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Survival of Adult Tree Swallows (*Tachycineta bicolor*) at a Site Contaminated by Mercury

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SURVIVAL OF ADULT
TREE SWALLOWS (*Tachycineta bicolor*) AT A SITE CONTAMINATED BY
MERCURY

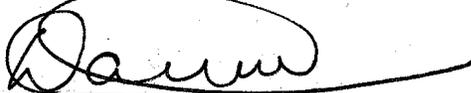
A thesis submitted in partial fulfillment of the requirement
for the degree of Bachelors of Science in Biology from
The College of William and Mary

by

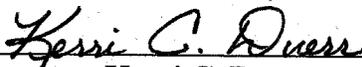
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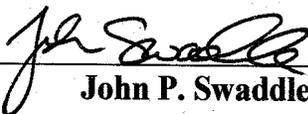
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Williamsburg, VA
May 6, 2009

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ABSTRACT

Mercury is a heavy metal that has become a ubiquitous contaminant in aquatic and terrestrial ecosystems worldwide, primarily as a result of human activity. While several studies have documented the serious physiological and neurological impairments caused by mercury exposure in birds, few have attempted to examine the effects of mercury on fundamental demographic parameters such as survival and reproductive success. The short time frame in which many ecotoxicological studies are undertaken cannot capture long-term changes in population structure and dynamics. To address these shortcomings, I conducted a multi-year mark-recapture study (2005-2008) to examine whether adult Tree Swallows (*Tachycineta bicolor*) breeding along the contaminated South River in Virginia, USA suffer reduced annual survival as a result of exposure to mercury. Over the course of the study, I individually marked 932 swallows and monitored their presence on our breeding sites in each year. I used Cormack-Jolly-Seber models for live recaptures of marked individuals to estimate apparent survival and probability of recapture. I evaluated 11 *a priori* linear models representing my hypotheses about the potential factors affecting survival, including sex, mercury treatment level, and mercury exposure for each individual. Three models containing univariate effects of these variables were supported in the data; the most parsimonious model assumed constant survival over time. Overall, survival declined with increasing individual mercury levels, although the effect of mercury exposure was not statistically significant. Post-hoc analyses suggested that females breeding on contaminated sites tended to exhibit reduced survival at older life stages relative to swallows breeding on reference sites. Because the number of individuals surviving to these older life stages is relatively small, it may be difficult to detect similar age-related differences in population-wide analyses of survival. In some passerine species, reproductive potential increases with age. Thus, reduced survival of the oldest birds could have serious consequences for population dynamics of songbirds in contaminated areas. Future research should attempt to integrate multiple demographic parameters and life history traits to effectively address how contaminants, such as mercury, may affect population dynamics of avian species over longer time scales than have traditionally been considered.

CHAPTER 1: INTRODUCTION AND STUDY APPROACHES

THE NATURAL HISTORY OF MERCURY

Chemical Forms

Mercury is a heavy metal which, in its elemental form, is a shiny, silvery white liquid at standard temperature and pressure. Although mercury is a naturally occurring substance, it has no known biological function, and can thus be considered potentially hazardous to any cells in which it is present (USNAS 1978, Eisler 2006). Mercury commonly exists in three oxidation states: 0 (elemental mercury), +1 (mercurous ion), and +2 (mercuric ion), all of which are capable of forming a variety of organic and inorganic compounds.

Although elemental mercury is relatively inert at room temperature (Anon. 1948b), there are a number of processes through which it is readily transformed into more reactive, and potentially toxic, species. Understanding factors influencing speciation is particularly important for two reasons. First, mercury is differentially toxic to organisms depending on its chemical form prior to and following exposure (UNEP 2002). For example, methylmercury, in addition to being the most common organic mercury compound in the environment, is also among the most toxic to wildlife due to its high stability, lipid solubility, and membrane permeability (Beijer and Jernelov 1979, Hamasaki et al. 1995).

The toxicity of a specific mercury compound depends largely on mechanisms of accumulation, bio-modification and detoxification within various target tissues, all of which are greatly influenced by chemical speciation (UNEP 2002). Second, speciation exerts a strong effect on the transport of mercury within and among environmental compartments, such as the atmosphere and ocean (UNEP 2002). For example, airborne mercury adsorbed to particulate matter or existing as an ionic compound will tend to

move short distances, often settling near the point of emission. In contrast, elemental mercury vapor is transported on a global scale, often traveling thousands of miles before finally coming to rest (UNEP 2002).

Transformation and Cycling of Mercury

Elemental mercury is relatively inert in the presence of many gaseous substances at room temperature (Anon. 1948b). However, there are a number of environmental processes by which it is readily transformed to more reactive species. In air, the oxidation of elemental mercury is thought to occur fairly rapidly, with an atmospheric lifetime estimated to be on the span of a few months (Sommar et al. 2001, Ariya et al. 2002).

Once oxidized, atmospheric mercury compounds show increased solubility, decreased volatility, and more rapid rates of both wet and dry deposition (UNEP 2002). In soils, conditions are often favorable for the formation of a number of inorganic and organic complexes. Because much of the mercury in soils is bound to bulk organic matter, wash-out into aquatic ecosystems can occur when the mercury is attached to suspended soil or humus (UNEP 2002). The retention time for mercury in soils is estimated to be on the order of hundreds of years; thus, contaminated soils may serve as a particularly potent source of mercury to surface waters and other media (Pirrone et al. 2001).

In aquatic environments, the chemical processes underlying speciation may be significantly more complex than in air or soil, and exactly which species form is dependent on a number of external factors. Of particular note are variables underlying the process of methylation. Methylmercury can be formed by both chemical and biological mechanisms, though biotic processes are thought to be much more common

(UNEP 2002). In particular, sulfate-reducing bacteria have often been implicated in the conversion of inorganic or elemental mercury to organic species. The efficiency of methylation depends on several factors, the most significant of which are microbial activity and amount of bioavailable mercury (Ullrich et al. 2001). These variables in turn are influenced by abiotic factors such as temperature, pH, redox potential, nutrient content, suspended sediment load, sedimentation rates, and the presence of organic and inorganic complexing agents, among others (USNAS 1978, Compeau and Bartha 1984, Berman and Bartha 1986, Callister and Winfrey 1986, Jackson 1986, Ullrich et al. 2001). In addition, certain bacteria are capable of reversing the methylation process (Marvin-DiPasquale et al. 2000, Bailey et al. 2001). These demethylating microbes are widespread in the environment (Clarkson et al. 1984) and may limit the continual build-up of methylmercury (Marvin-Disquale et al. 2000, Bailey et al. 2001).

The total amount of mercury present in global reservoirs is estimated at 334.17 billion metric tons (Clarkson et al. 1984). Because mercury is an element, it cannot be broken down into less harmful constituent parts. Thus, the total amount of mercury on Earth neither increases nor decreases; rather, the mercury simply cycles between global compartments and among chemical forms. Of the 334.17 billion tons of mercury in global reservoirs, 98.75% is contained within oceanic sediments (Clarkson et al. 1984). Other prominent reservoirs of mercury include oceanic waters (1.24% of total) and soils (0.0063% of total). Despite their relative centrality in ecotoxicological studies, living aquatic organisms account for only 7 metric tons of the total mercury present in the biosphere (Clarkson et al. 1984).

From these reservoirs, mercury can be released in a number of forms, most notably as a gas, in lava, in solution, or in particulate form (Eisler 2006). Following release, atmospheric transport occurs, after which time the mercury is deposited back onto land or water at some distance from its original source (Eisler 2006). The exact distance traveled and ultimate location for deposition is dependent on a variety of factors. For example, the chemical form of the mercury upon release strongly influences atmospheric dispersal: elemental mercury vapor is capable of being transported tens of thousands of kilometers, while Hg (+2) moves only a few hundred kilometers at most (Schroeder and Munthe 1998). The potential for long-range transport is evidenced by samples from locations such as the high Arctic, where actual mercury levels far exceed those predicted from local emissions. Rather, much of the mercury in the high Arctic is a result of activity in Europe, Russia, and North America (AMAP 1998).

Sources of Mercury

Natural Sources. Mercury is a naturally-occurring element which, despite having large oceanic and sedimentary repositories, has an average crustal abundance of only 0.05 ug/g (UNEP 2002). More than 25 mercury-containing minerals are known to occur in Earth's mantle, the most common of which, cinnabar (HgS), has been in continual human use for 2300 years (Schroeder and Munthe 1998). This mercury is released naturally from a wide variety of sources and by several different geochemical processes. One of the largest natural sources of mercury emissions is the degassing of mercury from the surface of the planet; this process alone is estimated to account for 30,000 tons of mercury emitted annually (Clarkson et al. 1984). Interestingly, a large amount of the variance in

the release of mercury is thought to be attributable to primary production by oceanic phytoplankton, with greater amounts of planktonic activity yielding more significant volatilization events (Kim and Fitzgerald 1986). A second major source of natural emissions is volcanic activity, which releases mercury and a number of other metals into the atmosphere (Hinkley et al. 1999). Terrestrial vegetation may also play a significant role in mercury emissions by serving as a conduit for the efflux of mercury from soils to the atmosphere (Leonard et al. 1998a). Such plant-mediated releases are influenced by many factors, including air temperature, irradiance, soil mercury concentrations, and leaf area (Leonard et al. 1998b). Finally, it should be noted that many of the biological and chemical processes involved in the transformation of mercury from one species to another may also play a critical role in the natural emission of mercury. For example, mercury bound to particulate matter in soil, where it is relatively unavailable to the surrounding ecosystem, may be released during methylation by sulfate-reducing bacteria in the sediment (e.g. Sundolf et al. 1994); in the Florida Everglades, nearly all natural emissions are the result of biological and chemical transformations of mercury in the soil (Sundolf et al. 1994).

Anthropogenic Sources. Global anthropogenic emissions of mercury have increased 1.5- to 3-fold since pre-industrial times. In some industrial areas, local deposition rates are presently 2 to 10 times higher than 200 years ago (Lindqvist et al. 1984, Bergen et al. 1999). Anthropogenic sources of mercury fall under two broad categories: intentional use of mercury and incidental release through the mobilization of impurities in high-volume materials. Fossil fuels, particularly coal, are excellent examples of the latter, as

they contain trace amounts of mercury that are released during combustion processes. In fact, coal-fired power production is the single largest source of anthropogenic mercury, accounting for 75% of anthropogenic emissions worldwide (Pacyna and Pacyna 2002). Unfortunately, in addition to comprising the single largest source of environmental mercury in the United States, coal-fired power plants are also the only significant source of mercury that remains unregulated (Maas et al. 2004). An attempt was made to model the economic cost to the United States in lost intelligence resulting from mercury exposure attributable to coal-fired power plants (Trasande et al. 2005); the authors arrived at an estimate of \$8.7 billion annually. While such a model may be subject to a high degree of skepticism and scrutiny, it does serve to demonstrate the magnitude and the potential severity of the problems caused by atmospheric mercury.

In addition to the incidental release of mercury resulting from the mobilization of impurities in high-volume materials, there are also a number of intentional uses and processes which may contribute significantly to local and global mercury emissions. Elemental mercury is widely used in a number of commercial products including batteries, electric lighting, paint residues, fever thermometers, thermostats, pigments, dental amalgam, and special paper coating, among others (USEPA 1992). Not only are organisms directly exposed through the use of certain mercury-containing products (i.e. dental amalgams), but the disposal and incineration of such products as waste is a major source of mercury emissions to the atmosphere (UNEP 2002). In addition, elemental mercury readily forms alloys with a number of metals (Au, Ag, Pt, Pd) (Schroeder and Munthe 1998), and is thus often used as an amalgam in the mining of precious metals (UNEP 2002).

One final indirect pathway through which humans are capable of affecting the release of mercury to the environment is through changes in land use practices (UNEP 2002). The creation of farmlands, recent clear-cuttings and water reservoirs may all contribute substantially to total mercury emissions (UNEP 2002). In addition, such changes in land use can significantly alter the chemical speciation and hence the bioavailability of mercury; in one recent paper, the authors demonstrated that the creation of artificial reservoirs served to enhance microbial methylation and thus methylmercury concentrations in the surrounding wildlife (Gerrard and St. Louis 2001).

History of Human Use

Mercury has had a long and varied tradition in human history dating back more than 2000 years. As early as 430 BCE, humans in the Almadén district of Spain began mining cinnabar (HgS), the principal ore of mercury (Martínez-Cortizas et al. 1999) for use as a red pigment. Eventually, refining processes for the recovery of elemental mercury were discovered, and large-scale mercury mining began worldwide. Such mining practices are thought to have significantly contributed to widespread contamination of various environmental media; in fact, many of the wastes from these mines continue to emit mercury for decades or even centuries after mining operations have ceased (Gosar et al. 1997, Ferrara 1999, Turner and Southworth 1999, Ganguli et al. 2000, Hines et al. 2000, Rytuba 2000, Trip and Allan 2000, Covelli et al. 2001, reviewed in Wiener et al. 2003).

The desirability of elemental mercury increased between 1550 and 1930 with the discovery that mercury could be used as an amalgamator for the extraction of gold and

silver (Averill 1946). Thus, mercury mining increased in tandem with major periods of gold and silver mining, such as occurred during the California gold rush in the United States during the mid 1800s (Domagalski 1998, Alpers and Hunerlach 2000, Rytuba 2000). Although the use of mercury for this purpose has generally declined, recent decades have witnessed a resurgence in the mercury-amalgamation process in gold mining, particularly in so-called artisanal operations, which are small, highly dispersed operations in South America, Southeast Asia, China, and Africa, where governmental regulations are difficult to enforce (Lacerda 1997a, Lacerda 1997b, Lacerda and Salomons 1999, Heemskerk 2001, Kambey et al. 2001).

During this same period, mercury came into wide use in a number of additional capacities, most notably as a medicinal agent, and in the manufacture of mirrors and felt hats. In the hat-making industry, people began to recognize “mad hatter” syndrome, a condition in which affected workers displayed signs of tremors, excessive salivation, irritability, and excitement, symptoms that are clearly recognizable today as those associated with inorganic mercury poisoning (Anon. 1948a, Norton 1986, Eisler 2006).

In 1892, the process of producing chlorine and caustic soda from brine (sodium chloride) was developed (Paine 1994). In its initial stages of use, this chlor-alkali process was carried out principally using liquid mercury as a cathode in the electrolysis reaction (Paine 1994). This mercury-mediated process came into wide use in a number of industrial settings throughout the world and caused a substantial increase in the release of mercury to the biosphere (Paine 1994). As recently as 1968, 33% of the US demand for mercury was related to its use in the chlor-alkali process (USEPA 1980). Although approximately a half-dozen chlor-alkali plants are still in operation in the United States,

the obvious hazards associated with a mercury cathode have led to its gradual replacement by mercury-free components (Paine 1994).

In fact, this gradual phase-out of mercury in industrial applications is part of a much wider trend that began to take shape in the 1950s and 1960s, after several instances in which humans were poisoned by industrial and agricultural mercury, the most publicized of which occurred at Minamata Bay in Japan (Tsubaki and Irukayama 1977). Such events brought mercury pollution to the forefront of the political arena in the United States, Canada, and other industrialized nations, and several new efforts became focused on identifying and controlling the release of mercury into the environment (Lacerda 1997b, Turner and Southworth 1999). For example, in the United States, mercury is no longer used as a biocide in seed grain or in antifouling paints (USEPA 1980, Weiner et al. 2003). Due largely to the subsequent decrease in price and demand, most large-scale mercury mining operations were discontinued (Wiener et al. 2003); in fact, the last U.S. mine to produce mercury as its main product closed in 1990 (Jasinski 1995).

Despite recent efforts to curb the manufacture and use of mercury, and its consequent release to the environment, much of the damage that resulted from past emissions is difficult to reverse. Even at sites where no new mercury is being produced, a legacy of contamination may remain. Once released, mercury is capable of being recycled in the environment, undergoing a series of chemical transformations to move between both biotic and abiotic components of ecosystems. Thus, mercury often remains a serious problem long after the direct sources of release have been terminated. In fact, many of the chemical and physical properties that made mercury a valuable metal in industrial settings also make it one of the most difficult to contain and recover from the

environment (Turner and Southworth 1999, Wiener et al. 2003). Despite these apparent difficulties, the magnitude of the problem has received considerable international attention and future decades may offer new solutions to this ever-burgeoning problem. In fact, in February 2009, the United Nations Environment Programme announced plans for a global treaty to reduce mercury emissions worldwide by 2013 (UNEP 2009).

EFFECTS OF MERCURY ON WILDLIFE

Mercury Methylation in the Environment

The methylation of inorganic mercury to produce methylmercury is the most toxicologically important transformation of mercury, as methylation greatly enhances the bioavailability and toxicity of mercury to humans and wildlife (Wiener et al. 2003). In fact, methylation is often a necessary precursor to the entrance of mercury into a food web. Although mercury can be methylated through both biotic and abiotic processes, methylation by sulfate-reducing bacteria in anaerobic sediments and wetlands is thought to be most significant (Wiener et al. 2003). In order for methylation to occur, mercury must first cross the cellular membrane of a methylating bacterium as a neutral dissolved species (Benoit et al. 1999a, 1999b). Thus, the amount of inorganic mercury available for methylation is greatly influenced by the presence of other elements or ions that may be capable of complexing inorganic mercury, thereby making it more or less accessible to the methylating microbes (Benoit et al. 1999a, 1999b). For example, at certain concentrations, chloride and sulfide ions bind Hg (II) to create neutrally charged species which are more capable of crossing the bacterial cell membranes (Barkay et al. 1998, Benoit et al. 1999a). However, at high enough concentrations, chloride and sulfide ions

will form charged compounds, making the inorganic mercury less available for methylation (Wiener et al. 2003). Similarly, if mercury becomes bound to large molecules of particulate matter, it becomes unavailable for methylation (Rudd et al. 1983). Once inside the bacterial cell, inorganic mercury can be methylated through either enzymatic or non-enzymatic pathways, both of which are driven by the presence and activity of the methyl donor, methylcobalamine (Ridley et al. 1977, Wood 1984).

Bioaccumulation, Biomagnification, and Trophic Transfer

Although organisms may be exposed to airborne and terrestrial sources of mercury, the most common pathway through which exposure and subsequent bioaccumulation occurs is aquatic in origin. The major routes of mercury exposure for organisms in aquatic environments are through food, water, and sediment (Wiener et al. 2003). Because rates of mercury uptake tend to greatly exceed rates of elimination, bioaccumulation occurs (Huckabee et al. 1979). Although the majority of mercury present in surface waters and sediment exists as inorganic mercury, most of the mercury in the tissues of fish and higher trophic levels of aquatic food webs is methylmercury (Thompson and Furness 1989, Thompson et al. 1991, Kim et al. 1996, Scheuhammer et al. 1998, Evans et al. 2000). This apparent discrepancy can be resolved by the fact that methylmercury binds to tissues much more readily than inorganic mercury. In fact, Scheuhammer (1987) reported intestinal absorption of methylmercury to be nearly 100 percent; in contrast, absorption of inorganic mercury was limited to a few percent. The absorption and selective retention of methylmercury in tissues can lead to concentrations of methylmercury in fish that exceed those in surface waters by a factor of 10^6 to 10^7

(Watras et al. 1994, Boudou and Ribeyre 1997, Kim and Buggraaf 1999, Bowles et al. 2001).

Biomagnification, the tendency for the concentration of a substance to increase with increasing trophic level, has been widely documented for methylmercury in aquatic food webs (Francesconi and Lenanton 1992, Watras et al. 1998, Bowles et al. 2001). Patterns of mercury biomagnification are similar across ecosystems regardless of differences in ecosystem type, mercury source, and pollution intensity (Wiener et al. 2003). Several rules appear to govern biomagnification of methylmercury in food webs. First, the concentration of methylmercury increases within a food web as one moves from water to lower trophic levels to top predators such as fish. Second, the fraction of total mercury that is present as methylmercury increases along a similar gradient (Wiener et al. 2003). The greatest increase in mercury concentration occurs between phytoplankton and water, for which bioaccumulation factors routinely reach values of 10^5 or 10^6 (Plourde et al. 1997, Watras et al. 1998, Bowles et al. 2001, Miles et al. 2001). In contrast, bioaccumulation factors between organisms at higher trophic levels tend to be much lower (MacCrimmon et al. 1983, Suns et al. 1987, Cope et al. 1990, Kim and Burggraaf 1999).

Several factors are thought to influence the build-up of methylmercury in individuals, the most notable of which is trophic position. Trophic position is thought to account for much of the variation in mercury concentration, both among and within species (Cabana and Rasmussen 1994, Cabana et al. 1994, Bowles et al. 2001) Trophic position can vary substantially, even within a species; thus, a single individual may experience different methylmercury concentrations across life stages (Vander Zanden and

Rasmussen 1996). In addition, due to the slow rate of elimination, methylmercury concentrations tend to change with age, with older individuals having higher mercury concentrations than younger individuals that have experienced shorter durations of exposure (UNEP 2002). Finally, mercury concentrations are strongly influenced by the length of the food web. Longer food webs with a greater number of trophic levels provide more opportunities for biomagnifications to occur. Thus, individuals at the top of long food webs tend to have higher concentrations of methylmercury relative to organisms that have fewer trophic levels below them (Wiener et al. 2003).

In contrast to methylmercury, inorganic mercury is not readily assimilated by wildlife and does not bioaccumulate or biomagnify to high concentrations. Consequently, although acute inorganic mercury poisoning can occur with severe effects for afflicted individuals, methylmercury is, in general, thought to pose a much greater threat to wildlife.

Health Effects

Intestinal absorption of methylmercury is nearly complete (Scheuhammer 1987). In addition, methylmercury readily crosses both the placental and blood-brain barriers (Wolfe et al. 1998, Eisler 2006), accounting in large part for its subsequent toxicity to organisms. In particular, its ability to cross the blood-brain barrier has earned it a well-established reputation as a potent neurotoxin (Wolfe et al. 1998). Within the brain, methylmercury is thought to primarily affect the cerebrum and cerebellum (Nixon 1994), possibly by inhibiting membrane Na^+/K^+ -ATPase (Clarkson 1987, Aschner et al. 1990b). Especially vulnerable are glial cells, which possess neutral amino acid carrier systems

that appear to enhance the uptake and transport of methylmercury in cells (Aschner et al. 1990a). The signs and symptoms of neurotoxicity resulting from such exposure are complex and varied. In adult mammals, typical neurological signs indicative of methylmercury poisoning include difficulty standing and moving and neurasthenia (Eaton et al. 1980, Wren et al. 1987, Heinz 1996). Such clinical symptoms often are accompanied by lesions in the cerebral and cerebellar cortexes (Wolfe et al. 1998).

In addition to producing obvious motor and sensory impairment, methylmercury is also capable of causing much more subtle neurological effects. In particular, several studies have examined the role of mercury in causing deficits in learning and memory. Inouye et al. (1985) found that mice and rats prenatally exposed to methylmercury displayed impaired maze, avoidance, and operant learning abilities, retarded swimming ability and righting reflex, and decreased spontaneous activity. Primates have been especially well studied with respect to neurological teratology. Gunderson et al. (1988) reported that crab-eating macaques (*Macaca fascicularis*) exposed to methylmercury during development showed significant deficits in visual recognition memory. Burbacher et al. (1990) found that these same primates also tended to exhibit less social play and more non-social passive behavior. In one long-term study, Rice and Gilbert (1992) exposed macaques to methylmercury from birth to seven years of age. At age 14, auditory tests were administered; exposed macaques showed impaired auditory abilities, but only in the upper threshold of the frequency range, demonstrating both the subtlety and latency with which mercury can act.

Mercury also acts at the level of the cell to produce effects that could have obvious consequences for many higher order processes. For example, Dieter and Ludke

(1975) found that cholinesterase activities declined in *Coturnix* quail following dietary exposure of 5 ppm methylmercury for 18 weeks. However, the relationship is not so straightforward, as several authors have failed to find any significant effect of methylmercury exposure on cholinesterase activity (Great Blue Herons (*Ardea Herodias*): Wolfe and Norman 1998; Rhesus Monkeys (*Macaca mulatta*): Petruccoli and Turillazzi 1991). Other reported biochemical effects of mercury include impairment of the immune system (Dieter et al. 1983, Ilback et al. 1991, Shenker et al. 1992, Shenker et al. 1993, Tan et al. 1993) and genotoxicity (reviewed in De Flora et al. 1994). In addition, mercury compounds are among the strongest known inhibitors of cell division (Birge et al. 1979), with obvious negative implications for growth and maintenance of organisms, especially during embryogenesis (Eisler et al. 2006).

Interactions That Alter Effects

Mercury is known to interact with a wide variety of biochemical species within organisms in ways that may alter its bioavailability or toxicity. Of particular note are its interactions with selenium and glutathione. Mercury and selenium share many properties: both bioaccumulate, bind to organothiol groups, and cause greatest toxicity through dietary exposure (USDI 1998). Because numerous instances of interactions between methylmercury and selenium have been documented (i.e. Cuvin-Aralar and Furness 1991, Sorensen 1991), much interest has centered on determining the nature and consequences of this relationship. A fairly extensive body of research in this area has produced conflicting results. While El-Begearmi et al. (1977) found that co-administering sodium selenite reduced the toxicity of methylmercury and increased

survival of Japanese Quail (*Coturnix japonica*), results obtained by Heinz and Hoffman (1998) were more ambiguous. They found that, although co-administration of the two metals did reduce toxicity in adults compared to the administration of either metal alone, Mallards (*Anas platyrhynchos*) that received both metals were more likely to suffer reproductive impairments.

Interactions between methylmercury and glutathione have received similar attention. Aschner et al. (1990b) reported that the neuronal swelling caused by methylmercury was greatly relieved with co-administration of a glutathione conjugate. Similar results were obtained by Ornaghi et al. (1993). Di Simplicio et al. (1993) measured glutathione activity under various conditions of exposure to methylmercury and selenium and found an interaction suggesting simultaneous actions of tissue damage by mercury and repair by glutathione. Thus, there is fairly substantial evidence implicating glutathione in a protective mechanism against methylmercury poisoning.

MERCURY IN BIRDS

Risks of Mercury Exposure to Birds

Aquatic food webs tend to exhibit much greater complexity and length than their terrestrial counterparts, providing greater opportunity for biomagnification in top predators to occur. In addition, conditions that are favorable for methylation are often found in aquatic ecosystems. These observations led, until recently, to the general conclusion that the risk of mercury to birds was largely aquatic in nature (Eisler 2006, Scheuhammer et al. 2007). As a result, although there is an extensive literature on the accumulation and effects of mercury in birds, most field and laboratory studies to date

have focused largely on the threat of mercury to large predatory or piscivorous birds, while neglecting potential risks to terrestrial insectivores or granivores (but see Rimmer et al. 2005, Shriver 2006, Cristol et al. 2008).

Routes of Elimination

Excretion in the feces and deposition into growing feathers represent major routes of mercury elimination in birds (Furness et al. 1986, Lewis and Furness 1991, Condon and Cristol 2009). Feathers, in particular, offer a significant mode of detoxification. All species of birds undergo periodic episodes of molt, during which time some or all of their feathers are replaced. In many species, including most songbirds, feathers are molted in over a discrete period of time; in contrast, seabirds often undergo continuous molt, in which feathers are replaced more gradually. During periods of molt, developing feathers are connected to blood vessels in the body which supply the nutrients necessary for growth (Furness et al. 1986). Methylmercury has a high affinity for disulfide bonds, and thus, circulating mercury will readily accumulated in the disulfide-rich keratin proteins in feathers (Crewther et al. 1965, Stettenheim 2000). Once growth ceases, the blood vessels atrophy, causing the newly formed feathers to become physiologically separate from the blood supply and any further influxes of mercury (Voitkevich 1966, Lewis and Furness 1991). The mercury deposited in feathers may be significant, in some cases relieving as much as 70-93% of the body burden (Burger 1993); virtually all of the mercury sequestered in feathers is in the form of methylmercury (Thompson and Furness 1989). Elimination of mercury in feathers may be a particularly important protective mechanism

for nestlings and chicks that are rapidly molting in thousands of feathers at once (Monteiro and Furness 2001, Condon and Cristol 2009).

In females, another important route of mercury excretion may be provided by deposition into eggs (Tejning 1967, Heinz 1979, Monteiro and Furness 2001). Just as mercury in mammals is capable of crossing the placental barrier to affect the developing fetus, so too, mercury readily passes from female bird to egg (Tejning 1967, Fimreite 1971, Heinz 1979). Furthermore, nearly all of the mercury transferred into eggs is methylmercury (Tejning 1967, D. A. Cristol, unpublished data). Approximately 85 to 95% of this mercury is deposited in the albumen and is believed to be reflective of dietary uptake in the period directly preceding laying (Tejning 1967, Walsh 1990).

There is also evidence to suggest that some species of seabirds may have the ability to demethylate mercury internally to reduce its toxicity (Kim et al. 1996, Scheuhammer et al. 1998). Kim et al. (1996) found unimpaired seabirds with extremely high liver mercury levels; however, further analysis revealed that the majority of this mercury was in the inorganic form. Similarly, Scheuhammer et al. (1998) observed a 1:1 molar ratio of Hg:Se in the livers of Common Mergansers (*Mergus merganser*) and Common Loons (*Gavia immer*), highlighting one potential route by which stable seleno-mercury complexes may act to reduce the toxicity of methylmercury. Furthermore, the researchers noted that, as the concentration of total mercury increased in the liver and kidneys, the proportion of that mercury present as methylmercury tended to decline, suggesting the occurrence of demethylation in these tissues.

Effects of Mercury in Birds

Reproduction. Reproduction is one of the most sensitive endpoints of mercury poisoning in birds (USDI 1998, Wolfe et al. 1998). In fact, it has been suggested that the concentrations of methylmercury required to cause reproductive impairment are only one-fifth of those believed to cause acute toxicity in adults (Scheuhammer 1991). Numerous examples of both field and laboratory studies attest to the potentially severe consequences of elevated mercury levels on avian reproduction. Typically, it has been thought that mercury levels in the egg are most predictive of reproductive risk (Wolfe et al. 1998); however, several studies have also examined reproductive impairment with respect to mercury concentrations in prey and in juvenile and adult tissues.

Heinz (1979) fed three generations of Mallards mercury doses equivalent to 0.5 ppm dry weight and observed the subsequent reproductive and behavioral impairments of both adults and juveniles. He found that dosed female Mallards laid more eggs outside of nest boxes, laid fewer eggs overall, and produced fewer ducklings. Those ducklings that did survive showed a reduced response to simulated maternal warning calls and were hypersensitive to fright stimuli. Hoffman and Moore (1979) treated Mallard eggs with externally applied methylmercury chloride and observed decreased embryo weights, developmental abnormalities, and embryonic death; based on their results, they estimated that the lowest concentration of methylmercury above which adverse effects might reasonably be expected to be 0.5 ppm in eggs. Heinz and Locke (1976) observed an even more subtle effect of mercury contamination on avian reproduction. They fed adult female Mallards diets containing 3.0 ppm methylmercury for two breeding seasons. The eggs of these females contained between 5.5 and 7.2 ppm mercury (fresh weight). Those

ducklings that hatched were found to have brain lesions which significantly reduced survival. Clearly, much of the experimental work examining the effects of mercury on avian reproduction has been conducted on Mallards. While this work has been instrumental in uncovering many of the subtle complexities of methylmercury poisoning, caution should be exercised in applying the results of these studies to other species as variation in sensitivity to mercury may be high (Heinz et al. 2009). For example, Heinz et al. (2009) found that the concentrations of mercury that were required to produce teratogenic effects were much lower in songbirds than in waterbirds like the Mallard.

There is also a large body of literature examining the effects of mercury on the reproductive capabilities of free-living birds. One of the most widely-cited field studies is that of Barr (1986), who found that Common Loons breeding in Ontario had severely elevated levels of mercury in their eggs and tissues. He examined different regions of the Wabigoon-English River system, each with a different degree of mercury contamination. In the most contaminated areas, loons showed reduced territorial behavior, increased desertion and predation of clutches, and decreased hatching success. More recently, Evers et al. (2008) conducted an extensive analysis of multiple reproductive, physiological, and behavioral endpoints in Common Loons. They found a negative correlation between mercury levels and fledging success, which may be attributable to the fact that the most contaminated adults also showed a reduced tendency to incubate their clutches and forage for their chicks.

Physiology. In addition to impacting reproduction, mercury may also significantly impair a number of biochemical and physiological processes, many of which may ultimately

undermine the functioning and survival of both adult and juvenile birds. In particular, a growing body of research has examined the potential for mercury to compromise various aspects of the immune system. Some of the earliest work in this area was based on the observation that many severely diseased birds appeared to exhibit high levels of mercury in their tissues. Sundlof et al. (1994) reported that elevated mercury concentrations were found in 30-80% of birds of various species found dead in the Florida Everglades between 1987 and 1991. Likewise, Spalding et al. (1994) compared mercury concentrations in the tissues of Great White Herons (*Ardea herodias*) that had died of chronic disease with those that had died in good body condition. They found that mercury levels in the former group were significantly higher than those of the latter. Although the authors urge caution in the interpretation of their results, given that the nature of the relationship between mercury and chronic disease is unclear, such a pattern is suggestive of severe weakening of the immune system by mercury. More recent work has attempted to pinpoint the exact nature of mercury's effect on immune functioning through controlled laboratory experiments. Kenow et al. (2007) performed a series of immune measurements on Common Loon chicks dosed with various levels of mercury. At dietary concentrations of 0.4 ppm (wet weight), the authors noted a suppressed response of some immune components, while other components of the immune system, and the same components at other levels of mercury, remained unaffected (Kenow et al. 2007). Thus, it appears that under certain conditions, mercury is capable of altering immune system functioning.

There is also support for the hypothesis that mercury may serve as an endocrine-disruptor. Heath and Frederick (2005) reported that mercury levels were negatively

correlated with estradiol concentrations and number of nesting attempts in prebreeding White Ibises (*Eudocimus albus*). The authors speculated that the sub-acute hormonal effects of mercury may cause fewer birds to nest or more to abandon their nests, providing one potential mechanism by which mercury might reduce overall productivity of individuals. In other studies, mercury has been found to alter expression and synthesis of glucocorticoid hormones, such as corticosterone (Thaxton et al. 1981, Evers et al. 2003, Adams et al. 2008, Franceschini et al. *in press*, Wada et al., unpublished data, but see Heath and Frederick 2005).

Behavior. In contrast to reproduction and physiology, behavioral endpoints of mercury toxicity have received relatively little attention. However, behaviors may prove more indicative of toxicological problems than the more traditionally measured chemical, physical, or morphological parameters, as behaviors represent the culmination and integration of a large number of complex developmental and physiological pathways (Gorissen et al. 2005). The few studies that have examined behavioral abnormalities have yielded results that give credence to this view. Heinz (1979) found that adult Mallards exposed to dietary methylmercury were more likely to lay eggs outside of nest boxes. In addition, their chicks exhibited impaired responses to maternal warning calls and a heightened fright response. Nocera and Taylor (1998) found that Common Loon chicks spent less time back brooding and more time preening in high-mercury environments. Because back brooding is often important for protecting chicks from underwater predators, such aberrant behavior could easily lead to reductions in chick survival (Nocera and Taylor 1998). Further evidence of the indirect mechanisms by

which mercury may alter survival was provided by Bouton et al. (1999), who observed that juvenile Great Egrets (*Casmerodius albus*) exposed to high doses of dietary mercury had decreased overall activity, tendency to seek shade, and motivation to hunt. Clearly, the behavioral abnormalities produced by mercury poisoning may work in very subtle ways to alter a number of higher level processes important to the successful reproduction and survival of individuals.

Evaluating Tissue Concentrations and Effect Levels

One of the major obstacles faced by avian ecotoxicologists has been determining the risk of particular levels of mercury to free-living birds. Part of this task involves the integration of adverse effects levels obtained in the laboratory with those observed in the field. In general, this has not proven an easy feat, and many aspects of avian risk levels remain poorly understood, especially with respect to passerine birds. Complicating the situation even further, there is a great deal of variability among both species and individuals in sensitivity to mercury, as has been recently demonstrated by Heinz et al. (2009). The authors injected doses of methylmercury into the eggs of 26 species of birds and examined dose-response curves of embryo mortality. Based on these curves, they concluded that there is a large amount of variation in sensitivity to mercury poisoning among species. Some species commonly used in laboratory dosing studies, such as the Mallard, exhibited very low sensitivity to mercury (median lethal dose of mercury for Mallard: 1.79 ppm (wet weight)); in contrast, several species were highly sensitive, with a median lethal dose below 0.25 ppm. Such variability highlights the difficulties in assigning a single threshold concentration above which adverse effects might reasonably

be expected. Nevertheless, numerous attempts have been made to estimate the concentration of mercury in prey or tissues which might pose a threat to wild birds. One of the most extensive analyses conducted to date was performed by Evers et al. (2008). By synthesizing nearly 20 years of data on Common Loons breeding in North America and examining a number of discrete, biologically relevant endpoints, Evers et al. (2008) estimated that adverse effects of mercury can be expected above 3.0 ppm (wet weight) in blood and 40.0 ppm (fresh weight) in feathers. Although extrapolating these levels to passerine birds should be done with caution, these effect levels are the most comprehensive to date, and as such, provide a valuable foundation from which to examine the effects of mercury in other species.

LIFE HISTORY THEORY

Overview of Life History Theory

Because the present study examines survival in the face of mercury exposure, it is important to discuss the components of life history theory, of which lifespan is one. Life history theory is concerned with understanding the patterns and processes governing the reproductive profile of an individual or species. Attempts to understand an organism's life history include questions pertaining to how that organism allocates time and resources throughout its life to maximize total reproductive output in a given environment. Thus, commonly studied attributes include age at maturation, clutch size, clutch number, lifespan, growth, and parental investment. In its most fundamental framework, life history theory is rooted in the idea that individuals are faced with various evolutionary and environmental challenges, many of which are conflicting. In order to

meet these varied demands, an organism can employ a number of specific strategies related to when it mates or how much it invests in reproduction, but it cannot simultaneously satisfy every demand in an intrinsically optimal way. Thus, there are trade-offs. Natural selection acts on populations to balance these trade-offs in such a way as to create a suite of life history characteristics that are, in combination, optimal for a given individual in a specific environment. The challenge faced by life history theorists is to discern exactly how natural selection has shaped the various life history traits displayed within a particular species and how such a complement of characteristics helps to maximize the lifetime reproductive output of individuals in the face of a number of environmental insults and demands.

Reproductive Value

A key concept in life history theory is the idea of reproductive value (Fisher 1930, Williams 1966, Lessells 1991). Reproductive value is specified by two components – the current reproductive output of an individual and the residual (or future) reproductive value (Begon et al. 1996). Residual reproductive value thus takes into account the expected future survival as well as expected future offspring (Begon et al. 1996). Natural selection will tend to favor the life history strategy that results in the highest combination of current and future reproduction (Begon et al. 1996). In discussions of life history evolution, it is important to consider these two facets of reproductive value, as they represent the trade-off between current reproductive investment and future fitness gains. Reproductive value is also important in understanding the strength of and direction in which natural selection will act at different stages of life. Because the various ages of life

are intimately connected to one another, and a finite amount of resources are available to be distributed throughout the life cycle, if natural selection acts to maximize reproduction at one age or stage of life, it will necessarily be constraining future reproductive effort (Begon et al. 1996).

Life History Traits and Trade-Offs

Over time, natural selection will tend to favor those traits that allow individuals to leave the greatest number of descendants in subsequent generations. The exact strategy by which this is accomplished can vary greatly depending on the evolutionary history of the species and the specific environmental conditions an individual experiences. Because each individual is allotted a limited amount of time and resources with which to produce offspring, the key to maximizing representation in the next generation is to effectively balance competing energetic and physiological demands throughout the lifespan. While the specific trade-offs employed will differ depending on the unique attributes of the organism being considered, several consistent patterns of trade-offs have emerged in the life history literature, two of the most extensively studied of which are described below.

Age and Size at Maturation. One area of life history research that has received considerable attention concerns the age and developmental stage at which an organism decides to begin reproducing. There are several obvious benefits to early maturation and reproduction. Most notably, the earliest-breeding individuals reduce the risk of dying before reproducing and are able to produce offspring more quickly (Stearns and Hoekstra 2005). However, there are significant potential costs as well, many of which are related

to a reduction in future survival or reproductive output (Ricklefs 1993). For one, a female that attempts reproduction at a very early maturational stage may be ill-equipped or provisioned for raising many, high-quality offspring that will, themselves, survive to reproduce (Begon et al. 1996). As with all life history trade-offs, the relative importance of these benefits and costs varies according to the specific organism and environment being considered. For example, there were significant differences in life history strategy employed by two populations of guppies (*Poecilia reticulata*) facing very different types of predation pressure (Reznick and Endler 1982). In one population, the primary predator was a cichlid fish (*Crenicichla alta*), which feeds primarily on large, sexually mature guppies. In the other type of environment, killifish (*Rivulus hartii*) were the principal threat. In contrast to the cichlid fish, killifish prey mainly on small, sexually immature guppies. As predicted by life history theory, in the cichlid-dominated populations, guppies tended to mature earlier and produce many small offspring relative to their counterparts living in killifish-rich environments.

Some organisms, unlike guppies, are evolutionarily constrained in terms of the maximum size which can theoretically be attained. Most birds, for example, maintain a pattern of growth and development which is fairly inflexible, and for most species, growth will not occur after the first year (Ricklefs 1993). Birds still face the choice of whether to breed immediately following sexual maturation or to delay until subsequent breeding seasons, but here, the factors involved in such behavioral decisions involve weighing the potential benefits of gained experience against costs such as predation or senescence (Ricklefs 1993). In fact, among bird species, there is a strong positive correlation between age at maturity and annual adult survival, demonstrating how the

evolutionary trajectory of life history traits balances current and future reproductive investment (Ricklefs 1973).

Number and Quality of Offspring. A second branch of life history theory that has sparked significant interest is the evolution of clutch size and the distribution of resources among offspring. At its core, this life history trade-off centers on balancing the number of offspring with the quality of each. Numerous studies have documented, both within and among species, a negative correlation between the number and size of offspring produced (Werner and Platt 1976, Montague et al. 1981, Sinervo 1990). In one particularly interesting case, Sinervo (1990) removed yolk from the eggs of an iguanid lizard (*Sceloporus occidentalis*) after they had been laid, leading to the production of healthy, albeit smaller, offspring. He then measured the sprint speed of the offspring from manipulated and unmanipulated eggs and found that the smaller lizards were slower, most likely indicating a reduced ability to avoid predators, and hence, lowered fitness. There is a great deal of natural variation in both the number and size of eggs laid across the range of this species, and as expected, offspring size and number tend to negatively covary within populations (Sinervo 1990).

Much of the early theory concerning reproductive investment was described by David Lack (1947), who used an optimality framework to address the simple question of why different species of birds lay different numbers of eggs. In particular, he postulated that parents lay the number of eggs that grant them the maximal number of fledged offspring. Lack assumed that the probability that a given nestling will survive to leave the nest decreases linearly with the total number of chicks present. If this is the case,

then adults can optimize their reproductive output by producing the greatest number of nestlings for which they are able to provide sufficient parental care. One key prediction of Lack's hypothesis is that nests in which brood size is experimentally manipulated to contain either more or fewer nestlings should exhibit lower productivity than unmanipulated nests. This prediction has been tested many times with mixed results. In particular, numerous studies have reported realized clutch sizes that are smaller than would be predicted by the Lack clutch hypothesis (Stearns and Hoekstra 2005). In these cases, parents raising experimentally increased broods are more productive than those at unmanipulated nests.

In an effort to explain the apparent discrepancies between theoretical and empirical results, researchers have noted several potential weaknesses with Lack's original framework. Most notably, Lack based his predictions on the idea that reproductive success is defined by the number of nestlings that leave the nest in a single breeding attempt. Although it is often convenient to measure fledging success as a proxy for fitness, it is not always an adequate predictor of actual productivity. Here again, measures of offspring quality must be taken into account, perhaps using parameters such as fledgling survival, or subsequent reproductive success of offspring (Begon et al. 1996). Waage and Godfray (1985) found that members of the egg parasitoid, *Trichogramma evanescens*, raising enlarged broods appeared to be more productive than controls. However, such success was not borne out in the reproductive success of daughters from enlarged broods, whose fecundity was severely inhibited.

A related, though separate, criticism of the Lack clutch size hypothesis is that it fails to take a longer view of the situation. By examining reproductive success in only a

single nesting attempt, Lack failed to account for long-term reductions in residual reproductive value that might result from raising larger broods (Stearns and Hoekstra 2005). As of 1992, there had been 55 published studies testing the predictions of the Lack hypothesis (Stearns 1992). Despite the fact that many species were able to raise enlarged broods to fledging, a synthetic analysis of these 55 studies yielded the following additional insight: For adults attempting to raise experimentally enlarged broods, weight of fledglings was reduced in 68% of the studies, survival of fledglings was reduced in 53%, weight of parents was reduced 41% of the time, survival decreased in 36% of cases, and future reproduction of parents declined in 57% of all studies considered (Stearns 1992). There appear to be many additional trade-offs which should be taken into account in predicting the degree of reproductive investment expected in a particular breeding attempt.

Thus, there are well-documented advantages to examining life history traits in the larger context of the entire lifespan. Returning to the idea of reproductive value, it is the total lifetime reproductive success of an individual that determines its representation in subsequent generations, and it is this characteristic that ultimately defines the particular strategies pursued by individual organisms. Ardia (2005) examined the responses of adult Tree Swallows (*Tachycineta bicolor*) breeding in either a relatively mild Tennessee climate or a much harsher Alaskan environment. He experimentally altered brood sizes in both populations and examined the subsequent immune function of nestlings and adults. In Tennessee, adults raising enlarged broods maintained a high level of immune function, while that of their nestlings was depressed. In Alaska, where annual adult survival is expected to be lower, the opposite trend was observed – adults compromised

their own immune systems in order to successfully raise their offspring to maturity. The results of that study demonstrate the ways in which adults are capable of trading off current reproduction with probability of survival and future reproductive attempts. In Alaska, the reduced chance of adult survival to the next breeding season made the current reproductive investment of much greater relative importance to lifetime reproductive output. This was reflected in the adults' decision to sacrifice their own maintenance in order to divert resources to their current brood. In contrast, in Tennessee, where the adults could reasonably expect to breed again, less of an emphasis was placed on raising the current year's brood, since more opportunities would present themselves in subsequent years. Thus, it is not possible to examine a single life history trait in isolation in time or context. Because the ultimate objective of each organism, and the only one on which natural selection acts, is to maximize proportional representation of genes in subsequent generations, the strategies employed by individuals to achieve such ends must be considered holistically.

Effects of Environmental Contaminants on Life History Traits

Traditionally, the field of ecotoxicology has been dominated by studies of contaminant impacts on individuals (Newman and Unger 2003). Concentrations that produced any adverse effects on growth, development, survival, or reproduction in individuals were used to estimate population-level effects (Newman and Unger 2003). However, this paradigm has slowly been shifting, as researchers have recognized the need to incorporate life history traits into discussions of population viability and contaminant effects (Newman and Unger 2003). Thus, there are now a number of studies that have

examined the ways in which contaminants are capable of altering important life history characteristics (Sibly 1996). Often, these studies have used demographic techniques to identify those life stages that are most susceptible to contaminants (e.g. Kammenga et al. 1996) or they have examined how environmental insults may alter particular life history characteristics of a population (e.g. Marshall 1962). One topic that has received considerable interest concerns the optimal stress response (Sibly and Calow 1989, Holloway et al. 1990, Sibly 1996). The optimal stress response dictates that the allocation of resources devoted to various maintenance and reproductive activities should be adjusted when an organism experiences stress, in this context, due to a pollutant. This redistribution of resources may be either immediate (e.g. early maturation or delayed growth: Sibly 1996) or evolutionary (e.g. enhanced metal tolerance at the expense of reduced growth: Wilson 1988). In addition to an emphasis on individual effects, the vast majority of studies concerning contaminant effects on life history traits have been conducted on a few groups (arthropods, algae, gastropods, nematodes, and humans; Sibly 1996) and over short time periods; thus, contaminant effects on vertebrate life history tradeoffs are poorly understood, likely owing to the much greater difficulties of performing controlled, long-term studies.

For birds, as for most other vertebrates, there is a general paucity of research on the effects of contaminants on many important life history characteristics. While numerous ecotoxicological studies have examined reproductive endpoints, most of these have been concerned with a narrow view that considers only reproduction within a single breeding attempt without regard to the inter-play between life history parameters on the scale of an entire lifetime. Still fewer studies have examined such endpoints in the field

(but see Barr 1986, Evers et al. 2008). More recently, ecotoxicologists have begun to consider other life history traits, such as survival and lifespan. Custer et al. (2007) examined adult Tree Swallows breeding on a site polluted by polychlorinated biphenyls and found modest evidence suggesting reduced survival. Esler et al. (2000) also found support for depressed winter survival in adult female Harlequin Ducks (*Histrionicus histrionicus*) in Alaska following the 1989 Exxon Valdez oil spill. However, these studies represent exceptions to the current paradigm, as few comprehensive studies examining important life history traits across the entire life cycle have been conducted in birds (but see Peterson et al. 2003, Evers et al. 2008).

TREE SWALLOW LIFE HISTORY

Introduction

Tree Swallows are migratory, insectivorous songbirds that breed throughout the northern half of North America (Robertson et al. 1992). As secondary cavity nesters, Tree Swallows are severely nest site limited, a trait that has probably played a significant role in shaping much of their unique life history (Robertson et al. 1992). In addition, the shortage of nest sites means that swallows will readily adopt artificial nest boxes, making them a particularly accessible and tractable study organism. Thus, Tree Swallows have come into wide use in a number of studies, particularly those pertaining to fields such as life history theory, climate change, and environmental contaminants (Jones 2003). Jones (2003) argued that the relative ease with which Tree Swallow populations can be established and manipulated, as well as the thorough understanding of important life history traits in this species, have earned Tree Swallows a rightful place among such

classical model organisms as fruit flies, nematodes, and mice. In addition to their general tractability, Tree Swallows possess a number of characteristics that make them particularly well-suited to studies of environmental contaminants.

Diet and Foraging

Tree Swallows feed on a diet that consists primarily of emergent aquatic insects (Robertson et al. 1992). Studies of nestling diet have found food items to consist predominantly of Diptera (46%), Homoptera (26%), and Ephemeroptera (11%) (Blancher et al. 1987). During the breeding season, adults likely feed on a diet that is very similar to that of the nestlings (Robertson et al. 1992). Furthermore, nesting Tree Swallows tend to forage in an area no more than 400 m from their nest box (Mengelkoch et al. 2004). Therefore, highly localized estimates of contaminant bioavailability can be developed using this species. Because, at my study site, approximately half of the swallow diet is terrestrial, and half aquatic, in origin, they provide a different window into contaminants than the more traditionally studied aquatic-feeding kingfishers, loons, and eagles (Brasso and Cristol 2008).

Delayed Plumage Maturation

Tree Swallows are a rarity among birds in that females, but not males, exhibit delayed plumage maturation (Robertson et al. 1992). Females in their first year of breeding display a diagnostic dull brown plumage and are readily identified as second-year birds (hereafter, SY). In contrast, older females (after-second-year; hereafter ASY) possess the iridescent blue plumage characteristic of all adult males (after-hatch-year; hereafter

AHY). Tree Swallows are one of only two North American songbirds that display this pattern of delayed plumage maturation in females, but not males (Jones 2003). Such a trait is thought to serve as an intersexual signal to reduce male aggression towards younger females (Stutchbury and Robertson 1987b). Because of the significant shortage of suitable nest sites even under the best of circumstances, a large surplus of both male and female Tree Swallows exists as floaters. Experimental addition of nest boxes or removal of breeders has led to the estimation that approximately 25% of Tree Swallows in a given population employ this strategy (Stutchbury and Robertson 1985). By adopting a subordinate plumage, floating SY females may be able to prospect for future nest sites under a lower threat of harassment while they wait for reproductive opportunities to arise (Stutchbury and Robertson 1987a). In addition to being an integral aspect of Tree Swallow life history, delayed plumage maturation may also be an important consideration in studies of environmental contaminants. First, there is some evidence that SY females tend to suffer from lower reproductive success than ASY females, probably due to their relative inexperience; for example, SY females have been shown to produce lighter eggs (Robertson et al. 1992). This may prove relevant in the context of toxicology if contaminants tend to affect SY females differently than ASY females. Second, the ability to distinguish SY females means that birds breeding for the first time can be clearly distinguished within a population, allowing for long-term studies in which the fate of an individual female is known completely.

Reproduction

Tree Swallows winter mainly in Florida and the Gulf Coast of Mexico, but have also been observed along the Caribbean coast of South America (Robertson et al. 1992). In mid-March or early April, they arrive on their breeding grounds, soon after which the process of pair formation begins (Robertson et al. 1992). Tree Swallows primarily nest in open habitat with easy access to bodies of water over which they can feed (Robertson et al. 1992). Males tend to arrive first to begin defending nest sites (Robertson et al. 1992). Nest construction typically begins in late April and continues into early May (Robertson et al. 1992). Nests are built initially by females and consist of a grass cup (Kuerzi 1941, Stocck 1970) lined with contour feathers from waterfowl, gulls, and domestic fowl which are collected by males (Austin and Low 1932, Kuerzi 1941). It has been hypothesized that feathers may act as a barrier to moisture (Mertens 1977) or ectoparasites (Cohen 1988), although support for the latter hypothesis has been mixed (Robertson et al. 1992). Tree Swallows typically have been thought to be single-brooded, although recent evidence has suggested that a portion of the population may raise two broods successfully in a single season (Monroe et al. 2008). In particular, at my study site, it appears that the earliest breeders are more likely to become double-brooded (Monroe et al. 2008), a fact that is not surprising given that laying date has repeatedly been shown to be one of the most important determinants of Tree Swallow reproductive success (Winkler and Allen 1996). Most often, clutches consist of five or six eggs which a female will incubate for 14-15 days, although clutches ranging in size from two to seven eggs are not uncommon (McCarty 2001). Upon hatching, nestlings are fed by both parents, with the male and female sharing approximately equal duties in parental care (Robertson et al. 1992). At

about 21 days of age, Tree Swallow nestlings fly from the nest, although their parents may continue to provide some care (Robertson et al. 1992).

Dispersal, Philopatry, and Survival

Annual adult survival in the Tree Swallow has been estimated to be approximately 40-60% (Chapman 1955, Houston and Houston 1987), with little evidence of differences by age or sex (Robertson et al. 1992). Average lifespan is thought to be 2.7 years (Butler 1988), but Tree Swallows as old as 11 years have been observed under natural conditions (Hussell 1982, Houston and Houston 1987). In contrast, mortality in the first year of life may reach 75-80%, meaning that only 20-25% of fledglings survive to reproductive age (Robertson et al. 1992, Shutler and Clark 2003). However, estimates of nestling survival must be interpreted with caution, as nestling Tree Swallows are thought to disperse away from their natal sites. For example, of all relocated yearlings (banded as nestlings the year before) at a site in Colorado, Cohen et al. (1989) found that only 23% bred on the natal site, while 77% nested at least 5 km outside the study area. Most relocated nestlings bred within 20 km of their natal site, and nearly all were observed within a 40 km radius (Cohen et al. 1989). This having been said, mortality in the first year of life is often very high across a number of songbird species, and 75-80% mortality of nestling Tree Swallows would not be unexpected.

In stark contrast, and perhaps contributing to the high natal dispersal exhibited by nestlings, breeding adult Tree Swallows have been shown to be highly philopatric. Winkler et al. (2004) conducted an extensive analysis of Tree Swallow inter-annual dispersal around Ithaca, New York. Due to a large network of nest boxes and a

substantial citizen science effort, the researchers had the ability to potentially detect Tree Swallows breeding in a radius hundreds of kilometers around their study site. However, even with such high rates of detection, only 14% of females and 4% of males were discovered breeding at a different site than in a previous year (Winkler et al. 2004). While other estimates have indicated weaker philopatry of adult breeders (13-60%: Robertson et al. 1992), the weight of evidence suggests that breeding adult Tree Swallows show a high degree of site fidelity. This characteristic is useful in studies of environmental contaminants as it allows adult birds to be individually marked and followed for several years, greatly facilitating studies of both individual and population-level survival and reproductive success.

Summary

In addition to being highly accessible and tractable study organisms, Tree Swallows possess a number of characteristics that make them particularly well-suited to the study of environmental contaminants. Since many contaminants originate in aquatic ecosystems, Tree Swallows' direct links to aquatic food webs place them in a category of high risk of exposure. Furthermore, their breeding site fidelity coupled with the ability to discriminate SY females means that individual Tree Swallows can be reliably tracked throughout their entire reproductive careers. Because effects of environmental pollutants may emerge only after several years of exposure, this ability to detect and monitor specific birds enables one to determine with greater certainty exactly what effects a contaminant like mercury may be having on a given individual on the scale of the entire lifespan.

SOUTH RIVER

History of Mercury Contamination on the South River

On April 14, 1977, representatives of E. I. DuPont de Nemours announced that the South River, a tributary of the South Fork Shenandoah River, was heavily contaminated with mercury as a result of chemical processes associated with one of that company's industrial plants in Waynesboro, Virginia (Carter 1977). An inquiry into the problem had begun in the previous September when workers in the factory stumbled upon minute globules of metallic mercury lying beneath the "old chemical building" of the plant (Carter 1977). Mercuric sulfate had been used between 1929 and 1950 as a catalyst in the manufacture of acetate fiber, after which time all processes requiring the use of mercury had been abandoned (Carter 1977). Despite the fact that no new mercury was known to have entered into the river in over 25 years, subsequent analysis of sediment samples taken from downstream of the plant revealed significant contamination with mercury (Carter 1977). In fact, several sediment samples exceeded concentrations of 240 ppm mercury (compared to 1 ug/g just upstream of the plant). The one fish that DuPont officials analyzed contained 0.86 ug/g, above the Food and Drug Administration (FDA) action level of 0.50 ug/g. On June 6, 1977, on the basis of mercury levels in sediment and fish analyzed by the State Water Control Board (now known as the Virginia Department of Environmental Quality: VDEQ), Governor Mills E. Godwin declared the South River downstream of Waynesboro and the entire South Fork of the Shenandoah River closed to the taking of fish for eating (Carter 1977). Bass caught as far as 77 miles

downstream from the plant had been found to have mercury levels twice as high as the FDA standard (Carter 1977).

Since the initial discovery of mercury in the South River, several plans and programs have been implemented to encourage a more thorough examination of patterns of accumulation and exposure. In the early 1980s, DuPont and the VDEQ created a trust fund designed to promote the monitoring of mercury in water, fish, and sediment throughout the Shenandoah River basin for a 100-year period (VDEQ 2000, Murphy 2004). In 2000, the South River Science Team (SRST), a collaborative group composed of individuals from state and federal agencies, citizen groups, academia, and industry, was created with the explicit intent of performing damage assessments on the South River (Murphy 2004). To date, the SRST has conducted a number of studies concerning the distribution and cycling of mercury as well as accumulation and effects in a wide variety of organisms including clams, insects, mammals, and amphibians (Brasso 2007). In 2005, Daniel Cristol began a study of mercury accumulation and associated effects on birds breeding along the South River. While several studies performed by the SRST had focused on various species in the aquatic ecosystem, none had yet attempted to examine whether terrestrial organisms, such as songbirds, might also be at significant risk of exposure (Brasso 2007).

Mercury Contamination in South River Birds

Traditionally, mercury has been viewed as an aquatic problem (e.g. Wiener et al. 2003). As a result, most research on mercury in birds has tended to focus exclusively on large piscivorous or predatory species at the top of long aquatic food webs. In contrast, the

threat of aquatic mercury to terrestrial songbirds was long ignored. By sampling 18 species of birds breeding along the South River and nearby reference tributaries, Cristol et al. (2008) discovered that many terrestrial songbird species accumulated even higher levels of mercury than piscivorous species such as the Belted Kingfisher (*Ceryle alcyon*). Furthermore, the mercury levels on the South River were some of the highest ever reported in any species of songbird, terrestrial or aquatic. Dietary analysis revealed that terrestrial species appear to be primarily exposed through the consumption of contaminated spiders, which often accumulated higher levels of mercury than those in the fish consumed by kingfishers (Cristol et al. 2008). Thus, aquatic mercury may pose a significant risk to purely terrestrial organisms, which were long thought to be immune to harm.

In 2005, as part of an initial attempt to examine mercury accumulation and possible effects in birds breeding along the South River, Masters student Rebecka Brasso established a nest box trail along the South River as well as two nearby tributaries with no history of mercury contamination, the North and Middle Rivers (see site descriptions below). In 2005 and 2006, Brasso sampled and monitored Tree Swallows breeding in the area (Brasso 2007). Over the two years of her study, the average blood mercury level of adult Tree Swallows was 3.66 ± 2.41 ug/g Hg (n = 79) on contaminated sites compared to 0.17 ± 0.13 ug/g Hg (n = 94) on reference sites (Cristol et al. 2008). These levels are essentially equivalent to those observed in Belted Kingfishers breeding in the same area, and are the highest ever reported in free-living Tree Swallows (Brasso and Cristol 2008). Despite these significantly elevated mercury levels, adverse effects were difficult to detect. In 2005, the first year of the study, no adverse effects on reproduction were

detected (Brasso and Cristol 2008). In 2006, the authors found that inexperienced SY females were differentially affected by high levels of mercury. Although ASY Tree Swallows breeding on contaminated sites bred just as successfully as ASY females on reference sites, contaminated SY females produced smaller eggs and exhibited lower rates of hatching and fledging success than their uncontaminated counterparts (Brasso and Cristol 2008). This led Brasso and Cristol (2008) to conclude that SY females may have been more susceptible to the stress of mercury in their first year of breeding, especially since 2006 was marked by a severe drought (Brasso and Cristol 2008). In addition to producing more hostile conditions for breeding, dietary shifts or changes in methylation rates due to the drought likely accounted for average adult blood mercury levels being twice as high as in the previous year (Brasso and Cristol 2008). A concurrent study documented suppression of some components of the immune system in adult female swallows breeding on the South River (Hawley et al. *in press*). Hawley et al. (*in press*) examined response of both humoral and cell-mediated axes of the immune system to mercury contamination. The authors found evidence of impairment of the humoral, but not cell-mediated, immune response.

Despite the subtle nature of these impairments, these two studies (Brasso and Cristol 2008, Hawley et al. *in press*) detected more adverse effects than most. The use of Tree Swallows in ecotoxicological studies has steadily been increasing due to their many advantages described above. Yet, many such studies have failed to find any effects, despite apparently high concentrations of contaminants (McCarty 2001). In fact, McCarty (2001) argued that Tree Swallows may simply be less sensitive to the effects of pollutants than most other species. Thus, even at high levels, adverse effects may be

difficult to observe. However, it is also possible that the relative paucity of effects is partly a result of the narrow time span over which most ecotoxicological studies have been conducted. Indeed, those studies that have been carried out over longer periods of time have often been most successful in observing biologically meaningful effects, many of which may only become apparent with prolonged exposure after several years on a contaminated site (e.g. Evers et al. 2008). Thus, in order to realistically evaluate the effects of mercury on Tree Swallows breeding on the South River, it is necessary to examine effects on the scale of the lifetime. Fundamental higher-order traits such as survival and lifetime reproductive success may prove to be more reliable metrics of contaminant impact.

OBJECTIVES

While the ecotoxicological literature is replete with both laboratory and field studies examining patterns of bioaccumulation and associated effects of mercury in birds, there are few long-term studies of free-living populations that have attempted to address how contaminants like mercury may alter biologically meaningful traits over the course of an entire lifetime. The short window of time over which most ecotoxicological studies are conducted may prove insufficient for realistically assessing the impact of contaminants. The goal of the present study was to effectively address such uncertainties by examining the effects of mercury on the long-term survival of adult Tree Swallows breeding along the contaminated South River in Virginia.

GENERAL METHODS AND ANALYTICAL APPROACHES

Study Sites

Nest Box Trail. The South River in Virginia was contaminated with industrial mercury between 1929 and 1950 (Carter 1977). In 2005, a nest box trail was established at contaminated sites along the South River and South Fork Shenandoah River as well as nearby sites with no history of mercury contamination on the Middle River, North River, and South River upstream of the point of contamination (Cristol et al. 2008; Figure 1). From 2005-2008, nest boxes were monitored at 36 different sites at various points along the South, Middle, and North Rivers (centroid of study area: 38°10'N, -78°59'W). The exact composition of sites and number of nest boxes located at each site varied by year (Table 1). Because all nest boxes were removed from the South Fork Shenandoah River prior to the 2006 field season, birds nesting at these sites were not included in any analyses and will receive no further attention here (for a full description of South Fork Shenandoah River sites, see Brasso 2007).

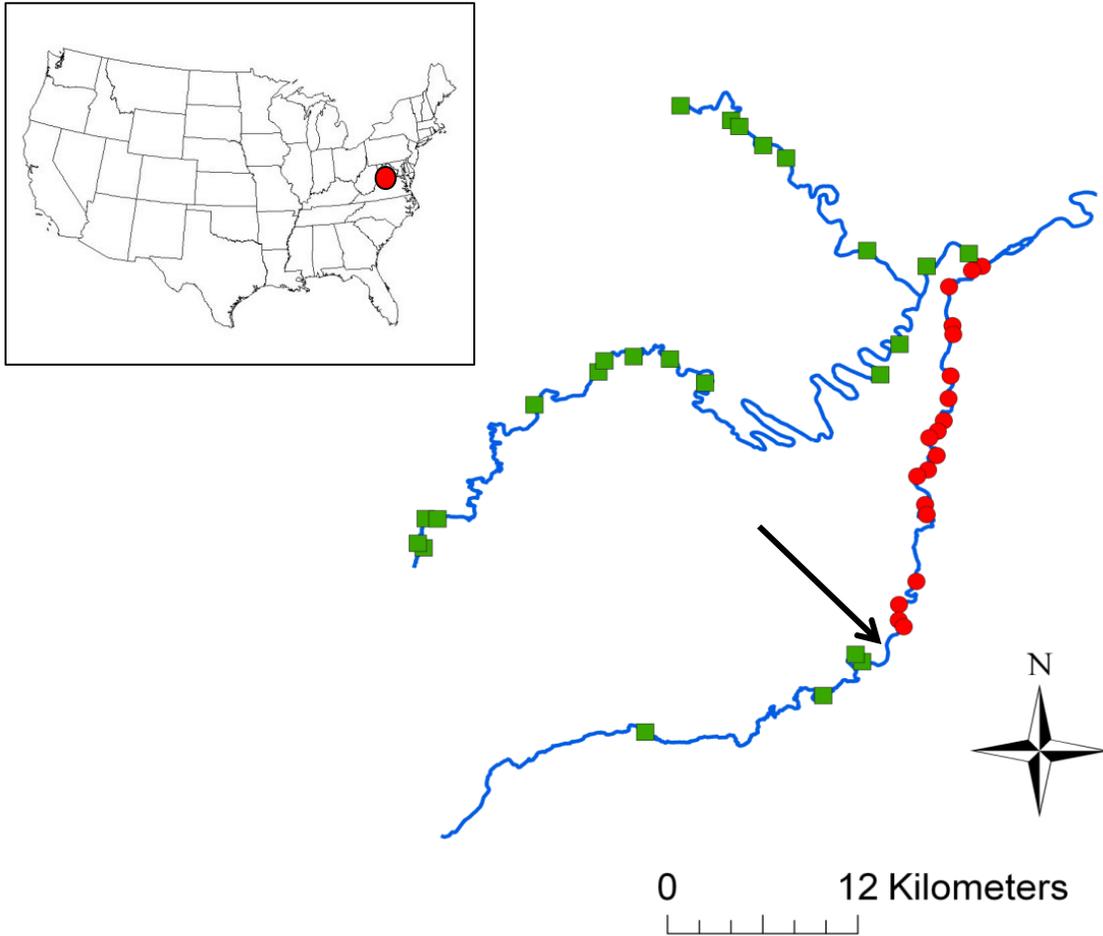


Figure 1: Map of study area in Virginia, USA. Contaminated sites along the South River are indicated by red circles. Reference areas are represented by green squares. The arrow points to the location of the chemical plant in Waynesboro, from which the mercury originated (taken from Cristol et al. 2008).

Table 1a: Contaminated (C) site and box locations in each year of study.

Site	River	Hg Status	# Boxes 2005	# Boxes 2006	# Boxes 2007	# Boxes 2008
BAPA	South	C	5	9	15	14
WH2O	South	C	8	10	10	10
GENI	South	C	8	14	14	27
DOOM	South	C	5	9	8	20
CRIM	South	C	2	3	10	10
WERT	South	C	0	0	7	8
WERTN	South	C	0	0	5	6
AUFC	South	C	21	31	50	83
DUBA	South	C	0	0	1	1
DESP	South	C	0	0	3	12*
BOES	South	C	0	2	6	4
WAMP	South	C	0	6	6	5
HARI	South	C	0	0	0	0
HARR	South	C	0	7	7	7
RENK	South	C	0	8	12	11
COMI	South	C	0	3	9	11
GRCA	South	C	2	7	12	15
GRCP	South	C	10	16	19	20
BRAD	South	C	0	4	0	0
TOTAL C			61	129	194	264

*8 nest boxes erected by local landowner

Table 1b: Reference (R) site and nest box locations in each year of study.

Site	River	Hg Status	# Boxes 2005	# Boxes 2006	# Boxes 2007	# Boxes 2008
SCOW	South	R	12	16	15	15
PBUC	South	R	0	11	13	13
SRDG	South	R	6	11	12	15
SLOC	South	R	7	7	7	7
MWHI	Middle	R	15	20	20	39**
MOPW	Middle	R	0	17	17	25
MGOD	Middle	R	8	14	13	13
MSMP	Middle	R	10	17	17	37
MFOR	Middle	R	4	4	4	4
MDOR	Middle	R	6	6	8	15
MRBR	Middle	R	0	8	8	8
MSHA	Middle	R	3	5	5	5
NCRA	North	R	10	10	10	12
NSBP	North	R	0	4	2	9***
NWWP	North	R	0	6	6	8
NAUC	North	R	4	4	3	8
276B	North	R	0	7	7	7
TOTAL			85	167	167	240
R						

19 nest boxes erected by local landowner; *2 nest boxes erected by local landowner

Tree Swallows typically nest in open fields with nearby bodies of water over which they can forage (Robertson et al. 1992). Nest boxes for Tree Swallows were thus placed in areas which fit these criteria. However, as part of a larger study, additional nest boxes were provided in other kinds of habitat for Carolina Chickadees (*Poecile carolinensis*), Carolina Wrens (*Thryothorus ludovicianus*), House Wrens (*Troglodytes aedon*), and Eastern Bluebirds (*Sialia sialis*). Tree Swallows occasionally nested in boxes intended for these other species. Although such boxes were similarly monitored, they are not included in the nest box totals calculated in Table 1. In 2005 and 2006, all nest boxes were located within 50 meters of each river. However, in 2007 and 2008, nest boxes were placed at distances up to 450 meters from the shoreline, and many of these attracted Tree Swallows. Prior to 2005, very few, if any, Tree Swallows nested in the study area due to the lack of suitable wetland habitat. Following the beginning of this study, a small number of Tree Swallows were observed in nest boxes put up by local landowners for Eastern Bluebirds; in nearly all cases, such boxes were subsequently adopted into the study and monitored in exactly the same manner (Table 1).

Nest boxes were placed in cropland or pasture within 450 meters of each river (see above) and approximately 25 meters apart. Boxes were constructed using a popular bluebird nest box design (North American Bluebird Society 2009), and were fitted with “stovepipe” predator guards (Erva Tool, Chicago, Illinois) in an attempt to reduce failure due to snake and mammalian predation (Gowaty and Plissner 1998). Each box was 23.8 cm deep with a floor-hole height of 16.5 cm. The floor area was 16 cm² and the entrance hole was 3.8 cm in diameter. As nest box dimensions have been shown to affect clutch

size in Tree Swallows (Rendell and Robertson 1993), this standardization of box dimensions across all sites was important.

Site Characteristics. Of the 36 sites used in this study, most consisted of local parks or privately owned properties adjacent to each river. In order to document variation in the specific habitat characteristics possessed by each site, I used Geographic Information Systems (GIS) software (ArcView 9.2, Environmental Systems Research Institute, Inc., Redlands, Calif.) to analyze habitat structure. I examined digital orthophoto quarter quad images and delineated sites by creating circular buffers centered on the midpoint of the most upstream and most downstream nest box at each site. Buffers were of variable size such that each consisted of the minimum radius necessary to enclose every nest box at a particular site. Although sites were operationally defined as separate if they possessed different access points (see Table 1), it was often the case that two or more sites were separated in space by only a few hundred meters or were across a river from one another. Thus, in describing habitat characteristics in GIS, I grouped those sites that were geographically and biologically contiguous. Such groupings resulted in the creation of 27 distinct sites as defined by GIS buffers. Relative proportions of each major habitat type were then determined using the National Land Cover Data layer (Multi-Resolution Land Characteristics Consortium, 2008; Table 2).

Table 2: Proportion of major habitat types at each site. Land cover habitat types were grouped according to the following scheme: % Forest (deciduous, mixed, and evergreen), % Developed (developed: high, medium, or low intensity, and open space), % Fields (cultivated crops and pasture/hay), % Open Water, and % Other (emergent herbaceous wetlands). The total site area enclosed by the GIS buffer is also provided. Hg Status refers to contaminated (C) or reference (R).

Site	Hg Status	% Forest	% Developed	% Fields	% Open Water	% Other	Total Area (m ²)
276B	R	10.7%	24.2%	17.0%	48.2%	0.0%	15364
AUFC	C	8.8%	9.7%	80.9%	0.6%	0.0%	1738672
BAPA/WH2O	C	17.7%	62.8%	17.0%	2.5%	0.0%	515130
BOES	C	86.9%	0.0%	13.1%	0.0%	0.0%	90720
BRAD	C	16.6%	33.4%	50.0%	0.0%	0.0%	635985
CRIM/WERTN	C	26.2%	16.9%	49.7%	7.3%	0.0%	237467
DESP	C	47.1%	8.4%	44.4%	0.0%	0.0%	183124
DOOM	C	14.4%	40.2%	45.4%	0.0%	0.0%	188470
GENI	C	18.1%	54.5%	27.3%	0.0%	0.0%	635985
GRCA/COMI	C	48.5%	31.4%	16.1%	4.1%	0.0%	534388
GRCP	C	30.1%	23.0%	39.8%	7.1%	0.0%	816914
HARI	C	88.7%	0.0%	11.3%	0.0%	0.0%	24847
HARR	C	0.0%	82.1%	17.8%	0.0%	0.0%	12442
MFOR	R	12.6%	20.0%	48.9%	18.6%	0.0%	49035
MGOD/MSMP	R	2.0%	4.8%	88.7%	4.5%	0.0%	1480601
MRBR/MDOR	R	43.6%	3.8%	52.5%	0.0%	0.0%	789907
MSHA	R	64.2%	4.0%	31.8%	0.0%	0.0%	24291
MWHI/MOPW	R	13.5%	10.7%	75.8%	0.0%	0.0%	530509
NSBP	R	0.0%	20.9%	70.0%	9.1%	0.0%	52630
NWWP/NCRA/ NAUC	R	15.0%	31.7%	44.1%	9.2%	0.0%	782048
PBUC	R	13.6%	7.4%	73.5%	5.5%	0.0%	717603
RENK	C	30.9%	19.9%	44.9%	4.3%	0.0%	197818
SCOW	R	13.9%	6.6%	78.7%	0.0%	0.7%	512589
SRDG/SLOC	R	0.0%	100.0%	0.0%	0.0%	0.0%	839495
WAMP	C	25.5%	1.2%	73.3%	0.0%	0.0%	141107
WERT	C	27.2%	3.3%	63.2%	6.3%	0.0%	543497

Capture and Sampling of Tree Swallows

Capture of Adults. Adult Tree Swallows were captured in their boxes either during incubation or the nestling stage by one of several methods. Some females (and a few males) were caught by hand on the nest directly during incubation or brooding. More often, neither adult was initially found inside the nest box so one of two trapping methods was employed. In the first, a small metal door was affixed to the interior of the nest box with a piece of duct tape and propped up inside the box with a small twig or piece of grass (Stutchbury and Robertson 1986; Figure 2). A second method was devised during this study (Friedman et al. 2008; Figure 2), in which the trap door was controlled by an observer holding an attached piece of 4-6 lb monofilament while stationed 30-50 meters away (Figure 2). The advantage of this latter method is that the visual stimulus of the prop is removed, making a particularly wary adult more likely to enter the nest box unperturbed. It should be noted that there was considerable variation in trapping effort across years and between the sexes. In 2005, only breeding females were targeted for capture while males were sampled opportunistically. In 2006-2008, a much greater emphasis was placed upon catching adults of both sexes and an effort was made to capture all females breeding in the population. In the majority of cases, capture effort at each nest was limited to one hour. If one or both adults were not captured in this time, further attempts were made on successive days.

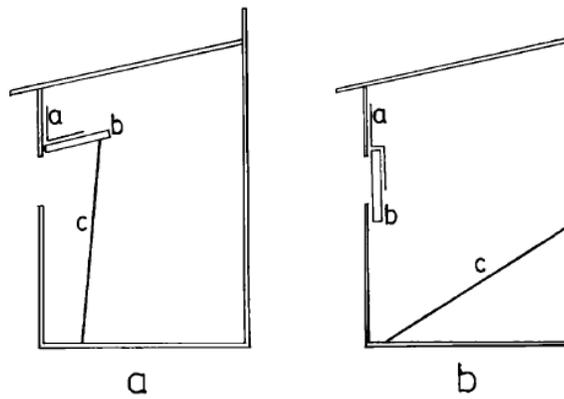


Figure 2a: Simple nest box trap described by Stutchbury and Robertson (1986). The two nest boxes represent the trap prior to (a) and following (b) entry of the bird. Within each image, a = duct tape, b = trap door, c = twig (taken from Stutchbury and Robertson 1986).

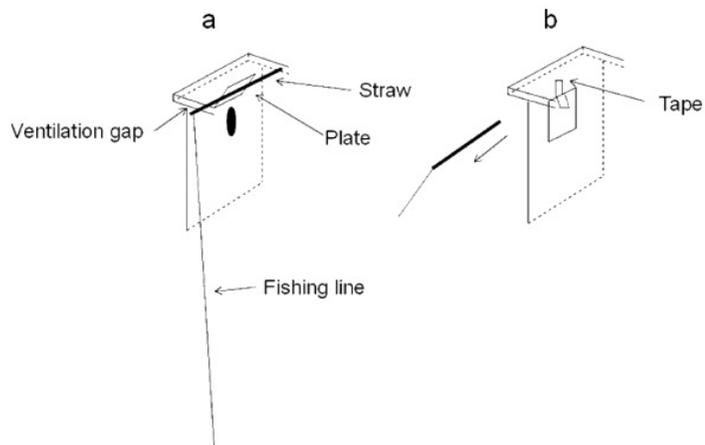


Figure 2b: Improved simple nest box trap described by Friedman et al. (2008). The two nest boxes represent the trap prior to (a) and following (b) entry of the bird (taken from Friedman et al. 2008).

Sampling of Adults. Upon capture, each adult was uniquely banded with a USGS metal band or, in the case of a returning bird, the band number was recorded. Sex was determined by the presence of a brood patch (in females) or cloacal protuberance (in males). Females were aged by plumage as either SY or ASY (see above) and wing chord and body mass were recorded. Following capture, a blood sample was extracted by puncturing the cutaneous ulnar (brachial) vein using a small gauge (26G ½) needle. Approximately 100 µL of blood was then collected in two or three 75 µL heparinized capillary tubes which were subsequently sealed with Critocaps[®]. Capillary tubes were then placed in a 10 cc BD[®] vacutainer to prevent breakage. Each vacutainer was stored inside a labeled Ziploc[®] bag which was placed on ice for a maximum of 8-10 hours before undergoing storage at -25°C until analysis. Additionally, 8-10 back feathers were plucked and the innermost primary (P1) was pulled (2006-2007) or clipped (2008) from each wing. These feathers were similarly placed in a labeled Ziploc[®] bag and stored following the same protocol as for blood. Blood mercury levels reflect recent dietary uptake while feather mercury levels tend to be more diagnostic of long-term exposure and overall body burden (Evers et al. 2005). Thus, sampling both tissues allows for an examination of current as well as historical patterns of accumulation. Following sampling, adult Tree Swallows were released and allowed to return to their nests. Typically, one or both parents reentered the nest box within minutes of release.

Several permutations in sampling occurred across the four years of study as a result of related projects occurring on the South River. In 2005, the two second-to-outermost rectrices (tail feathers) were plucked from each adult and no primaries were removed. In 2006, 8-10 chest feathers were taken from each returning adult for use in a

separate study on pigmentation. Additionally, in 2006-2007, a subset of breeding females (n = 41 in 2006, n = 51 in 2007) was used in a study of the effects of mercury on humoral and cell-mediated components of the avian immune system (Hawley et al. *in press*). In 2006, each female was captured initially to determine whether she was a banded bird from 2005. If the female was found to be unbanded, an observer returned after 2-3 days (when nestlings were approximately 4 days old), at which time the female was captured again. Upon capture, the right patagium (wing web) was injected with 30 μ L of a 0.15 mg/mL phytohaemagglutinin (PHA) suspension (Sigma-Aldrich, St. Louis, MO, USA) in phosphate-buffered saline (PBS). Immediately prior to and 24-26 hours after injection, patagial width was measured to 0.01 mm using a micrometer (five measurements taken each time). Following the final PHA measurement, 25 of these females were intra-abdominally injected with 5×10^7 sheep red blood cells (SRBCs) (MP Biomedicals, Irvine, CA, USA) suspended in 50 μ L of PBS. Blood samples were collected just prior to and eight days after SRBC injection. Such procedures have been carried out on adults and nestlings in numerous studies with no detectable prolonged stress to the birds (Ardia 2005). To verify this, Brasso (2007) performed analyses of reproductive success with and without Tree Swallows used in the immunocompetence study in 2006 and found no differences in results. In 2007, the PHA response of 51 additional Tree Swallows was tested in a manner similar to that described for 2006; however, no SRBC assays were performed on any birds. Because the immunocompetence tests did not appear to negatively impact swallows (Brasso 2007), all 92 females sampled across the two years are included in my analyses.

Collection of Eggs. In 2006 and 2007, several clutches of eggs were collected in order to assess the relationship between female blood and egg mercury levels. Eggs were collected and nests removed shortly following the onset of incubation. Swallows that had lost their clutches often renested either in the same box or elsewhere on our study sites. Any swallows for which eggs were collected during the course of this study were excluded from subsequent analyses of survival. In contrast, swallows that renested after experiencing natural failure remained in such analyses.

Laboratory Analyses

Mercury Analysis. Traditionally, most mercury analysis has been performed by cold vapor atomic absorption spectroscopy (CVAA), in which Hg^{2+} is transformed into Hg^0 by way of a strong reducing agent, exchanged into the gas phase, and then moved into an absorption cell for analysis. Prior to analysis, this method requires a digestion that releases particle-bound Hg^{2+} to solution, freeing it for subsequent conversion to Hg^0 . More recently, a method has been developed that allows one to forego the digestion step. In this method, a researcher combusts samples, traps released mercury on a gold surface, and then releases the trapped mercury as a concentrated slug for subsequent analysis by atomic absorption. Such a method is particularly well suited to samples of small mass (0.1-0.2 grams), meaning that its greatest utility is found in analysis of tissues or sediments (for which appreciable amounts of mercury may be found in small samples, as opposed to atmospheric or water samples).

In the present study, all tissue samples were analyzed on a Milestone Direct Mercury Analyzer (DMA) 80 at either the Trace Elements Research Laboratory at Texas

A&M University (TERL) or at the College of William and Mary (WM). Samples were weighed into pre-combusted boats which were then placed into slots on a 40-position autosampler carousel. Each boat was moved into the instrument in turn by a pneumatic arm and subjected to a sequence of heating steps while under a constant flow of oxygen gas (O_2). During this stage, samples are first dried and then combusted. The combusted gas then passes through a heated catalyst followed by a gold trap, which binds the gaseous mercury present in the sample. Following adhesion, the gold trap is heated, causing it to release the bound mercury into the gas stream, at which point the concentrated slug is moved into a two-stage absorption cell. Inside this cell, free mercury (Hg^0) atoms absorb light from a mercury vapor lamp. The amount of light that is absorbed is directly proportional to the amount of mercury present in the sample. Thus, one can quantify total mercury content by examining the two absorbance peaks produced for each sample, one from the highly sensitive long path cell, and the other from the far less sensitive short cell. By comparing the sample peak absorption with those of calibrated standards, it is possible to determine total mercury content with a high degree of accuracy.

Quality Assurance/Quality Control. A number of quality assurance/quality control (QAQC) metrics were regularly assessed in order to evaluate the performance of the DMA-80s in both laboratories. Prior to the first and following the last set of samples run each day, a standard suite of blanks and certified reference materials (CRMs) was analyzed. Blanks consisted of empty carousel slots and cleaned boats which should possess no mercury, while CRMs are substances containing known quantities of mercury,

the exact values of which have been previously verified by multiple independent laboratories. In the present study, the CRMs used were DOLT-2 (2140 ppb Hg) and DORM-2 (4640 ppb Hg), which are typically used for studies involving tissue samples. Blanks and CRMs were placed at the beginning and end of each batch of samples in order to provide a continuous evaluation of instrument performance.

In addition, a number of duplicate and “spiked” samples were run for QAQC. In the field, blood was sampled in duplicate or triplicate capillary tubes. Thus, it was often possible to run two or three different samples collected from the same bird at the same time in order to check the accuracy of the DMA. At both TERL and WM, one duplicate sample was analyzed for every 15 to 20 samples run. One can then compare the percent difference between the original and duplicate sample to determine the precision of the instrument according to the following equation:

$$RPD = (D1-D2)/((D1 + D2)/2) \times 100$$

where RPD is the relative percent difference, and D1 and D2 are the mercury concentrations of the original and duplicate samples, respectively. Typically, a DMA is considered to be performing adequately when 95% of the duplicate samples have an RPD less than or equal to 20%.

A “spike” is a normal sample, usually with a low mercury value, to which a known amount of mercury has been added. Samples were spiked using a known mass of one of the CRMs. By running spikes, one is able to evaluate the accuracy of mercury levels being reported by the DMA. This provides more information than simply running

a CRM sample, because the efficiency at extracting mercury from the tissue matrix is also factored in. As with duplicate samples, one spike was run for approximately every 20 samples analyzed. For spiked samples, one is typically concerned with the amount of mercury added to the sample that is subsequently detected by the instrument. Thus, one can examine percent recovery:

$$\% \text{ Recovery} = (\text{Spiked value} - \text{Unspiked value}) / (\text{Spike amount}) \times 100$$

Sample Preparation. Blood samples required no preparation and could be directly transferred to DMA “boats” following extraction from freezers. All blood mercury levels in the present study are reported as wet (i.e. not freeze-dried) weight values.

Mark-Recapture Analysis

One of the major objectives of ecological research is to accurately describe properties influencing the abundance and distribution of populations, such as population size, birth and death rates, and dispersal. In an ideal world, one could measure these variables directly. However, such direct measurement requires complete knowledge of the fate and whereabouts of every individual in a population, something which is often not available. Thus, in most cases, important demographic variables must be estimated using alternative sampling methods. One sampling method that has come to the forefront of ecological research is mark-recapture analysis, in which a representative subset of the population is sampled in order to infer population-level phenomena. Briefly, individuals are captured and marked before being released back into the population, at which point they are able

to move about freely. However, because it is often impossible to capture all individuals, only a subset of the population will actually be marked. At a later time, the population is again sampled and some marked individuals will be among those captured. Such analyses depend on the assumption that the proportion of marked individuals recaptured on the second occasion is representative of the proportion of all individuals marked on the first occasion. In other words, if one measures the number of marked (R) and unmarked (C) individuals caught during the resampling effort, and the original number of marked birds (M), then it should be possible to estimate the original population size (N) according to the following relationship:

$$C/R \approx N/M$$

While such an approach might appear straightforward, there are significant problems with employing the use of return rates (shown in the equation above) as a proxy for estimating survival. Such deficiencies stem from the fact that a marked individual may fail to be recaptured for one of two reasons. First, an individual may have died or permanently emigrated from the population. However, a second possibility is that the individual was alive and present in the population, but was simply not detected during the sampling effort. Thus, there are actually two criteria that must be satisfied in order for an individual to be recaptured: the individual must be alive at the time of resampling, and given that it is alive and present, it must be detected. Therefore, differences in return rate between two populations could reflect differences in rate of either survival or recapture.

Survival is not interchangeable with return rate; rather, it is one component nested within it. Stated another way,

$$\text{Return Rate} = P(\text{Survival}) \times P(\text{Encounter} \mid \text{Survival})$$

Several statistical modeling programs have been developed to estimate rates of survival and recapture. Program MARK (White and Burnham 1997) is one such program that has been used extensively to estimate the survival of marked populations under a suite of environmental conditions and evolutionary constraints. For the standard Cormack-Jolly-Seber (CJS) model, which is used for studies involving live recaptures, there are three different types of data which must be specified for each marked individual. First, the basic structure upon which MARK operates is the encounter history. The encounter history consists of a series of 0's and 1's which describe the sampling history of an individual over the course of the study. These encounter histories can be further sub-divided into groups (e.g. age classes, sex, or sites) or constrained according to individual covariates (e.g. morphological measurements or contaminant levels). For example, encounter history 10010 indicates that the animal was captured and marked on the first sampling occasion, was not seen on either the second or the third occasions, was detected again on the fourth occasion, and finally, was absent during the fifth period of sampling. This example serves to demonstrate why it would be inaccurate to use return rate to approximate survival. Although the animal was alive on occasions 2 and 3, as evidenced by its reappearance on occasion 4, simply counting 0's and 1's would render the animal dead on the second and third sampling occasions and alive again on the

fourth. To avoid such obvious errors, MARK incorporates both survival (Φ) and detection (p) probabilities into its description of each encounter history. Let Φ_t equal the probability that an animal that is alive at time t is still alive at time $t+1$, and p_t equal the probability that an animal alive in the study area at time t is captured. For the preceding example, the probability of encounter history 10010 is given by the following:

$$P(10010) = \Phi_1(1-p_2) \cdot \Phi_2(1-p_3) \cdot \Phi_3p_4 \cdot [(1-\Phi_4) + \Phi_4(1-p_5)]$$

This same formula can be derived for every possible encounter history over five sampling occasions. By examining the specific distribution of encounter histories present in a given sample, it is then possible to estimate Φ and p according to maximum likelihood theory. Maximum likelihood theory is based on the premise that the most likely solution is the one which best fits the data at hand. In the context of a survival analysis performed in MARK, one could calculate which combination of values for Φ and p would best explain the specific distribution of encounter histories observed for a marked population. In this way, MARK derives estimates of Φ and p that are most likely correct given the constraints of the data and the specific *a priori* hypotheses being tested.

Alternative hypotheses can be represented as statistical models such that each model represents a different group of factors that could be exerting an effect on either survival or encounter rate. Such factors can take the form of classes, such as age or sex, or individual covariates, such as mercury level. In addition, each model can specify whether survival or encounter rate varies or is constant over time. The exact suite of models chosen (i.e. the model set) is at the discretion of the particular researcher and

should be carefully chosen *a priori* with biological plausibility and relevance in mind. In order to determine which of the candidate models are best supported by the empirical data, one can estimate Kullback-Leiber information (the information lost when a model is used to approximate reality). One quantity commonly used for estimating Kullback-Leiber information is Akaike's Information Criterion (AIC). AIC values are calculated through the integration of two different pieces of information. The first is the maximized log-likelihood ($\log_e(L\theta | data)$) over the unknown parameters (θ). While maximized log-likelihoods are the basis for describing the fit of any particular model to the data, alone, they are insufficient to accurately assign priority to candidate models. This is because of the statistical phenomenon that as a greater number of parameters are added to any model, a greater proportion of the variance in the data is explained simply as a result of having more factors to account for such variation. In order to correct for this problem, AIC values assign a penalty to log-likelihoods with greater numbers of estimable parameters (K). These "corrected" log-likelihoods can then be assessed to determine which model(s) has the greatest explanatory power. Thus, AIC scores are calculated according to the following formula:

$$AIC = -2\log_e(L\theta | data) + 2K$$

where lower scores are considered to have greater support in the data. If K is large relative to the sample size (n), a small-sample version called AIC_c may be used:

$$AIC_c = -2\log_e(L\theta | data) + 2K + ((2K(K + 1))/(n - K - 1))$$

Regardless of the specific version employed, several standard rules exist for interpreting models ranked by AIC values. First, an AIC score is calculated for each candidate model in the model set. The model with the lowest AIC score is considered to have the greatest empirical support. For ease of interpretation, the best supported model (e.g. lowest AIC score) is rescaled such that its Δ_i value is 0.0. All subsequent models are rescaled as simple differences relative to this model:

$$\Delta_i = AIC_i - \min AIC$$

where Δ_i equals the difference between the AIC score for model i and the minimum AIC score among the candidate models ($\min AIC$). Typically, Δ_i values less than 2 are considered to have substantial support in the data, those between 3 and 7 are considered to have moderate support, and Δ_i values greater than 10 are thought to have very little support. It is possible to further quantify the relative support assigned to each model by using a simple transformation, $\exp(-\Delta_i/2)$. These transformed values can then be normalized such that they sum to one:

$$w_i = \exp(-\Delta_i/2) / (\sum \exp(-\Delta_i/2))$$

where the Akaike weights (w_i) equal the ‘weight of evidence’ in favor of any particular model, i . Because they sum to one, Akaike weights can be interpreted as probabilities, with higher values indicating greater empirical support. By comparing Akaike weights, it

is thus possible to determine whether specific factors, such as mercury contamination, may be exerting a differential effect on survival.

CHAPTER 2: SURVIVAL OF ADULT
TREE SWALLOWS (*Tachycineta bicolor*) AT A SITE CONTAMINATED BY
MERCURY

INTRODUCTION

Mercury is a heavy metal that has become a ubiquitous contaminant in aquatic and terrestrial ecosystems worldwide, primarily as a result of human activity. Although most mercury that is released into the environment is inorganic, it is readily transformed into the more toxic methylmercury by sulfur-reducing bacteria in aquatic sediments.

Methylmercury accumulates in animal tissue and concentrates at the top of food webs, potentially causing severe health problems in wildlife (Wolfe et al. 1998). In birds, the list of mercury-related impairments is substantial, with several studies documenting reproductive deficits (Heinz 1979, Barr 1986, Brasso and Cristol 2008), suppression of the immune system (Kenow et al. 2007, Hawley et al. *in press*), and behavioral abnormalities (Nocera and Taylor 1998, Bouton et al. 1999), among others. Although an adverse effect on any one of these traits may be relatively inconsequential for the organism as a whole, in combination, such impairments may act to significantly reduce higher-order life history traits such as survival or lifetime reproductive success.

However, few studies have examined the long-term effects of mercury on these important demographic parameters (but see Evers et al. 2008, Mitro et al. 2008). Indeed, the short time frame in which many ecotoxicological studies are undertaken often precludes analysis of long-term changes in population structure and function.

The Tree Swallow (*Tachycineta bicolor*) is an insectivorous, migratory songbird that breeds throughout the northern half of North America (Robertson et al. 1992). In recent years, Tree Swallows have come into wide use in a range of ecotoxicological

studies, especially those concerning polychlorinated biphenyls (PCBs) and mercury (McCarty 2001). Several characteristics make Tree Swallows particularly well-suited as a model organism in studies of environmental contaminants. They readily adopt artificial nest cavities and tend to forage within 400 m of their nests (Mengelkoch et al. 2004) on a diet comprised of both terrestrial and aquatic emergent insects (Robertson et al. 1992). Thus, one can obtain highly localized estimates of aquatic or terrestrial contaminants from a large number of individuals at a site of the researcher's choosing. Annual adult survival of Tree Swallows is estimated to be approximately 40-60% (Chapman 1955, Houston and Houston 1987) with little evidence of differences according to age or sex (Robertson et al. 1992). In general, adult philopatry to breeding sites is thought to be high. Winkler et al. (2004) reported that only 4% of males and 14% of females moved between study sites across years, most commonly in response to reproductive failure. Thus, adults are unlikely to permanently emigrate from a study area, greatly facilitating long-term studies of marked individuals across multiple breeding seasons.

Despite their general tractability, many studies involving Tree Swallows have failed to detect adverse impacts of contaminant exposure, leading to some speculation that Tree Swallows may be unusually resilient in the face of environmental perturbation (McCarty 2001). However, there are several alternative explanations for the apparent lack of effects. First, several of these studies have reported contaminant concentrations in the range of background levels (e.g. Gerrard and St. Louis 2001), suggesting that the paucity of effects may be, in some cases, due to insufficient exposure. Second, even at higher concentrations, researchers may fail to detect effects if such impairments emerge only after sustained exposure. Finally, the complex nature of ecosystems may make

pinpointing actual causal relationships between contaminant exposure and effects far more difficult in field studies than in controlled laboratory settings. The goal of the present study was to effectively address such uncertainties by examining the impact of high levels of mercury on the long-term survival of a free-living population of Tree Swallows.

The South River, a tributary of the South Fork Shenandoah River in Virginia, was contaminated with mercury from an industrial source between 1929 and 1950 (Carter 1977). From 2005-2006, Cristol et al. (2008) documented significantly elevated mercury levels in nearly all species of birds sampled along the contaminated portion of the South River, including Tree Swallows. Additionally, in 2005, a nest box trail was established along the South River and two adjacent uncontaminated tributaries, the North and Middle Rivers, in order to monitor the reproductive success of Tree Swallows breeding in the area. Preliminary work by Brasso and Cristol (2008) suggested reduced reproductive success in yearling (second-year, hereafter “SY”) females breeding on the contaminated sites. However, this pattern was only observed in one of the two years of the study, and was largely attributed to increased mercury levels and a severe drought in 2006 coupled with the reproductive inexperience of the SY females (Brasso and Cristol 2008). A concurrent study conducted by Hawley et al. (*in press*) found that adult female Tree Swallows exhibited suppression in one, but not in another, component of the immune system. Such results not only suggest that mercury could be exerting a strong effect on other higher-order life history traits, such as survival, but the annual variation and subtlety of response further highlight the necessity for the long-term monitoring of marked individuals over the course of an entire lifetime. To this end, I continued to

monitor the population for two additional years, yielding a contiguous four-year data set of banding records (2005-2008), from which inference concerning rates of annual survival could be made.

One of the primary goals of ecological research is to accurately describe those factors and demographic properties governing the abundance and distribution of populations. Estimating parameters such as birth and death rates, population size, and dispersal can be particularly important for understanding how populations will change over time, especially since many species are currently contending with a battery of anthropogenic stressors such as mercury. Ideally, one could measure these parameters directly; however, this is often not possible since direct measurement requires complete knowledge of a population, something which is rarely feasible, particularly for highly mobile, widely dispersed, and migratory animals such as swallows. As a result, a number of sampling techniques have been developed to allow researchers to estimate these important demographic properties. One of the most commonly employed is capture-mark-recapture analysis, in which a portion of the population is sampled and individually marked before being released back into the population. One can then make inferences about population-level parameters based on the proportion of marked and unmarked individuals captured on subsequent sampling occasions. In this way, it is possible to examine how various types of stressors, such as contaminants, may be impacting the structural and functional integrity of populations. My objective was to use capture-mark-recapture techniques to assess whether adult Tree Swallows breeding on the South River were suffering reduced survival as a result of mercury exposure.

MATERIALS AND METHODS

Study Sites and Nest Boxes

Beginning in 2005, nest boxes were erected at 36 sites along the South, Middle, and North Rivers in Augusta and Rockingham Counties, Virginia, USA (centroid of study area: 38°10'N, -78°59'W). Boxes were constructed following a popular bluebird nest box design (North American Bluebird Society 2009) and each was fitted with a “stovepipe” predator guard that almost entirely eliminated snake and mammalian predation (e.g. nest failure due to predation, abandonment, and disruption by House Sparrows [*Passer domesticus*] was < 10% in 2005-2007). Boxes were placed approximately 25 m apart in cropland or pasture, within 50 m of river shoreline in 2005-2006, and up to 450 m thereafter. In 2005, 146 nest boxes were available. This number was increased to 296, 361, and 504 before the breeding seasons of 2006, 2007, and 2008, respectively. There is no natural wetland habitat suitable for Tree Swallow nesting in the study area, and prior to the establishment of the nest box trail, few, if any, Tree Swallows were nesting on or near any of the sites.

Capture and Sampling

Adult Tree Swallows were captured in their nest boxes during incubation or the nestling period either by hand or using one of two trapping methods (Stutchbury and Robertson 1985, Friedman et al. 2008). Sex was determined by the presence of a brood patch (in females) or cloacal protuberance (in males). Tree Swallows are a rarity among birds in that females, but not males, exhibit delayed plumage maturation (Robertson et al. 1992). Thus, adult females could be aged as either SY or after-second-year (hereafter, “ASY”)

(Robertson et al. 1992). In contrast, all adult males have a similar plumage and can only be aged as after-hatch-year (hereafter “AHY”). Upon capture, each individual was uniquely banded with a USGS aluminum band or the band number was recorded (in the case of returning birds). In addition, a small blood sample was extracted by puncturing the cutaneous ulnar (brachial) vein using a small gauge (26 ½) needle. Approximately 100 uL of blood was collected in two or three 75 uL heparinized capillary tubes which were subsequently sealed with Critocaps[®] and placed in a 10 cc BD[®] vacutainer to prevent breakage. Each vacutainer was then stored inside a Ziploc[®] bag and placed on ice for a maximum of 8-10 hours before being transferred to a -25°C freezer for permanent storage. Additionally, 8-10 back feathers were plucked and the innermost primary (P1) was removed from each wing for use in another study. Blood mercury levels are thought to reflect short-term dietary uptake of mercury on the order of a few weeks (Evers et al. 2005) and should thus provide an accurate metric of exposure during the current breeding season. Following sampling, adults were released and usually returned to their nests within a matter of minutes.

Mercury Analysis

Mercury analysis was conducted at either the Trace Elements Research Laboratory (TERL) of Texas A&M University or at the College of William and Mary (WM). Blood samples were analyzed for total mercury on a Milestone[®] DMA 80 using cold vapor atomic absorption spectroscopy (as in Cristol et al. 2008). Since approximately 95% of the mercury present in avian blood is in the organic form (Evers et al. 2005, D.A. Cristol, unpublished data), total mercury concentration should yield a reasonable approximation

of methylmercury present in tissues. All mercury values are presented as wet/fresh weight (ug/g) concentrations.

Statistical Analyses

Mercury Accumulation. I constructed a general linear model (GLM) designed to examine factors important in influencing bioaccumulation of mercury in Tree Swallow blood. I used mercury status (contaminated or reference), age class (ASY, SY, or AHY), and year as fixed effects and included all two-way interactions in the model. Only those terms that were significant in the overall model are reported. I hypothesized that probability of survival was affected by previous, rather than current, mercury exposure. Therefore, blood samples collected in the final year of the study (2008) were not considered as factors in any mark-recapture analyses and were similarly omitted from the mercury bioaccumulation GLM.

Mark-recapture Analysis. I used Cormack-Jolly-Seber (CJS) models in Program MARK (White and Burnham 1997) to examine patterns of survival in mercury-contaminated swallows. The Cormack-Jolly-Seber model allows one to estimate rates of apparent survival and detection for studies involving live recaptures of marked individuals. The basic code upon which MARK operates is the encounter history. Each encounter history is unique to an individual and consists of a series of '1's and '0's signifying either the presence or absence of that individual on each sampling occasion. For example, the encounter history '1010' would represent a bird that was marked for the first time on occasion 1, was alive but remained uncaught on occasion 2, was recaptured on the third

occasion, and was absent on the fourth and final sampling occasion. Such an example serves to demonstrate why it is often inaccurate to use return rates as an approximation of survival. Although the bird was alive on occasion 2, the use of return rates would lead one to erroneously conclude that the individual was dead during the second sampling interval before reemerging on the third. In order to avoid such obvious errors, MARK uses the basic structure of the encounter history to estimate two different parameters: survival (Φ) – the probability that a bird sampled on occasion t is still alive at time $t+1$; and recapture (p) – the probability that an individual that is alive on occasion t is actually detected during this same interval.

I built 11 *a priori* selected candidate models representing my hypotheses regarding factors that may have affected adult survival across four annual sampling occasions (2005-2008). Each model was constructed to test different combinations of factors that might be important in affecting survival or recapture probability. Specifically, I investigated the possibility that apparent survival of adult Tree Swallows was influenced by sex, mercury status (contaminated or reference), or individual cumulative mercury exposure. I defined cumulative exposure as the sum of all past and present blood mercury levels for a particular individual at a given point in time. Prior to initial banding, each bird was assigned a blood mercury level of zero. Once a bird was detected in either the contaminated or reference area, it was assumed to remain in that same area on all subsequent sampling occasions, even if it was undetected in a particular year. In such cases, banded birds that were uncaught or unsampled were assumed to have accumulated an amount of mercury equal to their average exposure over the other years in which they were present. I used a cumulative index of mercury exposure because this

most accurately reflects the additive mechanism of mercury accumulation which has been documented in a number of studies (e.g. Evers et al. 2008). I also included sex*status, sex*Hg level, and status*Hg level as interaction terms in the models. For recapture probability, all models assumed an effect of sex since a greater emphasis was placed on capturing females throughout the study. Using data from 2005-2008, I was able to construct encounter histories for 932 adult Tree Swallows. Any banded nestlings that returned to breed on our study sites (n = 83) were included in the models beginning with their first adult year and were thereafter treated similarly to birds captured for the first time as adults.

I used maximum likelihood theory to estimate parameters and Akaike's Information Criterion (AIC) to evaluate the relative support for each candidate model (Burnham and Anderson 2004). AIC_c scores calculated using the effective sample size (N_{ess}) were analyzed in order to correct for small sample size. I evaluated overall goodness-of-fit by applying the median \hat{c} procedure to the most parameterized model ($\Phi_{sex + status + time + sex*status + sex*time + status*time, p_{sex}}$). The quasi-likelihood variance inflation factor (\hat{c}) was calculated and applied to all AIC_c scores in order to correct for overdispersion in the data. This correction generated a series of $QAIC_c$ scores. I ranked candidate models in such a way that those with lower $QAIC_c$ scores were considered to have greater support in the data. All models with $\Delta QAIC_c$ scores < 2 were considered to have significant support. I report model-averaged estimates for both annual survival and recapture probability for all sex and treatment groups. Additionally, MARK allows one to derive beta parameter estimates for each factor or covariate included in the models. Beta estimates can be interpreted as slopes, with positive values indicating a positive

effect on survival or recapture and negative values indicating a negative effect. Beta values for which the 95% confidence interval does not overlap zero can be considered to represent variables which are statistically significant in influencing survival or recapture probability.

Return Rates, Lifespan, and Population Age Structure. Although return rates must be interpreted with caution for the reasons discussed above, they may offer additional insight into long-term patterns in survival across multiple breeding seasons. While the annual survival rates provided by MARK are an excellent gauge of the overarching population-wide effects of mercury on annual survival rate, they are less well-equipped to offer insight into the cumulative impacts of mercury over several seasons of exposure. Therefore, I examined return rates of different cohorts of female Tree Swallows one, two, or three years after initial capture. I then used contingency tables to compare the proportion of birds alive on contaminated and reference sites at each interval of cumulative exposure. For all analyses involving return rates, individuals known to be alive during a particular sampling occasion were counted as such, regardless of whether they were actually detected.

In order to examine whether mercury might be differentially affecting birds of different ages, I again used contingency tables to compare survival of adult females from one age to the next. Because sampling of females was nearly complete in three of the four years of the study (2006-2008), most adult females could reliably be followed throughout their entire lives. In addition, since female Tree Swallows exhibit delayed plumage maturation, I was able to unambiguously identify one-year-old swallows. Any

female banded for the first time in adult (ASY) plumage was assumed to be two years old. The high site fidelity of breeding adults makes it unlikely that birds three years or older in age would be entering my study area for the first time. Rather, it is likely that these swallows had been SY floaters that were unable to secure a nesting site in the previous year (D. A. Cristol, unpublished data).

Finally, I examined the average life span of adult female Tree Swallows breeding on either contaminated or reference sites. Because measurements of adult life span could be influenced by age at initial banding, it was not sufficient for me to simply compare age at last recapture. Therefore, I defined adult life span as the number of years following banding that a female was detected on our sites. Swallows still alive at the end of the study were included in this analysis and were assumed dead in the following year. I compared mean adult life span of contaminated and reference females using a 2-sample t-test. All means are presented \pm SE.

RESULTS

Mercury Accumulation

A general linear model revealed significant effects of mercury status, year, and the status*year interaction as factors influencing mercury accumulation in Tree Swallows (treatment: $F_{1,741} = 614.29$, $P < 0.001$; year: $F_{1,741} = 23.20$, $P < 0.001$; treatment*year: $F_{1,741} = 29.70$, $P < 0.001$; Table 3; Figure 3). Post-hoc two-way comparisons indicated that blood mercury levels were higher in swallows breeding on contaminated sites (Tukey HSD: $t = 24.78$, $p < 0.0001$). Additionally, concentrations of mercury were significantly higher in 2006 than in either 2005 or 2007 (2005: Tukey HSD: $t = 4.02$, $P = 0.0002$;

2007: Tukey HSD: $t = 6.504$, $P < 0.0001$). However, these annual differences in mercury levels were only apparent in contaminated swallows, as evidenced by a significant treatment*year interaction.

Table 3: Blood mercury levels of adult Tree Swallows breeding on either contaminated (C) or reference (R) sites in each of the first three years of study (2005-2007).

Year	Treatment	Sample Size	Blood Mercury Concentration (ug/g: mean \pm SE)
2005	C	27	2.25 \pm 0.26
	R	48	0.18 \pm 0.03
2006	C	111	3.71 \pm 0.21
	R	167	0.15 \pm 0.01
2007	C	201	2.48 \pm 0.08
	R	188	0.17 \pm 0.02

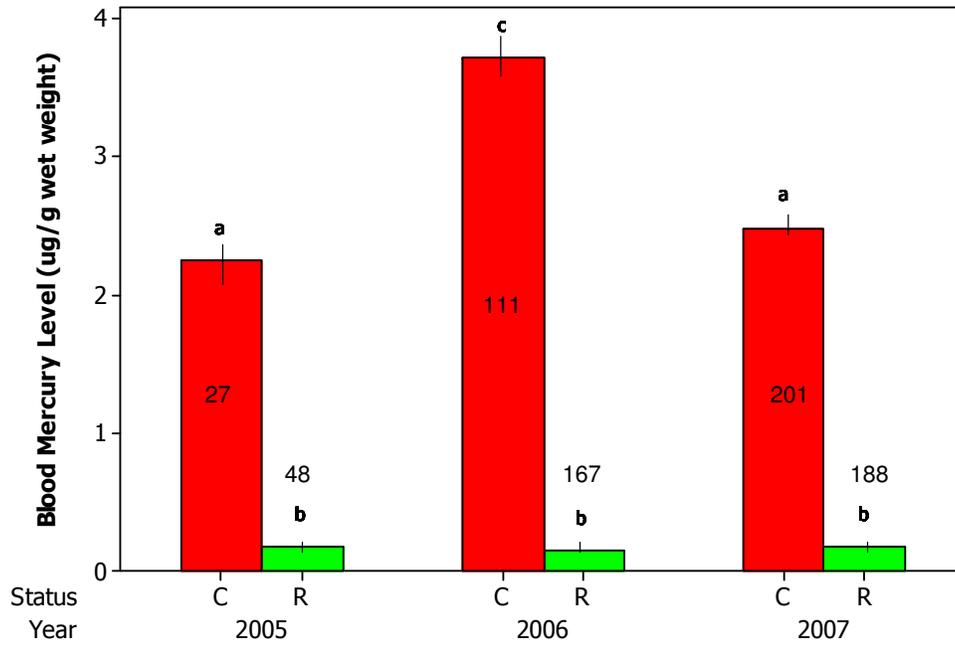


Figure 3: Mean (\pm SE) blood mercury levels of adult Tree Swallows breeding on either contaminated (C) or reference (R) sites in each of the first three years of the study (2005-2007). Sample sizes are represented as numbers on or above bars. Significant differences between groups are indicated by different letters above bars.

Mark-recapture Analysis

I constructed 11 *a priori* models in Program MARK to examine the influence of sex, mercury status, and cumulative mercury exposure on apparent survival of Tree Swallows breeding along the South River. Three univariate models in which apparent survival varied according to sex, status, or cumulative mercury exposure received support; model $\Phi \cdot p_{\text{sex}}$, in which survival was constant across all groups and time intervals, was the most parsimonious (Table 4). All 95% confidence intervals for covariate beta parameters overlapped zero, indicating the lack of a significant relationship between apparent survival and any covariates (Table 5). However, among the nine candidate models that included either status or cumulative mercury as factors, 11 of 12 beta estimates indicated a negative effect of mercury on survival (Figure 4; Table 5). A sign test revealed that this result differs significantly from chance ($P = 0.006$). Model-averaged estimates of apparent survival ranged from 0.48-0.49 and 0.47-0.48 for adult female Tree Swallows breeding on reference and contaminated sites, respectively (Table 6). For reference and contaminated males, the corresponding model-averaged estimates ranged from 0.45-0.46 and 0.44-0.45 (Table 6). As anticipated, recapture probability was greater for females than for males (model-averaged estimates of p : Females: 0.89; Males: 0.72; Table 6).

Table 4: Model selection for the estimation of apparent survival (Φ) of adult Tree Swallows. Subscripts indicate model structure: \cdot = time-constant survival, s = sex, Hg = cumulative mercury exposure, st = contamination status (contaminated or reference). Capture probability (p) varied by sex in every model. “K” refers to the number of estimable parameters. ΔQAIC_c scores were calculated using an effective sample size (ESS) = 754 and a variance inflation factor (\hat{c}) = 1.28.

Model	Description	K	ΔQAIC_c	Weight
$\Phi \cdot p_s$	Φ constant, sex variation in p	3	0.000	0.252
$\Phi_s p_s$	Sex variation in Φ , sex variation in p	4	0.887	0.162
$\Phi_{\text{Hg}} p_s$	Hg variation in Φ , sex variation in p	4	1.343	0.129
$\Phi_{\text{st}} p_s$	Status variation in Φ , sex variation in p	4	1.392	0.126
$\Phi_{s + \text{Hg}} p_s$	Sex and Hg variation in Φ , sex variation in p	5	2.260	0.081
$\Phi_{s + \text{st}} p_s$	Sex and status variation in Φ , sex variation in p	5	2.355	0.078
$\Phi_{\text{st} + \text{Hg}} p_s$	Status and Hg variation in Φ , sex variation in p	5	3.273	0.049
$\Phi_{s + \text{st} + s*\text{st}} p_s$	Sex by status interactive variation in Φ , sex variation in p	6	3.796	0.038
$\Phi_{s + \text{Hg} + s*\text{Hg}} p_s$	Sex by Hg interactive variation in Φ , sex variation in p	6	3.827	0.037
$\Phi_{s + \text{st} + \text{Hg}} p_s$	Sex and status and Hg variation in Φ , sex variation in p	6	4.225	0.030
$\Phi_{\text{st} + \text{Hg} + \text{st}*\text{Hg}} p_s$	Status by Hg interactive variation in Φ , sex variation in p	6	5.159	0.019

Table 5: Beta estimates (\pm SE) for each model. Subscripts indicate model structure: \cdot = time-constant survival, s = sex, Hg = cumulative mercury exposure, st = contamination status (contaminated or reference). Groups and covariates were coded such that a positive beta estimate indicates higher survival of swallows that were female (sex), that were on contaminated sites (status), or that had high mercury levels (Hg).

Model	Beta Estimate \pm SE (Φ)						
	intercept	sex	status	Hg	sex*status	sex*Hg	status*Hg
$\Phi_s P_s$	-0.11 \pm 0.12						
$\Phi_s P_s$	-0.34 \pm 0.22	0.30 \pm 0.26					
$\Phi_{Hg} P_s$	-0.05 \pm 0.13			-0.03 \pm 0.04			
$\Phi_{st} P_s$	-0.05 \pm 0.14		-0.15 \pm 0.18				
$\Phi_{s+Hg} P_s$	-0.29 \pm 0.24	0.29 \pm 0.26		-0.03 \pm 0.04			
$\Phi_{s+st} P_s$	-0.28 \pm 0.24	0.29 \pm 0.26	-0.14 \pm 0.18				
$\Phi_{st+Hg} P_s$	-0.04 \pm 0.14		-0.08 \pm 0.25	-0.02 \pm 0.05			
$\Phi_{s+st+s*st} P_s$	-0.37 \pm 0.26	0.42 \pm 0.31	0.05 \pm 0.23		-0.29 \pm 0.38		
$\Phi_s + Hg + s*Hg P_s$	-0.22 \pm 0.26	0.19 \pm 0.30		-0.07 \pm 0.07		0.06 \pm 0.08	
$\Phi_{s+st+Hg} P_s$	-0.28 \pm 0.24	0.29 \pm 0.26	-0.07 \pm 0.25	-0.02 \pm 0.05			
$\Phi_{st+Hg+st*Hg} P_s$	0.02 \pm 0.20		-0.14 \pm 0.30	-0.27 \pm 0.67			0.26 \pm 0.67

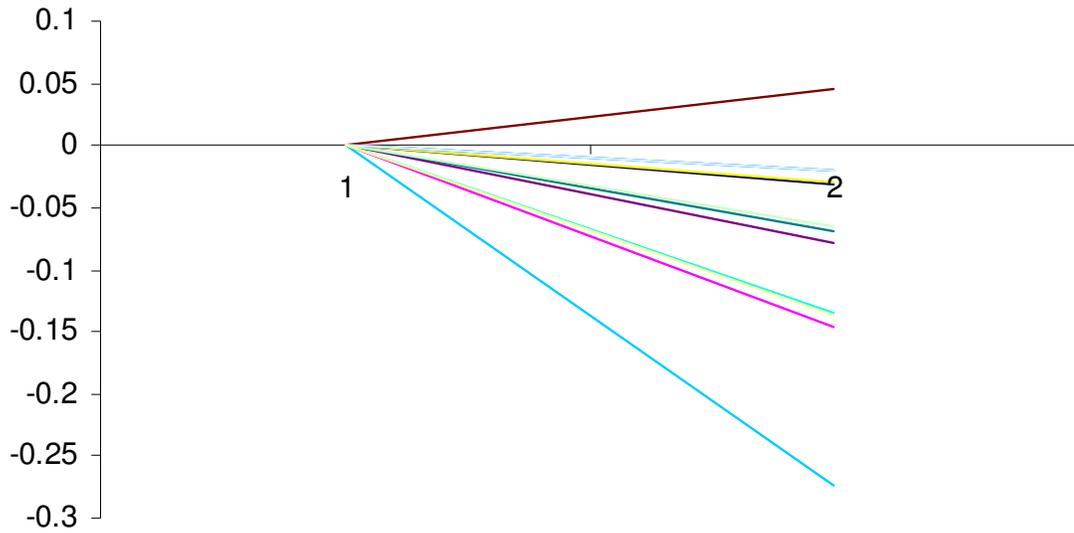


Figure 4: Beta slope estimates for mercury status or cumulative exposure terms in all candidate models. Groups and covariates were coded such that a negative slope indicates a negative effect of mercury on survival and a positive slope indicates a positive effect of mercury on survival. See Table 5 for beta estimates and associated error. None of the beta slopes represented here differed significantly from zero.

Table 6: Model-averaged estimates of apparent survival and recapture probabilities for adult Tree Swallows. C and R refer to swallows breeding on contaminated or reference sites, respectively.

Parameter	Estimate	SE	95% CI
Apparent Survival: Female R 2005	0.488	0.035	0.419 – 0.557
Apparent Survival: Female R 2006	0.486	0.036	0.417 – 0.556
Apparent Survival: Female R 2007	0.483	0.046	0.395 – 0.572
Apparent Survival: Female C 2005	0.477	0.041	0.398 – 0.557
Apparent Survival: Female C 2006	0.475	0.039	0.399 – 0.553
Apparent Survival: Female C 2007	0.473	0.038	0.401 – 0.547
Apparent Survival: Male R 2005	0.457	0.054	0.354 – 0.564
Apparent Survival: Male R 2006	0.455	0.054	0.352 – 0.561
Apparent Survival: Male R 2007	0.451	0.060	0.338 – 0.570
Apparent Survival: Male C 2005	0.448	0.056	0.342 – 0.559
Apparent Survival: Male C 2006	0.447	0.055	0.343 – 0.556
Apparent Survival: Male C 2007	0.444	0.054	0.343 – 0.551
Recapture: Female	0.889	0.047	0.760 – 0.953
Recapture: Male	0.723	0.100	0.495 – 0.874

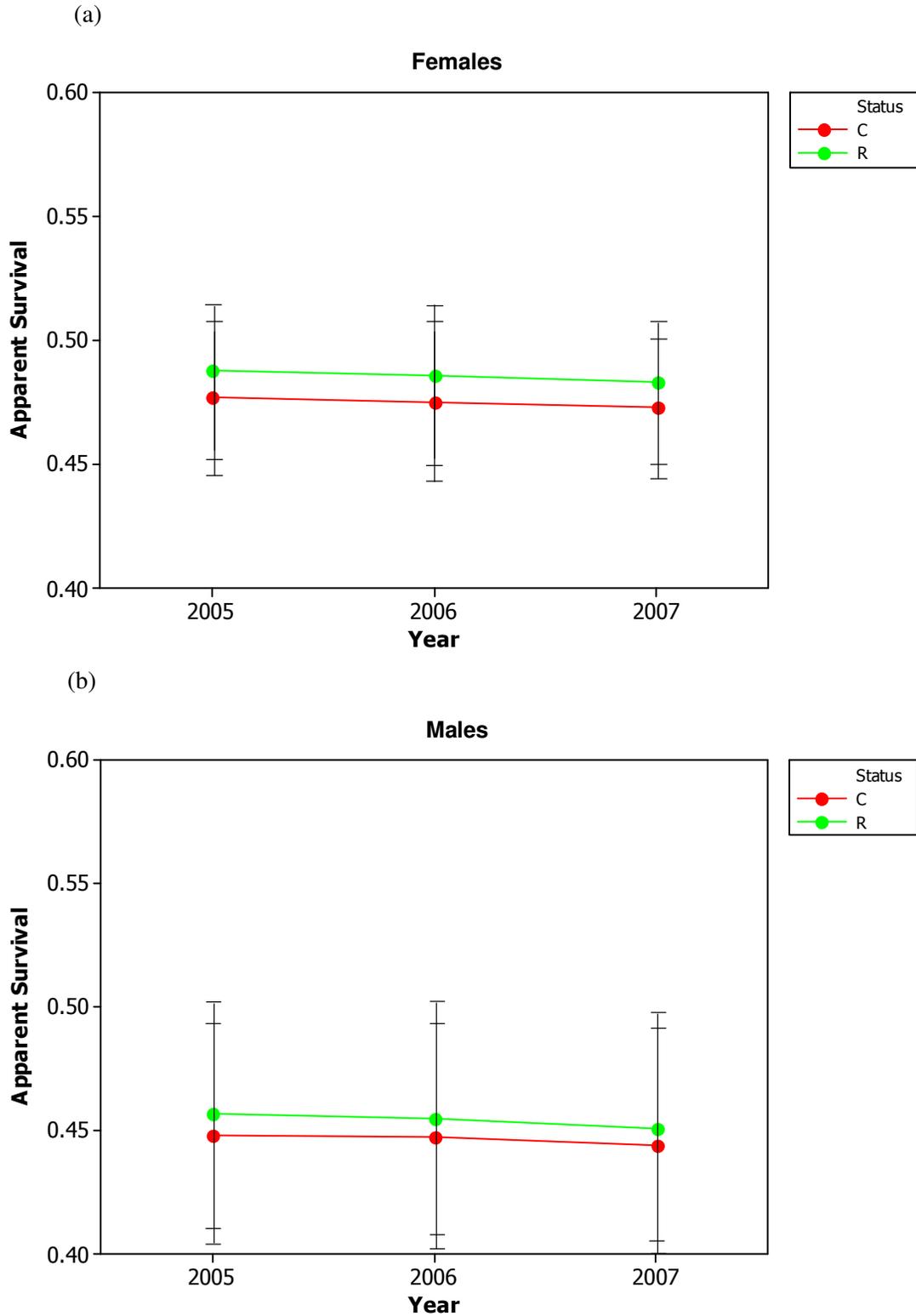


Figure 5: Model-averaged apparent survival for (a) female and (b) male Tree Swallows. Error bars represent one SE. Year refers to time t for the survival interval measured from t to $t+1$. C and R code for swallows breeding on contaminated or reference sites, respectively.

Return Rates, Lifespan, and Population Age Structure

Although the Cormack-Jolly-Seber models in Program MARK provide very robust estimates of apparent survival from one sampling occasion to the next, they are less well-equipped to allow examination of patterns of long-term, cumulative survival. Therefore, I conducted post-hoc analyses on Tree Swallow return rates across the four years of my study. Although return rates must be interpreted with caution, model-averaged estimates of recapture probability were very high for adult females ($p = 0.89$). Thus, I made the assumption that return rates of females would be an appropriate approximation of survival. I compared the return rate of females that had been banded one, two, or three years earlier in order to examine whether contaminated birds were less likely than reference birds to still be alive after a specified period of time. I found no significant differences in return rates of contaminated and reference swallows for any of the time periods considered (one year: $\chi^2 = 0.329$, $P = 0.566$, $df = 1$; two years: $\chi^2 = 1.280$, $P = 0.258$, $df = 1$; three years: $\chi^2 = 1.054$, $P = 0.305$, $df = 1$). Although the differences were not statistically significant, there was a clear trend in the predicted direction, such that reference swallows were twice as likely as contaminated swallows to return three years after banding (Figure 6). In order to document whether these patterns in return rate were related to age, I compared the probability of a swallow of age t surviving to reach age $t + 1$ on either reference or contaminated sites. This analysis similarly yielded non-significant trends suggesting that the probability of surviving into the following year decreased more steeply with age on contaminated sites (survival from age: 1-2: $\chi^2 = 0.019$, $P = 0.892$, $df = 1$; 2-3: $\chi^2 = 1.307$, $P = 0.253$, $df = 1$; 3-4: $\chi^2 = 0.931$, $P = 0.335$, $df = 1$; Figure 7). Finally, I examined the average adult lifespan of female Tree Swallows

breeding in either contaminated or reference areas. For this analysis, I defined adult lifespan as the total length of time after first detection that a female was present on a site. Swallows on reference sites were significantly older upon initial banding ($t_{330} = 2.21$, $P = 0.028$). When I controlled for this initial difference, reference swallows tended to live longer adult lives than contaminated conspecifics ($t_{342} = 1.60$, $P = 0.111$).

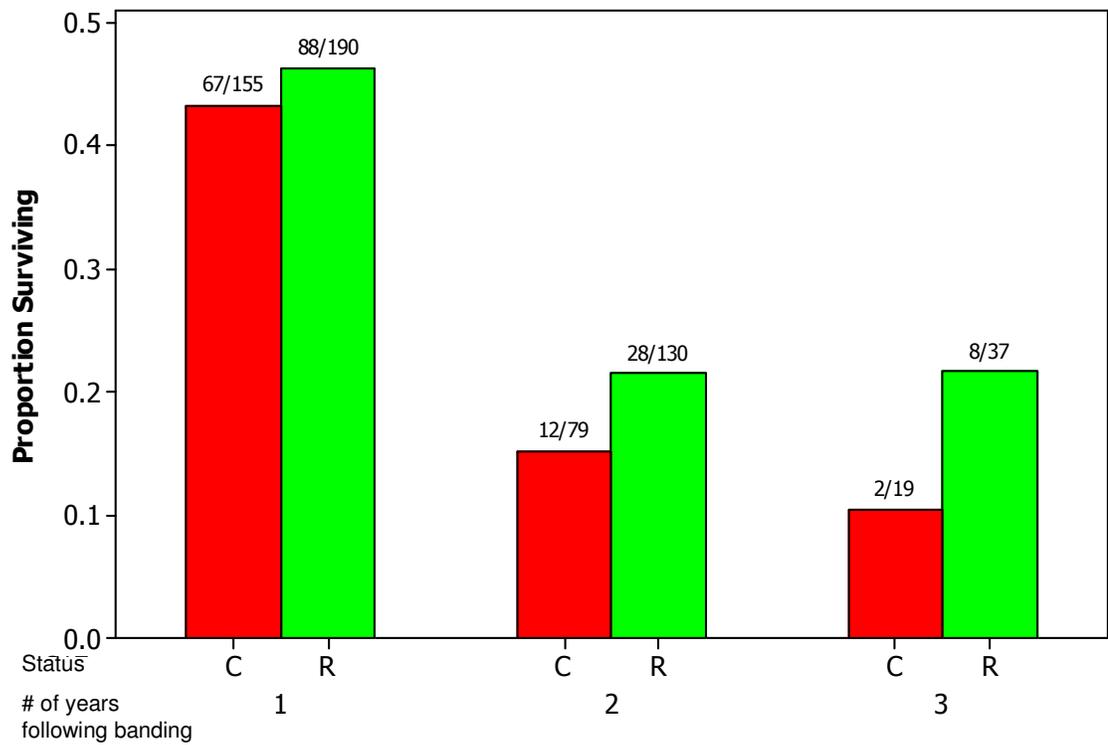


Figure 6: Proportion of adult female Tree Swallows returning to the study area to breed one, two, or three years after initial banding. Sample sizes are given above each bar. C and R refer to contaminated and reference, respectively.

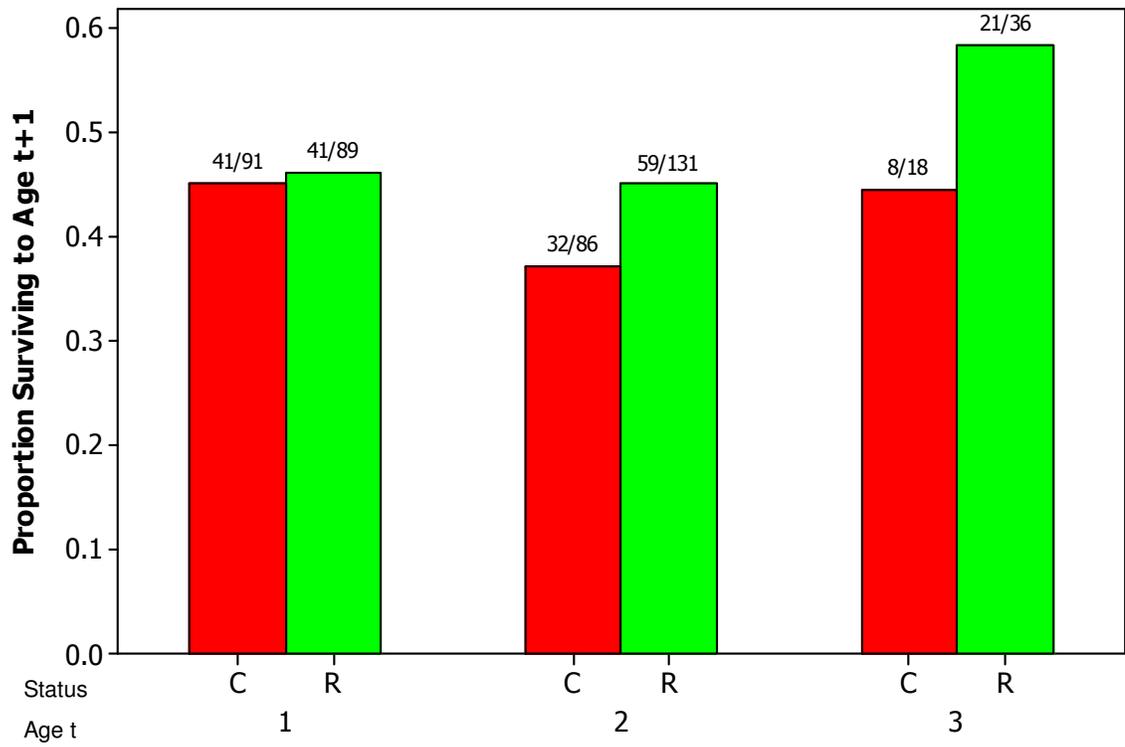


Figure 7: Proportion of adult female Tree Swallows surviving from age t to age t+1. Samples sizes and given above each bar. C and R refer to contaminated and reference, respectively.

DISCUSSION

Adult Tree Swallows were exposed to significantly elevated levels of mercury while breeding along the South River. Although mercury accumulation on contaminated sites varied by year, blood mercury levels of contaminated swallows were uniformly higher than those of reference swallows in all years of the study. In fact, the levels of mercury in these Tree Swallows are among the highest ever reported in a free-living passerine (Brasso and Cristol 2008). Yet, despite the high degree of exposure on contaminated sites, mark-recapture analysis indicated that annual survival of adult Tree Swallows was relatively unaffected by mercury. I used Program MARK to construct 11 a priori models designed to investigate the influence of sex, mercury status, and individual cumulative mercury exposure on annual adult survival. Of these candidate models, my data were best supported by a model in which survival was constant across all groups and time intervals. This suggests that the factors included in my models failed to account for the great majority of variation in survival probability. It is likely that differences in individual quality or environmental conditions, two variables that were not investigated in the present study, may have been more important determinants of survival that were not accounted for in any models.

Although it may seem surprising that such high levels of mercury could fail to produce detectable effects on survival, such equivocal findings actually represent the rule in ecotoxicological field studies, rather than the exception. While long-term studies of free-living populations of birds are still relatively uncommon in the ecotoxicological literature, the few studies that have been conducted have often failed to find any effects of contaminants on survival, even in cases where exposure is similarly high. Mitro et al.

(2008) examined annual survival in Common Loons (*Gavia immer*) breeding in areas with high atmospheric deposition of mercury. Although 14% of loons sampled were identified as ‘at risk’ based on previously described indices of exposure (Evers et al. 2008), the authors failed to detect any effect of mercury on survivorship. Interestingly, a post-hoc power analysis indicated an inability to detect “small” differences (<3%) in survival, even though survivorship reductions as small as 3% could be critical to the population viability of this long-lived piscivore. One possibility that requires investigation is whether expected mercury-related differences in survival were more subtle than my analysis had the power to detect. In fact, there is some evidence to suggest that such a mechanism may be at work; 11 out of the 12 mercury status or covariate beta estimates indicated a negative effect of mercury on survival. Thus, despite the fact that the influence of mercury was not statistically significant in any of the individual candidate models, the overall pattern may suggest a marginal negative effect of mercury on survival.

Alternatively, it could be the case that Tree Swallow survival on the South River was truly unaffected by mercury exposure. Again, such a result would fall squarely within the realm of past observations. Among songbirds, the Tree Swallow is the most widely-used model organism in ecotoxicological field studies, owing largely to its general tractability and ease of use (McCarty 2001). However, those same characteristics which make the Tree Swallow particularly well-suited to such studies may also render it less sensitive to environmental perturbation. Indeed, the vast majority of contaminant studies involving Tree Swallows have failed to detect any adverse effects, leading to some speculation that Tree Swallows may be unusually resilient in the face of

environmental stressors (McCarty 2001, but see Heinz et al. 2009). If this were the case, then Tree Swallows breeding along the South River may simply have failed to respond to mercury in any significant way even though exposure was high. However, caution should be taken in concluding that no mercury-related survival impairments existed as it is possible that the birds that I studied, successful nest box occupants and breeders, were a particularly resilient subset of the entire population. Individuals that are most sensitive to mercury may have died or failed to attempt breeding, and thus been excluded from my data. This is a major problem with any field study of a contaminated population and one that can only be addressed with experimental dosing studies.

Finally, it is possible that mercury-related impairments exist, but only manifest themselves after several years of exposure on a contaminated site. In order to investigate this possibility, I examined return rates of adult female swallows that had been present in the study area for various periods of time. Although the differences in return rate one, two, or three years after initial banding did not differ significantly between contaminated and reference swallows, there was a clear trend in which the disparity in return rate between the two groups became larger over time. Thus, reference swallows were twice as likely as contaminated swallows to be present in the study area three years following banding. If survival probability decreases only with prolonged exposure, then one might expect that the oldest individuals in the contaminated population would be most at risk of dying. To test this idea, I investigated the probability that female swallows of age t would survive to reach age $t+1$. This analysis indicated a trend in which the probability of surviving from one year to the next decreased more sharply with age on contaminated sites than on reference sites. Finally, I examined the average lifespan of adult Tree

Swallows in the population and found that swallows on reference sites tended to live longer than those on contaminated sites, independent of their age at first banding.

Taken together, such results suggest that mercury may be having a cumulative effect on the oldest members of the population. Because the number of individuals surviving to these older life stages is relatively small, it may be difficult to detect such differences in large, population-wide analyses of survival, such as those performed in Program MARK. However, impaired survival in the oldest subset of breeding individuals could have a number of important implications for Tree Swallows breeding in mercury-contaminated areas. First, in many species, reproductive potential increases with age (reviewed in Robertson and Rendell 2001). Thus, although the oldest females make up only a small proportion of all breeding individuals, their contribution to subsequent generations may be disproportionately important to the viability of the whole population. Cutting short the lives of the highest quality, longest-lived individuals, even by a small amount, could, over time, lead to declines in the overall health and stability of the population. Although senescence has been shown to occur in Tree Swallows (Robertson and Rendell 2001), it usually manifests itself at an older age than that at which contaminated swallows appear to be affected, thus providing the potential for serious population-level consequences to occur.

Furthermore, such age-related impairments may have interesting implications for the life history trade-offs and the selective pressures faced by individuals on contaminated sites. Previous studies on this population of swallows have documented mercury-related impairments in reproduction (Brasso and Cristol 2008) and immunocompetence (Hawley et al. *in press*). It is interesting to note that such sublethal

effects could manifest in contaminated swallows without strongly impacting survival. Perhaps contaminated Tree Swallows are investing more in survival than in self-maintenance and reproduction. Such a pattern seems maladaptive, given that the Tree Swallow is a relatively short-lived migrant that would be expected to invest more fully in current reproduction than in future survival. This is especially true if the expected lifespan of contaminated swallows is shortened, as suggested by my data. If swallows in mercury-polluted areas are suffering a reduced lifespan, one might expect selection to favor individuals that invest more in reproduction earlier in their lives at the expense of their continued survival. This could lead to birds that exhibit higher short-term reproductive success or increased immunocompetence, but a shorter lifespan relative to conspecifics in reference areas. This population was established in 2005 for the sole purpose of examining mercury contamination in birds breeding along the South River. Thus, it is unlikely that I would have seen the effects of this selective pressure translating into detectable evolutionary changes in the population. However, if the same patterns observed in Tree Swallows hold for other animal species that have been present on the South River over several decades of mercury exposure, and gene flow is very limited between contaminated and uncontaminated areas, then it is possible that such counter-intuitive life history strategies may have evolved.

While I am presently unable to definitively address the evolutionary consequences of a reduction in the lifespan of contaminated swallows, such potential implications speak to the importance of considering effects of environmental stressors in a more holistic way that fully encompasses the entire life history of both individuals and populations. Future work should attempt to simultaneously integrate many life history traits to effectively

address how contaminants such as mercury may be affecting the lifetime reproductive success of individuals. Documenting lifetime reproductive success and uncovering its contributing factors are major goals of ecological research and, given the permutations described thus far in South River tree swallows (Brasso and Cristol 2008, Hawley et al. *in press*, this thesis), should clearly become a primary focus of future ecotoxicological studies.

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