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Impact of Mercury Exposure on Birds and the Effect of Molt on Mercury Depuration in Songbirds

Margaret Crossley Whitney
College of William & Mary - Arts & Sciences

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Margaret Crossley Whitney

Mountainville, New York

Bachelor of Science, Bucknell University, 2009

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Margaret Crossley Whitney

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Committee Chair
Professor Daniel Cristol, Biology
The College of William and Mary

Assistant Professor Matthias Leu, Biology
The College of William and Mary

Assistant Professor Harmony Dalgleish, Biology
The College of William and Mary

COMPLIANCE PAGE

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ABSTRACT

Mercury is a ubiquitous environmental contaminant known to accumulate in and impact fish-eating bird species, and recently demonstrated to impact small songbirds to a comparable extent. It can bioaccumulate to concentrations of >1 ug/g in tissues of prey organisms such as fish and insects. At high enough concentrations, exposure to mercury is lethal to birds. However, environmental exposures are usually far below the lethal concentrations established by dosing studies.

Chapter 1:The objective of this review is to better understand the effects of sub-lethal exposure to mercury in birds. We restricted our survey of the literature to studies with exposures < 5 ug/g. The majority of sub-lethal effects were subtle and some studies of similar endpoints reached different conclusions. In general, there was no evidence that mercury affects longevity, but several of the sub-lethal effects identified likely result in fitness reductions that could adversely impact populations. Strong support exists in the literature for the conclusion that mercury exposure reduces reproductive output, compromises immune function, and causes avoidance of high-energy behaviors. For other endpoints, notably some measures of reproductive success, endocrine function, and body condition, there is weak or contradictory evidence of adverse effects and further study is required.

Chapter 2: Because mercury binds strongly to the keratin of feathers, molt is an important avenue for mercury elimination from birds. This paper is the first investigation of the depuration of mercury from songbird tissues and we attempt to quantify the impact of molt on the speed with which mercury is eliminated from tissues. We exposed two passerine species (European starling [*Sturnus vulgaris*] and zebra finch [*Taeniopygia guttata*]) to environmentally relevant dietary mercury for extended periods of time, and then allowed them to depurate on a mercury-free diet for 20 weeks while either molting or not molting. Molting birds depurated mercury from their blood significantly more rapidly than non-molting birds. The effect of molt on mercury retention in organ tissues was harder to decipher, but followed the same pattern for one species (finches). A subset of birds allowed to depurate for an additional 20 weeks exhibited slower mercury depuration among those individuals exposed to mercury for their entire lifetime as opposed to birds exposed only as adults. Our results confirm that molting expedites depuration, and suggest that migration/dispersal behavior and molt timing must be taken into consideration during risk assessment.

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Chapter 1: Impacts of Environmentally Relevant Mercury Exposure on Birds

Introduction

Mercury (Hg) concentrations are predicted to continue to increase worldwide, while climate change is expected to exacerbate the impact of this ubiquitous environmental contaminant (Sunderland et al. 2009, Stern et al. 2012). The effects of Hg on wildlife have been studied extensively (Fig. 1a), but until recently a vast majority of bird species investigated have been either piscivorous or domesticated, such as the white leghorn chicken. Current experiments and field studies have began to include passerines, as it has recently been discovered that Hg is not restricted to aquatic environments, but also impacts terrestrial species (Cristol et al. 2008). Additionally, many more experiments have used lower concentrations of Hg in an effort to understand the sub-lethal impacts that most exposed wildlife are experiencing (Fig. 1b).

This review incorporates the majority of known literature discussing the effect of sub-lethal doses of Hg on birds of all taxa. Experimental groups of birds fed 40 ppm Hg experienced 30% mortality within as few as 6 days (Finley et al. 1979). Chronic exposure to 5 ppm dietary Hg resulted in 25% mortality within 10 weeks, making it a lethal dose (Scheuhammer 1988). However, Hg concentrations in wild prey items, both fish and terrestrial arthropods, rarely exceed 2 ppm (Cristol et al. 2008, Burgess & Meyer 2008, Henny et al. 2002, Merrill et al. 2005). Experimental studies were included in this review only if birds were exposed to 5 ppm Hg or less. The following review provides a summary of effects of environmentally-relevant Hg concentrations on birds.

Reproduction

Overview: Depressed reproductive success is the most widely investigated and reported consequence of mercury exposure, but the endpoints measured vary widely between studies, from eggshell structure to timing of breeding to parenting behaviors. Dozens of different species have been studied, both in the field and in laboratories (all experimental dosing results are denoted as such throughout this paper). Despite the wide range of mercury concentrations and methodologies, mercury exposure clearly has some deleterious impacts on reproduction.

Fledglings: Reduction in the number of nestlings or fledglings is the effect of mercury exposure with the most robust support, and includes reports of fewer common loon chicks (Evers et al. 2008, Burgess & Meyer 2008), a lower number of tree swallows fledging (Brasso & Cristol 2008, Hallinger & Cristol 2011), and reduced fledgling output by free-living bald eagles, and dosed American kestrels and zebra finches (DeSorbo & Evers 2005, Albers et al. 2007, Varian-Ramos et al. 2014) (see Appendix 1 for scientific names). There was a nonsignificant trend towards fewer fledglings among white ibis dosed in an aviary (Frederick & Jayasena 2010) and an uncertain relationship between fledgling numbers and mercury exposure in free-living American dippers (Henny et al. 2005). The only study not reporting reduced numbers of offspring was one on great skua fledglings exposed to mercury in their natural fish diets (Thompson et al. 1991), however a recent study of long-term data from Antarctic colonies of two species of skuas indicates an effect of tissue mercury concentration in one year on reproductive success the following year, of a severity that is predicted to lead to population declines (Goutte et al

2013).

Hatchlings: In a series of landmark dosing studies on female mallards, Heinz (1974, 1976a, 1976b, 1979), reported a reduction in the number of ducklings, findings he recently replicated (Heinz et al. 2010b). Mercury exposure also resulted in fewer nestlings for free-living common loons (Meyer et al. 1998) and snowy egrets (Henny et al. 2002), and dosed black ducks (Finley & Stendell 1978), American kestrels (Albers et al. 2007), and white ibises (Frederick & Jayasena 2010). Anthony et al. (1999) reported fewer nestlings from free-living bald eagles, but Bowerman et al. (1994) and Weech et al. (2006) reported no correlations between environmental mercury exposure and number of bald eagle nestlings. Contamination from mercury mining did not correlate with number of black-crowned night-heron nestlings either (Henny et al. 2002), and Elbert & Anderson (1998) reported an unclear relationship for western grebes in the same situation.

Clutch Size: The number of eggs laid in a clutch also appears to be impacted by methylmercury in some species. Mercury contamination was associated with reduced numbers of eggs in black-legged kittiwakes (Tartu et al. 2013), as well as dosed white leghorn chickens (Lundholm 1995), American kestrels (Albers et al. 2007), and mallards (Heinz 1974). However, no differences were detected in the number of eggs laid by reference and environmentally exposed tree swallows (Brasso & Cristol 2008, Gerrard 2000), or dosed black ducks (Finley & Stendell 1978) and zebra finches (Varian-Ramos et al. 2014).

Other Measures of Reproductive Output:

The literature suggests that mercury may impact a number of other reproductive endpoints, but there are fewer examples of each of these. Nestlings from contaminated sites were more sensitive to high ambient temperatures (Hallinger & Cristol 2011), and primary sex ratios of offspring on mercury-contaminated sites were female-biased in belted kingfishers, tree swallows, and eastern bluebirds, relative to reference sites (Bouland et al. 2012). There was a mercury-related decline in the proportion of eggs hatching in tree swallows (Hallinger & Cristol 2011), laughing gulls (Jenko et al. 2012), and dosed zebra finches (Varian-Ramos et al. 2014) and American kestrels (Albers et al. 2007). In a set of experimental studies on mallards, hatching success declined in two studies (Hoffman & Moore 1979, Heinz et al. 2009) but improved in another (Heinz et al. 2010a). This apparent case of hormesis, perhaps based on a mild antibiotic effect of mercury, was reproduced in an egg injection experiment (Heinz et al. 2012). Mercury-related changes in hatching rate were not observed for great skuas (Thompson et al. 1991), or dosed black ducks (Finley & Stendell 1978) or mallards (Heinz 1976a, Heinz et al. 2010b).

Other metrics of reproductive success have yielded equivocal results. A model for Carolina wrens developed from field results indicated reduced nest survival, due primarily to nest abandonment, with even small increases in maternal blood mercury concentration (Jackson et al. 2011). However, for bald eagles, nest success, as defined by the percent of breeding territories producing at least one fledgling (Bowerman et al. 1994) or reproductive success in terms of the number of nestlings per nest (Weech et al.

2006), did not relate to mercury contamination.

Eggs and Embryos: Eggshell thinning is one of the most commonly observed effects of mercury contamination, seen in free-living snowy egrets (Olivero-Verbel et al. 2013) and domestic white leghorn chickens (Lundholm 1995). Bald eagle eggshells were found to be thinner by Wiemeyer et al. (1984), but no difference was detected by Anthony et al. for the same species (1999). Mallards maintained on a methylmercury-contaminated diet eggs did not show eggshell thinning (Heinz 1974, 1976A, 1976B) until the third generation (Heinz 1979). Lundholm (1995) also reported eggshell defects and shorter egg length in chickens, while Olivero-Verbel (2013) observed wider eggs with decreased weight in free-living egrets, and Heinz (1974) reported decreased egg weight in dosed mallards. Egg volume was lower for contaminated tree swallows in one study (Brasso & Cristol 2008), but did not differ in a larger study on the same population (Hallinger & Cristol 2011).

A small number of studies have found effects of Hg on embryos as well. Applying mercury to the surface of mallard eggs caused teratogenicity including skeletal defects and incomplete ossification (Hoffman & Moore 1979). When injected into eggs, mercury caused teratogenicity to varying degrees in up to 22 of 25 different species (Heinz et al. 2011). Eggs of dosed mallards experienced increased embryo mortality (Heinz 1974), with fewer viable eggs produced (Heinz 1979). Forster's tern eggs collected from the wild showed a positive relationship between number of malpositioned embryos and mercury concentration, but no relationship between embryo deformities and mercury. There was no relationship between mercury concentration and either

malpositioned or deformed embryos for black-necked stilts and American avocets (Herring et al. 2010), or embryonic development in white-tailed sea eagle eggs (Helander et al. 1982).

Timing of Breeding: Study of the effects of mercury on relative timing of reproductive events, such as egg laying and fledging dates, has yet to produce any consensus. The potential effect of mercury on laying date is especially uncertain, with two studies of dosed birds reporting an increased latency to renest (zebra finches, Varian-Ramos et al. 2014) or a delay in onset of egg laying (American kestrels, Albers et al. 2007), but another on free-living tree swallows reporting earlier onset of laying on contaminated sites (Hallinger & Cristol 2011). However, the onset of laying in the same population of tree swallows was reported to be unaffected on mercury-contaminated sites (Brasso & Cristol 2008). Neither great skuas (Thompson et al. 1991), black-legged kittiwakes (Tartu et al. 2013), nor dosed black ducks (Finley & Stendell 1978) exhibited a relationship between mercury concentration and onset of egg-laying. Blood mercury concentration was negatively related to date of hatching in Forster's terns (Ackerman et al. 2008a), while a positive relationship between mercury and interval between laying and hatching was observed for dosed American kestrels (Albers et al. 2007).

Longevity

It appears that mercury does not directly decrease longevity at environmentally relevant concentrations. No differences were found in post-fledging survival probability of Forster's terns (Ackerman et al. 2008a), re-sight probability of dosed and released white

ibises or free-living common loons (Frederick et al 2011, Mitro et al. 2008), annual adult return rate of common loons or great skuas (Meyer et al. 1998, Thompson et al. 1991), or survival of great egrets (Sepulveda et al. 1999). Survival probability of American avocet and black-necked stilt chicks at more contaminated sites dropped 1.4% and 3.0%,, respectively, but mercury had low predictive power in the models used (Ackerman et al. 2008b). Similarly, predicted tree swallow survival at mercury-contaminated sites dropped 1-2% but mercury exposure had weak explanatory power (Hallinger et al. 2011). Mercury concentration in tissues was related to lower recapture probabilities for white-winged scoters, but not king eiders (Wayland et al. 2008). Further studies of a long-lived birds observed over many years of mercury exposure may yet reveal a significant effect on survivorship, but thus far there is no evidence to this effect.

Behavior

Parental Behaviors: Parental behavior may be altered in a variety of ways after exposure to mercury. White ibises dosed in captivity made fewer nesting attempts (Heath & Frederick 2005) and even exhibited same-sex pairing among males (Frederick & Jayasena 2010). Both free-living common loons (Evers et al. 2008) and dosed American kestrels (Albers et al. 2007) spent less time incubating, while mercury was also related to decreased provisioning effort in loons (Merrill et al. 2005). Male American kestrels were observed cannibalizing their offspring in a dosing study (Fallacara et al. 2011b).

Behavior of Dependent Young Birds: A number of abnormal chick behaviors have also been reported. Common loon chicks with higher mercury exposure spent more time

preening and less time back-riding, although they did not change their swimming or diving habits in response to mercury contamination (Nocera & Taylor 1998). Loon chicks were also less capable of righting themselves after dietary exposure, and experimental *in ovo* mercury exposure resulted in other behavioral changes in captivity, including crossing a platform faster, spending more time on platforms and in sunlight, and exhibiting decreased responses to parental wails and frightening stimuli (Kenow et al. 2010). Dosed mallard ducklings did not alter their response to maternal calls (Heinz 1975, 1976a, 1976b) until the third generation of exposed birds, when they exhibited a reduced response (Heinz 1979). Ducklings also ran further from frightening stimuli (Heinz 1975, 1976b, 1979), except in one experiment in which their response to a frightening stimulus did not change (Heinz 1976a). When mercury was injected into white leghorn chicken eggs, the resulting chicks did not differ in their response to frightening stimuli, but they did take longer to right themselves (Rutkiewicz et al. 2013).

High-energy Behaviors: Mercury appears to impact behaviors requiring a large energy input. Carolina, house wrens, and song sparrows sang less complex, lower-frequency songs (Hallinger et al. 2010). Free-living common loons spent less time preening and swimming in the wild (Evers et al. 2008). In dosing studies that included both lethal concentrations and lowest doses of 5 ug/g, great egrets were less active (Bouton et al. 1999) and exhibited ataxia (Spalding et al. 2000b), while zebra finches became lethargic and had difficulty balancing or landing on perches (Scheuhammer 1988). Domestic pigeons also exhibited ataxia, pecked at food less accurately and at a slower rate (Evans et al. 1982), and made fewer, slower responses in operant conditioning tests (Laties et al.

1980). Mercury also impacted American kestrel motor skills, but only at concentrations above 5 ug/g (Bennett et al. 2009).

The relationship between foraging behaviors and mercury concentration is more complicated. Common loons with higher mercury exposure spent less time foraging for themselves and their chicks (Evers et al. 2008) and exhibited an increased diving frequency (Olsen et al. 2000), which may indicate that they were having difficulty foraging. However, dosed white ibises foraged more efficiently (Adams et al. 2008) and great egrets performed as well as birds on control diets, although they had a reduced appetite (Bouton et al. 1999). Food consumption of common loons dosed in captivity was not related to mercury concentration (Kenow et al. 2003).

Neurological Function

Although fewer studies of neurotoxicity in avian models have been done in recent years, there exists a body of evidence indicating that Hg results in axonal degeneration and other neurological problems. In mallards dosed with mercury, adults developed degenerated axons (Pass et al. 1975), and chicks exhibited demyelination and neuronal shrinkage (Heinz & Locke 1976). Pigeons also exhibited demyelination, but instead had neuronal swelling (Evans et al. 1982). American kestrels developed brain lesions, but only when fed concentrations above 5 ug/g, and all groups showed axonal degeneration (Bennett et al. 2009). Double-crested cormorants had axonal degeneration and swollen myelin sheaths (Loerzel et al. 1999). Red-tailed hawks, however, did not show axonal degeneration unless they were fed very high concentrations of Hg (Fimreite & Karstad 1971).

Several researchers have examined neurotransmitter function. Decreased binding to NMDA receptors was related to mercury concentration in free-living bald eagles and common loons (Scheuhammer et al. 2008), and dosed white leghorn chickens (Rutkiewicz et al. 2011). However, no change in binding to NMDA receptors was observed for domestic quail or chickens in one experiment while increased binding to NMDA in chickens was observed in another (Rutkiewicz et al 2013). Glutamine synthasewas found to increase in dosed chickens (Rutkiewicz et al 2011), but only at the high concentration of 6.4 ug/g, and another study found no change in glutamine synthase (Rutkiewicz et al. 2013). Glutamic acid decarboxylase has been found to either increase, remain the same (Rutkiewicz et al. 2013), or decrease with administration of inorganic mercury (Rutkiewicz et al. 2011) in chickens. Gamma-aminobutyric acid either showed no change, for chickens or quail, increased in chickens fed 6.4 ppm mercury, or decreased in chickens exposed to methylmercury-cysteine (Rutkiewicz et al. 2013). Muscarinic cholinergic receptor activity was related to mercury in free-living bald eagles and common loons, but no differences were found for cholinesterase, or MAO (Scheuhammer et al 2008).

Endocrine Function

Overview: While there is no evidence that mercury is a classic endocrine disrupting chemical that mimics or competes with specific hormones, there is data suggesting that mercury exposure is associated with alterations in profiles of several hormones. Much more work is needed in this area because the results are equivocal and no studies have

been replicated with the same mercury doses and species.

Corticosterone (CORT): Despite a considerable body of literature, the impact of mercury exposure on CORT is very unclear. The stress-induced increase in CORT was weaker for nestling tree swallows at contaminated sites (Wada et al. 2009) and dosed adult zebra finches (Moore et al. 2014), but did not relate to mercury level in free-living common eiders (Wayland et al. 2002), and was actually stronger in dosed white ibises (Jayasena 2010). Baseline CORT was also elevated in tree swallow nestlings (Wada et al. 2009) as well as in dosed juvenile white ibises, although this latter response exhibited a nonlinear relationship with dose (Adams et al. 2009). However, in adult lesser scaup, baseline CORT was only related positively to mercury in individuals with larger body size, while the relationship was reversed in smaller individuals (Pollock & Machin 2009). For nestling and adult tree swallows, a nonsignificant positive relationship was reported between feather mercury concentration and CORT, but a negative relationship was found between baseline CORT and both egg and blood mercury in the same birds (Franceschini et al. 2009). Baseline CORT was also depressed in nestling Forster's terns (Herring et al. 2012) and dosed adult white ibises (Jayasena 2010). Finally, no significant relationship was found between mercury and baseline CORT in adult (Heath & Frederick 2005) or nestling dosed white ibises, dosed zebra finches (Moore et al 2014), or nestling great egrets (Herring et al. 2009).

Testosterone (T): No clear patterns have yet emerged about the relationship between mercury and baselineT levels. In dosed adult white ibises, Jayasena et al. (2011) found no change in breeding males, although males paired to other males had depressed levels

during laying and elevated T levels during incubation, in contrast with males paired to females. Heath & Frederick (2005) found elevated T levels in males incubating nests in the wild. In adult black-legged kittiwakes, neither baseline T nor gonadotropin-releasing hormone (GnRH)-induced T were related to mercury level in breeding birds, but in birds that skipped breeding, baseline T was negatively related to mercury and GnRH-induced T was elevated (Tartu et al. 2013). In dosed juvenile white ibises, no effects of mercury on T were observed (Adams et al. 2009).

Other Hormones: Few other hormones have been studied as extensively as CORT or T. Other hormones related to reproduction have been the most studied, but like CORT and T, they are highly dependent on an individual's breeding stage and thus a relationship with mercury concentration is hard to detect. The emerging relationships between mercury exposure and hormone level are correspondingly complex. A significant relationship between mercury and luteinizing hormone (LH) was found in black-legged kittiwakes that skipped breeding, but not in birds that bred. Baseline LH levels were negatively associated with Hg in males but positively associated in females, while LH induced by GnRH increased with increasing mercury levels (Tartu et al. 2013). However, both baseline and GnRH-induced LH was suppressed in male and female snow petrels (Tartu et al. 2014). Prostaglandin synthesis declined after exposure to a high dose of 5 ppm in a homogenate eggshell mucosa from chickens (Lundholm 1995). Dosed white ibises had a nonsignificant increase in progesterone during incubation (Heath & Frederick 2005). Thyroid hormones, T3 and T4, were lower in nestling tree swallows exposed to mercury at contaminated sites (Wada et al. 2009), but T4 had no relationship

to mercury in lesser scaup (Pollock & Machin 2009).

More information is available regarding estradiol. In dosed female white ibises, estradiol levels decreased significantly with mercury during pre-breeding, nonsignificantly during the display stage (Heath & Frederick 2005), and in a dose-dependent manner during breeding. In male white ibises, estradiol levels were higher in dosed birds than controls during courtship but lower during other stages. Differences between dosed and control birds were amplified in males that paired, abnormally, with other males (Jayasena et al. 2011). Estradiol levels in juvenile white ibises increased in a dose-dependent manner with mercury dose (Adams et al. 2009).

Immunocompetence

Overview: The impact of mercury on immune function is relatively understudied. There has been little replication for many endpoints, and field investigations are often limited to non-specific measures of immune response, such as the swelling response phytohaemagglutinin (PHA) assay, which leave considerable room for interpretation. However, a general picture is forming that mercury is challenging to the immune systems of birds.

Blood Cells: The most widely reported white blood cell endpoints relate to heterophils and lymphocytes. The number of heterophils increased with mercury in dosed great egrets (Spalding et al. 2000a) and American kestrels (Fallacara et al. 2011a), while the percentage of heterophils increased with mercury in free-living western grebes (Elbert & Anderson 1998). Only one study reported a different trend, namely a nonsignificant

decrease in the number of heterophils in great egrets. The number of lymphocytes also exhibited a nonsignificant decrease associated with mercury in that study (Sepulveda et al. 1999), but this result corroborates other results, including a significant decrease in the number of lymphocytes in dosed American kestrels (Fallacara et al. 2011a) and decreased B cell proliferation in dosed zebra finches (Lewis et al. 2013). Dosed great egrets, however, have also displayed an increase in number of lymphocytes (Spalding et al. 2000a). In accordance with the majority of conclusions, the heterophil-to-lymphocyte ratio increased for dosed American kestrels (Fallacara et al. 2011a) and common loons (Kenow et al. 2007).

Fewer results have been published regarding other white blood cells. The number of eosinophils showed a nonsignificant decrease with mercury level in great egrets (Sepulveda et al. 1999), and a significant percentage decrease in environmentally exposed western grebes (Elbert & Anderson 1998). Macrophage activity decreased with mercury level in free-living black-footed albatross (Finkelstein 2003), and macrophage suppression was also observed in dosed American kestrels (Fallacara et al. 2011a). However, Holloway (2001) only observed a change in phagocytic activity when white blood cells from chickens were isolated and exposed to mercury *in vitro*, reporting no change when ringed doves or common loons were exposed to mercury *in vivo*. Number of monocytes increased with mercury in dosed great egrets (Spalding et al. 2000a), but did not change in dosed American kestrels (Fallacara et al. 2011a).

A small amount of information is available on how mercury impacts other aspects of blood. Hematocrit decreased in response to mercury in black-crowned night herons,

snowy egrets (Henny et al. 2002) and dosed great egrets (Spalding et al. 2000a). Sepulveda et al. (1999) also observed a nonsignificant decrease in hematocrit with mercury exposure in great egrets during one year, but an increase in another year. Plasma proteins in general may decrease, as observed in dosed and environmentally exposed great egrets (Hoffman et al. 2005, Sepulveda et al. 1999, Spalding et al. 2000a). However, the response is likely more complicated, as common loons displayed an increase in globulin and a decrease in albumin (Kenow et al. 2007).

Immune Responsiveness: A considerable body of literature shows that mercury decreases general immune response in birds, although there are variable results from different assays. PHA-induced swelling was lower for dosed great egrets (Spalding et al. 2000a), and American kestrels (Fallacara et al. 2011a and b), and environmentally exposed tree swallows (Hawley et al. 2009). Antibody response to sheep red blood cells (SRBC) was lower in dosed American kestrels (Fallacara et al. 2011a) and common loons (Kenow et al. 2007). However, Kenow et al. (2007) also reported no change in PHA-induced swelling in common loons, and Fallacara et al. (2011a) and Hawley et al. (2009) found no significant differences in antibody response to SRBC. In common eiders, no relationship was found between mercury and PHA-induced swelling (Wayland et al. 2002).

Other evidence for a generally compromised immune response includes a greater rate of bacterial infections in common loons (Kenow et al. 2007). Great white herons found dying of disease had higher body burdens of mercury than birds dying of acute causes, e.g., injuries (Spalding et al. 1994).

Other Physiological Endpoints

Oxidative Stress: A growing body of evidence indicates that mercury exposure induces oxidative stress. Although no changes in glutathione (GSH), glutathione disulfide (GSSG), or the ratio of oxidized GSSG to reduced GSH were found in dosed laughing gulls (Jenko et al. 2012), evidence of mercury-related oxidative stress has been observed in a number of other species. GSH was negatively related to mercury level in the livers of greater scaup, surf scoters, ruddy ducks (Hoffman et al. 1998), and Forster's terns (Hoffman et al. 2011). GSSG increased in the liver, brain, and kidney of dosed common loons (Kenow et al. 2008), and was also positively related to mercury in the livers of surf scoters, ruddy ducks (Hoffman et al. 1998), and great egrets (Hoffman et al. 2005), and in the kidney of Forster's terns, (although the opposite trend was found with mercury level in their brains; Hoffman et al. 2011). GSSG:GSH, which represents the ratio of available to unavailable antioxidant, decreased in kidneys of great egrets (Hoffman et al. 2005) and livers of common loon, but increased in their brains (Kenow et al. 2008), as well as in the livers of greater scaup (Hoffman et al. 1998), Forster's tern (Hoffman et al. 2011), and double-crested cormorant. GSH peroxidase, which converts oxidized to reduced glutathione, declined in great egret livers, kidneys, plasma, and brains (Hoffman et al. 2005), cormorant livers (Henny et al. 2002), and common loon brains, but increased in loon kidney and liver (Kenow et al. 2008), as well as in surf scoter liver (Hoffman et al. 1998). In addition to these chemical changes, Hg exposure increased the expression of two cellular stress-related genes, glutathione peroxidase 3 and glutathione *S*-transferase μ3 in female double-crested cormorants (Gibson et al. 2014).

Oxidative stress may be responsible for reports of damage to livers and other internal organs in birds with high mercury levels. European starlings dosed unintentionally in captivity showed extensive nephritic lesions (Nicholson & Osborn 1984), and black-crowned night herons, snowy egrets, and double-crested cormorants experienced hepatotoxicity and nephrotoxicity with higher exposure to mercury (Henny et al. 2002). Henny et al. (2002) also found that young snowy egrets had enlarged livers and kidneys (and smaller brains), and double-crested cormorants had enlarged spleens, which may have been the result of organ damage rather than initial growth.

Metabolism: Very few studies have investigated changes in metabolism in response to environmentally relevant mercury contamination, and none of these have been replicated. In western grebes, blood potassium and phosphorus decreased with increasing tissue mercury concentration (Elbert & Anderson 1998), and plasma phosphate also decreased in great egrets (Hoffman et al. 2005), although plasma potassium did not change in Japanese quail fed methylmercury (Hill & Soares 1984). After dietary mercury exposure at the upper limit of our defined environmentally relevant concentrations (5 ppm), white leghorn chickens had decreased calcium content in their blood plasma (Lundholm 1995). No changes were observed in plasma triglyceride levels of northern waterthrushes (Seewagen 2013), or in blood glucose levels of lesser scaup (Pollock & Machin 2009).

Growth and Condition: Mercury exposure does not appear to strongly impede overall growth, but may result in some potentially significant changes in size. No changes were observed in overall body mass of dosed American kestrels or common loons, tarsus length of American kestrels or tree swallows (Fallacara et al. 2011b), tarsus or primary

feather length of tree swallows (Wada et al. 2009), or body length and asymptotic mass of common loons (Kenow et al. 2003). However, common loons from lakes with low pH, which may be more vulnerable to mercury, did have lower asymptotic mass (Kenow et al. 2003). Meanwhile, dosed great egrets did reduce their food intake and had lower weight index scores (Spalding et al. 2000b). Similarly, young nestling tree swallows at sites with higher mercury also had a decreased linear growth rate, although wing and tail growth were not affected (Longcore et al. 2007).

A multitude of other indices have been used to assess body condition after mercury exposure, ranging from size-corrected body mass to feather growth rate. These wide-ranging definitions make categorizing the effect of mercury difficult. Body weight, as well as liver and heart weight, decreased in surf scoters, and the liver-to-body weight ratio increased in ruddy ducks (Hoffman et al. 1998). Male American kestrels dosed with mercury also had lower body weight, but only in one treatment group (Albers et al. 2007). Meanwhile, no change in body or organ weight was seen in greater scaup (Hoffman et al. 1998), in the body weight of bald eagles (Weech et al. 2006), or in terms of body mass, body size, or organ mass in common eiders (Wayland et al. 2002). Great white herons dying of chronic disease, and with elevated mercury in tissues, had less body fat, although this was dependent on age (Spalding et al. 1994).

Using more sophisticated measures of body condition presents a more ambiguous picture. California clapper rails had lower body condition as defined by a ratio of mass to structural size (Ackerman et al. 2012), but using the same metric, white ibises showed a nonsignificant trend of improved body condition with mercury level (Heath & Frederick

2005). Using body mass-to-body length and body mass-to-keel length as a measure of body condition resulted in a positive relationship between body condition and mercury in common mergansers (Kalisinska et al. 2010), but the interpretation of this result may not be straightforward. In terms of feather growth, common loons had increased flight feather asymmetry, but only at environmental exposures resulting in 40 ug/g in feathers (Evers et al. 2008). Neither these loons, nor glossy ibises or double-crested cormorants, exhibited the same response at lower Hg levels (Clarkson et al. 2012). However, daily feather growth as a nutritional condition index, measured through ptilochronology, had a negative relationship with mercury exposure in glossy ibises.

Conclusion

Overall, Hg does indeed negatively impact nearly all aspects of avian physiology (Fig. 1a and b). Reproduction is by far the most well-studied category of endpoints because of its overt relation to fitness, and Hg exposure clearly reduces the number of offspring. Although the reproductive phenology does not appear strongly altered by Hg, the reduction in number of offspring may be a result of eggshell malformation, teratogenicity, or nestling or fledgling mortality. Meanwhile, parental and chick behavior can be abnormal.

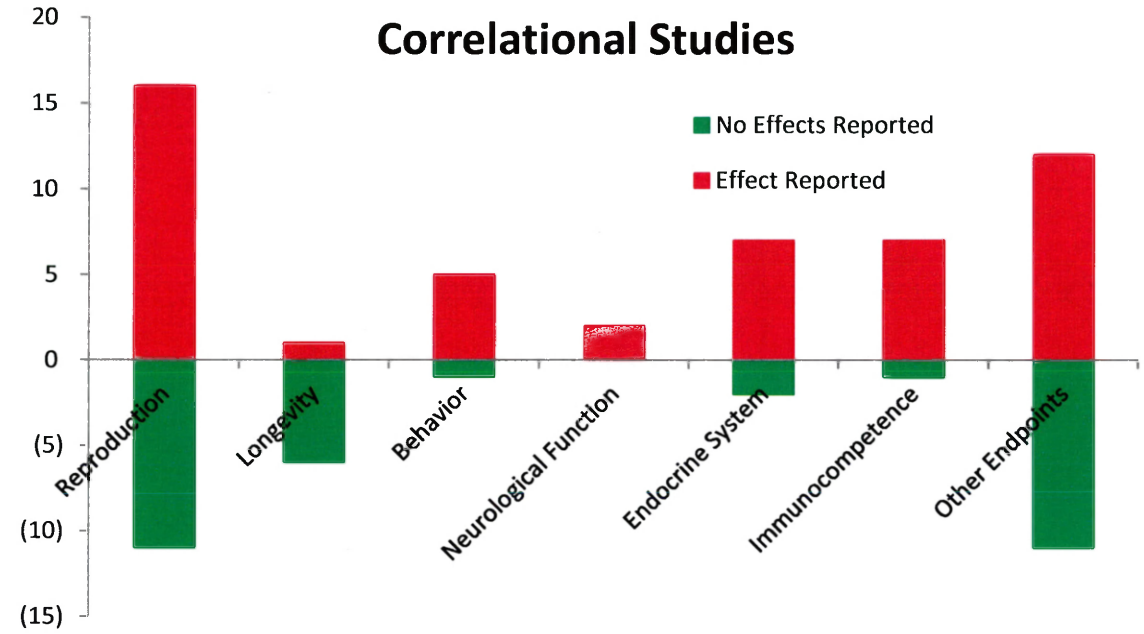
While offspring survival appears to be affected, longevity after leaving the nest does not decline detectably due to Hg exposure. Rather, exposed individuals face behavioral shifts away from higher energy activities. Hunting and foraging efficiency may be relatively resistant to the negative effects of Hg, with little consensus among

published results, and similarly there is no clear pattern regarding growth and body condition. However, immune function has frequently been found to be compromised, in addition to a number of changes in white blood cell counts.

Among endpoints with a small but rapidly growing body of evidence that reveal the deleterious effects of Hg are oxidative stress and neurological function, including axonal degeneration. But many endpoints remain understudied. There is currently too little information to make conclusions regarding neurotransmitters, or general metabolism. Many researchers have investigated various hormones, especially CORT and T, but together the results have not posed a meaningful explanation for how Hg is impacting the endocrine system. For most other hormones, there has been little to no investigation.

The only truly definitive conclusion to be drawn is that to understand how Hg is affecting birds, more experiments are required that focus on many physiological endpoints. The mechanisms for many of the observed results remain nearly a complete mystery, and similarly, some important traits, such as molt and migration behavior, have received disproportionately little attention. To collect meaningful data on most of the endpoints that remain inconclusive, especially endocrine function, great care must be taken to design appropriate experiments. The resources invested will be well spent to improve our understanding of how Hg is impacting avian wildlife.

a.



b.

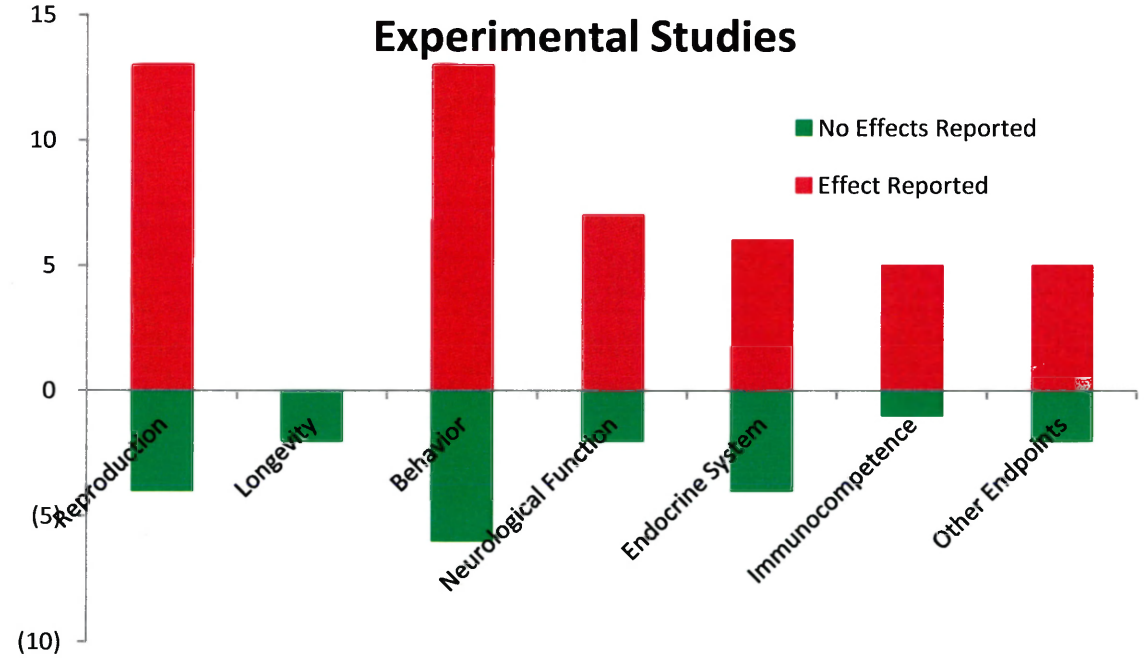


Figure 1. Number of published papers reporting negative impacts of Hg exposure (red) and number of papers reporting no effects or positive effects (green) for (a) correlational studies and (b) experimental studies.

Appendix 1. Common and scientific names of all species referenced, with citations.

Common Name	Scientific Name	Citation
American Avocet	*Recurvirostra americana*	Ackerman et al. 2008b
American Dipper	*Cinclus mexicanus*	Henny et al. 2005
American Kestrel	*Falco sparverius*	Albers et al. 2007 Bennett et al. 2009 Fallacara et al. 2011a Fallacara et al. 2011b
Bald Eagle	*Haliaeetus leucocephalus*	Anthony et al. 1999 Bowerman et al. 1994 DeSorbo & Evers 2005 Rutkiewicz et al. 2011 Wiemeyer et al. 1984 Scheuhammer et al. 2008 Weech et al. 2006
Belted Kingfisher	*Megaceryle alcyon*	Bouland et al. 2012
Black-Crowned Night-Heron	*Nycticorax nycticorax*	Henny et al. 2002
American Black Duck	*Anas rubripes*	Finley & Stendell 1978
Black-Footed Albatross	*Phoebastria nigripes*	Finkelstein 2003
Black-Legged Kittiwake	*Rissa tridactyla*	Tartu et al. 2013
Black-Necked Stilt	*Himantopus mexicanus*	Ackerman et al. 2008b
California Clapper Rail	*Rallus longirostris obsoletus*	Ackerman et al. 2012
Carolina Wren		Jackson et al. 2011 Hallinger et al. 2010
Common Eider	*Somateria mollissima*	Wayland et al. 2002
Common Loon	*Gavia immer*	Scheuhammer et al. 2008 Burgess & Meyer 2008 Evers et al. 2008 Kenow et al. 2003 Kenow et al. 2007 Kenow et al. 2008 Kenow et al. 2010 Merrill et al. 2005 Meyer et al. 1998 Mitro et al. 2008 Nocera & Taylor 1998 Olsen et al. 2000 Holloway 2001

Common Merganser	*Mergus merganser*	Kalisinska et al. 2010
Double-crested Cormorant	*Phalacrocorax auritus*	Clarkson et al. 2012 Gibson et al. 2014 Henny et al. 2002
Eastern Bluebird	*Sialia sialis*	Bouland et al. 2012
European Starling	*Sturnus vulgaris*	Nicholson & Osborn 1984
Forster's Tern		Ackerman et al. 2008a Herring et al. 2012 Hoffman et al. 2011
Glossy Ibis	*Plegadis falcinellus*	Clarkson et al. 2012
Great Egret	*Ardea alba*	Bouton et al. 1999 Hoffman et al. 2005 Sepulveda et al. 1999 Spalding et al. 2000a Spalding et al. 2000b
Greater Scaup	*Aythya marila*	Hoffman et al. 1998
Great Skua	*Stercorarius skua*	Thompson et al. 1991
Great White Heron	*Ardea herodias occidentalis*	Spalding et al. 1994
House Wren	*Troglodytes aedon*	Hallinger et al. 2010
Japanese Quail	*Coturnix japonica*	Hill & Soares 1984 Rutkiewicz et al. 2013
King Eider	*Somateria spectabilis*	Wayland et al. 2008
Laughing Gull	*Leucophaeus atricilla*	Jenko et al. 2012
Lesser Scaup	*Aythya affinis*	Pollock & Machin 2009
Mallard	*Anas platyrhynchos*	Heinz 1974 Heinz 1975 Heinz 1976a Heinz 1976b Heinz 1979 Heinz et al. 2010a Heinz et al. 2010b Heinz et al. 2011 Heinz & Locke 1976 Hoffman & Moore 1979 Pass et al. 1975
Northern Waterthrush	*Parkesia noveboracensis*	Seewagen 2013
Pigeon	*Columba livia*	Evans et al. 1982 Laties & Evans 1980

Red-Tailed Hawk	*Buteo jamaicensis*	Fimreite & Karstad 1971
Ruddy Duck	*Oxyura jamaicensis*	Hoffman et al. 1998
Snow Petrel	*Pagodroma nivea*	Tartu et al. 2014
Snowy Egret	*Egretta thula*	Olivero-Verbel et al. 2013 Henny et al. 2002
Song Sparrow	*Melospiza melodia*	Hallinger et al. 2010
Surf Scoter	*Melanitta perspicillata*	Hoffman et al. 1998
Western Grebe	*Aechmophorus occidentalis*	Elbert & Anderson 1998
Tree Swallow	*Tachycineta bicolor*	Brasso & Cristol 2008 Franceschini et al. 2009 Hallinger & Cristol 2011 Hallinger et al. 2011 Hawley et al. 2009 Longcore et al. 2007 Wada et al. 2009 Gerrard 2000 Bouland et al. 2012
White Ibis	*Eudocimus albus*	Adams & Frederick 2008 Adams et al. 2008 Frederick et al. 2011 Frederick & Jayasena 2010 Heath & Frederick 2005 Herring et al. 2009 Jayasena et al. 2011 Jayasena 2010
White Leghorn	*Gallus gallus domesticus*	Lundholm 1995 Holloway2001 Rutkiewicz et al. 2013
White-Tailed Sea Eagle	*Haliaeetus albicilla*	Helander et al. 1982
White-Winged Scoter	*Melanitta deglandi*	Wayland et al. 2008
Zebra Finch	*Taeniopygia guttata*	Lewis et al. 2013 Moore et al. 2014 Scheuhammer 1988 Varian-Ramos et al. 2014

Chapter 2: Rapid Feather Molt in Songbirds Leads to Effective Depuration of Mercury

Introduction

As environmental mercury (Hg) availability continues to increase worldwide (Sunderland et al. 2009), an increasing number of studies have suggested that methylmercury (MeHg) exposure affects physiology, behavior, and reproductive success of birds (Varian-Ramos et al. 2014). A few studies have addressed the issue of Hg kinetics and feather molt, but these have been limited to a small subset of avian taxa (large, fish-eating species; Monteiro et al. 2001a, Bearhop et al. 2000, Fournier et al. 2002). However, Hg accumulates in and impacts terrestrial species as well, including the small songbirds that comprise most of avian biodiversity. Hg is a potential problem for all birds and not restricted to piscivorous food webs (Cristol et al. 2008).

In both terrestrial and aquatic species, Hg exposure can impair immune function, disrupt the endocrine system, and damage organs (Lewis et al. 2013, Moore et al. 2014, Snelgrove-Hobson et al. 1988, Spalding et al. 2000) within weeks or months of exposure. Whether or not these effects persist after Hg exposure has ceased and birds have excreted their bodily Hg load has not been examined, and is an especially relevant question when assessing the risk of harm to migratory or dispersive species exposed for only part of the annual cycle. Because Hg binds to the keratin in growing feathers, molt serves as an important route of Hg depuration for birds (Condon & Cristol 2009). Most songbirds molt their flight feathers rapidly, often once a year between breeding and

migration/dispersal, whereas other species, including many seabirds, molt gradually throughout the annual cycle.

The elimination of Hg from adult aquatic birds appears to be best described by a 2-compartment model incorporating a 1-d rapid phase and a slow phase of many months. The biological half-life of Hg in adult Cory's shearwaters (*Calonectris diomedia*) that were not molting when dosed with Hg was 44–65 d for the slow phase (Montiero et al. 2001a). Fournier et al. (2002) found the half-life of Hg in juvenile common loons (*Gavia immer*) that were no longer molting to be 116 d during the slow phase. Juvenile great skuas (*Catharacta skua*) dosed before and during their first adult molt exhibited a half-life of 31–63 d for the slow phase (Bearhop et al. 2000).

In general, younger chicks that were rapidly growing their full plumage demonstrated considerably faster elimination of Hg, described by single-compartment models. For young shearwater chicks, the half-life of dosed mercury was 5.5–6.3 d (Montiero et al. 2001b), and for young loon chicks it was 3 d (Fournier et al. 2002).The shorter half-life relative to full-grown birds was attributed to the increased growth of body tissues and feathers. Feathers are well documented as Hg sinks (Furness et al. 1986), so a similar pattern emerges for adults that are molting compared to adults that are not. In adult Cory's shearwaters, birds dosed immediately prior to molt depurated Hg with a half-life of 38–46 d, compared to birds dosed 3–4 mo before molting, which had a half-life of up to 65 d (Montiero et al. 2001a).

In songbirds, feather growth reduced the concentration of Hg in blood in rapidly molting juvenile eastern bluebirds (*Siala sialis*, Condon & Cristol 2009), with Hg

concentrations rising to adult levels only after the completion of feather growth. To isolate the role of feather growth from other changes that occur during development, we measured the depuration of mercury from blood and organs in two species of songbirds, one that molts rapidly (European starling, *Sturnus vulgaris*) and one that molts gradually throughout the year (zebra finch *Taenopygya guttata*). For the starling we compared depuration rates during molt and when not molting, and in the finch we measured depuration during normal, gradual molt as well as during experimentally-induced rapid molt. Molting birds were expected to depurate more rapidly and to retain less Hg in organ tissues than non-molting birds.

Methods

European starlings

Rapid molt. We captured 54 young-of-the-year European starlings near the campus of the College of William & Mary (Williamsburg, VA, USA) in baited walk-in traps in May-July 2011 and maintained them in large outdoor aviaries on *ad lib.* poultry starter pellets (Bartlett Milling Company) and drinking/bathing water. They were divided into 3 groups and placed on a pelletized diet containing low (0.75 ppm) or high (1.5 ppm) concentrations of MeHg for14 mo, during which time they underwent one complete molt of all adult feathers. Each batch of food was produced by mixing pellets thoroughly with aqueous MeHg-Cysteine and testing to ensure that it was within 10% of the nominal dose on a wet weight basis (see Varian-Ramos et al. 2014 for details of MeHg-Cysteine preparation). Because the diet contained 18% moisture when consumed, the equivalent concentration on a dry weight basis was 0.92 ppm or 1.83 ppm. Immediately prior to the

second adult molt (May 2012), half of the birds in each treatment were sacrificed by rapid decapitation, and the rest were switched to the control diet for 20 weeks while they completed molt and depurated mercury. Blood samples were collected approximately monthly from all birds, and then weekly during depuration, to track Hg concentrations. Molt was quantified by examining all nine primary feathers weekly and scoring each feather's state from 0–5: 0 indicating that the old feather was present and feather replacement had not begun and 5 indicating that the new feather was fully grown (Ginn & Melville 1983). Remaining birds were sacrificed at the end of 20 weeks. Upon sacrifice, samples of blood, brain, pectoral muscle, kidney, and liver were collected for immediate freeze-drying and total Hg analysis.

No-molt. European starlings (n=11) were maintained as described above but on a mercury-free diet. After completing molting, they were placed on a mercury diet as prepared above containing 1.5 ppm MeHg-Cysteine wet weight for 11 weeks (October – December 2013), at which point their blood Hg concentration had stabilized and they were switched to a mercury-free control diet to depurate Hg for 20 weeks in the absence of molt (December 2013–May 2014). Blood was sampled bimonthly prior to depuration, then weekly during depuration. All birds were sacrificed to collect organ tissues prior to the beginning of their annual molt, and tissues were freeze-dried and assayed for mercury as described above. This experimental group provided a comparison with the molting starlings for rate of depuration in the absence of feather molt, however, it should be noted that these starlings had been exposed to mercury for only 3 mo prior to depuration, as opposed to 14 mo for the molting treatment group.

Zebra finches

Rapid molt. We maintained 15 zebra finches throughout adult life on pelletized diets containing 1.2 ppm MeHg-Cysteine wet weight. They were then placed on control diets and allowed to depurate for 20 weeks (October 2013–February 2014). Blood was sampled monthly prior to depuration, then weekly during depuration. Molt was scored weekly, as above. To simulate a rapid molt we plucked one primary or secondary feather each week from each wing until 9 primary feathers and 9 secondary feathers had been replaced. No more than 3 primary or secondary feathers and no more than 4 feathers total, per wing, were growing at a given time.

No molt. Zebra finches undergo a continuous molt that is so gradual that it approximates a non-molting condition. Captive-bred zebra finches (n=64) were maintained throughout adult life on *ad lib.* pelletized diets (ZuPreem, Shawnee, KS) prepared as above containing either 0.6ppm or 1.2ppm MeHg-Cysteine wet weight, along with water for drinking/bathing, vitamins and calcium supplements. (Food had moisture content of 13.9% when consumed so dry weight equivalents were 0.7 ppm and 1.4 ppm, respectively). Half of these birds had begun mercury dosing upon reaching sexual maturity (approximately 150 d after hatching, hereafter "adult exposure"), whereas the others had been exposed *in ovo* through parental dosing and then throughout their lives (hereafter "lifetime exposure"). Finches ranged in age from 14–34 mo (22 ± 5.3 mo) at the start of depuration in July 2013, when one-third of the birds in each treatment group were sacrificed to sample organ tissues and the rest placed on mercury-free control diets.

All finches had their blood sampled monthly for mercury throughout their lives,

and then weekly during depuration. After 20 weeks of depuration, an additional one-third of the finches were sacrificed for organ tissues, as with the second set of European starlings described above. Because we expected depuration to be slower in the gradually molting species, the final 28 finches were sacrificed after 40 weeks of depuration to sample organ tissues. Molt was scored weekly and tissues were prepared and analyzed for Hg as above.

Each week when finches were bled, all eggs in each cage were counted because egg-laying is a potential form of Hg depuration. Because eggs could not be attributed to specific female birds, the numbers were not used in any statistical tests but differences between groups of females were noted.

In order to avoid confusion, hereafter, rapid molt finches will be referred to as "molting" finches, and the 1.2 ppm and 1.5 ppm concentrations will both be classified as the "high" dose, while the 0.6 ppm and 0.75 ppm concentrations will be referred to as the "low" dose. Therefore, we had molting starlings on the low and high doses, non-molting starlings in the high dose, molting finches on the high dose, and non-molting finches on the low and high doses. There were lifetime exposure and adult exposure finches in both dose levels among the non-molting birds.

Data Analysis

Blood. Blood Hg concentrations were linearized using log10 transformation. The slope for each bird was calculated using each week's value until the week when the concentration had decreased by 98%, and this slope was considered the rate of depuration of Hg from the blood. Using ANOVA, these rates were compared between the two

dosing levels and exposure groups for the non-molting finches. A t-test was used to compare the two dosing levels for the molting starlings, and molting to non-molting finches, after combining the non-molting finch rates into a single group with both types of exposure (lifetime and adult). Rates for molting and non-molting starlings were compared using ANOVA for all groups, and a t-test between the high-dose birds. The following equation was used to calculate the biological half-life of Hg in the blood for each bird:

$$T_{1/2} = \frac{\ln 2}{-b \times \ln 10}$$

where b is the slope.

For each group, the curve for the concentration of Hg in the blood was visually inspected to determine fast and slow compartments, and the asymptotic phase was excluded. For each bird, the slope was found for these two subsets of weeks using the untransformed data. Using ANOVA, these rates were compared between the two dosing levels and exposure groups for the non-molting finches. A t-test was used to compare the two dosing levels for the molting starlings, molting to non-molting finches, and molting to non-molting starlings in the high-dose group.

Organs. Brain, liver, kidney, and pectoral muscle tissue Hg concentrations were transformed using the natural log and analyzed using MANOVA to compare Hg treatment effects, molting to non-molting finches from the high-dose groups, and molting starlings from the high-dose group to non-molting starlings. Tissue concentrations for finches depurated for 20 weeks were compared to those depurated for 40 weeks after natural log transformation using MANOVA.

Pre-depuration blood Hg was unexpectedly elevated in the molting finches compared to non-molting finches ($t = -2.8403$, $p = 0.011$), so raw organ Hg concentrations were not comparable. Therefore, we compared the percentage Hg remaining in each tissue for the molting and non-molting finches. To obtain pre-depuration tissue concentrations for the molting finches, a group in which we did not sacrifice a portion of the birds at the start of depuration, samples were collected from finches with comparable blood Hg concentrations that had already been sacrificed for a previous experiment. The average Hg concentration for brain, liver, kidney, and muscle was calculated from the pre-depuration samples for both groups. Percentages were then calculated using this number and the concentration of Hg in each individual post-depuration finch's tissues. These percentages were natural log-transformed, and this number was used in the MANOVA. All statistical analyses and modeling were conducted using the R statistical language v. 3.0.2 (R Development Core Team 2013).

All tissue samples were analyzed for total Hg using a Milestone DMA-80 (Shelton, CT) at the College of William & Mary. Tissues were freeze-dried for 48 h prior to Hg analysis, except blood samples, which were thawed and analyzed on a wet weight basis. The instrument was calibrated every 1-2 months or as needed. The calculated minimum detectable concentration during the period including these analyses was between 0.0181 and 0.0275 ppm. Two samples each of two solid standard reference materials were run with every 20 samples (DORM-3 or DORM-4 and DOLT-4, National Institute of Standards, Canada), along with one duplicate, two blank sample containers and two blanks. Relative percent difference of duplicate starling blood was $3.66 \pm 5.28\%$,

and relative percent difference of duplicate finch blood was 3.88 ± 5.29%. Standard recovery was 99.89 ± 3.51% for DORM-3, 100.01 ± 7.92% for DORM-4 and 99.68 ± 3.72% for DOLT-4. When we spiked 10 samples of blood with standard reference material our recovery was 96 ± 0.79%.

Results

Half life of blood Hg

European starling. In molting starlings, the Hg cleared rapidly from the blood, reaching 50% of the starting concentration in 1.1 (± 0.12) –1.21 (± 0.12) weeks (Fig. 2). At 11 weeks, blood Hg stabilized at baseline level, where it remained until the birds were sacrificed 20 weeks after being taken off of mercury. In non-molting starlings Hg depurated from the blood more slowly than in molting starlings, reaching 50% of the starting concentration in 1.99 ± 0.26 weeks (Fig. 2). At 20 weeks, when the birds were sacrificed, blood Hg concentrations remained elevated above baseline.

Zebra finch. Molting finches reached 50% of their starting Hg concentration in 2.43 ± 0.34 weeks (Fig. 3). This was faster than for non-molting finches, in which blood Hg reached 50% of the starting concentration in 3.17 ± 0.59 weeks (Fig. 3). After 11 weeks, when molting starlings (see above) had reached baseline, the molting finches' blood was still at 10% of starting level. At 20 weeks, when the second group was sacrificed, blood Hg concentrations remained elevated from baseline, and continued to decrease until 40 weeks when the final group was sacrificed.

Rate of depuration of blood Hg

European starling. The average rate of depuration from the blood, using log10 transformed values by week, was -0.28 ± 0.03 for the high-dose molting starlings, -0.25 ± 0.02 for the low-dose molting starlings, and -0.15 ± 0.02 for the non-molting starlings. The rate did not differ significantly between the high-dose molting birds and those fed the low dose ($t=2.1072$, $p=0.054$), and therefore the two groups were pooled for comparison with the non-molting starlings. This average rate for molting starlings, -0.26 ± 0.03, was significantly faster than the rate for the non-molting starlings ($F=124.62$, $p<0.001$), but a significant effect of dosing level also emerged ($F=20.98$, $p<0.001$), and so the comparison was repeated using only the molting starlings from the high-dose group. The molting high-dose starlings depurated significantly faster than non-molting starlings on the same dose ($t=10.5617$, $p<0.001$).

For the first compartment, the rates differed significantly for molting birds in the high- and low-dose groups ($t=4.6311$, $p=0.002$, Table 1a). The average first-compartment rate for the molting starlings was significantly faster than the non-molting starlings ($t=2.9669$, $p=0.01$). For the second compartment, the rates also differed significantly for molting birds in the high- and low-dose group ($t=3.1201$, $p=0.01$, Table 1a). The average second-compartment rate for the molting starlings was not significantly different from the non-molting starlings ($t=1.226$, $p=0.238$) (Fig. 4).

Zebra finch. The average rate of depuration from the blood for non-molting finches, using log10 transformed values by week, was -0.10 ± 0.02 for low-dose adult exposure birds, -0.09 ± 0.02 for low-dose lifetime exposure birds, -0.09 ± 0.02 for high-dose adult

exposure birds, and -0.11 ± 0.02 for high-dose lifetime exposure birds. These rates did not differ significantly, and therefore all non-molting finches were pooled for comparison with molting finches. The average rate for all non-molting finches, -0.10 0.02, differed significantly from the average rate for molting finches, -0.13 ($t=5.0635$, $p<0.001$).

For non-molting finches, the rate was significantly faster for high-dose birds than for low-dose ($F= 31.138$, $p<0.001$), and for lifetime exposure over adult exposure birds ($F=4.701$, $p=0.0358$, Table 1a). The average first-compartment rate for the molting finches was significantly faster than the high-dose non-molting finches, -2.54 ppm Hg/week ($t=3.1708$, $p=0.005$, Table 1b). For the second compartment, the rate was significantly higher for high-dose finches ($F=12.448$, $p<0.001$). The average second-compartment rate for the molting finches was not significantly different from the non-molting finches, -0.77 ppm Hg/week ($t=0.5673$, $p=0.575$, Table 1b) (Fig. 5).

Organ mercury

European starlings. After depuration, non-molting starlings had significantly lower concentrations of Hg in their brain, muscle, and liver compared to the molting starlings (Fig 6). Kidney Hg concentration did not differ (Pillai's Trace = 0.71, F = 7.24, *df* = 15, $p < 0.05$) (Table 2).

Zebra Finches. Post-depuration Hg concentrations in the brain, liver, and muscle were significantly lower in molting than non-molting finches (Fig 7). Kidney Hg concentration did not differ between molting and non-molting finches (Pillai's Trace = 0.39, F = 3.68, *df*= 19, $p < 0.05$) (Table 3, Table 4).

Despite the MANOVA showing a significant effect of dose when all tissues were combined (Pillai's Trace = 0.72, F = 5.68, *df*= 12, $p < 0.05$), no differences were found between the lifetime and adult-exposed finches or between the non-molting high-dose group and the low-dose group in the brain, liver, kidney, or muscle tissue for the birds sacrificed at 20 weeks in the univariate analysis (Fig. 8a). For the finches sacrificed at 40 weeks, the high-dose group retained more Hg than the low-dose group in their brain, muscle, and liver (Pillai's Trace = 0.65, F = 9.65, *df* = 24, $p < 0.01$) (Table 5a), and the lifetime exposure group retained more Hg than the adult exposure group in their liver (Pillai's Trace = 0.44, F = 4.15, *df* = 24, $p < 0.05$) (Table 5b, Fig. 8b).

Finches sacrificed at 40 weeks retained a significantly lower percentage of their starting Hg concentrations in their brain, muscle, kidney, and liver compared to those sacrificed at 20 weeks (Pillai's Trace = 0.75, F = 25.17, *df* = 36, $p < 0.001$) (Table 6a). For muscle and liver, percentages dropped less over time for birds in the high-dose groups than for birds in the low-dose groups between 20 and 40 weeks (Pillai's Trace = 0.28, F = 3.14, *df* = 36, $p < 0.05$) (Table 6b). Combining the data for birds at 20 and 40 weeks, birds in the high-dose groups retained a higher percentage of their starting concentration in their brain and liver than those in the low-dose groups (Pillai's Trace = 0.53, F = 9.36, *df* = 36, $p < 0.001$) (Table 6c), and lifetime exposure birds retained a higher percentage of their starting Hg concentrations in their brain, muscle, and liver than the adult exposure birds (Pillai's Trace = 0.39, F = 5.29, *df* = 36, $p < 0.01$) (Table 6d).

Discussion

The rates of depuration of Hg from the blood that we calculated are markedly faster than those reported in previous literature. The biological half-life for the non-molting zebra finches was 3.17 weeks (22 d); about half as long as the shortest reported half-life in the literature (44 days for non-molting adult Cory's shearwaters, Monteiro et al. 2001a). Molting zebra finches exhibited a blood Hg half-life of 2.43 weeks (17 d), also roughly half of the shortest reported half-life (31 days for juvenile great skuas, Bearhop et al. 2000). The molting and non-molting starlings depurated faster still (molting half-life: 1.1-1.21 weeks or 7-8.5 d; non-molting half-life: 1.99 weeks or 14 d). The faster half-lives of the birds we studied may be due in part to the considerable size difference between the large fish-eating birds and the small songbirds we studied. There are also methodological and analytic differences between each of the studies. In particular, our rates are based on percent decline of previously incorporated mercury as opposed to disappearance of a known mercury dose.

Similarly, the half-life for Hg in other tissues was shorter for our songbirds than for adult mallards (Stickel et al. 1977). Dosed mallards (presumed non-molting) had a half-life for tissue mercury of about 8 weeks for kidneys and 10 weeks for livers. Based on the reduction observed after 20 weeks of depuration, even the non-molting finches exceeded that rate.

The finches followed the expected pattern of non-molting birds retaining a higher concentration of Hg in organ tissues than molting birds did, but the starlings deviated starkly from it. We hypothesize that this was due to the much shorter duration of dosing

for the non-molting starlings, 11 weeks compared to 14 months for the molting starlings. Although blood Hg concentrations had reached an asymptote, the tissues may not yet have been saturated. Therefore, we likely did not perform valid comparison of molting and non-molting starlings because of different organ starting mercury concentrations.

In support of this interpretation, the half-life in livers, muscle, and kidney in pheasants, chickens, and commercial ducks was about 1–2 weeks (Gardiner 1972), which is comparable to or faster than the half-lives for the zebra finches and starlings. This may be in part because the larger commercial birds were dosed with Hg for only 5 weeks, compared to a minimum of 11 weeks for the songbirds, but the mallards mentioned above were fed Hg for only 2 weeks (Stickel et al. 1977). Therefore, the difference in exposure period is an incomplete explanation at best, both when comparing our data to the literature and explaining why the non-molting starlings had lower post-depuration tissue Hg concentrations. The non-molting starlings depurated during winter and early spring, and the higher metabolic demands compared to summer, when the molting starlings depurated, was another difference between treatments.

However, our results are consistent with previous studies in terms of the appreciably increased depuration rate in molting as opposed to non-molting birds. Molting starlings nearly doubled their blood depuration rate. The difference was less pronounced for the finches, but this could be explained by the fact that "non-molting" finches were actually replacing a relatively small number of feathers, and "molting" finches did not replace their primary feathers as quickly as the starlings and were not replacing their contour feathers in significant numbers. The molting starlings replaced all

of their feathers while the non-molting starlings replaced virtually no feathers.

Lifetime versus adult exposure had no effect on the overall rate of depuration from the blood or the rates of elimination of the second compartment. There was a significant effect on the rate for the first compartment, and the more rapid rate of depuration in the high lifetime exposure group is immediately apparent. However, this may have been the result of increased excretion of Hg into eggs compared to the other groups. The high lifetime exposure group laid approximately 100 more eggs than either adult exposure group or the low lifetime exposure birds during the first 20 weeks. No such extreme difference in the number of eggs was observed in the final 20 weeks.

However, no differences were found between the adult and lifetime exposure groups in their tissues after 20 weeks of depuration, whereas after 40 weeks lifetime exposure birds retained significantly more Hg in their livers. Furthermore, when all birds were analyzed together, the lifetime exposure birds retained significantly higher Hg in their livers, brains, and muscle tissue. This might suggest that the excessive egg-laying obscured differences in the group sacrificed at 20 weeks. The number of eggs was not used as a covariate in any analyses because it was unknown which birds in the cage laid the eggs.

As expected, molt significantly accelerated the rate of depuration of Hg from blood and tissues. Migratory birds may be relatively insulted from the impact of Hg compared to birds residing in contaminated areas year-round. Breeding in a contaminated site puts developing young at risk, but molting shortly before or after departing the site would allow individuals to reduce their body burdens more rapidly than migrants

wintering in contaminated sites. Migrants leaving contaminated wintering areas to breed may not have the benefit of molt accelerating the depuration of Hg from their bodies, and may deposit higher levels of Hg into eggs.

Birds exposed *in ovo* retained more Hg in their organs than birds dosed as adults, but we did not investigate whether there was a difference for birds exposed *in ovo* versus those exposed as nestlings. Although Hg exposure during early development is potentially more problematic than Hg exposure throughout later development, birds exposed throughout later development likely deposit more Hg into their organs. These birds would therefore be more likely to retain higher concentrations in body tissues compared to birds exposed only during early development.

While a typical explosive molt is beneficial for migrants leaving a contaminated site, continuously molting might be more useful for resident birds. An explosive molt would temporarily reduce their body burden, but molting continuously could prevent a large accumulation of Hg or maintain a generally lower body burden. Regardless of molt schedule or whether migrants are wintering on or breeding in a contaminated site, migrants clearly have an advantage over resident species for mitigating the deleterious effects of Hg contamination.

The fact that Hg is impacting terrestrial communities is a relatively new discovery (Cristol et al. 2008), and songbirds are clearly suffering negative impacts. However, songbirds are potentially more capable of mitigating Hg contamination because they depurate comparatively rapidly. Depending on how resilient other terrestrial organisms

are, songbirds may not be the most effective bioindicators for assessing Hg risk in these environments.

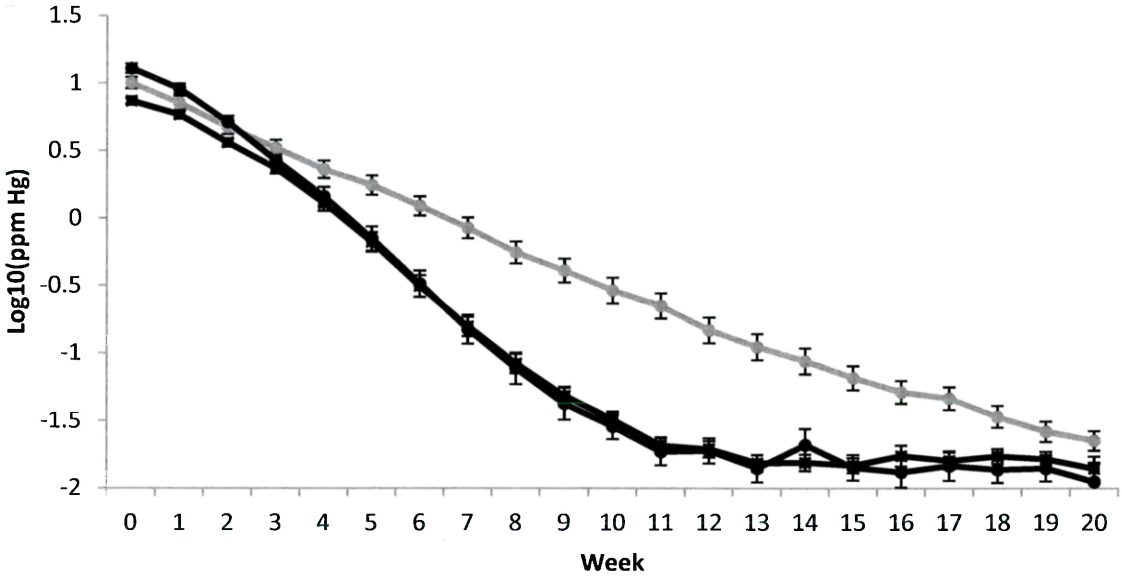


Figure 2. Blood Hg concentrations of molting (black) and non-molting (gray) starlings during depuration, transformed with log base 10. Circles represent high doses, squares represent low doses. Error bars represent standard error.

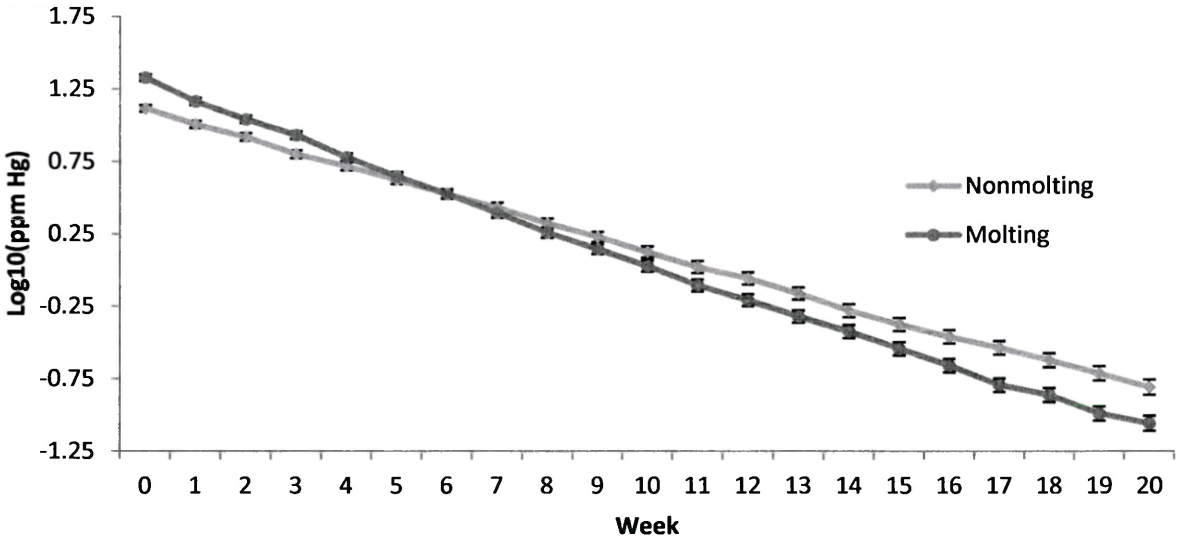
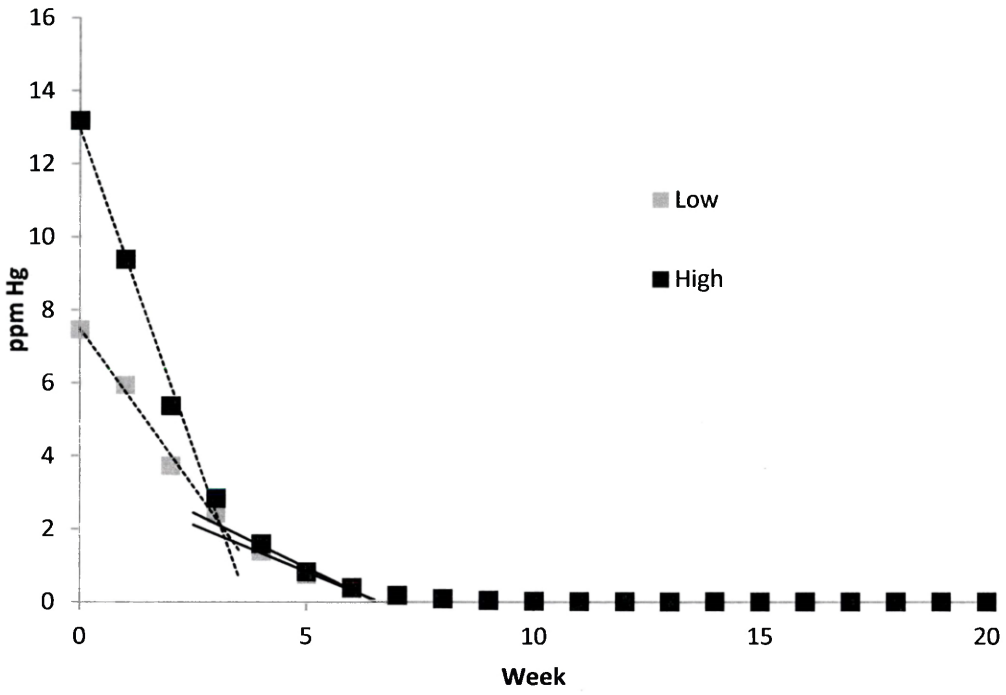


Figure 3. Blood Hg concentrations of molting and non-molting finches during depuration, transformed with log base 10. The low, high, adult exposure, and lifetime exposure groups of non-molting finches have been grouped. Error bars represent standard error.

a.



b.

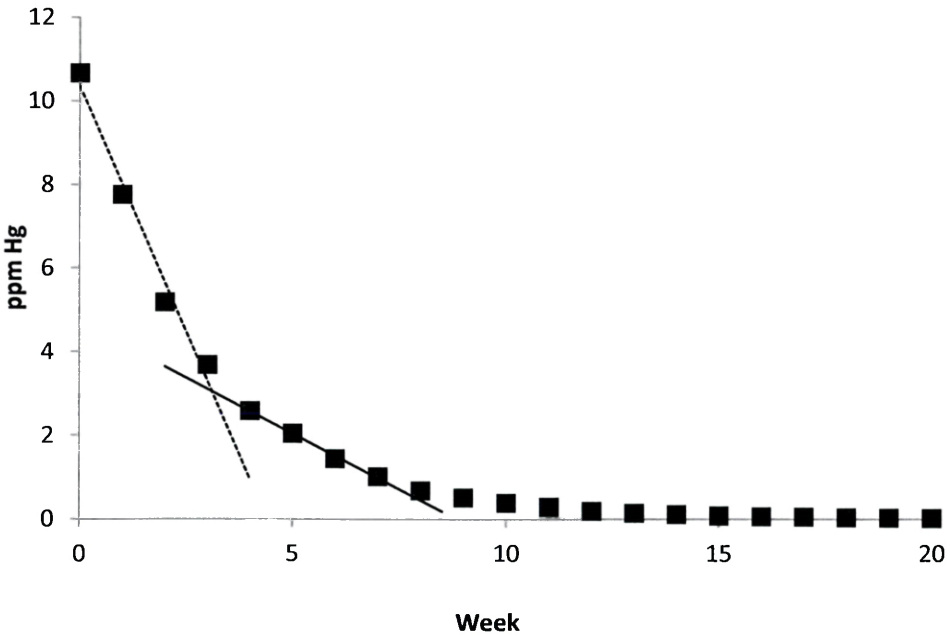
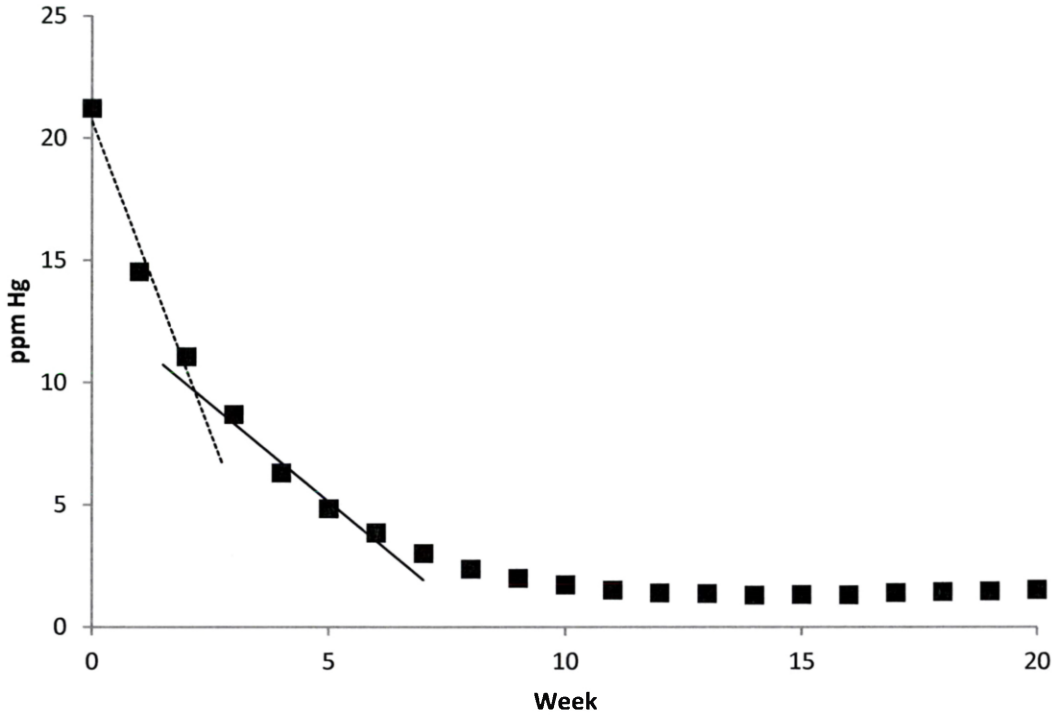


Figure 4. Starling blood Hg concentration during depuration for (a) molting and (b) non-molting birds. Dashed lines represent the first compartment and solid lines represent the second compartment.

a.



b.

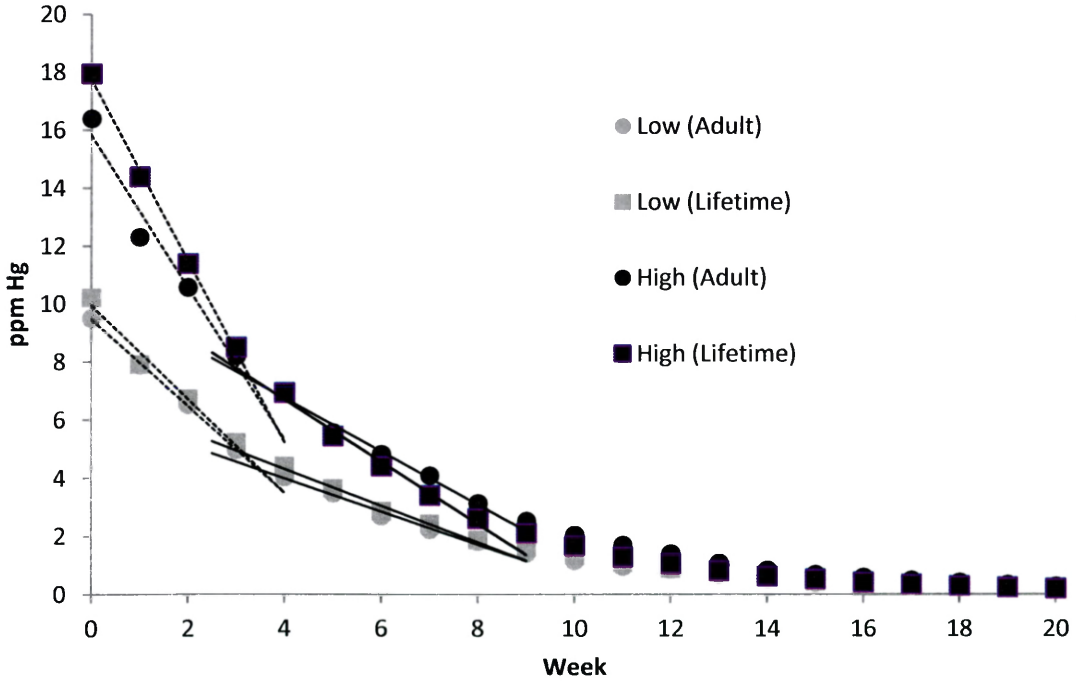


Figure 5.Finch blood Hg concentration during depuration for (a) molting and (b) non-molting birds. Dashed lines represent the first compartment and solid lines represent the second compartment.

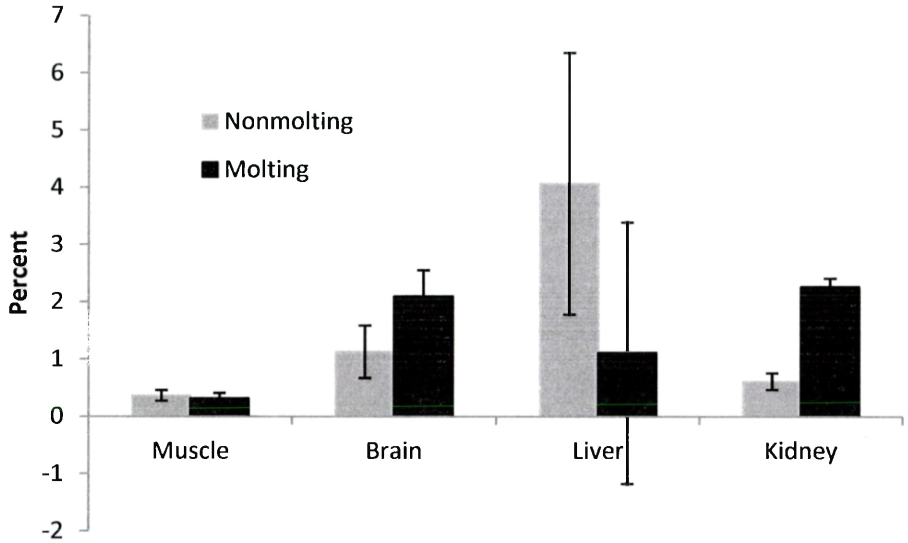


Figure 6. Percentage of the pre-depuration Hg concentration remaining in the muscle, brain, liver, and kidney for molting (black) and non-molting (gray) starlings from the high-dose groups. Error bars represent standard error.

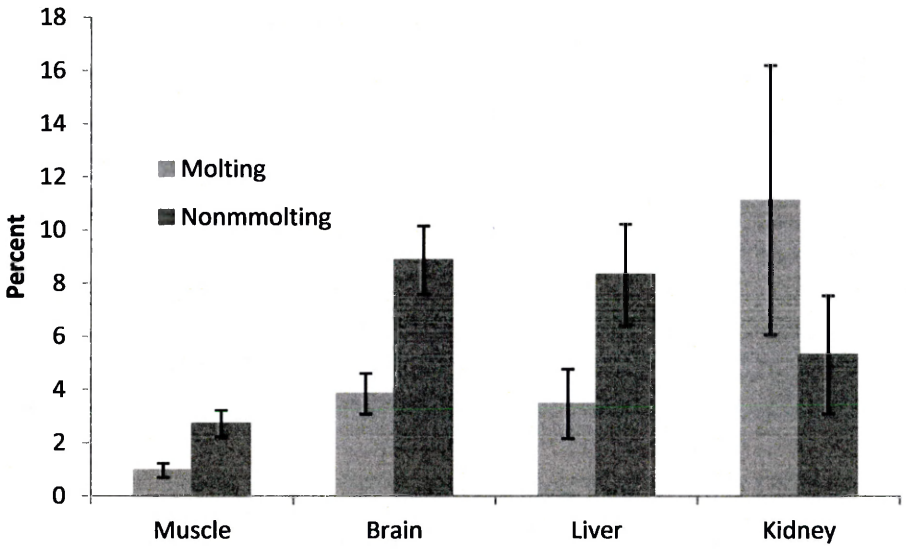
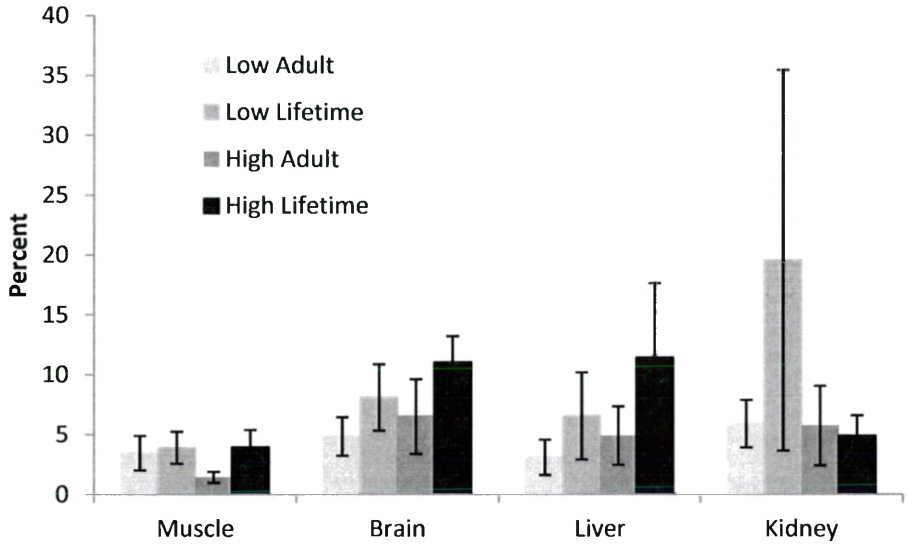


Figure 7. Percentage of the pre-depuration Hg concentration remaining in the muscle, brain, liver, and kidney for molting (light gray) and non-molting (dark gray) finches from the high-dose groups. Error bars represent standard error.

a.



b.

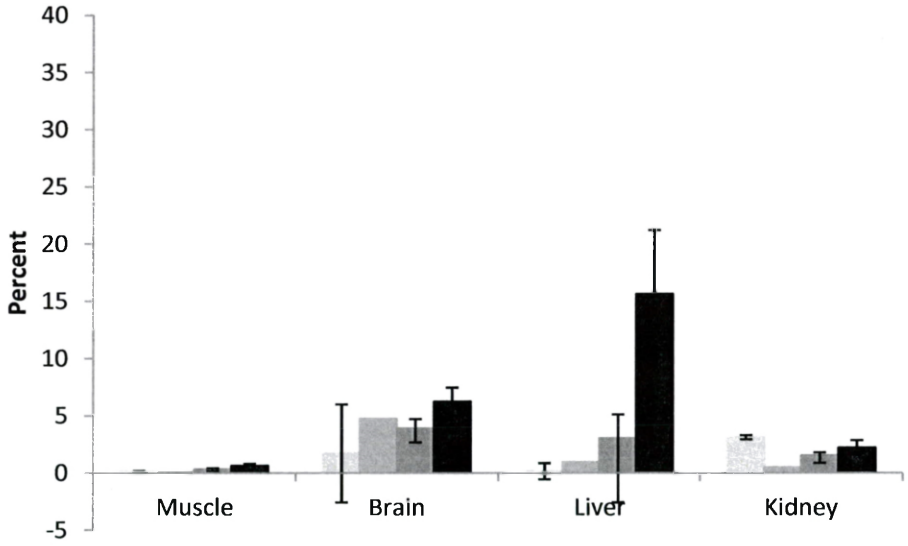


Figure 8. Percentage of the pre-depuration Hg concentration remaining in the muscle, brain, liver, and kidney after (a) 20 weeks and (b) 40 weeks. Error bars represent standard error.

Table 1. Mean and standard deviation for the first and second compartment rates (ppm/week) of Hg loss from blood tissue in (a) European starlings (b) and zebra finches.

a.

Group	Dose	First	Second
Molting	Low	-1.57 ± 0.19	-0.29 ± 0.15
	High	-3.37 ± 1.09	-0.60 ± 0.25
Non-molting	High	-2.02 ± 0.81	-0.45 ± 0.27

b.

Group	Dose	First	Second
Molting	High	-3.87 ± 1.48	-0.82 ± 0.31
Non-molting	Low (Adult)	-1.38 ± 0.53	-0. 50 ± 0.22
	High (Adult)	-2.29 ± 0.68	-0.78 ± 0.26
	Low (Lifetime)	-1.5 ± 0.28	-0.43 ± 0.34
	High (Lifetime)	-2.77 ± 0.87	-0.75 ± 0.33

Table 2. Significant univariate effects of molt for starlings. Means and confidence intervals are represented by the natural log of the Hg concentration in ppm.

Dependent Variable	*df*	Error *df*	*F*	Group	Means	95% Confidence Intervals	
						Upper	Lower
Brain	1	15	12.83	Molting	0.19	-0.57	0.95
				Non-molting	-1.30	-1.56	-0.65
Muscle	1	15	10.84	Molting	-1.54	-1.03	-1.03
				Non-molting	-2.42	-2.04	-2.04
Liver	1	15	24.54	Molting	2.95	3.46	2.44
				Non-molting	0.61	1.50	-0.27

Table 3. Significant univariate effects of molt for zebra finches. Means and confidence intervals are represented by the natural log of the percentage Hg concentration remaining in tissues.

Dependent Variable	*df*	Error *df*	*F*	Group	Means	95% Confidence Intervals	
						Upper	Lower
Muscle	1	19	8.70	Molting	-0.32	0.08	-0.73
				Non-molting	0.70	1.27	0.13
Liver	1	19	4.54	Molting	0.60	1.24	-0.03
				Non-molting	1.66	2.33	0.98
Brain	1	19	5.73	Molting	1.16	1.49	0.82
				Non-molting	1.92	2.51	1.34

Table 4. Concentration of Hg in ppm before (pre) and after (post) a depuration period of 20 weeks in body tissues. Reported are mean ± standard deviation and sample size in parentheses.

			Pre	Post	Pre	Post
Species	Group	Dose	Muscle		Brain	
Starlings	No Molt			0.11 ± 0.09 (11)		0.49 ± 0.65 (11)
Starlings	Molt	Low	17.04 ± 6.29 (9)	0.05 ± 0.02 (9)	25.16 ± 9.30 (9)	0.36 ± 0.25 (9)
	Molt	High	30.35 ± 7.44 (8)	0.27 ± 0.2 (8)	42.93 ± 9.21 (8)	1.96 ± 1.82 (8)
Finches	No Molt	Low (Adult)	21.08 ± 0.83 (4)	0.73 ± 0.61 (4)	20.78 ± 2.19 (4)	1.01 ± 0.67 (4)
	No Molt	Low (Lifetime)	21.3 ± 6.83 (4)	0.84 ± 0.57 (4)	25.05 ± 5.25 (4)	2.03 ± 1.39 (4)
	No Molt	High (Adult)	37.36 ± 9.1 (4)	0.54 ± 0.34 (4)	45.91 ± 9.2 (4)	2.99 ± 2.87 (4)
	No Molt	High (Lifetime)	39.01 ± 3.51 (4)	1.54 ± 1.13 (4)	50.31 ± 9.17 (4)	5.56 ± 2.21 (4)
Finches	Molt		53.32 ± 13.71 (10)	0.52 ± 0.50 (13)	70.4 ± 16.57 (10)	2.71 ± 1.95 (13)
			Liver		Kidney	
Starlings	No Molt			5.59 ± 10.42 (11)		0.74 ± 0.58 (11)
Starlings	Molt	Low	82.11 ± 42.4 (9)	0.19 ± 0.09 (9)	75.05 ± 27.93 (9)	0.39 ± 0.16 (9)
	Molt	High	137.43 ± 34.85 (8)	22.78 ± 11.63 (8)	120.29 ± 19.43 (9)	2.45 ± 4.31 (8)
Finches	No Molt	Low (Adult)	49.39 ± 5.31 (4)	1.54 ± 1.46 (4)	39.37 ± 5.01 (4)	2.37 ± 1.59 (4)
	No Molt	Low (Lifetime)	43.16 ± 12.89 (4)	2.84 ± 3.14 (4)	41.6 ± 10.39 (4)	8.16 ± 13.25 (4)
	No Molt	High (Adult)	71.48 ± 18.38 (4)	3.52 ± 3.5 (4)	75.29 ± 17.40 (4)	4.34 ± 5.02 (4)
	No Molt	High (Lifetime)	78.83 ± 16.75 (4)	9.02 ± 9.83 (4)	78.59 ± 12.51 (4)	3.86 ± 2.69 (4)
Finches	Molt		129.98 ± 53.27 (10)	4.52 ± 6.12 (13)	129.08 ± 60.7 (10)	14.4 ± 23.6 (13)

Table 5. Significant univariate effects of (a) dose and (b) exposure for non-molting finches sacrificed after 40 weeks. Means and confidence intervals are represented by the natural log of the Hg concentration in ppm.

a.

Dependent Variable	*df*	Error *df*	*F*	Dose	Means	95% Confidence Intervals	
						Upper	Lower
Brain	1	24	11.80	Low	-4.15	-3.61	-6.92
				High	-3.15	-2.86	-3.86
Muscle	1	24	18.24	Low	-6.76	-6.44	-7.08
				High	-5.60	-5.19	-6.00
Liver	1	24	31.33	Low	-6.38	-5.72	-7.04
				High	-3.56	-2.68	-4.44

b.

Dependent Variable	*df*	Error *df*	*F*	Exposure	Means	95% Confidence Intervals	
						Upper	Lower
Liver	1	24	10.97	Adult	-5.68	-4.89	-6.46
				Lifetime	-3.56	-2.36	-4.76

Table 6. Significant univariate effects of (a) time, (b) the interaction of time and dose level, (c) dose level, and (d) exposure group for non-molting finches sacrificed after 20 and 40 weeks. Means and confidence intervals are represented by the natural log of the Hg concentration in ppm.

a.

Dependent Variable	*df*	Error *df*	*F*	Time	Means	95% Confidence Intervals	
						Upper	Lower
Brain	1	36	15.43	20 Weeks	-2.67	-2.30	-3.04
				40 Weeks	-3.58	-3.24	-3.92
Muscle	1	36	104.82	20 Weeks	-6.10	-3.33	-4.13
				40 Weeks	-3.73	-5.76	-6.44
Liver	1	36	16.90	20 Weeks	-3.22	-2.74	-3.71
				40 Weeks	-4.77	-3.99	-5.54
Kidney	1	36	17.34	20 Weeks	-3.06	-2.54	-3.58
				40 Weeks	-4.53	-4.10	-4.95

b.

Dependent Variable	*df*	Error *df*	*F*	Group	Means	95% Confidence Intervals	
						Upper	Lower
Muscle	1	36	10.27	20 Weeks, Low	-3.56	-2.99	-4.13
				40 Weeks, Low	-6.76	-6.44	-7.07
				20 Weeks, High	-3.90	-3.33	-4.47
				40 Weeks, High	-5.60	-5.19	-6.00
Liver	1	36	7.78	20 Weeks, Low	-3.49	-2.81	-4.18
				40 Weeks, Low	-6.38	-5.72	-7.04
				20 Weeks, High	-2.95	-2.27	-3.63
				40 Weeks, High	-3.56	-2.68	-4.44

Table 6. (continued)

c.

Dependent Variable	*df*	Error *df*	*F*	Dose	Means	95% Confidence Intervals	
						Upper	Lower
Brain	1	36	11.39	Low	-3.67	-3.22	-4.12
				High	-2.90	-2.60	-3.19
Liver	1	36	25.72	Low	-5.23	-4.44	-6.02
				High	-3.36	-2.72	-3.99

d.

Dependent Variable	*df*	Error *df*	*F*	Exposure	Means	95% Confidence Intervals	
						Upper	Lower
Brain	1	36	4.86	Adult	-3.51	-3.14	-3.89
				Lifetime	-2.93	-2.54	-3.33
Muscle	1	36	9.00	Adult	-5.56	-5.01	-6.10
				Lifetime	-4.85	-4.20	-5.50
Liver	1	36	16.63	Adult	-4.97	-4.29	-5.66
				Lifetime	-3.29	-2.52	-4.06

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