MERCURY AND SELENIUM CONCENTRATIONS IN LIVERS AND EGGS OF MINNESOTA COMMON LOONS (*GAVIA IMMER*)

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COMMON LOONS (GAVIA IMMER)

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Chapter I: Introduction and Literature Review

Loons

Introduction

The common loon (*Gavia immer*) is part of an ancient genus of birds. This genus separated from its closest relatives approximately 50 million years ago and emerged 10 million years ago in a form similar to what is present today. Loons are top carnivores, feeding primarily on fish. As top carnivores in the aquatic ecosystem, they are especially likely to bioaccumulate environmental contaminants (McIntyre 1988). Such contaminants include mercury and selenium.

Loon Distribution and Reproduction

Loons overwinter along the coast of North America, from California to Alaska in the Pacific and from the Gulf of Mexico to Newfoundland in the Atlantic. During the summer, their nesting range extends through the northernmost United States and most of Canada and Alaska. Loons are still found in Maine, New Hampshire, Vermont, New York, Massachusetts, Michigan, Wisconsin, Minnesota, Montana, Idaho, Washington, and Wyoming in the United States during breeding season. At

one time, breeding loons could also be found in Connecticut, Pennsylvania, Illinois, Nebraska, Indiana, Ohio, Oregon, California, and North Dakota. The southernmost border of the breeding range has been moving north over the past century. Loons prefer to nest on deep or shallow clear lakes, which allows for visualization of prey when hunting (O'Brien *et al.l* 1995, McIntyre 1998).

Decreased reproductive success has been reported in loons from northern Minnesota, east central Alberta, northern Lake Ontario, and other areas. Factors that may affect loon populations include loss of habitat, human disturbance, increased predation of eggs, and acidification of lakes (Haseltine *et al.* 1983).

Mercury

Mercury in the Environment

Mercury is a major heavy metal contaminant in aquatic ecosystems (Evers *et al.* 1998, Thompson and Furness 1989). Some geographic areas, including parts of Ontario, have naturally high background mercury levels (Barr 1986). Environmental mercury deposition has increased 3.7 times in Minnesota since pre-industrial times (Swain *et al.* 1992). Globally, mercury deposition increases by 1% each year (Evers *et al.* 1998). Natural background mercury levels account for approximately 25% of current mercury deposition

in northern Minnesota lakes. Mercury concentrations in fish from some lakes in Minnesota are above the tolerable limit for human consumption (Meyer *et al.* 1995, Swain *et al.* 1992).

Anthropogenic sources of environmental mercury have historically included methylmercury seed dressing and effluents from chlor-alkali plants and paper pulp mills (Barr 1986, Goyer 1995, Haseltine *et al.* 1983, Meyer *et al.* 1995, Pass *et al.* 1975). Most of these point sources of mercury pollution have been eliminated in the United States (Meyer *et al.* 1995). However, other sources, such as industrial and municipal wastes, fossil fuel combustion, and the mining and smelting industries continue to be a concern (Goyer 1995, Meyer *et al.* 1995, Thompson and Furness). Contamination in the area of the Great Lakes comes mostly from industrial pollution (Evers *et al.* 1998). Metallic mercury can become airborne and contaminate distant ecosystems through atmospheric transport and deposition (Barr 1986, Evers *et al.* 1998, Goyer 1995, Meyer *et al.* 1995).

Acidification of the environment associated with acid precipitation increases the bioavailability of mercury in the aquatic system and mercury accumulation in the biota (Barr 1986, Daoust *et al.* 1998, Meyer *et al.* 1995, Meyer *et al.* 1998, Scheuhammer *et al.* 1994, Scheuhammer *et al.* 1998b, Burge *et al.* 1976). Acidified lakes, which tend to have increased water clarity, may be preferred by nesting loons.

Loons on such lakes may be detrimentally affected by elevated mercury levels as well as decreased prey abundance (Daoust *et al.* 1998, Meyer *et al.* 1998, Scheuhammer *et al.* 1998a).

Methylmercury is the most significant form of mercury in aquatic ecosystems. Inorganic mercury is methylated by sediment bacteria at a rate which is inversely proportional to water pH (Meyer *et al.* 1995, Pokras *et al.* 1998, Scheuhammer *et al.* 1994). The methylmercury produced is highly lipophilic and readily bioaccumuated (Pedersen *et al.* 1998, Thompson and Furness 1989). It is the primary form of mercury found in fish and can be taken up by fish through the gills or the gastrointestinal tract (Pedersen *et al.* 1998, Pokras *et al.* 1991). More than 80% of the mercury in freshwater fish is methylmercury (Meyer *et al.* 1998, Scheuhammer *et al.* 1994, Scheuhammer *et al.* 1998a, Thompson and Furness 1989). In a study of fish in Ontario, 20% of the lakes sampled had small fish with muscle mercury concentrations greater than 0.5 ppm wet weight, and 30% of lakes had small fish with mercury levels greater than 0.4 ppm (Meyer *et al.* 1998, Pass *et al.* 1975). A similar study was conducted in Wisconsin and found that 36% of lakes had small fish containing mercury at concentrations higher than 3 ppm wet weight (Meyer *et al.* 1995). Total mercury concentration tends to increase proportionally with fish size (Meyer *et al.* 1995, Meyer *et al.* 1998, Scheuhammer *et al.* 1994). Other macroscopic aquatic organisms living in

contaminated lakes, such as crayfish, are known to bioconcentrate methylmercury (Barr 1986).

Biomagnification of mercury is believed to occur in aquatic ecosystems and top aquatic predators are likely to accumulate high tissue mercury levels (Coppock 1990, Daoust *et al* 1998,. Evers *et al*. 1998, Frank *et al*. 1983, Fox *et al*. 1980, Meyer *et al*. 1995, Meyer *et al*. 1998, Scheuhammer *et al*. 1998b, Stroud and Lang 1983, Thompson and Furness 1989). Incidents of methylmercury poisoning in top predators, including wild birds, have been reported (Heinz and Locke 1975, Thompson and Furness 1989). However, in some aquatic predators, including marine mammals and some birds, only a small proportion of the total mercury is methylmercury (Augspurger *et al*. 1998, Scheuhammer *et al*. 1998b, Thompson and Furness 1989).

Mercury Toxicokinetics and Toxicodynamics

Methylmercury uptake in aquatic predators is mostly through dietary exposure. Gastrointestinal absorption of methymercury is 90 to 95% complete in humans (Goyer 1995). Upon absorption, methylmercury can be carried by blood cells or bind to sulfhdryl groups on albumen or other serum proteins. Methylmercury can cross the blood brain barrier and eventually concentrates in the liver, kidneys, and central nervous system (Pedersen *et al*. 1998, Pokras *et al*. 1998).

Top predators may be able to detoxify methylmercury by demethylating it and storing it in an inorganic form (Daoust *et al.* 1998, Scheuhammer *et al.* 1998b, Thompson and Furness 1989). The proportion of mercury as methylmercury tends to decrease as total mercury levels increase in marine birds such as albatross and petrels (Daoust *et al.* 1998, Thompson and Furness 1989). These birds may accumulate high tissue mercury levels with no apparent adverse effects. Common loons with elevated tissue mercury levels accumulate mostly inorganic mercury in the liver and kidney while muscle mercury is mostly the methyl form. It has been suggested, as an alternate mechanism for accumulation of inorganic mercury, that birds with long life spans accumulate inorganic mercury from the diet but excrete methylmercury rapidly (Daoust *et al.* 1998).

The half-life of methylmercury in avian tissues is 2 to 3 months (Evers *et al.* 1998, Scheuhammer *et al.* 1998a). Mercury binds to sulfhydryl groups in feather keratin and 60 to more than 90% of the total body burden of methylmercury may be incorporated into growing feathers which are later molted (Barr 1986, Becker 1992, Burge *et al.* 1976, Burger *et al.* 1994, Evers *et al.* 1998, Forrester *et al.* 1997, Gochfeld 1997, Scheuhammer *et al.* 1998a, Thompson and Furness 1989). Penguins may excrete 60% of their methylmercury burden into feathers, and Bonaparte's gulls may excrete 93% in this way (Becker 1992). Female birds also excrete methylmercury into eggs (Barr 1986, Evers *et al.* 1998, Burger *et al.* 1994,

Becker 1992, El-Begearmi *et al.* 1977, Heinz and Hoffman 1998, Pokras *et al.* 1991, Gochfeld 1997). Methylmercury is the predominant form of mercury in eggs and the level of methylmercury in the egg or embryonic brain may exceed the level in the brain of the female parent (Barr 1986, Becker 1992).

Methylmercury interacts with RNA and DNA, affecting transcription and translation. Its affinity for sulfhydryl groups allows it to bind proteins, including microtubular proteins in neurons. Methylmercury may cause focal necrosis in neurons (Goyer 1995). It may also cause fibrinoid degeneration of blood vessel walls and consequently ischemia (Pass *et al.* 1975). Sublethal mercury toxicosis may be associated with increased susceptibility to infection. Elevated tissue levels were detected in heavily parasitized loons recovered from a mass mortality in the Gulf of Mexico (Barr 1986).

Mercury Toxicosis and Residues

Clinical signs of mercury intoxication in wild birds are mostly related to the central nervous system and reproduction. The primary central nervous sign is motor impairment (Heinz and Locke, 1975, Heinz and Hoffman 1998, Pokras *et al.* 1991). Flaccid paralysis has been reported in adult mallard ducks, red-tailed hawks, and goshawks experimentally intoxicated with mercury (Pass 1975, Heinz and Hoffman 1998). Experimentally induced mercury toxicosis

in mallards was manifested by weight loss, head tremors, ataxia, and incoordination early. This was followed by torticollis, opisthotonus, and clonic convulsions. Appetite remained normal throughout the study (Heinz and Hoffman 1998). Similar signs have been reported in mercury poisoning in humans, and the damage is considered irreversible. Decline in eyesight, which has been reported in mercury intoxicated humans, may be associated a decline in hunting ability in predatory birds, such as loons (Barr 1986, Goyer 1995).

Decline in egg production may be one of the first signs of mercury toxicosis in birds (Barr 1986, El-Begearmi *et al.* 1977, Meyer *et al.* 1998, Scheuhammer *et al.* 1998a). Reduced egg production occurs before teratogenicity and hatchling mortality. Peregrines with feather mercury levels greater than 42 ppm failed to produce eggs (Barr 1986). Quail fed methylmercury had decreased fertility and egg production. Fifteen ppm mercury as methylmercury in the diet of female quail was associated with reduction in egg hatchability to zero. Female quail were more tolerant of high dietary levels of methylmercury than males, possibly due to excretion in eggs (El-Begearmi *et al.* 1977). Egg color may also be affected by mercury toxicosis in the female bird (Barr 1986). When adult female mallard ducks were fed methylmercury, their ducklings suffered from poor balance and tremors (Heinz and Locke 1975). Teratogenic effects have been reported when eggshells were treated with mercury

(Heinz and Hoffman 1998). Experimental mallards fed a diet containing 0.5 ppm mercury had behavioral changes that may have influenced reproduction (Barr 1986).

The primary pathologic lesions of mercury toxicosis are emaciation and a peripheral neuropathy. Emaciation, muscle atrophy, neuronal necrosis, and severe, diffuse myelin degeneration caused by elevated levels of dietary methylmercury have been reported in adult birds fed high dietary levels of methylmercury (Barr 1986, Heinz and Locke 1975, Goyer 1995).

Lesions in experimental mallards with induced peracute methylmercury toxicosis include serous atrophy of fat, atrophy of skeletal muscle; petechial hemorrhages in skeletal muscle, cardiac muscle, visceral serosal surfaces, and cerebellum; subcutaneous edema of the lower neck, wing, and thigh; and thickening of the brachial and sciatic nerves. The major histopathologic lesion in these birds was Wallarian degeneration in the central and periferal nervous systems. Shrunken, eosinophilic neurons with pyknotic nuclei were observed in the telencephalon. Similar lesions were also detected in sciatic nerves, brachial nerves, and the dorsal root ganglia. Additionally, hypertrophy of astrocytes was associated with blood vessels in the cerebral grey matter. White matter tracts between the cerebellum and medulla had vacuolation, microgliosis, astrogliosis, and neuronal degeneration. Additionally, necrosis and fibrinoid degeneration of arterial walls with thromobosis, edema, and

hemorrhage were reported. Lastly, renal tubular necrosis was seen in the kidneys (Pass *et al.* 1975).

Adult female mallard ducks were fed dietary levels of 3 ppm mercury as methylmercury and lesions were detected in their ducklings. Neuronal shrinkage and necrosis were associated with the cerebellar folia and the cerebral optic lobes. Status spongiosus was described the optic lobes. Congestion and extensive hemorrhage was associated with the cerebellar meninges and folia (Heinz and Locke 1975).

Mercury sensitivity in individual birds may depend upon a number of factors including species, level and duration of exposure, age, and physiological and environmental stressors. Liver tends to accumulate higher total mercury levels than kidney, and kidney accumulates higher levels than muscle. Brain total mercury levels may be one-tenth those in the liver, however, brain tends to accumulate higher concentrations of methylmercury than liver (Barr 1986, Coppock *et al.* 1990). Liver mercury levels of 10 ppm wet weight or greater are reported to be associated with reproductive impairment in some avian species (Pokras *et al.* 1991). Reduced hatchability was seen in eggs from female pheasants with experimental mercury poisoning. Liver mercury levels were 3 to 13.7 ppm (Barr 1986). Liver levels in excess of 30 ppm have been associated with neurologic signs and levels of 50 ppm or higher have been associated with lethal mercury toxicosis in some avian species (Augspurger *et al.* 1998, Daoust *et al.* 1998, O'Brien *et al.*

1995). However, liver mercury levels in excess of 1,000 ppm have been detected in apparently healthy albatross (Thompson and Furness 1989).

Two separate studies were conducted in which diets containing 3 ppm mercury as methylmercury were fed to experimental black ducks and female mallard ducks. The range of liver mercury levels measured in the black ducks was from 40 to 140 ppm, dry matter. Kidney mercury levels ranged from 30 to 100 ppm dry matter in black ducks and approximately 60 ppm dry matter in mallard ducks (Scheuhammer *et al.* 1998b). Mallard ducklings had CNS signs and decreased survival (Gochfeld 1997).

Tissue mercury levels in wild birds have been studied (see table 1). In a study of Franklin's gulls, feather mercury levels were lower in birds from Minnesota than those from Montana or North or South Dakota (Gochfeld 1997). Common loons found near Lake Michigan and Illinois had detectable levels of mercury in their livers (Coppock *et al.* 1990). In a study of loons from Ontario and Wisconsin, birds from both states had similar blood mercury levels (Scheuhammer *et al.* 1998a). Three Wisconsin loons from another study had liver mercury levels of 30, 33, and 90 ppm wet weight (Meyer *et al.* 1995, O'Brien *et al.* 1995). Several studies have been performed on Ontario loons. Liver and kidney mercury levels in one study remained below 7 ppm wet weight (Frank *et al.* 1983). Another study found an average liver mercury concentration of 29.7 ppm wet weight

(Meyer *et al.* 1995). One Ontario loon was reported to have 90.5 ppm mercury in the liver, wet weight (O'Brien *et al.* 1995). Loons from Maritime Canada had levels of 0.16 to 61.0 ppm mercury (wet weight) in kidneys with an average of 18.80 ppm (Daoust *et al.* 1998). A study of liver mercury levels in New England loons found a range of 0.106 to 187.0 ppm with an average of 41.06 ppm mercury, wet weight. Total mercury levels were significantly higher than methylmercury levels that tend to remain below 10 ppm. Liver methylmercury levels ranged from 0.26 to 9.10 ppm, with an average of 3.55 ppm (Pokras *et al.* 1998). Mercury toxicosis may have contributed to the death of a New England loon, which had a liver mercury level of 55.1 ppm, wet weight (Pokras *et al.* 1991).

In general, male loons have higher tissue mercury levels than females (Barr 1986, Becker 1992, Evers *et al.* 1998, Meyer *et al.* 1995, Meyer *et al.* 1998, Pokras *et al.* 1998, Scheuhammer *et al.* 1998a). Male loons in the New England study had liver total mercury levels ranging from 11.300 to 187.000 ppm mercury, wet weight, with an average of 56.060 ppm, whereas the range for females was from 10.000 to 100.500 ppm with an average of 45.900 ppm (Pokras *et al.* 1998). Male loons tend to be larger than females, and may eat larger fish, which are likely to contain higher levels of mercury. Breeding females have the ability to excrete mercury into eggs (Barr 1986, Becker 1992, Meyer *et al.* 1995, Meyer *et al.* 1998, Scheuhammer *et al.* 1998a).

Interestingly, male hatchlings tend to have higher mercury levels than female hatchling chicks (Barr 1986).

Tissue mercury levels are higher in adult than in immature loons (Burger *et al.* 1994, Evers *et al.* 1998, Frank *et al.* 1983, Pokras *et al.* 1991). In a study of loons from Vermont, Massachusetts, and Maine, adult feather mercury levels were two times those in immature loon feathers. Adult loons tend to eat larger fish than immature loons and have had more time to accumulate mercury. Immature loons feed relatively close to the nest site, therefore tissue mercury levels in loon chicks reflect the amount of contamination in the natal lake (Burger *et al.* 1994).

Female birds are able to excrete mercury into their eggs. Abnormalities may occur in mallard eggs containing 0.85 ppm mercury wet weight (Heinz and Locke 1975). Egg mercury levels above 1 to 2 ppm are considered toxic and may cause clinical effects in eggs from various species of birds, including mallards (Custer *et al.* 1997, Gochfeld 1997, Haseltine *et al.* 1983). Eggs from experimental female mallards on control diets contained 0.78 to 1.07 ppm mercury wet weight with an average of 0.94 ppm. Mallards fed an experimental diet containing 3 ppm mercury produced eggs containing 4.05 to 7.59 ppm mercury with an average level of 5.46 ppm (Heinze and Locke 1975). Experimentally, female pheasants had liver mercury levels ranging from 3 to 13.7 ppm wet weight, and produced eggs containing 0.5 to 1.5 ppm mercury. Chick survival was not affected, and female birds

appeared healthy but had histopathologic neuronal lesions in the CNS (Barr 1986).

Eggs from wild mallards have been reported to contain 2.7 ppm wet weight mercury and eggs from common terns have been reported to contain 6.25 ppm (Barr 1986). Numerous studies on mercury levels in herring gull eggs have been published. In gulls, egg levels greater than 16 ppm wet weight are associated with reproductive impairment (Custer *et al.* 1997). Reports state that 0.1 ppm mercury, wet weight, has been detected in Norwegian gull eggs, and 1.0 ppm wet weight has been detected in German gull eggs. New York herring gulls eggs may contain up to 1.47 ppm mercury wet weight. Great Lakes herring gull eggs average 0.50 ppm mercury, wet weight (Gochfeld 1997). In a study of gull eggs from Minnesota, Mississippi, Wisconsin, Illinois, and Iowa, mercury concentrations averaged 0.49 ppm dry matter, with a range of 0.24 to 2.82 ppm (Custer *et al.* 1997).

A few studies have documented mercury levels in common loon eggs collected in Canada and the United States. Almost 100% of the mercury in loon eggs is in the form of methylmercury (Barr 1986). Loon egg mercury levels sometimes exceed levels that would be lethal to eggs of other avian species (Barr 1986, Meyer *et al.* 1998). A report from northwest Ontario examined lakes that had perch containing 0.36 ppm or more of mercury, wet weight and found that loon eggs found near those lakes had reduced hatchability. Loon reproduction was inhibited in lakes

where perch contained 0.4 ppm mercury. One loon chick that died at hatching had a total body burden of 2.44 ppm mercury, wet weight. The average loon egg mercury level reported in that study was 0.26 ppm wet weight (Barr 1986). A study of east Ontario loon eggs determined that the average level of mercury was 0.47 ppm wet weight. The reported average mercury level for south Ontario loon eggs was approximately 1 ppm with little change over a period of 9 years (Frank *et al.* 1983). A Saskatchewan report found a mean mercury level of 0.35 ppm in loon eggs, which had hatching failure rate of only 5% (Fox *et al.* 19 1980, Haseltine *et al.* 19 1983). Loon eggs collected in Wisconsin had mercury levels ranging from 0.5 to 1.6 ppm, with a mean of 0.9 ppm wet weight (Fox *et al.* 19 1980). A New Hampshire study found that two loon eggs contained more than 1 ppm mercury, wet weight, and up to 47% of eggs analyzed contained more than 0.5 ppm mercury. Loon egg mercury concentrations ranged from 0.22 to 1.60 ppm mercury in that study (Haseltine *et al.* 19 1983).

Selenium

Selenium in the Environment

Selenium is an essential cofactor of glutathione peroxidase (Goyer 1995, Yoneda and Suzuki 1997). Selenium deficiency associated with low soil selenium levels is endemic in some parts of the world where nutritional muscular dystrophy in calves and lambs, liver necrosis in rats, bleeding disorders in poultry, and cardiomegaly in humans occur (Goyer 1995, Heinz 1996). Elevated levels of selenium in the diet may cause selenium toxicosis. Diseases that are believed to be caused by selenium toxicosis in mammals include alkali disease in horses and other livestock (Goyer 1995, O'Tool and Raisebeck 1997).

Selenium toxicosis in birds has been associated with exposure to irrigation runoff water in Kesterson Reservoir, San Joaquin Valley, California (Albers *et al.* 1996, Fairbrother *et al.* 1994, Heinz 1996, Lemly and Smith 1987, Ohlendorf *et al.* 1986). Reservoir water contained 300 ppb selenium and small fish contained 26 to 31 ppm, wet weight (Ohlendorf *et al.* 1990, Hoffman and Heinz 1988). Selenium concentrations of 3 to 8 ppm in the diet may be toxic to wildlife (Lemly and Smith 1987). Other sources of selenium pollution in the environmental include sewage sludge, fly ash, and metal smelting plant emissions (Heinz 1996). High

soil selenium levels are naturally present in some areas of the western United States (Albers *et al.* 1996).

The most biologically relevant form of selenium in the environment is selenomethionine, an organic form produced by plants. Selenomethionine tends to accumulate in animals through the food chain (Heinz and Hoffman 1998, Goyer 1995, Heinz 1996, Lemly and Smith 1987). Selenomethionine is embryotoxic in mallards and believed to present a hazard to wild birds (Heinz 1996).

Selenium concentrations in plants and animals associated with the aquatic ecosystem may be up to 300 times higher than the water level. The biomagnification factor for selenium is usually between 2 and 6 (Lemly and Smith 1987). Most organisms absorb little selenium directly from the water. Most uptake occurs through the food chain (Rudd *et al.* 1980).

Selenium Toxicokinetics and Toxicodynamics

Most selenium accumulation in fish and aquatic birds is due to dietary exposure (Pedersen *et al.* 1998). Experimental mallards on high selenium diets were found to accumulate selenium rapidly in the liver (Heinz *et al.* 1990, Heinz 1996, Ohlendorf *et al.* 1990). Selenium is distributed to the liver, kidneys, feathers, brain, and muscle (O'Toole and Raisebeck 1997). Selenium is also found in red blood cells, where it is associated with glutathione peroxidase

(Goyer 1995). Methylmercury and selenium are mutually antagonistic (Albers *et al.* 1996, Goyer 1995, Heinz 1996).

The half-life of selenium is about 19 days in mallard liver and 27 days in chicken blood (Heinz *et al.* 1990, Ohlendorf *et al.* 1990). In mammals, twice as much selenium is excreted in the urine compared to the feces (Goyer 1995). In female birds, selenium becomes concentrated in egg albumen (Lemly and Smith 1987). Selenomethionine concentrates in eggs ten times more readily than selenite (Hoffman and Heinz 1988).

The mechanism of selenium toxicosis in birds is not fully understood. Tissue necrosis occurs in the avian embryo and may be due to oxidative stress from elevated selenium levels in the egg (Hoffman and Heinz 1988). Selenium may replace sulfur in the amino acid methionine, present in keratin, causing characteristic claw and feather lesions seen in intoxicated birds (O'Toole and Raisebeck 1997). Selenium is also immunotoxic (Fairbrother *et al.* 1994).

Selenium Toxicosis and Residues

The integumentary system and reproduction are affected by selenium toxicosis in wild birds. Adult grebes and coots at Kesterson were emaciated and had depterylation of the heads (Albers *et al.* 1996, Ohlendorf *et al.* 1990, Pokras *et al.* 1991). Depterylation of the crown and dorsal midline of the neck has been reported in mallards, as well as

onychoptosis and degeneration of the maxillary nail. Poor quality of feathers has been reported. Anorexia, muscle atrophy, onychoptosis, and depterylation have been produced experimentally in birds fed diets containing 40 ppm selenium. Emaciation is also a consistent clinical finding in selenium poisoned birds (Albers *et al.* 1996, O'Toole and Raisebeck 1997).

Lesions in birds poisoned with selenium include smaller, nodular livers, which may be pale to bronze and contain multiple foci of necrosis (Albers *et al.* 1996, Ohlendorf *et al.* 1990). Microscopically, liver lesions include hemosiderosis, hepatic necrosis, and bile duct hyperplasia (Albers *et al.* 1996, Heinz 1990, O'Toole and Raisebeck 1997). The gastrointestinal tract is usually empty and the intestines may be hemorrhagic. The kidneys may be enlarged and the spleen may be atrophic (Albers *et al.* 1996).

Reproductive effects may occur in birds in the absence of other clinical signs or lesions (Lemly and Smith 1987). Reproductive effects range from decreased fertility and sterility to embryonic death and teratogenesis (Albers *et al.* 1996, El-Begearmi *et al.* 1977, Heinze and Hoffman 1998, Lemly and Smith 1987, Scheuhammer *et al.* 1998b). At Kesterson Reservoir, 46% of nests studied contained dead embryos, and 19.6% of nests contained deformed embryos. Deformities were usually bilateral and included microphthalmia, anophthalmia, micromelia, amelia,

ectrodactyly, club foot, missing, reduced, or crossed beaks, hydrocephaly, exancephaly, and liver and heart defects (Ohlendorf *et al*. 1990, Albers *et al*. 1996). Most of these lesions have been reproduced experimentally by feeding diets containing elevated levels of selenium to mallards. Experimentally, it has been determined that 25 ppm sodium selenate or 10 ppm selenomethionine in the diet of adult mallards causes a 40 to 44% decrease in egg hatchability. Ten ppm sodium selenate in the diets of hens produced 100% reduction in egg hatchability, although no teratogenic effects were detected. Hatchability was reduced nearly 90% by feeding female mallards a diet containing 16 ppm selenomethionine. Selenomethionine is more teratogenic to mallards than selenite (Hoffman and Heinz 1988).

Factors that may influence selenium toxicity in birds include nutritional status, disease status, species, environmental stress, and gender (Lemly and Smith 1987, Ohlendorf *et al*. 1990). Other metals such as silver, copper, cadmium, lead, and arsenic, as well as mercury, may influence the bioavailability of selenium (Albers *et al*. 1996, Goyer 1995, Hoffman and Heinz 1988). In most species studied, selenium accumulates to the highest concentrations in the liver, followed by the kidney, then the feathers. The next highest selenium levels may be found in the brain and muscle (O'Toole and Raisebeck 1997). In some avian species, liver selenium concentrations above 3 ppm wet weight may be associated with impaired reproduction.

Experimental female mallards with liver selenium levels of 3.5 ppm wet weight had reduced reproductive performance. Teratogenesis occurred when parental liver selenium concentrations reached about 9 ppm wet weight. Dead Kesterson Reservoir birds had liver selenium levels between 8 and 26 ppm wet weight. However, selenium levels were similar in birds that were shot at the same location. Some experimental mallards fed diets containing 40 ppm selenium, wet weight, died with liver selenium levels of about 60 ppm, wet weight. Most male mallards fed a diet containing 32 ppm selenium, wet weight, survived with liver selenium levels of about 29 ppm. Liver selenium levels of 12.5 ppm, wet weight, were associated with pathologic changes (Heinz 1996).

Mercury and Selenium Interactions

Mercury and Selenium Toxicokinetics and Toxicodynamics

Mercury and selenium appear to be mutually antagonistic in adult aquatic birds (El-Begearmi *et al*.1977, Heinz and Hoffman 1998, Lourdes *et al*.1991, Rudd *et al*.1980, Scheuhammer *et al*.1998, O'Brien *et al*.1995). Dietary selenium decreases the toxicity of organic and inorganic forms of mercury in the diet, and dietary mercury decreases the toxicity of selenium in the diet. Toxic levels of selenium in the diet increased survival of quail on a diet

containing toxic levels of methylmercury (El-Begearmi *et al*.1977). Studies have found that similar mercury and selenium interactions occur in mammals. Selenite and selenomethionine are protective against mercury toxicosis in rats (Lourdes *et al*.1991).

Mercury and selenium tend to accumulate in a one to one molar ratio, or a 1 to 2.5 weight ratio in tissues of mammals including humans, rats, and wild marine mammals (Lourdes *et al*.1991, Rudd *et al*.1980, Yoneda and Suzuki 1997). Most marine fish, including marlin and tuna, also maintain a one to one molar ratio of mercury to selenium, however, most freshwater fish have a ratio greater than one part mercury to one part selenium (Lourdes *et al*.1991). The mercury to selenium ratio in birds may be one to one or greater (El-Begearmi *et al*.1977).

Mercury and selenium do not interact significantly in the water column (Pedersen *et al*.1998, Rudd *et al*.1980). The presence of selenium in water may or may not affect the uptake of mercury by fish. The presence of selenite or selenate in water did not increase gill uptake of methylmercury, however, the presence of selenate and selenite in tissues did increase gill mercury uptake by two and three times, respectively, and both increased the rate of methylmercury elimination (Pedersen *et al*.1998). In quail fed elevated dietary levels of methylmercury, increasing the dietary selenium concentration caused increased deposition of mercury in the brain in the absence

of clinical signs of mercury toxicosis. When quail were fed diets containing elevated selenium levels, increasing the methylmercury concentration in the diet led to an increase in brain selenium (El-Begearmi *et al*.1977). Liver mercury and selenium levels responded to dietary manipulations in a similar manner (El-Begearmi *et al*.1977, Heinz and Hoffman 1998). Selenium does not appear to enhance mercury excretion in quail (El-Begearmi *et al*.1977). Selenium may alter the distribution pattern of mercury in tissues, increasing distribution to the brain and decreasing kidney mercury concentrations (El-Begearmi *et al*.1977, Lourdes *et al*.1991, Yoneda and Suzuki 1997).

Elevated selenium concentrations in the diet of rats prevented mercury from binding to metallothionin. In some species, including rats, selenium and mercury may bind to a high molecular weight protein in a one to one molar ratio. In other species, including rabbits, methylmercury may complex with reduced selenium to form bis(methylmercuric) selenide (Lourdes *et al*.1991, Yoneda and Suzuki 1997). Mercury and selenium complex formation is believed to occur in the plasma (Yoneda and Suzuki). It is not known, but a protein may be involved in mercury and selenium interactions in bird tissues. This complex has a long tissue half-life (Scheuhammer *et al*. 1998).

Female mallards excrete mercury and selenium into eggs. Experimentally, mallards fed elevated dietary levels of methylmercury and selenomethionine had higher egg selenium

levels than mallards fed diets containing elevated selenium concentrations only (Heinz and Hoffman 1998). Mercury and selenium may act in an additive or synergistic manner in immature fish and birds (Burge *et al.* 1976, Lourdes *et al.* 1991). When mercuric mercury and selenate were injected into chicken eggs, the decrease in hatchability was greater than predicted based on an additive response (Burge *et al.* 1976). When female mallards were fed diets containing elevated levels of methylmercury and selenomethionine, decreased hatchability, decreased post-hatching duckling survival, and increased teratogenesis were reported. However, when female Japanese quail were fed diets containing elevated levels of selenite and methylmercury, hatchability of was not decreased below that of eggs from females fed elevated dietary levels of selenite or methylmercury alone. The difference in effect may be due to the different forms of selenium used, species differences, differences in liver function in immature versus mature birds, or some combination of these factors (Heinz and Hoffman 1998).

Mercury and Selenium Toxicosis and Residues

In an experiment where female mallards were fed variable dietary concentrations of methylmercury and selenomethioinine, approximately 6 percent of ducklings produced by mallards on control diets containing low levels of mercury and selenium had birth defects. About 36% of

ducklings from mallards on diets containing elevated levels of selenium had signs of teratogenesis, as did about 16% in ducklings from mallards on diets containing elevated mercury concentrations. Birth defects were present in approximately 73% of ducklings produced by mallards fed diets containing elevated concentrations of both mercury and selenium. Teratogenic effects associated with elevated mercury levels in the diet included missing bills, malformed bills, anophthalmia, microphthalmia, exophthalmia, and hydrocephaly. Birth defects associated with elevated selenium or selenium plus mercury in the diet included misshapen faces, hydrocephaly, anophthalmia, microphthalmia, exophthalmia, small or malformed wings, missing or malformed feet or legs, and small or missing toes. Certain teratogenic effects were only seen in hatchlings from mallards fed elevated dietary levels of both mercury and selenium. These include scoliosis, lordosis, dicephaly, exancephaly, and spina bifida (Heinz and Hoffman 1998).

Mammals and fish generally store mercury and selenium in a one-to-one molar ratio, or one to 2.5 weight ratio, as stated previously (Lourdes *et al.* 1991, Rudd *et al.* 1980, Yoneda and Suzuki 1997). The ratio in birds varies. Experimental quail fed high levels of dietary mercury and selenium had a mercury to selenium ratio of one to 2.8 based on weight, the ratio in the kidneys was one to 2.2. Increasing the dietary methylmercury increased the ratio in quail to one to 6.5 (El-Begearmi *et al.* 1977). Studies on

loon tissues have found mercury and selenium ratios ranging from approximately 1 to 2.5 to about 1 to 2.85, by weight (Barr 1986, Scheuhammer *et al.* 1998).

Recently, experimental mallards were fed diets containing 10 ppm mercury as methylmercury and 10 ppm selenium as selenomethionine. Male mallards accumulated average liver concentrations of 114 ppm mercury and 65 ppm selenium, wet weight. Females accumulated 9.2 ppm mercury and 21 ppm selenium, on average, in their livers. Males accumulated 0.039 ppm mercury and 1.0 ppm selenium in the liver on average, and female livers contained 0.011 ppm mercury and 0.49 ppm selenium. Mallards on diets high in mercury but not selenium tended to have increased tissue selenium levels. Males had average liver mercury levels of 71 ppm and selenium concentrations of 19 ppm. Females had average liver mercury concentrations of 22 ppm and selenium concentrations of 1.7 ppm. When mallards were fed diets containing elevated levels of selenium, liver mercury levels did not increase significantly. Male mallards had average liver mercury concentrations of 0.094 ppm and average liver selenium concentrations of 9.6 ppm. Females had liver mercury levels of 0.007 ppm and selenium concentrations of 6.0 ppm, on average (Heinz and Hoffman 1998).

Mercury and selenium concentrations in tissues of wild birds have been reported (see table 2). Wild merganser ducks in eastern Canada had elevations in tissue levels of both mercury and selenium. Liver mercury levels averaged 15

ppm and liver selenium averaged 9.7 ppm on a dry matter basis. Kidneys contained on average 11 ppm mercury and 8.5 ppm selenium. Liver and kidney total mercury and selenium concentrations correlated strongly, but liver and kidney methylmercury concentrations did not correlate significantly with selenium levels (Scheuhammer *et al.* 1998).

A few studies have investigated mercury and selenium levels in tissues of wild loons. Common loons in eastern Canada had elevated tissue levels of mercury and selenium. Average mercury and selenium levels in loon livers were 19 ppm and 15 ppm dry matter, respectively. The average kidney mercury concentration was 15 ppm, and the average selenium concentration was 15 ppm. Mercury levels tended to be about two times higher in emaciated loons than in healthy loons that came from areas considered `uncontaminated.' The elevated mercury levels in emaciated loons may have been a predisposing cause for the emaciation, or elevated mercury levels may have been due to redistribution of mercury in the sick loon. This study also found that the proportion of methylmercury in tissues decreased as total mercury increased, and tissue methylmercury concentrations always remained below 10 ppm. Liver total mercury and selenium were present in a molar ratio of approximately one to one, however there was no correlation between liver methylmercury and selenium concentrations (Scheuhammer *et al.* 1998). Another study investigated liver mercury and selenium levels in loons from Michigan. Liver mercury levels averaged 3.03

ppm wet weight, and ranged from 7.26 to 22.4 ppm. Liver selenium levels averaged 2.88 ppm wet weight, with a range from 2.48 to 6.28 ppm. One Michigan loon was reported to have concentrations of 1,410 ppm mercury and 522 ppm selenium wet weight in the liver (O'Brien *et al.* 1995). A study of loons from North Carolina found liver mercury levels averaging 10.9 ppm with a range from 2.08 to 84.3 ppm wet weight and selenium concentrations averaging 10.4 ppm with a range of 4.08 to 15.9 ppm (Heinz and Hoffman 1998). A study of New England common loons compared tissue mercury and selenium levels in adult and immature birds. Adult loons had liver mercury concentrations averaging 23.6 ppm with a range of 1.1 to 55.1 ppm wet weight, and selenium levels of 11.5 ppm on average with a range of 1.7 to 5.4 ppm. Immature loons had liver mercury levels averaging 8.0 ppm with a range of 3.0 to 7.8 ppm, and selenium concentrations of 3.6 ppm on average, with a range from 1.7 to 5.4 ppm. The two loons with the highest liver selenium levels (20.4 and 21.0 ppm) also had elevated liver mercury levels (Pokras *et al.* 1991).

Egg mercury and selenium concentrations were recorded in mallards fed methymercury and selenomethionine. Mallards fed dietary levels of 10 ppm mercury and 10 ppm selenium laid eggs containing on average 17 ppm selenium wet weight, and 9.5 ppm mercury. Mallards fed elevated levels of mercury but not selenium produced eggs containing an average of 16 ppm mercury and 0.39 ppm selenium. Eggs from mallards

fed elevated dietary levels of selenium but not mercury contained an average of 0.004 ppm mercury and 7.6 ppm selenium. Eggs produced by birds on control diets contained an average of 0.005 ppm mercury and 0.35 ppm selenium (Heinz and Hoffman 1998). A study of wild Adouin gulls found elevated mercury levels in eggs, but reproductive performance appeared unimpaired. Egg mercury levels averaged 5.06 ppm dry matter and egg selenium concentrations averaged 4.12 ppm. No correlation was found between mercury and selenium deposition. Eggs from New York Herring gulls were found to contain up to 1.47 ppm mercury and 4.038 ppm selenium (Morera *et al.* 1997). Again, no correlation between deposition of the elements was detected (Gochfeld 1997). Herring gull eggs collected from the upper Mississippi River area in another study were found to contain an average of 0.49 ppm mercury, with a range of 0.24 to 2.82 ppm, on a dry matter basis. Eggs contained an average of 3.00 ppm selenium with a range of 1.76 to 4.17 ppm (Custer *et al.* 1997). Eggs from red breasted mergansers successfully breeding near Lake Michigan were collected between 1977 and 1978. These eggs were found to contain, on average, 0.66 ppm selenium, wet weight, which is within normal limits for wild birds, and elevated mercury concentrations of 0.55 ppm. Mallard duck eggs, also collected from Lake Michigan, were found to contain 0.08 ppm mercury and 0.54 ppm selenium (Haseltine *et al.* 1981).

Table 1. Mercury levels in wild bird tissues and eggs.

Species	Location	Tissue	Hg Concentration (ppm)	Reference
Common loon	Wisconsin	liver	30 ww[a] 33 90	Gochfeld 1997
Common Loon emaciated healthy	Maritime Canada	kidney	 18.80 (mean) ww 3.07 (mean)	Daoust 1998
Common Loon	Lake Michigan	liver	0.25 ww	Coppock 1990
Common Loon males females	New England	liver	41.26 (mean) ww 56.060 45.900	Pokras 1998
Herring gull	New York	egg	1.47 ww	Gochfeld 1997
Herring gull	Minnesota, Mississippi, Illinois, Iowa	egg	0.49 (mean) dm[b]	Custer 1997
Common loon	Ontario	egg	0.26 (mean) ww	Barr 1986
Common loon	Ontario	egg	1.0 (ave.) wet	Frank 1983
Common loon	Saskatchewan	egg	0.35 (mean) ww	Haseltine 1983
Common loon	Wisconsin	egg	0.9 (mean) ww	Fox 1980

a. Wet weight
b. Dry matter

Table 2. Mercury and selenium concentrations in wild bird tissues.

Species	Location	Tissue	Mean Hg Concentration (ppm)		Mean Se Concentration (ppm)	Source
Common loon	East Canada	liver kidney	19.0 15.0	dm [a]	15.0 15.0	Scheuhammer 1998b
Common loon	Michigan	liver	3.03	ww [b]	2.88	O'Brien 1995
Common loon Adult Immature	New England	liver	 23.6 8.0	 ww	 11.5 3.6	Pokras 1991
Common loon	New Hampshire	egg	0.44	ww	0.42	Haseltine 1983
Adouin gull	Spain	egg	5.06		4.12	Morera 1997
Herring gull	New York	egg	1.47	dm	4.04	Morera 1997
Herring gull	Mississippi River	egg	0.49	dm	3.00	Custer 1997
Red breasted merganser	Lake Michigan	egg	0.52 0.51	ww	0.74 0.61	Haseltine 1981
Mallard	Lake Michigan	egg	0.08	ww	0.54	Haseltine 1981

a. Wet weight
b. Dry matter

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Chapter II

Mercury and Selenium Concentrations in Livers and Eggs of Common Loons (*Gavia immer*) from Minnesota

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Abstract

Mercury and selenium act as mutual antagonists in mature birds, but their toxicity is additive or synergistic in avian embryos and immature birds. Twenty eggs and livers from 18 mature and 9 immature loon carcasses found in Minnesota were collected. Livers and eggs were analyzed for mercury and selenium by atomic emission and absorption spectroscopy. Liver mercury concentrations were significantly higher in mature loons compared to those of immature loons and eggs. Liver selenium concentrations were

significantly higher in mature loons than those of immature loons, and selenium concentrations in immature loon livers were significantly higher than levels in eggs. There was a significant direct correlation between mercury and selenium in loon livers. There was a similar correlation between mercury and selenium in eggs.

Introduction

Mercury and selenium are important environmental contaminants (Lemly and Smith 1987, Thompson and Furness 1989). The environmental form of mercury, methylmercury, has been known to cause poisoning and decreased reproduction in wild birds and may be associated with population declines (Heinz and Lock 1975). Environmental mercury deposition has increased since preindustrial times (Swain *et al.* 1992).

Though an essential micronutrient, excess dietary selenium is toxic (Swain *et al.* 1992). Selenium toxicosis in wild birds is associated with reproductive effects and mortality. Selenium accumulation in wild birds has occurred in association with irrigation drain water (Heinz and Locke 1975).

Mercury and selenium accumulate in mammalian tissues in a molar ratio of one to one and there is evidence that both elements accumulate in a similar manner in some avian species (El-Begearmi *et al.* 1977, Yoneda and Suzuki 1997).

The two elements act as mutual antagonists in tissues of adult birds when both are present in the diet (El-Begearmi *et al.* 1977, Heinz and Hoffman 1998, Lourdes *et al.* 1991, O'Brien *et al.* 1995, Rudd *et al.* 1980, Scheuhammer *et al.* 1998b).

It has been reported that experimental and wild female birds ingesting high concentrations of mercury or selenium in the diet will excrete these elements into their eggs (Barr 1986, El-Begearmi *et al.* 1977, Heinz 1996, Heinz and Hoffman 1998, Lemly and Smith 1987). Evidence exists that birds consuming a diet high in both mercury and selenium will excrete both into their eggs. A few studies have shown that mercury and selenium have additive or synergistic toxic effects in immature or embryonic animals (Burge *et al.* 1976, Heinz and Hoffman 1998, Lourdes *et al.* 1991). In a recent study, female mallards on a diet containing elevated levels of mercury and selenium had decreased reproductive efficiency and embryo toxicity with teretogenesis (Heinz and Hoffman 1998).

Multiple studies have reported elevated mercury levels in tissues of loons and other wild birds (Daoust *et al.* 1998, Forrester *et al.* 1997, Meyer *et al.* 1995, Meyer *et al.* 1998 O'Brien *et al.* 1995, Stroud and Lang 1983, *et al*). Some studies have focused on mercury and selenium accumulation in tissues of common loons, and often have reported elevated levels of both elements (Augspurger *et al.* 1998, O'Brien *et al.* 1995, Pokras *et al.* 1991 Scheuhammer *et*

al. 1998). Mercury and selenium deposition in wild bird eggs, including those of merganser ducks, mallard ducks, and herring gulls, has also been investigated (Custer *et al*. 1997, Gochfeld 1997, Haseltine *et al*. 1981). No correlation has been found between egg mercury and egg selenium concentrations (Morera *et al*. 1997, Gochfeld 1997). This is the first study, to the author's knowledge, that looks at mercury and selenium in tissues from adult loons and in loon eggs from Minnesota.

The specific objectives of this study are: 1) to survey mercury and selenium levels in livers recovered from dead loons found in northern Minnesota, 2) to survey mercury and selenium levels in abandoned loon eggs found in northern Minnesota, 3) to determine if there is a correlation between mercury and selenium levels present in loon tissues, 4) to determine if there is a correlation between mercury and selenium levels present in loon eggs, 5) to determine if the tissue mercury and/or selenium levels in Minnesota loons are elevated, and 6) to determine if mercury and selenium levels in Minnesota loon eggs are elevated.

The liver was chosen for analysis in this study because it tends to store more total mercury than other avian tissues, with the exception of feathers (Barr 1986, Frank *et al*. 1983, Scheuhammer *et al*. 1998b). The mercury concentration in the liver may be ten-fold greater than the concentration in the major target organ, the brain (Barr 1986). Like mercury, selenium tends to concentrate in the

liver (Albers *et al.* 1996, Heinz *et al.* 1990, Heinz 1996, Ohlendorf *et al.* 1986, O'Toole, Raisebeck 1997).

Materials and Methods

Collection of Liver and Egg Samples

Twenty-seven common loons (*Gavia immer*) found dead in Minnesota during the years 1989 through 1995 were collected by the Minnesota Zoo in Apple Valley, Minnesota. The lake where each carcass was found was recorded. Loons were classified as mature or immature based on plumage. Immature loons included young chicks. Loons were necropsied to determine the cause of death. Fresh liver was saved for elemental analysis.

From 1997 through 1998, twenty loon eggs were found on abandoned or washed out nests and collected by the author or a team from Biodiversity, Inc. of Freeport, Maine. Eggshells were removed. If the embryo was readily visible, an attempt was made to determine it's age. Egg contents were emptied into I-Chem™ jars. Egg contents were homogenized in a Waring blender at 13,800 RPM and returned to I-Chem™ jars. Occasionally, egg content could not be homogenized sufficiently due to desiccation or large embryos. When necessary, distilled water was added to egg contents to increase the volume by up to 50 percent. One to two gram samples of each egg were weighed out and dried in a vacuum to determine dry matter content.

From 1997 through 1998, four loon carcasses and one egg recovered in Minnesota by the Minnesota Department of Natural Resources were sent to the Oklahoma Animal Disease Diagnostic Laboratory, Oklahoma State University, Stillwater, Oklahoma for analysis. The egg was treated in the same manner as previously noted. Carcasses were necrospied to determine the cause of death. Fresh liver was collected from each carcass for mercury and selenium analysis. One to two gram samples of each liver were dehydrated in a vacuum to determine percent dry matter.

Determination of Mercury and Selenium Concentrations in Liver and Egg by Atomic Absorption Spectrometery

Elemental analysis of loon tissues collected before 1996 were performed at the Animal Health Diagnostic Laboratory in East Lansing, Michigan using a Polyscan 61E® Inductively Coupled Argon-Plasma Emission Spectrometer (ICP) with a fixed cross-flow nebulizer under instrumental conditions described previously (Stowe et al 1985). Selenium analysis for loon eggs and tissue samples collected after 1996 were performed at Oklahoma Animal Disease Diagnostic Laboratory using a Perkin-Elmer Zeeman Graphite Furnace Atomic Absorption Spectrometer® (model 4110 ZL) at 196.0 nm. Mercury analysis for livers and eggs collected after 1996 were performed at Texas Veterinary Medical Diagnostic Laboratory in Amarillo, Texas on a ThermoJarrell®

Ash S11 Atomic Absorption Spectrophotometer-Cold Vapor Generator using single beam absorption at a wavelength of 253.7 nm.

Mercury and selenium concentrations for individual livers are presented in table 1. Mercury and selenium concentrations for individual eggs are presented in table 2.

ICP Analysis: One gram samples of liver were dried in a convection oven and weighed to the nearest 0.1 mg on an analytical balance. Dried tissues were placed in 15 mL screw-capped vials to which 2 ml of analytic grade concentrated (34 N) nitric acid was added. NISST bovine liver SRM 1577a (National Institute of Standards and Technology, Gaithersburg, MD) was placed in a similar flask and use as a control. Samples were digested overnight at 95°C. Samples were transferred to 10 mL volumetric flasks, to which 100 µg of yttrium in 1 mL 20% nitric acid was added as an internal standard, and diluted to volume with distilled water. Mercury and selenium concentrations were determined using ICP.

Graphite Furnace Atomic Absorption Spectrometry: **Approximately** 1 gram of each sample was weighed into an acid-washed Erlenmeyer flask. A blank flask was also prepared. Ten mL of 17N nitric acid and 2 mL of 1% nickel nitrate were added to each flask containing tissue and to the blank. Flasks were placed on hot plates set to low heat

(150 to 200°C) and allowed to digest for 4 or more hours. Tissue digests were filtered into a volumetric flask and diluted with distilled water to a volume of 50 mL. Standards containing 25, 50 and 100 ppb selenium were prepared from a selenium reference solution produced by Fisher Chemicals-Fisher Scientific. Aliquots of diluted digest and the blank were analyzed

Cold Vapor Atomic Absortion Spectrometry: Approximately 2 grams of each egg was placed in a royal blue capped Vacutainer® serum tube for trace element analysis to be transported to the Texas Veterinary Diagnostic Laboratory in Amarillo, Texas. Approximately 1 gram of each subsample was measured into an acid-washed quartz 250 mL volumetric digestion tube. A quality control tube containing 0.5 grams of dogfish liver certified reference material DOLT-2™ from the National Research Council in Canada was prepared. A second tube that contained no tissues was prepared as a blank. 20 mL of a solution of 10 N analytic grade nitric acid, 5 N analytic grade sulfuric acid, and 10% analytic grade perchloric acid was added to each tube. Two or three Teflon boiling chips were placed in each tube and digestion tubes were placed in a stainless steel tube rack and held at room temperature for four hours. Tubes were then heated at 50°C for 2 hours, 75°C for 1 hour, 110°C for one hour, 160°C for 2 hours, and 200°C for two hours. Each tissue digest, the quality control sample, and the blank was placed

in separate 150 mL reaction fleakers to which was added 20 mL of 1N hydrochloric acid. A stock solution containing 10 ppm mercury was diluted with distilled, deionized water to produce 0, 20, 40, 50, 80, and 100 ppb working standard solutions. These were used to generate a standard curve. Tissue samples, the quality control sample, and the blank were analyzed by cold vapor atomic absorption spectrometry.

Statistical Analysis of Data

Statistical analysis was performed using SAS® software. Means and standard deviations for mercury and selenium levels in liver and egg were computed. Linear regression was used to compare mercury and selenium levels independently in livers and eggs. Analysis of variance was used to determine significance of the correlation. Student t-test was performed to determine significant differences in metal concentrations between mature birds, immature birds, and eggs. Results were considered significant when $p < 0.05$.

Results

Gross Pathologic Findings

Eighteen mature and nine immature birds were examined. The cause of death was determined for 24 out of 27 loons collected in Minnesota (Table 1). Trauma was the leading cause of death, affecting 13 of 27 birds. Likely causes of

trauma included fighting among loons and gunshot wounds. Several loons died from ingesting fishing gear or becoming entangled in fishing line or nets (4/27). Three loons had no significant lesions.

Gross Examination of Egg Content

Visible embryos were present in 5 out of 20 eggs collected in Minnesota (Table 2). One embryo was determined to be approximately 22-days-old, two were 24-days-old, and two were 26-days-old.

Dry Matter Analysis of Liver and Egg

Loon livers contained 27.1% dry matter on average. Loon eggs contained an average of 22.8% dry matter.

Mercury Concentrations in Liver

The mean liver mercury concentration for all loon livers was 28.8 ppm with a standard error of 5.4 and range from 3.4 to 103.0 ppm dry matter. The mean liver mercury concentration for all mature loons was 39.3 ppm with a standard error of 29.2 and a range from 3.4 to 103.0 ppm. The mean concentration of mercury present in livers of immature loons was 8.0 ppm with a standard error of 1.9 and a range from 3.6 to 23.1 ppm (table 1).

Mercury Concentrations in Egg Content

The mean egg mercury concentration was 1.7 ppm with a standard error of 0.20 and a range from 0.38 to 3.73 ppm (table 2).

Comparison of Mercury Concentrations in Mature Liver, Immature Liver, and Egg Content

Mature loon liver contained significantly higher concentrations of mercury than immature loon liver ($p < 0.01$). Immature loon liver tended to contain a higher concentration of mercury than egg content, but the difference was not significant.

Selenium Concentrations in Liver

The mean liver selenium concentration for all loons was 20.3 ppm, dry matter, with a standard error of 2.7 and a range from 3.4 to 43.7 ppm. The mean liver selenium concentration for mature loons was 24.3 ppm with a standard error of 3.7 and a range from 3.4 to 43.7. The mean liver selenium concentration for immature loons was 12.4 ppm with a standard error of 0.8 ppm and a range from 7.8 to 15.0 ppm (table 1).

Selenium Concentrations in Egg Content

The mean egg selenium concentration was 1.91 ppm with a standard error of 0.22 and a range from 0 to 3.7 (table 2).

Comparison of Selenium Concentrations in Mature Liver, Immature Liver and Egg Content

Significant differences existed between selenium concentrations in mature loon livers, immature loon livers, and eggs ($p < 0.01$). Mature loons had the highest selenium concentrations, followed by immature loons. Eggs had the lowest selenium concentrations.

Mercury and Selenium Interactions

There was a significant ($p < 0.01$) direct linear correlation between mercury and selenium concentrations in loon livers (Figures 1 and 2). This correlation is represented by the following equation: $Hg = 0.31(Se) + 11.13$. A similar significant ($p < 0.01$) correlation between mercury and selenium existed in eggs (Figure 3). This correlation was represented by this equation: $Hg = -0.61(Se) + 2.94$. Mercury and selenium remain significantly correlated ($p < 0.01$) in combined liver and egg data (Figure 4). The equation becomes $Hg = -0.42(Se) + 5.16$ when the data is combined.

Table 1: Case number, year collected, age based on plumage, cause of death based on necropsy data, and liver mercury and selenium concentrations in ppm from each Minnesota common loon (*Gavia immer*).

No.	Year	Age & Location (Lake)	Location (lake)	Cause of Death	Liver Hg (ppm)	Liver Se (ppm)
1	1991	Mature	Big Trout	Trauma	19.4	43.7
2	1991	Mature	Agate	Unknown	24.4	16.9
3	1991	Mature	Cross	Trauma	22.7	17.4
4	1991	Immature	Pokegma	Gout	6.68	13.3
5	1991	Mature	Bass	Impaction	46.8	30.6
6	1991	Mature	Miltona	Emaciation	62.9	37.6
7	1991	Immature[a]	Lake John	Trauma	6.11	12.2
8	1991	Immature[a]	Lake John	Trauma	7.24	14.4
9	1989	Immature	Serpent	Emaciation	7.54	15.0
10	1991	Immature	Mille Lacs	Trauma	7.27	14.2
11	1991	Mature	Mille Lacs	Lead poisoning	30.9	25.9
12	1991	Mature	Rush	Trauma	7.14	14.2
13	1991	Mature	Bay	Trauma	80.6	29.5
14	1991	Mature	Mink	Trauma	103.0	68.1
15	1990	Immature	Rush	Air sacculitis	5.18	11.6
16	1991	Mature	Leech	Drowned	13.5	11.9
17	1991	Mature	Cass	Trauma	26.8	28.6
18	1993	Mature	Horseshoe	Trauma, aspergillosis	24.6	15.0
19	1993	Mature Upper Mission	Upper Mission	Trauma, parasites	13.2	14.2
20	1993	Mature	Unknown	Trauma	53.1	40.6
21	1990	Immature	Partridge	Gastroenteritis	23.1	13.3
22	1990	Immature	Turtle River	Unknown	4.84	9.68
23	1990	Immature	Turtle River	Metabolic bone disease	3.59	7.81
24	1997	Mature	Clarks	Emaciation (fishing line)	90.23	11.70
25	1997	Mature	Pokegma	Unknown	55.19	7.956
26	1998	Mature	Deer	Emaciation (fishing line)	29.23	19.439
27	1998	Mature	Little Moose	Trauma	3.448	3.422

a. Denotes a chick.

Table 2: Case number, year of collection, location, and mercury and selenium concentration for each Mionnesota commmon loon (*Gavia immer*) egg.

Number	Year	Location/ID	Mercury (ppm)	Selenium (ppm)
1	1997	Echo Trail, Ed Shave	0.476	3.117
2	1997	Echo Trail, Fenske	0.615	1.900
3	1997	South of GR, Moulton[b]	1.516	2.608
4	1997	Kabatogama, Ek's Bay	2.307	0.526
5	1997	Echo Trail, Kabustasa	0.883	2.261
6	1997	Echo Trail, Kabustasa	0.672	2.349
7	1997	Vermillion Lake[c]	1.384	3.682
8	1997	Sandpoint, Grassy Bay #1	0.969	1.109
9	1997	Kabatogama, Ek's Bay[b]	1.860	1.432
1	1997	Deer River[a]	1.542	2.547
11	1997	Namakan, Blind Pig Island	1.552	2.942
12	1997	Bass Lake, Big Island	1.929	1.786
13	1997	Lake Kabatogama, Mukooda	2.402	0.000
14	1997	Sandpoint, Grassy Bay	3.141	0.810
15	1997	Sandpoint, Mukooda	3.732	0.478
16	1998	Dunning[c]	2.169	2.683
17	1998	Echo	0.382	2.068
18	1998	Echo	2.879	1.423
19	1998	Kabatogama	1.4512	1.884
20	1998	Kabetogama	1.911	2.659

a. 22 day old embryo b. 24 day old embryo b. 26 day old embryo

Figure 1: Mercury concentration vs. selenium concentration in 18 mature Minnesota common loon (*Gavia immer*) livers. Mean mercury concentration = 39.3 ppm, standard error = 29.2. Mean selenium concentration = 24.3 ppm, standard error = 3.7. Mercury concentrations greater than 10 ppm may be associated with reduced reproductive efficiency in some avian species, and levels greater than 18 ppm may be associated with neurologic lesions. Selenium concentrations greater than 10 ppm may be associated with reproductive deficiencies in some avian species.

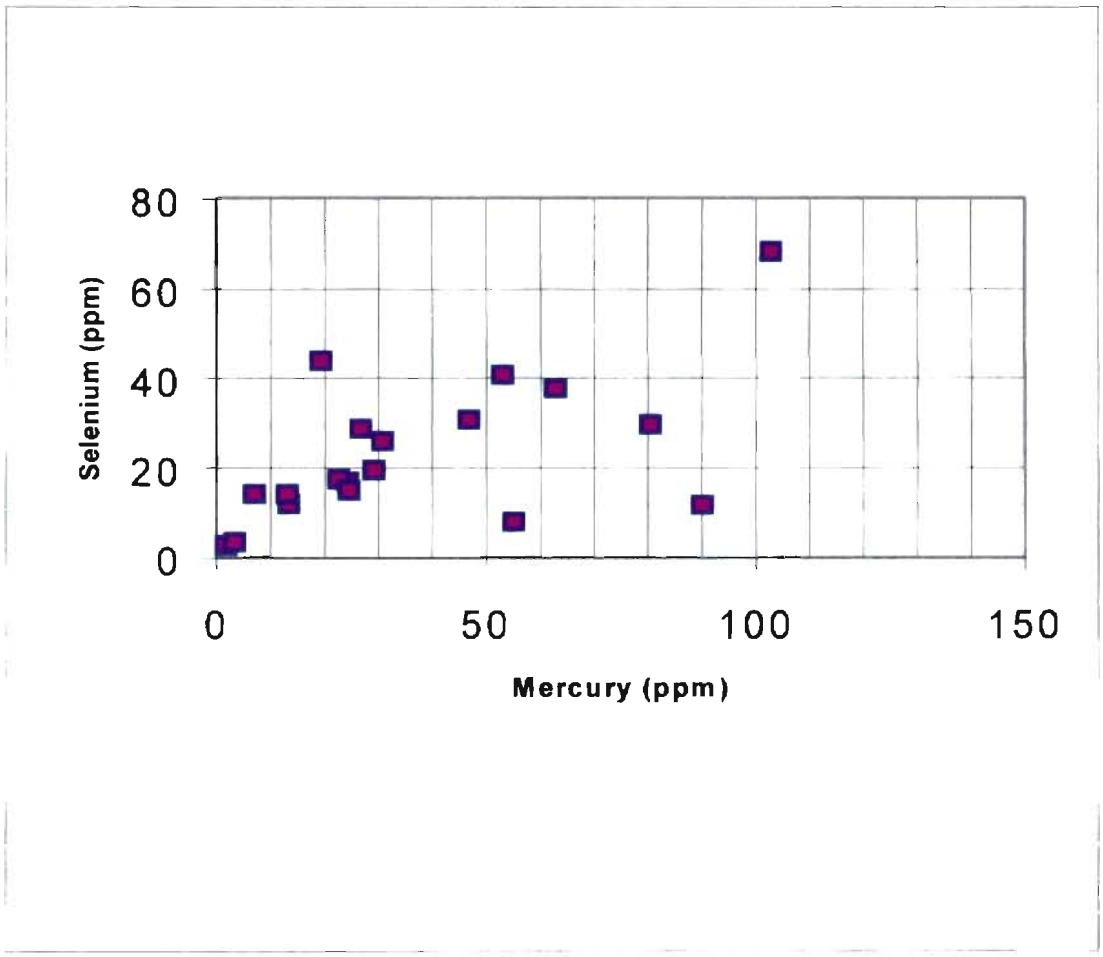


Figure 2: Mercury concentrations vs. selenium concentrations in 9 immature Minnesota common loon (*Gavia immer*) livers. Mean mercury concentration = 8.0 ppm, standard error = 1.9. Mean selenium concentration = 12.4 ppm, standard error = 0.8. Mercury concentrations greater than 18 ppm may be associated with neurologic lesions in some avian species.

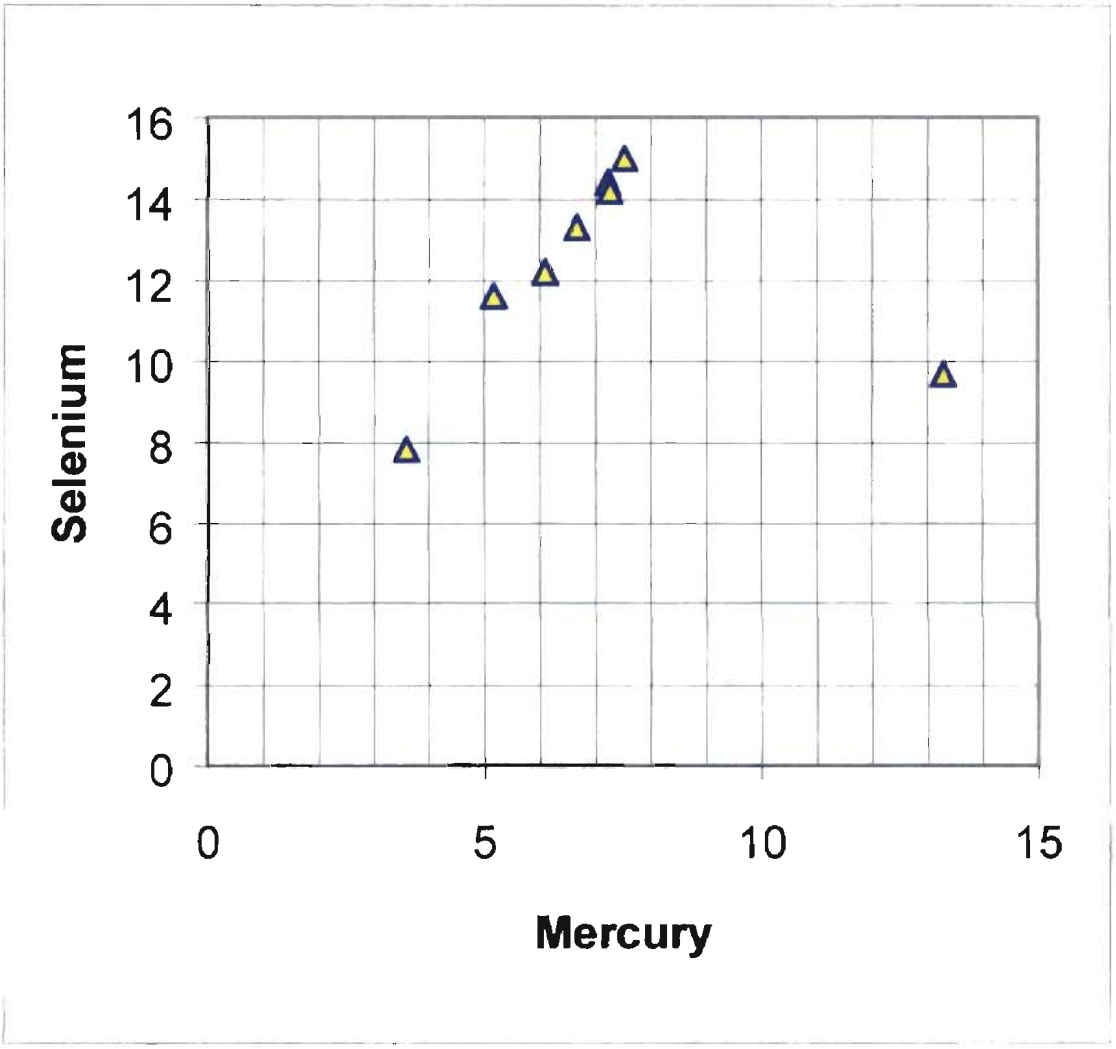
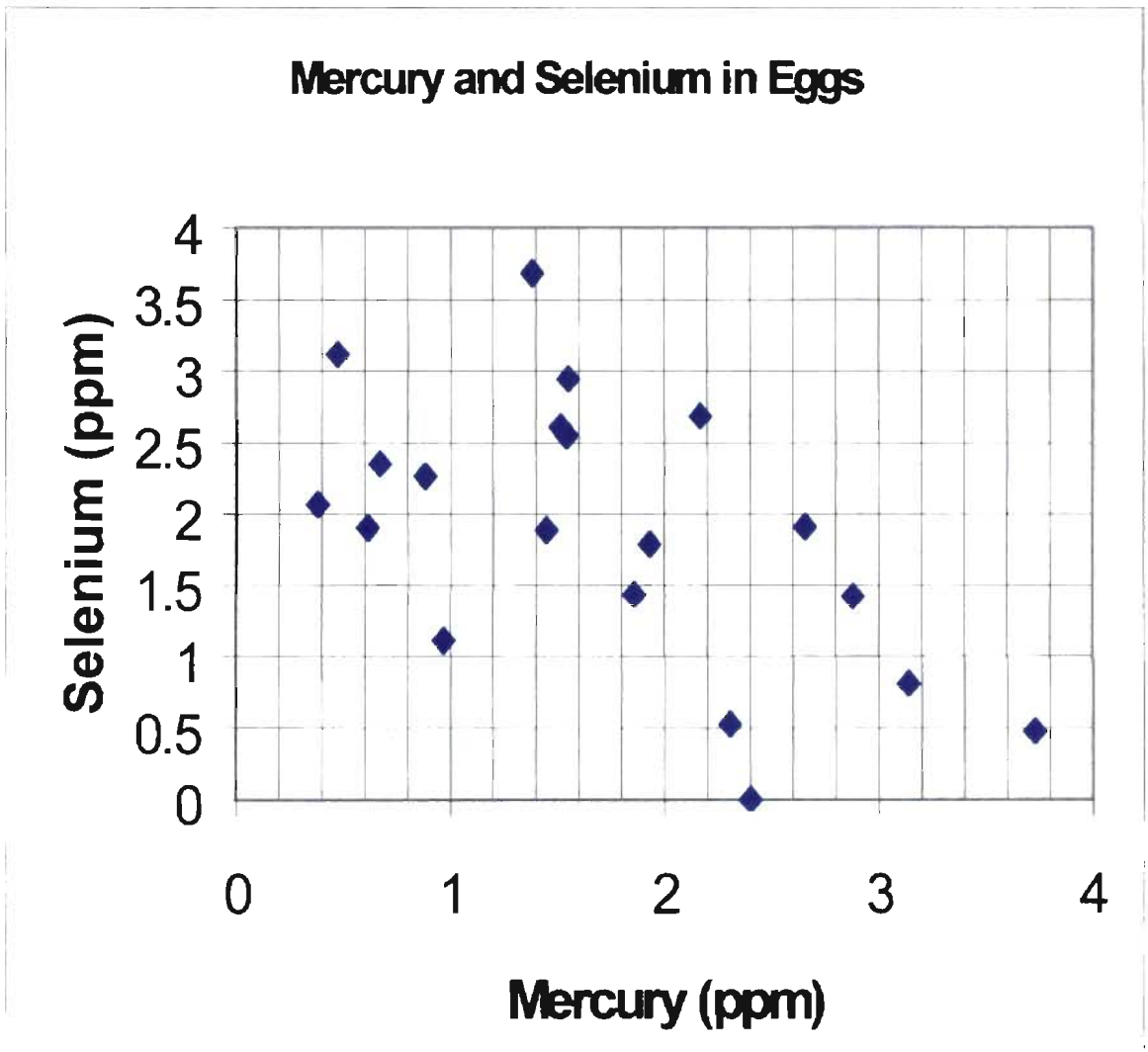
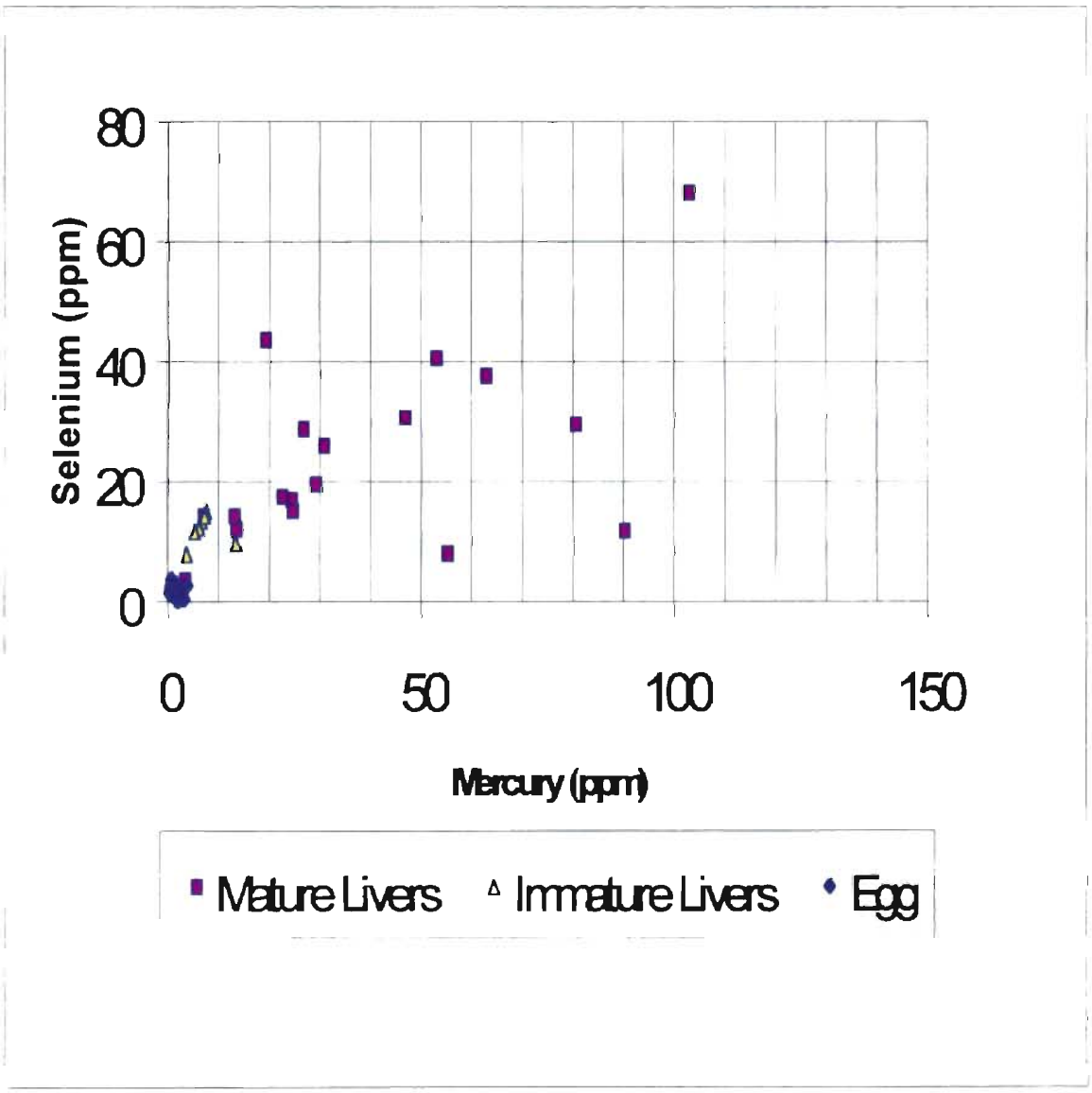


Figure 3: Mercury concentration vs. selenium concentration in the content of 20 Minnesota common loon (*Gavia immer*) eggs. Mean mercury concentration = 1.7 ppm, standard error = 0.2. Egg mercury concentrations exceeding 2 ppm and selenium concentrations exceeding 13 ppm may be associated with embryotoxicity.



Mean selenium concentration = 1.9, standard error = 0.2.

Figure 4: Mercury concentration vs. selenium concentration in 18 mature common loon (*Gavia immer*) livers, 9 immature loon livers, and 20 loon eggs. Mercury concentrations greater than 10 ppm in livers and 2 ppm in eggs may be of toxicologic significance. Selenium concentrations exceeding 10 ppm in liver and 13 ppm in egg may be of toxicologic significance.



Discussion

Mercury Concentrations in Loon Liver

The mean liver mercury concentration for adult loons in this study was 39.3 ppm, dry matter. One study of east Canadian loons reported a mean liver mercury concentration of 19.0 ppm on a dry matter basis (Scheuhammer *et al.* 1998b). The wet weight mean mercury concentration for mature loons was approximately 10.6 ppm. Several studies found liver mercury concentrations in loons that were much higher than this. Gochfeld (1997) reported common loon liver mercury concentrations of 30, 33, and 90 ppm wet weight in Wisconsin. Pokras *et al.* (1998) reported mean liver mercury concentrations of 41.26 ppm in common loons from New England. Other studies have found lower loon liver mercury levels. A study of common loons from Lake Michigan found one loon with a liver mercury concentration of 0.25 ppm (Coppock *et al.* 1990). Haseltine *et al.* (1983) studied loons from New Hampshire and found a mean liver mercury concentration of 0.44 ppm, and O'Brien *et al.* (1995) found a mean of 3.03 ppm mercury in livers of Michigan loons. Minnesota loons appear to have relatively moderate liver mercury concentrations. No previous studies have looked specifically at livers of loons from Minnesota.

Liver mercury concentrations from mature loons in this study were approximately double the 5 ppm liver mercury associated with clinical signs in experimentally poisoned

mallards (Pass *et al.* 1975) and triple the 3 ppm level known to cause reproductive deficiencies in female pheasants (Barr *et al.* 1986). However, many aquatic birds are able to concentrate higher levels of mercury in their livers. Albatross with liver mercury levels in excess of 1000 ppm (0.1%) dry matter were apparently healthy (Thompson and Furness 1989). As total mercury concentrations in marine birds increase, the proportion of methylmercury, a more bioactive form of mercury, decreases and the proportion of less biologically active inorganic mercury increases (Scheuhammer *et al.* 1998b, Thompson and Furness 1989). Mercury in common loon liver is mostly in the inorganic form (Daoust *et al.* 1998). The reason for this is unknown, but some aquatic birds may be able to reduce the toxicity of mercury by demethylating it for storage (Evers *et al.* 1998). Selenium may also be involved in the detoxification of mercury (Forrester *et al.* 1997, Meyer *et al.* 1995). This mechanism will be discussed later.

A significant difference was detected between liver mercury concentrations in mature versus immature loons. The mean liver mercury concentration for mature loons was nearly five times that of immature loons. Liver mercury concentrations for mature and immature birds in this study were approximately equal to 10.66 ppm and 2.17 ppm, respectively, on a wet weight basis. This agrees with a previous report by Pokras *et al.* (1991), which found a mean liver mercury concentrations in mature loons 23.6 ppm

compared to immature loons 8.0 ppm, on a wet weight basis. Burger *et al.* (1994) reported that feathers from mature loons contained approximately twice as much mercury as feathers from immature loons. Other studies have also found higher tissue mercury levels in mature compared to immature common loons (Frank *et al.* 1983, Evers *et al.* 1998). Causes for this difference in mercury accumulation have been suggested. Mature birds are larger and, thus, eat larger fish, which are likely to contain higher levels of mercury. Furthermore, mature birds have had more time to accumulate mercury. Lastly, immature loons feed near the nesting site, whereas mature loons may retain mercury from their overwintering site (Burger *et al.* 1994).

Mercury Concentrations in Egg Content

We chose to analyze egg contents in this study because early life stages may be more sensitive to mercury than mature stages (Barr 1986, Meyer *et al.* 1998). Most mercury deposited in eggs is present in the egg content rather than the shell (Morera *et al.* 1997). The mean wet weight mercury concentration for loon egg content in this study was 0.39 ppm. This egg mercury concentration is consistent with the literature. Barr (1986) reported a mean mercury level of 0.26 ppm wet weight from eggs recovered from Lake Ontario, and Frank *et al.* (1983) reported a mean mercury concentration of 1.0 ppm wet weight for Ontario loon eggs. Haseltine *et al.* (1983) analyzed loon eggs recovered in

Saskatchewan and found a mean mercury concentration of 0.35 ppm wet weight. Fox *et al.* (1980) reported a mean of 0.9 ppm mercury, wet weight, in loon eggs from Wisconsin. Few eggs in this study contained mercury above concentrations associated with decreased hatchability. Previous reports state that waterfowl and tern eggs containing more than 1 ppm mercury wet weight and pheasant eggs with 0.5 ppm have decreased hatching success. (Barr 1986, Custer *et al.* 1997, Haseltine *et al.* 1983).

Mercury concentrations present in egg contents were significantly lower than those present in livers of mature birds but not significantly different from liver mercury concentrations in immature birds. Female birds sequester mercury in their eggs (Barr 1986, Evers *et al.* 1998, Burger *et al.* 1994, Becker 1992, El-Begearmi *et al.* 1977, Gochfeld 1997, Heinz and Hoffman 1998, Pokras *et al.* 1991). Barr (1986) reported that the loon egg mercury concentrations correlate significantly with maternal brain mercury concentrations. However, in this study, the mean egg mercury concentration was more than ten-fold lower than the mean mature loon liver mercury concentration. It is possible that liver mercury was not mobilized prior to egg production. This would suggest that only dietary mercury is deposited into the egg. Morera *et al.* (1976) have speculated that mercury levels in Adouin's gull eggs reflect mercury contamination in the nesting area, suggesting that mercury stored in the tissues may not influence egg concentrations.

Since loons in this study were probably unrelated to eggs tested it is also possible that female birds that have accumulated high liver concentrations of mercury do not produce many eggs. Lastly, liver is a single tissue known to concentrate mercury, whereas egg content is analogous to a whole organism. It would be expected that a whole organism would have a lower overall mercury concentration than a particular tissue that stores mercury.

Selenium Concentrations in Loon Liver

The mean selenium concentration for mature loon livers was approximately 6.6 ppm wet weight. Unlike mercury, selenium is an essential micronutrient, thus, it is expected to be present in the tissues. Results from this study fall within the reported normal range of selenium concentrations in avian liver, between 2 and 16 ppm, wet weight (Hoffman and Heinz 1988, O'Toole and Raisebeck 1997). A study by Ohlendorf *et al.* (1990) found that stilts from areas not polluted with selenium containing agricultural wastewater had liver selenium levels below 10 ppm wet weight. However, some mature loon liver selenium concentrations exceed the 7.4 ppm wet weight reported to be associated with adverse effects. Most mature loons had liver mercury levels greater than 3 ppm wet weight reported to cause a decrease in reproductive success in some species (Heinz *et al.* 1990, Heinz 1996). Liver selenium concentrations in this study were moderate compared to those detected by researchers

studying loons in other areas. Scheuhammer *et al.* (1998b) found a mean liver selenium concentration of 15 ppm, dry matter, in loons from eastern Canada. Loons recovered during a die-off in North Carolina had a mean liver selenium concentration of 10.4 ppm wet weight (Augspurger *et al.* 1998). Other researchers have also reported elevated selenium concentrations in aquatic birds (Albers *et al.* 1996, O'Tool and Raisebeck 1997). O'Brien *et al.*(1995) reported a mean liver mercury concentration of only 2.88 ppm wet weight in Michigan loons.

Loons may be able to sequester elevated liver selenium concentrations without toxic effects. Selenium and mercury are mutually antagonistic, therefore selenium may be involved in mercury detoxification, as will be discussed in detail later (Haseltine *et al.* 1981, Heinz and Hoffman 1998, Lourdes *et al.* 1991, O'Brien *et al.* 1995, Pokras *et al.* 1991, Rudd *et al.* 1980).

Immature loons had significantly lower liver selenium concentrations than did mature loons. The mean liver selenium concentration for immature loon livers was approximately half that of mature loon livers. This result is similar to the findings reported by Pokras *et al.* (1991), found mean selenium concentrations of 11.5 ppm in mature loons and 3.6 ppm in immature loons, wet weight. Perhaps mature birds have had more time to accumulate selenium than immature birds.

Selenium Concentrations in Loon Egg

Eggs were analyzed for selenium because selenium, especially the organic form, selenomethionine, is known to accumulate in eggs (Albers *et al.* 1996, Heinz *et al.* 1990, Heinz 1996, Hoffman and Heinz 1988, Ohlendorf *et al.* 1986, Ohlendorf *et al.* 1990,). The mean loon egg selenium concentration in this study was a approximately 0.4 ppm, wet weight. Few previous studies have looked at the concentration of selenium in loon eggs. Haseltine *et al.* (1983) also found a mean selenium concentration of 0.4 ppm, wet weight, in loon eggs from New Hampshire.

The concentration of selenium in loon egg content was significantly lower than its concentration in mature loon liver, the difference being more than ten-fold. The egg selenium concentration was also significantly lower than that of immature loon liver. Similar to mercury, possible explanations for low selenium levels in eggs include inability to mobilize selenium from the liver during egg production and the reduced ability of female loons with elevated liver selenium concentrations to produce eggs. Lastly, liver is a storage site for selenium in an organism, whereas an egg is analogous to a whole organism, therefore liver would be expected to have higher selenium levels than egg content.

Mercury and Selenium Interactions in Loon Liver

There was a significant linear correlation between liver mercury and selenium concentrations in all loon livers. Mercury and selenium are believed to be mutually antagonistic in mature birds when present together in the diet (El-Begearmi *et al.* 1977, Heinz and Hoffman 1998, Lourdes *et al.* 1991, O'Brien *et al.* 1998, Rudd *et al.* 1980, Scheuhammer *et al.* 1998b). The mechanism for this interaction in birds has not been fully elucidated. Selenium and mercury have been shown to bind in an equimolar ratio to a plasma protein in rats (Yoneda and Suzuki 1996). Indeed, many species are known to accumulate tissue mercury and selenium in approximately a 1:1 molar ratio, (equal to a 2.5:1 weight ratio,) including rats, humans, seals, some fish, and quail (El-Begearmi *et al.* 1977, Lourdes *et al.* 1991, Yoneda and Suzuki 1996). Though a linear correlation was detected, the ratio of liver mercury to selenium was not determined in the present study.

Mercury and Selenium Interactions in Loon Egg

This study found a significant correlation between mercury and selenium concentrations in loon eggs. This is unlike previous studies of Adouin gull eggs by Morera *et al.* (1997) and herring gull eggs (Gochfeld 1997). However, both mercury and selenium concentrations in loon eggs were significantly lower than concentrations in mature loon

livers. Mean concentrations of mercury and selenium were lower in eggs than in mature livers by factors of more than ten. It is unfortunate that, due to the opportunistic method of collection, maternal liver mercury and selenium could not be directly compared to egg mercury and selenium. This would have allowed for a better estimate of how much of the maternal mercury and selenium load is mobilized during egg production and deposited into egg content.

Mercury and selenium interactions in egg content is of particular interest because, though mutually antagonistic in mature birds, mercury and selenium may act in an additive or synergistic manner in embryonic birds and hatchlings. Immature birds may not be able to metabolize selenium and mercury in the same way as mature birds (Burge *et al.* 1976, Heinz and Hoffman 1998, Lourdes *et al.* 1991). Female birds can sequester both mercury and selenium in eggs (Albers *et al.* 1996, Barr 1986, Becker 1992, Burger *et al.* 1994, Evers *et al.* 1998, Gochfeld *1997,* Heinz *et al.* 1990, Heinz 1996, Ohlendorf *et al.* 1986). Heinz and Hoffman (1998), in a recent study, fed female breeding mallard ducks diets containing elevated concentrations of mercury at a level of 10 ppm as methylmercury, selenium at a concentration of 10 ppm as selenomethionine, or both. Eggs produced by the females had a decrease in hatchability similar to that caused by elevated dietary methylmercury alone. The level of teratogenic effects detected in the embryos was significantly higher than that caused by mercury or selenium

alone. Certain teratogenic effects were only present when female mallards were fed both methylmercury and selenomethionine. However, El-Begearmi *et al.* (1977) fed diets containing elevated levels of methylmercury and selenite to breeding female quail and found that egg hatchability was greater than for breeding quail fed either selenite or methylmercury alone. The different results may be caused by species differences or differences in the form of selenium used (Heinz and Hoffman 1998). Since mercury and selenium concentrations were not usually elevated in Minnesota loon eggs analyzed in this study, it seems unlikely that mercury and selenium interaction would cause embryotoxicity.

Summary

Liver mercury concentrations were elevated in adult loons. This finding agrees with many previous studies. It was expected that immature birds would have lower mercury concentrations than mature birds because mature birds eat larger fish and have had more time to accumulate mercury. Egg mercury levels were lower than mature liver concentrations by more than ten-fold. This suggests that either mature female loons are not excreting mercury from tissues into eggs, or that mature females with high liver mercury concentrations do not lay eggs. However, one would expect a tissue which concentrates mercury such as the liver

to have a higher mercury concentration than an egg, which may, for our purposes, be looked at as a whole organism.

Liver selenium concentrations were also elevated in adult loons. Immature loons had significantly lower selenium concentrations compared to mature loons, but significantly higher concentrations than eggs. Again, eggs had less than one tenth the amount of mercury as mature loon livers. Reasons could be similar to those given for mercury: inability to mobilize liver selenium into eggs, inability of females with high liver selenium levels to produce eggs, and difference between concentrations in a specific tissue versus a `whole organism.'

Mercury and selenium concentrations correlated in a linear manner in loon livers and also in loon eggs. This correlation was expected in the livers. Mercury and selenium are reported to accumulate in animal tissues in a 1:1 molar ratio. This is believed to be part of a mercury detoxification mechanism, but it is not fully understood. However, previous reports did not find a correlation between mercury and selenium concentrations in eggs of wild birds.

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Chapter 3

Summary and Conclusions

Introduction

The purpose of this study was to compare mercury and selenium concentrations in livers and eggs of loons from Minnesota. Mercury and selenium are known to accumulate in a 1:1 ratio in some avian species. Both elements are present in high concentrations in the liver and female birds may excrete them in eggs. Though mercury and selenium are antagonistic toxins in mature birds, they may behave in an additive or synergistic manner in embryo and immature birds. This study compared mercury and selenium levels in loon livers and loon eggs, detected a correlation between mercury and selenium in loon livers and eggs, and found that mercury and selenium concentrations were elevated in Minnesota common loons, but not in eggs.

Results

The mean liver mercury level was 28.8 ppm dry matter for mature and immature loons, 39.3 ppm for mature loons, 8.0 ppm for immature loons, and 1.7 ppm for eggs. The mean mature liver mercury concentration was significantly higher

than that of immature loons and eggs, however, immature liver and egg levels were not significantly different.

The mean selenium concentration was 20.3 ppm dry matter for all livers, 24.3 ppm for mature livers, 12.4 ppm for immature livers, and 1.91 ppm for eggs. Mature livers had a significantly higher mean selenium concentration than immature livers, which had a significantly higher concentration than eggs.

There was a significant linear correlation between liver mercury and selenium concentrations. Mercury and selenium concentrations in eggs also correlated significantly.

Conclusions

Liver mercury concentrations were elevated in adult loons. This finding agrees with many previous studies. It was expected that immature birds would have lower mercury concentrations than mature birds because mature birds eat larger fish and have had more time to accumulate mercury. Egg mercury levels were lower than mature liver concentrations by more than ten-fold. This suggests that either mature female loons are not excreting mercury from tissues into eggs, or that mature females with high liver mercury concentrations do not lay eggs.

Liver selenium concentrations were also elevated in adult loons. Immature loons had significantly lower selenium concentrations compared to mature loons, but

significantly higher concentrations than eggs. Again, eggs had less than one-tenth the amount of mercury as mature loon livers. Reasons could be similar to those given for mercury: inability to mobilize liver selenium into eggs and inability of females with high liver selenium levels to produce eggs.

Mercury and selenium concentrations correlated in a linear manner in loon livers and also in loon eggs. This correlation was expected in the livers. Mercury and selenium tend to accumulate in animal tissues in a 1:1 molar ratio. This is believed to be part of a mercury detoxification mechanism, but it is not fully understood. However, previous reports did not find a correlation between mercury and selenium concentrations in eggs of wild birds.

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