THE REPRODUCTIVE HEALTH OF SELECT AVIAN SPECIES RESIDING NEAR AN ACTIVE IRON MINE IN MARQUETTE COUNTY, MICHIGAN

By

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ABSTRACT

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In Marquette County, Michigan, Cliffs Natural Resources (Cliffs) operates two iron mines that extract magnetite and hematite for steel production (Cliffs Natural Resources 2012). These mining operations have introduced selenium (Se) into the local watershed through processing outflows and surface water runoff. In 2008 the Environmental Protection Agency (EPA) promulgated more stringent standards for Se emissions in water basins. This required Cliffs to reduce the amount of Se released to the environment. The Michigan State University Wildlife Toxicology Laboratory was tasked to determine the Se and Hg exposure to select avian species and if there were any negative effects associated with the presently bioavailable Se to birds residing in the Cliffs Mining Operations (CMO)-associated watersheds.

A multiple lines of evidence approach was used to assess the exposure and potential for effects from mine-associated Se exposure. These lines of evidence included measuring reproductive effectiveness through hatching and fledging success rates, measuring concentrations of Se and Hg in avian eggs and prey items, and assessing avian embryos for teratogenic effects associated with Se toxicity. Results indicated that nest abandonment rates were greater than expected in waterfowl. Se exposure as measured by egg tissue concentrations exceeded those expected to cause adverse effects and teratogenic effects were observed in two of 287 reproductive attempts observed.

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I dedicate this thesis to my wonderful parents Willy and Sheila Kaulfersch for always encouraging me to follow my passion for science.

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KEY TO ABBREVIATIONS

15C- Fifteen Creek

ADD_{pot}- Potential average daily dose

AEI- Aquatic emergent insect

ANOVA- Analysis of variance

BW- Body weight

C- Dietary concentrations of prey items

CMO- Cliffs Mining Operations

CNR- Cliffs Natural Resources

DCPAH- Diagnostic Center for Population and Animal Health

DEQ- Department of Environmental Quality

dw- Dry weight

EC10- Toxicity effect threshold for 10% of sample

EPA- Environmental Protection Agency

Fe- Iron

FIR- Food intake rate

GC1- Green Creek One

GCD- Green Creek Downstream

GCR- Green Creek Reference

GL2- Goose Lake Two

GLD- Goose Lake Downstream

GLI- Goose Lake Inlet

GLL- Goose Lake

GLO- Goose Lake Outlet

GRR- Greenwood Reservoir

GRS- Greenwood Reservoir Stream

GSH- Glutathione

Hg- Mercury

L40-Lost Forty Tributary

LMR- Lake Minnie Reference

MCL- Lake Mary Charlotte

MeHg- Methylmercury

MSU-WTL- Michigan State University Wildlife Toxicology Laboratory

NOAEL- No observed adverse effect level

NPDES- National pollutant discharge elimination system

Se- Selenium

TLP- Tilden Pond

TRV- Toxicity reference value

USFWS- United States Fish and Wildlife Service

WAC- Warner Creek

WCP- Warner Creek Park

WEFH- Wildlife Exposure Factors Handbook

ww- Wet weight

CHAPTER ONE

THE EFFECTS OF SELENIUM AND MERCURY ON AVIAN REPRODUCTION

Abstract

In Marquette County, Michigan, Cliffs Natural Resources operates two proximal large open pit iron mines and processing facilities that extract magnetite and hematite for steel production (Cliffs Natural Resources 2012). These mining operations introduced selenium into the local watershed through processing outflows and surface water runoff. In 2008 the Environmental Protection Agency (EPA) promulgated more stringent standards for selenium emissions in water basins. This required Cliffs to reduce the amount of selenium released to the environment.

The toxic effects of selenium (Se) and mercury (Hg) are difficult to predict due to variations in exposure and toxicity from one ecosystem to the next. Selenium and mercury toxicity is dependent on speciation, whereas exposure is dependent on available Se and Hg along with ecosystem-specific bioaccumulation factors (Haudin et al. 2007). The Michigan State University Wildlife Toxicology Laboratory was tasked to determine if there are any ecosystem-specific negative effects associated with Se and Hg exposure of birds residing in the Cliffs Mining Operations (CMO) associated watersheds with the understanding that the collected data would capture the maximal exposure and effects potential. Avian species were selected as receptors of concern because of their great sensitivity to Se and Hg exposure. Excess Se or Hg can negatively impact reproduction in avian species by decreasing hatching success and increasing the rate of embryonic defects and chick mortality (Ohlendorf et al. 1986).

A multiple lines of evidence approach was used to assess the potential for effects from mine-associated Se and Hg exposure. These multiple lines of evidence included measuring

reproductive effectiveness through hatching and fledging success rates, assessing concentrations of selenium in avian eggs and prey items, and observing avian embryos for teratogenic effects associated with Se toxicity. Selected avian species included wood ducks (*Aix sponsa*), hooded mergansers (*Lophodytes cucullatus*), common mergansers (*Mergus merganser*), Canada geese (*Branta canadensis),* eastern bluebirds (*Sialia sialis),* black-capped chickadees (*Poecile atricapillus),* house wrens (*Troglodytes aedon),* tree swallows (*Tachycineta bicolor),* eastern kingbirds (*Tyrannus tyrannus),* and savannah sparrows (*Passerculus sandwichensis).* Objectives of the study included assessing the selenium concentrations in aquatic insects as avian dietary items, the bioavailability of Se and Hg as measured by dietary exposure of birds to insects, the rate of teratogenic effects, and egg hatchability in selected avian species.

Selenium and Mercury in the Environment

Natural Occurrence of Selenium and Mercury

The mineral form of selenium (Se) is present in great concentrations in sedimentary rocks of marine origins (Presser et al. 1994). In North America, considerable concentrations of Se are found in soils and shale in the western United States and Mexico, as well as in the iron ranges of upper Wisconsin, Minnesota, and Michigan. In these geological deposits, selenium can be in an inorganic or organic form (Yudovich and Ketris 2006).

Mercury is found naturally in the earth's crust and is released atmospherically through anthropogenic activities that include coal burning and natural events such as forest fires (U.S. Environmental Protection Agency 2014). Mercury vaporizes at a low temperature, which extends areas surrounding coal combustion activities to the risk of particulate mercury. Water sources which collect particulate mercury are responsible for the bioaccumulation of methylated mercury throughout aquatic food webs (New Hampshire Department of Environmental Services 2010).

The Effects of Mining on Selenium and Mercury in the Environment

Mining for semi-precious metals such as iron (Fe) greatly increases the environmental surface area exposure of Se-containing rock, resulting in a great potential for environmental weathering. Iron mineral processing produces great quantities of water that are often selenium-enriched. This Se is difficult and expensive to remove from waste-water, resulting in the discharge of selenium to the environment. Large volumes of selenium-bearing waste rock must be removed and stored to gain access to the discreet bands of mineral-bearing ore. These large piles of unconsolidated rock are subject to enhanced weathering that can leach Se into nearby bodies of water as a result of precipitation runoff and surface water contact. Pit lakes, which are bodies of collected rain and surface water resulting from inactive open pit mines, accumulate elevated selenium concentrations after mining activity ceases (Pond et al. 2008).

Iron mining in particular has a great potential to impact environmental Se concentrations due to iron-bearing mineral elemental content and geochemistry. There are several Fe-Se minerals that can form, including ferroselenite and dzharkenite. These minerals form when elemental selenium reacts with pyrite under reduced conditions (Howard 1977). Selenate and selenite are adsorbed by these iron oxide minerals and released as particulates or colloids to the atmosphere or the water column (Johnson 2004).

Mercury was historically used as an amalgam in silver and gold mining. Residual mercury in the post-processed slurry can result in direct proximal releases of Hg, while the vaporization of mercury during the amalgamation process resulted in atmospheric mercury deposition that is more distant to the mining activity (Fernandez-Martinez et al. 2014). Mine tailings from iron ore and non-ferrous mines can increase mercury uptake into surrounding watersheds through leaching of weathered mercury-containing waste rocks (United Nations Environment Programme 2013).

Selenium and Mercury Speciation

Selenium and mercury both undergo speciation due to reduction/oxidation reactions that occur naturally in the environment. Whereas Se becomes toxic only in its organic forms, Hg is toxic in both organic and inorganic forms. However, for both elements bioaccumulation occurs from the organic forms of Se and Hg.

Selenium speciation is the fractioning of various Se compounds and associated ionic states from inorganic to organic forms depending on varying biogeochemical reactions. There are four main inorganic species of selenium consisting of elemental Se (0), selenate (VI), selenide (-II), and selenite (IV) (Haudin et al. 2007). Inorganic species are oxidized and cannot bioaccumulate within tissues until reduction occurs. The two main organic forms of selenium include selenocysteine and selenomethionine, which are reduced Se forms and are readily bioavailable (Chapman et al. 2010).

Varying inorganic forms of selenium are found within the environment. The conversion of particulated Se to selenite and organo-selenium is slow, resulting in settling into aquatic sediments and a potential to bioaccumulate over time (Cutter and Bruland 1984). The type of aquatic ecosystem determines how much Se is available to resident wildlife. Lentic systems such as small lakes, wetlands, and estuaries have greater biological activity and longer residence times, increasing bioaccumulative organo-selenium interactions (Orr et al. 2006). In lotic systems such as fast-moving streams and rivers, the oxidized environment favors the formation of selenate over selenite. Particulates containing selenate and selenite move through a lotic system more quickly, resulting in lesser availability of Se relative to lentic systems (Lemly 1999).

The bioavailability and subsequent level of Se toxicity is dependent upon whether Se is in an inorganic or organic form. Inorganic forms of selenium have a lesser potential to bioaccumulate compared to organic Se as a result of limited bioavailability and greater rates of elimination or shorter half-life (Heinz et al. 1990). Of the inorganic forms of Se, selenite has the greatest potential to undergo oxidation and bioaccumulate within an organism. (Canton 1999). When comparing the organic selenium species, selenomethionine is a non-specific selenoprotein and has a greater potential to bioaccumulate in tissues than selenocysteine, a specific selenoprotein (Hoffman 2002).

Mercury species can be separated into elemental mercury, monovalent mercury, and divalent mercury. Divalent mercury is most often associated with aqueous systems, with the organic divalent form (MeHg) being responsible for Hg bioaccumulation within organisms. The inorganic divalent form (Hg^{II}) is the most abundant inorganic form of Hg found in freshwater systems, and can be transformed into MeHg through biochemical pathways within individual organisms (Le Faucheur et al. 2013). Unlike selenium, both the inorganic and organic species of mercury are harmful to organisms. However, only methylated mercury becomes bioaccumulative (United Nations Environment Programme 2013).

Selenium and Mercury Toxicity

Absorption, Distribution, Metabolism, and Elimination

Avian species receive selenium almost exclusively through their diet. Once ingested, selenium compounds are absorbed by the small intestine and enter the circulatory system. Selenium in avian species accumulates in the feathers, ovaries, skeletal muscle, eggs, and liver (O'Toole and Raisbeck 1997).

In avian species, methylmercury is absorbed through the digestive system and has the relatively unique ability to cross the blood-brain barrier resulting in accumulation in the brain. Elsewhere in the organism methylmercury accumulates in liver, feathers, and muscle. Methylated mercury is also distributed to egg tissues (Kalisinka et al. 2014). Mercury can be eliminated through molting and fecal matter (DesGranges et al. 1998).

Mechanisms of Action

There are two proposed mechanisms of action for Se including enhanced superoxide production and Se displacement of sulfur functional groups in select amino acids. Selenium cellular toxicity can occur as the result of oxidative stress due to increased cellular superoxide exposure. At normal doses, Se reduces lipid peroxidation and decreases the amount of available free radicals. At elevated doses, selenium results in increased superoxide production due to glutathione (GSH) oxidation reactions and interferes with inflammatory signaling molecules, leading to oxidative stress (Chen et al. 2007). Oxidative stress leads to apoptosis, which results in damaged hepatocytes and death. Excess selenium is also known to adversely affect reproduction, including teratogenesis. The mechanism of action for this form of toxicity is likely insertion of Se into amino acids such as methionine and cysteine that typically contain sulfur. Selenium and sulfur share similar elemental properties, but selenium is more readily oxidized than sulfur. This enables Se to be an ideal nucleophile for methionine and cysteine (Rahmanto and Davies 2012). Selenium replacement of sulfur results in poorly functioning or non-functioning selenium analogs of important sulfur-containing enzymes and structural proteins. The replacement of sulfur by selenium in significant quantities can lead to Se toxicity within the cells and the organism as a whole (Hoffman 2002).

The elimination rate of selenium in birds is rapid once the source of excess Se is removed from the environment. Seleno-methionine administered to mallards (*Anas platyrhynchos)* had a half-life of selenium of 19 days in the liver and 30 days for muscle tissue after exposure ceased (Ohlendorf et al. 1990). Another laboratory study by Heinz and Fitzgerald (1993) showed that mallards switched from an excessive Se diet to a baseline Se diet lost most excess selenium in a period of two weeks.

Mercury toxicity occurs in ways similar to selenium toxicity due to the high-affinity binding of mercury to sulfhydryl groups. Specifically, methylmercury binds to the sulfhydryl groups in the microtubules associated with mitosis. This binding produces mercaptides, which inhibit the proteins necessary for cells to replicate. The production of mercaptides also increases oxidative stress by depleting glutathione peroxidase. In the brain, methylmercury inhibits protein synthesis, which leads to apoptosis (Gupta 2007).

Dietary Exposure

Birds that feed on aquatic-based dietary items are at greater risk of being exposed to selenium than are birds that have a terrestrial-based diet (Adams et al. 2003). Aquatic avian species are known to consume fish and/or aquatic emergent insects (AEIs) such as Diptera, Trichoptera, Ephemeroptera, Odonata, and Plecoptera (Andrahennadi et al. 2007). These AEIs can make up a significant portion of avian species' diets, thus selenium concentrations in AEIs are often determinant of avian exposure. Studies suggest an increase in the relative proportion of AEIs in aquatic birds' diets during egg production. This is considered to be the result of the relatively greater percentage of protein in AEIs as compared to other dietary items combined with the increased protein requirement associated with egg production (Swanson et al. 1985). Terrestrial avian species consume terrestrial grasses, fruits, and terrestrial insects such as Coleoptera and Lepidoptera that are less likely to bioaccumulate selenium (Foote et al. 2010, Gowaty and Plissner 1998, Johnson 1998, Mowbray 2002). As a result, avian species that consume aqueous dietary items most often have a greater exposure potential to selenium than are avian terrestrial insectivores (Santolo and Yamamoto 1999a).

Piscivorous avian species are at the greatest risk for exposure to methylmercury due to their diet of fish, which bioaccumulate mercury from lower-order organisms such as aquatic invertebrates (Hoffman et al. 2003). Fish receive approximately 85% of their MeHg load from aquatic invertebrates and other dietary items rather than directly from water, which increases the amount of MeHg available for bioaccumulation in fish tissues (MacRury et al. 2002). For freshwater invertebrates, methylmercury toxicity can occur at concentrations less than 0.04 µg/L, whereas for freshwater fish such as brook trout methylmercury toxicity occurs at concentrations of 0.29-0.93 (U.S. Environmental Protection Agency 1985). The combination of bioaccumulation and the lesser susceptibility to toxicity results in fish having relatively great tissue burdens as compared to lesser trophic organisms in most freshwater systems.

Given this relationship, species that are strictly piscivorous, such as the cormorant, generally should be exposed to relatively greater mercury concentrations than species that consume fish and invertebrates. In a site-specific comparative study by Houserova et al. (2007), the tissues of cormorants contained greater Hg concentrations than great crested grebes which consume both fish and invertebrates and Eurasian buzzards which are terrestrial scavengers.

Maternal Transfer of Selenium and Mercury

Female oviparous species may transfer excess selenium to their eggs. Avian embryos cannot metabolize or excrete excess nutrients prior to cell differentiation. Thus, the developing embryo is exposed to greater Se concentrations than the reproducing female. Selenium is concentrated in the albumin of the egg and to a lesser extent within the yolk sac (Chapman et al. 2010). Exposure of the embryo to selenium occurs primarily during development, but selenium exposure is possible for the first few days post-hatch due to the final resorption of the yolk sac.

Mercury is transferred to avian eggs through the female's consumption of aquatic invertebrates and/or fish that have been exposed to mercury. Due to increased energy requirements during egg production, ingestion of prey items increases; this combination leads to excess Hg being exported to egg tissues. Specifically, prey items that contain calcium such as fish are more likely to be consumed due to the increased calcium requirement needed for avian females to create egg structures. The shift away from lower-order organisms and towards higher-order organisms increases the concentration of MeHg in the female and egg tissues (Morrissey et al. 2010).

Toxicity of Different Selenium and Mercury Forms in Birds

Numerous laboratory studies have examined the effects of various selenium compounds in avian species. Mallards fed 10 ppm selenium as seleno-L-methionine or seleno-DL-methionine had lesser hatching success than the control group. Mallards fed selenized yeast, which was a feed additive containing 100 ppm selenium, had a greater percentage of deformed embryos than the control group, and those fed seleno-L-methionine or seleno-DL-methionine produced more deformed embryos than those on the selenized yeast diet (Heinz and Hoffman 1996). In a second mallard study, a diet containing 15 or 30 ppm seleno-L-methionine resulted in greater mortality of embryos than diets containing seleno-DL-methionine, selenized yeast, or selenized wheat (wheat grown in soil containing elevated concentrations of Se) at the same doses (Hoffman et al. 1996).

Methylated mercury is the main form of mercury responsible for bioaccumulation within avian tissues. In some tissues such as the intestines, MeHg comprises nearly 94% of the mercury species present. Inorganic forms are also present in avian tissues, sometimes accounting for over 57% of the mercury acquired in the liver (Houserova et al. 2007).

Species Specific Selenium and Mercury Toxicity

Avian species vary in their sensitivity to selenium toxicity depending on their diet and detoxification mechanisms. Aquatic avian species are at high risk of developing selenium toxicity in a Se-rich environment due to an increased potential of exposure in the aquatic food web as compared to the terrestrial food web combined with the sensitive reproductive characteristics of oviparous species (O'Toole and Raisbeck 1997). Some avian species that can distinguish between different Se-containing amino acids during protein synthesis are able to tolerate greater exposures of selenium as a result of lesser concentrations of selenium being incorporated into developing embryos (Fry 2000).

Previous studies suggest mallards and tree swallows are sensitive to selenium-induced teratogenesis. Mallards were more sensitive than chickens (*Gallus gallus domesticus)* and double-crested cormorants (*Phalacrocorax auritus)* in a laboratory study by Heinz et al. (2012). Injection of mallard eggs with seleno-L-methionine at a concentration of 0.1 µg/g resulted in teratogenic effects in greater than 63% of embryos and hatchlings. Chickens and double-crested cormorants did not exhibit teratogenesis at the same exposure concentration. A second laboratory study that demonstrated increased mallard embryo teratogenesis used the organic Se compound seleno-DL-methionine at 10 mg/kg dry weight in the diet. In comparison, the black-crowned night-heron (*Nycticorax nycticorax)* and eastern screech-owl (*Megascops asio)* receiving the same dose did not show signs of teratogenesis (Wiemeyer and Hoffman 1996). A field-based study of tree swallows exposed to Se noted egg concentrations of 5.2 to 9.5 mg/kg dw resulted in a significant increase in teratogenic effects as compared to reference site birds. This suggested that tree swallows are also particularly sensitive to selenium (Weech et al. 2012).

Teratogenic Effects

Selenium toxicity in avian embryos leads to several different teratogenic effects including anophthalmia and microphthalmia, micromelia and amelia of the wings or legs, ectrodactyly (absence of digits) and clubfoot, abnormal beaks, hydrocephalus and encephaly, and various skeletal deformities. Heart and liver anomalies can also be present and include thin ventricular walls and abnormally sized hearts and livers (Ohlendorf et al. 1986). Teratogenic effects can be severe enough that embryos do not hatch or hatchlings only live for a few hours (Franson et al. 2002).

Ectromelia or amelia is the shortening or absence of long bones and is a defect associated with selenium toxicity. In avian embryos, the apical ectodermal ridge develops into wings and legs. If this ridge is not present in the embryo, the result is amelia (Oppenheimer and Lefevre 1984). Eastern screech-owls fed seleno-DL-methionine produced hatchlings with shortened femurs (Wiemeyer and Hoffman 1996). Birds experiencing Se toxicity from the Kesterson Reservoir included black-necked stilts that experienced a greater than 2% occurrence of ectromelia or amelia. Ducks (*Anas* species) also had these defects, but at a lesser percentage (0.4%) compared to stilts (Hoffman et al. 1988).

Other teratogenic effects associated with selenium toxicity include anophthalmia and microphthalmia, which are the absence of or abnormally small eyes, respectively. In a study that involved female ring-necked pheasants (*Phasianus colchicus*) that were accidentally overdosed with Se through contaminated feed, over half of the developed embryos from affected hens had teratogenic defects. Of the embryos that hatched, about 10% had defects that included anophthalmia and microphthalmia (Latshaw et al. 2004). In laboratory studies involving mallards, females fed a diet that included 10 ppm selenomethionine laid eggs that contained elevated concentrations of selenium. Over 36% of the developed embryos from these females exhibited teratogenic effects. Almost 10% of the embryos had defects related to anophthalmia (Heinz and Hoffman 1998).

While selenium is well-known to produce teratogenic effects, methylmercury is more likely to reduce hatching success than cause deformities. However, teratogenic effects do occur with exposure to MeHg. Some effects observed include hydrocephaly, lordosis/scoliosis, and deformities of the eyes, bill, neck, and legs/feet (Heinz et al. 2012).

Avian Toxicological Endpoints

Toxicological assessment endpoints for selenium toxicity in avian species include embryo mortality, teratogenesis, and reproductive success. In this study, measurement parameters for these endpoints included Se egg tissue concentrations, rate of embryonic defects, and hatching and fledging success rates (Chapman et al. 2009). Embryo mortality is the most sensitive endpoint, but it is difficult to assess in a field study due to unpredictable factors such as disease and predation. Although teratogenesis is not as sensitive as embryo mortality, it is easier to calculate in the field (Fairbrother et al. 1999). Reproductive parameters such as reduced hatching success are appropriate endpoints when determining whether mercury toxicity is occurring in avian tissues. Behavioral parameters are also used, which include motor impairment. In this study, reproductive parameters including hatching and fledging success and abandonment rates were used to determine if mercury toxicity was occurring within avian eggs.

Avian Toxicity Thresholds

A lowest observed adverse effect level (LOAEL) signifies the concentration at which the lowest observed adverse effect takes place. Threshold concentrations for selenium-related toxicity in avian egg tissues are dependent upon exposure duration, study type (laboratory or field), avian species sensitivity, selenium bioavailability, and measurement endpoint (USDOI 1998, Santolo et al. 1999b). The current consensus toxicity effect threshold (EC10) for egg tissues is between

16 and 24 mg/kg dw Se for chick mortality (Adams et al. 2003, Fairbrother et al. 1999). Table 1.1 compares Se concentrations necessary for teratogenesis to occur in different avian species. For mercury, the benchmarks for reduced hatching success and neurological impairment in eggs differ according to avian species, but a consensus is held at less than 2 µg/g wet weight (Heinz and Hoffman 2003) (Table 1.2).

Table 1.1. An abbreviated review of studies that have resulted in differing values at which teratogenic effects are observed in avian egg tissues.

Author	**Teratogenic Effects**	**Species**	**Significance Determination***	**Se Compound Quantified**	**Field/Lab Study**
Adams et al. 2003	21 mg/kg dw	Mallard (*Anas platyrhynchos*)	EC_{10}	Overall Se	Lab
Adams et al. 2003	16-24 mg/kg dw	Black-Necked Stilt (*Himantopus mexicanus*)	EC_{10}	Overall Se	Field
Wiemeyer and Hoffman 1996	10 mg/kg dw	Eastern Screech-Owl (*Megascops asio*), Black-Crowned Night-Heron (*Nycticorax nycticorax*)	NOAEL	Seleno-DL-Methionine	Lab
Harding 2008	22 mg/kg dw	Red-Winged Blackbird (*Agelaius phoenicus*)	NOAEL	Overall Se	Field

*EC_{10} is defined as the effect concentration threshold at which 10% of embryos exhibit teratogenesis. The no observed adverse effect level (NOAEL) is defined as the greatest level of exposure that no adverse effects are detected.

Table 1.2. An abbreviated review of studies that have resulted in differing values at which reproductive failure in eggs is observed in avian egg tissues.

Author	**Reproductive Effects**	**Species**	**Significance Determination***	**Hg Compound Quantified**	**Field/Lab Study**
Depew et al. 2012	1.3 µg/g ww	Common Loon (*Gavia immer*)	LOAEL	MeHg	Field & Lab
Burgess et al. 2005	0.23 µg/g ww	Common Loon (*Gavia immer*)	EC_{50}	MeHgCl	Lab
Heinz et al. 2009	1.79 µg/g ww	Mallard (*Anas platyrhynchos*)	LC_{50}	Overall Hg	Lab
Heinz et al. 2009	0.97 µg/g ww	Canada Goose (*Branta canadensis*)	LC_{50}	Overall Hg	Lab
Heinz et al. 2009	1.23 µg/g ww	Hooded Merganser (*Lophodytes cucullatus*)	LC_{50}	Overall Hg	Lab
Heinz et al. 2009	0.32 µg/g ww	Tree Swallow (*Tachycineta bicolor*)	LC_{50}	Overall Hg	Lab

* The lowest observed adverse effect level (LOAEL) is defined as the least level of exposure at which adverse effects are detected. EC_{50} is defined as the median effect concentration threshold at which reproductive success is inhibited. LC_{50} is defined as the median lethal concentration at which reproductive failure occurs.

Site Information

Study and Reference Areas

The Michigan State University Wildlife Toxicology Laboratory (MSU-WTL) began conducting this study in 2010 to determine hatching and fledging success of select avian species and correlating their success with concentrations of Se present in avian eggs. The spatial boundary of the study consisted of 17 sites near the city of Ishpeming within Marquette County, each covering µto 7 km of aquatic shoreline within or proximal to Cliffs Natural Resources (CNR) mining operations (Figures 1.1 and 1.2). Nest boxes were installed at all sites to monitor avian species and their reproductive health. There were 12 study sites and five reference sites. All study sites received water that was associated with the Cliffs Mining Operation (CMO) through processing water outfalls and/or surface water runoff. Reference sites were nearby sites upstream of operations and did not receive water from the CMO. The various sites were grouped by the eight watersheds described in detail below. Study watersheds included Green Creek Study, Goose Lake, Warner Creek, and Ely Creek. Reference watersheds were Green Creek Reference, Lake Minnie, Greenwood Reservoir, and Fifteen Creek.

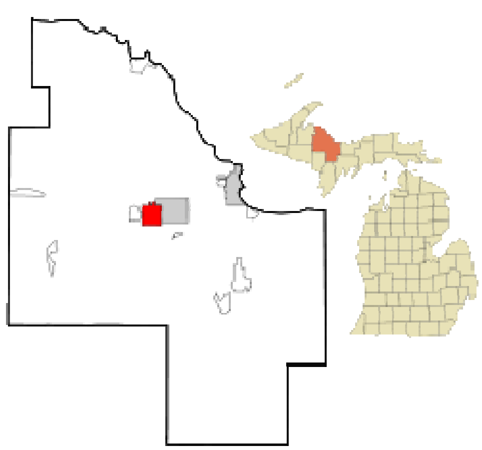

Figure 1.1. The city of Ishpeming within Marquette County, Michigan.

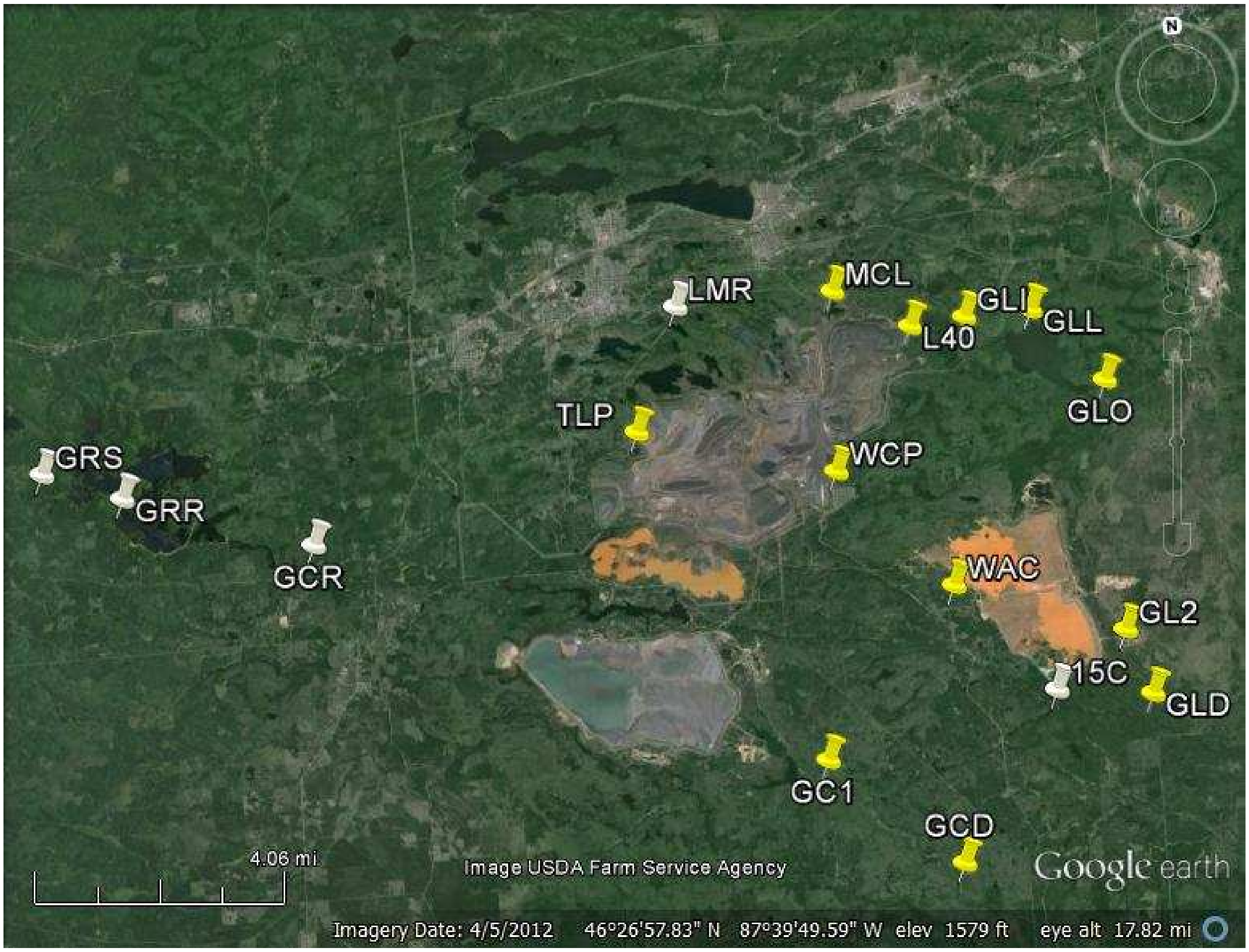

Figure 1.2. The locations of study sites (in yellow) and reference sites (in white) within Marquette County, Michigan.

Study Watershed- Green Creek Study

Green Creek receives water from the national pollutant discharge elimination system (NPDES) of Cliffs Natural Resources' Empire mine and flows to the middle branch of the Escanaba River. This watershed contained the sites Green Creek 1 (GC1) and Green Creek Downstream (GCD). Nest boxes at GC1 were located approximately half of a mile downstream of where a permitted tailings basin was allowed to enter Green Creek. Green Creek Downstream nest boxes were placed at the edge of CMO property.

Study Watershed- Goose Lake

Goose Lake is located outside the village of Palmer and receives discharged water from the NPDES outflow and rainwater from Empire's rock stockpiles. There were seven study sites in this watershed. Lake Mary Charlotte (MCL) is a lake that was formed by the collapsed Mary Charlotte mine. Lake Mary Charlotte drains water from Empire mine into Goose Lake. MCL nest boxes were placed around the edges of the lake and nearby ponds. Lost 40 tributary (L40) receives rainwater from Empire and drains into Goose Lake Inlet. The area is composed of a series of small ponds. Lost 40 nest boxes were placed on the edges of several beaver ponds. Goose Lake Inlet (GLI) is a slow-moving stream that receives water from L40 and the Empire rock pile. GLI nest boxes were placed along either side of the stream. The Goose Lake site (GLL) was located at Goose Lake and boxes were placed on the northern side of the lake in a heavily wooded area. Goose Lake Outlet (GLO) is located at the southern tip of Goose Lake where it forms a stream and later flows into the east branch of the Escanaba River. GLO nest boxes were placed between the stream's edge and the Lake Superior and Ishpeming Railroad track bed. The Goose Lake 2 (GL2) site was located between the GLL and GLO sites and had nest boxes in a cedar forest with interconnecting small streams. Goose Lake Downstream (GLD) is located where GLO ends and features a stream of varying size and speed. GLD nest boxes were placed on one side of the stream.

Study Watershed- Warner Creek

Warner Creek receives water from the Empire rock stockpile. Sites for this watershed included Warner Creek Park (WCP) and Warner Creek (WAC). WCP was located within the village of Palmer. Nest boxes were placed in a small park that is separated from the CMO by a series of railroad tracks and small ponds. The entry road to CMO was within walking distance of WCP.

WAC was outside of Palmer in an area where Warner Creek goes through a large sedge marsh. Nest boxes were placed along the edge of the marsh.

Study Watershed- Ely Creek

Unlike the other study watersheds that receive water from the Empire mine, Ely Creek receives water from Tilden's mine stockpile and enters the Schweitzer Reservoir. There was one site at this watershed, Tilden Pond (TLP). Tilden Pond consists of a series of ponds that are located next to the Tilden rock pile. TLP nest boxes were placed in open and wooded areas surrounding two of these ponds.

Reference Watershed- Green Creek Reference

The reference site at Green Creek (GCR) was located approximately 10 miles upstream from the NPDES outflow. Next boxes were placed in both heavily wooded and open areas on either side of Green Creek.

Reference Watershed- Lake Minnie

Lake Minnie (LMR) is a shallow lake located in Ishpeming that receives ground and surface water not associated with mining activities. The site at Lake Minnie had boxes placed around half of the lake that coincided with a walking trail.

Reference Watershed- Greenwood Reservoir

Greenwood Reservoir is a man-made reservoir that receives water from the middle branch of the Escanaba River. The Empire and Tilden mines use water from Greenwood Reservoir, which can make the water levels fluctuate. The reservoir consists of 1,400 acres and is a recreational area. Two sites in this watershed included Greenwood Reservoir (GRR) and Greenwood Reservoir

Stream (GRS). Greenwood Reservoir next boxes were placed on the banks of an open part of the reservoir. Greenwood Reservoir Stream nest boxes were placed near a small unnamed stream and a beaver pond that flows away from the reservoir.

Reference Watershed- Fifteen Creek

Fifteen Creek (15C) is a tributary of the east branch of the Escanaba River located outside of Palmer. Site 15C had nest boxes on either side of Fifteen Creek in a heavily forested area off of a forest service road.

Species Information

Species Selection

Selected species for this study included five waterfowl species- the wood duck (*Aix sponsa*), hooded merganser (*Lophodytes cucullatus*), Canada goose (*Branta canadensis*), common merganser (*Mergus merganser),* and mallard (*Anas platyrhynchos*) and six passerine species- the eastern bluebird (*Sialia sialis*), black-capped chickadee (*Poecile atricapillus*), house wren (*Troglodytes aedon*), tree swallow (*Tachycineta bicolor*), eastern kingbird (*Tyrannus tyrannus*), and savannah sparrow (*Passerculus sandwichensis*). These species were chosen because cavity-nesting females are attracted to the nest boxes provided. Some species were exposed to Se through their diet of aquatic emergent insects while others consumed fish or plants. Diets and foraging methods of the waterfowl and passerines differed, providing varying selenium concentrations and exposure pathways.

The study focused mainly on cavity-nesting waterfowl and passerines. All sites contained habitats suitable for one or more selected avian species and included forests, wetlands, riverine systems, open grasslands, and scrub/shrub habitats. Wood ducks, hooded mergansers, and common mergansers were provided waterfowl boxes while eastern bluebirds, black-capped chickadees, house wrens, and tree swallows were provided passerine nest boxes. Natural nests of select species that do not use cavities but are prevalent in the study and reference sites were also monitored. These species included Canada geese, mallards, eastern kingbirds, and savannah sparrows.

Study Information

Objectives

There were four main objectives of this study. The first was to determine the concentrations of selenium and mercury in the eggs of targeted avian species. The second objective was to determine the presence of terata in collected embryos and correlate the incidence of teratogenic effects with selenium concentrations in the embryos. Thirdly, reproductive parameters were assessed to determine the nesting success of targeted species that were being exposed to Se and Hg. The selection of cavity-nesting species that readily use nest boxes made it possible to closely monitor each nest and determine hatch and fledge rates, as well as to identify any abandonment or predation issues. The final objective involved determining if bioaccumulation of selenium in the food web was occurring. We expected whole body tissue selenium concentrations in AEIs to be greater than avian embryo Se concentrations.

CHAPTER TWO

SELENIUM AND MERCURY CONCENTRATIONS IN AVIAN EGGS AND AQUATIC INVERTEBRATES RESIDING NEAR AN ACTIVE IRON MINE IN MARQUETTE COUNTY, MICHIGAN

Abstract

Birds that occupy ecosystems near Ishpeming, Michigan are exposed to elevated concentrations of selenium (Se) and mercury (Hg) as a result of mine-associated surface water discharges. There is significant uncertainty as to whether concentrations of Se and Hg are great enough to result in adverse effects in avian species. Selenium is a biologically essential mineral that has the potential to cause teratogenicity when beneficial exposures are exceeded. Mercury is a heavy metal that causes neurological damage and death at elevated concentrations. Organic forms of Se and Hg are more prevalent in the aquatic food web, thus birds with a diet consisting of fish, aquatic vegetation, and aquatic invertebrates have a greater potential for exposure. To determine if avian species were being exposed to Se or Hg at concentrations expected to cause adverse effects, we collected eggs from active clutches of waterfowl and passerine species, which included hooded mergansers, wood ducks, common mergansers, tree swallows, eastern bluebirds, black-capped chickadees, house wrens, Canada geese, eastern kingbirds, and savannah sparrows. Embryos were examined for gross deformities and total Se and Hg concentrations were determined. Selenium concentrations that exceeded the 16 to 24 μg/g dw toxicity threshold value for teratogenicity (Adams et al. 2003, Fairbrother et al.1999) were found in wood duck and hooded merganser eggs among nine of 12 study sites. Mercury concentrations in avian eggs exceeded the accepted threshold for reproductive toxicity of 2.0 μg/g ww in hooded mergansers,

common mergansers, and wood ducks among all reference sites and nine of the 12 study sites (Heinz and Hoffman 2003).

Introduction

The aquatic ecosystems surrounding the Cliffs Natural Resources (CNR) mining operations have concentrations of selenium and mercury greater than those of other local watersheds (Michigan Department of Environmental Quality 2009). Expected sources included leaching from waste rock piles and discharges of processing water. The presence of selenium and mercury in the same aquatic environment has been shown to be antagonistic to selenium toxicity in organisms (Koeman et al. 1973). Selenium and mercury exposure pathways in organisms occur mainly through the diet, although for lower order organisms such as aquatic invertebrates, uptake can occur through water directly (Ogle and Knight 1996).

Selenium Toxicity

Selenium is an essential micronutrient and is needed for proper protein structure and cellular processes. Selenium has a narrow therapeutic range for all organisms, which can lead to toxicity at concentrations exceeding 16 to 24 mg/kg dry weight in avian chicks (Adams et al. 2003, Fairbrother et al. 1999). Clinical signs of selenium toxicity in adult birds and hoof stock include feather or hoof loss, edema of the body cavity, and emaciation (Pond et al. 2005). Selenium toxicity also causes teratogenic effects that include shortened or absent limbs, crossed bills in birds, lordosis or scoliosis, and undersized or absent eyes (Hoffman et al. 1988).

Mercury Toxicity

Mercury is a heavy metal that causes neurological and reproductive impairment in birds, fish, and invertebrates. Like selenium, mercury accumulates in lower-order organisms and becomes toxic when consumed by higher-order predators. Aquatic invertebrates reside in sediments where bacteria convert elemental mercury to the more biologically available methylmercury, which bioaccumulates in fish and birds. Once internalized, methylmercury is transformed to the divalent cation Hg^{++}, where it concentrates in the central nervous system. This leads to modified neuron function, resulting in tremors, reduced visual field, and reduced reproductive success (Young 1992). In avian embryos, concentrations of mercury from 0.5 to 2.0 μg/g wet weight can cause teratogenic defects (Heinz and Hoffman 2003).

Dietary Exposure in Avian Species

Invertebrates are a common food source for aquatic avian species such as wood ducks, hooded mergansers, common mergansers, tree swallows, and eastern kingbirds. Invertebrates are essential during the reproductive season when females require greater protein levels for egg production and brooding. Aquatic insect orders consumed include Diptera (flies), Trichoptera (caddis flies), Ephemeroptera (mayflies), Odonata (dragonflies and damselflies), and Plecoptera (stoneflies). Considering that aquatic invertebrates have a greater tolerance for selenium and heavy metals without exhibiting adverse effects, elevated concentrations of selenium and mercury are transferred from invertebrates to higher order species such as birds where bioaccumulation can cause toxic effects (Lemly 1993).

Materials and Methods

Study Spatial and Temporal Boundaries

Study and Reference Areas

The spatial boundary of the study consists of 17 sites in Marquette County, Michigan, each covering two to seven kilometers of aquatic shoreline within or proximal to Cliffs Natural Resources (CNR) mining operations. In 2010 there were four reference sites and 11 study sites. In 2011, the sites GCR and WAC were added, which brought the total number of reference sites to five and the total number of study sites to 12 for both 2011 and 2012 (Figure 1.2). All study sites received water that was associated with the Cliffs Mining Operation (CMO) through processing water outfalls and/or surface water runoff. Reference sites were nearby and upstream of CMO operations.

Nest Boxes

Two types of nest boxes were installed at each of 12 study and five reference locations. One box design was known to support the reproduction of cavity nesting waterfowl and the other was designed for cavity nesting passerines (songbirds). There were 120 waterfowl boxes placed 6-17 meters off the ground in a variety of tree species. The boxes were made of cedar and lined with pine shavings. All boxes were placed within 300 meters of surface water at a minimum of 100 meters apart. An infrared camera was placed inside the box and a cable connected to the camera ran down the length of the tree where connectors for the attachment to a monitor were placed approximately four feet from the ground. Connectors were compatible with mobile monitors and video cameras to allow for remote observations and recordings of nest box activity. Remote monitoring allowed for the collection of many reproductive measurement parameters such as incubation date and hatch date without the need to physically climb the tree. This minimized nest disturbance and damage to the tree. Four weeks after the last fledging event, box entrances were sealed with wire mesh and not reopened until the following spring to keep other wildlife from establishing residence during the winter months.

There were 251 passerine boxes mounted on 8 foot T-posts within 100 meters of surface water. Passerine boxes were also made of cedar but were not lined. A side panel allowed easy monitoring of passerine activity. Typically, two passerine boxes were placed between a waterfowl box and open water, located three to seven meters apart. A wire mesh predator guard was placed around the box entrance and non-toxic lithium-based grease was applied to the length of the T-posts in an effort to keep predators from reaching the boxes and their contents.

Monitoring

Nest boxes were monitored every other day on a rotational basis. Waterfowl boxes were monitored remotely using the in-box camera. Tree climbing equipment was utilized to access the waterfowl boxes as needed for maintenance or sample collection. Passerine boxes were checked by opening a side panel and visually inspecting the box interior. Field notebooks documented the daily status of each active nest box. Photo documentation including time, date, and nest box number was initiated and noted when there were any signs of activity including presence of nest material or any nest construction, breeding pairs near or in the box, or the presence of eggs and/or hatchlings.

For passerines, the breeding pairs were trapped and banded with a U.S. Fish and Wildlife Service (USFWS) leg band. All nestlings were banded after three to four days of age. Information containing the band number, sex of the individual (if applicable), and age was recorded, along with pictures of brood patches and primary feathers.

Collection

Avian Egg and Whole Body Collections

Post clutch completion, a pre-specified number of eggs were collected from each nest. Fresh eggs were collected on or before the fourth day of incubation. For waterfowl species, two randomly selected eggs were collected per clutch. Due to their smaller clutch sizes, one randomly selected egg per clutch was collected from passerine species. All addled/unhatched eggs were salvaged five days after the last egg from the nest hatched or ten days after the last adult activity was observed. Eggs were wrapped in a chemically cleaned cloth and placed in glass I-Chem® jars. Information concerning the location, date, time, temperature, species, egg weight, egg length, egg width at three locations, site, and team members present was recorded. Jars containing egg samples were placed in a cooler in the field and later transferred to a refrigerator at 4°C until analysis. Any deceased adults, nestlings, hatchlings, or fledglings that were recovered underwent the same collection procedure as egg collection and were stored at -23°C. All sampling events were documented in field notebooks and with photos. Copies of notebooks and photos are available upon request.

Aquatic Emergent Insect Collections

Aquatic emergent insects were collected as key dietary components for the selected avian receptors and as indicators of lower trophic level Se and Hg bioavailability. Insects were collected from sites that had active nests. Collections occurred twice; the first collection occurred within a 20-day window during egg production, and the second occurred during a 20-day window during brooding. Sixteen samples from reference sites and 45 samples from study sites were analyzed for selenium and mercury.

A metal halide bulb illuminated pre-cleaned white sheets stretched proximal and perpendicular to the shoreline in a 3.96m x 5.03m area. Insects were aspirated using pre-cleaned insect vacuums or were caught mid-flight using clean sweeps nets. Once collected by vacuums or nets, the insects were transferred to certified clean glass I-Chem® jars and frozen at -23°C.

Analysis

Eggs were opened using a scalpel to cut around the widest part of the egg. Egg contents were weighed and examined macroscopically for physical deformities. Photos were taken of each embryo before the contents were homogenized and submitted for metals analysis. Deceased nestlings, fledglings, and adults were also analyzed for metals. Deceased birds underwent necropsies for signs of disease, malnourishment, and/or abnormalities. Photos were taken pre- and post-necropsy and deceased whole-bodied individuals were homogenized and divided into appropriate aliquots for analysis.

Aquatic emergent insects from each sampling effort were thawed and sorted according to taxonomic order in an effort to determine if certain aquatic invertebrate orders bioaccumulated greater concentrations of Se or Hg than other orders. Taxonomic orders of interest included Diptera (flies), Trichoptera (caddis flies), Ephemeroptera (mayflies), Odonata (dragonflies and damselflies), and Plecoptera (stone flies). Each AEI sampling effort was taxonomically sorted twice; once for initial separation and again for quality assurance. Individual orders from each sampling event were homogenized separately and divided into appropriate aliquots for analysis.

Homogenized egg, deceased bird, and AEI samples were sent to the Michigan State University Diagnostic Center for Population and Animal Health (DCPAH) for metals analysis. Samples were analyzed using the U.S. Environmental Protection Agency's (EPA) method 6020. Analyzed metals and micronutrients included boron, magnesium, vanadium, chromium, manganese, iron, copper, zinc, arsenic, selenium, molybdenum, cadmium, antimony, barium, mercury, thallium, and lead.

Statistical analysis was performed using SAS 9.2. Analysis of variance (ANOVA) tests were conducted to determine significant differences between Se and Hg concentrations in birds among reference and study sites and among avian species. For aquatic emergent insects, ANOVA was utilized to test for significant differences in Se and Hg concentrations among species and site. A Pearson correlation, Chi-squared test, and Mann-Whitney test were run to determine if there was a correlation between nest abandonment rates and elevated Hg concentrations and any correlations between Se and Hg concentrations in individual samples of egg tissues.

Potential average daily doses were calculated for both aquatic and terrestrial avian species to determine if a difference in Se concentrations existed between the two diet types. Exposure to selenium in adult birds was calculated using information provided in the U.S. Environmental Protection Agency (USEPA) Wildlife Exposure Factors Handbook (WEFH; USEPA 1993). Factors affecting selenium exposure included body weight (BW kg), dietary concentrations (C) of prey items, time spent foraging on site, and food intake rate [FIR; g wet weight food/g body weight (BW)/day]. For each species, mean body weight and an analysis of dietary items was based on previous literature. Food intake rates were taken directly from the WEFH for applicable species or were calculated using WEFH equations 3-4 or 3-5. The potential average daily dose (ADD_{pot}) was calculated using WEFH equation 4-3 (USEPA 1993).

Aquatic invertebrates that were collected by MSU-WTL along with plants and fish that were collected at the same sites as avian eggs by Cardno-Entrix Inc. were used to calculate reach-specific concentrations for those dietary items (Michigan Department of Environmental Quality 2009). Reference area aquatic invertebrate maximum and minimum values were sued as a conservative surrogate for terrestrial insect tissue concentrations, which were not available. Reach-specific concentrations of Se in dietary items were multiplied by their contribution to the diet. A 100% time spent foraging at sites was considered to be the most accurate based on the published foraging range of each species, the surface area of the sites, and nest locations.

Results

Dietary Exposure and Effects Assessment

Selenium Concentrations in Aquatic Emergent Insects

Insects were collected from 8 of 15 sites during 2010 and 14 of 17 sites in 2011. For each site and sampling event, insects were sorted and analyzed by taxonomic order. Resulting in a total of 45 AEI data points (n=16 from reference sites and n=29 from study sites), study area AEI Se concentrations ranged from 2.64-144 µg/g dw. Ten samples (22.2%) collected from study sites had concentrations of selenium greater than 30 µg/g dw with 4 of those being from the order Trichoptera. Odonata generally had the least selenium concentrations, averaging 12.4±1.13 µg/g dw.

Samples collected from reference sites in 2010 and 2011 had selenium concentrations that ranged from 0.87 to 3.39 µg/g dw Se. The reference site sample with the greatest Se concentration was from the order Ephemeroptera (3.39 µg/g dw). A sample of the order Trichoptera had the least elevated Se concentrations of those collected in the reference sites, averaging 0.96 with a standard deviation of 0.14 µg/g dw.

Comparisons of the mean concentrations of Se in orders between reference and study sites indicated significant differences for both Diptera and Ephemeroptera. For Diptera, samples from study site TLP had significantly greater Se concentrations than for all other sites where Diptera were collected (Table 2.1). TLP selenium concentrations in Diptera ranged from 36.4 to 103 µg/g dw. On average, the order Trichoptera had the greatest selenium concentrations (49.01±39.33 µg/g dw) when compared to all other orders. Trichoptera samples from study sites TLP, WAC, and WCP were individually significantly greater compared to all other sites where

Trichoptera were collected with Se concentrations ranging from 57.1 to 144.0 µg/g dw (Table 2.2).

Table 2.1. Mean Se concentrations and standard deviations in Diptera by site.

Site	Se Concentration (µg/g) dw
TLP*	66.53 ± 34.52
GCR	1.39 ± 0.19
GRR	1.66 ± 0.39
LMR	2.25 ± 0.15
GC1	10.62 ± 5.33
GCD	3.48 ± 1.18
GL2	16.75 ± 7.03
GLD	11.6 ± 0
GLO	21.08 ± 12.99
MCL	15.78 ± 4.21
WCP	20.15 ± 4.74
15C	2.47±0.61
GLL	11.42 ± 3.22
L40	13.5 ± 2.97
WAC	3.95 ± 0.19

*Site TLP contains significantly greater concentrations of Se in Diptera compared to all other sites where Diptera were collected.

Table 2.2. Mean Se concentrations in Trichoptera by site.

Site	Se Concentration (µg/g) dw
TLP*	144 ± 0
WAC**	70.5 ± 0
WCP***	65.25 ± 11.53
GLO	26.75 ± 3.46
GLD	22.7 ± 0
GC1	28.5 ± 0
GL2	20.2 ± 0
GCR	0.96 ± 0.13

*Site TLP contains significantly greater concentrations of Se in Trichoptera compared to all other sites where Trichoptera were collected.
** Site WAC contains significantly greater concentrations of Se in Trichoptera compared to all other sites where Trichoptera were collected.
*** Site WCP contains significantly greater concentrations of Se in Trichoptera compared to all other sites where Trichoptera were collected.

In general, mean Se concentrations were greater among study sites as compared to reference sites both within and among orders. However, Diptera and Ephemeroptera were the only orders that had significantly greater concentrations of selenium among study sites compared to reference sites (Table 2.3). Diptera and Ephemeroptera collected among study sites had approximately a ten-fold greater whole body concentration than those collected from reference sites.

Table 2.3. Mean concentrations of Se (μg/g dw) ± standard deviation in selected orders among reference and study sites.

	Order				
	Diptera	Ephemeroptera	Odonata	Trichoptera	Plecoptera
Reference	1.91 ± 0.56	3.39 ± 0	1.48 ± 0	0.96 ± 0.14	NA
Study	21.11 ± 22.78	28.63 ± 2.65	12.4 ± 1.13	49.01 ± 39.33	16.5 ± 0
p value	0.0061	0.0144	0.0804	0.1273	NA

Potential Average Daily Doses

Potential average daily doses were calculated for both aquatic and terrestrial avian species to compare dietary exposure-based toxicity reference values (TRVs) and to determine if a difference in Se concentrations existed between the two diet types. The diets of each target species varied, and included aquatic invertebrates, terrestrial invertebrates, fish, other animals, plants, and fruit. Since the selenium exposure is occurring through the water source and not inland soils, it was assumed that terrestrial insects would not be bioaccumulating excess amounts of Se and would contain Se concentrations less than or equal to reference area aquatic invertebrates (Ecological Risk Assessment for Kesterson Reservoir 2000). A 100% time spent foraging at sites was considered to be the most accurate based on the published foraging range of each species, site area, and nest location.

Common mergansers were calculated to have the greatest study area ADD_{pot} with a mean and maximum value of 18.81 and 64.0 mg/kg day, respectively. Mallards had the second greatest study area mean and maximum ADD_{pot} of 8.86 and 45.57 mg/kg day, with wood ducks and hooded mergansers following with mean and maximum ADD_{pot} values of 3.84 and nearly 20 mg/kg day and 2.55 and 11.54 mg/kg day, respectively. Canada geese had the least maximum ADD_{pot} of the waterfowl species with 2.58 mg/kg day. Of the passerine species, the eastern bluebird had the greatest maximum ADD_{pot} with 0.07 mg/kg day. Tree swallows and eastern kingbirds had maximum ADD_{pot} values of 0.04 and 0.02 mg/kg day, respectively. Black-capped chickadees and house wrens both had maximum ADD_{pot} values of 0.01 mg/kg day. Savannah sparrows had the least maximum ADD_{pot} of 0.03 mg/kg day. (Table 2.4).

Table 2.4. Potential average daily dose (ADD_{pot}) of selenium in target species

Species	Reference Mean (mg/kg day)	Reference Maximum (mg/kg day)	Study Mean (mg/kg day)	Study Maximum (mg/kg day)
Common Merganser	8.78	12.97	18.81	64.0
Mallard	0.86	1.70	8.86	45.57
Wood Duck	0.33	0.64	3.84	19.77
Hooded Merganser	1.25	2.82	2.55	11.54
Canada Goose	0.34	0.74	1.38	5.81
Eastern Bluebird	0.001	0.002	0.01	0.07
Tree Swallow	6.83×10^{-4}	0.001	0.01	0.04
Savannah Sparrow	3.40×10^{-4}	6.68×10^{-4}	0.01	0.03
Eastern Kingbird	0.001	0.003	0.02	0.02
Black-Capped Chickadee	2.17×10^{-4}	4.12×10^{-4}	0.003	0.01
House Wren	1.95×10^{-4}	3.56×10^{-4}	0.003	0.01

Selenium dietary exposure in study areas based on both maximum and mean ADD_{pot} values exceeded dietary TRVs for all species. Calculated dietary exposures among reference sites exceeded TRVs for common and hooded mergansers. For all aquatic and terrestrial avian species among reference sites, ADD_{pot} values were lesser than dietary-based TRVs (Table 2.5).

Table 2.5. Estimated toxicity reference values (TRVs) based on dietary concentrations from previous studies known to cause adverse effects in adult avian species

Species	**Estimated TRVs (mg/kg day)**
Common Merganser	4.08-5.99
Mallard	2.44-3.58
Wood Duck	0.97-1.43
Canada Goose	0.77-1.13
Hooded Merganser	0.56-0.82
Eastern Bluebird	0.004-0.01
Eastern Kingbird	0.004-0.01
Tree Swallow	0.003-0.004
Savannah Sparrow	0.001-0.002
Black-Capped Chickadee	6.60×10^{-4}-9.69×10^{-4}
House Wren	6.48×10^{-4}-9.50×10^{-4}

Mercury Concentrations in Aquatic Emergent Insects

Mercury concentrations in samples collected from reference sites in 2010 and 2011 ranged from 0.09 to 0.41 μg/g ww. Odonata had the greatest mean mercury concentrations from reference sites at 0.23 ± 0 µg/g ww. Ephemeroptera had the least mean concentrations of Hg at 0.16 ± 0 µg/g ww.

Mercury concentrations in samples collected from study sites in 2010 and 2011 ranged from 0.35 to 11.44 µg/g ww Hg. On average among study sites, Diptera had the greatest mean mercury concentrations at 2.56 ± 2.4 µg/g ww. Ephemeroptera had the least mean Hg concentration at 1.09 ± 0.64 µg/g ww.

There were statistically significant differences in mercury concentrations between reference and study sites for the orders Diptera and Trichoptera. Mean Hg tissue concentrations were lesser in reference sites as compared to study sites in all cases (Table 2.6).

Table 2.6. Average concentration of mercury (µg/g) ww in selected insect orders among study and reference sites.

	Order				
	Diptera	Ephemeroptera	Odonata	Trichoptera	Plecoptera
Reference	0.19 ± 0.09	0.16 ± 0	0.23 ± 0	0.21 ± 0.05	NA
Study	2.56 ± 2.4	1.09 ± 0.64	1.56 ± 0.21	1.77± 0.87	1.26 ± 0
p value	0.0015	0.3359	0.1193	0.0348	NA

Tissue Based Exposure and Effects Assessment

Selenium Concentrations in Avian Species

Avian eggs were collected from 3 of 15 sites in 2010. In 2011, eggs were collected from all five reference sites and nine of 12 study sites. In 2012, eggs were collected from all reference sites and 11 of 12 study sites. Between 2010 and 2012 a total of 460 eggs were sampled and evaluated for embryonic defects and 287 were analyzed for elements of interest.

Additionally, eggs with embryos at a species-specific development stage of greater than 1/3 and appropriate tissue integrity were assessed for gross abnormalities. Samples for Se analysis included eggs from wood ducks, hooded mergansers, Canada geese, and common mergansers. Egg tissue concentrations of 16-24 mg/kg dw were considered as consensus toxic threshold values for aquatic birds based on previous field and laboratory data and signifies the EC10 for teratogenicity (Adams et al. 2003, Fairbrother et al. 1999). A range for the EC10 exists because several species were taken into consideration and signifies the range of sensitivity across avian species (Adams et al. 2003).

Out of a total of 287 waterfowl egg tissue samples analyzed from 2010 to 2012, 43 samples (15%) exceeded 16 mg/kg dw Se and 8 samples (2.8%) exceeded the 24 mg/kg dw threshold. Of the egg tissues between 16 and 24 mg/kg dw Se, 22 were hooded mergansers and 13 were wood ducks. Of the eight samples exceeding 24 mg/kg dw Se, seven were wood ducks and one was a hooded merganser. The greatest concentration of selenium in an egg tissue sample was 32.7 µg/g dw and the sample was from a wood duck. Of 49 avian egg tissues collected from reference sites, one sample had a concentration of selenium that exceeded 16 mg/kg dw Se at 17.6 µg/g dw. Approximately 36% of the wood duck samples from study sites had Se concentrations which exceeded 16 mg/kg dw and 12.7% exceeded 24 mg/kg dw. Hooded merganser egg tissues from study sites (n=175) exceeded 16 mg/kg dw Se in 12.6% of the samples, and less than 1% were above 24 mg/kg dw. Canada goose embryos among study sites (n=8) had selenium concentrations of less than 6 µg/g dw. No mallard or common merganser egg tissues were collected at study sites despite significant effort.

Wood duck mean egg tissue concentrations exceeded the 16 µg/g dw toxicity threshold for Se at the GLO study site. There were significant differences in wood duck and hooded merganser selenium concentrations between reference and study sites, with birds from study sites having greater concentrations of Se (Table 2.17). Selenium concentrations in Canada geese were elevated in study sites compared to reference sites but were not significant. Wood duck mean egg tissue concentrations in combined study sites was 5 fold greater than reference sites while hooded mergansers and Canada geese were 2-fold greater. The upper confidence limits of Se for each species and site combined by study and reference type are presented in Table 2.8.

Table 2.7. Average selenium concentrations (μg/g dw) ± standard deviation in egg tissues of avian species among study and reference sites

	Wood Duck	Hooded Merganser	Canada Goose	Common Merganser
Reference	1.95 ± 0.43	3.54 ± 4.32	1.07 ± 0.49	3.24 ± 2.01
Study	11.66 ± 9.89	7.25 ± 6.3	2.48 ± 1.9	NA
p value	0.0202	0.0009	0.2569	NA

Table 2.8. Upper confidence limits of Se in egg tissues

	Wood Duck	Hooded Merganser	Canada Goose	Common Merganser
Reference	2.4 μg/g dw	5.00 μg/g dw	5.52 μg/g dw	5.73 μg/g dw
Study	14.33 μg/g dw	8.19 μg/g dw	4.58 μg/g dw	NA
EC10 threshold	16-24 μg/g dw	16-24 μg/g dw	16-24 μg/g dw	16-24 μg/g dw

Among reference sites, selenium concentrations in eggs of all species ranged from 0.72 to 17.6 μg/g dw. On average, hooded merganser eggs had the greatest mean concentrations of Se at 3.54 ± 4.32 μg/g dw. Common merganser eggs had the next greatest mean Se concentration at 3.24 ±

2.01 µg/g dw. Wood duck eggs were intermediate in their mean selenium concentration at 1.95 ± 0.43 µg/g dw. Canada goose eggs averaged 1.07 ±0.49 µg/g dw Se.

Selenium concentrations among study sites for all species of egg tissues ranged from <0.1 to 32.7 µg/g dw. Among study sites, wood duck eggs had a mean selenium concentration of 11.66 ± 9.89 µg/g dw. Hooded merganser eggs had a mean of 7.37 ± 6.19 µg/g dw Se and Canada goose eggs had a mean of 2.47 ± 2.04 µg/g dw Se. When comparing selenium concentrations of all species among study sites, Canada goose and hooded merganser eggs had statistically lesser Se concentrations compared to wood duck eggs.

When statistically comparing Se tissue concentrations of the three aquatic species (wood duck, hooded merganser, and common merganser) with the terrestrial species (Canada goose), we expected the Canada goose to have lesser concentrations of selenium due to less exposure to the water-based food web. This expectation was verified at study sites where Se concentrations in Canada geese were lesser than concentrations of Se in the aquatic species ($p=0.0207$). There were no significant differences between terrestrial and aquatic species among reference sites ($p=0.9706$). Among reference sites, aquatic avian species had an average Se concentration of 3.31 ± 3.85 µg/g dw, whereas terrestrial avian species had an average of 1.07 ± 0.49 µg/g dw Se. Among study sites, the average concentration of selenium in aquatic and terrestrial species was 8.31 ± 6.87 µg/g dw and 2.48 ± 2.04 µg/g dw Se, respectively.

Dietary exposure as predicted by the potential average daily dose (ADD_{pot}) values were compared against selenium concentrations present in egg tissues. Maximum exposures based on both approaches exceeded thresholds for effects while minimum exposures did not, and both measures of exposure had comparable rank orders (Ohlendorf et al. 2008) (Table 2.9). Maximum egg tissue concentrations were about two fold greater than the calculated ADD_{pot}.

Table 2.9. Dietary versus tissue exposure of selenium based on species

Species	Maximum Dietary Exposure (mg/kg day)	Mean Dietary Exposure (mg/kg day)	Maximum Egg Tissue Exposure (mg/kg dw)
Wood Duck	19.77	3.84	32.7
Hooded Merganser	11.54	2.55	24.8
Common Merganser	64.0	18.81	6.8
Canada Goose	2.58	1.38	5.81
Mallard	45.57	8.86	NA

Mercury Concentrations in Avian Species

Of all avian egg tissue samples, 47.7% exceeded the threshold of Hg at 2 µg/g ww. The sample with the greatest concentration of mercury was 44.93 µg/g ww. The sample with the least amount of Hg was 0.08 µg/g ww.

Out of 49 avian embryos analyzed from reference sites, 35 samples (71.4%) had concentrations greater than the threshold of 2 µg/g ww Hg. All common merganser samples (n=5) and 30 of 36 hooded merganser samples (83.3%) from reference sites contained mercury concentrations that exceeded 2 µg/g ww. No wood duck or Canada goose samples (n=8) exceeded 2 µg/g ww Hg concentrations. The greatest concentration of Hg in a reference site sample was 12.07 µg/g ww.

Out of 238 avian egg tissues from study sites, 132 samples (55.5%) contained mercury concentrations greater than 2 μg/g ww. In the samples containing Hg concentrations greater than 2 μg/g, 121 samples were hooded mergansers and 11 samples were wood ducks. Forty-three embryo samples, which were all hooded mergansers, were above 10 μg/g ww Hg, with the greatest concentration being 44.93 μg/g ww Hg. The greatest concentration of mercury in a wood duck was 4.71 μg/g ww. Of the 55 wood duck embryos analyzed from study sites, 20% of the samples had Hg concentrations above 2μg/g ww. Of the hooded merganser embryos in study sites (n=175), 44.6% were between 2 and 10 μg/g ww Hg and 24.6% were greater than 10 μg/g ww Hg.

When comparing Hg concentrations for a particular species between reference and study sites, Canada geese among reference sites (n=2) had significantly greater concentrations of Hg than those among study sites (n=8) (Table 2.10). No significant differences were found when aquatic birds (hooded merganser, common merganser, and wood duck) (n=187) were compared against the terrestrial species (Canada goose) (n=10) among both study and reference sites.

Table 2.10. Average concentration of mercury (μg/g ww) with standard deviations in avian species among study and reference sites

	Wood Duck	Hooded Merganser	Canada Goose	Common Merganser
Reference	1.65±0.15	5.22±3	1.35±0.09	7.43 ± 1.13
Study	1.42 ± 1.04	6.48±8.18	0.24 ± 0.37	NA
p value	0.5942	0.3694	0.004	NA

Teratogenic Effects

Out of 287 waterfowl egg and whole body tissues examined from 2010 to 2012, two individuals were noted to have deformities. A hooded merganser embryo from a study site in 2010 exhibited anophthalmia, which is defined as a partial or complete absence of eye tissue. In 2012, a hooded merganser nestling from a study site had a club-foot and was unable to leave the nest. Gross deformities were not observed for any other individual or embryo. Hatchlings that were able to climb the interior of the nest box and exit the nest were assumed to be normal.

Nest Abandonment

When mercury concentrations at abandoned nests were compared to non-abandoned nests among the same site and of the same species, some significant differences were found. At the study site Goose Lake (GLL), both fresh and addled hooded merganser eggs from abandoned nests contained Hg concentrations that were greater than hooded merganser eggs from non-abandoned nests.

When comparing fresh eggs versus addled eggs from both abandoned and non-abandoned nests, no significant differences were found in selenium concentrations (Figure 2.1). Fresh eggs had greater concentrations of selenium than addled eggs in both abandoned and non-abandoned nests; however these concentrations were not significantly different.

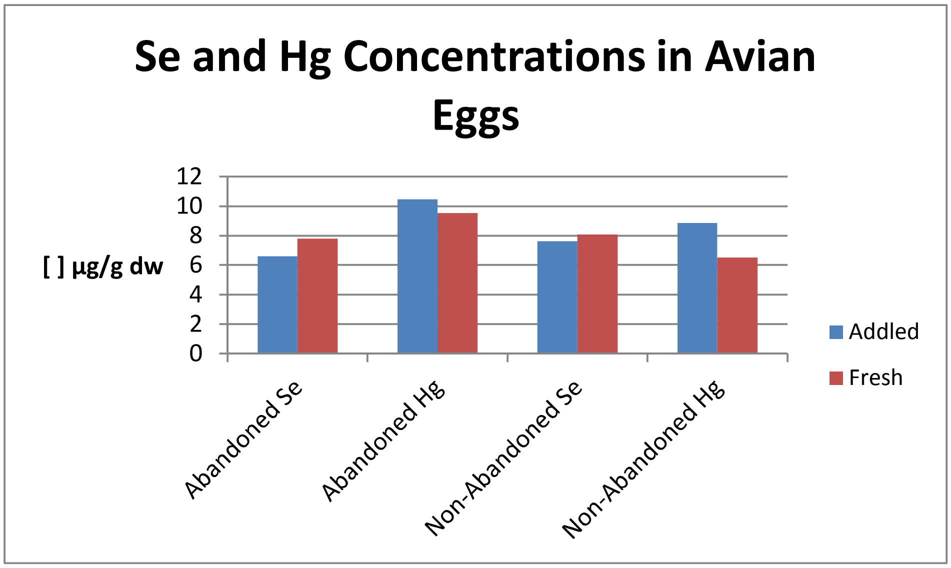

Figure 2.1. Comparison of Se and Hg concentrations in abandoned and non-abandoned nests for fresh and addled eggs.

When mercury concentrations were compared between fresh and addled eggs from the same nest, there was a single incidence in an abandoned nest of addled eggs having significantly greater Hg concentrations in the addled eggs than in the fresh eggs. All other nests exhibited no difference in Hg concentrations between fresh and addled eggs. When all eggs from abandoned or non-abandoned nests were compared, addled eggs had significantly greater concentrations of mercury than fresh eggs. A comparison of eggs from either nest type (abandoned or non-abandoned) by species showed no significant differences for hooded merganser or wood duck eggs (Table 2.11). However, mercury concentrations in eggs were significantly greater in hooded merganser eggs than wood duck eggs in both abandoned and non-abandoned nests.

Table 2.11. Mercury concentrations in eggs from abandoned and non-abandoned nests in two species.

	Hooded Merganser	Wood Duck
Eggs from Non-Abandoned Nests	5.5 µg/g ww Hg	1.3 µg/g ww Hg
Eggs from Abandoned Nests	7.2 µg/g ww Hg	2.6 µg/g ww Hg

Selenium-Mercury Interactions

Selenium has been shown to reduce the amount of mercury available for uptake in a 1:1 ratio (Koeman et al. 1973). There was not a strong correlation between Hg and Se concentrations in eggs among reference sites (Figure 2.2) or study sites (Figure 2.3). Selenium and mercury concentrations in eggs from abandoned nests were not significantly greater when compared to eggs from non-abandoned nests.

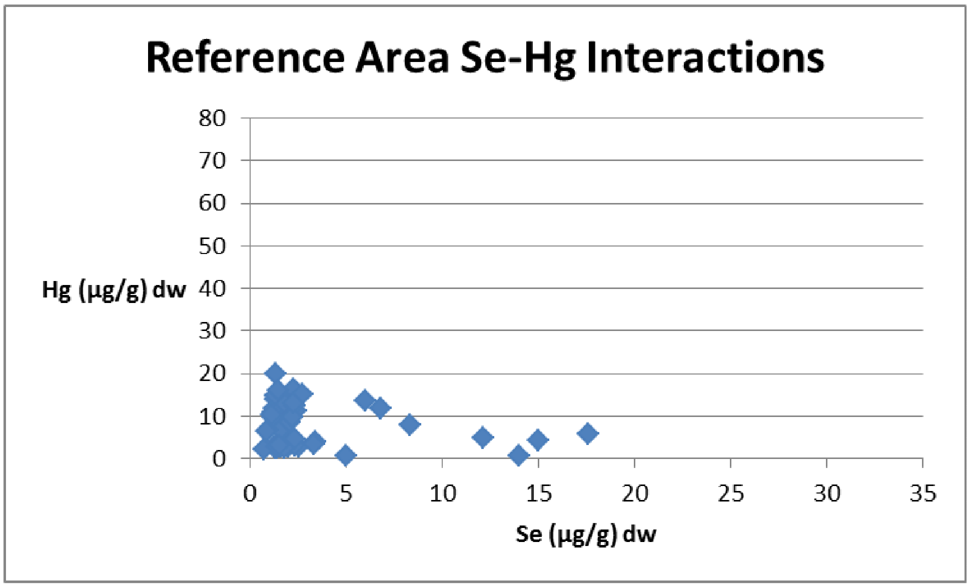

Figure 2.2. Correlation of Se concentrations with Hg concentrations in individual avian eggs collected from reference sites.

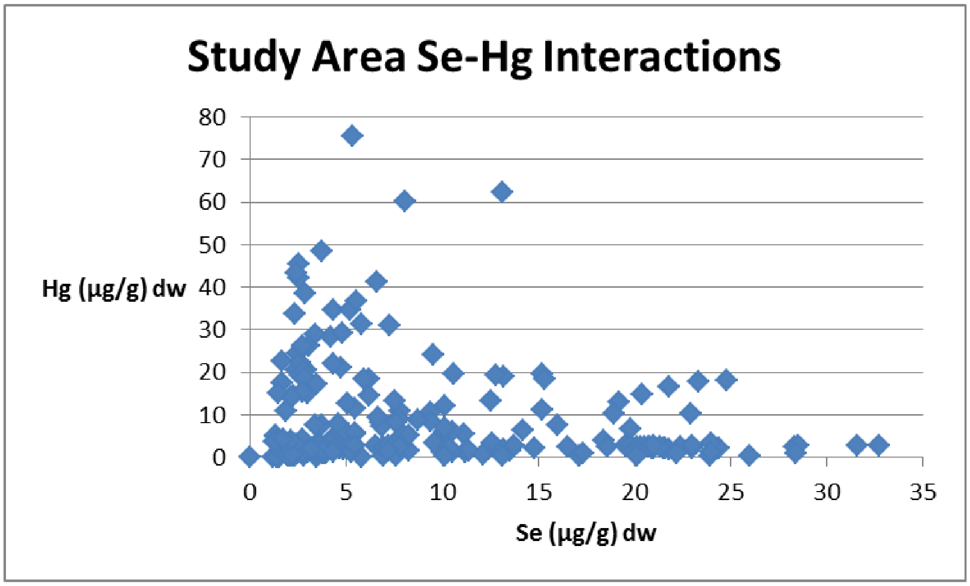

Figure 2.3. Correlation of Se concentrations with Hg concentrations in individual avian eggs collected from study sites.

Discussion

Dietary Exposure and Effects Assessment

Selenium Concentrations in Aquatic Emergent Insects

Aquatic insects contained varying concentrations of selenium depending upon site and species. Some species of Trichoptera feed on biofilm, which makes them greatly exposed to selenium that has bioaccumulated in lentic systems (Arribere et al. 2010). Due to the elevated concentrations of selenium in Trichoptera collected, it was assumed that the species of Trichoptera collected were omnivorous or detritivorous, where they could bioaccumulate selenium from biofilm, detritus, and sediment. Due to elevated Se concentrations in Diptera, it was assumed that the species of Diptera in this ecosystem were a mix of predators and detritivores, with detritivore species accumulating elevated selenium concentrations through sediments in lentic systems (Wayland and Crosley 2006).

The three sites (TLP, WAC, and WCP) that contained Trichoptera with elevated concentrations of selenium were lentic systems and were the sites with the closest proximity to the CMO. Ephemeroptera were collected at two study sites and Plecoptera were collected at one study site (GL2) due to a lack of abundance at other sites. Previous studies have shown a decrease in Ephemeroptera at coal-mined sites versus unmined sites (Pond et al. 2008). This trend may explain why there was a lack of Ephemeroptera and possibly Plecoptera at the mine-associated target sites.

Potential Average Daily Doses

Calculated selenium ADD_{pot} values paralleled those measured in egg tissues. For both maximum and minimum ADD_{pot} values, aquatic avian species had greater potential average daily doses than terrestrial avian species. Birds residing within the study area had a greater calculated exposure than those in the reference area and the relationship between ADD_{pot} Se concentrations and that measured in tissues was comparable. Maximum and mean tissue exposures for wood ducks, hooded mergansers, and Canada geese were approximately 2 times greater than the maximum and mean dietary exposures. This ratio is consistent with previous data which states that egg tissue concentrations may be up to 4 times greater than the dietary concentration (Ohlendorf et al. 2011). Recommended TRVs for selenium can range from 6 to 10 mg/kg dw depending on species (Lemly 1993, Ohlendorf et al. 2008). Based on these TRVs, wood ducks, hooded mergansers and Canada geese are averaging lesser Se concentrations per day than those known to cause adverse effects. Common mergansers are above the TRV range, which may indicate that they are at greater risk for developing adverse effects due to selenium toxicity.

The five waterfowl species in this study had three distinct diets allowing the evaluation of a range of potential selenium exposures and food web interactions. Potential average daily doses in hooded mergansers paralleled their expected trophic status due to their risk of greatest exposure to Se based on their dietary composition of fish and to lesser extent aquatic invertebrates (Dugger et al. 2009, White 1957). Wood ducks and mallards were predicted to have a lesser exposure consuming aquatic vegetation and invertebrates (Hepp and Bellrose 1995, Swanson et al. 1985) and tree swallows even lesser consuming primarily aquatic emergent insects. Since the excess Se in this study was being released through water sources, selenium concentrations in aquatic prey were likely to be greater than concentrations in terrestrial prey. Whereas wood ducks and mallards consume aquatic vegetation, Canada geese consume terrestrial grasses and grains (Mowbray et al. 2002). This reduced the Canada goose's exposure to waterborne Se, making them the least likely of our waterfowl species to receive excess selenium from the aquatic ecosystem.

Mallards were included as a study species due to their known sensitivity and presence on site, however, no eggs were sampled. Adult female mallards were often observed at sites, but located nests did not contain eggs. Mallard fledglings were observed in study areas albeit rarely.

For the passerine species in this study, two are aquatic insectivores and four are terrestrial insectivores. Tree swallows (*Tachycineta bicolor)* and eastern kingbirds (*Tyrannus tyrannus)* have nesting sites over water due to their consumption of aquatic insects (Murphy 1996, Winkler et al. 2011). Black-capped chickadees, eastern bluebirds, house wrens, and savannah sparrows nest in open fields or dense forests further from a water source as their preferred prey are terrestrial insects (Foote et al. 2010, Gowaty and Plissner 1998, Johnson 1998). Tree swallows and eastern kingbirds were considered aquatic insectivores because of their dietary items, and their exposure to selenium was likely greater than terrestrial insectivores.

Mercury Concentrations in Aquatic Emergent Insects

Invertebrate samples collected from both reference and study sites had concentrations of mercury that were above effect threshold limits of 0.001 to 0.01 µg/g ww (Utah DEQ 2007). It was unknown whether the samples contained methylmercury, which is bioavailable for uptake by organisms. Despite this, the presence of excess mercury is a cause for concern. Aquatic avian species may be experiencing bioaccumulation of mercury through their prey of aquatic emergent insects. When aquatic avian species have a diet that includes AEIs along with fish, their risk of exposure to selenium is greater than an avian species that has a strictly insectivorous diet.

Tissue-Based Exposure and Effects Assessment

Selenium Concentrations in Avian Species

Avian tissues contained varying concentrations of selenium depending upon site and species. Avian tissues at one reference site (15C) had significantly greater Se concentrations than the other four reference sites. The 15C site connects directly to the Escanaba River, which ultimately receives surface water discharge from the mine-influenced watershed. Because fish are mobile, it is possible that some fish in 15C could be moving between mine-influenced waters and unaffected waters. Therefore, piscivorous birds feeding from the 15C site could be consuming fish that spend their time in either watershed. Samples from study sites with the greatest concentrations of selenium came from lentic systems. Slow-moving water allows for a greater period of time to accumulate excess Se within aquatic sediments, plants, and invertebrates, leading to greater bioaccumulation in avian tissues (Orr et al. 2006).

Of the four waterfowl species analyzed, hooded mergansers and wood ducks were the two species to exhibit elevated selenium concentrations. Selenium concentrations exceeded thresholds in 1 of 175 study area egg tissues for hooded mergansers and 7 of 55 study area eggs for wood ducks. It should be noted that mallards were included as a stud species due to its presence on the site and known sensitivity to Se exposure. However, no mallard eggs were sampled. Adult female mallards were often observed at sites, and significant effort was expended to locate nests but none contained eggs. Presently it is unclear why all of the located mallard nests were unoccupied. Possibilities include poor breeding rates, significant predation rates, inability to locate active nests, or earlier than expected hatch dates. The last two possibilities have merit as mallard fledglings were observed in study albeit rarely.

Mercury Concentrations in Avian Species

Mercury concentrations varied in avian tissues by site and species. When avian species were grouped by site, all reference sites and eight study sites had average concentrations of Hg that exceeded 2 μg/g ww. Elevated mercury concentrations in avian samples were unexpected. The region of Michigan where this study took place has been involved in iron and precious metal mining for over 150 years. It is possible that residual mercury was deposited in former mining locations or locations not associated with mining activity through atmospheric and water deposition over an extended period of time. The fact that mercury levels in the prey items sampled from reference areas contained significantly lesser mercury than those from the study area yet no differences in tissue concentrations were observed is inexplicable.

Teratogenic Effects

Our percentage of deformities in embryos examined was less than 1%, which is less than some background levels of deformities reported for unexposed white leghorn chickens, Japanese quail, and common pheasants (Cohen-Barnhouse et al. 2011). In order to more directly compare Se exposure related deformity rates, we need to determine the number of reproductive events observed at which concentrations meet or exceed those at which deformities would be expected. In a study involving mallards fed selenomethionine, eggs produced from females consuming the 8 ppm Se diet had a 6.8% deformity rate (Heinz et al. 1989). Between 2010 and 2012, we observed 18 and 57 wood duck and hooded merganser nests, respectively. Out of all wood duck and hooded merganser nests in our study, two deformities were observed. Differences in species sensitivity between mallards, wood ducks, and hooded mergansers, or differences in other environmental factors could be a possible explanation for the difference in deformity rates. The two deformities observed, anophthalmia and club foot, are associated with selenium toxicity (Ohlendorf et al. 1986). However, these defects are also naturally occurring in avian species, which makes it difficult to determine if the single incidences of anophthalmia and club foot in the present study were due to excess selenium. Overall, it appeared that some individuals may be at a greater risk of teratogenesis at the most contaminated sites, but there was a low risk in the population.

Nest Abandonment

Selenium concentrations did not vary significantly between eggs from abandoned and non-abandoned nests or between fresh and addled eggs. The hypothesis that females abandon their nest because they can detect excess Se concentrations in eggs was not supported by results from the present study. The results showed that 50% of abandoned eggs contained less selenium than eggs that hatched, contrary to the hypothesis stated earlier. This indicated that females are not abandoning nests due to elevated selenium within the eggs. It is unlikely from these results that nest abandonment occurred from elevated amounts of Se within the embryos.

A significant difference was found in mercury concentrations between eggs from abandoned nests versus eggs from non-abandoned nests with abandoned nests having increased egg Hg concentrations. When comparing addled versus fresh eggs, there was an individual sample where an addled egg contained elevated concentrations of mercury compared to fresh eggs from the same nest. However, there was no correction factor for dehydration in addled eggs. The site where these eggs were located was considered a high-risk site (TLP) due to its close approximation to the Cliffs Mining Operation (CMO). Some of the greatest selenium concentrations in avian egg tissues were found at site TLP. Because this was an isolated example, it was unlikely that elevated selenium concentrations caused the presence of addled eggs. However, the presence of a significant increase of mercury in eggs from abandoned nests may lead to the possibility that excess mercury levels are interfering with female incubation behavior.

Selenium-Mercury Interactions

When comparing avian mercury and selenium concentrations on a nest-by-nest and site-by-site basis, there did not appear to be a 1:1 ratio of Se to Hg. Antagonistic effects may have accounted for the low rate of teratogenesis despite elevated concentrations of Se and Hg in avian embryos. It appeared that hooded mergansers and common mergansers were exposed to excess mercury and selenium through their piscivorous diet, whereas wood ducks were exposed to excess selenium and mercury through aquatic invertebrates.

While the egg tissue samples taken from reference sites contained less selenium than the egg tissue samples from the study sites, the same pattern was not observed for mercury. Some egg tissues from reference sites contained greater concentrations of Hg than egg tissues from study sites. This may be caused by the reference sites being involved historically with gold mining, which uses mercury amalgamation to extract gold ore (Environmental Protection Agency 2012). The Ropes Gold Mine in Ishpeming, MI was located within the study area and extracted gold using amalgamation processes from 1883-1897 (Broderick 1945). After 1897, the mine extracted gold periodically until 1991. Excess mercury and background levels of selenium in the egg tissues from reference sites may have led to an imbalanced ratio of Se: Hg and caused an increase in methylated mercury. Hg levels were above the recommended level in over half of the egg tissue samples, whereas Se levels were above the recommended level in less than 20% of the egg tissue samples. This bias towards elevated mercury in egg tissues may be indicative of antagonistic effects between Hg and Se. Selenium may be reducing or slowing the methylation of mercury, making Hg less bioavailable and less likely to cause teratogenic effects in embryos or nestlings despite the elevated Hg concentrations.

Past studies have shown antagonistic interactions between selenium and mercury in freshwater environments where both elements occur, with a decrease in Hg concentrations present in fish tissue being inversely correlated with an increase in Se concentrations (Chen et al. 2001; Paulsson and Lundberg 1989; Turner and Swick 1983). Some explanations for this antagonistic relationship include the reduced formation of methylmercury due to selenium (Se^0 or Se^{2-}) interacting with mercury (Hg^0 or Hg^{2+}) to form an insoluble compound, respectively. Selenium compounds can bind strongly to methylmercury before Hg can cause cellular damage, which leads to a rapid elimination of Se-Hg compounds from the organism before toxicity can occur (Yang et al. 2008). At the Tilden and Empire mines where the study sites were located, most watersheds have been contaminated with mercury for over 150 years due to extensive anthropogenic activities related to the mining industry. The relatively recent discovery of excess concentrations of Se to these study sites may actually be helping to decrease the amount of methylmercury in fish tissues and bird egg tissues already present in these aquatic systems. The CMO's subsequent process to eliminate or decrease selenium concentrations in these watersheds may increase the bioavailable methylmercury levels to food web biota by reducing the protective actions of Se against Hg.

Conclusions

For aquatic emergent insects (AEIs), selenium concentrations often exceeded threshold concentrations. However, mercury concentrations were less than threshold concentrations. For egg tissues, both selenium and mercury concentrations exceeded threshold concentrations. Despite elevated concentrations of both elements, teratogenic effects were less than control deformity rates in other studies. The lack of teratogenesis may have been influenced by the antagonistic relationship between selenium and mercury. Nest abandonment was an issue that may have been caused by mercury toxicity or a different physiological response.

CHAPTER THREE

HATCHING AND FLEDGING SUCCESS OF SELECT AVIAN SPECIES RESIDING NEAR AND ACTIVE IRON MINE

Abstract

Birds that spend their breeding season near Ishpeming, MI were exposed to elevated concentrations of selenium (Se) and mercury (Hg) as a result of mine-associated surface water discharges. There is significant uncertainty as to whether these concentrations of selenium and mercury are great enough to result in adverse reproductive effects in avian species. To determine if avian species were experiencing individual or population level adverse effects due to exposure to Se or Hg above recommended toxicity thresholds, we monitored reproductive parameters including abandonment rates, embryo morphology and hatching and fledging success of waterfowl and passerine species including hooded mergansers, wood ducks, common mergansers, tree swallows, eastern bluebirds, black-capped chickadees, house wrens, Canada geese, eastern kingbirds, and savannah sparrows. Hatching and fledging success in all species were within normal ranges and abandonment rates in most species were normal. Abandonment rates in wood ducks were greater than expected, with egg tissues from abandoned wood duck nests averaging 2.55 µg/g ww Hg. Despite elevated concentrations of selenium and mercury in the environment, overall reproductive success of the avian species monitored did not appear to be negatively impacted.

Introduction

In Marquette County, Michigan, Cliffs Natural Resources operates the proximal open pit Tilden and Empire iron mines and processing facilities that extract magnetite and hematite for steel production (Cliffs Natural Resources 2012). These and other mining operations have introduced selenium and mercury into local watersheds through processing outflows and surface water runoff. This has increased the amount of Se and Hg available for uptake by lower trophic level organisms and subsequently increased the rate of bioaccumulation of Se and Hg in birds (Kaulfersch et al. 2014). At the time of this study, the U.S. Environmental Protection Agency (USEPA) had promulgated more rigorous standards for selenium emissions in water basins (5 μg/L), thus requiring Cliffs to reduce the amount of Se released to the watersheds (Kohlhepp 2010). The Michigan State University Wildlife Toxicology Laboratory (MSU-WTL) was tasked as an independent party to assess hatching and fledging success and abandonment rates in select avian species residing in the Cliffs Mining Operations (CMO) associated watersheds.

Materials and Methods

Nest Boxes

There were 371 waterfowl and passerine (songbird) nest boxes installed at 12 study and 5 reference locations for 3 breeding seasons (2010-2012). Nest boxes included 120 waterfowl boxes that were placed 6-16 meters off the ground in tree species that included maple, oak, birch, and pine. Waterfowl boxes were made of cedar wood, lined with pine shavings, and placed within 300 meters of surface water at a minimum of 100 meters apart laterally along the shoreline. An infrared camera was placed inside each waterfowl box and a cable connected to the camera ran down the length of the tree where connectors for a monitor could be attached. This allowed the field team to remotely monitor nest activity without physically climbing the tree and disturbing the nest. At the time of egg tissue sample collection, tree climbing equipment was utilized to access the nest box. During the winter months, the entrances to nest boxes were covered with wire mesh to exclude habitation by non-target species.

There were 251 passerine boxes mounted on 8 foot T-posts within 100 meters of surface water. Passerine boxes were also made of cedar but were not lined. A locking side panel could be lifted to monitor passerine activity. Typically, two passerine boxes were placed proximal to a waterfowl box and within sight of open water. The T-posts were coated with non-toxic lithium-based grease and a wire mesh predator guard was installed around the box entrance in an effort to keep predators from reaching the boxes and their contents.

Spatial and Temporal Study Boundaries

The spatial boundary of the study consisted of 17 sites located in Marquette County, Michigan, each covering 2-7 kilometers of aquatic shoreline within or proximal to Cliffs Natural Resources

(CNR) mining operations. All twelve study sites received water that was associated with the Cliffs Mining Operation (CMO) through processing water outfalls and/or surface water runoff. Conversely, all five reference sites were just upstream of mine operations. Greater site details are presented in Kaulfersch et al. 2014.

Monitoring

Nest boxes were monitored every other day and activity was documented in field notebooks and with digital images. Activity was defined as the presence of nest material or any nest construction, adult birds, eggs, incubation, and/or hatchlings. Waterfowl nest boxes were fitted internally with an infrared video camera and a video monitor or recorder was plugged into the cable that was located at the base of the tree. Passerine boxes were checked by opening a side panel and visually inspecting the interior of the box. Passerine breeding pairs and nestlings were banded with a U.S. Fish and Wildlife (USFWS) leg band. Information containing the band number, sex of the individual, and age was recorded. Pictures of brood patches and primary feathers were also taken.

Tissue Collection

Once a clutch had been completed, the permitted numbers of fresh eggs were collected for Se and Hg analysis. Fresh eggs were collected on or before the fourth day of incubation. For waterfowl species, 2 eggs per clutch were randomly collected by accessing the nest box and contents using tree-climbing equipment. Passerines generally have smaller clutch sizes, so one egg per clutch was randomly collected to minimize population impacts. All unhatched eggs were collected (salvaged) for all species. Eggs were considered addled if they remained unhatched five days after the last egg from the clutch hatched. Eggs were considered abandoned if they remained cold for 10 days post incubation initiation. All eggs were wrapped in a chemically cleaned cloth and placed in glass I-Chem® jars. Information concerning the location, date, time, temperature, species, site, egg weight, egg length, egg width at three locations, and team members present was recorded. Jars containing egg samples were placed in a cooler in the field and transferred to a refrigerator at 4°C until analysis. Any deceased adults, nestlings, hatchlings, or fledglings that were recovered underwent the same collection procedure as eggs but were stored at -23°C.

Tissue Analysis

Collected eggs were opened using a scalpel to cut around the widest part of the egg. Egg contents were weighed and examined macroscopically for physical abnormalities. Evidentiary photos were taken of each embryo before the contents were homogenized for contaminant analysis. Deceased nestlings, fledglings, and adults that were collected were also homogenized for contaminant analysis. Deceased birds underwent necropsies for signs of disease, malnourishment, and/or disfigurement. Specimens were photographed during necropsies and homogenized for contaminant analysis.

Statistical Methods

All statistical analysis was performed using SAS 9.2. Differences in hatching success for each species between reference and study sites were determined by conducting an analysis of variance (ANOVA). ANOVA was also used to test for differences in fledging success in each species between reference and study sites. Statistical significance was established at $p<0.05$. For hatching and fledging success, nests that underwent complete abandonment or predation were not included in the statistical data. Fresh eggs collected from each nest were subtracted from the total number of eggs laid. A Pearson correlation was used to determine if there was a correlation between decreased hatching success and elevated Hg and Se concentrations.

Results

Selenium and Mercury Concentrations in Avian Eggs

Concentrations of Se and Hg were quantified for Canada goose, hooded merganser, wood duck, and common merganser eggs (Table 3.1). Selenium concentrations ranged from 0.72 to 17.6 μg/g dw among reference sites and <0.1 to 32.7 μg/g dw among study sites. Mercury concentrations ranged from 0.73 to 19.9 μg/g dw among reference sites and 0.14 to 75.6 μg/g dw among study sites (Kaulfersch et al. 2014).

Table 3.1. Mean Se and Hg concentrations and standard deviations in selected avian species among reference and study sites (Kaulfersch et al. 2014).

Species	Se (μg/g) dw	Hg (μg/g) ww
Wood Duck- Reference	1.95 ± 0.43	1.65 ± 0.15
Wood Duck- Study	11.66 ± 9.89	1.42 ± 1.04
Hooded Merganser- Reference	3.54 ± 4.32	5.22 ± 3
Hooded Merganser- Study	7.25 ± 6.19	6.48 ± 8.18
Canada Goose- Reference	1.07 ± 0.49	1.35 ± 0.09
Canada Goose- Study	2.48 ± 2.04	0.24 ± 0.37
Common Merganser- Reference	3.24 ±2.01	7.43 ± 1.13
Common Merganser- Study	NA	NA

Hatching Success

Hatching success was calculated as the number of eggs that hatched divided by the number of eggs incubated to hatch day. Abandoned and predated nests were not included in hatching quantifications. Hatching success was within normal ranges for each species studied (Table 3.2). There were no significant differences in hatching success between species residing in reference sites as compared to study sites or among the 17 individual sites. Furthermore, hatching success for aquatic-based consumers (wood ducks, hooded mergansers, and tree swallows) as compared to terrestrial-based consumers (eastern bluebirds, black-capped chickadees, and house wrens) were not different (Table 3.3).

Table 3.2. Mean hatching success and standard deviation of each species among reference and study sites as compared to previous studies.

Hatching Success			
Species	Reference Site	Study Site	Literature
Wood Duck	87% ± 12	88% ± 13	83% [a]
Hooded Merganser	88% ± 11	90% ± 15	87% [a]
Eastern Bluebird	92% ± 14	79% ± 25	75%[b]
Black-Capped Chickadee	92% ± 11	93% ± 9	86.3%[c]
House Wren	NA	87% ± 22	85%[d]
Tree Swallow	96% ± 8	96% ± 9	85%[d]

a- Mallory et al. 2002
b- Cornell et al. 2011
c- Mahoney et al. 1997
d- Custer et al. 2007

Table 3.3. Mean hatching success and standard deviation of aquatic and terrestrial species among reference and study sites.

Species	Reference Site	Study Site
Aquatic	91% ± 10	90% ± 14
Terrestrial	92% ± 11	88% ± 18

Fledging Success

Fledging success was quantified as the number of fledglings divided by the total number of hatchlings per clutch. Fledging success was within species norms as described in the literature (Table 3.4). There were no significant differences in fledging success between species residing in reference sites versus study sites. Fledging success among sites was similar. There were no significant differences between aquatic-based consumers and terrestrial-based consumers (Table 3.5).

Table 3.4. Mean fledging success of each species among reference and study sites as compared to previous studies

Fledging Success			
Species	Reference Site	Study Site	Literature
Wood Duck	100% ± 0	98% ± 7	52.1%[a]
Hooded Merganser	100% ± 0	99% ± 4	50%[b]
Eastern Bluebird	100% ± 0	94% ± 13	80.4%[c]
Black-Capped Chickadee	93% ± 13	100% ± 0	100%[d]
House Wren	NA	86% ± 38	98%[e]
Tree Swallow	98% ± 6	84% ± 36	90%[f]

a- Heusmann 2000
b- Jackson 1996
c- LeClerc et al. 2005
d- Bronson et al. 2005
e- Robinson and Rotenberry 1991
f- Hallinger and Cristol 2011

Table 3.5- Mean fledging success and standard deviation in aquatic and terrestrial species among reference and study sites.

Species	Reference Site	Study Site
Aquatic	99% ± 4	97% ± 12
Terrestrial	95% ± 11	93% ± 24

Nest Abandonment

Nest abandonment rates continually decreased from 2010 to 2012 for both passerines and waterfowl. Overall, waterfowl had greater abandonment rates than passerines. Nest abandonment rates were greater in study sites compared to reference sites for wood ducks, black-capped chickadees, and hooded mergansers (Table 3.6). Wood ducks exhibited a significant difference in abandonment rates between reference and study sites ($p=0.0116$). There were no significant differences in all other species between reference and study sites. Eastern bluebirds had the greatest percentage of abandoned nests among reference sites (25%), followed by hooded mergansers at 8%. There were no nesting attempts at reference sites by house wrens. No abandonment events occurred at reference sites for wood ducks, black-capped chickadees, or tree swallows. Among study sites, wood ducks had the greatest percentage of abandoned nests (33%), followed by black-capped chickadees (30%), eastern bluebirds (20%), hooded mergansers (19%), house wrens (13%), and tree swallows (0%).

Table 3.6. Rates of abandonment in each species among reference and study sites

Species	Reference Site	Study Site	Previous Literature
Wood Duck	0%	33%	43%[a]
Hooded Merganser	8%	19%	18.2%[b]
Eastern Bluebird	25%	20%	22%[c]
Black-Capped Chickadee	0%	30%	5%[d]
House Wren	NA	13%	11%[e]
Tree Swallow	0%	0%	7%[e]

a- Semel and Sherman 1986
b- Morse et al. 1969
c- Bass 2011
d- Fort and Otter 2004
e- Finch 1990

There were 98 salvaged hooded merganser and wood duck eggs to assess embryo development. Out of 63 salvaged hooded merganser eggs, 35 were fertilized (55.6%), with development ranging from 1-17 days post incubation. For wood ducks, 23 of 35 eggs were fertilized (65.7%). Development ranged from 1-7 days post incubation.

When correlating hatching success with selenium and mercury concentrations in waterfowl egg tissues (hooded mergansers and wood ducks), the only significant correlation was a decrease of selenium as hatching success increased in wood ducks among reference sites. (Figures 3.1.1-3.1.4).

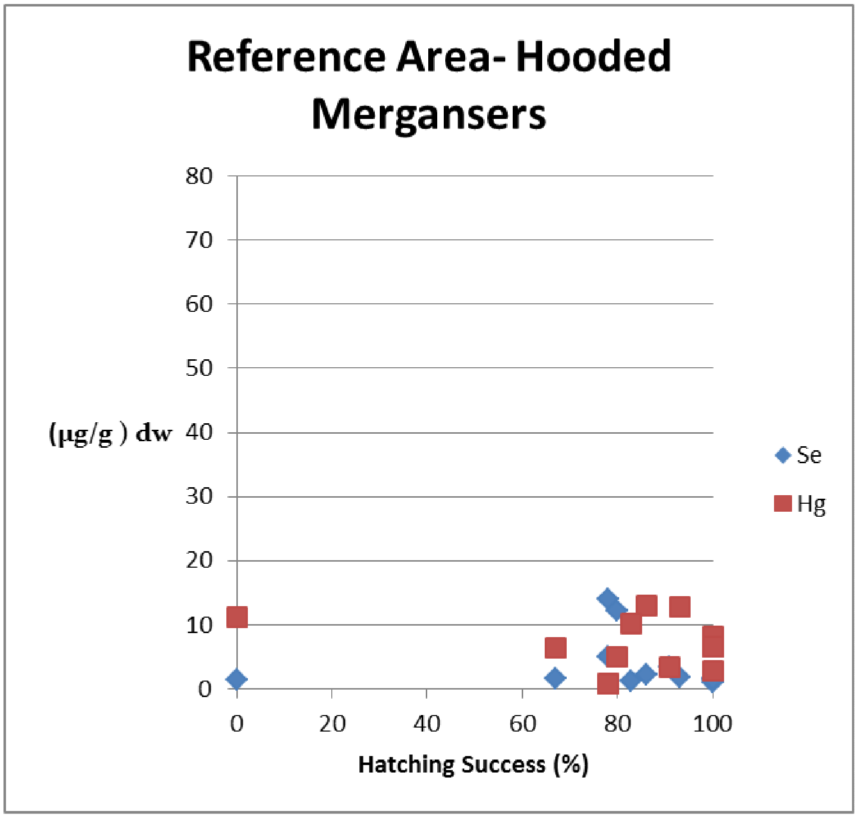

Figure 3.1.1. Correlations between hatching success and exposure concentrations in hooded mergansers from reference sites.

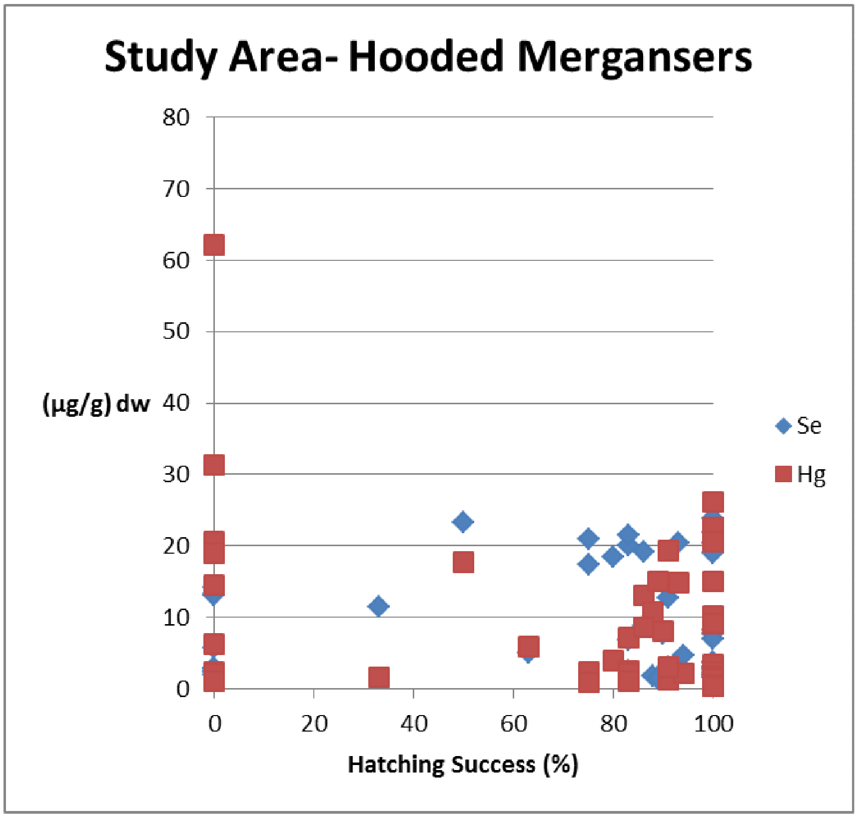

Figure 3.1.2. Correlations between hatching success and exposure concentrations in hooded mergansers from study sites.

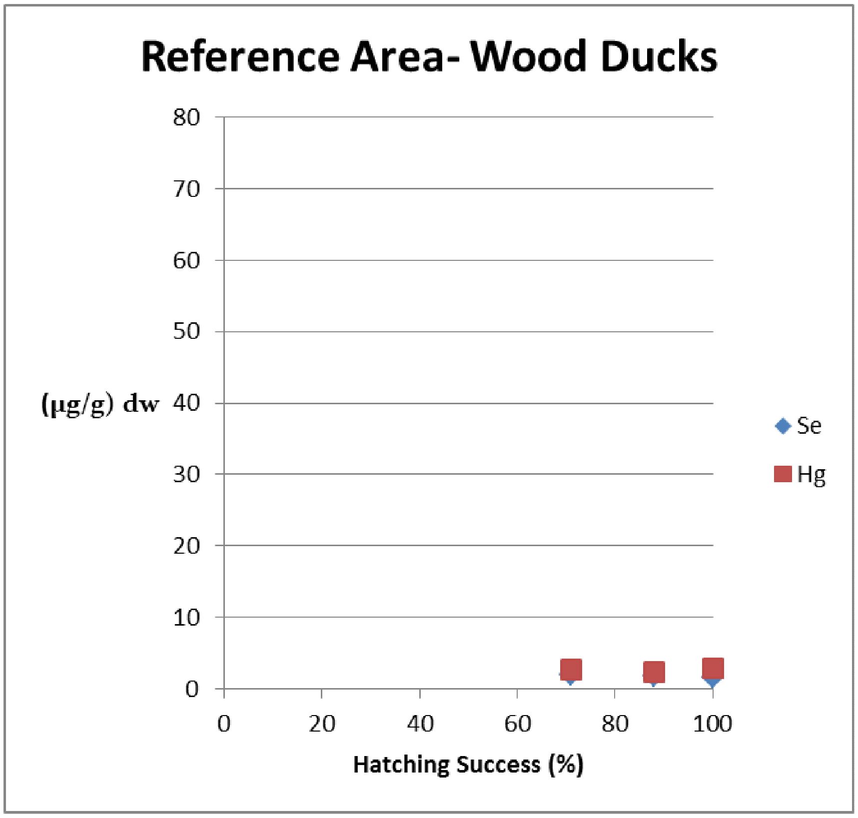

Figure 3.1.3. Correlations between hatching success and exposure concentrations in wood ducks from reference sites.

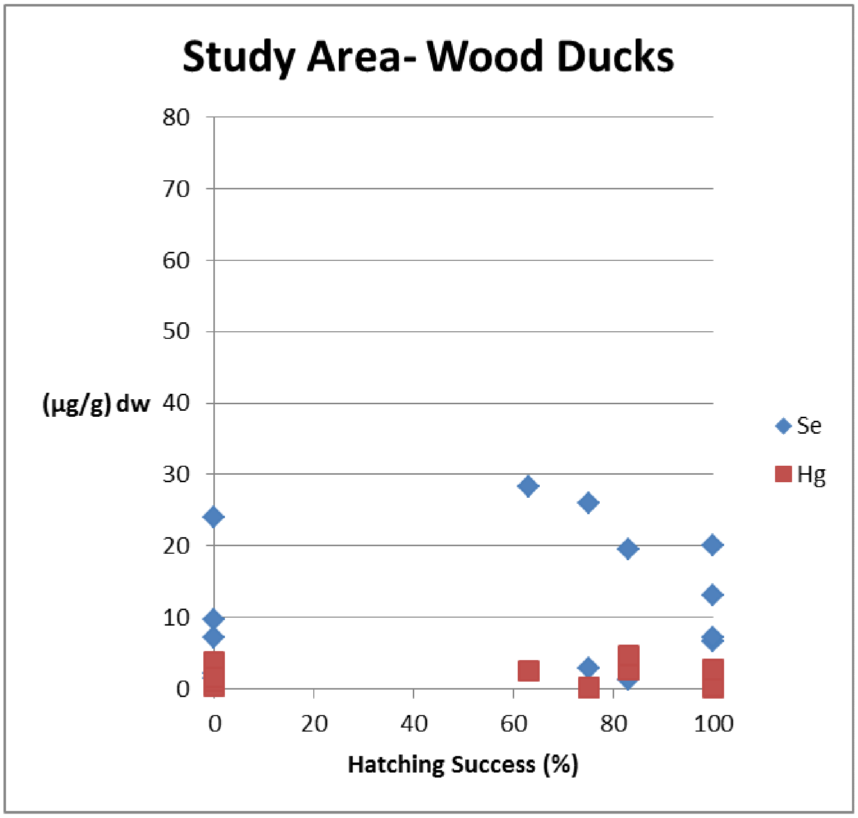

Figure 3.1.4. Correlations between hatching success and exposure concentrations in wood ducks from study sites.

Discussion

Hatching and Fledging Success

Hatching and fledging success rates for all species examined were within species norms and the rates were not different among areas with greatly differing selenium exposure, suggesting that reproductive health was not significantly affected by elevated selenium or mercury. The dietary and tissue-based exposure assessments showed that the elevated Se and Hg concentrations in egg tissues exceeded concentrations expected to cause adverse effects. In fact, 12.7% and 3.6% of wood duck egg tissues exceeded the threshold for effects of Se and Hg, respectively. Hooded mergansers exceeded the threshold for Se and Hg in 0.6% and 46.3% of egg tissues. The presence of Se and Hg in the same aquatic environment produces antagonistic actions, which may be responsible for the lack of obvious adverse effects. Selenium can reduce the concentration of mercury available for uptake by embryonic tissues. A study found that selenium at concentrations of 0.25 mg/kg in anaerobic sediments could stimulate the methylation of mercury, making it more harmful to the surrounding wildlife. However, concentrations of selenium between 0.25 µg/g and 2.5 µg/g decreased the methylation of mercury (Jin et al. 1997). Studies have indicated that when the ratio of selenium: mercury is equal to 1:1, the harmful effects from either compound are reduced (Koeman et al. 1973). Accelerated glutathione peroxidase activity may help remove excess Se from cells, decreasing the risk for bioaccumulation to occur. Because selenium binds strongly to methylated mercury, excretion of selenium includes excretion of methylmercury (Yang et al. 2008).

Nest Abandonment

Nest abandonment rates were elevated in study area waterfowl. Probable causes include inexperienced females, nest parasitism, study area, or mercury contamination (Conomy et al. 1998, Guigeno and Sealy 2011, Pascual and Peris 1992, Jackson et al. 2011).

Young female birds are more likely to inhabit newly placed nest boxes and demonstrate a greater chance of abandonment due to a lack of experience (Hepp and Kennamer 1993). Inexperienced females may also complete egg laying in more than one nest box (Semel and Sherman 1986). It was observed that nest boxes proximal to a nest that failed were often occupied and produced viable clutches. However, we could not determine if the female associated with the failed nest was the same female in a successful proximal nest box. While it is likely that some abandonment was due to inexperienced females, this does not explain the difference between study and reference areas as nest boxes were placed at all locations simultaneously. Therefore, female wood duck demographics should have been similar.

Nests that are parasitized by another female are more likely to be abandoned. Parasitism is associated with dump nests, where a large number of eggs (>15) are placed in a single nest. Females will abandon these large nests due to their inability to incubate all eggs evenly (Nielsen et al. 2006). Nest parasitism occurs in species that include wood ducks and hooded mergansers, both of which were present in this study. The rate of nest parasitism in this study was not significant and it was an unlikely cause of elevated abandonment in the waterfowl species.

One of the main reasons for nest abandonment is food availability. Insufficient prey items increases the female's time spent foraging and causes greater periods of time away from the nest (Pinkowski 1979). This leads to decreased incubation and eventual inviability of the eggs and abandonment of the clutch. It is unknown how much time females spent foraging and incubating in this study. Dietary items were collected from each active site and differences in the relative abundance of specific insect orders were noted (Kaulfersch et al. 2014). More specifically, the order Ephemeroptera was absent from study sites. This may be due to water quality issues since a lack of mayflies is a strong indication of an unbalanced water system (Wayland and Crosley 2006). Besides Ephemeroptera, there appeared to be an abundance and variety of aquatic emergent insects available for consumption.

Exposure to excess concentrations of mercury affects abandonment rates in avian species. Nest abandonment increased in Carolina wrens that were exposed to elevated concentrations of mercury through their food source (Jackson et al. 2011). When mercury concentrations between eggs from abandoned nests and eggs from non-abandoned nests were compared, abandoned nests had increased egg Hg concentrations. A possible explanation for mercury-associated nest abandonment is that elevated mercury concentrations leads to decreased estradiol concentrations, making females less likely to reproduce or to incubate eggs (Jayasena et al. 2011). Mercury concentrations were elevated in a greater percentage of hooded mergansers than wood ducks, while more abandonment events occurred with wood ducks than hooded mergansers. Therefore, it is unlikely that mercury is the main cause of most abandonment events.

Conclusions

Hatching and fledging success was within normal ranges for all targeted species in this study. Nest abandonment rates were increased in wood ducks, which may be due to the presence of inexperienced females or elevated Hg concentrations causing behavioral anomalies. Although eggs from abandoned nests contained on average more mercury than eggs from non-abandoned nests, there was no significant difference in Hg concentrations.

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