2009

EFFECTS OF DIETARY SELENIUM ON THE HEALTH AND SURVIVAL OF WINTERING LESSER SCAUP

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EFFECTS OF DIETARY SELENIUM ON THE HEALTH AND SURVIVAL OF WINTERING LESSER SCAUP

(Spine title: Effects of Selenium on Health and Survival of Lesser Scaup)
(Thesis Format: Monograph)

by

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Graduate Program in Biology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

School of Graduate and Postdoctoral Studies
The University of Western Ontario

ABSTRACT

The acquisition and accumulation of selenium (Se) in the Lower Great Lakes (LGL) could be contributing to the continental decline of lesser and greater scaup (*Aytha affinis, A. marila*). I exposed lesser scaup to background (0.8 μg/g), moderate (8.1 μg/g) and high (20.7 μg/g) levels of dietary Se, measured survival rates and several indices of health in relation to hepatic Se concentrations. There was 100% survival of scaup exposed to Se for 10-weeks (staging duration) but birds in the high treatment group had lower lipid reserves. There was 93% survival after 23-weeks (wintering duration), but no differences among treatment groups in body composition or body mass. There were no effects of Se on indices of oxidative stress and cell-mediated immunity, but immuno-stimulatory effects on antibody production. Elevated Se in scaup on the LGL do not likely impact overall health and survival, and future research efforts should focus on alternative hypotheses for the scaup decline.
Keywords: Scaup, contaminants, Se, selenium, staging, wintering, health, survival

AKNOWLEGMENTS

Funding for this research study was provided by Long Point Waterfowl (LPW), the Metals in the Human Environment Strategic Network, the Waterfowl Research Foundation, and Ducks Unlimited Canada. I would like to thank my supervisor, Scott Petrie for this opportunity, his insight, encouragement, hunting opportunities and his humor. I would also like to thank my co-supervisor Bob Bailey for his statistical support, and simplistic way of explaining the world of stats to me, as well as those on my UWO advisory committee, Dr. Chris Guglielmo, Dr. Jack Millar, and Dr. Sheila Macfie. Many thanks to those on my LPW scientific advisory committee, including Dr. Shannon Badzinski, Dr. Ken Abraham, Dr. Dave Ankney, Darrell Dennis, Dr. George Finney, Dr. Mark Gloutney, and Shawn Meyer. I'd like to thank Dr. Bob Clarke, Kirsty Gurney, and Steve Leach for including me in the collection of the scaup eggs, housing, and invaluable insight. I would also like to thank Dr. Anne Fairbrother for her endless knowledge and guidance of the avian immune system, and Dr. Jean-Michelle DeVink for providing me with his insight, opinions, and suggestions on the ins and outs of captive projects.

I am forever grateful for the help, advice and practical jokes of Glenn Howe. You provided me with a place to keep my scaup (and myself), endowed endless information on the proper way to raise & maintain a healthy flock of ducks, and provided me with the best tasting strawberries and squash in town. I would also like to thank the rest of the Howe family, including Monica, Ryan, Jill, Rick, and Kevin

for incorporating me into their family during and after my field season, and the countless delicious dinners (Monica).

I am grateful for the students in my lab, Dave Messmer, Rob Baden, Jeremy Stempka and Robin Churchill, their dogs Lance and Rusty, and other LPW employees, Kate England, Ted Barney and Jim Cook for the hours put in for pen construction, capture, sampling and euthanization of my ducks, as well as your company and humor (most of the time). I can't express how much I enjoyed working with a group of people who share the same passion for hunting and the outdoors. Thanks to my Western friends Katie Stammler, Sonja Teichert, Alissa Bults, Dara Orbach, and Kate England for their female companionship and support during the past two years. Lastly I'd like to thank my friends and family for their continued support and encouragement throughout this process.

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CHAPTER 1. INTRODUCTION

1.1 Scaup population status

The North American Waterfowl Management Plan (NAWMP) is an international action plan designed to conserve migratory birds throughout the continent. NAWMP population objectives were established for several North American waterfowl species to aid in setting sustainable harvest limits and conservation strategies. During the 1980s, several North American duck populations declined, presumably due to poor water conditions on breeding areas. Although several waterfowl populations rebounded following the drought, the combined continental greater (*Aythya marila*) and lesser (*A. affinis*) scaup (hereafter scaup) population continued to decline markedly between the mid-1980s and late 1990s (Figure 1.1) (Afton and Anderson 2001). The 2009 scaup population estimate (4.2 ± 0.2 million) was 18% below the long-term average (U.S. Fish & Wildlife Service 2009) and 33% below the NAWMP population goal. Several hypotheses have been proposed to explain the scaup decline, such as the loss and degradation of breeding habitat, changes in food resources, and increased contaminant burdens (Austin et al. 2000). My study focused on several key aspects relating to the contaminant hypothesis.

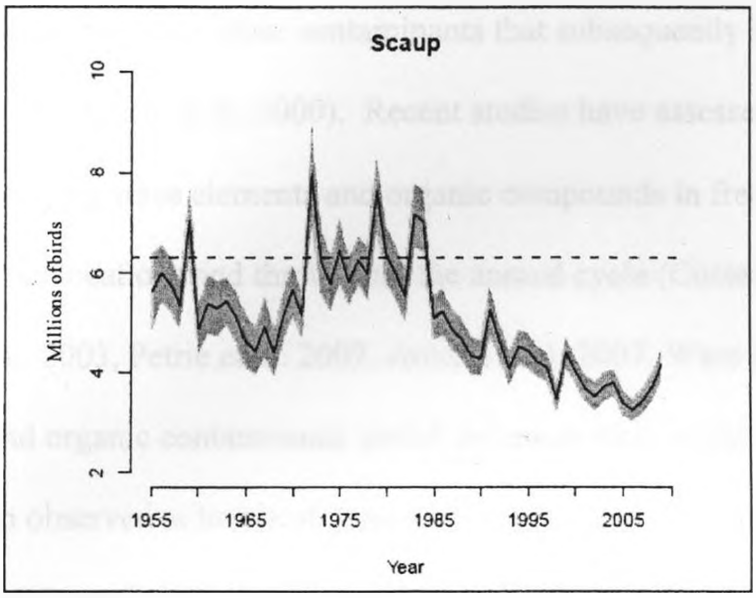

Figure 1.1 Breeding population estimates, 95% confidence intervals, and North American Waterfowl Management Plan population goal (dashed line) for Greater and Lesser Scaup in the traditional survey area; strata 1-18, 20-50, 75-77 (U.S Fish and Wildlife Service 2009).

1.2 The contaminant hypothesis

The contaminant hypothesis states that non-breeding scaup are acquiring levels of trace elements and/or other contaminants that subsequently impact survival and/or reproduction (Austin et al. 2000). Recent studies have assessed hepatic concentrations of many trace elements and organic compounds in free-ranging scaup collected at various locations and throughout the annual cycle (Custer and Custer 2000, Custer et al. 2003, Petrie et al. 2007, Anteau et al. 2007, Ware 2008). Of all the trace elements and organic contaminants tested, selenium (Se) is one that has consistently been observed at levels of potential concern in staging and wintering scaup (Custer and Custer 2000, Custer et al. 2003, Petrie et al. 2007, Ware 2008), with consistent reports of elevated Se in scaup from the Lower Great Lakes (LGL). Therefore, the contaminant hypothesis was of particular interest with respect to Se burdens in scaup that stage and winter on the LGL.

1.3 Scaup use of the Lower Great Lakes

The LGL (including lakes Ontario, Erie and St. Clair as well as the Niagara, Detroit and St. Clair Rivers) have an abundance and diversity of wetland complexes (lacustrine and palustrine) that provide staging and wintering habitat for migratory birds (Prince et al. 1992, Petrie 1998). Historically, the LGL has been used by large numbers of staging and moderate numbers of wintering scaup (Bellrose 1976, Petrie and Knapton 1999, Badzinski and Petrie 2006a). However, with a trend towards annually decreasing ice cover (Assel and Rodionov 1998), and increased food supply (Custer and Custer 1996, Petrie and Knapton 1999), scaup distribution, abundance

and diets have changed substantially over the past 20 years (Custer and Custer 1996, Petrie and Knapton 1999, Badzinski and Petrie 2006a, CWS & LPW unpublished data).

Non-native zebra (*Dreissena polymorpha*) and quagga (*D. bugensis*) mussels (hereafter dreissenid mussels) were introduced to the LGL in the 1980s and 1990s, respectively, and reached substantial densities (Neary and Leach 1992, Kovalak et al. 1993, Leach 1993). This novel, readily available food source permitted scaup to shift from native foods to a diet dominated by dreissenid mussels (Custer and Custer 1996, Petrie and Knapton 1999, Badzinski and Petrie 2006a). Since dreissenid mussels have become prevalent there also has been a substantial increase in the number of scaup staging and wintering on the LGL in some years (Wormington and Leach 1992, Petrie and Knapton 1999, Petrie and Schummer 2002, CWS & LPW unpublished data), despite the continental population decline (Allen et al. 1999, Wilkins et al. 2005). For example, waterfowl days (each day a bird spends in the given area) for scaup in the Long Point area on Lake Erie increased from 38,500 in 1986, before dreissenid mussel colonization, to 3.5 million in 1997 (Petrie and Knapton 1999). Therefore, scaup have altered their diets and their staging and wintering durations and distributions in response to dreissenid mussel colonization of the LGL.

Dreissenid mussels filter large quantities of water, zooplankton, and phytoplankton, and can thus accumulate contaminants in their tissues (deKnock and Bowmer 1993). This filtering capacity makes dreissenid mussels efficient at bio-accumulating Se, especially if Se is elevated in the aquatic/pelagic environment (Mills et al. 1993). High levels of Se have been detected in dreissenid mussels from

the LGL and in molluscivorous waterfowl, suggesting that the mussels facilitate the bioaccumulation and trophic transfer of Se (Custer and Custer 2000, Kwan et al. 2003, Petrie et al. 2007). Little is known, however, regarding specific threshold concentrations or the impacts of Se acquisition on staging and wintering scaup.

1.4 Selenium

Selenium is a naturally occurring trace element, which can also occur as an environmental pollutant due to anthropogenic activity (Ohlendorf 2003, Lemely 2004). Specific sources of Se input into the LGL have not been widely documented, but in general, excess Se in the environment can occur due to smelting of ore, burning of fossil fuels, and run-off from agriculture and industry (Ohlendorf 2003). The Great Lakes region (GLR) is highly populated and industrialized (Manninen 1993), and human activities such as power generation (coal mining and combustion), nickel mining, metal smelting, transportation, and agricultural practices likely contribute to increased Se in the watershed.

Selenium is an essential semi-metallic trace element that is required for normal body function by birds in small concentrations but can become toxic at elevated concentrations (Heinz 1996, Irwin et al. 1997). High Se burdens have been associated with increased mortality (Heinz and Fitzgerald 1993a), impaired reproduction (Heinz et al. 1989, Heinz and Hoffman 1998), suppressed immune system function (Fairbrother and Fowles 1990, Hoffman 2002, Spallholz and Hoffman 2002), oxidative stress (Custer et al. 2000, Hoffman 2002), and reduced body condition (Takekawa et al. 2002, Franson et al. 2007) in waterfowl.

Although Se occurs in several different chemical forms, most of the laboratory data used to derive threshold concentrations have been based on the toxic form, selenomethionine, believed to be the most bio-available Se species to wildlife. Based on studies of captive mallards (*Anas platyrhynchos*), hepatic Se concentrations ≥10 μg/g (unless otherwise stated, concentrations are reported on a dry tissue weight [dw] basis) can cause reproductive impairment (e.g., deformities of embryos and hatching failure), concentrations ≥33 μg/g can cause sub-lethal effects (e.g., decreased body weight, and histopathological lesions), and concentrations exceeding 60 μg/g can cause adult and juvenile mortality (Heinz et al. 1989, Heinz and Fitzgerald 1993a, b, Heinz 1996). However, these thresholds may be unsuitable for scaup because Se tolerance can vary among waterfowl species (Skorupa 1998). For instance, scaup may be less sensitive to Se than mallards because they normally winter in areas and eat foods that contain higher Se concentrations (Haygarth 1994, Ohlendorf 2003).

1.5 Selenium burdens in scaup

1.5.1 Breeding areas

Reproductive success is a more sensitive endpoint to Se exposure than is the health and survival of young and adult birds (Heinz 1996). For instance, elevated Se burdens in aquatic birds have been associated with embryotoxicity, teratogenesis, and reduced offspring growth and survival (Heinz et al. 1987, Ohlendorf et al. 1988, Heinz et al. 1989, Hoffman 2002). However, studies conducted on pre-breeding and breeding scaup reveal that Se is near background levels (Fox et al. 2005, DeVink 2007, Badzinski et al. 2009) suggesting that elevated Se burdens acquired on winter and staging areas may be depurated prior to ovulation (Heinz and Fitzgerald 1993b,

DeVink 2007, DeVink et al. 2008). Furthermore, Badzinski et al. (2009) found no evidence for an effect of Se on the ability of female scaup to initiate rapid follicle growth. Reduced burdens in breeding birds might be attributed to the relatively short half-life of Se in waterfowl (18.7 days in liver tissue of mallards, Heinz et al. 1990) and the fact that there can be a considerable amount of time between departure from the GLR and arrival on breeding areas (LPW, unpublished data). Currently there is no evidence suggesting that scaup reproduction is being impacted by Se (Fox et al. 2005, DeVink et al. 2007, DeVink et al. 2008, Badzinski et al. 2009). However, it is not known if Se acquisition is compromising scaup survival or condition prior to arrival on breeding areas (Anteau et al. 2007, DeVink 2007, Ware 2008). If Se is adversely impacting non-breeding scaup, then it is likely to be most problematic for birds that spend prolonged periods of time in a contaminated area, such as scaup that winter on the LGL.

1.5.2 Staging and wintering areas

Several studies have assessed Se burdens in non-breeding scaup across North America, including the Pacific and Mississippi flyways and the GLR (Hothem et al. 1998, Custer and Custer 2000, Takekawa et al. 2002, Custer et. al. 2003, Petrie et al. 2007). Normal liver Se levels for birds living in freshwater habitats range from 4.0-10.0 μg/g (Ohlendorf 1989), while 95% of wintering and fall staging scaup sampled from lakes Erie, St. Clair, and Michigan in 1991-1993 had elevated Se concentrations ($\geq$10 μg/g), with a mean liver concentration of 21.7 μg/g (Custer and Custer 2000). A subsequent study showed that 75% of spring collected lesser scaup had Se levels $\geq$10 μg/g, with Se values ranging from 7.40 – 59.7 μg/g, and a mean liver concentration of

22.6 μg/g (Petrie et al. 2007). Petrie et al. (2007) also found a correlation between hepatic Se levels and collection date, suggesting that spring migrant lesser scaup were acquiring elevated Se burdens while on the LGL. Hepatic Se levels in scaup collected between November 1999 and May 2000 from sites throughout the Mississippi flyway ranged from 4.23 – 10.7 μg/g (Custer et al. 2003). Lastly, Se concentrations in scaup from the San Francisco Bay region increased from 20.7 μg/g in early winter to 35.5 μg/g in late winter (Hothem et al. 1998), and ranged from 3.5-11.9 μg/g at other sites along the coast of California (Takekawa et al. 2002).

These studies suggest that hepatic Se burdens in scaup vary regionally within North America. GLR scaup had higher Se burdens than Mississippi flyway birds, possibly due to increased bioaccumulation and trophic transfer via introduced dreissenid mussels. Birds sampled from the GLR had Se burdens comparable to those from the San Francisco Bay area, which also have a diet dominated by filter-feeding bivalves, such as *Potamocorbula amurensis* and *Macoma balthica* (Hothem et al. 1998, Poulton et al. 2002). It appears that scaup that stage and winter in areas with a combination of high human population density (which would infer a high anthropogenic Se input) and the availability of filter-feeding bivalves tend to have higher hepatic Se concentrations.

1.5.3 Depuration of selenium

Waterfowl acquire Se quickly once exposed to elevated dietary concentrations, but can depurate Se to background levels after leaving a contaminated area (Heinz et al. 1990). Depuration rates likely differ interspecifically as well as by tissue type within species. For example, Heinz et al. (1990) calculated Se

accumulation and elimination rates in mallards fed 10 μg/g selenomethionine for 6-weeks and found the trace element was assimilated and depurated more quickly in the liver than in muscle tissue. DeVink (2007) calculated half-life to be 16 and 22 days for blood Se in lesser scaup fed 15 and 7.5 μg/g, respectively, a slower depuration rate than the 9.8-day half-life reported for blood of captive mallards by Heinz et al. (1990). However, this variation could have been due to differences in diet concentrations.

1.6 Sub-lethal health effects

Chronic health effects from Se exposure have been documented in several bird species, including the American coot (*Fulica americana*), pied-billed grebe (*Podilymbus podiceps*), common moorhen (*Gallinula chloropus*), American kestrel (*Falco sparverius*), common eider (*Somateria mollissima*), and mallard (Ohlendorf et al. 1988, Hoffman et al. 1991, Heinz and Fitzgerald 1993a, Albers et al. 1996, Yamamoto et al. 2000, Franson et al. 2007). Adult birds with Se poisoning can exhibit various conditions such as oxidative stress, hepatic lesions, abnormal feather loss, and immune function impairment (Heinz et al. 1987, Ohlendorf et al. 1988, Heinz et al. 1989, Hoffman 2002), but can often show no signs other than emaciation or weight loss (O'Toole and Raisbeck 1997).

1.6.1 Body condition

Body condition is a general indicator of health in birds (Heinz and Fitzgeral 1993a, Debacker et a. 2000, Spalding et al. 2000, Yamamoto and Santolo 2000, Takekawa et al. 2000), and is an important determinant of fitness and survival

(Bergan and Smith 1993, Newton 2004). Selenium can affect body condition of waterfowl by reducing organ and body mass (including fat and protein stores), damaging organs, and altering metabolism (Ohlendorf et al. 1988, Albers et al. 1996, Heinz and Hoffman 1998, Hoffman 2002, Franson et al. 2007). Therefore, if Se burdens negatively affect body condition of staging and/or wintering scaup, it is possible that overall health, survival or future reproductive output could be compromised.

1.6.2 Oxidative stress

Oxidative stress is the condition of increased oxidant production in animal cells and is characterized by the release of free radicals, resulting in cellular damage. Oxidative stress has been associated with contaminant toxicity in aquatic animals and birds (DiGiulio et al. 1989, Custer et al. 2000, Hoffman 2002, Franson et al. 2007) and can be quantified by measuring levels of antioxidants such as glutathionine (GSH) (LaBoeuf et al. 1985, Ganther 1986) and endpoints of lipid peroxidation (LPO) such as malondialdehyde (MDA) (Hoffman et al. 1989, Hoffman et al. 1991, Hoffman 2002). Increases in antioxidant activity and increased LPO due to consumption of dietary Se have been associated with negative health effects in waterfowl (Hoffman et al. 1989, Hoffman et al. 1991), including diminished immune function, histopathological lesions and reduced growth in ducklings (Hoffman et al. 1989, Fairbrother and Fowles 1990, Hoffman 2002). Thus, monitoring cellular damage by measuring oxidative stress may be useful in identifying Se effects on overall health of scaup.

1.6.3 Avian immune system

The avian immune system is comprised of two interacting components: nonspecific immunity and specific immunity. Specific immunity refers to immune responses that are inducible, are specific to particular antigens, and have memory (Fairbrother et al. 2004). Specific immunity is further subdivided into two components - cell-mediated immunity (CMI) and humoral (or antibody)-mediated immunity (HMI). Cell mediated immunity acts through the development and proliferation of T-cells, which regulate the function of HMI as well as nonspecific immune responses by either enhancing or suppressing the immune response (Fairbrother et al. 2004).

The HMI response is characterized by the production of antibodies by B cells. Birds have three types of antibodies, primary (IgM), secondary (IgG), and mucosal (IgA) antibodies. This study focused on IgM and IgG antibodies, which animals mount in response to foreign antigens, such as sheep red blood cells (SRBC). Naive B cells produce IgM antibodies when a foreign antigen is first encountered, while at the same time producing memory B cells. When the same protein antigen is encountered again (eg. booster shot), these memory B cells proliferate and differentiate producing large quantities of IgG. Depending on function, location within the body, and time course of infection, birds employ different types of antibodies (Fairbrother et al. 2004).

Both high Se exposure and Se deficiency are known to impair the immune system. While low levels of Se (that are nutritionally adequate) tend to enhance immune function response (Kiremidjian-Schumacher and Stotzky 1987), excessive

amounts may lead to undesirable immunological effects. Measuring immune system responses can provide early warning signals of toxic effects of metals (Weeks et al. 1992), as well as aid in overall assessment of scaup health, because changes in immune response can affect susceptibility to disease.

1.7 Study approach & objectives

Effects of long-term exposure to elevated Se burdens are currently unknown, but it is plausible that birds that stage and winter on the LGL are being adversely impacted by a 2-6 month exposure to this trace element. A previous study of greater scaup wintering on the LGL during 2006 and 2007 showed that birds had elevated Se burdens (10-32 μg/g) but were not emaciated and did not show external or internal signs of Se poisoning (Ware 2008). Even though 99% of the scaup collected by Ware (2008) had hepatic Se levels above 10 μg/g, only 6% had levels above the 33 µg/g threshold that is associated with sub-lethal effects in mallards (Heinz 1996). Therefore, if Ware's (2008) sample of birds was representative of scaup wintering on the LGL, it is unlikely that Se is impacting their health or survival. However if Se levels $\geq$33 μg/g adversely affects health or survival of scaup, as in captive mallards, then birds with high Se burdens may be under-represented in field collections (e.g., Ware 2008). Therefore a captive study was justified to examine the effects of Se on the health and survival of scaup in a controlled setting.

Currently there are no data for: 1) hepatic Se thresholds for scaup (i.e., is it possible for them to acquire and survive with levels above 33 μg/g), and 2) effects of Se burdens on the health and body condition of scaup. Therefore, I exposed birds to dietary Se levels similar to, and much higher than, those found in mussels on the LGL

for periods simulating scaup staging and wintering duration on the LGL. My objectives were to determine survival rates and effects of Se-dosing on wild-strain lesser scaup in captivity using several indicators of health (e.g. body condition, oxidative stress, immune function). I hypothesized that scaup which acquired Se levels above background concentrations would experience reduced health and survival in comparison to control birds. I predicted that if Se affects the overall health of scaup there would be a negative relationship between hepatic Se concentrations and body condition, nutrient reserves, immune response, and survival, but conversely, a positive relationship between hepatic Se and indices of oxidative stress. Results from this study will help determine if the Se hypothesis is plausible and as such, if more scientific effort should be focused on this hypothesis or alternative hypotheses for the scaup decline.

CHAPTER 2. METHODS

2.1 Study design

I investigated the effects of Se on the health and survival of captive lesser scaup exposed to a background concentration (0.19 μg/g), a dose similar to the maximum reported concentration in zebra mussels from the Lower Great Lakes (LGL) (11.5 μg/g) (U.S. Department of Commerce 2007), and an extreme dose (23.0 μg/g). Duration of exposure to treated diets was based on maximum time scaup spend staging (8-12 weeks) or wintering (up to 24 weeks) on the LGL. This study took place at the Glenn Howe captive facility south of Aylmer, Ontario (42.7379°, -81.0104°), and was approved by the University of Western Ontario's Animal Care & Use Committee (protocol #2008-044-03).

2.1.1 Egg collection

A total of 70 eggs were collected from 43 lesser scaup nests at Waterhen Marsh, Saskatchewan (N 52.83 and W -105.03) between 8 and 17 of July 2008 (Canadian Wildlife Service permit # CWS08-S003). Upon collection, eggs were uniquely marked and placed in an incubator until hatched; hatch dates ranged from 17 July - 2 August 2008. The hatch rate of collected eggs was 79%, with a total of 55 eggs hatching. Six ducklings died from an *E. coli* infection, leaving 49 surviving ducklings for the study. Once ducklings reached 60-70 days old, they were crated and flown to Toronto, Ontario, along with nine after-hatch-year lesser scaup for a total of 58 birds. Prior to the onset of the study, four hatch year ducks died; two drowned due to nasal disc entanglement, and two were diagnosed with pulmonary

congestion and edema; neither bird had evidence of an infectious process or disease. At the onset of the study there were a total of 54 scaup.

2.1.2 Captive facility, treatment groups and exposure durations

Birds were housed in outdoor pens approved by University of Western Ontario's Animal Care Committee, south of Aylmer, Ontario (protocol #2008-044-03). Pen dimensions were ~30.5 by 30.5 meters and enclosed with chain-linked fencing and netting. Each pen was sod-lined, with an in-ground fresh water pond and a rain-protected food dish. Birds were randomly assigned to control or one of two Se enriched diet treatment groups (control, 0.19 μg/g; moderate, 11.5 μg/g; high, 23.0 μg/g), as well as to staging (10-weeks) and wintering (23-weeks) exposure durations. The treatments for both durations began 3 November 2008 and ended 9 January and 9 April 2009, for the staging and wintering durations, respectively.

2.1.3 Diet preparation

Selenomethionine is proposed to be the main form of Se available to wildlife (Heinz 1996), and is the form that was used to enrich waterfowl diets in this study. During a three-week acclimation period, birds were fed untreated Mazuri Sea Duck Food. At commencement of the study scaup were fed *ad libitum* Mazuri Sea Duck Food, which was treated with either distilled water (control, $n = 18$) or one of two Se treatments targeted to increase diet concentrations to 11.5 µg/g (moderate, $n = 17$) and 23.0 µg/g (high, $n = 19$). To increase Se concentrations to desired levels in each treatment diet, the feed was first ground from pellets into crumbs, then L-selenomethionine was dissolved in distilled water and sprayed directly onto the food. The food was then re-pelleted and dried at the Arkell feed mill in Guelph, Ontario.

The concentration applied for the 23.0 μg/g diet was 1456.6 mg L-Selenomethionine in 0.5 L distilled H_2O per 25 kg bag of food. Half this concentration was applied for the 11.5 μg/g diet. I calculated dietary Se concentration values based on the weight of the food and the amount of water I used to dissolve the selenomethionine.

MW Se = 78.96 g/mol
MW Selenomethionine = 196.1 g
25 kg food + 0.5kg water = 25.5 kg (food/water)
23.0 mg/kg (trt) × 25.5 kg = 586.5 mg Se
586.5 × (196.1/78.96) = 1456.6 mg L-Selenomethionine
Note: mg/kg = μg/g

Se analyses, conducted by the Central Analytical Facility in the Department of Chemistry and Biochemistry at Laurentian University, indicated treatment concentrations were 0.84 ± 0.06 μg/g (n = 23), 8.08 ± 0.92 μg/g (n = 23), and 20.66 μg/g ± 2.92 (n = 22) for control, moderate and high treatment groups respectively. Treatment groups were observed daily to ensure birds were eating treated diets, and although it was not possible to record individual food consumption, group intake was measured as frequency of refills with a 750 g scoop. Food samples were collected and frozen on a weekly basis, and analyzed for Se concentration to determine if Se content in the food remained constant over time.

2.2 Hepatic Se concentrations

2.2.1 Monitoring and collection of samples

Birds were euthanized on their predetermined endpoints (day 67 and day 158), by cervical dislocation (protocol #2008-044-03) and were then double bagged and frozen. Prior to dissection, birds were partially thawed, and ovaries collected. Also, if present, approximately 0.25-1.0 g of pinfeathers were plucked from the breast

regions (ovary and feather samples were collected for the 23-week duration groups only). This was done to examine Se allocation in reproductive tissues, as well as depuration that may have been occurring during feather molt. Right and left liver lobes were separated, bagged and frozen for future analysis. The right lobe of each liver, pinfeather samples, and food samples were packed in dry ice and sent to the Central Analytical Facility in the Department of Chemistry and Biochemistry at Laurentian University for Se analysis. The left lobe was sent to the Ecotoxicology laboratory in the Department of Biological Sciences at the University of Lethbridge and was used to assess oxidative stress endpoints.

2.2.2 Biological sample preparation & Se determination

Each liver, ovary, and feed sample was prepared by first freeze-drying at -15°C. Liver and ovary samples were then ground into a fine powder using a mortar and pestle, while feed samples were ground using a laboratory blender and mortar and pestle. Feather samples were first washed and sonicated three times for five minutes in 200 mL detergent (1-2 drops of Sunlight dish soap per 200 mL water) and deionized water (trace metal free), then rinsed twice with deionized water for five minutes to remove any dirt or impurities. The clean feathers were then dried in an oven at 70°C and cut into small pieces with scissors before being ground to a fine powder.

Teflon digestion vials for all biological samples were washed in concentrated HNO_3, rinsed with deionized water, and then dried in an oven to ensure sterility. A subsample of 0.2 g of each liver, feed and feather sample was placed on weighing paper and transferred to a Teflon vial. Then, 2.0 mL of 30% (w/w) analytical reagent

grade H_2O_2 and 8.0 mL of concentrated (15 M) analytical reagent grade HNO_3 were added to each vial and carefully mixed. The lid of each vial was securely fastened and put into a Milestone ETHOS microwave oven with an HPR-1000/10 rotor.

Total Se in samples was measured by hydride generation atomic fluorescence spectrometry using a PSA 10.055 Millennium Excalibur. The machinery was equipped with a continuous flow hydride generation system and a boosted discharge hollow cathode Se lamp as the radiation source of the atomic fluorescence detector. Contaminant concentrations were calculated as μg/g (dw) and the detection limit for Se was 1.00 μg/g.

2.2.3 Quality assurance

Reference materials and blank reagents were analyzed once every eight samples for quality control. Both DOLT-2 (dogfish liver certified reference material 6.06 ± 0.49 μg/g) and DORM-2 (dogfish muscle certified reference material 1.40 ± 0.09 μg/g) were used as standard reference material for liver and pinfeather samples. DORM-2 alone was used for feed samples; recovery from all reference materials was within the certified variation range (National Research Council Canada 2009).

2.3 Body condition

2.3.1 Body mass and survival

Survival of individual scaup was recorded daily during feeding and general maintenance of the pens. All birds were weighed when treatments started and after 4, 6, 7, 10, 13, 15, 17, 18, 19, 20, 21, 22, and 23 weeks. Birds were caught with dip nets and weighed to the nearest 0.01 g with a Mettler-Toledo digital scale.

2.3.2 Dissections and carcass analysis

Prior to dissection, birds were partially thawed, and inspected for any external abnormalities that could be associated with chronic Se exposure (sloughed or broken claws, bill abnormalities and plumage abnormalities/damage). Then the following structural measurements were taken using digital calipers (± 0.01 mm): 1) body length – taken from the tip of the bill to the base of the longest retrix, 2) culmen – taken from the tip of the bill to the feather line at base of the bill, 3) skull width – taken from behind eyes, 4) bill width – taken at the widest point, 5) total tarsus, and 6) keel length – taken from top of the breast to the base of the breast (internal). During dissections, the liver, kidneys, heart, pancreas, and gastrointestinal tract were examined and weighed to the nearest 0.01 g, wet weight (ww).

Body condition was determined by proximate analysis (total lipid and protein content). Fat extraction and protein determination was conducted at the Avian Energetics Laboratory in Port Rowan, Ontario; methods followed Badzinski and Petrie 2006b. Frozen, dissected, ingesta-free carcasses (with feathers) were weighed to the nearest 0.01 g on a Mettler-Toledo digital scale (CARCASS WET), chopped into approximately 2.5 cm chunks with a hatchet and re-weighed (HOMOGENATE WET). Chopped birds were dried in an oven to a constant mass (HOMOGENATE DRY) at approximately 80° C. This took roughly one week, during which carcass pieces were rotated and stirred to promote even drying. Dried pieces were ground to a fine powder using a blender followed by a coffee grinder. Finally, the homogenate was put through a 1.5 mm sieve. Carcass dry mass (CARCASS DRY) was determined using the equation:

CARCASS DRY= (HOMOGENATE DRY / HOMOGENATE WET) × CARCASS WET

To measure lipid content, a 10 g sample of the dry, ground homogenate was placed into a pre-weighed and dried cellulose thimble and dried in an oven to a constant mass (SUBSAMPLE). Fat was extracted from each sample using petroleum ether in a modified Soxhlet apparatus. After fat extraction, thimbles with the lean sample were dried to a constant mass and re-weighed (LEAN SUBSAMPLE). Total lipid content (CARCASS FAT) was calculated using the equation:

CARCASS FAT =

[(SUBSAMPLE – LEAN SUBSAMPLE) / (SUBSAMPLE)] × CARCASS DRY

Each lean dry sample was then placed in a dry, pre-weighed Coors porcelain crucible, weighed, and placed in a muffle furnace at 550°C overnight. The resulting ash was then re-weighed (SUBSAMPLE ASH). Total protein was determined using the following calculations:

CARCASS LEAN DRY= CARCASS DRY – CARCASS FAT DRY

TOTAL ASH = (SUBSAMPLE ASH / LEAN SUBSAMPLE) × CARCASS LEAN DRY

TOTAL PROTEIN = CARCASS LEAN DRY – TOTAL ASH

2.4 Oxidative stress in liver tissues

2.4.1 Sample collection and preparation

Frozen left liver lobes were sent to the Ecotoxicology laboratory in the Department of Biological Sciences at the University of Lethbridge to quantify

oxidative stress. Oxidative stress was assessed by measuring malondialdehyde (MDA) concentration - an endpoint of lipid peroxidation (LPO). Antioxidant capacity was determined by measuring glutathionine (GSH) (Miller et al. 2007). A portion of the liver was homogenized in 10 volumes of 50 mM potassium phosphate buffer with 1 mM EDTA, pH 7.4 for GSH, and LPO determination. Metaphosphoric acid was added to a portion of the homogenate for GSH analysis and butylated hydroxytoluene (BHT) was added to a portion of the homogenate for LPO analysis. Homogenates were centrifuged at 4°C at 3270 *g* for 20 minutes. The resulting supernatants were removed and used for analysis. GSH was assayed within one hour, and supernatants for LPO were kept at −80°C for a maximum of two days before analysis.

2.4.2 Malondialdehyde analyses and determination

Lipid peroxidation was determined using the Bioxytech LPO-596 kit (Catalogue #21012) purchased from Medicorp (Montreal, Quebec). The kit measured the reaction of MDA and 4-hydroxyalkenals (4-HA), end products of the LPO process, with *n*-methyl-2-phenylindole at 45°C and 586 nm. Lipid peroxidation was expressed as μM/mg protein, where one unit was one μM MDA and 4-HA.

2.4.3 Glutathione analyses and determination

Glutathionine was determined using the Bioxytech GSH 400 kit (Catalogue #21011) purchased from Medicorp (Montreal, Quebec). Glutathionine forms a thioether that reacts with a reagent forming a thione, which was measured on a UV–vis absorption spectrophotometer at 400 nm. Glutathionine was expressed as μM GSH/mg protein.

2.5 Immune system challenges

2.5.1 Cell mediated immunity & phytohemagglutinin skin test

Scaup were tested for T-cell-mediated immunity using the phytohemagglutinin-P (PHA-P) skin test. When injected into the skin, PHA stimulates T-lymphocytes to proliferate and differentiate, resulting in an inflammatory response that can be measured (Stadecker et al. 1977). On day 66 (staging) and day 144 (wintering) the thickness of the right wing web was measured to the nearest 0.01 mm with a pressure sensitive micrometer. Then 0.1 mL PHA-P in phosphate buffered solution (PBS) (1 mg/mL) was injected intradermally into the right wing web, while PBS alone (0.1 mL) was injected into the left wing web. The wing web was measured 24 hours later and the response to PHA-P was calculated as the difference between the pre- and post-injection measurements. The response of the test was calculated as follows:

$$(RW_{\text{post-injection}} - RW_{\text{pre-injection}})$$

$$- \quad (LW_{\text{post-injection}} - LW_{\text{pre-injection}})$$

Where RW and LW refer to PHA-P injected right wing web, PBS injected left wing web (Fairbrother and Fowles 1990).

2.5.2 Humoral mediated immunity & protein antigen inoculations

Scaup were tested for anti-body-mediated immunity using sheep red blood cell (SRBC) inoculations. The SRBCs act as a foreign protein antigen once intravenously injected into the bird, thereby triggering antibody production. The amount of antibodies produced in response can be measured in blood plasma. Three blood samples were taken to quantify baseline (pre-SRBC injection), primary (post

SRBC injection #1), and secondary (post SRBC injection #2) antibody response. Blood was collected on day 40, 47 and 67 (10-week duration), and day 124, 131 and 145 (23-week duration).

Scaup were inoculated intravenously (brachial vein) with 1mL of 10% SRBC for every 1 kg of body weight. Blood was drawn from the brachial vein with a 23-gauge heparinized needle, centrifuged and the plasma frozen within six hours of collection. Antibodies (IgG and IgM) produced in response to SRBC injections were collected in plasma samples and titrated for hemagglutination; methods followed Wayland et al. 2002.

In the lab, plasma samples were thawed and heated in a water bath at 56°C for 30 minutes to inactivate complement. Fifty microliters of PBS was added to each U-shaped well of 96-well plastic microtiter plates. After samples were heated, 50 μL plasma was added in duplicate across the entire first row of microtiter wells. Serial two-fold dilutions were carried out by transferring 50 μL from well to well down each column. The last 50 μl was discarded and the highest dilution was 1/256. Plates were then incubated at 37°C for one hour. Fifty microliters of 1% SRBC in saline solution was then added to each well in a row, covered and left overnight at room temperature.

Presence of antibodies was indicated by the lattice pattern that SRBCs form at the bottom of the well. When there are no antibodies present, the SRBC collect at the base of the well, making a "button" shape. The highest dilution that causes agglutination, or the lattice pattern (absence of a button) was the reported endpoint (Fairbrother and Fowles 1990, Wayland et al. 2002). I scored dilution ratios by using the inverse of the highest dilution achieved in the titer by each individual (e.g. if an

individual had enough antibodies in their plasma to achieve agglutination at a dilution of 1/128, then their antibody score was recorded as 128).

2.6 Statistical analyses

With the exception of the body mass trend evaluation, all analyses (body composition, organ mass, oxidative stress, immune challenges, pinfeather and hepatic Se concentration) were separated by exposure duration (10-week vs. 23-week). Therefore PERIOD was not included in these models. Two birds died during the course of the study and were excluded in all analyses. I used a significance level of $p \leq 0.05$ for all tests.

2.6.1 Selenium concentrations in biological samples

Se concentrations in biological samples were compared for each treatment group using a one-way Analysis of Variance (ANOVA). I performed three separate ANOVAs to test whether concentrations of Se in scaup livers, pinfeathers, and ovaries varied among treatment groups (Se diet treatments: control [0.84 ± 0.06 μg/g], moderate [8.08 ± 0.92 μg/g] and high [20.66 ± 2.92 μg/g]); among-group differences were identified in a step-wise manner using a post hoc Tukey's test (SPSS 16.0). The pattern of among-group differences in liver Se concentrations (see section 3.1) provided support that differences among treatment groups (TRT_GROUP) in subsequent analyses were due to acquired Se burdens. Thus, I used TRT_GROUP instead of individual Se concentrations as an independent variable in all subsequent analyses, with the exception of the PHA and oxidative stress linear regression analysis.

2.6.2 Trends in body mass

Body mass was measured over time for each individual, and analyzed using a factorial repeated measures ANOVA in SAS 9.0. The model included treatment group, date, gender, and all two-way and three-way interactions (Y [mass] = TRT_GROUP, DATE (ordinal), GENDER (male, female), TRT_GROUP × GENDER, TRT_GROUP × DATE, GENDER × DATE, TRT_GROUP × DATE × GENDER). Individual birds were nested within treatment groups to account for repeated mass measurements, and band numbers were assigned to individuals in the model to account for random effects. On weigh-in dates where groups differed significantly, I compared among-group variations in a step-wise manner using a post hoc Tukey's test (SPSS 16.0)

2.6.3 Body composition and organ mass

Principal components analysis was used to account for individual differences in body size using the following variables in the correlation matrix: tarsus length, head length, skull width, culmen, keel, and body length (PC-ORD 4.0). The PC1 scores were then used as a covariate in body composition and organ weight analyses.

Body composition and organ weights were analyzed using a MANCOVA with PC1 scores as a covariate (Y [body composition (TOTAL_FAT, TOTAL_PROTEIN)] = PC1, TRT_GROUP) and (Y [organ weight (LIVER, KIDNEY, HEART, PANCREAS, GASTROINTESTINAL)] = PC1, TRT_GROUP). I justified examining differences among class variable levels using a Tukey's HSD test, by evaluating the approximated F statistics from the MANCOVA output (SPSS 16.0).

2.6.4 Immune challenges

2.6.4.1 Cell mediated immunity & PHA skin test

I used ordinary least squares regression to model the relationship between the response of subcutaneous PHA injections (indexed by swelling response of the wing web in mm) and the hepatic Se concentrations (μg/g) of each bird.

2.6.4.2 Humoral mediated immunity & SRBC inoculations

The data collected from SRBC hemagglutination titers were analyzed with a Chi-Square test (SPSS 16.0). The scores (SCORE) were grouped categorically into low (2-8), medium (16-32), and high (128-256) antibody presence to give the analysis more power. Data were analyzed separately by date representing the three points in time blood samples were taken; date/sample 1 measured baseline antibody levels (baseAB), date/sample 2 primary (IgM) antibodies, and date/sample 3 gauged secondary (IgG) antibodies. Therefore I conducted a Chi-square for each blood sample date to represent groups of antibodies (Y [antibodies] = TRT_GROUP × SCORE).

2.6.5 Oxidative stress

I used an ordinary least squares regression to model the relationship between endpoints of oxidative stress (MDA and GSH) and the hepatic Se concentrations (μg/g) of each bird. Total GSH and MDA concentrations were also compared for each treatment group using a one-way ANOVA (SPSS 16.0).

CHAPTER 3. RESULTS

3.1 Hepatic Selenium concentrations

At the end of the staging period (10-weeks), mean (± SD) hepatic Se concentrations for control (n = 9), moderate (n = 9), and high (n = 9), groups were significantly different (ANOVA: $F_{2, 26.}$ = 105.59, P =. 001), and exhibited expected Se levels based on dietary concentrations (Table 3.1). At the end of the wintering period (23-weeks), mean hepatic Se concentrations for control (n = 9), moderate (n = 9), and high (n = 7) treatment birds fell into the recognized threshold ranges established by mallard studies (Table 3.1), with liver concentrations significantly different among treatment groups (ANOVA: $F_{2, 24.}$ = 246.77, P =. 001).

Table 3.1 Mean (±SD) concentrations (μg/g, dry weight), and ranges of hepatic Se in captive lesser scaup exposed to dietary Se for 10 and 23-week periods.

Treatment Group	10-week Exposure		23-week Exposure	
	Mean	Range	Mean	Range
Control	5.65 ± 1.19	3.86 – 7.81	4.77 ± 0.49	4.36 – 5.92
Moderate	39.63 ± 7.37	27.48 – 49.37	26.10 ± 1.65	23.11 – 27.85
High	75.31 ± 15.95	57.07 – 103.63	60.53 ± 9.35	50.50 – 72.98

3.2 Se concentrations in pinfeathers and ovaries

Pinfeathers were collected from the 23-week exposure group only, as they were molting when euthanized. The amount of Se depurated into new feather growth was significantly different (ANOVA: $F_{2, 22} = 86.34$, P =. 001), among control ($n = 9$), moderate ($n = 9$), and high ($n = 7$) treatment groups (Table 3.2). Ovaries collected from females in control ($n = 3$), moderate ($n = 3$), and high ($n = 2$) treatment groups exposed to dietary Se for 23-weeks were significantly different (ANOVA: $F_{2, 5.} = 93.471$, P =. 001), following a trend similar to that of other biological samples (Table 3.2).

3.3 Survival

There was a 100% survival of scaup in 10-week exposure duration groups. Two out of nine birds died in the high Se treatment group during the 23-week duration, making the total wintering ($n = 27$) survival 93%. However cause of death was due to drowning under ice cover and it was not possible to link this directly to Se burdens. Thus they were excluded from all analysis. Combined survival of both exposure durations ($n = 54$) was 96%.

Table 3.2 Mean (±SD) concentrations (μg/g, dry weight), and ranges of Se depurated into pinfeathers and ovaries of captive lesser scaup exposed to dietary Se for a 23-week period.

Treatment Group	Pinfeathers		Ovaries	
	Mean	Range	Mean	Range
Control	1.40 ± 0.16	1.17 – 1.63	2.5 ± 0.45	2.19 – 3.01
Moderate	25.01 ± 2.82	22.47 – 31.03	15.44 ± 0.89	14.60 – 16.37
High	61.81 ± 20.50	25.38 – 86.57	34.06 ± 5.48	30.19 – 37.94

3.4 Body condition

3.4.1 Trends in body mass

There was no significant three-way interaction between treatment × gender × date ($F_{2, 28.} = 1.05$, P = .401). There was a significant two-way interaction between treatment × date ($F_{2, 28} = 6.97$, P = 0.001), which suggests that treatment groups were losing and gaining weight differently throughout the study. Figure 3.1 illustrates that in general, all three groups had similar mean weights from late-October to mid-December and from early-March to early-April. However, birds in the high, and occasionally the moderate, treatment groups had lower body mass in January to February. The largest reduction of mean body mass occurred between 8 January and 30 January 2009 and was 54 g, 121 g and 87 g for control, moderate and high treatment groups, respectively. Notably, these changes coincided with the coldest temperatures that birds experienced during the study, with average high/low air temperatures of -6° and -12°C (Figure 3.2). During extreme temperatures birds reduced food consumption (all groups reduced food intake by 40-50% from overall average) and spent the majority of time resting, thereby implying they were conserving energy at that time. There was also a significant interaction between gender × date ($F_{2, 28} = 2.31$, P = 0.0046), which implies that male and female scaup lose and gain weight differently throughout the season, but there was no interaction between gender and treatment ($F_{2, 28} = 0.75$, P = 0.47).

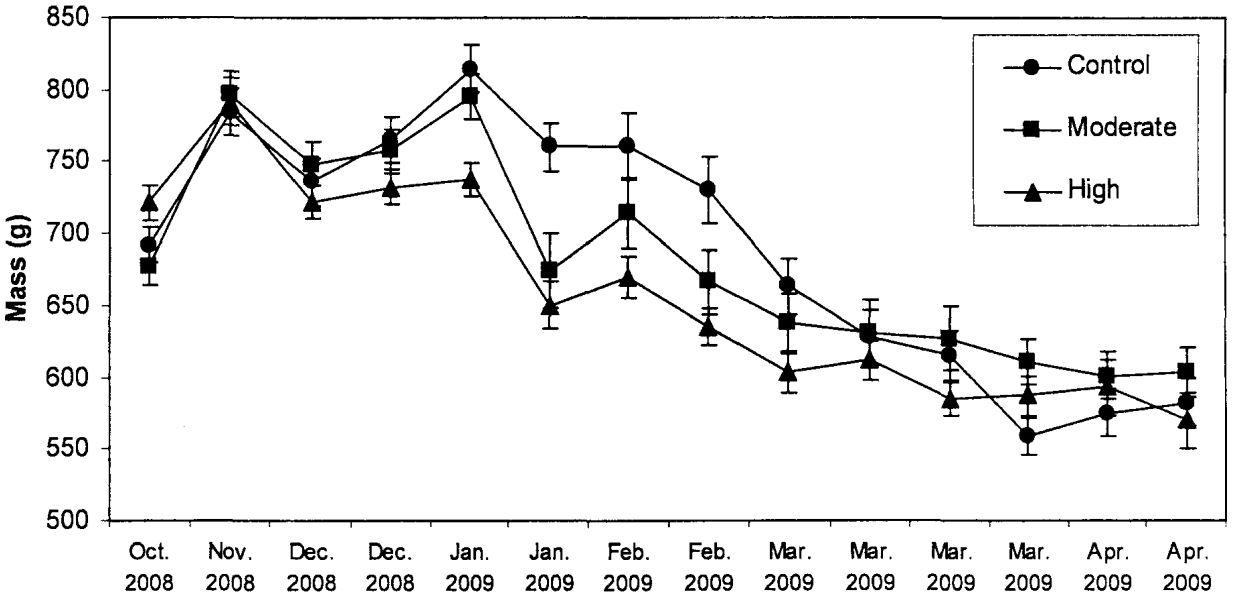

Figure 3.1 Mean (±SD) mass of lesser scaup from control, moderate, and high treatment groups. Treatment duration was November 3rd 2008 – April 9th 2009.

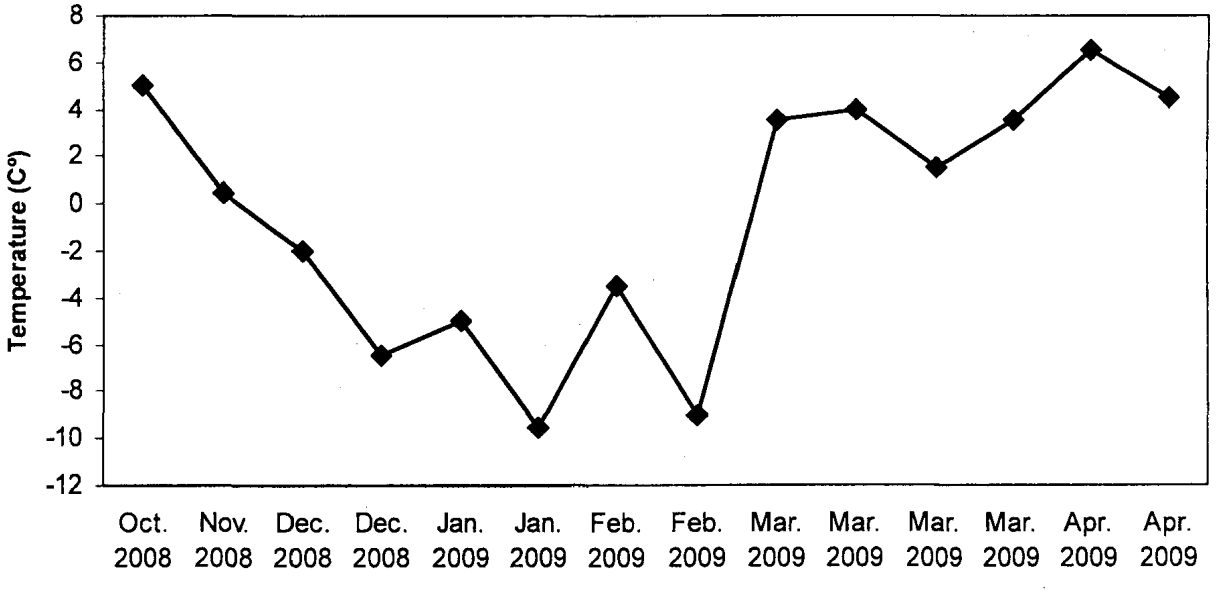

Figure 3.2 Average high/low air temperatures (C°) during weigh in periods in Aylmer Ontario October 30th 2008 – April 9th 2009.

3.4.2 Body composition

To account for individual differences in body size, I used principal components analysis, and principal component 1 (PC1) accounted for 46% (Eigenvalue = 2.753) of the variation in structural measurements in lesser scaup. Eigenvectors had similar loadings, ranging from -0.3257 to -0.5273, suggesting that they describe variation in overall body size. For scaup collected at the end of the 10-week duration, there was a weak overall effect of Se on nutrient reserves (MANCOVA: Wilks' $\lambda = 0.37$, $P = 0.063$). Scaup in treatment groups differed significantly in total lipid reserves ($F_{2, 26} = 7.87$, $P = 0.001$), but not total protein ($F_{2, 26} = 1.96$, $P = 0.15$). For scaup euthanized at the end of the 23-week exposure, Se concentration had no significant effect on body composition (MANCOVA: Wilks' $\lambda = 5.34$, $P = 0.487$) (Table 3.3).

Table 3.3 Mean (±SD) weight (g) and ranges of total lipid and total protein of captive lesser scaup exposed to dietary Se for 10 and 23-week periods.

	10-week Exposure				23-week Exposure			
Treatment Group	**Total Lipid**		**Total Protein**		**Total Lipid**		**Total Protein**	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Control	264.95 ± 35.58	204.16 – 310.00	134.99 ± 16.76	112.06 – 164.58	58.25 ± 15.39	43.42 – 90.36	153.0 ± 7.1	117.53 – 132.01
Moderate	257.82 ± 53.59	187.16 – 347.63	149.54 ± 23.31	122.46 – 181.14	64.47 ± 33.11	31.76 – 141.81	152.38 ± 28.71	66.79 – 216.55
High	180.33 ± 40.77	124.59 – 250.62	149.23 ± 24.2	87.82 – 179.47	45.41 ± 13.31	35.24 – 72.92	154.36 ± 9.56	110.68 – 132.62

3.4.3 Organ mass

There was no overall effect of Se concentration on organ mass among treatment groups in the 10-week exposure (MANCOVA: Wilks' $\lambda = 0.33$, $F = 1.64$, $P = 0.142$), or 23-week exposure (MANCOVA: Wilks' $\lambda = 0.26$, $F = 1.63$ $P = 0.163$). However, there was a significant difference in pancreas mass (ANOVA $F_{2, 26} = 3.75$, $P = 0.038$), but only among birds in the control and high treatment in the 10-week exposure (Table 3.4).

Table 3.4 Mean (±SD) weight (g, wet weight) of organs collected from captive lesser scaup exposed to dietary Se for 10 and 23-week periods.

	10-week Exposure			23-week Exposure		
Organs	**Control**	**Moderate**	**High**	**Control**	**Moderate**	**High**
Liver	13.18 ± 2.18	13.76 ± 3.41	11.17 ± 1.21	17.12 ± 1.44	16.88 ± 2.64	19.27 ± 1.88
Kidney	4.16 ± 0.53	3.82 ± 1.63	4.28 ± 0.56	5.25 ± 0.46	5.20 ± 0.70	6.41 ± 0.87
Heart	5.52 ± 0.91	5.28 ±0.50	5.18 ± 0.41	4.40 ± 0.36	4.36 ± 0.49	4.43 ± 0.34
Pancreas	1.64 ± 0.74	1.28 ± 0.33	1.02 ± 0.24	1.89 ± 0.51	2.08 ±0.37	2.04 ± 0.16
Gastro-Intestinal	25.40 ± 8.15	21.70 ± 5.14	20.64 ± 3.75	26.48 ± 2.08	32.54 ± 5.62	29.37 ± 3.95

3.5 Oxidative stress

There was a positive correlation between hepatic Se concentrations and MDA concentration in the 10-week exposure group ($R^2 = 0.188$, $F_{1, 26} = 5.794$ $P = 0.024$) (Figure 3.3), but no correlation with GSH levels ($R^2 = 0.005$, $F_{1, 26} = 0.129$ $P = 0.722$). The amount of variability appeared to increase from control to high treatment birds, which led to the lack of significance reported by the ANOVA in both MDA (ANOVA: $F_{2, 26.} = 2.031$, $P = 0.153$), and GSH (ANOVA: $F_{2, 26.} = 1.236$, $P = 0.308$) concentrations.

There was no correlation between hepatic Se concentrations and MDA concentration in the 23-week exposure group ($R^2 = 0.002$, $F_{1, 24} = 0.040$ $P = 0.842$), but a positive correlation with GSH levels ($R^2 = 0.170$, $F_{1, 24} = 4.716$ $P = 0.04$) (Figure 3.4). However, ANOVA results detected no significant differences among treatment groups in either GSH concentrations (ANOVA: $F_{2, 24.} = 1.641$, $P = 0.217$) or MDA concentrations (ANOVA: $F_{2, 24.} = 0.092$, $P = 0.913$).

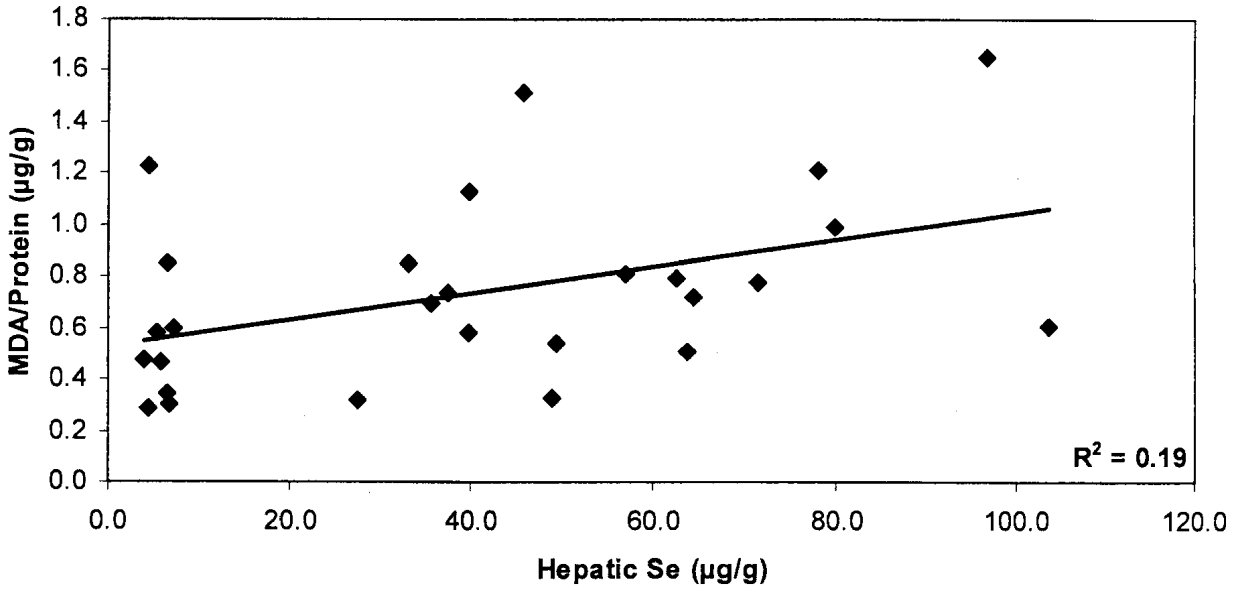

Figure 3.3 Relationship between hepatic MDA/protein (μmol/mg) concentrations and increasing hepatic Se concentrations in captive lesser scaup exposed to dietary Se for a 10-week period.

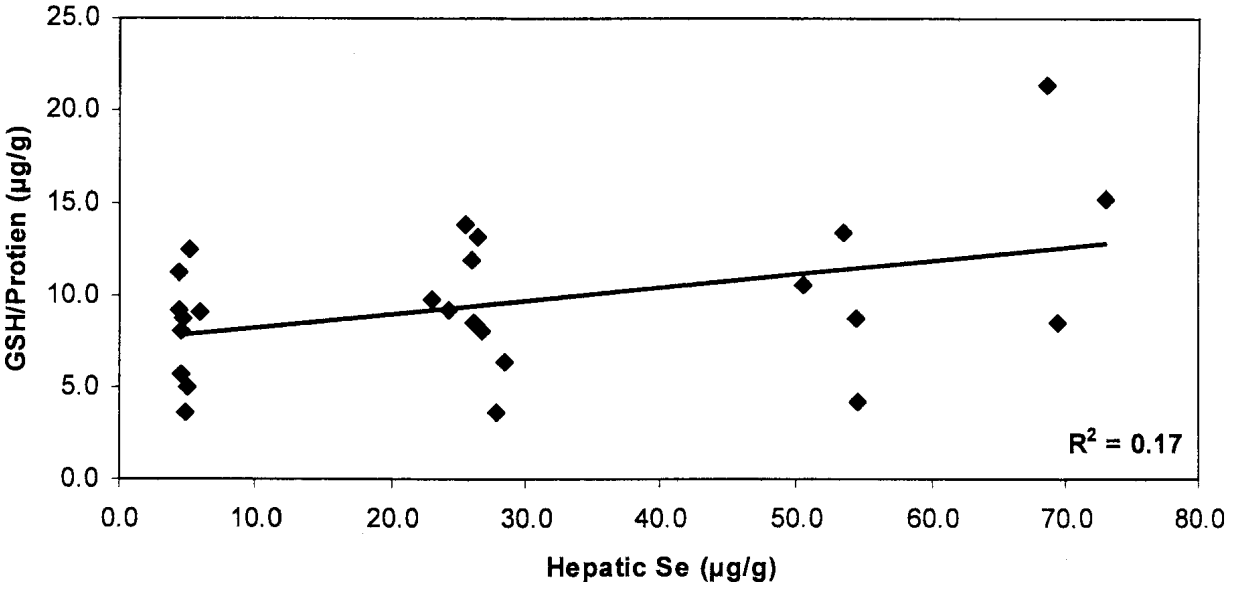

Figure 3.4 Relationship between hepatic GSH/protein (μmol/mg) concentrations and increasing hepatic Se concentrations in captive lesser scaup exposed to dietary Se for a 23-week period.

3.6 Immune system challenges

3.6.1 Cell mediated immunity & PHA skin test

T-cell response, as indexed by amount of wing-web swelling after injection of PHA did not correlate with hepatic Se during the 10-week ($R^2 = 0.019$, $F_{2, 26} = 0.480$ $P = 0.233$) or 23-week ($R^2 = 0.012$, $F_{2, 24} = 0.278$ $P = 0.603$) exposure periods.

3.6.2 Humoral mediated immunity & SRBC inoculations

There was no difference in amount of baseline ($\chi^2 = 3.06$, df = 4, $P = 0.508$) or secondary (IgG) ($\chi^2 = 2.770$, df = 4, $P = 0.597$) antibodies among groups in the 10-week exposure. However, birds in the high treatment group had higher primary (IgM) antibody titers relative to birds in the control group ($\chi^2 = 14.218$, df = 4, $P = 0.01$) (Figure 3.5). During the 23-week period, there was no difference in amount of baseline antibodies ($\chi^2 = 4.894$, df = 4, $P = 0.298$), but birds in the moderate and high treatment groups had more IgM ($\chi^2 = 8.466$, df = 4, $P = 0.015$) and IgG ($\chi^2 = 13.21$, df = 4, $P = 0.010$) antibodies than did control birds in response to SRBC inoculations (Figure 3.6).

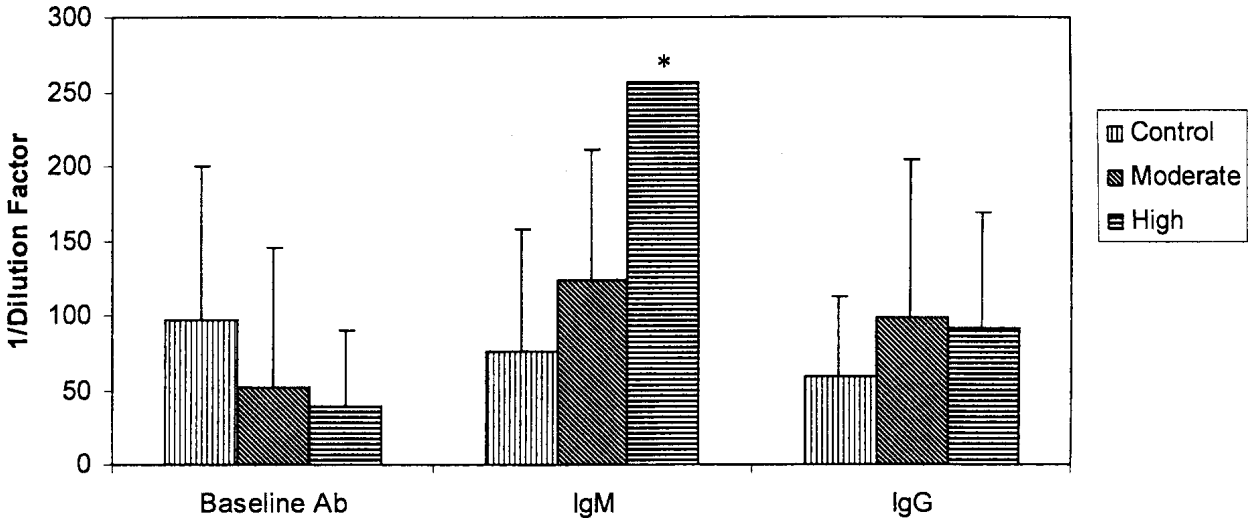

Figure 3.5 Mean (±SD) of plasma hemagglutination antibody titers from captive lesser scaup prior to, and 7, and 27 days following SRBC inoculations during a 10-week Se exposure period. * All individuals in treatment group achieved the same level of agglutination. Therefore these categorical variables resulted in no standard error.

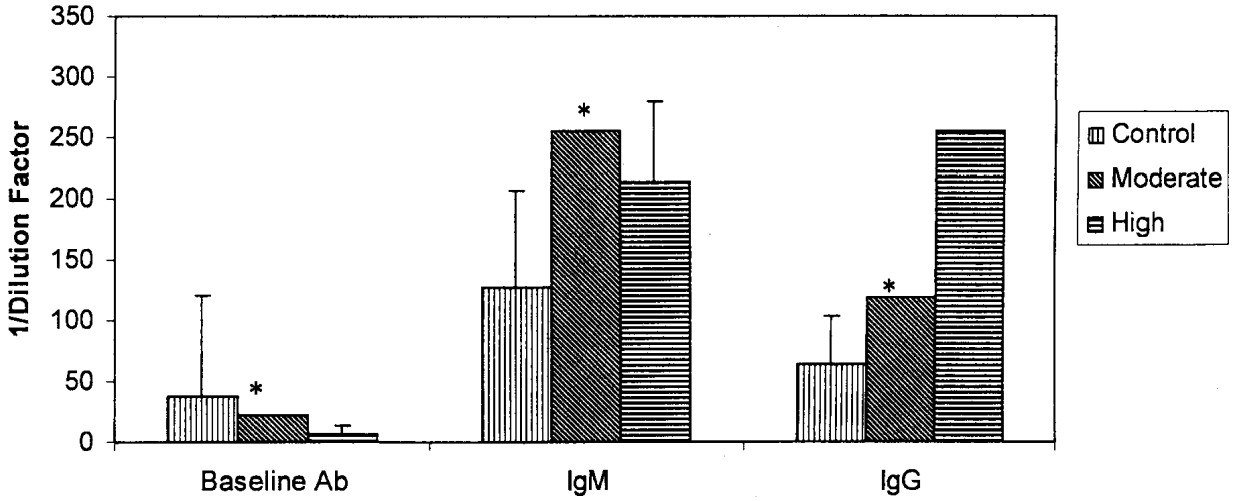

Figure 3.6 Mean (±SD) of plasma hemagglutination antibody titers from captive lesser scaup prior to, and 7, and 21 days following SRBC inoculations during a 23-week Se exposure period. * All individuals in treatment group achieved the same level of agglutination. Therefore these categorical variables resulted in no standard error.

CHAPTER 4. DISCUSSION

4.1 Overview

The contaminant hypothesis proposes that the continental scaup population decline is due to increased exposure to contaminants and/or trace elements leading to decreased fitness (Austin et al. 2000). Previous studies have identified Se as a trace element of concern for scaup (Custer and Custer 2000, Custer et al. 2003, Petrie et al. 2007, Anteau et al. 2007, Ware 2008). Subsequent research provided no evidence that concentrations of Se acquired by scaup have major impacts on reproduction (Fox et al. 2005, DeVink 2007, DeVink et al. 2008, Badzinski et al. 2009). However, it remains unclear whether Se burdens are affecting health and/or survival of staging and wintering scaup. Field studies have detected elevated concentrations of hepatic Se in scaup staging and wintering on the LGL (Custer and Custer 2000, Petrie et al. 2007, Ware 2008), but indices of body condition and oxidative stress of greater scaup wintering at eastern Lake Ontario were not correlated with hepatic Se concentrations (Ware 2008). It is unknown, however, whether individuals in Ware's (2008) study that had Se concentrations high enough to cause sub-lethal effects (e.g., loss of body mass, oxidative stress, etc) or possibly death were under-represented in field collections due to behavioral changes and/or reduced survival.

My thesis addressed three major questions regarding possible effects of Se acquisition on lesser scaup using the LGL during migration or winter. First, can hepatic Se burdens that greatly exceed background concentrations reduce survival of lesser scaup? Second, do these elevated Se concentrations compromise health and body condition? Third, is the Se threshold for impaired health/survival [>33 μg/g]

derived from mallards (Heinz 1996), and commonly applied to all waterfowl, appropriate for this species?

4.2 Hepatic selenium concentrations and survival

All birds in the 10-week exposure group survived to their previously assigned termination date, despite the fact that scaup in the moderate and high treatment groups had acquired hepatic Se concentrations that exceeded thresholds associated with sub-lethal and lethal effects in mallards (Heinz 1996). Similarly, DeVink et al. (2008), reported no mortality in lesser scaup fed 7.5 and 15.0 μg/g Se for 6-weeks. This further reinforces the hypothesis that hepatic Se thresholds for reduced fitness vary interspecifically (Skorpua 1998) and that established thresholds should be used with caution when applied to species other than mallards (Wayland et al. 2002, Franson et al. 2007, DeVink 2007). Alternatively, determining species-specific, or even genus or family-specific thresholds would aid in more accurate interpretation of toxic effects of Se, thereby increasing our understanding and ultimately leading to better management and conservation of wildlife in which Se is a problem.

Mallards fed 20 μg/g Se for 16-weeks experienced 25% mortality (Heinz and Fitzgerald 1993a), while the lesser scaup in this study fed 20.7 μg/g for 23-weeks had 7% mortality. During the 23-week exposure period, two high treatment birds became trapped under ice and drowned, but the ultimate cause of death could not be specifically linked to Se. It is possible that Se contributed to the death of the two birds, but evidence supporting this is anecdotal. For example, several individuals within the 23-week exposure, high treatment group were noticeably less active

relative to birds in both the control and moderate treatment groups (personal observation). It also was notable that individuals in the high treatment group were most easily captured for processing; it typically took less than 0.5 hour to capture those birds whereas capture time ranged from 1 to 2 hours for birds in the control group. This delay in reaction time to a perceived threat could result in higher predation/harvest rates for scaup with sub-lethal Se levels in the wild. So, although it is possible that Se contributed to the death of two individuals, winter survival rates were high (93%) and Se burdens were extreme (53.3-73.0 μg/g).

4.3 Seasonal trends in mass and body composition

The influence of elevated Se burdens on the body condition in birds remains unclear (Yamamoto and Santolo 2000, Wayland et al. 2002, Anteau et al. 2007, Franson et al. 2007, Ware 2008, DeVink et al. 2008). For example, scaup with high hepatic Se burdens (Se range 57.0 – 103.6 μg/g) had lower lipid reserves than did those with lower hepatic Se concentrations (this study). In contrast, a positive correlation was identified between lipid reserves and hepatic Se concentrations in wintering and spring migrating lesser scaup (range 3.7-52.3 μg/g) from the Mississippi flyway (Anteau et al. 2007) as well as in nesting common eiders (range 8.5-75.9 μg/g)(Wayland et al. 2002). There was also no relationship between hepatic Se and body composition in greater scaup wintering on the LGL (Ware 2008) or in breeding lesser scaup collected from the boreal region (DeVink et al. 2007b). However, the hepatic Se burdens within birds from the aforementioned studies were lower than the concentrations in this study.

Conversely, some studies have found reduced body mass in adult birds exposed to Se, with anorexia most frequently cited as the probable cause in the mallard (Heinz and Fitzgerald 1993a,b, Albers et al. 1996), common eider (Franson et al. 2007), black crowned night heron (*Nycticorax nycticorax*) (Smith et al. 1988), and eastern screech-owl (*Otus asio*) (Wiemeyer and Hoffman 1996). In those studies, body mass reduction occurred at dietary Se concentrations higher than those used in this study (e.g., 20–80 μg/g versus 20.7 μg/g) and was often associated with other symptoms and effects of Se poisoning, including mortality. Birds in the high and moderate treatment groups in this study did not exhibit food avoidance behavior or other symptoms of Se toxicity, so it is likely that some other mechanism was responsible for the observed effects on body condition.

The body mass decline throughout the study within all three treatment groups reflects a seasonal pattern observed in many migratory birds. Typically, lowest fat levels occur after the reproductive season, followed by rapid increase before fall migration (King and Farner 1965). Whyte and Bolen (1984) reported that lipid reserves in mallards increased from autumn to mid-winter, declined in late winter, and then increased again in early spring. Increasing fat reserves and body mass may be a slow process in scaup ingesting Se during winter. Thus, it is possible that birds, which experienced prolonged elevated and sub-lethal Se burdens, would not recover lipid reserves at the same rate as those with background Se concentrations, thereby possibly impacting migration and reproduction.

Heinz and Fitzgerald (1993a) suggested that the Se dietary threshold causing weight loss and mortality in adult mallards is lower in colder weather. High hepatic

Se concentrations had an effect on timing/rate of weight loss in comparison to control birds. But despite having lower body weight and energy reserves throughout winter, birds with high Se burdens were in similar condition to other treatment groups by spring, implying that birds with elevated and sub-lethal Se burdens lost the same amount of mass as those with background levels, but earlier in the season. This pattern suggests that high hepatic Se burdens compromise body condition in winter, but scaup may be able to compensate once ambient temperatures increase.

It is these differences in the timing of body mass reduction among groups that could have negative implications for the survival of migrating and wintering scaup. Birds generally show a positive relationship between body condition and survival, especially in times of harsh environmental conditions (wintering on the LGL) or physically demanding activities (migration) (Haramis et al. 1986, Anteau and Afton 2009). Extrapolation of observed effects in the high Se treatment group to wild scaup (which are faced with greater energetic stress than captive birds, and are not provided with food *ad libitum*), suggests potential for disruption of normal seasonal lipid dynamics. While reduced body condition of wild birds may not immediately compromise health and/or survival, it could have long-term consequences. For instance, in some bird species, winter body condition or fat storage is an indicator of whether breeding will be successful (Newton 1979, Alisauskas and Ankney 1985, Wiebe and Bortolotti 1995). However, since treatment and control birds were in similar condition by spring, it is unlikely that Se burdens acquired throughout the winter impact reproduction or post-winter survival, but survival could be compromised in birds with high Se burdens during harsh winters.

4.4 Oxidative stress

Excess dietary Se causes oxidative stress at different stages of the life cycle of birds (Ohlendorf et al. 1988, Smith et al. 1988, Fairbrother et al. 1994, Hoffman et al. 1998, Hoffman and Heinz 1998) including several species of waterfowl (Fairbrother and Fowles 1990, Hoffman et al. 1991, Hoffman et al. 1996, Hoffman et al. 1998, Custer et al. 2000, Ji et al. 2006, Franson et al. 2007). However, Ware (2008) reported that there was no correlation between hepatic Se concentrations and MDA concentrations in greater scaup. In my study, the correlation between hepatic Se concentrations and MDA in 10-week period birds suggests that scaup with sub-lethal Se burdens were experiencing a greater degree of LPO in comparison to scaup with elevated and background Se levels. It should be noted that the variability of MDA concentrations within groups appeared to increase from control to high treatments, and average MDA concentrations did not differ among groups.

The lack of correlation between Se burdens and GSH levels in 10-week period birds, but positive relationship in 23-week birds, suggests that perhaps a longer exposure time to Se was required for scaup to begin producing significantly more antioxidants. Ware (2008) did not measure GSH levels, but speculated that lack of high MDA concentrations in greater scaup was due to increased GSH activity acting as protectant. The relationship between hepatic Se and GSH levels found in the 23-week period birds implies that scaup with high Se burdens were producing more GSH, possibly to counteract free radicals produced by excess Se, but there was no difference among treatment groups in average GSH levels present in the liver. This in combination with the absence of correlation between MDA levels, and hepatic Se

burdens in 23-week birds suggests that GSH activity was indeed protecting against the oxidative properties of Se. Furthermore, scaup in this study did not show other signs of oxidative stress, such as suppressed immune response, emaciation, or decreased survival (Hoffman et al. 1991), suggesting that there are Se/oxidative stress thresholds.

4.5 Immune system challenges

4.5.1 T-cell response

The PHA skin test is a reliable indicator of T-cell function, however results of this test in relation with Se have been inconsistent among waterfowl species, and even within species. For example, hepatic Se levels of common eiders have been reported as positively correlated with PHA response (Wayland et al. 2002) as well as unrelated to PHA response (Wayland et al. 2003). Franson et al. (2007) documented that common eiders fed 60 μg/g Se had significantly decreased response to PHA skin test, and finally, mallards exposed to Se in drinking water exhibited no effect on amount of swelling caused by PHA (Fairbrother and Fowles 1990). T-cell response to PHA injections was unrelated to the hepatic Se burdens acquired by scaup in this study. This implies that Se concentrations in treatment groups never reached a point of causing cell-mediated immune response impairment. However, although T-cell response was not affected by Se toxicity in this study, disease and other stresses in nature, such as migration, breeding, and food shortages could possibly change the dietary threshold at which disruption of T-cell function could occur.

4.5.2 Antibody response

The production of antibodies against foreign red blood cells has proven to be a sensitive indicator of immunotoxicity in birds and antibody response can vary with environmental stressors (Fairbrother and Fowles 1990, Grasman and Scanlon 1995, Svensson et al 1998, Fair and Ricklefs 2002). For example Svensson et al. (1998) demonstrated that cold temperatures suppress antibody production in blue tits (*Parus caeruleus*). In mallard ducks, antibody titers against SRBC were unaffected by Se exposure (Fairbrother and Fowles 1990) whereas lead ingestion suppressed (Grasman and Scanlon 1995), or had no effect (Fair and Ricklefs 2002) on antibody responses in quail (*Coturnix coturnix*). The dietary Se treatments in this study had no effect on baseline antibodies present prior to SRBC inoculations. During the 10-week period, birds with sub-lethal Se burdens produced more primary (IgM) antibodies relative to birds with elevated and background Se levels. During the 23-week period, birds with sub-lethal and elevated Se burdens had more IgM and secondary antibodies (IgG) than did control birds in response to SRBC inoculations. This suggests that for scaup with sub-lethal Se burdens acquired over 10-weeks, Se acted as an immunostimulator for IgM antibodies. As exposure time increased from 10 to 23-weeks, Se continued to act as an immunostimulator for birds with sub-lethal Se concentrations, as well as those with elevated burdens. Thus, scaup with elevated Se levels likely needed longer exposure (>10-weeks) to achieve immunostimulatory effects from Se and birds with sub-lethal Se concentrations needed longer exposure to stimulate IgG antibodies. These findings are consistent with Fairbrother and Fowles (1990) where mallards

given high Se doses via drinking water for 12-weeks produced more IgM and IgG antibodies than control birds.

Each component of the immune system has its own inherent costs and protective values, and it may be argued that the energetic cost of producing more antibodies may have negative implications. However, although the developmental costs of the humoral immune system (which occurs during developmental life stages) may be high, the cost of using the HMI in later life stages is relatively low compared to innate and cell-mediated immunity (Klasing and Leshchinsky 1999, Lee 2006). Thus I can concluded that Se was actually acting as an immunostimulator, and Se concentrations used in this study never reached the point of causing any signs of immunosuppression.

The complexity of the immune system becomes evident with the magnitude, span, and inconsistency of responses across assays within and among species (Matson et al. 2006). Relationships between immune response and infection do not likely follow a general pattern, but instead probably depend on several factors, such as type, range and strength of exposure, an individuals health at time of exposure, other natural stressors (seasonal or niche related), and exposure to additional contaminants (Fairbrother et al. 2004, Matson et al. 2006, Mendes et al. 2006).

4.6 Depuration of selenium in pinfeathers and ovaries

Molting is an intense physiological change that includes the synthesis of keratin, increased amino acid metabolism, and daily cycling of body protein among many other physiological changes (Dolnik and Gavrilov 1979, Murphy and King

1991). In this study scaup were incorporating excess Se into new feather growth, which supports the hypothesis that, when present, selenomethionine will replace methionine protein structures (Beilstein and Whanger 1987, Kigawa et al. 2002). However, it should be noted that despite the high levels of Se that were integrated into new feather growth, anecdotally there appeared to be no difference in the quality of feathers among treatment groups. Conversely, Albers et al. (1996) found a 47% frequency of feather loss in mallards fed 40 μg/g for 16-weeks, but these differences are likely due to variation in dietary concentrations used in either study. Therefore, Se allocation into new feather growth could possibly be a safe avenue of depuration that scaup may employ to reduce Se burdens to less toxic levels.

Previous studies have found that as dietary Se increases, so does Se concentration in various tissues (Heinz et al. 1989, Hoffman et al. 1991, Albers et al. 1996, section 3.2). Scaup in this study were allocating Se into tissues, in this case only ovaries were examined but it is likely that other organs also stored excess Se. Using Se concentrations of undeveloped ovaries has limitations in foreseeing reproductive impairment. While egg laying is a known route of Se depuration, little is known about the actual amount of Se that gets transferred to eggs (Heinz 1996), and likely varies among individuals. Nonetheless, this avenue of depuration could have negative implications for molluscivorous waterfowl that breed on or near the LGL, as they would have limited time to depurate Se.

4.7 Conclusions

The potential toxic effects of Se assessed in this study, including reduced survival, changes in seasonal body mass and organ weight, body composition, changes in immune response, and oxidative stress have been identified in both laboratory and field studies of aquatic birds with high hepatic Se concentrations (Ohlendorf et al. 1988, Fairbrother and Fowles 1990, Heinz and Fitzgerald 1993a,b, Albers et al. 1996, O'Toole and Raisbeck 1997, Franson et al. 2007, DeVink 2007b, DeVink et al. 2008). Despite high Se burdens detected in scaup on some staging and wintering areas, the significance of these reports to the continental population decline is unclear. Scaup in the present study achieved a range of elevated to sub-lethal Se burdens (4.36-103.63 μg/g) and some adverse health affects were detected. For example, birds with elevated and sub-lethal burdens acted lethargic and lost body fat during the coldest period of the study. However, these birds did not exhibit other symptoms of compromised health, such as suppressed immune response, enlarged organs, oxidative stress, or extreme emaciation. In fact, excess Se burdens actually provided immunostimulatory effects for antibody-mediated immunity.

Captive waterfowl studies enable experimental manipulation of treatments, but often do not account for other factors that individuals experience in the wild. Even though I found that elevated Se can affect rate or timing of seasonal weight loss, there was no evidence to suggest that exposure to Se levels similar to, and much higher than, those found in mussels on the LGL influenced spring body mass. However, the weight loss experienced by scaup with elevated and sub-lethal Se burdens during fall and early winter is of concern because poor winter body condition

can negatively affect survival, migration and reproduction (Heinz et al. 1993, Anteau and Afton 2004).

Despite the reduced body mass experienced by birds in the high Se treatment, the amount of Se provided would never be biologically available to free-ranging scaup, except for in an acute poisoning situation, as seen in Kesterson Refuge in the late 1980s (Ohlendorf et al. 1988). Reduced body mass observed in the moderate treatment group should also be viewed with scrutiny because the likelihood of wild scaup consistently ingesting foods with Se concentrations of 8.1 μg/g is unlikely. Even though it was reported in 1996 (Custer and Custer 1996) that scaup diets on the LGL consisted of 54.4-98.6% dreissenid mussel, the National Center for Coastal Monitoring and Assessing reported that in 2005 dreissenid mussels sampled from 8 out of 12 Great Lakes Region sites had an Se range of 3.42-4.22 μg/g, while only 2 sites sampled mussels with Se above 4.22 μg/g (U.S. Department of Commerce 2007).

Although results from this study do not support the contaminant hypothesis, it has advanced our knowledge of the impacts, or lack thereof, of Se burdens on lesser scaup. It is necessary to continue monitoring of aquatic systems that are susceptible to anthropogenic Se input, and the aquatic birds and wildlife that could be impacted. In conclusion, this study suggests that, while there was no evidence for most of the health effects tested, there is the possibility that elevated Se burdens could compromise the condition of scaup during extreme winter conditions. Based on results and the amount of Se acquired by scaup during this study, it is unlikely that Se acquisition on the LGL is contributing to the continental scaup decline or lack of

recovery. However, it may be impacting some birds under extreme conditions but should not be of conservation concern. These results, combined with recent breeding and wintering ground studies (Fox et al. 2005, DeVink 2007, Ware 2008, Badzinski et al. 2009), suggest that future research should focus on alternative hypotheses for the decline of scaup.

CHAPTER 5. LITERATURE CITED

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