to the findings of proportion of nestlings fledged, there was a significant effect of treatment group ($F_{1,173}$=8.00, p=0.005). Although the interaction of female age and year on the proportion of eggs that fledged was not significant, there appears to be a weak a relationship ($F_{1,173}$=3.50, p=0.06). Post-hoc comparisons indicated a significant difference between the contaminated and reference areas in 2006 (p<0.0001); however, no corresponding difference was detected in 2005 (p=0.81). In 2006, the proportion of eggs fledged by ASY females in contaminated areas, as indicated by post-hoc comparisons, was significantly higher than that of SY females in contaminated areas (Fig. 17, p=0.0006). The proportion of eggs fledged by SY females in contaminated areas was also significantly lower than SY (p=0.0002) and ASY (p<0.0001) females in reference areas. ASY females in contaminated areas appeared to have a smaller proportion of eggs fledge than ASY females in reference areas; however the difference was not significant as these females had similar success as both ASY (p=0.31) and SY (p=0.99) in reference areas.

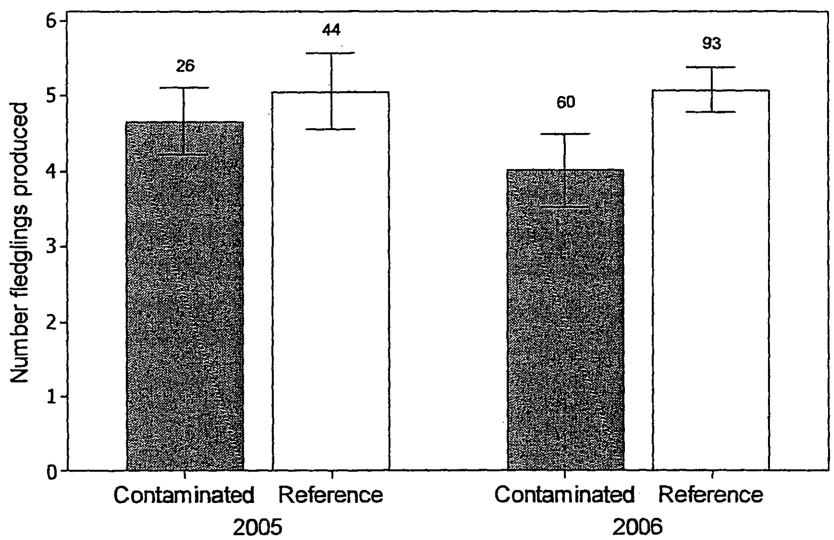
Number of fledglings produced There was a significant effect of treatment group ($F_{1,173}$=9.54, p=0.002) and female age ($F_{1,173}$=6.34, p=0.01) as well as a significant interaction of year and female age ($F_{1,173}$=4.34, p=0.04) on the number of fledglings produced. Post-hoc comparisons indicated a significant difference in the number of fledglings produced in contaminated and reference areas in 2006 (p=0.0002); no corresponding difference was found in 2005 (p=0.44) (Fig. 18a). SY females in contaminated areas produced significantly fewer fledglings than ASY females in contaminated areas in 2006, as indicated by post-hoc comparisons (p=0.0001). SY females in contaminated areas also produced significantly fewer fledglings than both

ASY ($p<0.0001$) and SY ($p=0.004$) females in reference areas in 2006. While there was a tendency for SY females in reference areas to have fewer fledglings than ASY females in reference areas, the difference was not significant ($p=0.06$). ASY females in contaminated areas had a similar number of fledglings as ASY ($p=0.48$) and SY ($p=0.98$) females in reference areas in 2006. Overall, in 2006, SY females in contaminated areas fledged approximately two fewer nestlings than ASY females in contaminated and reference areas, and approximately one less nestling fledged than SY females in reference areas (Fig. 18b). No differences were found in the number of fledglings produced in 2005.

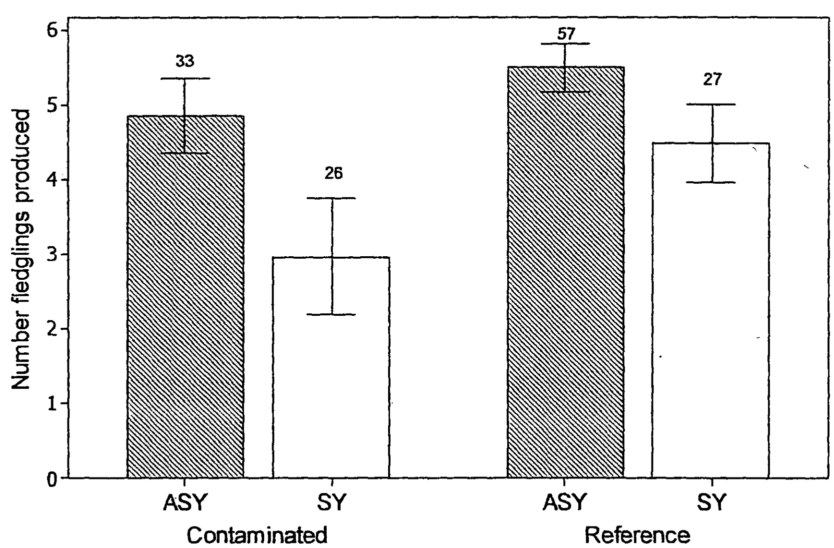
Summary Despite elevated mercury levels on contaminated sites in 2005, I did not detect any differences between contaminated and reference areas in success at any of the six measured reproductive effect parameters. The lack of female age as a significant effect in 2005 may have been due to the small number of ASY females in contaminated ($n=3$, 20%) and reference ($n=6$, 25%) areas in this year. In 2006, mercury levels were higher and I found significant differences in the proportion of nestlings and eggs fledged in contaminated and reference areas. Ultimately, both of these parameters are measures of the number of fledglings produced; in 2006 fewer fledglings were produced in the contaminated areas. However, post-hoc comparisons indicated that the SY females in contaminated areas produced fewer fledglings than all ASY females, and SY females in reference areas. It appears that only the inexperienced breeders in contaminated areas were impacted by mercury.

FIGURE 18

NUMBER OF FLEDGLINGS PRODUCED



a) In 2006, females in contaminated areas produced significantly fewer fledglings than females in reference areas. No corresponding difference was found in 2005. Error bars represent the 95% CI of the mean.



b) In 2006, SY females in contaminated areas produced significantly fewer fledglings than ASY females. The number of fledglings produced by ASY females in the contaminated areas did not differ significantly from SY or ASY females in reference areas. Error bars represent the 95% CI of the mean.

Chick growth (2005)

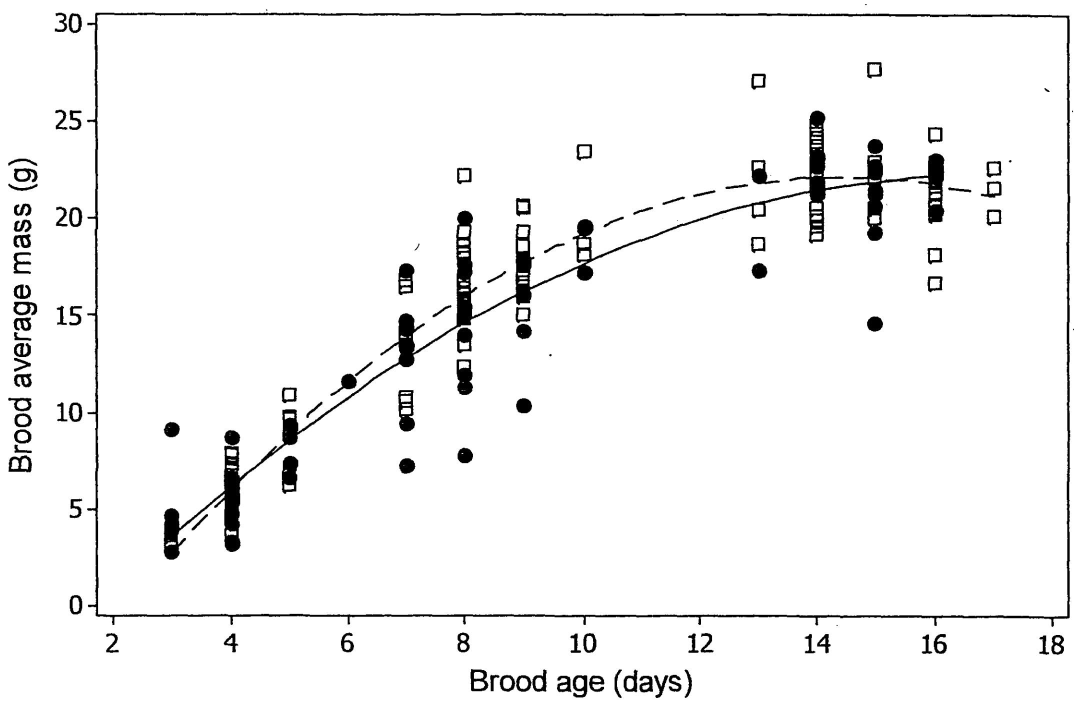
To compare nestling growth over time in contaminated and reference areas in 2005, the mass of all nestlings in a brood were averaged at each of the three measurement intervals (Fig. 19). Because the day on which I measured the nestlings varied within a few days of the target age, I first compared the day on which I measured chicks in the contaminated and reference areas to be sure I was not measuring chicks in one treatment group at a younger age. There was no difference in the age at which measurements were taken in the contaminated and reference areas (Table 10). As there was no relationship between brood mass and tarsus length at day 15 (see Methods section *Pre-fledging condition,* Fig. 20), I used the pre-fledging mass of nestlings as a measure of condition (De Steven 1980) to compare broods in contaminated and reference areas. Although broods in contaminated and reference areas were, on average (Table 10), measured on the same day for the final pre-fledging measurement, I used a Mann-Whitney U test to compare the residuals of brood mass on day measured to control for any small amount of variation in brood age. Pre-fledging condition, based on brood mass at the final measurement, was not significantly different in contaminated or reference areas ($W=335.0$, $p=0.83$).

Egg volume

In 2006, I measured eggs in the contaminated and reference areas to assess any impacts of mercury on egg volume. If females with high mercury were in poor condition they could have laid eggs of lower quality (smaller) than birds with low mercury (Evers et al. 2003). When eggs from the contaminated sites were compared to reference sites,

FIGURE 19

NESTLING GROWTH



Brood mass in the contaminated (black circles, solid line) and reference (open squares, broken line) areas appeared to increase at similar rates. Most importantly, there was no difference in pre-fledging condition, based on mass on Days 13-17, between broods in contaminated and reference areas.

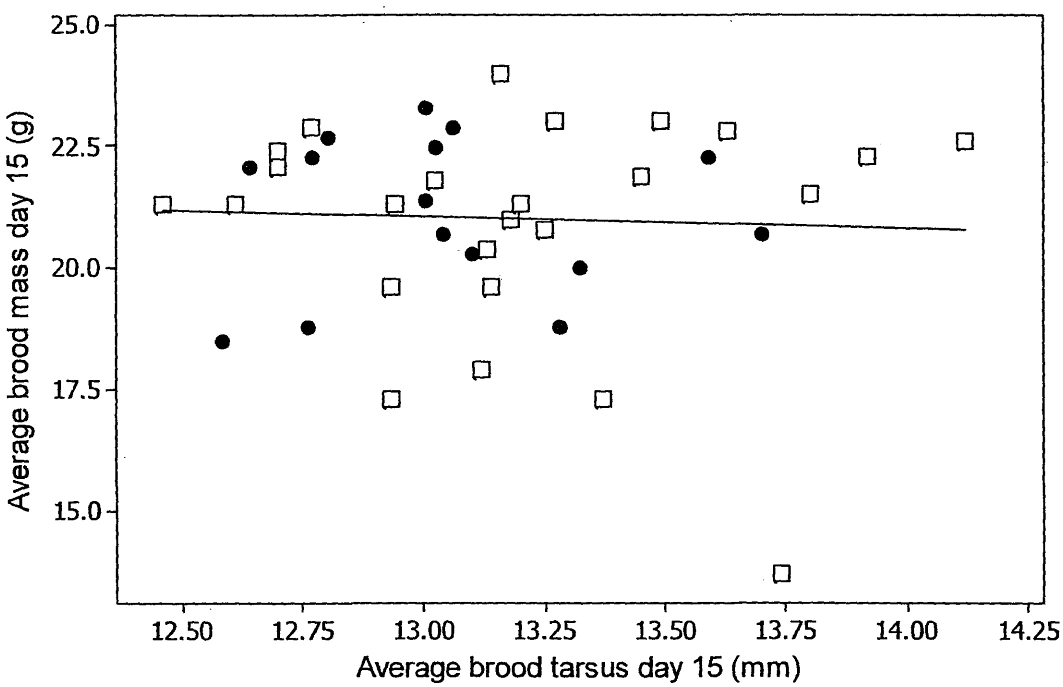
TABLE 10

AGE AT WHICH NESTLING MEASUREMENTS WERE TAKEN

	Site	N	mean	SE	SD	range	F	p
Measurement 1	Contaminated	24	4.0	0.13	0.62	3.0 - 5.0	0.67	0.42
	Reference	27	4.0	0.13	0.70	3.0 - 5.0		
Measurement 2	Contaminated	26	8.0	0.14	0.74	7.0 - 10.0	1.39	0.24
	Reference	31	8.0	0.18	0.98	7.0 - 10.0		
Measurement 3	Contaminated	47	15.0	0.25	1.26	13.0 - 17.0	0.35	0.56
	Reference	31	15.0	0.16	0.92	13.0 - 16.0		

FIGURE 20

RELATIONSHIP BETWEEN BROOD MASS AND TARSUS LENGTH



There was no relationship between average mass and average tarsus length in broods across all study sites. The lack of a clear relationship between these two morphometrics precluded further analysis of pre-fledging condition in this manner.

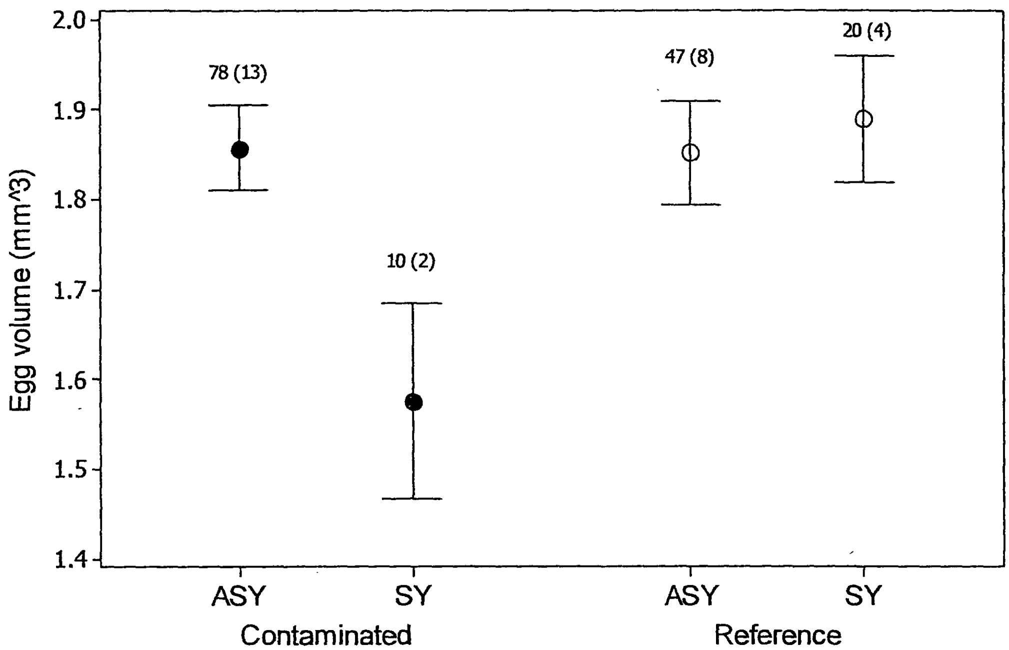
eggs in the reference areas were, on average, larger than in contaminated areas ($F_{1,151}$=13.57, p<0.0001); however, there was a significant effect of female age ($F_{1,151}$=14.41, p<0.001). SY females in contaminated areas laid smaller eggs than ASY females (Fig. 21). Post-hoc comparisons indicate that SY female eggs in contaminated areas were significantly smaller than both SY (p=0.0003) and ASY (p=0.0005) eggs in reference areas. There was no difference in egg size in the reference areas between the age classes (p= 0.89). Eggs from ASY females in contaminated areas were not significantly different in size from SY (p=0.91) or ASY (p=0.99) eggs in reference areas. I also compared eggs laid in the "hot-zone" to those upstream in the contaminated areas. Eggs were of similar volume in the "hot-zone" and upstream ($F_{1,85}$=0.42, p=0.52); however, there were no eggs sampled from SY females upstream so I was not able to test for an effect of female age.

Did the location of the nest along the South River determine its success?

Nest success was also compared between nests in the "hot zone" (river miles 9.9-22.3) and miles 1-9 upstream. As mercury levels were higher between Crimora and Grottoes City Park than upstream of Crimora, birds nesting in this area of high mercury may have experienced more effects from elevated mercury levels. For these analyses, I only used nests at which the age of the female was known. There was no significant effect of zone for any of the five reproductive parameters tested (Table 11). Nesting in the mercury "hot zone", compared to sites upstream, did not appear to impact nesting success.

FIGURE 21

COMPARISON OF EGG VOLUME BETWEEN FEMALE AGE CLASSES



SY females in the contaminated areas laid smaller eggs than all other females in the study population. Circle indicates the mean; error bars represent the 95% CI of the mean. Sample size, total number of eggs measured, and number of clutches measured, in parentheses, provided above interval bar.

TABLE 11

EFFECT OF "ZONE" ON NESTING SUCCESS

Parameter	Location	N	mean	SE	SD	median	range	F	p
Clutch size	Hot zone	42	5.79	0.13	0.87	6.0	4.00 - 7.00	0.72	0.40
	upstream	34	5.82	0.14	0.83	6.0	4.00 - 8.00		
Proportion eggs hatched	Hot zone	42	0.85	0.03	0.17	0.86	0.25 - 1.00	0.10	0.76
	upstream	34	0.84	0.03	0.19	0.83	0.17 - 1.00		
Proportion nestlings fledged	Hot zone	41	0.81	0.04	0.29	1.00	0.00 - 1.00	0.66	0.42
	upstream	33	0.88	0.05	0.29	1.00	0.00 - 1.00		
Proportion eggs fledged	Hot zone	41	0.69	0.04	0.28	0.80	0.00 - 1.00	0.30	0.58
	upstream	33	0.75	0.05	0.30	0.83	0.00 - 1.00		
Number fledglings	Hot zone	41	4.00	0.28	1.81	4.00	0.00 - 7.00	0.00	0.97
	upstream	33	4.30	0.32	1.81	5.00	0.00 - 6.00		

Nests from 2005 and 2006 were combined in these analyses.

Were female mercury and nesting success correlated?

In 2005, I did not detect any impacts of mercury on nesting success; however, in 2006 higher mercury may have led to decreased nesting success in contaminated areas in some females. To determine whether an individual female's mercury level was predictive of her nesting success, I used regression analyses to compare each female's nest success to the amount of mercury in her blood. I combined all females from 2005 and 2006 as well as contaminated and reference areas to compare the nesting success of females across a wide range of mercury levels.

Four of the seven reproductive parameters had significant, negative relationships with female mercury level (Table 12). The four parameters that indicated a significant relationship with female mercury level were ultimately all measures of the number of fledglings produced. I did not detect a relationship between female mercury level and clutch initiation date, clutch size, or average egg size. While the relationships between female mercury level and the proportion of eggs fledged and number of fledglings produced were significant, the R^2 values were low, indicating much unexplained variation (Figs. 22 and 23). The proportion of eggs fledged had the strongest relationship based on the R^2 value.

Did mercury have a cumulative effect on nesting success?

Females banded in 2005 that returned to the study site in 2006 experienced two years of mercury exposure on the breeding grounds. If mercury exposure has a cumulative effect, returning birds should have experienced decreased nesting success in the second year of breeding in a contaminated area. Using a one-way ANOVA, I compared the nesting success of ASY females recaptured on contaminated sites in 2006

TABLE 12

RELATIONSHIP OF FEMALE MERCURY LEVELS MEASURED

REPRODUCTIVE PARAMETERS

Parameter	**N**	**R^2**	**F**	**p**
Clutch initiation	118	0.002	0.24	0.62
Clutch size	118	0.009	1.11	0.30
Proportion eggs hatched	118	0.07	8.37	0.005*
Proportion nestlings fledged	114	0.04	4.49	0.04*
Proportion eggs fledged	114	0.12	15.09	<0.001*
Number fledglings produced	114	0.08	9.05	0.003*
Average egg size	13[†]	0.07	0.88	0.37

* indicates a significant relationship was found.
[†] sample size is number of broods.

FIGURE 22

RELATIONSHIP BETWEEN FEMALE MERCURY LEVEL AND

PROPORTION EGGS FLEDGED

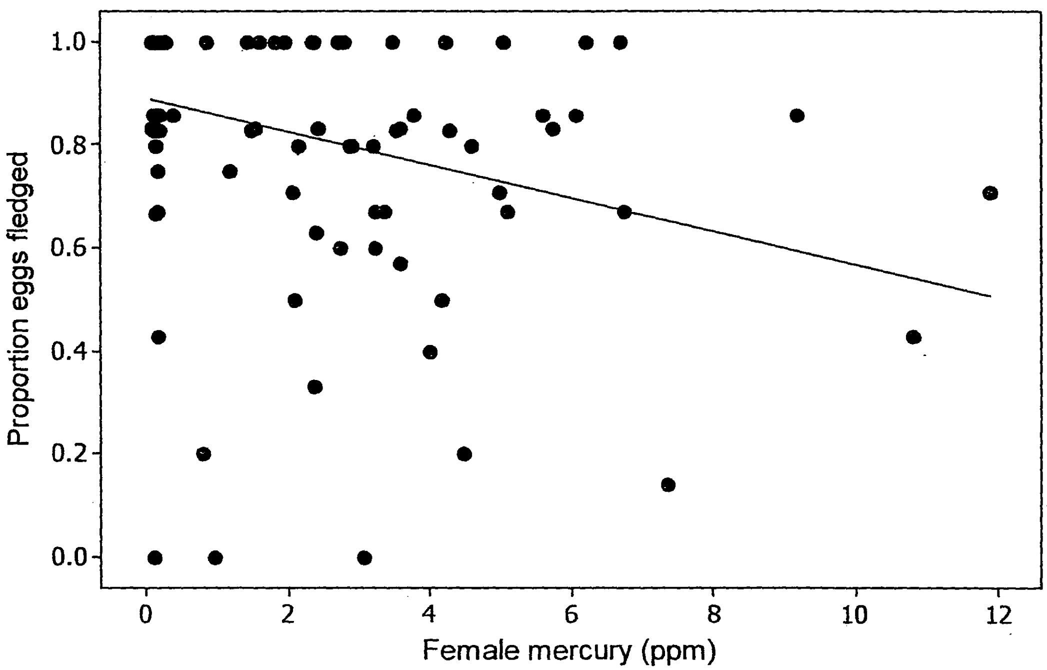
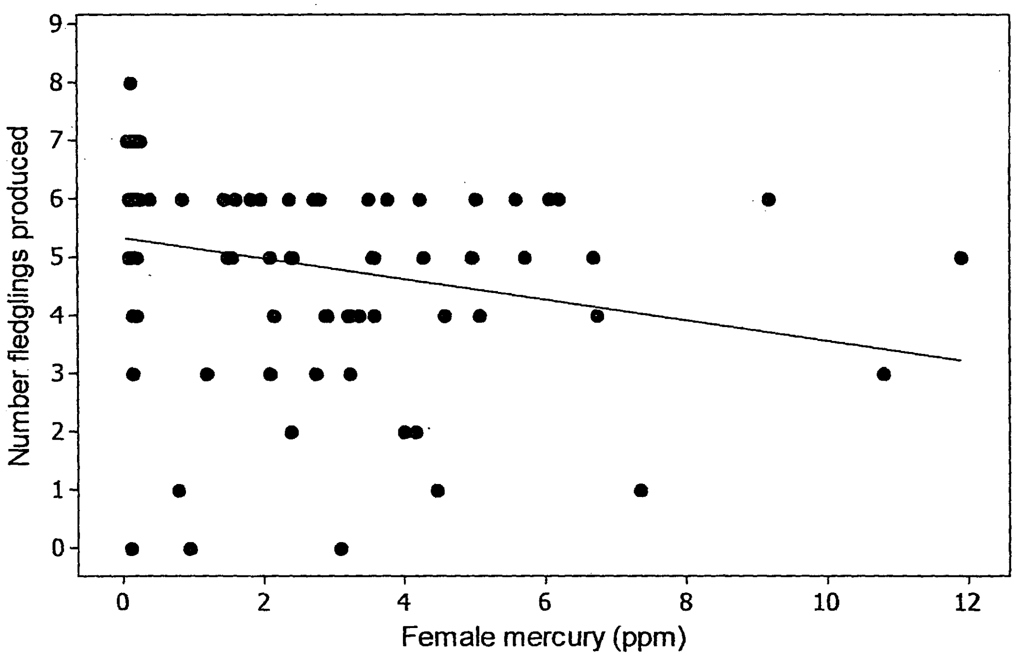


FIGURE 23

RELATIONSHIP BETWEEN FEMALE MERCURY LEVEL AND NUMBER OF FLEDGLINGS PRODUCED



to the nesting success of the new ASY females on contaminated sites. This comparison allowed me to determine if birds breeding in a contaminated site for two years had lower nesting success than birds breeding on the site for the first time. Based on these analyses, nesting in a mercury contaminated area across two breeding seasons did not appear to impact a female's reproductive success. The success of recaptured and new females did not differ for any of the six measured reproductive parameters (Table 13). It should be noted, however, that a "new" female was one that had not bred in one of our nest boxes in 2005, but this does not preclude her having been a non-breeding floater who was exposed to mercury on the site.

DISCUSSION

Objective 1: Was mercury accumulated by tree swallows, a non-piscivorous species, nesting along the South River?

Tree swallows nesting along the contaminated portions of the South River had mercury levels an order of magnitude higher than birds sampled in reference areas in both 2005 and 2006. The availability of mercury to insectivorous birds nesting in this watershed was comparable to that of a piscivorous species. The belted kingfisher (*Ceryle alcyon*), an obligate piscivore, nesting along the South River had an average blood mercury level of 3.35 (±2.67 SD) ppm (N= 21) across both years (unpublished data). The average tree swallow blood mercury level was 3.69 (±2.40 SD) ppm (N=75) indicating similar risk and exposure to mercury for aquatic insectivorous and piscivorous species. While it is widely recognized that fish are a route of mercury exposure for wildlife feeding in aquatic systems (e.g., loons, kingfishers, eagles), this may be the first case in

TABLE 13

COMPARISON OF NESTING SUCCESS BETWEEN RECAPTURED AND NEW BIRDS

Parameter	Status	N	mean	SD	F	p
Clutch initiation date	New	22	16.2	9.09	0.29	0.59
	Recap	11	14.5	7.59		
Clutch size	New	22	5.86	0.83	2.97	0.10
	Recap	11	6.36	0.67		
Proportion eggs hatched	New	22	0.87	0.19	0.02	0.90
	Recap	11	0.88	0.14		
Proportion nestlings fledged	New	22	0.93	0.18	0.10	0.75
	Recap	11	0.92	0.18		
Proportion eggs fledged	New	22	0.81	0.24	0.10	0.78
	Recap	11	0.80	0.18		
Number fledglings produced	New	22	4.73	1.52	0.48	0.50
	Recap	11	5.09	1.22		

which an insectivorous species has been found to have comparable bioaccumulation to a piscivore in the same area.

Adult tree swallow levels in 2005 (2.28 ppm ± 1.38 SD) were an order of magnitude higher than nestling levels (0.23 ppm ± 0.17 SD). This finding is consistent with the literature, as mercury levels reported in nestling birds are typically lower because they are eliminating large amounts of mercury into their growing feathers (Evers et al. 2005). As with adult tree swallows, nestlings had mercury levels similar to those found in nestling kingfishers (0.26 ± 0.16 SD, unpublished data).

I did not detect any differences between male and female mercury level in either year. In 2006, male mercury levels appeared higher than female levels; however, only one sample from a male swallow was collected upstream of the "hot-zone" in this year. Because I collected blood samples from adults more than two weeks after the eggs were laid, any effect of mercury elimination into the eggs by the female was minimal. Male common loons have been reported to accumulate higher mercury levels than females, but this is most likely due to the larger males eating larger fish with higher mercury concentrations (Evers et al. 2005). Tree swallows are not size dimorphic and are not known to partition foraging niches and therefore should have accumulated mercury at similar rates. However, the tendency for ASY females to have more elevated mercury levels than SY females has not been previously investigated. While tree swallows have not been shown to defend feeding territories (Robertson et al. 1992), it is possible that SY females, which are easily identified by their immature plumage, were excluded by older birds from higher quality foraging areas. Older birds consuming larger insects, or more insects, could accumulate mercury at a higher rate than young birds in lower quality foraging habitats. Further, unbanded ASY females captured on the study site in 2006 may have been present on the site in 2005 as undetected floaters and may have had higher body burdens of mercury than SY females for that reason.

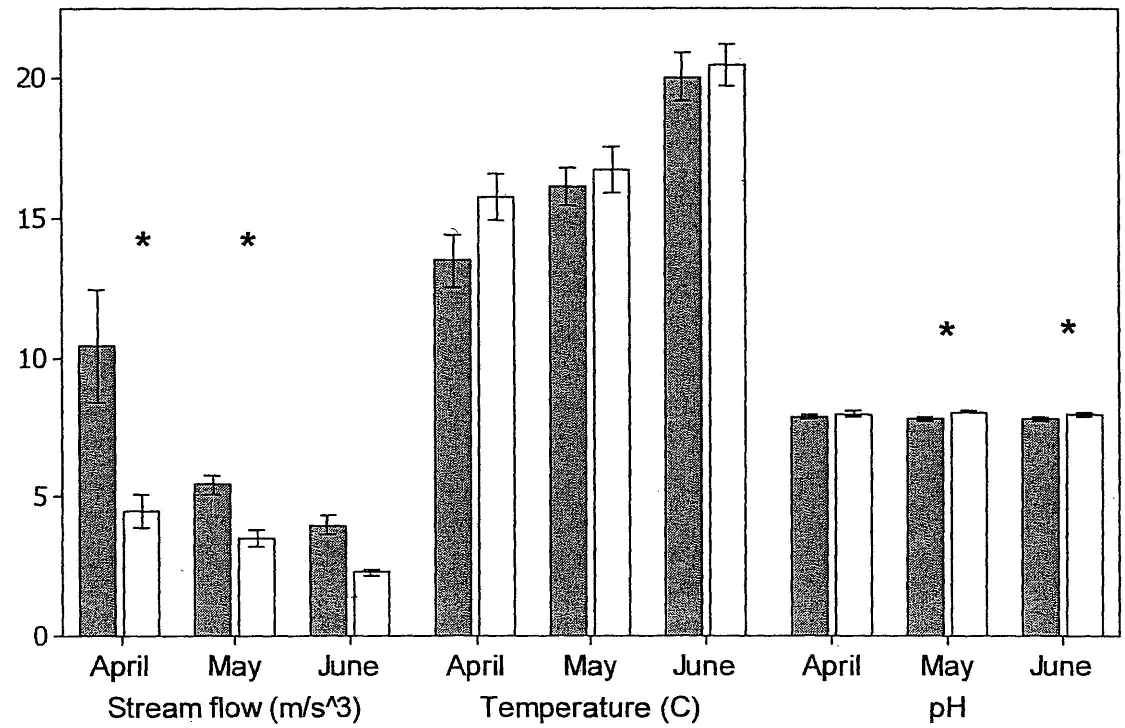
Why were mercury levels higher in 2006?

Mercury levels in adult birds in contaminated areas in 2006 were significantly higher than levels found in 2005 ($p<0.001$). There was no change in the mercury levels of birds nesting in reference areas between 2005 and 2006 ($p=0.96$) indicating mercury level change was not higher due to an increase in regional atmospheric mercury deposition. If atmospheric mercury deposition had been higher in the Shenandoah Valley in 2006 than in 2005, mercury levels would have increased in reference as well as in contaminated areas. As the addition of nest boxes, new sites, and a larger proportion of older birds, including those returning to breed for a second year, on the study site in 2006 did not appear to be sufficient to drive the higher mercury levels detected in contaminated areas, it is possible that methylmercury availability was higher in 2006. The conversion of inorganic mercury to methylmercury is facilitated by changes in oxygen levels, temperature, and pH in aquatic sediment which alters bacterial methylation rates (Andersson 1979). Changes in stream flow (river discharge, as a measure of changing O_2 level), water temperature, and/or pH in the South River in 2006 could have lead to an increase in mercury methylation, thus increasing mercury bioavailability.

To look for potential differences in these factors between years, I compared the monthly stream flow (discharge m^3/s), surface water temperature, and water pH using data from the Harriston and Dooms water gauges in the South River (USGS Hydrologic Unit Code: 01627500 and 01626920). Water temperature and pH data were only available from the Dooms gauge for the time periods requested; the Harriston gauge provided the stream flow data. I compared stream flow from April-June (1-22 only) in 2005 and 2006 as these months span the tree swallow nesting period (from egg laying to chick fledging). Data after June 22 were not included as most nests in both years were fledged by this date. I used a GLM to run an ANOVA to compare stream flow, surface water temperature, and pH in 2005 and 2006 with month and year as factors, and month*year as an interaction term (Fig. 24). Stream flow during the nesting season in 2005 was significantly faster, by approximately 2.38 m^3/s, than in 2006 ($F_{1,148}$=59.45, p<0.0001). As indicated by post-hoc comparisons, flow was faster in April and May of 2005 than in April and May 2006; although stream flow appeared slower in June 2006 than June 2005, the difference was not significant (Table 14). There was a significant effect of year on surface water temperature ($F_{1,114}$=8.20, p=0.005); post-hoc comparisons indicate surface water temperature was warmer in 2006. However, there was not a significant interaction between month and year. There was also a significant effect of year on pH ($F_{1,113}$=36.88, p<0.0001), as well as a significant interaction of month and year (Table 14, $F_{2,113}$=3.19, p=0.05). pH was higher in May and June 2006 compared to May and June 2005; no difference was found between April 2005 and 2006. Methylation typically increases under more acidic conditions (lower pH) (Andersson 1979), therefore, the slight increase in pH in 2006 was likely not a cause of increased mercury bioavailability in 2006. Decreased stream flow could have led to lower oxygen availability and with increased water temperature, methylation rates may have been higher during the breeding season in 2006. Therefore, due to the absence of any rain in

FIGURE 24

COMPARISON OF STREAM FLOW, SURFACE WATER TEMPERATURE, AND pH BETWEEN THE 2005 AND 2006 BREEDING SEASONS



Stream flow was lower and temperatures were higher in 2006† (white bars) compared to 2005 (grey bars). pH was slightly higher in 2006 than in 2005.

* indicates a significant differences in monthly flow, temperature, and pH ($p<0.05$). Error bars represent the 95% CI of the mean. Data provided by USGS (http://va.water.usgs.gov/projects/south_river_hg.html).

† Data from 2006 were provided as provisional and may be subject to revision by USGS, data from 2005 were approved.

TABLE 14

MONTHLY COMPARISONS OF STREAM FLOW (M^3/S), SURFACE WATER TEMPERATURE (°C), AND pH BETWEEN 2005 AND 2006

	April	**May**	**June**
Stream flow	<0.0001	0.03	NS
Temperature	NS	NS	NS
pH	NS	<0.0001	0.005

Values indicate p values, NS indicates $p>0.05$.

the 2006 nesting season, methylmercury may have been more bioavailable potentially explaining why mercury levels in tree swallows increased from 2005.

Where was mercury the highest along the South River?

In 2005 and 2006, mercury levels varied with river mile downstream of the contamination source in Waynesboro, VA. Perhaps surprisingly, mercury levels were not highest closest to the contamination source. Mercury levels in birds, fish, and sediment increased downstream from the contamination source being highest after river mile 10 near Crimora and the Augusta Forestry Center. Mercury levels remained elevated until river mile 22 (Grottoes City Park) and then decreased downstream of the confluence in Port Republic at mile 24. For birds and sediment, mercury levels remained elevated from river mile 10-22 in the area labeled as the "hot-zone". Fish mercury levels also increased at Grand Caverns; however, the peak was less extreme compared to that in sediment and birds. Bird mercury appeared to decline sharply near mile 16, at Harriston Crossing, but was most likely a quirk of nest box placement; the swallows at this site nested in boxes facing away from the river. The thick riparian buffer at this site precluded feeding over the river and a large pasture located near the nest boxes, away from the river, was used as a foraging area by birds at this site (pers. obs.). Although mercury levels were lower at this site than anticipated, mercury was still elevated above the levels of birds nesting upstream of the contamination source.

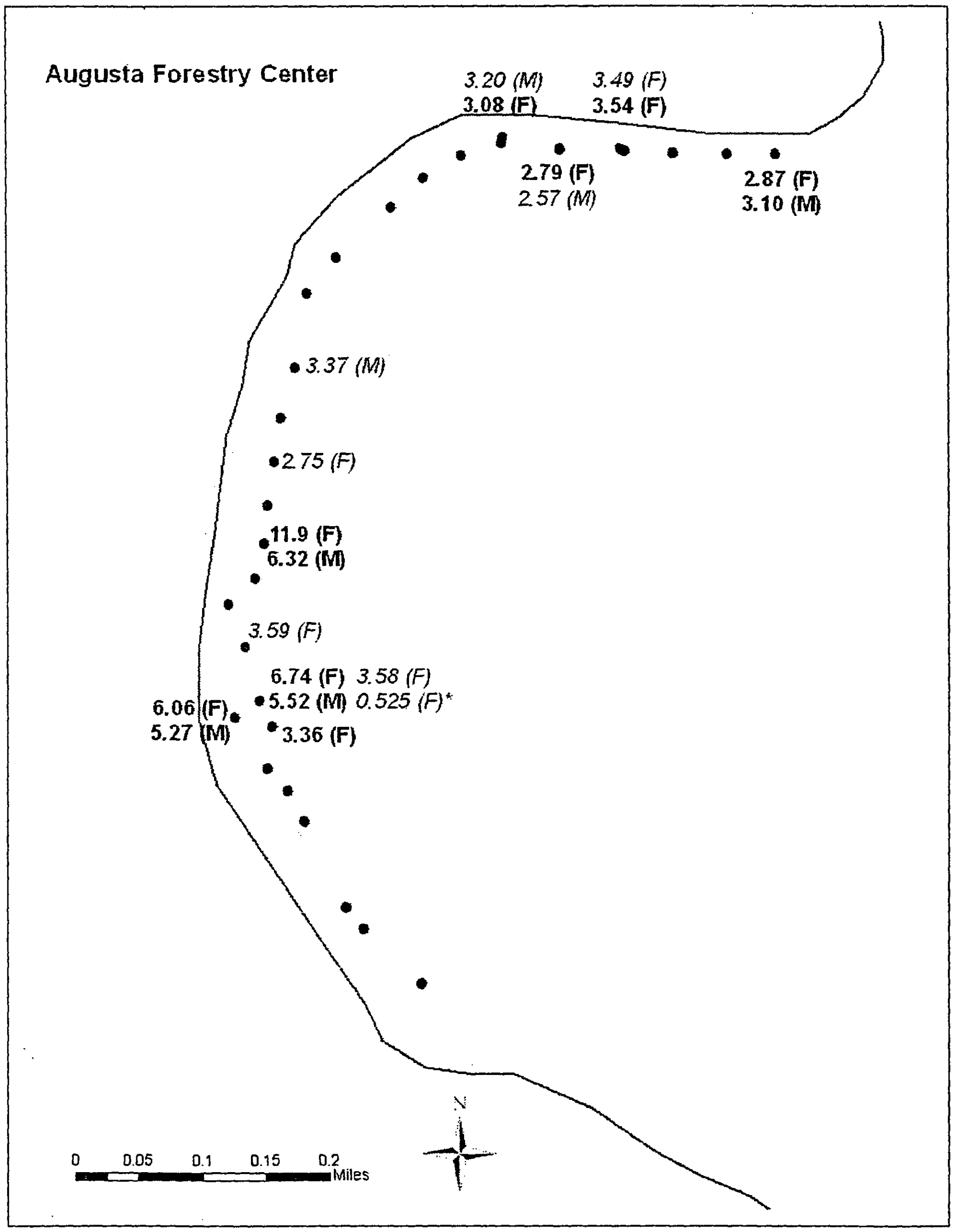
The similar patterns of mercury in birds, fish and sediment could be explained by varying aquatic conditions favoring increased bacterial methylation of mercury at certain sites or movement of mercury in sediment from one part of the river to another. Work by the SRST is ongoing in determining the sources of mercury into the South River.

Possible pathways of historical mercury into the South River include inputs from floodplain soils entering the river through bank erosion and drainage channels, storm sewers holding residual deposits, or backwater areas such as wetlands and oxbows. Understanding the spatial distribution of these potential historical inputs could explain the peaks in mercury at various points along the South River. Hypothesized current inputs of mercury into the river include atmospheric deposition, point and non-point source discharges, and fertilizers. The dramatic peak in mercury availability to swallows and predatory fish, and the lack of a clear explanation for this pattern, underscores the current lack of a complete understanding of the dynamics of mercury availability in the South River.

Variation in mercury levels among neighboring boxes There was a large amount of variation in the amount of mercury an adult tree swallow had in its blood from one box to the next at some locations. To consider spatial variation, I looked at the distribution of mercury in the area with the largest number of nest boxes. In 2006, mercury in adult blood at the Augusta Forestry Center, the largest property on the South River, ranged from 2.79–11.9 ppm (N=12) representing samples collected across five weeks (Fig. 25). Although the downstream-most nest boxes at the Forestry Center appeared to have lower mercury levels (<4.00 ppm) compared to the nest boxes further upstream (>5.00 ppm), samples have not been analyzed at nest boxes between the two sides to determine if this trend is consistent across the whole site. The amount of the site the nest box trail covered at the downstream and upstream ends of the field was similar, approximately 175 m and 150 m respectively; the gap between the upstream and downstream boxes sampled was approximately 250 m. It is possible that the adults at one end of the field were not

FIGURE 25

MERCURY LEVELS OF ADULT TREE SWALLOWS AT NEST BOXES AT THE AUGUSTA FORESTRY CENTER



Mercury samples from 2005 (*italic text*) and 2006 (**bold text**). M=male, F=female.

* It should be noted that this individual was sampled in the late nesting season in 2005, all other samples represent the early nesting season in both years.

foraging in the exact same areas as birds at the other end of the field. However, foraging over the center of this large field most likely occurred for all individuals sampled.

The variation in mercury levels of individuals in this large field was extreme; a male and female sampled at box 194 differed in their mercury levels by 5.58 ppm and the samples were only collected two days apart. A second pair sampled at the Forestry Center only differed by 0.23 ppm and were also sampled two days apart. Within a four day period at Grand Caverns, a smaller area with only 75 m between the two nest boxes sampled, mercury levels ranged from 6.27–10.8 ppm (N=4); a male-female pair sampled on the same day differed in mercury level by 4.53 ppm. Though high levels of variation between the male and female in a pair existed, it should be noted that one sex was not consistently higher than the other across all sites (T=-1.37, p=0.20). Another location close to the contamination source, the Waynesboro Water Treatment Plant, had a difference of only 1.03 ppm between the highest and lowest individuals (N=5) across three weeks of sampling. However, it is important to consider the biological significance of the differences being discussed. The difference between a female with 11.9 ppm and her mate with 6.32 ppm in a contaminated area is probably not as biologically relevant as if there was difference of >0.20 ppm (reference area value) and 2.00 ppm. While a difference of nearly 6.0 ppm may seem large, it may well be that the physiological effects of 11.9 ppm and 6.32 ppm are not much different, whereas the biological effectiveness of the < 2.0 ppm difference between a reference bird with 0.2 ppm and a contaminated bird with 2.0 ppm may be great. Until more is known about the dose-response of songbirds to mercury contamination outside of the lab, this issue cannot be resolved.

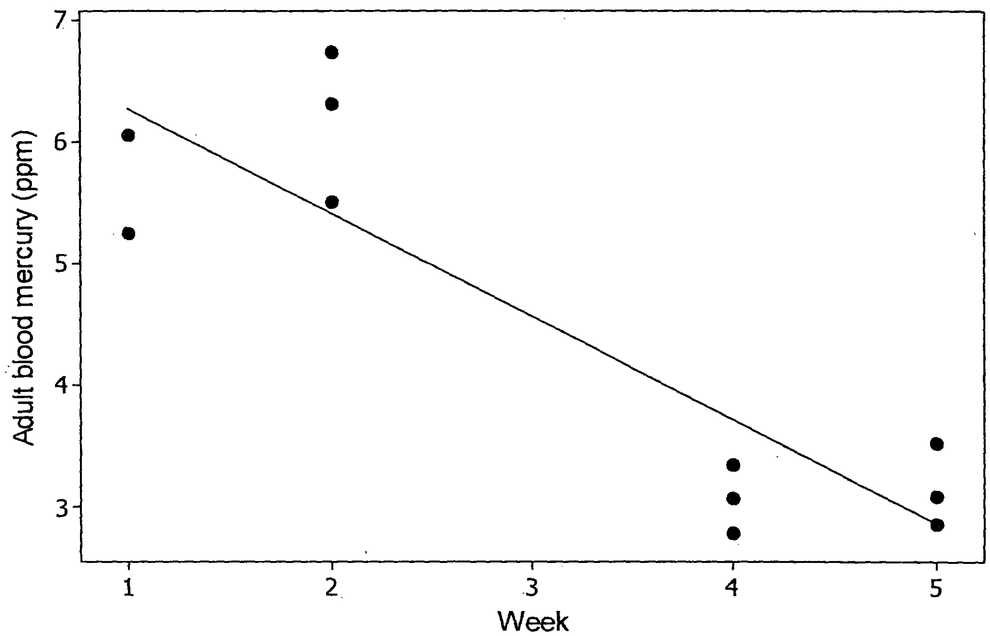
Impact of sampling date on mercury level There appeared to be a trend in contaminated areas of decreasing mercury levels across the breeding season as a whole; however, samples need to be collected at each site throughout the season to test this assumption. This is necessary to control for not only potential variation in mercury availability, but also for the variation present among individuals at a site regardless of date. It should be noted that in both 2005 and 2006, mercury levels of adult birds in reference areas were not affected by date; this indicates that atmospheric deposition was likely constant throughout the breeding season in both years. While there appeared to be a weak relationship between mercury and sampling day in reference areas, it was most likely due to an individual with higher than average mercury (1.29 ppm) sampled late in the season. This individual may have arrived on a reference site after spending time in a contaminated area earlier in the season; when the analysis was run without this individual, the relationship between mercury level and sampling day in reference areas disappeared. While there was a significant relationship ($p<0.0001$) between mercury level and sampling day in contaminated areas across both years, the relationship was weak ($R^2=0.16$). In both years combined, only seven samples were collected after Julian day 30 (10 June); more samples need to be collected late in the season to determine whether this apparent trend is real.

Fortunately, I was able to collect samples from the Augusta Forestry Center across four weeks to determine whether mercury availability changed within a site over time. At the Augusta Forestry Center, which is the largest site on the South River, mercury levels did decrease significantly from week one to week five (Fig. 26). Though only a few samples are available from each week, it appears that adult mercury levels

FIGURE 26

SAMPLES COLLECTED AT THE AUGUSTA FORESTRY CENTER

WITH SAMPLING DATE



Mercury levels were higher during the first two weeks of sampling than during weeks four and five. However, samples collected during weeks one and two were from the upstream-most nest boxes while 5/6 samples collected during weeks four and five were collected at the downstream-most nest boxes at this site.

decreased over time ($F_{1,10}$=7.91, R^2=0.44, p=0.02). No other sites allowed for this type of analysis as not enough samples were collected across enough weeks to observe a trend. However, it should be noted that five of the six samples collected in weeks four and five were from the downstream end of the Forestry Center and the samples collected during weeks one and two were from the upstream-most end. Therefore, even this apparent pattern of decreasing mercury levels with date could be a product of spatial difference in mercury availability across this large site. It is also possible that this apparent spatial trend at the Augusta Forestry Center is a product of the timing of sampling. Samples need to be collected from other sites along the South River across the entire season to determine whether or not temporal trends in mercury exist.

At this time, I do not believe one factor was driving the differences found in the range of mercury at each site, but do hypothesize that it is the relationship among several factors including changes in weather conditions, food availability, foraging, differences in parental care, and brood age, within one day or one week. No studies have looked at the influence of daily foraging activities on blood mercury levels. Consuming a large meal immediately before a blood sample was taken or daily differences in metabolic rate could alter the level of mercury; the time course for change is simply not known.

Insectivorous birds as biomonitors of environmental contamination

Previous notions of mercury contamination as solely an aquatic problem (Weiner et al. 2003) have lead to the use of piscivorous species as biomonitors. The use of insectivorous species as biomonitors is growing as they have been shown to also accumulate heavy metals at high levels, are abundant with limited home ranges, and have characterized life histories (Eens et al. 1999, Adair et al. 2003, Janssens et al. 2003, Dauwe et al. 2005). Understanding the life history of a species is critical to understanding how any contaminant may be altering reproductive behaviors and species with small home ranges allow identification of local contaminant availability (Dauwe et al. 2005, Hollamby et al. 2006). Tree swallows have been described as model organisms, based on their foraging and life history characteristics (nest box use, resistance to disturbance, small foraging range, etc.), for studies of environmental contamination (McCarty 2001, Jones 2003). My results support the fact that tree swallows make good biomonitors of mercury availability in river systems as the spatial distribution of swallow mercury levels along the South River were similar to fish and sediment. Other insectivores, such as great tits, blue tits (*Parus caeruleus*), and prothonotary warblers (*Protonotaria citrea*) have been used as biomonitors of heavy metal contamination as they readily use nest boxes and are residents with small home ranges (Adair et al. 2003, Janssens et al. 2003). While tree swallows have been used in many studies of PCB and organochloride contamination (Bishop et al. 1995, Custer et al. 1998, Bishop et al. 2000, Custer et al. 2003, Mayne et al. 2004), fewer studies have employed this species to determine heavy metal availability.

The accumulation of mercury in tree swallows has been documented in several studies in Canada and New England. However, the mercury levels reported from the destructive sampling of nestling birds and eggs in these studies does not allow for direct comparison with the blood mercury levels sampled along the South River. Based on a tissue ratio suggested by Evers et al. (2005) for common loons, mercury levels in feather and liver are six and 15 times higher, respectively, than levels present in the blood. Female blood mercury was estimated to be 2.5 times higher than the mercury found in her eggs (Evers et al. 2005). As no ratio is currently available for insectivorous or passerine birds, I have used the tissue ratios for common loons to convert the liver, feather, and egg mercury levels from the literature to approximate blood mercury levels. This ratio does provide an accurate conversion based on mercury levels reported from bald eagles (*Haliaeetus leucocephalus*) in a mercury contaminated lake in British Columbia. Adult blood mercury levels reported averaged 6.70 ppm ww and 40.0 ppm ww in feathers. The average feather mercury levels were almost exactly six times higher (5.9) than in the blood, as predicted by the ratio presented by Evers et al. (2005). Using this ratio allowed for a more direct comparison between the levels and effects found in other studies compared with tree swallows nesting along the South River.

Mercury accumulation in tree swallows has been demonstrated in pre- and post-flood situations at the Experimental Lakes Area Reservoir Project (ELARP) in Alberta, Canada (Gerrard and St Louis 2001), in the Great Lakes basin (Bishop et al. 1995), and the Agassiz National Wildlife Refuge in northwestern Minnesota (Custer et al. 2006). The flooding of wetlands has been shown to increase methylmercury availability (Zillioux et al. 1993) creating the potential for increased bioaccumulation by tree swallows foraging in these habitats. In all three of these studies mercury was analyzed from whole nestling body, nestling feather, or egg. Using the tissue ratio for adult common loons, I estimated adult female blood mercury from the egg mercury values reported in these manuscripts (Table 15).

In both Minnesota and the Great Lakes, the eggs were most likely laid by females with less than 2.00 ppm ww mercury in their blood. Whole nestling body mercury levels reported were similar to the blood mercury levels from the South River, which averaged

TABLE 15

COMPARISON OF PUBLISHED MERCURY LEVELS IN FREE-LIVING TREE SWALLOWS

Location	**Hg (tissue)**	**Estimated* blood Hg**	**Author(s)**
ELARP, Canada	0.13 ppm dw (whole nestling body)	-	Gerrard and St Louis (2001)
ELARP, Canada	1.21 ppm dw (nestling feather)		Gerrard and St Louis (2001)
ME	0.13 - 0.24 ppm dw (whole nestling body)		Custer et al. (2006)
ME	0.25 ppm ww (egg)	0.63 ppm	Custer et al. (2006)
Great Lakes	0.66 ppm ww (egg)	1.65 ppm	Bishop et al. (1995)
MA, ME	0.41 ppm ww (blood)	-	Evers et al. (2005)
South River, VA	**3.69† ppm ww (blood)**	-	

* Approximate blood mercury levels were calculated using the tissue ratio in Evers et al. 2005.
† Average adult mercury level for 2005 and 2006 combined

0.23 (±0.17 SD) ppm. As blood mercury levels should be lower than any body tissue, nestling tree swallows at these sites were exposed to lower dietary concentrations of mercury compared to nestlings on the South River. Mercury in adult tree swallows sampled in areas with point-source contamination in Maine and Massachusetts averaged 0.41 (±0.21 SD, N=53) ppm ww. Across both years of my study, only eight out of 76 (11%) adult birds sampled along the contaminated portions of the South River had blood mercury less than 1.00 ppm; only two (3%) had levels below 0.50 ppm. With an average of 2.28 ppm in 2005 and 4.44 ppm in 2006 in adult tree swallow blood, birds along the South River have accumulated the highest levels of mercury ever reported for this species.

Comparisons of tree swallow blood mercury with uptake in other passerines

Though the use of insectivores as biomonitors is growing, there is currently only a small amount of literature presenting mercury levels in these birds (Table 16). Several studies have addressed the impacts of a heavy-metal smelter in Antwerp, Belgium on great tits, a small insectivorous passerine (Dauwe et al. 1999, Janssens et al. 2003, Dauwe et al. 2005). The concentration of mercury in the blood of female great tits ranged from 0.08 – 2.73 ppm dw. The conversion to the wet weight measurement involves dividing by the fresh weight of the sample, so these values (0.08-2.73 ppm dw) would be considerably lower as wet weight values. Therefore, tree swallows along the South River were accumulating mercury at a higher rate than great tits near the heavy-metal smelter.

A similar study was conducted on pied flycatcher nestlings, also insectivorous passerines, near a sulfide ore smelter in northern Sweden. The average nestling blood mercury along the South River (0.23 ppm ww) was similar to the liver mercury levels

TABLE 16

COMPARISON OF PUBLISHED AVERAGE MERCURY LEVELS IN OTHER INSECTIVOROUS PASSERINES

Species	Hg (tissue)	Location	Author(s)
Great tit	0.26 ppm dw (blood)	Antwerp, Belgium	Dauwe et al. (2005)
Pied flycatcher	0.25 ppm ww (nestling liver)	northern Sweden	Nyholm (1995)
Prothonotary warbler	0.93 ppm ww (kidney)	Alabama	Adair et al. (2003)
Tree swallow	**3.69† ppm ww (blood)**	**South River, VA**	

† Average adult mercury level for 2005 and 2006 combined

(0.25 ppm ww) found in nestlings near the metal smelter in Sweden (Nyholm 1995). As liver levels are much higher than blood, nestling tree swallows along the South River were likely exposed to higher concentrations of mercury. Lead, copper, and zinc were found in extremely high quantities in the livers of nestlings at this site in Sweden indicating mercury was not as available for uptake compared to the other heavy metals at the smelter. Unlike mercury availability along the South River, heavy metal contamination in nestling pied flycatchers closely reflected the contamination gradient with nestlings having highest metal contamination closest to the smelter and progressively lower levels with increasing distance from the source (Nyholm 1995).

The only study on mercury accumulation in a passerine bird in the southern United States looked at mercury in adult and nestling prothonotary warblers and their prey. Prothonotary warblers are small, migratory, insectivorous passerines and consume more predatory spiders than tree swallows (Adair et al. 2003). Therefore these warblers should accumulate mercury at higher rates than tree swallows as they feed higher on the trophic ladder. The average mercury in adult warbler kidney was 0.93 ppm ww (Adair et al. 2003). While there is currently no conversion factor to estimate blood levels to correlate with kidney mercury, kidney and liver mercury values are typically similar (Evers et al. 2005). Therefore, warblers in Alabama had lower mercury in their kidneys than 89% of tree swallows along the South River had in their blood, despite foraging at a higher trophic level. Overall, mercury levels in tree swallows along the South River were not only higher than in other studies on tree swallows, but were higher than in any other passerine species nesting near a point source of heavy metal contamination.

Comparison of insectivore and piscivore mercury levels

To compare the high mercury levels found along the South River to levels from another foraging guild, I return to the bald eagle levels previously discussed. Eagles nesting in Pinchi Lake, British Columbia were exposed to mercury via a mercury mine point source (Weech et al. 2006). The average blood mercury in eagles, feeding primarily on fish during the sampling period, was 6.70 (±2.5 SD) ppm ww in adults and 0.57 (±0.16) ppm ww in nestlings (hereafter, eaglets). While the average blood mercury in eagles was higher than in tree swallows (4.44 ppm in 2006); the levels in adult eagles ranged from 4.70 – 9.40 ppm (Weech et al. 2006). The median mercury level of adult tree swallows nesting along the South River across both years of the study was 3.21 ppm, indicating a similar range of accumulation of mercury. The average mercury in eaglets was slightly higher than tree swallow nestlings on the South River (0.23 ± 0.17 SD ppm, median 0.17 ppm). The range of exposure in eaglets was 0.37 – 0.79 ppm ww, within which nestling tree swallows levels did fall.

As previously mentioned, tree swallow and belted kingfisher blood mercury levels were similar across both years indicating similar exposure to mercury in an insectivore and a piscivore nesting along the South River. Tree swallows also had similar mercury levels to a large piscivore, the bald eagle, nesting in an area of point source mercury contamination. These findings imply that though the mercury problem in birds was once considered "largely an aquatic one" (Weiner et al. 2003), it is equally problematic for insectivores foraging over these contaminated systems. The exposure and risk to mercury toxicity and its effects must be considered in other species besides top piscivores in aquatic systems because at least one other foraging guild, insectivores, is accumulating the contaminants at similar rates.

Objective 2: Determining the impacts of mercury on the nesting success of tree swallows along the South River.

I compared the nesting success of tree swallows breeding along the contaminated South River with the success of birds on reference rivers to determine any impacts mercury may have been having on avian reproduction. Nests were compared based on clutch initiation date, clutch size, the proportion of eggs hatched, and the number of fledglings produced (also measured as proportion of nestlings and eggs fledged).

Reported effects of mercury on avian reproduction

Among species, the tolerance for heavy metal contamination varies widely. Mercury levels reported to cause mortality or significant decreases in reproductive success in one species may have little to no effect in another. Studies have found grackles, red-winged blackbirds (*Agelaius phoeniceus*), and European starlings (*Sturnus vulagris*) to experience LD33 (lethal dose for 33% of individuals) at levels of 54.5 ppm, 126.5 ppm, and 30.0 ppm ww in the liver (USDI 1998). By converting these levels to approximate blood mercury levels, LD33 was common at blood mercury levels between 2.00 and 8.43 ppm, well within the range of adult tree swallows successfully breeding along the South River. While some individuals experience mortality at high mercury levels, others may only have slight impacts on their reproductive success, or no effects at all.

In an attempt to establish risk levels for mercury in the eggs of free-living common loons, Evers et al. (2003) reported that females with blood mercury levels >3.00

ppm ww experienced significant decreases in reproductive success in laying smaller clutches that experienced low hatching success. Females with >3.00 ppm blood mercury were reported to lay eggs with >1.30 ppm mercury which was in the high risk category for reproductive impairment (Evers et al. 2003). However, the risk categories presented by Evers et al. (2003) were established based on common loons and are not generally applicable across species as tolerance levels vary. In osprey, mercury levels as low as 0.30 – 0.60 ppm ww in the eggs were reported to decrease the number of young fledged (USDI 1998). Herring gulls have been shown to exhibit a wide range of tolerance with no signs of decreased hatchability with egg mercury ranging from 2.00 – 16.0 ppm ww, while common terns with >3.65 ppm ww in the eggs experienced only 27% hatching success and only 10-12 % of the nestlings fledged (Fimreite 1974). Adult mercury levels correlated with these effects in common terns were >20.0 ppm in the liver (Fimerite 1974), or greater than 1.33 ppm in the blood based on the tissue ratio.

Risk levels based on feather mercury levels should also be interpreted with caution. The amount of mercury in a feather varies with molt pattern, type of feather sampled, the age of the individual, and the species (Monteiro and Furness 1995). The timing of feather growth can also impact the levels found as exposure typically varies between the wintering and breeding grounds (USDI 1998). Across various bird species, adverse effects from mercury have been reported between 5.00 – 40.0 ppm dw in feathers (Burger and Gochfield 1997). Female mallards dosed with 0.5 ppm mercury (average 11.17 ppm in feathers) laid more eggs outside of the nest and produced fewer one-week old ducklings than control birds (Heinz 1979). However, care needs to be taken when extrapolating effects from laboratory dosing experiments to effects in free-living birds. A study on lead exposure in herring gulls found wild birds to experience similar impacts as dosed birds, but wild birds were better able to recover and experienced fewer behavioral problems than laboratory birds (Burger and Gochfield 1997b).

Bald eagles from Pinchi Lake, BC had an average of 40.0 (± 22.0 SD) ppm mercury in their feathers and showed no signs of methylmercury toxicity (no abnormal behavior or behavioral impacts) or impacts on their reproductive success (Weech et al. 2006). In fact, the adult with the highest mercury successfully raised two chicks each season making it one of the most productive in the study area (Weech et al. 2006). Using the tissue ratio (Evers et al. 2005), I estimated the median feather mercury for tree swallows along the contaminated South River across both years to be 19.3 ppm. Similar to the bald eagles on Pinchi Lake, few impacts of mercury exposure were apparent and the female tree swallows with the highest mercury values successfully raised their broods. Though it is important to establish LOAEL (lowest observable adverse effects level) of contaminants in birds, the differences among species, relationships among tissue types, and the potential for extreme variation among individuals must be taken into consideration.

Impacts of mercury on tree swallows along the South River

Clutch initiation date and clutch size In 2005 and 2006, I did not find any differences in clutch initiation date or clutch size when I compared nests in the contaminated and reference areas. As this was similar across both years, these two parameters may not be affected by mercury uptake in female tree swallows. In this species, first egg dates are not necessarily correlated with arrival dates, but do coincide with favorable weather and food abundance (Stutchbury and Robertson 1987). By timing the onset of laying with food availability and good weather conditions, incubation and caring for nestlings can occur under the most favorable conditions, helping to increase reproductive success for the season (Stutchbury and Robertson 1987). Tree swallows may also coordinate breeding with increased food abundance to allow females to reach peak body condition before laying eggs rather than timing laying to match seasonal peaks in food availability for feeding nestlings (Nooker et al. 2005). Food abundance is positively correlated with onset of egg laying (Nooker et al. 2005), nestling growth, condition, and survival (Quinney et al. 1986). The difference in clutch initiation dates between 2005 and 2006 in my study was most likely due to differences in insect emergence and favorable weather conditions. In 2006, the first egg date (4/18/2006) was the earliest ever reported for tree swallows in the state of Virginia (R. Clapp pers. comm.). Earlier egg dates suggests early onset of warm temperatures and insect emergence in the Shenandoah Valley in 2006 compared to 2005. The lack of a difference in clutch initiation dates between contaminated and reference areas across both years may have been due to similar weather patterns across the Shenandoah River Valley and therefore similar timing of insect emergence.

In 2006, similar clutch initiation dates in the contaminated and reference areas could also have been influenced by the larger number of returning, ASY females. Across all sites, the first SY female did not initiate a clutch until day 10 of the 2006 nesting season; the 18 previously initiated nests belonged to ASY females (10 contaminated, eight reference), of which half were returning birds banded in 2005. Familiarity with the breeding site for birds banded in 2005, and even perhaps the unbanded ASY females who may have been undetected floaters in 2005, may have led to an early return and early clutch initiation dates across all sites. Within the first 10 days of the 2005 breeding season, 13 nests were from SY females and nine were from ASY females. The larger number of young females nesting early in 2005 may have been due to the fact that it was the first year of the study and nest boxes only became available in this area as of February 2005.

Clutch size was not different in contaminated or reference areas across both years of this study. Like first egg dates, clutch size in tree swallows is also related to food abundance (Quinney et al. 1986). When food availability is higher, larger clutches are laid as more resources are available and the chances of raising more nestlings in better condition are greater (Quinney et al. 1986). Therefore, food availability across all three rivers used in my study may have been the driving force behind clutch size.

Other studies on the impacts of contaminants on tree swallow reproduction have found similar results in clutch initiation dates and clutch size. Tree swallows contaminated with PCBs in Green Bay, WI had similar clutch completion dates and clutch sizes as birds in reference, or PCB-free areas (Custer et al. 1998). For tree swallows and eastern bluebirds nesting in a pesticide (organochlorines) sprayed apple orchard in Ontario, Canada individuals with high pesticide loads had similar clutch sizes as birds with lower levels (Bishop et al. 2000). Great tits nesting along a contamination gradient in Belgium that were closest to the source (elevated mercury) had similar clutch initiation dates and clutch sizes to birds nesting farthest from the source with lower mercury levels (Janssens et al 2003). Therefore, it seems that clutch initiation date and clutch size are robust reproductive parameters more strongly influenced by food availability and weather patterns than by contaminants.

Proportion of eggs fledged and number of fledglings produced While the data collected on the nesting success of tree swallows were analyzed in several ways (proportion eggs hatched, nestlings fledged, and eggs fledged), these parameters were ultimately measuring one similar factor: how many fledglings were produced. I felt that overall, the two most meaningful measures of nest success were the proportion of eggs fledged and the number of fledglings produced. The proportion of eggs fledged provided a big picture observation of the overall reproductive success of individual birds relative to their investment; out of all of the eggs laid, how many hatched and survived to leave the nest? As far as an absolute fitness estimate, however, it is the comparison of the number of fledglings produced that indicates whether reproduction is being negatively impacted in a contaminated area. I therefore focused on these two parameters.

In 2005, I did not detect any differences in nesting success between contaminated or reference areas. In 2006, nests in contaminated areas did have a significantly smaller proportion of eggs fledge ($p>0.0001$) and produced fewer fledglings ($p=0.0002$) than nests in reference areas. On average, females in contaminated areas produced one fewer fledgling than females in reference areas. However, ASY and SY females had significantly different responses in terms of the proportion eggs fledged within the contaminated areas. ASY females did not have a significantly different proportion of eggs fledged compared to all females in the reference areas. It was only the SY females in contaminated areas that had a lower proportion of eggs fledge than all other females regardless of treatment group. This indicates that the success regarding overall reproductive effort by SY females in contaminated areas was significantly lower than all other breeding females.

Another way to look at the overall reproductive effort was to simply compare the number of fledglings produced by both age classes in each treatment group. SY females also produced fewer fledglings than ASY females in contaminated areas (p=0.0001). ASY females in the contaminated areas produced similar numbers of fledglings as both SY and ASY females in the reference areas. Ultimately, perhaps due to a combination of mercury contamination and inexperience, SY females in contaminated areas produced approximately two less chicks than ASY females in both treatment groups and one less chick than reference SY females.

Egg size Eggs laid by females in contaminated areas were significantly smaller than eggs laid in reference areas (p<0.0001); however, there was a significant effect of female age (p <0.001). Preliminary results indicate SY females in contaminated areas laid smaller eggs than all other females regardless of treatment group. Results are described as preliminary due to the smaller number of clutches of SY females measured. Older females have been shown to lay larger eggs than SY females (Robertson et al. 1992, Ardia et al. 2006); however, SY females in the contaminated areas in my study laid eggs smaller than SY females in reference areas. Egg size and egg quality are directly related to offspring survival; larger eggs have higher hatching success and hatch larger nestlings that grow faster (Ardia et al. 2006). In order to determine whether the tendency for SY females in contaminated areas to lay smaller eggs is a true pattern, additional clutches need to be measured as only two clutches from SY females in contaminated areas were sampled in 2006. SY females in contaminated areas experienced lower nesting success (smaller proportion of eggs fledged and fewer fledglings produced) which could have been a product of laying smaller, lower quality eggs.

Differences between age classes Any differences between the success of SY and ASY females in contaminated areas was most likely not due to differences in mercury levels. In 2006, ASY females tended to have higher mercury levels than SY females, though the difference was not significant. No comparison could be made from 2005 as there were only three ASY females. Two possible explanations for the differences in nest success between these two classes could be: the effect of mercury on inexperienced breeders in favorable conditions versus unfavorable conditions. As SY females in reference areas had similar success to ASY females, high quality mates, food abundance, and/or favorable weather conditions could have leveled the playing field for less experienced breeders. In contaminated areas, the elevated mercury levels of SY females may have impaired their condition or ability to reproduce in spite of favorable food and weather conditions. The other possibility was that food availability and weather conditions were less favorable causing competition over resources. SY females in poor condition (elevated mercury) may not have been able to compete with ASY females for the resources needed to successfully reproduce. Despite elevated mercury levels, ASY females may have bred successfully due to previous experience. In either scenario, being an inexperienced breeder with elevated mercury was a harmful combination.

Nesting success of returning birds Ten females returned as ASY birds to breed in 2006 on the South River after having been banded in 2005. These females experienced two years of breeding in a mercury-contaminated area. Compared to other ASY females, that were new to the study site in 2006, I expected returning birds to have lower nest success as they most likely had higher body burdens of mercury than birds new to the site. Mercury levels did not differ between recaptured females and females new to the study site; however, this was expected as blood only indicates dietary uptake over the past few weeks. Though blood mercury levels were not different, returning females likely had higher body burdens. Based on this assumption, I was surprised to find that returning females had similar breeding success to ASY females new to the study site for all six parameters. I expected clutch initiation dates to be earlier for returning females as a result of presumed early arrival by returning birds. Clutch initiation dates were not different. I also assumed returning females would have lower success at all other measured parameters; however, being experienced breeders returning to a familiar area may have allowed returning females to breed as successfully despite higher body burdens of mercury. The returning females may have also had high quality mates to provide excellent parental care. Further, "new" females may have previously been floaters and had some amount of experience on the site and similar body burdens of mercury as returning birds.

Was female mercury level correlated with nest success? Although few differences were detected between nest success in the contaminated and reference areas, the question remained as to what levels of mercury in female tree swallows caused reproductive impairment. ASY females tended to have higher mercury than SY females, but SY females experienced significant impacts on their nest success. However, not all ASY females had successful breeding attempts. To determine whether a mercury level threshold existed, above which all females' experienced decreased reproductive success, I used regression analysis to look for any potential relationships. No relationship was found between female mercury level and clutch initiation date or clutch size. This was not surprising as I have established those two parameters as robust to female mercury and more dependent on environmental conditions. The relationships between the proportion of eggs hatched, nestlings and eggs fledged, and the number of fledglings produced were all significantly, negatively correlated with female blood mercury. However, mercury level did not explain much of the variation in these parameters, based on the low R^2 values. The females with the highest mercury levels (between 9.00 and 11.9 ppm) had success at each parameter similar to that of females with mercury levels less than 1.00 ppm. Within the range of 1.00 – 6.00 ppm, many females demonstrated complete nest success while others experienced decreases at each measured parameter. Instead of finding a mercury threshold above which females began to experience decreased nest success, I found that there is extreme variation in an individual's response to mercury contamination.

This finding, that a female's contaminant level does not predict her nesting success, was not novel. Bald eagles nesting at a lake with similar mercury levels as tree swallows on the South River did not show a strong relationship of eaglet mercury level with reproductive success (Weech et al. 2006). As adult eagle and eaglet blood were highly, positively correlated (Weech et al. 2006), one can deduce that there was also no relationship between adult blood mercury and productivity. That study found adult eagles with high blood mercury levels (>10.0 ppm) to reproduce as successfully as birds in reference areas. While PCBs impact avian reproduction via different pathways compared to mercury, few studies record both contaminant levels and effects. As two studies of PCBs on tree swallows report both levels and effects, I felt the comparison was worth noting. In a study on the effects of PCBs on tree swallow reproductive success, PCB levels did not differ in clutches where all, none, or some of the eggs hatched (Custer et al. 1998). There was no difference in the number of dead embryos in a clutch or ultimate hatching success at sites that differed in PCB concentrations (Custer et al. 1998). Another study of PCBs in tree swallows that took place along the Housatonic River in Massachusetts found, as with tree swallows along the South River, that although there was significant decrease in the number of eggs that hatched as PCB increased, the fit of the model was poor (Custer et al. 2003). This study did find hatching and nest success to decrease with increasing contamination, but nests with the highest recorded levels of PCB were as successful as nests with low levels of contamination (Custer et al. 2003). Tree swallows nesting along the South River have the highest reported blood mercury levels ever reported for an insectivorous passerine; however, much like the studies in Green Bay and the Housatonic River, there is a large amount of variation in what levels result in decreased reproductive success.

Conclusions

Tree swallows nesting along the South River in Virginia had the highest blood mercury levels ever reported in a free-living insectivorous passerine. The exposure to adult tree swallows was equal to that of piscivorous species, such as the belted kingfisher, nesting along this polluted tributary. Perhaps due to increased bacterial methylation of mercury in the river, mercury levels in 2006 were higher than in 2005. While no effects of mercury on nesting success were discovered in 2005, SY females in contaminated areas in 2006 experienced significant impacts on their nesting success as measured by the proportion of eggs fledged and number of fledglings produced. ASY females in contaminated areas, perhaps due to previous breeding experience, were able to breed as successfully as females in reference areas despite elevated mercury levels. Similar to other studies, the amount of mercury in a female's blood did not predict her nesting success. There was no apparent difference in the mercury levels of females that had success or failure at each of the measured parameters.

My findings were similar to other recent studies regarding extreme variation in individual tolerance to contaminants (see Custer et al. 2003, Weech et al. 2006). The differences found in the tolerance and impacts of mercury between age classes have previously been overlooked. Though SY and ASY female tree swallows are easily distinguishable from each other, no study has discussed, or found, differences in mercury accumulation or impacts based on age (aside from the comparison between nestlings and adults). Further, no studies have attempted to address the differences in response to mercury exposure on experienced versus inexperienced breeders. Knowing an individual's age is most important when food resources are partitioned and foraging strategies may be different in various age cohorts; however, niche partitioning between SY and ASY female tree swallows has never been reported. ASY females appeared to have higher mercury levels than SY females along the South River, but experienced fewer impacts. The potential for contaminants to impact first time breeders compared to experienced breeders needs to be further investigated. Gaining experience through breeding in the first year may allow females to return and breed successfully in spite of elevated contaminant levels the next year. However, this poses an interesting question regarding a female's lifetime reproductive success. By experiencing decreased success in the first year of breeding, are females able to compensate for reproductive losses in successive years? For example, if first year breeding females in contaminant free areas could produce four chicks in their first year of breeding and six in their second year, they would have a lifetime total of ten chicks. As SY females have been shown to have lower success in their first year, an increase in reproductive output in the second year would be predicted (McCarty and Secord 2000). However, in a contaminated area a first year breeder may only produce one chick and return the next year and produce five, a lifetime total of only six chicks. Ultimately, birds in contaminated areas may have lower lifetime productivity than birds in contaminant-free areas due to lower productivity in the first year.

To determine whether SY females experience decreased reproductive success compared to ASY females, mercury dosing studies need to be done on free-living birds. This type of study could control the amount of mercury accumulated by both age classes to determine whether the same amount of dietary mercury has different effects in first-time breeders. If first-year breeders consistently fledge fewer young when exposed to contaminants, populations in contaminated areas may be ultimately less productive than those in pristine, contaminant free areas. As the tree swallow population in the Shenandoah Valley is an artificial one, discovering population level impacts would be unlikely. However, based on their life history characteristics and easily distinguishable age classes, tree swallows could act as model organisms for potential population level impacts of mercury on less abundant or even endangered species. If effects levels can be established by dosing studies, the potential risk to birds in areas of environmental contamination could be predicted and remediation strategies set up to prevent population level impacts.

CHAPTER 2

THE EFFECTS OF MERCURY ON THE RETURN RATE OF TREE SWALLOWS

Philopatry and survivorship

Tree swallows are secondary cavity nesters, relying on primary cavity nesters to excavate nest cavities, or use nest boxes, and are highly philopatric once they begin to breed (De Steven 1980, Robertson et al. 1992, Winkler et al. 2004). As the availability of nest cavities, natural or man-made, is limiting to tree swallows competition for them is strong. A site at which a pair was able to establish a territory and successfully raise a brood in the past may have a high value (Barber and Robertson 1998, Shutler and Clark 2003). The value of a successful nest site, as well as the well-demonstrated phenomenon of prior residence advantage in competition for resources (Cristol et al. 1990), should motivate individuals to return in following years (Barber and Robertson 1998). Philopatry of females to nest sites has been reported in many other song birds, including the pied flycatcher (*Ficedula hypoleuca*), barn swallow (*Hirundo rustica*), and bobolink (*Dolichonyx oryzivorus*) (Winkler et al. 2004).

To return to a breeding site from one year to the next, a tree swallow must migrate to the wintering grounds, survive the winter, and then migrate back to the breeding grounds. Based on band recoveries, approximately 79% of nestlings die within the first year of their life (Butler 1988, Robertson et al. 1992). Annual survivorship after the first year ranges, on average, from 40%-60% (Butler 1988, Robertson et al. 1992), although lower values have been reported (see Table 21). Extreme winter or migration conditions, as well as individual variation in tolerance to adversity, cause this variation in survivorship. Toxins such as mercury, which can decrease neurological function and physiological condition, might be expected to reduce survivorship below the 40-60% expected , for example by lowering the likelihood of successful navigation or surviving winter food stress. The objective of this portion of the study was to compare the return rate in 2006, as an estimate of short-term survivorship for swallows from contaminated and reference areas in 2005.

Natal vs. breeding dispersal Dispersal is defined as the movement of a bird from a natal or previous breeding site to a new one (Winkler et al. 2004). Natal dispersal, refers to a bird's movement from birthplace to site of first breeding (Winkler et al. 2004). Breeding dispersal, change of location between breeding attempts, can occur within or between breeding seasons (Winkler et al. 2004). Nestling birds typically disperse from their natal site because parents survive to occupy the site in the following year; reported philopatry of nestling tree swallows to study sites is 0.8-12% (Butler 1988) compared to near 40% for breeding adults (Chapman 1955). It should be noted that only 20% of nestlings from one season are expected to be alive in the following season (Butler 1988), in which case 12% philopatry is considerable. Of the returning nestlings that were detected by researchers in Saskatoon, Saskatchewan, most were detected within 40 km of their natal site (Houston and Houston 1987). The average dispersal distance of nestling tree swallows is not known, but has been estimated to be within 20-40 km of the natal site

(Roberston et al. 1992). Few studies have attempted to describe natal dispersal (Shutler and Clark 2003).

Variables impacting return rates

In general, the return rates reported for adult tree swallows range from 13% to 60% (DeSteven 1980, Robertson et al. 1992). Estimated survival rates of other swallow species are similar to those of tree swallows. Return rates of cliff swallows (*Hirundo pyrrhonota*) range from 27% to 50%, bank swallows (*Riparia riparia*) 35-40%, and barn swallows (*H. rustica*) 43% (Butler 1988).

Detection and recapture Nest boxes function, in a sense, as traps collecting individuals upon their return to a study site. However, care must be taken when interpreting recapture rate of individuals in nest boxes as survivorship. Recapture rates, or apparent survivorship, can be limited by observers' ability to detect a returning bird. For example, in Saskatchewan, the recapture rate of adult birds one year after banding was 12.8% (Houston and Houston 1987). However, the estimated survivorship, based on recaptures across the 17-year study, was near 40%. Because the study took place across many years, individuals that were missed one year were typically recaptured in subsequent years allowing for an overall estimate of survival (Houston and Houston 1987).

As study sites represent finite areas, individuals that disperse beyond a pre-determined distance are typically undetectable (Koenig et al. 1996). Individuals can also go undetected within a finite area; in the case of tree swallows, any individual that returns to a natural cavity, as opposed to a nest box, in the study area is likely to go undetected. No studies have reported recaptures of tree swallows using natural cavities that used nest boxes in previous years. One study in Saskatchewan estimated, based on 12 years of surveys, that no more than 20% of the tree swallow population in Saskatchewan used natural cavities due to the lack thereof (Shutler and Clark 2003). This may also be true for the population in the Shenandoah Valley as intensive agriculture has eliminated most dead trees, and beaver dams or flooded wetlands with dead trees are absent. No nesting outside of nest boxes was observed during the two years of the study. Clearly, it would not be prudent to consider an undetected individual as a certain mortality event, especially after only one year. But, while there are limitations to detecting individuals in any study system, nest box use by tree swallows and their established philopatric behavior do allow survival estimates to be made based on recapture rates.

Sex and age Dispersal in adult tree swallows is highly correlated with breeding success during the previous year (Robertson et al. 1992, Winkler et al. 2004). Of the surviving females who had a successful breeding season (defined as fledging ≥1 young) only 5% did not return compared to 28% of females who failed (Winkler et al. 2004). Historically, male and female tree swallows were thought to have similar return rates (Chapman 1955); however, more recent studies have found that females were more likely than males to change sites from one breeding season to the next (Shutler and Clark 2003, Winkler et al. 2004). Females tend to show lower site fidelity than males; especially after an unsuccessful breeding season (Robertson et al. 1992). Emigration rates (dispersal from the previous nest site) increase for females after breeding failure, possibly explaining this difference in philopatry (Winkler et al. 2004). However, a few studies on other songbirds have suggested that only females who experience nest failure in low-quality breeding sites disperse and that breeding dispersal from high-quality sites is not related to nesting success (Bollinger and Gavin 1989, Shutler and Clark 2003). The definition of what makes a high-quality site may vary between studies; Bollinger and Gavin (1989) considered low-quality habitat to be an area where nests were lost to frequent cutting of hay. The mechanism behind female dispersal after the loss of a nest site (Bollinger and Gavin 1989) versus decreased fledgling production (Shutler and Clark 2003) may differ and should be considered.

Return rates can also be influenced by the age of the female. In one study, first time breeding ("Second Year", hereafter SY) females tended to have higher return rates than older females ("After Second Year", or ASY); however the difference reported was not significant (DeSteven 1980). Another study reported lower dispersal in older females (Winkler et al.2004). SY females tend to have lower reproductive success than ASY females (McCarty and Secord 2000) which would predict a higher rate of dispersal in younger birds.

Nestling return to the natal site Very few studies have looked at the return rate of nestling tree swallows to their natal site, most likely owing to the fact that mortality in the first year is near 80% (Butler 1988). As the sex of nestling tree swallows cannot be determined in the field before fledging, it is difficult to estimate differences in survivorship or return rate between males and females. Based on an assumption of equal sex ratios within a nest, one study reported that female nestlings recruited to the study site at lower rates than males (Shutler and Clark 2003); another study suggests no difference in male or female nestling return rates (Robertson et al. 1992). Additionally, natal dispersal distance of nestlings can have a significant effect on their detection rate and estimated survivorship. In a 17-year banding effort in Saskatoon, Saskatchewan only

0.8% returned to the study site each year (Houston and Houston 1987). On average, only four out of 472 nestlings banded each year were detected on the study site the next year. Of the six nestlings that returned in multiple years, the average distance from their natal site to the point of recapture was approximately 19 km. After the initial dispersal from the natal site to the first breeding site, these six individuals moved, on average, 1.3 km each year they returned. Another study in Saskatchewan reported that 4.9% of the nestlings, banded across six years, were recruited to breed on the study site (Shutler and Clark 2003). In this study, the average natal dispersal was 0.83±0.50 km, similar to the findings of Houston and Houston (1987).

An interesting point to note from this study was that the return of nestlings, or their detection, was typically delayed (Houston and Houston 1987). While a number of recruits were detected the following year, similar numbers of recruits were detected for the first time two to three years later. This is important to consider when estimating survivorship based on return rates of swallows to nest boxes. Though yearling birds can reproduce, the proportion of a given breeding population made up of SY females can range from 10% to 80% (Robertson et al. 1992). Thus, a large proportion of female recruits may be on the study site but go undetected as they did not obtain a nest site. There are no studies that report on the success of gaining a breeding territory in the first year for male nestlings.

Potential for mercury to impact survivorship and philopatry

Acute mercury poisoning is characterized by severe neurological dysfunction causing physiological and behavioral problems. Symptoms can include loss of strength in legs and wings, weight loss due to appetite suppression, and an inability to coordinate muscle movements during walking and flying (Wolfe et al. 1998). Neurological impacts of mercury in birds result in brain lesions, spinal cord degradation, and other dysfunctions of the central nervous system (Wolfe et al. 1998). Abnormal feather loss, brain lesions, and mortality have been attributed to mercury levels between 3-14 ppm in the liver (Wolfe et al. 1998, see Chapter 1 for details on impacts of mercury on birds). Any decrease in neurological and/or physiological condition could impact a bird's survivorship and/or ability to return to their previous nesting site, for example through inability to remember spatial information during migration or foraging, or decreased function of kidney or liver.

Impact of mercury on survivorship of tree swallows nesting along the South River, VA

For 20 years, mercury was released by an industrial source into the South River, a tributary to the South Fork Shenandoah River, in Virginia (see Chapter 1 for details). Beginning in 2005, my study became the first to assess the impacts of mercury contamination on birds nesting along this polluted tributary. I chose to use tree swallows as biomonitors because they are aquatic insectivores and readily use man-made nest boxes. Nest boxes were erected along the South, Middle, North, and South Fork Shenandoah Rivers to study the impacts of mercury contamination on tree swallow nesting success in 2005.

While mercury levels in the blood of adult tree swallows were an order of magnitude higher in contaminated compared to reference areas in 2005, nesting success was similar across all sites (see Chapter 1). I was unable to detect any impacts of overt mercury toxicity in adult or nestling tree swallows in the contaminated areas throughout the 2005 breeding season. It should be noted that outright mortality would not have been detected as I only sampled breeding adults, possibly the most mercury-resistant individuals in the population. Although nesting success was similar, survivorship after the breeding season could have been impacted by the accumulation of mercury over the three or more months spent on the breeding grounds. If mercury accumulation left birds in poor condition, especially after raising young, survivorship of birds in contaminated and reference areas from one breeding season to the next may have differed.

Objectives

Did birds nesting in contaminated sites in 2005 have lower return rates than reference birds?

Females that bred in mercury-contaminated areas in 2005 may have decreased survivorship compared to females who nested in areas free of mercury. Because of the highly philopatric behavior of female tree swallows, rate of return was used as an index of short-term survivorship. To assess any impacts of mercury on tree swallow survivorship from 2005 into 2006, I compared the return rates of females banded in 2005 from contaminated and reference areas. I predicted that if mercury decreased neurological or physiological condition, fewer females who had nested in mercury-contaminated sites would be detected on the study site than females from reference sites.

I also looked at the return rate of nestling birds to the study site in 2006. My study in 2005 compared only pre-fledging reproductive parameters in the contaminated and reference sites. However, the impacts of growing up in a mercury-contaminated area may only be apparent after fledging. Nestlings that consumed prey items with high mercury may have had lower post-fledging survivorship than nestlings from reference areas. To determine nestling survivorship, I compared the return rate, to anywhere in the study area, of nestlings that grew up in reference and contaminated areas.

Was movement within the study area between 2005 and 2006 different in contaminated and reference areas? My ability to detect returning birds relied on their use of the nest boxes provided along the three rivers. Even if birds banded in contaminated and reference areas returned to the study site at similar rates, differential movement within sites could confound their detection. For example, if birds from contaminated areas moved, on average, farther from their 2005 nest box than birds from reference areas, they would be more likely to move off of the study site and avoid detection. To test the assumption that birds from contaminated areas had similar within-study-site fidelity as birds from reference areas, the distance from the 2005 nest box to the nest box used in 2006 was estimated.

METHODS

This study took place in the same study areas described in Chapter 1. All nest boxes were designed and erected as previously described. Data collection on return rate and site fidelity coincided with the 2006 breeding season and was collected as part of continuing research on the effects of mercury on nesting success of tree swallows that began in 2005.

Increased availability of nest boxes in 2006

Additional nest boxes were added to the study sites in 2006 in order to recruit returning juveniles banded in 2005. As tree swallows are highly philopatric, adult birds banded in 2005 were expected to return to the study site in 2006 and reclaim their nest boxes. Additional nest boxes increased the probability of a surviving, returning nestling being detected after settling in an available nest box.

Nest boxes were added to sites used in 2005 as well as at seven new sites. Twenty-two boxes were erected at three new reference sites along the South (upstream of the contamination), Middle, and North Rivers; 21 boxes were placed at four new sites along contaminated portions of the South River. Not all sites used in 2005 received new nest boxes due to limited space at smaller sites. Thus, at the beginning of the 2006 breeding season there were a total of 119 nest boxes in the contaminated areas and 167 boxes in reference areas; a total of 286 available boxes (Table 17).

Detection of returning swallows

Ninety-eight adult and 506 nestling tree swallows were banded with USGS bands in 2005, including birds nesting on the SFSR. Of the 98 adults banded, 74 were female and 24 were male. If dead, banded adults or nestlings were recovered before the end of the 2005 breeding season, the mortality was noted and those birds were removed from any analyses done on return rates. Return rates were based on the number of birds banded in 2005 that were detected on the study site in 2006. In this study, the term detection is synonymous with the ability to capture a bird in a nest box to read its band number. To prevent nest abandonment, I did not attempt to capture adult birds during the nest building or incubation periods. Nest boxes were checked frequently, as described in Chapter 1, to detect the presence of eggs or chicks. If an adult bird was found in the box during a nest box check, I looked at the right leg to see if it had a band or not. If banded, I wrote down the band number and returned the bird to the nest box.

TABLE 17

NUMBER OF NEW NEST BOXES ERECTED AND THEIR LOCATIONS IN 2006

	# Boxes 2005	# Added to previous sites	# Added to new sites	Total 2006
Contaminated	60	38 (63%)	21 (35%)	119
Reference	89	60 (67%)	22 (25%)	167*

Percentages were calculated as the number of new nest boxes divided by the total number of boxes in 2005, thus representing the percent increase in box availability.

* Total number of nest boxes in 2006 does not equal the sum of old and new nest boxes as one site (four boxes) was removed from the Flora property in 2006.

When I was unable to determine the status (banded or unbanded) of a pair during the incubation period, nest box traps were used to capture the adult birds (see trap design in Appendix B). Every female bird that nested in the boxes was caught, providing an exact, rather than estimated, return rate of female birds to the boxes on the study site. It is possible that a female could have remained undetected in the unlikely case that she settled in a nest box for a short time but abandoned before her clutch was completed – otherwise I examined all females breeding at the site (approximately four clutches were determined to have been abandoned in 2006). Each capture attempt lasted no longer than one hour each day so as not to disturb the nest for an extended period of time. If a female was not caught within that time frame, I returned within a few days for a second attempt, never needing a third. An effort was made to capture all males as well to aid in determining the return rate of male and nestling birds to the study site. During capture attempts, males were identified as banded or unbanded by using binoculars to look for a band on their right leg. If there was no band present, attempts to capture the male were typically cut short due to time constraints. If a male was noted to be banded, 1-3 hours of total effort were made to catch him.

Determining return rate

Return rate was compared between adult birds from contaminated areas along the South River and all reference areas to assess whether mercury had an impact on the survivorship of the birds from one breeding season to the next. The few birds that returned to the study site that were banded along the South Fork Shenandoah River were not included in the analyses as the SFSR study sites were cleared of nest boxes after the 2005 breeding season.

To determine female return rate, the number of banded females recaptured in the contaminated areas in 2006 was divided by the total number of birds banded in 2005, minus any known mortalities. This value provided a proportion used to describe the rate of return. The same was done for female birds in the reference areas. The return rate of males banded in 2005 was an estimate based on the proportion of males identified as banded or unbanded in 2006. However, a much smaller number off males were banded in 2005, so the sample size of potential returning birds was small. Previously, female philopatry was thought to depend heavily on her success in the previous year (Winkler et al. 2004); however, a recent study suggested philopatry may be based on the combination of a number of factors (Shutler and Clark 2003). Therefore, as only one female in the contaminated and one from the reference area did not produce at least one fledgling in 2005, all females were used in the analyses.

The return rate of nestling birds was calculated by dividing the number of banded nestlings caught in 2006 by the total number of nestlings banded in 2005, minus those that died in the nest after banding. Chi-square analysis was used to compare the proportion of birds that returned to the contaminated and reference areas for adult females. Chi-square analysis was also used to compare nestling return rate, but was corrected for continuity due to the large sample size. Fisher's exact was used to compare the return rate of males as the sample size was small ($N<15$).

Movement within study area

Because female tree swallows are highly philopatric, most will return to the same nest boxes or sites used in the previous year. However, the accumulation of mercury during the 2005 breeding season could have impacted fidelity. I compared the movement within sites of female birds banded in reference and contaminated areas in 2005. Site fidelity was defined as the distance the nest box used in 2006 was from the nest box used in 2005. Distance moved was estimated using the number of territories separating the 2005 and 2006 nest boxes; territory was defined as the distance from one nest box to the next closest box (on average, 25 m). Therefore, if in 2006 a female used the nest box next to the one used in 2005, she would have moved one territory, or 25 m. The distance moved by females on contaminated and reference sites was compared. If a female moved to a different site than the one used in the previous year, she was eliminated from this analysis (n=6).

Using regression analyses, adult blood mercury levels from 2005 were correlated with the distance each individual moved, within a site, from the nest box used in 2005 to the one chosen in 2006. Comparisons were made for birds across all sites and for only those birds that nested along the contaminated South River.

RESULTS

Number of returning birds

Forty-six birds banded in 2005 returned to the study site in 2006 (Table 18). Of these 46 birds, 37 were female and nine were male. Fourteen birds returned from the contaminated areas and 32 from reference areas. Ten of the recaptured birds were nestlings in 2005, eight were female and two were male. Only two of the 10 returning nestlings were from contaminated areas, both of which were female. The two female nestlings that returned from the contaminated areas were siblings from the same clutch in

TABLE 18

RETURNING BIRDS IN 2006

Sex	Age	Box 2005	C or R	Site	River	Box 2006	Site	C or R
F	ASY	169	C	Genicom	South	167	Genicom	C
F	ASY	105	C	Merck	SFSR	194	AFC	C
F	ASY	156	C	AFC	South	190	AFC	C
F	ASY	190	C	AFC	South	230	AFC	C
F	ASY	36	C	H20 Treatment	South	39	H20 Treatment	C
F	ASY	40	C	H20 Treatment	South	156	AFC	C
F	ASY	164	C	Genicom	South	241	Basic Park	C
F	ASY	165	C	Genicom	South	170	Genicom	C
F	ASY	12	C	Grottoes City Park	South	13	Grottoes City Park	C
F	ASY	19	C	Grand Caverns	South	19	Grand Caverns	C
F	ASY	184	C	Dooms	South	185	Dooms	C
F	SY	105	C	Merck	SFSR	133	Crimora	C
F	SY	55	C	Sheets farm	SFSR	339	Wampler property	C
F	SY	39	C	H20 Treatment	South	254	H20 Treatment	C
F	SY	39	C	H20 Treatment	South	160	AFC	C
M	AHY	157	C	AFC	South	154	AFC	C
M	AHY	101	C	Basic Park	South	100	Basic Park	C
F	ASY	149	R	Crawford annex	North	152	Crawford annex	R
F	ASY	151	R	Crawford annex	North	151	Crawford annex	R
F	ASY	172	R	Cowbane	South	176	Cowbane	R
F	ASY	174	R	Cowbane	South	173	Cowbane	R

(continued)

TABLE 18 (continued)

RETURNING BIRDS IN 2006

Sex	Age	Box 2005	C or R	Site	River	Box 2006	Site	C or R
F	ASY	75	R	Godfrey farm	Middle	87	Smith's Pond	R
F	ASY	89	R	Smith's Pond	Middle	8	Godfrey farm	R
F	ASY	87	R	Smith's Pond	Middle	88	Smith's Pond	R
F	ASY	60	R	Whitescarver farm	Middle	296	Whitescarver farm	R
F	ASY	61	R	Whitescarver farm	Middle	61	Whitescarver farm	R
F	ASY	96	R	Shapcot property	Middle	96	Shapcot property	R
F	ASY	93	R	Shapcot property	Middle	93	Shapcot property	R
F	ASY	73	R	Godfrey farm	Middle	74	Godfrey farm	R
F	ASY	74	R	Godfrey farm	Middle	4	Godfrey farm	R
F	ASY	75	R	Godfrey farm	Middle	75	Godfrey farm	R
F	ASY	78	R	Godfrey farm	Middle	78	Godfrey farm	R
F	ASY	80	R	Smith's Pond	Middle	79	Godfrey farm	R
F	ASY	145	R	Crawford annex	North	315	Sandy Bottom Park	R
F	ASY	129	R	Dories/Middle River Rd	Middle	304	Dories/Middle River Rd	R
F	ASY	69	R	Whitescarver farm	Middle	295	Whitescarver farm	R
F	SY	181	R	Cowbane	South	174	Cowbane	R
F	SY	83	R	Smith's Pond	Middle	28	Locust Street	R
F	SY	66	R	Whitescarver farm	Middle	290	Whitescarver farm	R
F	SY	86	R	Smith's Pond	Middle	322	Whitescarver farm	R
F	SY	89	R	Smith's Pond	Middle	317	Whitescarver farm	R
F	SY	79	R	Godfrey farm	Middle	289	Whitescarver farm	R

(continued)

TABLE 18 (continued)

RETURNING BIRDS IN 2006

Sex	Age	Box 2005	C or R	Site	River	Box 2006	Site	C or R
M	AHY	181	R	Cowbane	South	181	Cowbane	R
M	AHY	174	R	Cowbane	South	60	Whitescarver farm	R
M	AHY	77	R	Godfrey farm	Middle	76	Godfrey farm	R
M	AHY	59	R	Whitescarver farm	Middle	298	Whitescarver farm	R
M	AHY	177	R	Cowbane	South	177	Cowbane	R
M	AHY	66	R	Whitescarver farm	Middle	67	Whitescarver farm	R
M	AHY	79	R	Godfrey farm	Middle	89	Smith's Pond	R

2005. One of these two siblings was found freshly killed in a nest box in 2006, most likely from attack by a neighboring house sparrow. The remaining six females and two males were banded at reference sites in 2005. Of these eight birds, two were siblings from the same brood at the Godfrey farm in 2005.

One adult female captured in 2006 had been banded along the South Fork Shenandoah River in 2005 and was therefore not used in any further analyses. In addition, two nestlings that had been banded along the SFSR returned to the contaminated portions of the South River in 2006. One of these returned to Crimora Crossing; the other returned to the Wampler property, a new site in 2006. These individuals have been left out of all total counts and further analyses as the nest boxes at these sites were removed before the 2006 breeding season.

Return rate

Adult females Return rates were calculated for adult female birds banded along the South River and all reference sites in 2005. Of the 56 adult females banded in 2005, 29 (52%) returned to the study site in 2006. In 2005, 18 adult females were banded in contaminated areas and 38 were banded in reference areas. Of the 27 adult females recaptured in 2006, ten were from contaminated areas and 19 were from reference areas. Ten out of 18 (56%) females banded along the contaminated South River in 2005 returned in 2006. Nineteen of the 38 (50%) adult females banded in 2005 along the reference rivers returned to the study site in 2006. Based on the proportion of returning females detected in the reference and contaminated areas, there was no difference in return rate of adult female birds to the study site in 2006 (Table 19, χ^2=0.15, DF=1, p=0.70).

Estimated return of adult males Calculating the rate of return for banded males was less informative than calculating female return rate because males were sampled only opportunistically in 2005. Of the 80 tree swallow nests in the contaminated areas in 2006, the identity of the male was known at 47 (59%). There were 130 nests in the reference areas in 2006, the identity of the male was known at 71 (55%) of them. The proportion of males unidentified in 2006 was similar in the contaminated and reference areas (χ^2=0.047, DF=1, p=0.83). Because the proportion of unidentified males did not differ significantly between the contaminated and reference areas, the chance of missing a returning, banded male was similar.

Adult male return rate was estimated using the males of known status from contaminated portions of the South River and reference areas. Of the nine males banded in contaminated areas in 2005, two (22%) were recaptured in 2006. Of the 11 males banded in reference areas in 2005, five (45%) were recaptured in 2006. The number of banded males detected in contaminated areas was not significantly different from the number of banded males detected in reference areas (Table 19, Fisher's exact, p=0.13), but the sample size is too small to provide adequate statistical power for meaningful conclusions.

Estimated nestling return Nestling sex cannot be determined in the field and therefore the return rate of nestlings from 2005 was estimated for both sexes combined. Only seven of 131 nestlings banded in the contaminated sites in 2005 failed to fledge which left 124 nestlings that could have returned to the study site in 2006. Two nestlings (2%) returned to the study site from contaminated areas in 2006. Of the 294 nestlings banded in reference areas in 2005; four known mortality events left 245 nestlings to

TABLE 19

CHI-SQUARE TABLE: NUMBERS OF RETURNING BIRDS IN 2006

	Treatment group	# returned	# did not return	Total
Females	Contaminated	10	8	18
	Reference	19	19	38
	Total	29	27	56
Males	Contaminated	2	3	5
	Reference	5	6	11
	Total	7	9	16
Nestlings	Contaminated	2	122	124
	Reference	8	237	245
	Total	10	359	369

return to the study site in 2006. In 2006, 8/245 (3%) nestlings returned to the study site from reference areas. The return rate of nestling birds from contaminated and reference areas did not differ significantly (Table 19, χ^2=0.34, DF=1, p>0.50). However, the number of nestlings that returned was too small to warrant valid statistical comparison.

Nest site fidelity

Movement among rivers Birds that nested along the South, Middle, and North Rivers in 2005 returned to nest along those same rivers in 2006. Of course, birds moving among rivers that did not use nest boxes would not have been detected. Only two nestling birds returned to breeding sites other than their natal rivers. One male nestling banded at Cowbane Nature Preserve along the South River in 2005 nested on the Middle River in 2006, approximately 20 km away, as the swallow flies. The other male nestling returned to Smith's Pond in 2006 from its natal site on the Godfrey farm in 2005, which is immediately adjacent and separated by only narrow roadway.

Movement among sites Altogether, only six out of 27 (22%) returning adult females nested in a different site from the one used in 2005, and none moved from reference to contaminated sites or vice versa. Two females from the contaminated areas did not return to the same breeding site used in 2005. One female nested at the Waynesboro Water Treatment Plant in 2005, and returned to the Augusta Forestry Center to breed in 2006, a distance of nine river miles downstream. The second female nested at Genicom in 2005, and returned to Basic Park in 2006, less than one river mile upstream. From the reference areas, four females nested in different sites than they did in 2005. Three females moved among the three sites located in Swoope along the Middle River, all within the same river mile. The fourth returning female was from the North River and moved from the Crawford Annex in 2005 downstream to Sandy Bottom Park, a new site, in 2006.

Movement within a site A majority of the birds returned to the same nesting site used in 2005 in both contaminated and reference areas (33/46, 72%); however, very few used the same nest box (9/46, 20%). Of the 10 adult females banded along the contaminated stretches of the South River in 2005, eight (80%) returned to the same site used in 2005. Only one of those eight females used the same nest box that she used in 2005. This female nested at Grand Caverns and had the highest blood mercury level of all adult tree swallows in 2005 (5.72 ppm). One female returned to the same site, but used a new box erected for the first time in 2006; the remaining birds used old boxes that had been present at the site in 2005. The distance a female moved within a site ranged from 0-21 territories (~525 m; Table 20). The two males that were detected in the contaminated areas both returned to the same site used in 2006, but did not use the same nest box. One male from the Augusta Forestry Center moved four territories (100 m); the other was from Basic Park and moved only one territory (25 m).

In the reference areas, 15 of the 19 (79%) adult females banded in 2005 returned to the same site to breed in 2006 that they used in 2005. Of those, six (40%) used the same nest box as they did in 2005. The distance moved within a site by females in reference areas ranged from 0-13 territories (325 m) (Table 20). All five adult males recaptured in the reference areas returned to the same site used in 2005. The two males recaptured at Cowbane were in the same nest box they used in 2005. One male was recaptured at the Godfrey farm and moved 25 m, or one territory, from the box used in 2005. The remaining two males were recaptured on the Whitescarver farm; one moved

TABLE 20

NUMBER OF TERRITORIES AND DISTANCE MOVED FROM THE NEST BOX USED IN 2005 BY RETURNING ADULT FEMALES.

		N	median	range	W	p
Territories moved	Contaminated	7	2.0	1.0 - 21.0	42.0	0.61
	Reference	5	4.0	1.0 - 13.0		
Distance (m)	Contaminated	7	50.0	25.0 - 525.0	42.0	0.61
	Reference	5	100.0	25.0 - 325.0		

only 25 m from the box used in 2005, the other used a nest box 14 territories (~350 m) away, to a new box erected in 2006.

There was no difference between contaminated or reference areas in the proportion of adult female birds that returned to the same site used in 2005 (χ^2=0.004, DF=1, p=0.95). Sample sizes were too small to compare the number of females that used the same nest box as in 2005. Movement within a site did not differ between the contaminated and reference areas (Table 20). The small sample size of returning males or nestlings precluded analysis.

Impact of individual mercury level on distance moved within a site

I compared the amount of mercury in the blood of adult tree swallows (male and female) in 2005 to the distance they moved in choosing a 2006 nest site. There was no relationship between mercury level in 2005 and distance moved (F=0.72, R^2=0.04, p=0.41; Fig. 27). Within the contaminated areas along the South River, adults with higher mercury did not nest farther away from the nest box used in 2005 than birds with lower mercury (R^2=0.02, F=0.14, p=0.71). As mercury values were only available for one individual in the contaminated areas that returned to the same nest box, no comparison could be made regarding mercury levels of individuals that moved versus those that returned to the same box.

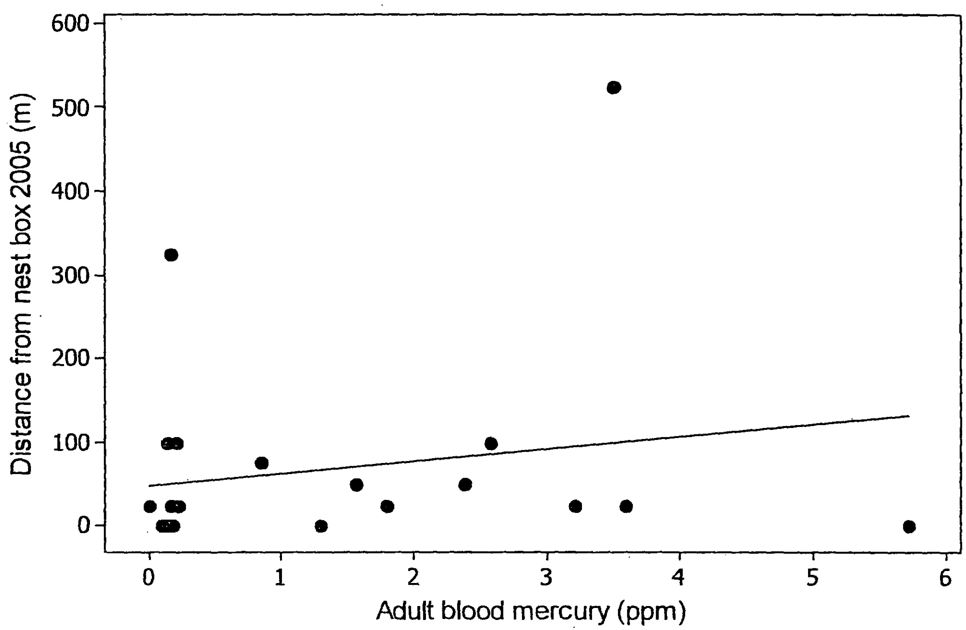
DISCUSSION

As part of a continuing study on the effects of mercury on the nesting success of tree swallows along the South River, I collected data on the return rate and site fidelity of birds banded in 2005. Mercury levels in the blood of adult tree swallows nesting in

FIGURE 27

RELATIONSHIP BETWEEN ADULT MERCURY LEVELS IN 2005

AND DISTANCE MOVED WITHIN A SITE



contaminated areas in 2005 were an order of magnitude higher than birds nesting in reference areas (see Chapter 1 for details). Although I was unable to detect any impacts of mercury on the nesting success of tree swallows in 2005, mercury accumulation on the breeding grounds could have had significant neurological or physiological impacts on adult birds not detectable during the breeding season. As tree swallows are highly philopatric (De Steven 1980, Robertson et al. 1992, Winkler et al. 2004), I used the return rate of birds to the study site in 2006 as an estimate of short-term survivorship. I predicted that swallows banded in contaminated areas would have lower return rates than birds from reference areas. To ensure equal detection of birds in contaminated and reference areas, I compared the dispersal distances of the adult birds to verify the validity of the return rate measure. Contrary to my predictions, adult tree swallows banded in contaminated and reference areas returned to the study site at similar rates. I did not detect any differences in nest box fidelity among birds that returned; birds banded in contaminated areas moved similar distances within a study site to birds from references areas and so detection of returning birds was equal in both treatment groups.

Impact of mercury on survivorship in birds

There are few published reports on the impacts of mercury on the survivorship, or return rate, of free-living birds. Conducting a study on the impacts of pollutants on survivorship requires two things, 1) the study must employ non-lethal sampling, and 2) the individuals used in the study must be banded (or otherwise uniquely marked) to allow for later identification. Additionally, using a species that will return to the study site in subsequent years (high philopatry) is important for detection. Cavity nesting birds, such as tree swallows, are ideal for studies on the impacts of contaminants on survivorship as they are unlikely to remain undetected within the study area.

There are few populations of free-living birds with a large enough proportion of uniquely marked individuals to consider the impacts of mercury on long term survival (Thompson et al. 1991). Several field studies on the uptake and impacts of contaminants in cavity-nesting birds have occurred across multiple years; however, the authors do not mention whether or not they banded their birds or attempted to determine recapture rates (Eens et al. 1999, Gerrard and St. Louis 2001, Adair et al. 2003, Custer et al. 2003, Janssens et al. 2003, Custer et al. 2006). In 1988, one study color marked 120 adult great skuas (*Catharacta skua*) in a mercury-contaminated area in Scotland (Thompson et al. 1991). The following year, 72% of the marked birds returned to the study area to breed, 10% returned but did not breed, and the remaining 18% did not return. Great skuas with higher mercury levels in 1988 were as likely to return and breed in 1989 as birds with low mercury levels (Thompson et al. 1991). Mercury levels in the feathers of the adult skuas used in this study averaged 7.0 ppm (fresh weight, ±5.1 SD) (Thompson et al. 1991). A study on common loons in northwestern Ontario suggested that brain mercury residues >2.0 ppm may have led to higher rates of nest desertion and decreased incubation success causing birds to abandon their territories (Barr 1986). Further, the author suggested nervous system dysfunction due to increased mercury levels may have led to reduced territorial drive which could also lead to abandonment of breeding territories (Barr 1986). This study employed lethal sampling and therefore it is unknown whether the adults that abandoned their territories in the spring would have returned the following year.

The 2003 study of PCB contamination in tree swallows along the Housatonic River in Massachusetts did employ non-lethal sampling and the birds were banded to allow for observation of return rates (C. Custer, pers. comm.). This part of the PCB study, currently unpublished, found that the return rate of adult tree swallows with high levels of PCB was higher or the same as the return rate of birds with lower levels of PCB (C. Custer, pers. comm). These findings are similar to those of Thompson et al. (1991) which found great skua return rate to be similar in birds with high and low mercury values. Like these studies, I also did not detect any difference in return rate between swallows in contaminated and reference areas (high and low mercury levels) after one year.

Return rate of tree swallows

If mercury had an impact on the return rate of tree swallows along the South River, the overall return rate of birds from my study would be lower than average return rates found in locations free of pollutants. Because I did not detect any differences in return rate between birds banded in contaminated and reference areas, I decided to combine birds from all sites to compare the return rate of tree swallows in the Shenandoah Valley with return rates reported in other studies. Of the 56 adult female tree swallows banded in 2005, 29 (52%) returned to the study site in 2006. Only seven male tree swallows were recaptured in 2006 (44%); however, males were sampled only opportunistically in 2005 and therefore fewer were banded. Males also proved more difficult to capture in 2006 and the identity of the male was known at 56% of the nests, while the identity of the female was known at 100% of the nests. For this reason, all discussion of adult return rate hereafter refers to adult female birds only. This is consistent with the literature as female return rate is commonly used to estimate survival in field studies.

Adults The total proportion of recaptured birds at my study site (52%) fits within the range for adult female tree swallows of 13% to 60% reported in the literature (Table 21). In fact, the return rate of birds in my study was higher than in four out of six of the studies that reported detection rate after one year. Two long-term banding studies reported average survivorship (based on recapture) of adult tree swallows to be near 40% (Chapman 1955, Houston and Houston 1987). As banded birds were often detected for the first time two to three years after banding, long term studies such as these allowed for the detection of birds across multiple years to calculate an overall recapture/survival rate.

Using the average 40% single-year recapture/survival rate I was able to compare the return rate of adult tree swallows in my study to the findings of these long term studies. Of the 56 birds banded in 2005, a 40% return rate would have predicted approximately 23 individuals returning for the 2006 breeding season. The return rate of tree swallows nesting in the Shenandoah Valley from 2005 to 2006 was 52%, or 29 individuals. The return rate of adult swallows in the Shenandoah Valley did not differ significantly from the predicted 40% return rate from long term studies (Table 22, χ^2=1.29, DF=1, p=0.26). This is the first time return rate has been estimated for a population of tree swallows in the southern United States; additional years of recapture are needed to determine whether or not differences in survivorship exist between northern and southern breeding populations.

Nestlings In 2005, a total of 369 banded nestlings were fledged from the study site. Of these nestlings, only 10 (2.7%) returned to the study site in 2006. In a synthesis

TABLE 21

PUBLISHED RECAPTURE RATES OF TREE SWALLOWS

	% recaptured after 1 year			
Location	**Nestlings**	**Adults**	**# years**	**Reference**
New Jersey	3.1[†]	39.6	15	Chapman (1955)
Saskatchewan	0.8	12.8	17	Houston and Houston (1987)
Pennsylvania	5.4*	51.3*	7	Stahura (1982)
Alberta	1.3	13.7	-	Pinel (1980)
Massachusetts	12.0	34.0	5	Low (1933)
Ontario	-	62.5	1	De Steven (1980)

*these values represent the overall return rates over the five years of the study rather than per year.

[†] Includes birds that returned to the natal study area as well as birds detected by another researcher in a nearby study area.

TABLE 22

CHI-SQUARE* TABLE: RECAPTURE RATE IN THE SHENANDOAH VALLEY COMPARED TO 40% AVERAGE SURVIVORSHIP

	Recaptured	**Undetected**	**Total**
Observed	29	27	56
Expected	23	33	56
Total	52	60	112

* $\chi 2$=1.29, DF=1, p=0.26

of band recoveries of nestling tree swallows from 1929-1985, the estimated mortality rate was calculated to be 79.1% in the first year of life, or 20.9% survival (Butler 1988). Therefore, return rates are often low due to decreased survivorship and difficulties in detecting returning nestlings if they do not nest. Reported return rates of nestling birds to the study site one year later range from 0.8% to 12% (Table 21). The return rate of nestling swallows to the study site after one year in the Shenandoah Valley was significantly higher than reported by Houston and Houston (Saskatchewan, 0.8%, 1987) (χ^2=3.84, DF=1, p=0.05); and was similar to the average return rate of nestlings found by Chapman (New Jersey, 3.1%, 1955) (χ^2=0.003, DF=1, p=0.95).

Estimating survivorship from recapture rate

Detection of returning adults In order for a bird to be recaptured, the researcher(s) must be able to detect the bird on the study site. Detection can be influenced by several factors including weather patterns, changes in census techniques between/among years, and the number of years the study has taken place. One study used the program MARK to predict the recapture rate of adult birds returning to their study site in Saskatchewan. They found that based on changing weather conditions and census efforts, program MARK predicted recapture as low as 20% in a cold, rainy year and as high as 85% under normal weather conditions and intensified, systematic capture (Shutler and Clark 2003). The percent of adult and nestling birds recaptured after one year by Houston and Houston (1987) was lower than in my study (12.8% for adults). However, it was noted by the authors that their sampling was at times inconsistent due to changes in the number and skill level of researchers working each year. Their long-term recapture rate (approximately 40%) was similar to mine as well as the rate determined by

Chapman (1955) as multiple years of the study allowed birds missed one year to be spotted in a subsequent year. If a bird nested on my study site, the chance that it went undetected was extremely low. Only the status (banded or unbanded) of birds that abandoned their nests before they hatched (n=4) was unknown. However, there may have been a number of surviving birds that returned to the study site but did not nest. These may appear in 2007 or future years, increasing the estimate of survivorship (see below).

Detecting returning nestlings Long-term studies are also necessary when discussing nestling survivorship as young birds may not appear in a nest box on the study site until several years later (Butler 1988, Shutler and Clark 2003). While a number of nestlings may return to their natal study area (for example, the South River), they may not return to the same nest site (for example, Basic Park), on average breeding 5-40 km away (Robertson et al. 1992). In Colorado, approximately 77% of the nestlings banded from 1976-1987 were recaptured outside of the natal study area by researchers in other study areas (within 30 km of the natal study area) (Cohen 1989). As natal dispersal is greater than breeding dispersal (Shutler and Clark 2003) many nestlings are not recaptured one year later because they return as floaters or disperse beyond the boundary of the study site, only moving closer in subsequent years. Of 203 recruits reported by Shutler and Clark (2003), 40% were detected one year after banding, 39% two years after, 17% three years after, and the remaining 4% were not detected until four or more years later.

SY female tree swallows may go undetected upon their return if they remain floaters due to competition for nest sites. Nest box availability upon the return of young birds could impact their detection rate by potentially decreasing the number of floaters. The previously discussed studies of tree swallow return rate did not mention providing additional nest boxes to recruit the returning nestlings. Successful adult tree swallows are highly philopatric; in one study 33% of females and 66% of males used the same nest box as the previous year and another 15% of females and 22% of males returned to a box within 100 m (Roberston et al. 1992). Therefore, if the adults return to the same box, or another box close by, only a small proportion of nest boxes may be available to recruits. The number of vacant boxes in a study site could influence the number of nestlings that return as floaters and go undetected. In my study, it is hard to say if the addition of nest boxes to the study site increased the detection of returning nestlings, especially because this was the first year for birds to return. As nestlings typically return to the study site later than older birds (Robertson et al. 1992), the large number of unbanded adults on the study site in 2006 may have usurped nest boxes erected to recruit juveniles. Thus, to use return rate as an index of survivorship for birds banded as nestlings, the age-related dispersal and migratory patterns of the study species must be considered to overcome bias against undetected young birds (Butler 1988).

Site fidelity and detection

Breeding dispersal distance could also influence the detection of adult birds. Though a large proportion of adult tree swallows return within 100 m of their nest box (48% females and 88% males, Robertson et al. 1992), any long distance dispersal could cause an individual to move off of the study site. Longer dispersal distances might occur due to nest site competition; more birds arrive at a study site than there are boxes and so not all birds will be successful at claiming a nest box. Returning birds with higher body burdens of mercury may not have been able to compete with new birds (lower body burdens) for the limited number of available nest boxes. Any impacts of mercury on memory could affect territoriality or site fidelity.

Movement within a study site The objective of this part of the study was not to compare dispersal distances of tree swallows in the Shenandoah Valley with dispersal in other populations, but to determine if there was an equal chance of detecting birds returning from contaminated and reference areas. Although I did not detect a difference in the return rate of adult birds banded in contaminated or reference areas, if birds in one treatment group were moving greater distances than the other, it could have influenced my ability to detect them. All adult birds returned to sites on the same river as in the previous year, approximately 80% of returning females were detected at the same site as in 2005. Only 18% of adult birds returned to the exact box used in 2005; box fidelity reported from other studies ranges from 21% to 66% (Robertson et al. 1992, Shuter and Clark 2003). To control for any effects of dispersal distance on detection, I compared the distance moved within a site from the nest box used in 2005 for birds in contaminated and reference areas. For females that returned to the same location used in the previous year, the distance from the nest box an individual moved ranged from 0-21 territories (525 m) in contaminated areas and 0-13 territories (325 m) in the reference areas, the difference in movement between treatment groups was not significant.

There was also no relationship between the distance an individual moved and their blood mercury level in 2005 ($p=0.41$); the adult female with the highest mercury in 2005 returned to the exact nest box she used the previous year. Overall, an individual's exposure to mercury in 2005 did not appear to impact their site fidelity as determined by the distance moved from the previous nest box. Because the distance moved by females returning from contaminated and reference areas did not differ, the chance of an individual being undetectable (outside of the study area) was equal for both treatment groups.

Conclusion

Adult female tree swallows nesting in contaminated areas in 2005 returned to the study area in 2006 at similar rates as birds from reference areas. Upon their return, the distance moved from the nest box used in 2005 was similar for birds in contaminated and reference areas. After one year, the return rate of adult and nestling tree swallows in the Shenandoah Valley was similar to the average return rates of other tree swallow populations in Canada and the northern United States. However, additional years of studies are needed to determine possible annual variation in return rate and to estimate the survivorship of birds in this population. Further study is especially needed to estimate survivorship of nestling birds as they may not return to breed (be detectable) until several years later. The average lifespan of tree swallows ranges from 2.7-8.0 years (Butler 1988); however, as body burdens of mercury increase with age in many species (Evers et al. 2005) the overall survivorship of birds along the South River may be lower than in reference areas during the swallows' later years.

Mercury levels in 2006 were twice as high as in 2005 and returning birds were exposed to increased amounts of mercury compared to the previous year. Significant differences between the nesting success in contaminated and reference areas were detected in 2006; SY females in contaminated areas experienced decreased nesting success compared to ASY and SY females in contaminated and reference areas. It is possible that SY females in contaminated areas were in poor condition and may return at lower rates than ASY females. Along the South River, annual variation in mercury availability may have a significant impact on the survival of tree swallows and continuing recapture efforts could lead to the discovery of differential survivorship between the female age classes.

APPENDIX A1

GPS POINTS OF UPSTREAM AND DOWNSTREAM-MOST NEST BOXES AT EACH SITE

Site	River	River mile	Upstream		Downstream	
Basic Park	South	1.8	38.08385262	-78.87560256	38.08622791	-78.87688734
Water Treatment Plant	South	1.8	38.08063568	-78.87235900	38.08314095	-78.87418200
Genicom	South	2	38.09211951	-78.87728301	38.09546095	-78.87647506
Dooms river crossing	South	5.2	38.10749398	-78.86301443	38.10812636	-78.86254917
Crimora river crossing	South	9.9	38.15590187	-78.85465083	38.15609293	-78.85443987
Augusta Forestry Center	South	11.5	38.1644371	-78.85986967	38.17383224	-78.85447236
Wampler property	South	13	38.19368333	-78.85113333	38.19580000	-78.85020000
Harriston river crossing*	South	14	38.21765000	-78.83688333	38.21765000	-78.83688333
Rankin property	South	16.4	38.22853512	-78.83403009	38.23197544	-78.83611636
Grand Caverns	South	19.9	38.25859094	-78.83144728	38.26105667	-78.83134771
Grottoes City Park	South	22.3	38.28054410	-78.83650556	38.28432745	-78.83426600
Bradburn Park	South	23.7	38.29407161	-78.81237571	38.29417325	-78.81184894
Port Republic	SFSR	24.1	38.29756771	-78.80494370	38.29837997	-78.80290204
Sheets farm*	SFSR	31	38.20317000	78.43918000	38.20317000	-78.43918000
Merck Plant	SFSR	37	38.38942354	-78.65595131	38.38615000	-78.63760000
Cowbane Nature Preserve	South	-13.7	38.02410189	-79.06346862	38.01972980	-79.05660700
P Buckley Moss Barn	South	-4.7	38.03993333	-78.93806667	38.04255000	-78.92791667
Locust Street	South	-1.7	38.06084590	-78.90293380	38.06283867	-78.90367281
Ridgeview Park	South	-1.5	38.06417323	-78.90533958	38.06417323	-78.90533958

(continued)

* only one GPS point was available for this site.

APPENDIX A1 (continued)

GPS POINTS OF UPSTREAM AND DOWNSTREAM-MOST NEST BOXES AT EACH SITE

Site	River	River mile	Upstream		Downstream	
Whitescarver Farm	Middle	-	38.13280100	-79.21773400	38.138929000	-79.22052700
Godfray Farm	Middle	-	38.15257300	-79.20716500	38.150309000	-79.20370800
Smith's Pond	Middle		38.15024500	-79.21827100	38.152637000	-79.21097000
Fort River Road	Middle		38.22978700	-79.01229800	38.228638000	-79.00998400
Dories/Middle River Rd	Middle		38.23133500	-79.09412500	38.237554000	-79.08587300
Shapcot property	Middle		38.24602100	-79.06211200	38.245172000	-79.06208000
Crawford annex	North		38.38514500	-78.98881100	38.380018000	-78.98134700
Flora property	North		38.38683800	-79.03716400	38.386699000	-79.03568200
Sandy Bottom Park	North	-	38.36947900	-78.96675000	38.369542000	-78.96540400
Rt. 276 river crossing	North	-	38.30610000	-78.89335000	38.305350000	-78.89213300

APPENDIX A2

PROPERTY DESCRIPTIONS

Site	River	Year used	City	County	Property type
Basic Park	South	2005-2006	Waynesboro	Augusta	Public city park
Water Treatment Plant	South	2005-2006	Waynesboro	Augusta	Waste treatment facility
Genicom	South	2005-2006	Waynesboro	Augusta	Hay field
Dooms river crossing	South	2005-2006	Dooms	Augusta	Cow pasture
Crimora river crossing	South	2005-2006	Crimora	Augusta	Public city property
Augusta Forestry Center	South	2005-2006	Crimora	Augusta	Open, cut grass habitat
Grand Caverns	South	2005-2006	Grottoes	Rockingham	Public city park
Grottoes City Park	South	2005-2006	Grottoes	Rockingham	Public city park
Wampler property	South	2006	Crimora	Augusta	Cow pasture
Rankin property	South	2006	Grottoes	Augusta	Residential property
Harriston river crossing	South	2006	Harriston	Augusta	Open field
Bradburn Park	South	2006	Grottoes	Rockingham	Public park
Port Republic	SFSR	2005	Port Republic	Rockingham	Residential properties (2)
Sheets farm	SFSR	2005	Island Ford	Rockingham	Cow pasture
Merck Plant	SFSR	2005	Elkton	Rockingham	Open, field habitat
Cowbane Nature Preserve	South	2005-2006	Stuart's Draft	Augusta	Undisturbed, open and marshy habitat
Ridgeview Park	South	2005-2006	Waynesboro	Augusta	Public city park
Locust Street	South	2005-2006	Waynesboro	Augusta	Residential properties (4)
P Buckley Moss Barn	South	2006	Waynesboro	Augusta	Residential property

(continued)

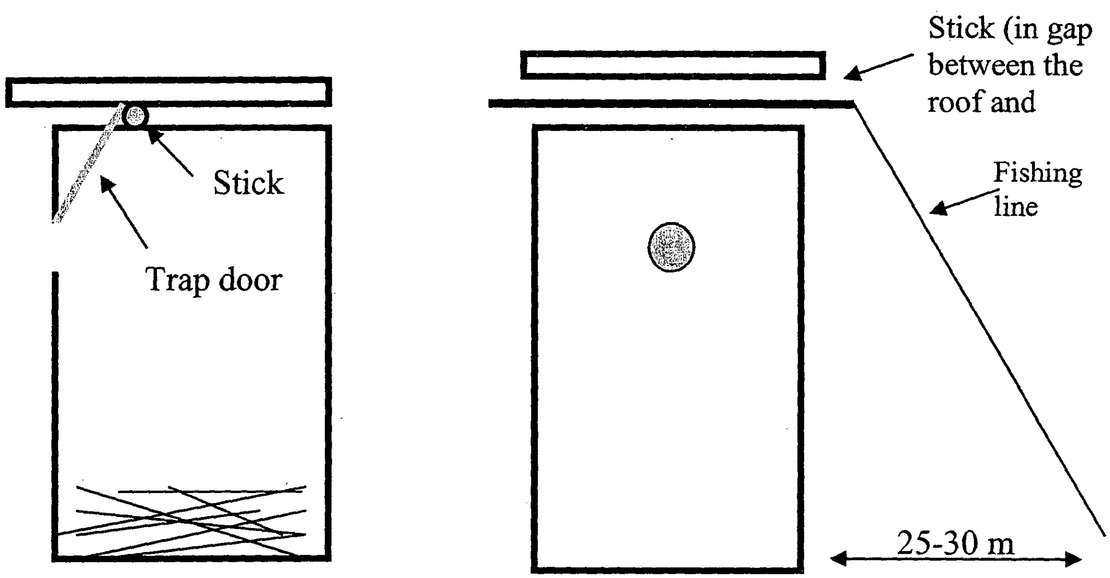
APPENDIX A2 (continued)

PROPERTY DESCRIPTIONS

Site	River	Year used	City	County	Property type
Whitescarver Farm	Middle	2005-2006	Swoope	Augusta	Cow pasture
Godfray Farm	Middle	2005-2006	Swoope	Augusta	Undisturbed pasture
Smith's Pond	Middle	2005-2006	Swoope	Augusta	Cut-grass field
Fort River Road	Middle	2005-2006	Verona	Augusta	Residential properties (4)
Dories property/Middle River Rd	Middle	2005-2006	Franks Mill	Augusta	Residential property
Shapcot property	Middle	2005-2006	Spring Hill	Augusta	Residential property
Auckerman property	North	2005-2006	Bridgewater	Rockingham	Residential property
Crawford annex	North	2005-2006	Bridgewater	Rockingham	Residential property/ Public city park
Flora property	North	2005	Bridgewater	Rockingham	Residential property
Sandy Bottom Park	North	2006	Bridgewater	Rockingham	Public Park/ Par 3 golf course
Rt. 276 river crossing	North	2006	Weyers Cave	Rockingham	Undisturbed river bank, corn field

APPENDIX B

MODIFIED NEST BOX TRAP DESIGN



Side-view (box interior)

Front-view (exterior)

APPENDIX C1

AVERAGE ADULT MERCURY LEVELS

a) Adult mercury levels by year and treatment group (sexes combined)

Year	C or R	N	Mean	SD	Min	Max
2005	C	26	2.28	1.38	0.00	5.72
2005	R	37	0.19	0.20	0.01	1.29
2006	C	49	4.44	2.50	0.80	11.90
2006	R	50	0.15	0.06	0.07	0.40

b) Adult mercury levels by year, treatment group, and sex

Year	C or R	Sex	N	Mean	SD	Min	Max
2005	C	F	19	2.29	1.48	0.002	5.72
2005	C	M	7	2.26	1.14	0.003	3.37
2005	R	F	29	0.20	0.22	0.01	1.29
2005	R	M	8	0.18	0.11	0.08	0.44
2005	SFSR	F	18	1.18	0.35	0.01	1.73
2005	SFSR	M	2	1.10	0.53	0.73	1.48
2006	C	F	39	4.14	2.53	0.80	11.90
2006	C	M	10	5.59	2.14	1.54	9.17
2006	R	F	39	0.16	0.06	0.07	0.40
2006	R	M	11	0.15	0.07	0.09	0.31

c) Adult mercury levels in 2006 by treatment group, sex, and recapture status*

C or R	Sex	Recap?	N	Mean	SD	Min	Max
C	F	N	9	3.77	1.71	1.50	6.20
C	F	Y	12	5.70	3.34	1.61	11.90
C	M	N	9	5.87	2.07	1.54	9.17
C	M	Y	1	3.10	-	3.10	3.10
R	F	N	11	0.15	0.03	0.09	0.19
R	F	Y	18	0.14	0.04	0.07	0.21
R	M	N	6	0.18	0.08	0.10	0.31
R	M	Y	3	0.11	0.02	0.09	0.12

*Recapture status: Y = recaptured bird banded in 2005, N = bird new to study site in 2006

APPENDIX C2

AVERAGE FEMALE BLOOD MERCURY LEVELS BY YEAR, TREATMENT GROUP, AND AGE CLASS

a) Female mercury levels by treatment group and age class (2005 and 2006)

C or R	Age	N	Mean	SD	Min	Max
C	ASY	24	4.64	2.77	1.50	11.90
C	SY	33	2.78	1.76	0.002	7.36
R	ASY	38	0.17	0.19	0.01	1.29
R	SY	29	0.17	0.04	0.10	0.27

b) Female mercury levels by year, treatment group, and age class

Year	C or R	Age	N	Mean	SD	Min	Max
2005	C	ASY	3	3.00	1.06	2.38	4.22
2005	C	SY	16	2.15	1.54	0.002	5.72
2005	R	ASY	9	0.27	0.39	0.01	1.29
2005	R	SY	20	0.17	0.04	0.10	0.25
2006	C	ASY	21	4.87	2.88	1.50	11.90
2006	C	SY	17	3.36	1.79	0.80	7.36
2006	R	ASY	29	0.14	0.03	0.07	0.21
2006	R	SY	9	0.18	0.04	0.12	0.27

APPENDIX D1

NESTING SUCCESS BY YEAR AND TREATMENT GROUP

Parameter	Year	C or R	N	Mean	SD
Clutch size	2005	C	27	5.56	0.85
	2005	R	46	5.80	0.83
	2006	C	67	5.76	0.89
	2006	R	98	5.80	0.92
Proportion eggs hatched	2005	C	26	0.93	0.09
	2005	R	45	0.93	0.17
	2006	C	67	0.76	0.28
	2006	R	98	0.87	0.22
Proportion nestlings fledged	2005	C	26	0.91	0.17
	2005	R	44	0.91	0.24
	2006	C	60	0.83	0.31
	2006	R	93	0.95	0.16
Proportion eggs fledged	2005	C	26	0.85	0.17
	2005	R	44	0.86	0.25
	2006	C	60	0.69	0.30
	2006	R	93	0.86	0.22
Number fledglings	2005	C	26	4.65	1.09
	2005	R	44	5.05	1.66
	2006	C	60	4.00	1.89
	2006	R	93	5.06	1.48

APPENDIX D2

NESTING SUCCES BY YEAR, TREATMENT GROUP, AND FEMALE AGE

Parameter	Year	C or R	Age	N	Mean	SD
Clutch initiation date	2005	C	ASY	3	6.33	3.51
	2005	C	SY	12	10.67	1.87
	2005	R	ASY	6	6.83	2.14
	2005	R	SY	18	14.56	10.33
	2006	C	ASY	33	15.61	8.54
	2006	C	SY	28	23.86	9.20
	2006	R	ASY	58	15.90	6.29
	2006	R	SY	28	21.75	7.93
Clutch size	2005	C	ASY	3	5.67	0.58
	2005	C	SY	12	5.75	0.75
	2005	R	ASY	6	6.50	0.55
	2005	R	SY	18	5.56	0.86
	2006	C	ASY	33	6.03	0.81
	2006	C	SY	28	5.57	0.92
	2006	R	ASY	58	6.03	0.72
	2006	R	SY	28	5.43	0.96
Proportion eggs hatched	2005	C	ASY	3	0.94	0.10
	2005	C	SY	12	0.92	0.10
	2005	R	ASY	6	0.97	0.07
	2005	R	SY	18	0.92	0.24
	2006	C	ASY	33	0.88	0.18
	2006	C	SY	28	0.76	0.19
	2006	R	ASY	58	0.92	0.15
	2006	R	SY	28	0.87	0.17
Proportion nestlings fledged	2005	C	ASY	3	0.78	0.39
	2005	C	SY	12	0.91	0.15
	2005	R	ASY	6	0.92	0.09
	2005	R	SY	17	0.93	0.24
	2006	C	ASY	33	0.92	0.17
	2006	C	SY	26	0.72	0.40
	2006	R	ASY	57	0.98	0.06
	2006	R	SY	27	0.94	0.12

(continued)

APPENDIX D2 (continued)

NESTING SUCCES BY YEAR, TREATMENT GROUP, AND FEMALE AGE

Parameter	Year	C or R	Age	N	Mean	SD
Proportion eggs fledged	2005	C	ASY	3	0.78	0.39
	2005	C	SY	12	0.84	0.15
	2005	R	ASY	6	0.89	0.13
	2005	R	SY	17	0.90	0.25
	2006	C	ASY	33	0.81	0.22
	2006	C	SY	26	0.54	0.33
	2006	R	ASY	57	0.90	0.16
	2006	R	SY	27	0.83	0.21
Number fledglings produced	2005	C	ASY	3	4.33	2.08
	2005	C	SY	12	4.83	1.11
	2005	R	ASY	6	5.83	1.17
	2005	R	SY	17	5.06	1.68
	2006	C	ASY	33	4.85	1.42
	2006	C	SY	26	2.96	1.93
	2006	R	ASY	57	5.49	1.21
	2006	R	SY	27	4.48	1.31

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