Comparative Reproductive Energetics and Selenium Ecotoxicology in Three Boreal-breeding Waterfowl Species

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ABSTRACT

Environmental conditions on wintering or spring-staging areas may influence subsequent reproductive performance in migratory birds. These cross-seasonal effects may result from habitat loss and degradation (e.g., via contamination) which in turn reduce reproductive success, particularly in waterfowl that use stored nutrients for reproduction. North American lesser scaup (*Aythya affinis*) and white-winger scoter (*Melanitta fusca*) numbers have declined over the past 20 years, particularly in the boreal forest, and remain well below conservation goals, whereas ring-necked duck (*A. collaris*) numbers have increased. Environmental changes on scaup and scoter wintering and staging areas have raised concern about possible cross-seasonal effects on birds arriving on breeding grounds. The spring condition hypothesis (SCH) purports that many female scaup fail to acquire sufficient nutrients in late winter and spring, causing a decrease in breeding propensity and productivity. The contaminant hypothesis proposes that increased exposure to contaminants (particularly selenium [Se]) on wintering and staging areas has decreased scaup productivity. Accordingly, I compared body condition and studied Se concentrations in scaup, scoters and ringnecks to test the condition and contaminant hypotheses.

Scaup had similar body condition to ringnecks, and had similar body mass compared to scaup collected near Yellowknife, NT, in 1968-70. There was no relationship between scaup and ringneck nutrient levels and claw tip carbon, nitrogen or hydrogen isotope values, suggesting that arrival body condition likely was not related to location or diet several months prior. Instead, scaup and ringnecks nutrient levels may be more affected by feeding or habitat conditions on or near the breeding grounds. Scaup had slightly higher liver Se concentrations than ringnecks, but levels in both species were below recognized harmful threshold concentrations; I found no relationship between Se and breeding propensity, or between Se and somatic lipid or protein stores. Scoters had much higher Se concentrations, yet contrary to predictions, there were positive relationships between Se and both lipid stores and breeding status. Follicle [Se] in scaup was below threshold concentrations; despite high liver Se in scoters, egg and follicle levels also were well below threshold concentrations. Using both body composition analysis and stable-isotope analysis I determined that scoters derive egg protein from

their breeding ground diet, which likely prevents Se deposition from somatic protein to eggs, and egg lipids are apparently derived from somatic tissues. In all three species, liver Se concentrations were significantly correlated with claw tip $\delta^{15}N$. As the claw tip likely represents assimilated diet from 2-5 months prior to sampling, this correlation suggests that Se in these boreal breeding species is carried over from wintering and staging areas.

Overall, results did not support either the spring condition or contaminant hypotheses. Scaup and scoters are late-nesting species, with highest pair densities occurring at the northern extent of their range. Maximum ring-neck pair densities occur at more southern latitudes. Ring-necks also nest earlier and appear to be more flexible in timing of nest initiation. Therefore, it is possible that due to climate change, early spring conditions alter the optimal timing of nest initiation to the detriment of late-nesting species such as scaup and scoters, and favour earlier nesters like ringnecks. Further research into this mismatch hypothesis is warranted.

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I was supported wholeheartedly in the decision to commit myself to this endeavour by my wife and friend Christiane Guérette and my families who live several time zones away. Without their unconditional support my success would have been less than certain - thank you! A true mentor has the ability to perceive and expose skills perhaps unknown or underutilized in their students, and they can assist students in problem solving by merely asking questions. They also provide moral support and advice to develop a student's professional abilities. My supervisor, Bob Clark, was such a mentor and friend to me throughout this journey – cheers, Bob. I wish to acknowledge the wonderful members of my committee: Ray Alisauskas, Stuart Slattery, Mark Wayland, and Gary Wobeser, all of whom provided thoughtful and challenging comments throughout this project, and also my external examiner, Dan Esler. In particular, thanks Stuart for sharing data and help with analyses and manuscript preparation. Thanks also go out to all the other students and staff at PNWRC and in Biology. Being part of this community has been a real pleasure for me and a wonderful learning experience.

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FOREWORD

Each data chapter of my thesis was written in the format of an independent manuscript for publication in peer-reviewed journals. Below, I have identified the journals in which I have published or submitted each chapter at the time the thesis was completed. Therefore, there will be some redundancy in the introduction and discussion sections of some chapters, though I have removed some redundancy in methods by cross-referencing among chapters. Although I always took the lead role in acquiring and analyzing data and writing all papers, I also wish to acknowledge the contributions of co-authors in preparing these manuscripts.

Chapter – manuscript status at time of thesis completion

2 – DeVink, J.-M., Clark, R.G., Slattery, S.M., Trauger, D.L. 2008. Are late-spring boreal lesser scaup (*Aythya affinis*) in poor body condition? Auk, in press (accepted, May 2007).

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4 – DeVink, J.-M., Clark, R.G., Slattery, S.M., Scheuhammer, A. 2007. Effects of dietary selenium on reproduction and body mass of captive lesser scaup. Environmental Toxicology and Chemistry, in press (accepted, June 2007)

5 – DeVink, J.-M., Clark, R.G., Slattery, S.M., Wayland, M., Scheuhammer, A. Selenium dynamics in white-winged scoter eggs: insights from reproductive energetics. Environmental Science and Technology, in preparation.

6 – DeVink, J.-M., Clark, R.G., Slattery, S.M., Scheuhammer, A. Cross-seasonal association between winter trophic status and breeding ground selenium levels in boreal white-winged scoters. Avian Conservation and Ecology – Écologie et Conservation des Oiseaux, resubmitted (June 2007)

7 – DeVink, J.-M., Clark, R.G., Slattery, S.M., and Wassenaar, L.I. Stable isotopes of lesser scaup and ring-necked duck claws: claw isotope relationships with spring nutrient levels and selenium concentrations. Canadian Journal of Zoology, in preparation.

8 – DeVink, J.-M., Slattery, S.M., Clark, R.G., Alisauskas, R.T. Body composition and stable isotope analyses reveal plasticity in scoter reproductive energetic strategies. Oecologia, invited for resubmission (July 2007)

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1. GENERAL INTRODUCTION

1.1 POPULATION DYNAMICS

Populations are dynamic, and factors affecting population growth rate are diverse. But, to study population growth and determine appropriate conservation action, it is helpful to consider the four components of a basic population growth model:

$$\text{Population}_{t+1} = \text{Population}_t + \text{birth}_t - \text{death}_t + \text{immigration}_t - \text{emigration}_t \quad (1.1)$$

In studies that examine total populations of species, one can justify eliminating the immigration and emigration components of this equation. This is the case with most of the estimates from the USFWS breeding waterfowl population surveys (NAWMP 2004). Consequently, research into waterfowl population growth rate at the continental (North America) level focuses on the birth and death components of this equation. While most waterfowl population modelling has focused on mallards, species of concern, such as lesser scaup (*Aythya affinis*) and northern pintails (*Anas acuta*), have received more attention recently.

Koons et al. (2006) recently developed a population dynamics model for scaup and found that the population growth rate was most sensitive to female survival during and outside the breeding season, and to a lesser extent productivity. These results are consistent with a similar model for greater scaup (Flint et al. 2006), though Flint et al. also found that productivity explained twice as much annual variation in predicted population size because of its greater variability. Hunting is likely the single most important cause of adult mortality in scaup with an average annual harvest of approximately 341,000 birds between 1961-1994 (range: 93,000 to 687,000; Austin et al. 1998). Yet, additional harvest restrictions since 1988 do not appear to have affected population growth. Furthermore, the mean age ratio of lesser scaup (mean ratio = 1.46 immature: 1 adult) had a 1% annual decline between 1961-1997 in the United States (Austin et al. 2000, Afton and Anderson 2001). This declining age ratio trend for scaup was similar in Canada; during 1969-1996, there was a 1.5% decline per year. The ratio of male: female lesser scaup in the U.S. harvest has also increased (Austin et al. 2000, Afton

and Anderson 2001). Afton and Anderson (2001) interpreted these findings as meaning that recruitment and female survival have decreased, particularly in the Canadian western boreal forest.

Fitness can be measured in the number of viable offspring surviving to reproductive age that an individual produces over its lifetime. In birds, recruitment of young (i.e., the birth component) is the result of a successful outcome at each of the successive stages in the reproductive cycle: breeding propensity, nesting (and renesting if applicable), incubation, hatching, brood rearing, and juvenile survival (Flint et al. 2006). With the possible exception of juvenile survival, each of these parameters also depends on female survival. Failure at any of these stages results in failed reproduction for that year. Therefore, variation in one of more of these parameters could potentially cause a decline in scaup productivity.

Since the first scaup research and management summit held in 1998 - and publication of its report (Austin et al. 2000) - two hypotheses about factors influencing productivity in scaup have been dominant: the spring condition hypothesis (SCH) and the contaminants hypothesis. The spring condition hypothesis purports that reproductive success is lower now than in the past due to a reduction in average body condition of females arriving on breeding grounds. The contaminants hypothesis postulates that acquired contaminants have negatively affected scaup survival or productivity. However, to date no studies have tested the SCH and only one study has tested the contaminants hypothesis for lesser scaup in the boreal forest. This thesis attempts to address these specific knowledge gaps.

1.2 SCAUP POPULATION STATUS

Waterfowl are an important natural resource from an economic and social perspective. To help ensure the sustainability of waterfowl populations, the North American Waterfowl Management Plan (NAWMP), a continental partnership, was enacted in 1986 (http://www.nawmp.ca/). In its first report, species population objectives were presented and based on historical estimates from breeding population surveys (NAWMP 1986). While most species have either surpassed or fluctuated around their

respective population objective, three of the 12 species with defined population objectives have exhibited a persistent declining population trend or remain well below population objectives (NAWMP 2004). Of notable concern is the population trend of scaup. Between 1978 and 1997, scaup declined by approximately 150,000 birds/year – a decline of roughly 3% annually (Afton and Anderson 2001). In the past decade, the rate of decline has been reduced, but population growth continues to be negative (Fig. 1.1) and the population remains well below NAWMP conservation objectives.

Both lesser scaup (*Aythya affinis*) and greater scaup (*A. marila*) are combined in counts during breeding population surveys because of the inability to differentiate the two species from the air. However, lesser scaup are estimated to comprise *ca.* 89% of the combined population (Bellrose 1980), and tundra breeding population estimates, which are assumed to represent primarily greater scaup, suggest that this biome has a stable population (Fig. 1.2; NAWMP 2004, Flint et al. 2006). Of the three scaup breeding biomes, the boreal forest is overwhelmingly the most important with 2/3 of the breeding population (Fig. 1.2). Hereafter, scaup refers only to the population of lesser scaup unless otherwise noted.

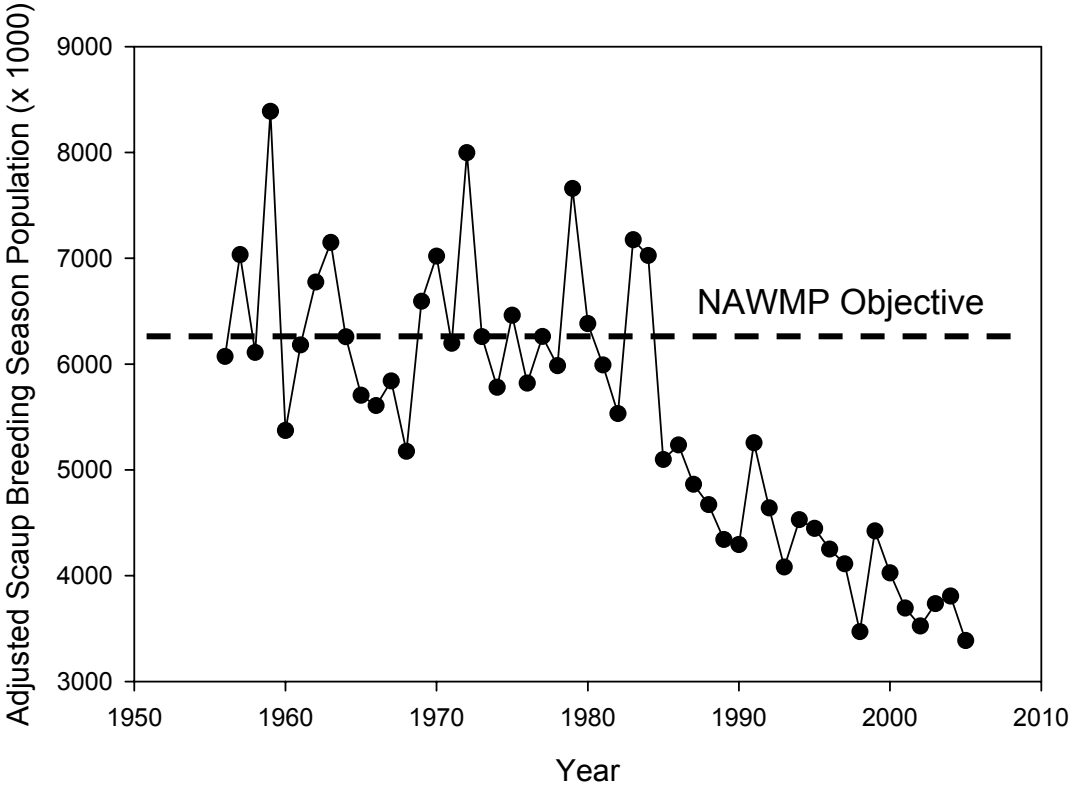

Figure 1.1 Scaup breeding season population (1956-1971, adjusted to include flocked birds; Stuart Slattery, IWWR, unpubl. data). The reference line indicates the North American Waterfowl Management Plan scaup population objective (6.3 million).

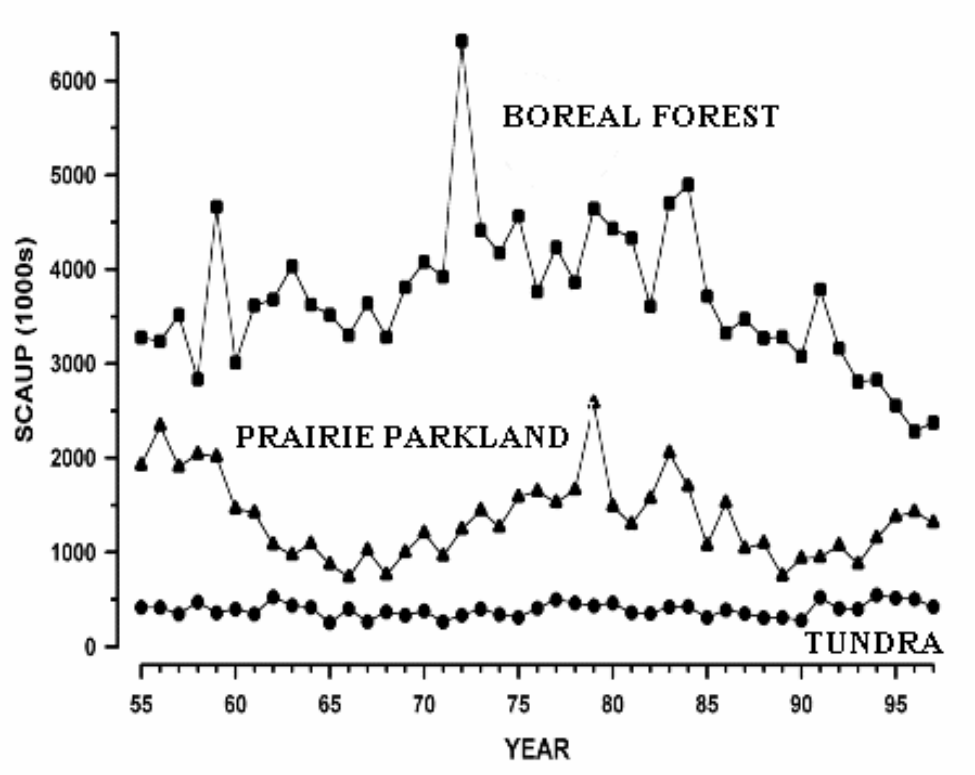

Figure 1.2. Annual estimates of breeding greater and lesser scaup (combined) in the Tundra (strata 8-11), Boreal Forest (strata 1-7, 12-25, 50, and 77), and Prairie-Parkland biomes (strata 26-49, and 75-76). (From Afton and Anderson 2001 in Austin et al. 1999).

1.3 REPRODUCTIVE ENERGETICS AND THE SPRING CONDITION HYPOTHESIS

Energy is the currency of life. Without sufficient energy to fuel basic maintenance and activity, organisms cease to function normally and die. Reproduction is the currency of fitness and is an activity that requires energy and nutrients in excess of those required for basic maintenance and activity. Prior to senescence and death, semelparous organisms that do not provide parental care to their offspring may translocate energy and nutrients into reproductive tissues that would otherwise be required for maintenance. For example, true salmon (*Onchorhynchus spp.*) die after reproduction and may maximize the transfer of nutrients into reproductive tissue. However, this strategy would not be possible in species, such as birds, that provide some degree of parental care to offspring; in birds,

adequate nutrients must be available for the production of offspring, for survival of parents, and subsequent care of the offspring.

Nutrients (lipids, protein, and minerals) used in reproduction (egg formation, incubation, maintenance) can be derived from two sources: endogenous nutrients are those stored in somatic tissues that can be metabolized or transferred as needed, and exogenous nutrients, which are nutrients assimilated from the diet and not stored in somatic tissues (Drent and Daan 1980). Larger species, such as most waterfowl are capable of storing greater quantities of nutrients and are more frequently reported using somatic nutrients in the formation of reproductive tissues than smaller species, such as passerines (Blem 1990). For females that rely on endogenous nutrients for reproduction it is logical to assume that threshold nutrient levels must be attained in order to attempt reproduction (Alisauskas and Ankney 1992). Lesser Scaup (hereafter scaup) appear to use endogenous nutrient stores for reproduction; somatic lipid stores decrease by approximately 0.50 to 0.67 $g \cdot g^{-1}$ of lipid invested in reproductive tissues (Afton and Ankney 1991, Esler et al. 2001). Thus, one explanation for the scaup decline, the SCH (Austin et al. 2000, Afton and Anderson 2001, Anteau and Afton 2004, Anteau 2006), purports that productivity has decreased due to a reduction in average body condition of females in spring. Anteau and Afton (2004) reported that females arriving in the Upper Mid-West (UMW) United States and in the parklands of Manitoba had lower lipid and protein levels in 2000-2001 than in 1977-1988. Anteau (2006) subsequently determined that poor habitat conditions in the UMW were likely the result of this poor condition scaup. Poor body condition could manifest itself through decreased breeding propensity, particularly among inexperienced females (Afton 1984), delayed nest initiation due to increased feeding time to acquire adequate stores (Esler et al. 2001), and reduced clutch sizes due to delayed nest initiation (Rohwer 1992, Guyn and Clark 1999), all of which could lower productivity and subsequently affect population dynamics.

1.4 SELENIUM AND THE CONTAMINANTS HYPOTHESIS

Contaminants are responsible for numerical declines of brown pelicans (*Pelecanus occidentalis*) (Keith 1996), double-crested cormorants (*Phalacrocorax*

auritus) (Peakall 1996), and bald eagles (Grier 1982), among others. In studies conducted on captive birds, elevated levels of trace elements such as cadmium (Cd), mercury (Hg), and selenium (Se) reduced body weight, damaged organs, altered metabolism, or caused behavioural changes (Heinz and Hoffman 1998; Wolfe et al. 1998; Furness 1996)**.** Contaminants could reduce wild populations through acute exposure causing mortality of adults and young. However, chronic effects of repeated exposure and accumulation of contaminants are more likely common and affect behaviour (Peakall 1996), organ function (Guillette 1995), and body condition (Wayland et al. 2002) in wildlife.

In scaup, the only contaminant that has consistently been observed at levels of concern is Se. High Se concentrations in livers of wintering or spring staging scaup have been detected in San Francisco Bay (Hothem et al., 1998), the southern Great Lakes (Custer and Custer, 2000), and the Mississippi alluvial valley (Custer et al., 2003). Elevated levels of Se in American coots (*Fulica americana*) caused emaciation, abnormal feather loss, and histopathological lesions (Ohlendorf et al. 1988). However, the most Se sensitive endpoint in oviparous species is embryonic development. Egg concentrations above 9 mg/kg dry weight have been associated with reduced hatchability and teratogenesis in mallards (Heinz 1996).

The potential for effects of high Se burdens on scaup population from wintering and staging areas remains unclear. This uncertainty is due to the lack of information about the number of birds exposed to contaminants and persistence of contaminants in tissues and thus potential for impacts on breeding propensity and reproductive success. Some information suggests that scaup are increasingly using the Great Lakes as a migratory staging area. Waterfowl days (peak count multiplied by average stopover duration) for lesser and greater scaup combined increased from 38,500 in 1986, just before the zebra mussel colonisation of Long Point, to 3.5 million in 1997 (Petrie and Knapton 1999). Though significant in terms of a local increase, this still represents a small percentage (0.05%; based on mean wintering estimates of 1504 ± 383 lesser scaup [Edmunds, unpubl. data, in Petrie et al. 2007] and a lesser scaup population of 3 million) of the total wintering population of lesser scaup.

However, studies on the breeding grounds suggest that Se is not affecting breeding scaup. Fox et al. (2005) did not detect high levels of either Hg or Se in lesser

scaup females and eggs taken from several locations in western Canada, including boreal forest sites. Fournier and Hines (2001) reported that recent estimates of mean clutch size and egg hatchability of lesser scaup in the Yellowknife area had not changed from those of 1969. The number of nests found in the Yellowknife study area had decreased, which could indicate a reduced number of females attempting to nest. Therefore, further examination of Se concentrations in boreal scaup are needed to better understand the potential impact of this contaminant on their productivity.

1.5 STUDY APPROACH

To date, there have been few studies of scaup or other waterfowl breeding in the boreal forest, yet it is in the western part of this biome that *c.a.* 2/3 of scaup are observed during spring surveys (Afton and Anderson 2001). That is why I chose to conduct this study in the western Canadian boreal forest. To evaluate and interpret the body condition and Se levels in boreal scaup, I chose an interspecific comparative approach using ring-necked ducks and white-winged scoters.

The ring-necked duck (*Aythya collaris*; hereafter ringneck), is a species closely related to scaup. It overlaps most scaup wintering areas (Austin et al. 1998, Hohman and Eberhardt 1998); has more omnivorous dietary preferences except during breeding when both species rely heavily on invertebrates (Austin et al. 1998, Hohman and Eberhardt 1998); has a similar reproductive energetic strategy (Alisauskas et al. 1990, Afton and Ankney 1991, Esler et al. 2001, this study); is similar in structural size, though slightly smaller (JMD unpubl. data); and yet, has an increasing northwestern boreal population (Ferguson 2006) (Fig. 1.3). The white-winged scoter (*Melanitta fusca*; hereafter scoter), is a species of seaduck. It overwinters primarily in coastal marine areas, with a small population wintering in the Great Lakes (Brown and Fredrickson 1997). Scoters do, however, overlap with scaup and ringnecks on breeding grounds; most a large majority of scoters breed in the Canadian western boreal forest (Brown and Fredrickson 1997). Like scaup, the boreal breeding population of scoters has declined by approximately 58% in the boreal forest since the late 1970s (Fig. 1.3) and declines are highly correlated ($r = 0.89$) with those of scaup (Alisauskas et al. 2004, Stuart Slattery, IWWR, personal

communication). Therefore, I attempted to test these two mutually non-exclusive hypotheses (SCH and Contaminants), and to identify possible mechanisms responsible for numerical declines of scaup by comparing energetic strategies and life-history attributes of these three species.

In general, my predictions for the SCH were that boreal collected ringnecks will be in better relative body condition, will have a greater proportion of females developing eggs than scaup, and that egg development will be inversely related to body condition. I predicted that based on the contaminants hypothesis scaup and scoters would have higher concentrations of Se than ringnecks, and there would be an inverse relationship between Se and nutrient stores, and that Se concentrations would be greater in females not producing eggs.

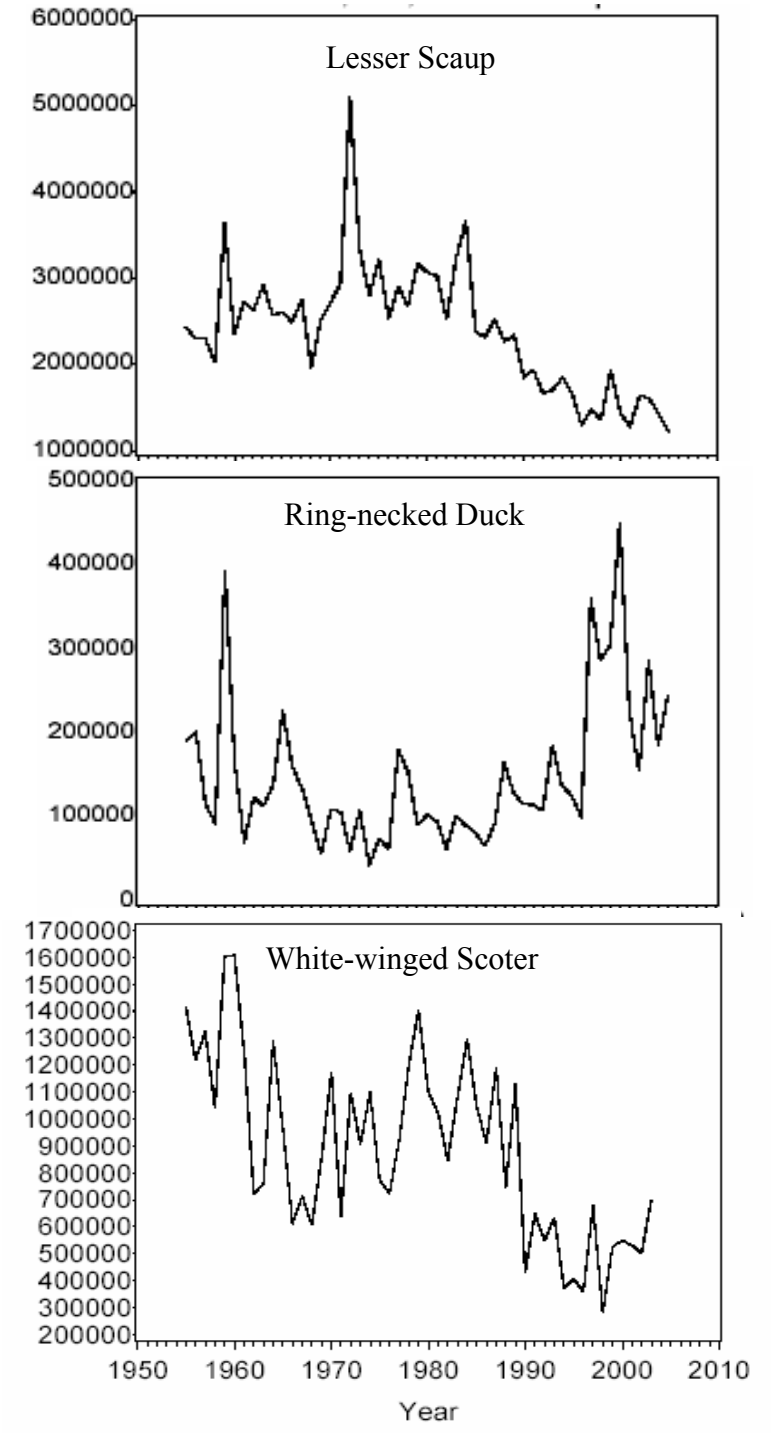

Figure 1.3. Breeding waterfowl survey population estimates of lesser scaup, ring-necked ducks and white-winged scoters from survey strata 13-18, 20 and 77 (Northwestern Canadian Boreal Forest; figures adapted from Ferguson 2006).

1.6 STUDY OBJECTIVES

At the outset of this research there were two non-mutually exclusive hypotheses about scaup declines that emphasized events occurring largely in the boreal forest and a lack of research in this core breeding area. This formed the basis for the first three objectives of this study:

1. To test the SCH and contrast body condition of boreal scaup and ring-necked ducks (Chapter 2)
2. To test the Contaminants Hypothesis and evaluate the relative burdens of selenium in scaup, ring-necked ducks and white-winged scoters (Chapter 3).
3. To compare isotopic signatures of stable-nitrogen, -carbon, and –hydrogen isotopes between scaup and ring-necked duck tissue grown on wintering and staging areas (Chapter 7).

As my research progressed, I identified, with the guidance of my committee, several gaps in my knowledge of selenium ecotoxicology that were essential to my understanding and testing of the contaminants hypotheses. These knowledge gaps led to three additional objectives for my study:

4. To determine the effects of selenium on scaup in a controlled captive setting (Chapter 4).
5. To study selenium dynamics in breeding white-winged scoters (Chapter 5, 6). Because scoters have much higher selenium levels than most other species of ducks, and because they migrate between isotopically distinct wintering and breeding areas, this species provided an ideal system to examine the seasonal dynamics of selenium in waterfowl.

The final data chapter of my thesis examines the reproductive energetics of scoters. There is currently limited information about the reproductive energetics of this species. This information is important for identification of habitats used in the acquisition of reproductive nutrients. I conducted this analysis using both body composition analysis and stable-isotope analysis techniques, and the objective of the study was:

6. To compare the interpretation of body composition and stable-isotope analyses, and to compare results with the only energetics study to date completed at the southern edge of their breeding range (Chapter 8).

2. ARE LATE-SPRING BOREAL LESSER SCAUP (Aythya affinis) IN POOR BODY CONDITION?

2.1 INTRODUCTION

Birds employ a variety of energetic strategies to allocate nutrients required to form eggs and offset other reproductive costs (Meijer and Drent 1999). Optimal reproductive energetic strategies of migratory species have presumably been shaped by costs of migration and food availability, as these and other factors may influence nutrient stores at arrival on breeding sites and the ability of females to obtain nutrients on the breeding grounds. Endogenous nutrients are important for reproductive success in many migratory waterfowl species, and poor body condition has been associated with decreased breeding propensity or reproductive failure (Drent and Daan 1988, Alisauskas and Ankney 1992).

The continental scaup (lesser (*Aythya affinis*) and greater (*A. marila*) combined) breeding population has declined by an estimated 150,000 birds per year between 1978 and 1997 (Afton and Anderson 2001), with a continued decline and lack of recovery over the last decade (Wilkins and Otto 2006). Likewise, the ratio of immatures:adults in harvest data has decreased, indicative perhaps of low breeding productivity (Afton and Anderson 2001). This decline has been attributed primarily to Lesser Scaup as they comprise *c.a.* 89% of the total scaup population (Afton and Anderson 2001), and my study has focused on this species. Lesser Scaup (hereafter scaup) appear to use endogenous nutrient stores for reproduction; somatic lipid stores decrease by approximately 0.50 to 0.67 $g \cdot g^{-1}$ of lipid invested in reproductive tissues (Afton and Ankney 1991, Esler et al. 2001). Thus, one explanation for the scaup decline, the spring condition hypothesis (SCH) (Austin et al. 2000, Afton and Anderson 2001, Anteau and Afton 2004), purports that reproductive success is lower now than in the past due to greater occurrence of poor-condition females arriving on breeding grounds. Poor body condition could manifest itself through decreased breeding propensity, particularly among inexperienced females (Afton 1984), delayed nest initiation or failure to initiate breeding due to increased feeding time to acquire adequate stores (Esler et al. 2001), and

reduced clutch sizes due to delayed nest initiation (Rohwer 1992, Guyn and Clark 1999), all of which could lower productivity.

Anteau and Afton (2004) found support for the SCH by collecting scaup at four locations (1 wintering, 2 staging, and 1 breeding) in 2000-01 and observed that at their two northern-most sites, fresh body mass (FBM) and nutrient content were lower when compared to those of scaup collected at the same locations during 1977-88. Anteau (2006) further suggested that changes in wetland habitats, and associated losses of key foods, in UMW staging areas as being the likely causes of poor scaup body condition. Breeding Waterfowl Population Surveys indicate that in late May approximately two thirds of Lesser Scaup are found in western Canadian boreal forest (Austin et al. 2000, Afton and Anderson 2001), much further north (approximately 800-3500 km) than sites visited by Anteau and Afton (2004), and where scaup may be able to compensate for possible food limitations further south. Survey data analyses also indicate that Canadian boreal forest is the region where declines have been most pronounced (Afton and Anderson 2001). Therefore, I evaluated body condition of boreal scaup using two comparative methods. First, I compared spring body condition of boreal-breeding scaup to that of Ring-necked Ducks (*Aythya collaris*; hereafter ringneck), a species whose main wintering range overlaps with that of scaup (Austin et al. 1998, Hohman and Eberhardt 1998); has more omnivorous dietary preferences except during breeding when both species rely heavily on invertebrates (Austin et al. 1998, Hohman and Eberhardt 1998); has similar reproductive energetic strategy (Alisauskas et al. 1990, Afton and Ankney 1991, Esler et al. 2001, this study); is similar in structural size, though slightly smaller (JMD unpubl. data); and yet, has an increasing northwestern boreal population (Ferguson 2006). If female scaup arriving on boreal breeding grounds are in poor body condition, then I expected that scaup would have lower lipid and protein stores and a lower proportion of breeding females when compared with simultaneously collected ringnecks. Second, I tested the SCH by comparing body masses of female scaup collected at Yellowknife, NT, in 1968-1970 (Trauger 1971), to those of scaup collected in 2003-04. Following the SCH (i.e., Austin et al. 2000, Afton and Anderson 2001, Anteau and Afton 2004), I reasoned that females collected in recent years would be lighter than those sampled 30 years earlier.

2.2 METHODS

2.2.1 Field Collections

In 2003-04, I collected 179 female scaup and 96 female ringnecks without the use of decoys to avoid bias (Pace and Afton 1999) at boreal sites (Slave Lake, Alberta [AB], 55^0N, 115^0W; Yellowknife, Northwest Territories [YK], 62^0N, 113^0W; and Inuvik, Northwest Territories [IN], 67^0N, 133^0W; Table 2.1) varying by 12 degrees in latitude (1800 km linear distance). For most sites/years, I sampled birds during pre-breeding (mid-May at AB to early June at IN) to early breeding (early June at AB to mid-June at IN). Local waterfowl specialists were consulted for information about migration progression, and I targeted small flocks (< 8 birds) and pairs to avoid collecting from migrant flocks. Ringnecks were not collected from IN as the population density was too low. Birds were immediately weighed (nearest 10 g using a 1000 g Pesola spring balance), and frozen before dissection and analysis. Hereafter, "collection" refers to a specific sampling location-period; for example, IN-1 refers to the first collection within a year at the Inuvik site.

Scaup collected on the Yellowknife Study Area during 1968-70 were shot with a 0.22 caliber rifle. Efforts were made to sample birds only from isolated pairs on small ponds to minimize collecting migrants. I used females collected in 1968 beginning on 15 May (day 135) and ending on 22 June (day 173). All birds sampled in 1969 and 1970 were collected beginning on 4 and 6 June, respectively (Table 2.1). Fresh carcasses were weighed to the nearest 5 g using a Chatillion 1000 g spring balance.

2.2.2 Carcass Analysis

I thawed birds overnight and reweighed them before taking the following structural measurements: body length (excluding retrices, birds placed on ruler and measured to tip of bill with neck extended; nearest 1 mm), wing chord (nearest mm with a 300 mm wing ruler), total tarsal length (nearest 0.1 mm with digital calipers), and exposed keel length (nearest 0.1 mm with digital calipers). I excised and weighed (nearest 0.01 g with a digital scale) one breast muscle, muscles of one leg, empty gizzard, abdominal fat, and gastro-intestinal tract contents (hereafter, ingesta).

I estimated individual lipid and protein content in subsamples of 35 scaup and 28 ringnecks systematically stratified across the range of body mass of each species. Liver, breast and leg muscles were dried to constant mass in a drying oven at 80^0C (Alisauskas et al. 1990). The remaining carcass, less reproductive organs (oviduct and ovaries/testes) and ingesta, was homogenized using a Hobart industrial bowl chopper and meat grinder with 5 mm plates. A sample of the homogenate was also dried to constant mass and reweighed. All dried samples were individually ground using an electric coffee mill, washed for 6 hr using petroleum ether in a modified Soxhlet apparatus to remove lipids, and then lean samples were dried and reweighed to estimate lipid mass. Total lipids were estimated based on extracted lipid masses, and I used lean dry breast muscle mass (two times excised breast), lean dry leg muscle mass (two times excised leg), and lean dry gizzard mass as an index of protein content as these are the major stores of metabolically active protein (mean dry muscle mass was 25% of fresh muscle mass). For each species, estimates of total lipids (somlip) and abdominal fat mass (square root transformed; squabfat) from the subsample of birds were used to derive predictive equations for the remaining birds not subjected to the above proximate analysis techniques (Fig. 2.1).

2.2.3 Gonad Analysis

Reproductive organs were removed during dissections and weighed (nearest 0.01 g with a digital scale). Ovaries were examined for signs of rapidly growing follicles (RFG), post-ovulatory follicles (POF) and damaged follicles. All RFG follicles and oviducal eggs were removed, weighed individually, and dried to constant mass as above. The remaining non-developing follicles and oviduct were individually weighed, dried and re-weighed. The dry oviduct was assumed all protein and discarded (Alisauskas et al. 1990). The ovaries but not follicles were ground with a coffee grinder and each was then washed with petroleum ether to extract lipids, and subsequently dried and reweighed to estimate lipid and determine lean dry mass (LDM), which I assumed was protein (Alisauskas et al. 1990). In laying females, I counted the number of eggs laid based on the number of POFs observed and assumed that each laid egg consisted of 6.82 g of lipids and 6.91 g protein in scaup (Afton and Ankney 1991) and 7.15 g of lipid and 6.79 g of protein in ringnecks (Alisauskas et al. 1990). In scaup, 38 of 347 developing follicles and

in ringnecks 27 of 153 were damaged during sampling. I determined the damaged follicle rank in the series based on size of the membrane and undamaged follicles. Then, using the following species-specific least-squares quadratic regressions of adjacent RFG follicles (where $f_{lipid+1}$ and f_{LDM+1} are the follicle lipid mass and LDM, respectively, and f_{lipid} and f_{LDM} are the lipid mass and LDM, respectively, of the next-smallest follicle), I estimated follicle lipid and LDM of damaged follicles:

Scaup,

$$f_{lipid+1} = 0.268 + 2.152*f_{lipid} - 0.159*f_{lipid}^2 \quad (n = 41, r^2 = 0.98, P < 0.001) \qquad (2.1)$$

$$f_{LDM+1} = 0.267 + 1.912*f_{LDM} - 0.112*f_{LDM}^2 \quad (n = 41, r^2 = 0.97, P < 0.001), \text{ and} \qquad (2.2)$$

Ringnecks,

$$f_{lipid+1} = 0.238 + 2.419*f_{lipid} - 0.278*f_{lipid}^2 \ (n = 40, r^{2=} 0.95, P < 0.001) \qquad (2.3)$$

$$f_{LDM+1} = 0.185 + 2.181*f_{LDM} - 0.246*f_{LDM}^2 \quad (n = 40, r^2 = 0.95, P < 0.001) \qquad (2.4)$$

I summed lipids and LDM of all reproductive tissues to estimate total reproductive lipids and protein.

Female breeding status (non-RFG vs. RFG) at time of collection was determined by the largest RFG follicle mass. As scaup and ringnecks produce eggs of near identical mass and composition, in both species follicles with dry mass > 0.1 g were assumed to have initiated RFG and be indicative of a female attempting reproduction (Alisauskas et al. 1990). Non-RFG females did not show signs of rapid follicular growth at the time of collection, but might have initiated follicular growth at a later date.

2.2.4 Statistical Analysis

I modeled lipid and protein content in scaup and ringnecks using ANCOVA in JMP (SAS Institute, Cary, NC, USA). All other statistical analyses were conducted using SPSS version 13.0 (Norušis 1990). The first principal component (PC1) extracted from principal component analysis (PCA) of the correlation matrix of the four structural measurements was used to derive an index of body size (size) for both species combined. To test the assumption that scaup and ringnecks have similar patterns of nutrient reserve use, I ran General Linear Models (GLM) of somatic nutrients stores over nutrient estimates of reproductive tissues (Alisauskas et al. 1990).

I tested for an interspecific difference in somatic lipid or protein investment in reproductive tissues between RFG scaup (n = 94) and ringnecks (n = 43) using an interaction term of species*repro lipid or species* repro protein, respectively, in a GLM while controlling for size differences. I use GLMs to test for effects of year, species, breeding status (RFG and non-RFG) and collection date (date standardized to year, 2004 was a leap-year) on endogenous lipid and protein estimates from scaup and ringnecks. I also used an interaction term, species*breeding status, to test for differences between species in RFG and non-RFG, and the interaction of species*collection date to test for differences in nutrient content over time between species. I also ran a full GLM including the above parameters as well as year and year*collection date, but found no significant effect (Ps > 0.05) of year or the year*collection date interaction, and so dropped these parameters to reduce model complexity. I estimated lipid and protein by breeding status for each species using least-square (LS) means based on the above GLM.

I used binary logistic regression to model the effect of species, collection date, protein, lipid and two-way interactions between species and the other covariates on breeding status in both species combined.

I used analysis of covariance (ANCOVA) to test for an effect of year or year*collection date interaction on FBM from 1968 (n = 59), 1969 (n = 9), and 1970 (n = 11), 2003 (n = 30) and 2004 (n = 36) while controlling for date of collection. I only used female scaup collected over the same range of dates (24 May – 22 June) for this analysis. Statistical significance was evaluated against an $\alpha = 0.05$.

2.3 RESULTS

Because of damage to tissues during collections, I was not able to take all structural measurements or assess reproductive status for 5 scaup and 9 ringnecks; as a result, these birds were not used in analyses controlling for size. All PC1 variable loadings were positive and ranged from 0.19 to 0.87. PC1 explained 50% of the total variation in the size measurements for scaup and ringnecks combined. Somatic lipid declined significantly with investment in reproductive tissues in both scaup ($\beta = -0.70 \pm 0.17$ [SE], $r^2 = 0.145$ $P < 0.001$) and ringnecks ($\beta = -0.834 \pm 0.35$ [SE], $r^2 = 0.189$ $P =$

0.006), but were similar between species. There was no decline in somatic protein with reproductive investment in either species scaup (β = -0.02 ± 0.03 [SE], r^2 < 0.001 P = 0.93) or ringnecks (β < 0.01 ± 0.04 [SE], r^2 < 0.001 P = 0.89). I found no effect of species*reproductive lipid on somatic lipid reserves (GLM; $F_{1,132}$ = 0.02, P = 0.90) or of species*reproductive protein on somatic protein reserves (GLM; $F_{1,132}$ = 0.22, P = 0.64). This indicated that there was no interspecific difference in the slope of somatic nutrient loss with investment in reproductive nutrients, lipid or protein.

My interspecific comparison of nutrient levels indicated that there was an effect of species, date of collection and breeding status on both lipid and protein stores (P's < 0.010), but no effect of species interactions (Ps > 0.61) after controlling for size (Table 2). LS mean estimates for lipid and protein contents from our GLMs indicated that scaup had on average 12.5 ± 3.7(SE) g greater lipid but 1.6 ± 0.5 g less protein than did ringnecks (Ps < 0.001). After controlling for date of collection, in each species RFG females had greater lipid and protein than did non-RFG birds, and scaup of either breeding status had more lipid but less protein than did ringnecks of similar breeding status (Ps < 0.001; Table 3).

Logistic regression of breeding status further indicated that follicular development was associated with females that had more somatic lipid (χ^2 = 9.74, P = 0.002) and protein (χ^2 = 9.02, P = 0.003), and that were collected at later dates. Rapid follicular growth in ringnecks began earlier than in scaup (Fig. 2.2), as indicated by the significant species*collection date interaction (χ^2 = 3.98, P = 0.046) term. The pattern of follicular development differed between scaup and ringnecks in that many female scaup appeared to have had adequate nutrient levels to start egg production before day 155 (3 June), but few (6%) had initiated follicular growth by that date; most (90%) had developing follicles after that date (Fig. 2). Ringnecks, however, had a larger variance in the timing of breeding and a large percentage of ringnecks were in RFG before and after day 155 (42% and 79%, respectively; Fig. 2).

When I restricted the analysis to the YK site and scaup only and controlled for collection date, no effects of year (ANCOVA; $F_{4,121}$ = 1.75, P = 0.14) or year*collection date (ANCOVA; $F_{4,121}$ = 1.49, P = 0.21) on FBM were detected for females collected in 1968-70 and 2003-04. Date of collection was a significant predictor (ANCOVA; $F_{1,121}$ =

5.35, $P = 0.023$) in this model; FBM increased over time (Figure 3). FBM includes reproductive tissues that, on average, began developing in scaup on 6 June (day 157) at our YK site in 2003 and 2004.

Table 2.1. Collection dates and sample size of female Lesser Scaup (LESC) and Ring-necked Ducks (RNDU) from three collections sites in 2003-04 and from Yellowknife, NT, in 1968-70.

		2003		2004		1968[a]	1969[a]	1970[a]
		1st	2nd	1st	2nd			
AB	LESC	16	.	20	14	.	.	.
	RNDU	22	.	21	.	.	.	.
	Dates[b]	139-141	.	136-142	161	.	.	.
YK	LESC	24	6	20	16	50	9	11
	RNDU	18	7	18	10	.	.	.
	Dates[b]	144-149	167-170	146-150	166-173	135-173	155-173	157-173
IN	LESC	24	.	20	19	.	.	.
	Dates[b]	159-163	.	155-158	167-169	.	.	.

[a] - All birds were collected on the Yellowknife study area, NT

[b] – Range of collection dates standardized for leap years (1968, 2004) - May 1 =121

Table 2.2. Results of general linear models testing for the effects of species, breeding status, year, collection date, size, and interactions on lipid and protein content of both Lesser Scaup (n = 174) and Ring-necked Ducks (n = 87). Significant model parameters are denoted with an asterisk.

Model	Predictor	*df*	*F*	*P*
Protein	Intercept	1	465.59	< 0.001*
	Species	1	13.49	<0.001
	Breeding Status	1	37.78	< 0.001*
	Julian Day[a]	1	6.72	0.010*
	Size[b]	1	10.63	<0.001
	Species*Breeding Status	1	0.17	0.682
	Species*Julian Day	1	0.01	0.905
Lipid	Intercept	1	113.10	<0.001*
	Species	1	12.08	<0.001
	Breeding Status	1	29.10	<0.001*
	Julian Day[a]	1	60.20	<0.001*
	Size[b]	1	0.25	0.614
	Species*Breeding Status	1	0.00	0.956
	Species*Julian Day	1	0.26	0.612

[a] - 2004 Julian Day was standardized for leap year

[b] - size was calculated using LESC and RNDU combined

Table 2.3. Least-squares mean lipid and protein estimates for non-RFG and RFG female Lesser Scaup and Ring-necked Ducks while controlling for year, collection date, and body size index.

		Lipid (g)	SE	n	Protein (g)	SE	n
Non-RFG	Scaup	61.7	2.8	80	51.7	0.4	80
	Ringneck	49.3	3.8	44	53.2	0.5	44
RFG	Scaup	80.5	3.2	94	54.2	0.4	94
	Ringneck	67.8	3.5	43	55.9	0.4	43

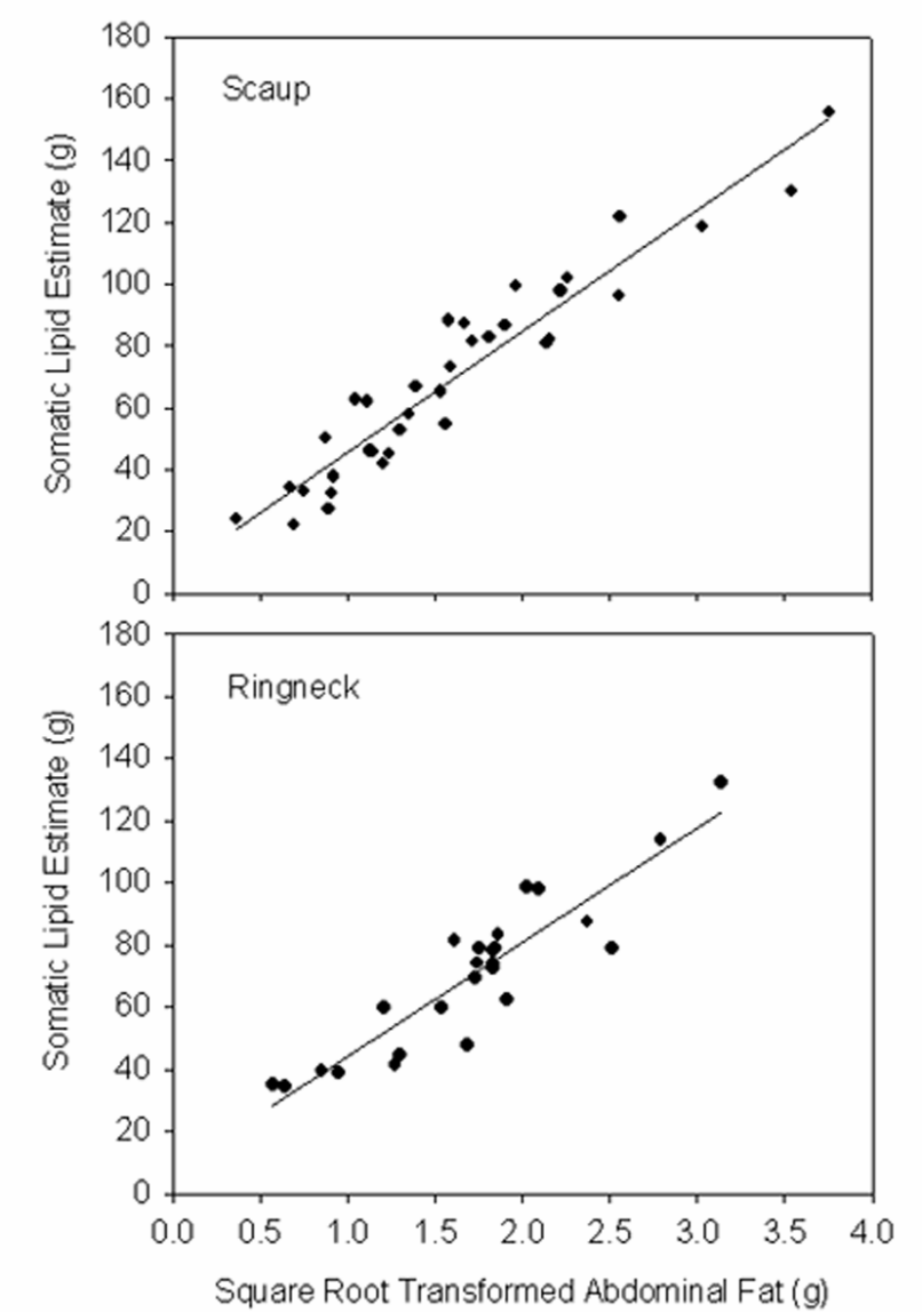

Figure 2.1. Scatterplots and linear regressions of square-root transformed abdominal fat as a predictor of total somatic lipids determined through lipid extraction techniques in Ring-necked Ducks (Somlip = 7.217 + 36.916*squabfat; $n = 28$, $r^2 = 0.83$, $P < 0.001$) and Lesser Scaup (Somlip = 6.898 + 38.980*squabfat; $n = 35$, $r^2 = 0.91$, $P < 0.001$).

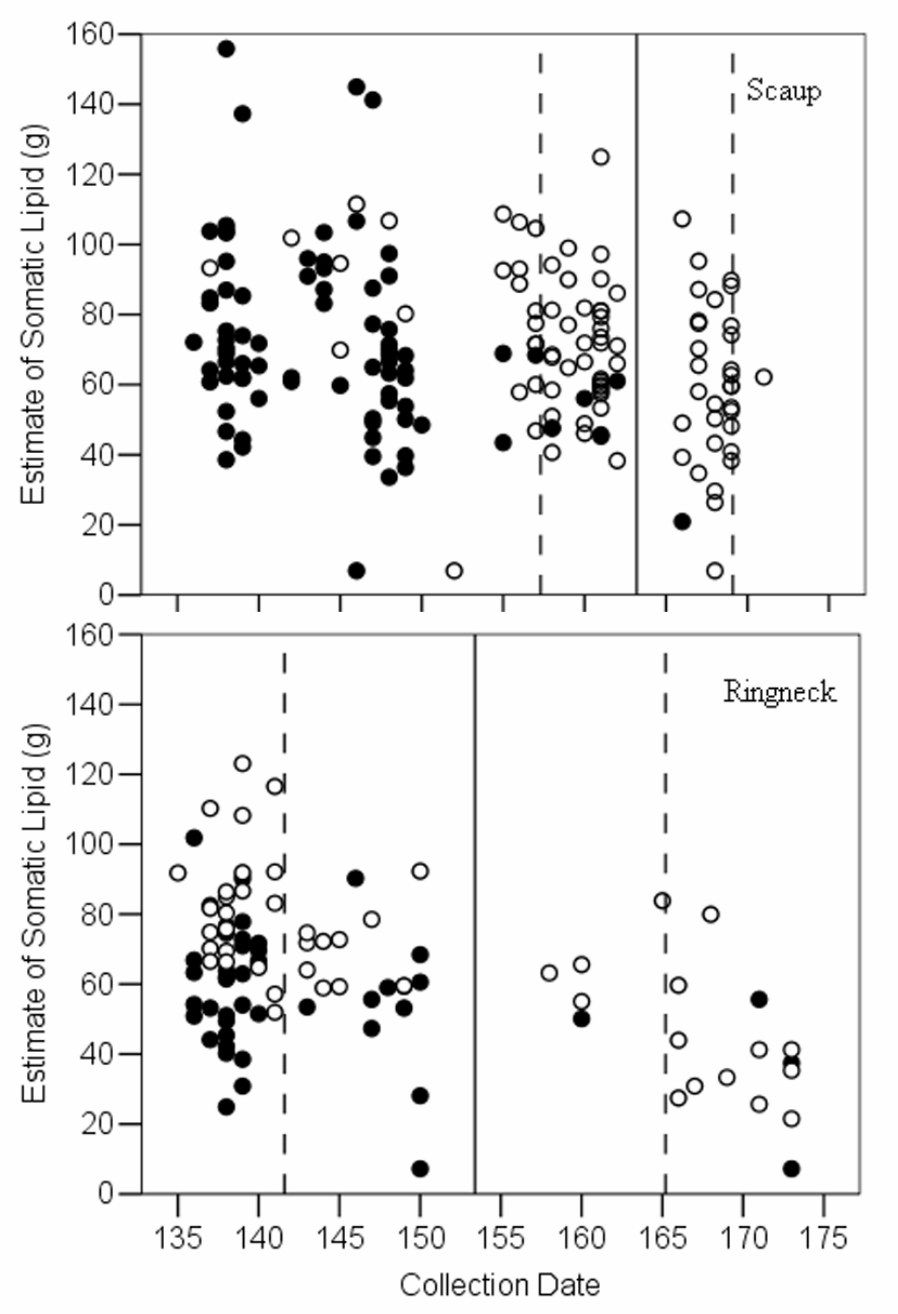

Figure 2.2. Estimates of somatic lipid mass for female Lesser Scaup and Ring-necked Ducks in rapid follicular growth (open circles) and not in non rapid follicular growth (closed circles) at the time of collection, 2003-2004. Reference lines denote mean nest initiation date (solid line) and SD (dashed lines) based on estimated first egg date of females with developing follicles.

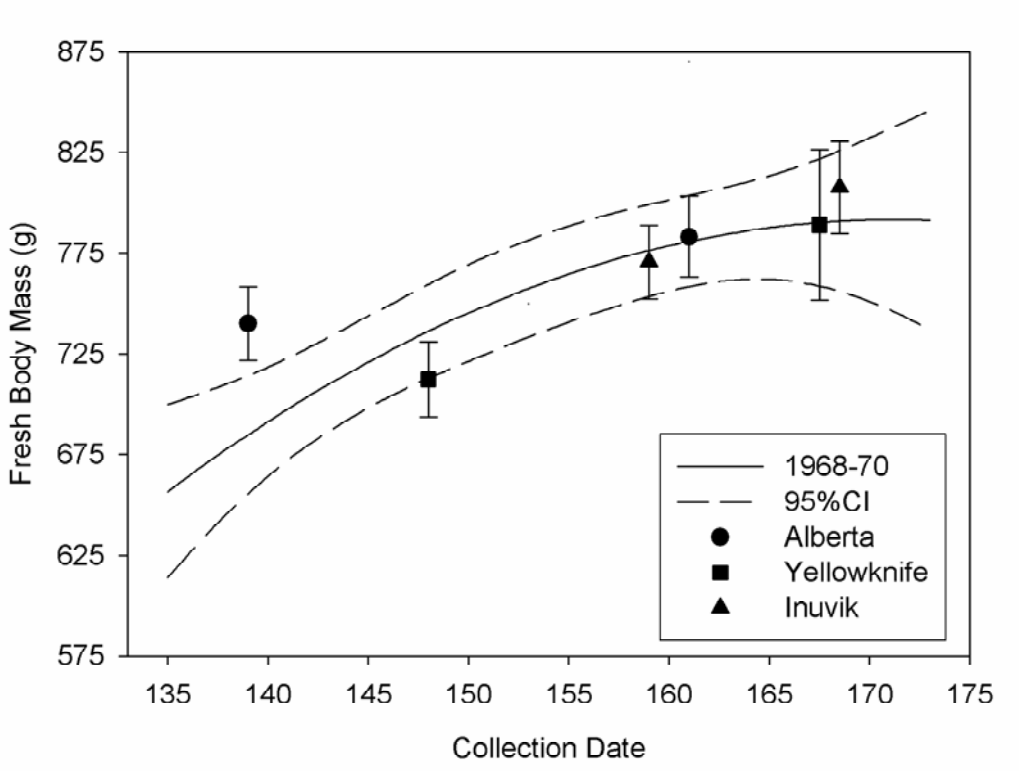

Figure 2.3. LSmean (± 95% CI) fresh body mass (controlled for collection date) by mean collection date of the 2003-04 females for the two collections from each of the three sites (Alberta, Yellowknife and Inuvik) and the quadratic regression and 95% CI (F = 12.78, P < 0.001, R^2 = 0.35, n = 70) of the 1968-70 Lesser Scaup fresh body mass from females collected between 15 May and 22 June at Yellowknife.

2.4 DISCUSSION

I used two approaches to evaluate the relative body condition of scaup from the boreal breeding grounds, an area where the greatest scaup declines have occurred and where the majority of scaup breed (Austin et al. 1998, Afton and Anderson 2001). First, body condition and breeding status of scaup and ringneck collected simultaneously at the same sites were similar (after controlling for collection date, breeding status and interspecific differences in structural size). Scaup had more lipid and slightly less protein than did ringnecks. I also found no difference between rates of lipid loss with reproductive investment in female scaup and ringnecks, consistent with previous reports of nutrient use for reproduction, though estimates were slightly higher than other reports (Alisauskas et al. 1990, Afton and Ankney 1991, Esler et al. 2001). Mean egg weight (48 g) and composition are virtually identical in scaup and ringnecks (Alisauskas et al. 1990,

Afton and Ankney 1991), and reported average clutch size in both species ranges from 8-10 eggs (Austin et al. 1998, Hohman and Eberhardt 1998). There was similar intraspecific difference in lipid and protein content in RFG and non-RFG females (Table 2.3), which suggests that, compared to RFG females in each species, non-RFG scaup and ringnecks had comparable body condition. Differences in nutrient levels among RFG and non-RFG females suggest a nutrient-limitation threshold for breeding (Esler et al. 2001), which is an important premise of the SCH (Anteau and Afton 2004). However, since there was little difference in lipid content between non-RFG scaup and RFG ringnecks, many non-RFG scaup likely had sufficient lipid to attempt reproduction given that this nutrient likely limits reproduction in scaup (Esler et al. 2001), yet they showed no physiological sign of breeding at the time of collection. The inability to predict whether these non-RFG females in good body condition would have later developed eggs is the greatest limitation of this study design. Despite carrying less protein on average, this nutrient likely does not constrain reproduction given that invertebrate diets have a estimated protein:lipid ratio of approximately 14:1 (Ankney and Afton 1988). Overall, my body condition results for boreal scaup do not suggest a nutrient constraint on breeding, and scaup were not in poor body condition compared to ringnecks.

I also found a similar ratio of RFG:non-RFG females in both species (Fig. 2.2). In my sample, ringnecks initiated RFG on average 9 d earlier than did scaup, but ringnecks also had a wider range of onset of RFG. I observed RFG female ringnecks in my first AB collections in mid-May and only one occurrence of RFG scaup, despite many scaup having equal or more somatic lipid reserves (Fig. 2.2). Given that non-RFG scaup had fewer nutrient reserves than RFG scaup and that few RFG females were collected in my earlier samples, nest initiation may have been later than normal due to insufficient nutrient stores. However, given that reported mean nest initiation in scaup is around mid-June regardless of breeding latitude, biome, and era (Murdy 1964, Hammell 1973, Hines 1977, Afton 1984, Grand 1995, Brook 2002, Koons and Rotella 2003, Stuart Slattery, IWWR, unpubl. data, JMD unpubl. data for captive scaup), perhaps it is not surprising that scaup I collected during May in AB and YK were not yet developing follicles. However, due to the destructive nature of the technique used, this information cannot be determined.

The inability to determine an individual's future breeding status and body condition once collected is the greatest limitation in destructive studies. I interpreted results based on the breeding status of the females at the time of collection and, as expected, my first collections were conducted just prior to the onset of RFG in most scaup (Fig. 2.2). Therefore, it is difficult for me to assess what proportion of early-collected scaup would have bred later on, a problem which applies equally to ringnecks. Because scaup nested later than ringnecks, I may have underestimated the number of breeding scaup to a greater extent than ringnecks. A second limitation is that I did not collect from large flocks of scaup in order to avoid sampling migrating birds, which could have biased my sample towards breeders. However, this criticism would also apply to ringnecks given that both species used the same wetlands, and that group size distributions of scaup and ringnecks were similar in spring boreal surveys (See Appendix A). Nonetheless, I caution against use of these data as true estimates of breeding propensity in either species.

I conducted a direct test of the SCH by comparing scaup body masses from my contemporary YK sample to those of scaup collected from the YK site in 1968-70 with the same range of collection dates (24 May – 22 June). Historical data were obtained when scaup population estimates were increasing, before peak levels were recorded in the 1970s, and should therefore be an appropriate period to use for comparisons to contemporary data. Contrary to the SCH, I found no effect of year or year*collection date interaction on FBM. I did, however, observe one exceptionally low mean FBM in the 2004 YK-1 collection. I believe the low body mass was directly related to cold, late spring conditions at Yellowknife in 2004 where many ponds remained frozen until late May. Mean daily temperatures for 31 days prior to my mean collection date at YK-1 (27 May) for 1968-70, 2003, 2004, and the long-term (1971-2000) average were 2.2, 3.0, -3.9, and 3.5^0C, respectively (Environment Canada's Meteorological Service Online Data). One limitation with comparing FBM is that it could also represent variation in levels of ingesta, lipid and protein stores, and plumage. However, FBM was a good predictor of lipid mass variation (linear regression; $F_{1,33} = 23.747$; $P < 0.001$, $r^2 = 0.42$, $\beta = 0.312 \pm 0.064$ SE), the nutrient that likely limits onset of egg formation in scaup.

There were some methodological differences in collection techniques employed between eras. In 1968-70, isolated scaup pairs were targeted and these were probably

breeding pairs. In 2003-04 I collected paired scaup and from groups of up to 8 birds. If this sampling difference had an effect on my results, it should have resulted in lower body mass in the 2003-04 collections given the mass differences between RFG and non-RFG females (Table 2.3). However, this was not the case.

One important assumption in my temporal comparison is that there has been no significant change to the spatial population composition of scaup where non-breeders no longer migrate to boreal breeding grounds. Although I am unaware of any evidence for this possibility, it should be examined in future work. One potentially confounding factor with the between era comparisons of Anteau and Afton (2004, 2006) is the difference in timing of collections at the Manitoba site. In Anteau and Afton (2004: Table 2.1) the range of collection dates for the contemporary sample (30 April – 15 May) is earlier than the historical data (13 May – 23 June). Given that I observed an increase in body mass over time (Fig. 2.3) and a positive relationship between FBM and nutrients, the reported difference in FBM and nutrients by Anteau and Afton (2004) could be attributable to differences in collection timing. A comprehensive assessment of all body mass data relative to a common biological process such as the timing of nest initiation would provide a better indication of the variability in FBM among studies, and may help determine if there have been consistent shifts in scaup nutrient levels.

When should birds deposit somatic nutrient stores? Our interspecific comparison of scaup and ringneck nutrient levels suggest scaup are not in poor condition and my temporal comparison of FBM failed to support the SCH. I therefore question the ability of this hypothesis to explain scaup declines in the boreal forest. While scaup staging habitat has undergone landscape level changes in the UMW (Anteau 2006), it is possible they have shifted to using other staging areas; a significant increase in staging scaup was observed on the Great Lakes between 1986-1997 (Petrie and Knapton 1999). It is also possible that scaup migrating through these poor habitats are capable of acquiring reproductive nutrients once on the breeding areas before they would normally breed in mid-June. I observed a substantial increase (Δ mean = 130 g; 6.5 g/day) in FBM between YK-1 and YK-2 collections in 2004. Given that 7 of 10 marked scaup observed during the first collection on the YKSA remained on site during my second collection (Robert

Clark, Environment Canada, unpubl. data), I believe the observed increase in mass was due to mass gain by females.

Despite scaup and ringnecks migrating earlier than they did historically (Murphy-Klassen et al. 2005), mean NID in scaup, though variable, has shown no pattern of directional change in locations where multiple studies have occurred over a period of decades (Yellowknife, NT: 7-13 June (Toft et al. 1984), 19, 21 June (Brook 2002), 12 June (this study); Erickson, MB: 25 June (Hammell 1973), 15 June (Afton 1984), 24 June (Koons and Rotella 2003). Yet, I estimated mean NID in YK collected ringnecks at 7 June (Fig. 2.2), which is much earlier than previously reported from 1962-65 (*c.a.* 18-30 June) for YK (Toft et al. 1984). Although data are limited, this earlier nest initiation suggests that ringnecks may be more flexible in the timing of reproduction.

Numerical declines of scoters (all three species combined) mirror that of scaup in the boreal forest (Ferguson 2006) and they are equally late nesters throughout their range (Brown and Frederickson 1997), yet scoters overwinter in different areas than scaup. If they share a common cause of decline, a breeding ground origin of this cause is the most parsimonious explanation. Photoperiod thresholds to initiate breeding have been described for several bird species (see Cockrem 1995). The limited latitudinal variation in mean NIDs (Murdy 1964, Hammell 1973, Hines 1977, Afton 1984, Grand 1995, Brook 2002, Koons and Rotella 2003, Stuart Slattery, IWWR, unpubl data, JMD unpubl. data) and the abrupt onset of rapid follicular growth in scaup from a range of sites (Fig. 2.2) is consistent with a fixed cue threshold for timing of reproduction (Cockrem 1995). This late nesting strategy in scaup was also proposed as a food dependent trait for ducklings (Dawson and Clark 1996). Therefore, a dependence on photoperiod as a breeding cue could limit the ability of scaup and possibly scoters to adjust timing of nest initiation with changes in climate and invertebrate phenology (Hogg and Williams 1996), thus leading to a mismatch in food requirements and availability (Cushing 1974, Thomas et al. 2001, Schlaepfer et al. 2002, Corcoran 2005). This "Mismatch Hypothesis" should be tested, particularly in the boreal forest where timing of food availability is critical to both hens and ducklings during the short breeding seasons.

3. IS SELENIUM AFFECTING BODY CONDITION AND REPRODUCTION IN BOREAL BREEDING SCAUP, SCOTERS, AND RING-NECKED DUCKS?

3.1 INTRODUCTION

The continental scaup (lesser (*Aythya affinis*) and greater (*A. marila*) combined) breeding population declined by c.a. 150,000 birds/year between 1978 and 1997 (Afton and Anderson 2001), and the 2006 breeding population estimate (3.2 ± 0.2 million) was the lowest since surveys began in 1955 (Wilkins and Otto 2006). The contaminant hypothesis, which purports that acquired contaminants have negatively affected scaup survival or productivity, has been proposed as one cause for population decline and lack of recovery (Afton and Anderson 2001, Austin et al. 2000).

Selenium (Se) is an essential micro-nutrient required in small quantities for normal biological function, but can be toxic to vertebrates at concentrations slightly over essential levels, although toxicity levels vary among species (Heinz et al. 1989, Ohlendorf 2003, Spallholz and Hoffman 2002). It can be enriched in the environment through burning of fossil fuels, smelting of ores, and irrigation of seleniferous soils (Barceloux 1999, Haygarth 1994, Ohlendorf 2003). Selenium has been identified as the cause of mortality and high reproductive failure in a variety of aquatic birds at the Kesterson National Wildlife Refuge, where it accumulated in evaporation ponds receiving irrigation drainage water from seleniferous soils (Hoffman et al. 1988, Ohlendorf et al. 1986a). Adult birds affected by selenosis can exhibit various conditions, but can often show no apparent signs other than emaciation (Ohlendorf 1996, O'Toole and Raisbeck 1997, Wobeser 1997). Developing embryos, however, are the most sensitive developmental stage (Heinz et al. 1989, Heinz and Hoffman 1998, Stanley et al. 1994).

High Se concentrations in livers of wintering or spring staging scaup have been detected in San Francisco Bay (Hothem et al. 1998), the southern Great Lakes (Custer and Custer 2000), and the Mississippi alluvial valley (Custer et al. 2003). Indeed, hepatic Se levels detected in wintering scaup were commonly above threshold levels for reproductive impairment in captive mallards (*Anas platyrhynchos*) (3 mg/kg wet wt or

approximately 11 mg/kg dry wt), and occasionally above adult physiological impairment levels (10 mg/kg wet wt, approximately 33 mg/kg dry wt) (Custer and Custer 2000, Custer et al. 2003, Heinz 1996). Yet, two recent studies conducted on scaup breeding grounds found normal or background (4-10 mg/kg dw in liver; Heinz, 1996) Se levels in blood, liver and eggs of breeding birds (Fox et al. 2005, Rocque et al., USFWS unpubl. data). This discrepancy between studies of wintering or staging birds and breeding birds, and uncertainty about whether non-breeding scaup have been adequately represented in breeding ground contaminant studies leaves the possibility that Se exposure at Se-enriched wintering and staging areas may be increasing the numbers of scaup that return to breeding areas but fail to reproduce.

Here I test the contaminants hypothesis by comparing Se levels in scaup on spring arrival at boreal breeding areas and during early egg laying with those of ring-necked ducks (*Aythya collaris*; hereafter ringneck) and white-winged scoters (*Melanitta fusca*; hereafter scoter). The latter two species were chosen for comparison because ringnecks are closely related to scaup, but their numbers are increasing in the boreal forest, and because scoters are distantly related, winter in different habitat than scaup and ringnecks, yet are declining at a rate that is highly correlated ($R = 0.89$) to that of scaup (Stuart Slattery unpub data). Scoters may also be affected by Se because they mostly winter in marine environments, which are naturally Se enriched (Haygarth 1994, Ohlendorf 2003). In addition to Se, I measured liver mercury (Hg) due to its ability to interact with and affect the toxicity of Se in-vivo (Cuvin-Aralar and Furness 1991, El-Begearmi et al. 1977). If Se is contributing to low scaup populations, I reasoned that Se concentrations in scaup and scoter livers and eggs/follicles would be above known effect levels, and greater than those of ringnecks. Further, if high Se concentrations reduce breeding propensity in adult females, I predicted a negative relationship between Se and breeding status of females and a negative relationship between Se and nutrient (lipid and protein) levels in scaup and scoters, because Se can cause emaciation and endogenous nutrients are important for waterfowl reproduction (Alisauskas and Ankney 1992).

3.2 METHODS

3.2.1 Field Collections

In 2003-04, I collected totals of 71 female scaup, 42 ringnecks, and 50 scoters without the use of decoys to avoid decoy bias (Pace and Afton 1999) in the vicinity of three boreal sites (Slave Lake, Alberta [AB]; Yellowknife, Northwest Territories [YK]; and Inuvik, Northwest Territories [IN]) varying by 11 degrees in latitude (1800 km linear distance). For most sites/years, I collected birds during pre-breeding (mid-May at AB to early June at IN) and early breeding (early June at AB to mid-June at IN). Local waterfowl specialists were consulted for information about migration progression, and I targeted small flocks (< 8 birds) and pairs to avoid collecting migrants. Ringnecks were not collected from IN, and scoters were not collected from AB or YK as population densities were too low. Birds were immediately weighed (nearest 10 g using a 1000 g Pesola spring balance), and frozen prior to dissection and analysis. Hereafter, "collection" refers to a specific sampling location-period; for example, IN-1 refers to the first collection within a year at the Inuvik site.

3.2.2 Carcass Analysis

I thawed birds overnight and reweighed them before taking the following structural measurements: length (nearest 1 mm with a ruler), wing chord (nearest mm with a 300 mm wing ruler), tarsal length (nearest 0.1 mm with digital calipers), total head length (0.1 mm with digital calipers), and keel length (nearest 0.1 mm with digital calipers). I excised and weighed (nearest 0.01 g with a digital scale) one breast muscle, muscles of one leg, empty gizzard, abdominal fat, and gastro-intestinal tract contents (hereafter, ingesta).

I estimated a female's lipid and protein contents (see methods below) in all scoters and in subsamples of 35 scaup and 28 ringnecks stratified across the range of body mass of each species. Liver, breast and leg muscles were dried to constant mass in a drying oven at 80^0C (Alisauskas et al. 1990). The remaining carcass, less reproductive organs (oviduct and ovaries/testes) and ingesta, was homogenized using a Hobart industrial chopper and meat grinder with 5 mm plates. An aliquot of the homogenate was also dried to constant mass and reweighed. All dried samples were individually ground

using an electric coffee mill, washed for 6 hr using petroleum ether in a modified Soxhlet apparatus to remove lipids, and then lean samples were dried and reweighed to estimate lipid mass (Alisauskas et al. 1990). Total lipids were estimated based on extracted lipid masses, and I used dry breast muscle mass (two times excised breast), dry leg muscle mass (two times excised leg), and dry gizzard mass as an index of protein content as these are the major stores of metabolically active protein (mean dry muscle mass was 25% of fresh muscle mass). For scaup and ringnecks, values of total lipids (totlip) and square root transformed abdominal fat mass, which I am calling squabfat, from the subsample of birds were used to derive predictive equations to estimate total lipids for the remaining females not subjected to the above proximate analysis techniques using the following equations:

Scaup, Totlip = 6.898 + 38.980*squabfat (n = 35, r^2 = 0.91, P < 0.001), and (3.1)

Ringnecks, Totlip = 7.217 + 36.916*squabfat (n = 28, r^2 = 0.83, P < 0.001) (3.2)

3.2.3 Gonad Analysis

Reproductive organs were removed during dissections and weighed (nearest 0.01 g with a digital scale). Ovaries were examined for signs of follicles undergoing rapid follicular growth (RFG), post-ovulatory follicles (POF) and damaged follicles. All yolky follicles and oviducal eggs were removed, weighed individually, and dried to constant mass as above. The remaining non-developing follicles and oviduct were individually weighed, dried and re-weighed. The dry oviduct was assumed to be all protein and was discarded (Alisauskas et al. 1990). The ovaries but not follicles were ground with a coffee grinder and were then washed with petroleum ether to extract lipids, and subsequently dried and reweighed to estimate lipid and determine lean dry mass (LDM), which I assumed was protein (Alisauskas et al. 1990). In laying females, I counted the number of eggs laid based on the number of POFs observed and assumed that each laid egg consisted of 6.82 g of lipids and 6.91 g protein in scaup (Afton and Ankney 1991) and 7.15 g of lipid and 6.79 g of protein in ringnecks (Alisauskas et al. 1990), and 13.7 g of lipid and 14.3 g of protein in scoters (based on regression equations in Alisauskas and Ankney 1988). As some follicles undergoing RFG were damaged during sampling, I determined their rank in the series based on size of the membrane and undamaged

follicles. I estimated follicle lipid and LDM of damaged follicles using the following species-specific least-squares quadratic regressions of adjacent RFG follicles, where $f_{lipid+1}$ and f_{LDM+1} are the follicle lipid mass and LDM, respectively, and f_{lipid} and f_{LDM} are the lipid mass and LDM, respectively, of the next-smallest follicle:

Scaup,

$$f_{lipid+1} = 0.268 + 2.152*f_{lipid} - 0.159*f_{lipid}^2 \quad (n = 41, r^2 = 0.98, P < 0.001) \tag{3.3}$$

$$f_{LDM+1} = 0.267 + 1.912*f_{LDM} - 0.112*f_{LDM}^2 \quad (n = 41, r^2 = 0.97, P < 0.001), \text{ and} \tag{3.4}$$

Ringnecks,

$$f_{lipid+1} = 0.238 + 2.419*f_{lipid} - 0.278*f_{lipid}^2 \ (n = 40, r^{2\,=}\ 0.95, P < 0.001) \tag{3.5}$$

$$f_{LDM+1} = 0.185 + 2.181*f_{LDM} - 0.246*f_{LDM}^2 \quad (n = 40, r^2 = 0.95, P < 0.001) \tag{3.6}$$

Scoters,

$$f_{lipid+1} = 0.732 + 1.966*f_{lipid} - 0.120*f_{lipid}^2 \ (n = 41, r^{2\,=}\ 0.95, P < 0.001) \tag{3.7}$$

$$f_{LDM+1} = 0.546 + 2.019*f_{LDM} - 0.165*f_{LDM}^2 \quad (n = 41, r^2 = 0.94, P < 0.001) \tag{3.8}$$

Follicles removed for Se analysis below were accounted for in reproductive nutrient estimates using the above equations. Female breeding status (non-breeding vs. breeding) at time of collection was determined by the largest RFG follicle mass. As scaup and ringnecks produce eggs of near identical mass and composition, in both species follicles with dry mass > 0.1 g were assumed to have initiated rapid follicular growth and be indicative of a female attempting reproduction (Alisauskas et al. 1990). I also used the 0.1 g threshold for scoters as apparent non-RFG follicles in scoters were of similar mass to those of scaup and ringnecks, approximately 0.05 g dry mass.

3.2.4 Contaminants Analysis

Total Hg and Se in liver and eggs (yolk and albumen)/follicles were analyzed at the National Wildlife Research Centre (NWRC), Environment Canada, Ottawa, Ontario. Briefly, tissue samples were homogenized and approximately 0.5 g placed into preweighed, acid-washed test tubes, freeze-dried, and their dry masses recorded. Deionized H_2O (0.5 ml) and HNO_3 (either 0.5 ml or 1.0 ml) were added to each test tube. For Hg analyses, samples were heated at 70°C for 1 h. After cooling, 1.0 ml of H_2SO_4 (95–97%) followed by 0.5 ml HCl (37%) was added to each sample. They were heated again at 70°C for 2 h. After cooling, the volumes were adjusted to 10 ml with 2 mM

$K_2Cr_2O_7$ in 3% HCl. Volumes were then adjusted to 20 ml with 9.9 ml HCl (1.5%) and 0.1 ml octanol. Total Hg was analyzed by cold vapour atomic absorption spectrometry (CVAAS) using a 3030 AAS by Perkin-Elmer equipped with a Varian VGA-76 hydride generator and a PSC-55 Varian autosampler. For Se in liver and follicles, samples treated with deionized H_2O and HNO_3 as described above, were allowed to sit overnight at room temperature. The following day they were heated at 100°C in dry baths for 6 h. Samples were allowed to cool overnight, volumes were then adjusted to 4.0 ml with deionized H_2O. Se was analyzed by graphite furnace atomic absorption spectrometry using the Perkin-Elmer 3030b equipped with a deuterium background corrector, HGA-300 graphite furnace, and AS-40 autosampler. All concentrations are reported on a dry weight (dw) basis.

3.2.5 Quality Assurance

Three reference materials (Tort-2, Dorm-2 and Dolt-2 from the National Research Council), five random duplicate samples, and two blank reagents were analysed to assess quality assurance. Recovery of analytes from reference materials ranged from 94-122% and averaged 109%; recoveries were all within confidence intervals. Repeatability, determined as percent residual standard deviation of duplicate samples, ranged from 0.3-11.3% and averaged 4.3%. Detection limits were 0.08 μg/g and 0.5 μg/g for Hg and Se, respectively.

3.2.6 Statistical Analysis

All statistical analyses were conducted using SPSS version 13.0 (Norušis 1990). I used ANOVA and LSD post-hoc comparisons of log-transformed Se and Hg values to test for differences in mean contaminant concentrations between years, sites and species. In the absence of year or year*site interaction effects, samples from both years were combined to reduce model complexity. I used species-specific ANCOVAs to test for a relationship between Se and somatic lipid or protein content of females while controlling for date of collection and lipid or protein deposited into reproductive tissues from endogenous sources. I controlled for date of collection because tissue levels of Se likely decline throughout the season after ducks leave wintering and migration areas where

selenium exposure is believed to be high, due to the natural process of depuration (Heinz 1993, Heinz et al. 1990, Ohlendorf 2003). I used binary logistic regression to model the relationship between liver Se and breeding status (RFG follicles vs. no RFG follicles) in each species separately while controlling for date of collection. Statistical significance was evaluated against an $\alpha = 0.05$.

3.3 RESULTS

Because liver Hg concentrations were between 5 and 30 fold lower than Se, across species and locations (Table 3.1), I considered the potential interaction of these two elements to be negligible.

There was no effect of year or interaction between year and other parameters on mean Se concentrations within site and species, so I pooled samples from 2003 and 2004 (GLM; *P*s > 0.44). Geometric mean (95%CI) concentrations of Se in female scaup, ringnecks and scoters from all sites and years combined were 6.2 (5.5-7.0), 4.6 (4.0-5.4), and 32.6 (28.4-37.3) mg/kg, respectively. Within locations, scoters had greater Se levels than did scaup, and scaup had greater Se levels than did ringnecks (ANOVA LSD post-hoc comparisons; *P*'s ≤ 0.05; Table 3.1). However, Se levels in all scaup and ringnecks were below 33 mg/kg (Table 3.1), the threshold for physiological impairment in captive mallards; 10% of scaup and 5% of ringnecks had liver Se levels above 11 mg/kg. Where egg concentrations in captive mallards were observed greater than 9 mg/kg, the teratogenic threshold (Heinz 1996). Selenium levels in AB were significantly greater than in IN (ANOVA LSD post-hoc comparisons; $P = 0.02$), but not YK ($P = 0.17$) for scaup, and Se levels in AB were higher than in YK for ringnecks ($P = 0.03$). Collections at higher latitudes occurred at later dates.

After controlling for date of collection and nutrients deposited into reproductive tissues, there was no relationship between liver Se and either total lipid or protein estimates in scaup or ringnecks (*P's* > 0.144; Table 3.2). In scoters, no relationship was observed between Se and somatic protein ($P = 0.467$), but a significant positive relationship ($P = 0.016$) was found between Se and total lipids (Table 3.2).

Liver Se did not contribute significantly to my logistic regression model of breeding status in scaup ($\chi^2 = 1.846$, $P = 0.174$, df = 68) or ringnecks ($\chi^2 = 0.191$, $P =$

0.662, df = 40). However, Se did significantly contribute to the model in scoters (χ^2 = 10.047, P = 0.002, df = 47); breeding females generally had greater Se levels than non-breeders (β = 0.09 ± 0.03 SE).

I recovered eight oviducal eggs from scoters and compared Se values of these eggs to those of the next largest follicle, but found no difference in mean Se values (*t*-test; t = 0.809, P = 0.427). Therefore, I used the largest developing follicle or oviducal egg for future comparisons. No scaup had oviducal eggs. I measured Se levels in scaup and scoters eggs/follicles and compared these values against embryonic malformation thresholds. Only one of nine scaup whose follicles were analysed for Se had liver concentrations above 11 mg/kg, the threshold for reproductive effects in captive mallards (Fig. 3.1); all scaup follicles had normal Se levels (mean [95%CI] = 2.3 [1.9-2.6] mg/kg) and were well below the 9 mg/kg reproductive impairment threshold for Mallards (Heinz, 1996). Although 95% of scoters with developing follicles had liver Se above 11 mg/kg, all scoter eggs/follicles also had normal levels of Se (2.4 [2.1-2.7] mg/kg).

Table 3.1. Geometric mean (95%CI) and range of selenium (Se) and mercury (Hg) concentrations (mg/kg dry weight) from livers of female lesser scaup, ring-necked ducks and white-winged scoters collected at Utikima, AB, Yellowknife, NT, and Inuvik, NT, in 2003 and 2004 combined.

		Location								
		Alberta			Yellowknife			Inuvik		
Species		Mean (95%CI)	Range	n	Mean (95%CI)	Range	n	Mean (95%CI)	Range	n
Scaup	Se	7.5 (6.2-9.2)	3.9-22.3	24	6.0 (5.0-7.3)	1.8-20.9	25	5.3 (4.3-6.5)	3.2-13.9	22
	Hg	1.1 (0.8-1.5)	0.6-4.0	12	1.3 (0.9-1.7)	0.5-6.4	15	1.2 (0.8-1.6)	0.6-2.6	12
Ringneck	Se	5.5 (4.4-6.7)	3.1-14.0	21	3.9 (3.2-4.9)	1.7-7.9	21	-[a]	-	-
	Hg	0.4 (0.3-0.5)	0.1-1.3	12	0.8 (0.6-1.1)	0.5-3.2	12	-	-	-
Scoter	Se	-[a]	-	-	-[a]	-	-	32.6 (28.4-37.3)	3.9-75.1	50
	Hg	-[a]	-	-	-[a]	-	-	1.0 (0.8-1.3)	0.5-1.9	14

[a] – denotes that too few birds occur in this area so none were collected.

Table 3.2. ANCOVA evaluating the relationship between selenium (Se) and endogenous nutrients while controlling for date of collection (Day) and nutrients deposited into reproductive tissues (Repro lipid and Repro protein) for lesser scaup, ring-necked ducks and white-winged scoters collected from 3 locations in Canada in 2003 and 2004. Parameters and estimates in **bold** indicate a relationship differs from zero.

Species	Model	Parameter	*B*	SE	*P*	R^2
Scaup	Lipid	Se	-0.61	0.80	0.449	0.04
		Day	-0.67	0.39	0.095	
		Repro[a] lipid	0.22	0.786	0.778	
	Protein	Se	-1.19	0.08	0.144	0.08
		Day	0.01	0.04	0.748	
		Repro[a] protein	0.08	0.08	0.327	
Scoters	Lipid	**Se**	**1.34**	**0.53**	**0.016**	**0.38**
		Day	**-5.37**	**2.03**	**0.011**	
		Repro[a] lipid	0.70	0.50	0.164	
	Protein	Se	0.06	0.08	0.467	0.09
		Day	-0.20	0.30	0.500	
		Repro[a] protein	0.12	0.07	0.096	
Ringneck	Lipid	Se	-0.12	1.28	0.925	0.05
		Day	-0.60	0.50	0.234	
		Repro[a] lipid	0.53	0.54	0.334	
	Protein	Se	0.13	0.18	0.485	0.08
		Day	0.03	0.08	0.750	
		Repro[a] protein	0.08	0.08	0.320	

[a] - Nutrient estimated from reproductive tissues (eggs, follicles, oviduct)

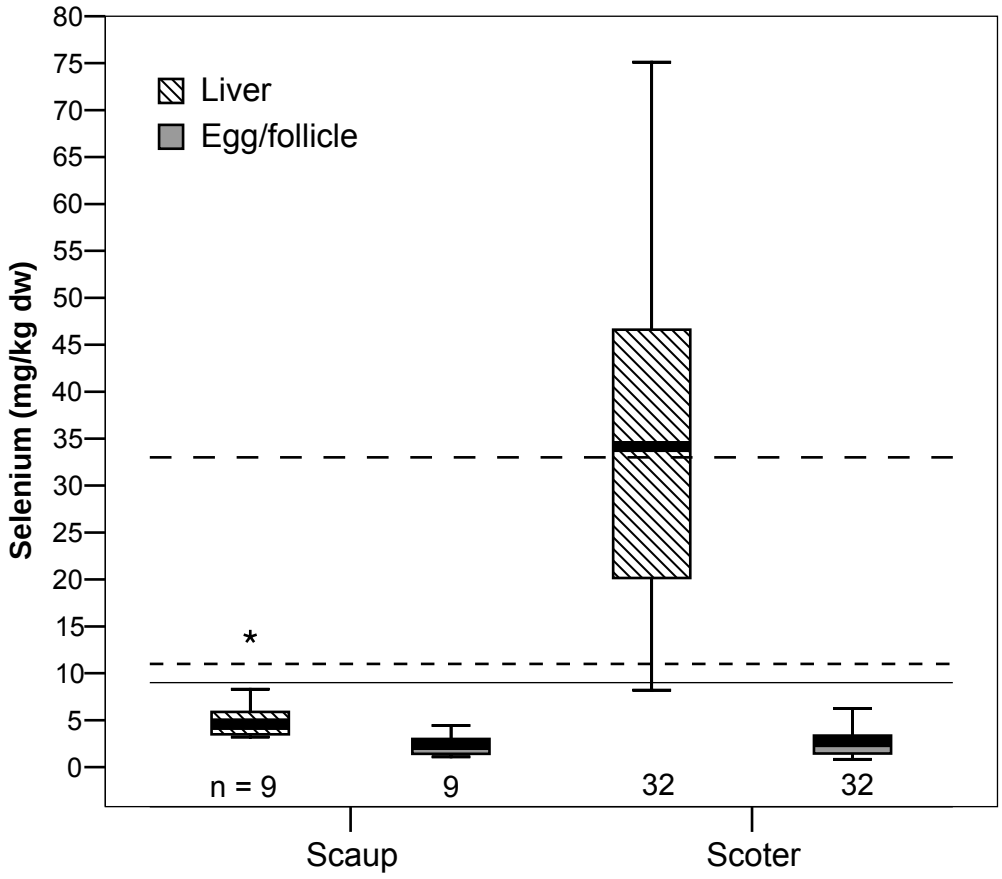

Figure 3.1. Boxplot of matched liver and egg/follicle selenium concentrations from scaup and scoters. Horizontal lines inside boxes indicate median values, ends of boxes show 25th and 75th percentile, whiskers show 10th and 90th percentiles and stars indicate values in the bottom and top 10 percentiles. The solid reference line indicates the avian embryonic malformation threshold for egg Se (~9 mg/kg dry weight at 67% moisture content; Heinz 1996), the short-dash line indicates the captive mallard liver threshold for reproductive impairment (11 mg/kg dw), and the long-dash line indicates the liver threshold for physiological impairment (33 mg/kg dw).

3.4 DISCUSSION

Consistent with one of my predictions from the contaminants hypothesis, Se levels were higher in scaup and scoters than in ringnecks. However, I did not observe any female scaup or ringnecks with levels above the observed physiological impairment threshold for captive mallards (33 mg/kg). I observed lower Se levels in birds collected at sites with higher latitude (Table 3.1). This was likely due to depuration of selenium over time since collections at higher latitudes occurred at later dates; mean collection date of birds analysed for Se were 18 May, 29 May and 9 June for AB, YK, and IN respectively.

Being an essential micro-nutrient, Se is regulated in vertebrates and is eliminated from the body following removal or a reduction in Se concentration of the dietary source (Heinz 1993, Heinz et al. 1990, Ohlendorf, 2003). Se concentrations in foods consumed by ducks in the boreal forest are likely much lower than those of foods they eat on wintering grounds due to less anthropogenic enrichment and lower natural occurrence in the boreal (Kubota et al. 1967). This is consistent with evidence that Se concentrations on the breeding grounds are lower than those observed on the wintering or staging areas (Hothem et al. 1998, Custer and Custer 2000, Custer et al. 2003, Fox et al. 2005, Anteau et al. 2007, Rocque et al. USFWS unpub data).

Recently, Anteau et al. (2007) found a positive correlation between liver Se and somatic lipids in wintering and staging scaup. However, after controlling for date of collection, there was no relationship between selenium levels and either nutrient levels or breeding status in either my scaup or ringnecks (Table 3.2). The absence of a relationship between selenium level, body condition and breeding status leads us to believe that the relatively small difference in average liver Se levels between scaup and ringnecks observed in this study cannot account for differences in scaup and ringneck population trends.

Selenium concentrations observed in my collected scoters were five times higher than in the other two species, which I assume is explained by their greater use of marine wintering habitat. Marine systems generally have higher Se than freshwater environments (Haygarth 1994, Ohlendorf 2003), and therefore scoters may normally carry, and be less impacted by, higher levels of endogenous Se. The levels I observed in scoters also are consistent with other breeding ground studies of seaducks (Braune and Malone 2006, Henny et al. 1991, Henny et al. 1995, Wayland et al. 2003), and are consistent with observations by Henny et al. (1995) of liver and egg concentrations (n = 5) in white-winged scoters from Alaska. In other waterbirds, the observed liver:egg relationship has been close to 2:1. Although there may be differences in Se tolerance among freshwater and marine birds, I propose two habitat-independent hypotheses about the observed liver: egg ratio in scoters: a) endogenous Se in scoters is in a form that is not readily deposited into eggs, or b) endogenous Se is not deposited into eggs because scoters utilize exogenous rather than stored nutrients for egg formation (Dobush 1986). Henny et al.

(1995) suggested that Se concentrations in livers of seaducks or other seabirds may not be a good indicator of potential reproductive problems and recommended further investigation of liver-egg Se relationships.

Heinz et al. (1990) reported that Se concentrations in the albumen of captive mallards were higher than those of yolk during the treatment phase of females fed 15 ppm selenomethionine. However, once removed from the treatment, yolk and albumen contained similar concentrations of Se. Given that whole oviducal eggs did not have higher Se concentrations than developing follicles of the same females ($P = 0.427$, $n = 8$), egg formation in my scoters were likely in a similar post-exposure period as I would expect with seaducks breeding in freshwater environments.

One of the greatest difficulties in studying Se in wildlife is the issue of threshold concentrations for impairment to adults and embryos. To date, my understanding of the effects of Se on waterfowl in controlled captive experiments has been based on mallards as surrogate species. Most studies have focused on one species of Se, selenomethionine, because it is likely the most common form ingested by birds (Fan et al. 2002, Heinz 1996, Heinz et al. 1989, Moreno et al. 2004, Olson et al. 1970). But, given the various forms of Se, the differences in their toxicity (Heinz 1996, Hoffman 2002, Spallholz and Hoffman 2002), interactions of Se with other elements and compounds (Cuvin-Aralar and Furness 1991, O'Toole and Raisbeck 1997, Stanley et al. 1994) and interspecific differences in sensitivity to Se even within families of birds (Ohlendorf et al. 1986b, Skorupa 1998), among other confounding factors, identifying broad threshold concentrations for birds is difficult. The liver Se concentration thresholds of 11 mg/kg for reproductive impairment and 33 mg/kg dw for physiological impairment outlined by Heinz (1996) have been used extensively to interpret levels in wild waterfowl (Braune and Malone 2006, Custer and Custer 2000, Custer et al. 2003, Hothem et al. 1998, Anteau et al. 2007, this study) despite cautions that egg concentrations are a more relevant endpoint, as these values are better correlated with potential embryotoxic effects (Heinz 1996, Ohlendorf 2003). However, there are inconsistent reports of a relationship between liver Se and either reproductive or physiological impairment in wild waterfowl where the majority of studies have found no significant correlation (Henny et al. 1991, Henny et al. 1995, Takekawa et al. 2002, Wayland et al. 2003). Furthermore, where a significant correlation was found,

there is no evidence for a cause-effect relationship, and, as I found in this study, there can also be positive relationships between Se and nutrient levels (Scoter lipid – Se; Table 3.2). Therefore, there is likely important variability in sensitivity to Se among waterfowl species and it is possible that scaup and scoters are less sensitive than are captive mallards because of greater concentrations normally found in wintering habitat (Haygarth 1994, Ohlendorf 2003). Skorupa (1998) suggested that birds have likely adapted to concentrations of Se found naturally in the environment, and that problems are likely only to occur when Se is anthropogenically enriched above normal environmental concentrations. Therefore, I caution the use of these threshold concentrations to interpret potential effects of Se on other species in the absence of evidence for such effects.

Contaminants accumulated on the wintering grounds may impact an individual's breeding propensity or its migration pattern if significant physiological effects occurred. This would bias my boreal breeding ground samples to healthy, uncontaminated individuals. However, given that scaup staging on the Great Lakes and implanted with satellite transmitters migrated to breeding grounds in 2005 (n = 6) and 2006 (n = 19) (Birds Studies Canada 2006), I believe that my sample was not biased away from birds exposed to higher environmental Se concentrations. The Se concentrations I observed are consistent with those reported in Fox et al. (2005) and Rocque et al. (USFWS, unpub data) from other boreal forest locations, so inferences made from my analyses on body condition and reproductive status may be representative of the boreal breeding season population and of birds on the Great Lakes and other important staging areas.

3.5 CONCLUSIONS

Several studies of wintering and staging scaup indicate that Se is the only contaminant consistently above levels that should cause concern, but birds sampled outside of the breeding season may generally be unreliable as predictors of potential reproductive efforts (Ohlendorf 2003). Indeed, in my study, Se did not appear to be at levels of concern in boreal scaup or ringnecks during the breeding season. Moreover, higher concentrations in scoters do not appear detrimental to female body condition or breeding propensity. Therefore, I believe that Se is likely not the cause of declines or lack

of population recovery of scaup or scoters. Given the variability in toxicity of Se due to its various forms, interactions with other elements and compounds, and interspecific differences in apparent threshold concentrations, future contaminant studies should be conducted to examine the sensitivity of scaup to Se in a controlled captive setting.

4. EFFECTS OF DIETARY SELENIUM ON REPRODUCTION AND BODY MASS OF CAPTIVE LESSER SCAUP

4.1 INTRODUCTION

Between 1978 and 1997 the breeding population of scaup (both lesser, *Aythya affinis* and greater, *A. marila*, combined) decreased by approximately 150,000 birds/year (Afton and Anderson 2001), and has continued to decrease in the last decade (Wilkins and Otto 2006). One of the hypotheses proposed to explain this decline is that exposure to contaminants on migration and wintering areas has reduced breeding propensity, reproductive success and/or survival of adults (Austin et al. 2000, Afton and Anderson 2001). The one contaminant that has been consistently reported at levels of potential toxicological concern in scaup collected from wintering and staging areas is the trace element selenium (Se) (Hothem et al. 1998, Custer and Custer 2000, Custer et al. 2000, Custer et al. 2003).

Selenium is a semi-metallic, nutritionally essential element in vertebrates that is also a potential environmental contaminant causing negative reproductive and physiological effects at elevated levels of exposure. Selenosis in adult birds can be expressed as hepatic lesions, abnormal feather loss, or simply as emaciation (Ohlendorf et al. 1988). Concentrations ≥11 μg/g (all concentrations are reported on a dry weight [dw] basis unless otherwise stated) in livers of captive mallards (*Anas platyrhynchos*) fed a diet containing ≥8 ppm Se during breeding was associated with malformation or death in chicks; when liver concentrations were ≥33 μg/g, sublethal effects occurred in juveniles and adults (Heinz et al. 1989, Heinz 1996). The applicability of these values to other waterfowl species is unknown.

Male lesser scaup collected from Indian Harbor Canal, Lake Michigan, in 1994 had high levels of Se in their liver; >50% (n = 16) of the birds had levels ≥33 μg/g (Custer et al. 2000). Custer and Custer (2000) found elevated Se concentrations (≥11 μg/g) in livers of 95% of lesser scaup sampled from Lake Erie, Lake St. Clair, and Lake Michigan in 1991 to 1993. Finally, a recent Great Lakes study found that 93% of spring collected greater scaup and 75% of lesser scaup had Se levels ≥11 μg/g (Petrie et al.

2007). Studies from locations outside the Great Lakes, such as San Francisco Bay and along the Mississippi alluvial valley, have also reported elevated levels (≥11 µg/g) of hepatic Se in scaup (Custer et al. 2003, Takekawa et al. 2002). On the breeding grounds, however, concentrations are much lower and rarely exceed 11 µg/g in females, and no eggs have been reported above the 9 µg/g threshold associated with teratogenic effects in mallards (Fox et al. 2005, DeVink et al. 2007b). Therefore, it is unknown whether females with high Se burdens are either not breeding, eliminating endogenous Se before laying eggs, or not depositing Se into developing eggs.

Here, I simulated scaup exposure to Se on staging areas in a captive scaup breeding experiment to determine effects on reproduction and physiology. Our objectives were to: 1) evaluate whether exposure to elevated dietary Se affects scaup survival, body condition and breeding propensity; 2) describe egg Se concentrations and determine the time required for concentrations to decline below the 9 µg/g toxicity threshold proposed by Heinz (1996); 3) test for intraclutch variation in egg Se; 4) describe the rate of decrease in blood Se levels after Se-supplemented diets were removed; and 5) compare liver concentrations post-experiment to determine whether an observable difference in liver Se among females persists long after exposure to Se.

4.2 METHODS

4.2.1 Study Design

This experiment was conducted at the Patuxent Wildlife Research Center in Laurel, MD, with approval of the local animal care committee. Twenty-three pairs of second-year captive-reared lesser scaup were placed in individual pens lined in three rows of eight pens. Each pen was approximately 6 m by 3 m equipped with a 2.5 m diameter and 1 m deep pool with circulating fresh water, two straw-lined nest boxes and a rain-protected food dish. Pens were lined with smooth gravel and enclosed with chain-link fencing. Pairs were randomly assigned to pens and treatments.

Birds were fed Mazuri Sea Duck Diet (0.65 ppm Se, 21.6% Protein, 6.5% Lipid, 8.4% Fiber, 10.9% Ash) in pellet form *ad libitum* treated with either distilled water (control; $n = 7$ pairs), or with one of two Se treatments to increase diet concentrations to

targets of 7.5 ppm (n = 8) and 15 ppm (n = 8) (analyses indicated actual treatment concentrations were 7.7 ± 0.2 (n = 3) and 14.9 ± 0.5 (n = 3), respectively). The 7.5 ppm treatment was based on the highest concentrations (7.4 μg/g) observed in zebra mussels (*Dreissena polymorpha*) at 14 sites in the St. Lawrence River (Kwan et al. 2003). The 15 ppm treatment concentration was chosen to simulate exposure above the maximum observed concentration in mussels from the Great Lakes (Lake Michigan – 11.5 μg/g; Center for Coastal Monitoring and Assessment's mussel watch; http://www8.nos.noaa.gov/cit/nsandt/download/mw_monitoring.aspx). Treatment diets were administered for a period of four weeks starting 19 April 2005, and ending 18 May 2005, after which all treatment birds were returned to a control diet until the end of the experiment (5 July 2005). To increase Se concentrations to desired levels in treatment food, I dissolved L-selenomethionine (99+% pure; molecular weight – 196.11 g/mol; Voigt Global Distribution Inc., Kansas City, MO) in distilled water and applied the solution using a one-liter atomizer spray bottle while stirring food in a 12 gallon container. The concentrations applied for the 15 ppm diet was 853.2 mg selenomethionine (343.5 mg Se; atomic weight 78.96 g/mol) in 463 g distilled H_2O per 22.9 kg bag of food. Half this concentration was applied for the 7.5 ppm diet. The solution was sprayed directly on the food only in order to coat the surface, stirred, then repeated until the entire quantity of solution was applied. The atomizer, stirring trowel, and mixing container were cleaned and rinsed between treatment preparations, which were conducted in the order of increasing concentration: control, 7.5 ppm, then 15 ppm.

4.2.2 Monitoring and Sampling

Because of possible aversion to selenomethionine-enriched diets by waterfowl (observed in common eiders (*Somateria mollissima*) fed 80 ppm, Franson et al. 2007), I monitored food dispensing during the treatment portion of the study. In my captive setting, it was impossible to monitor actual food consumption by individuals. Therefore, I recorded the frequency of food refills with an identical 1L dispenser for each diet (mean food weight per 1L = 355 ± 1 g, n = 5), and assumed that there was no significant loss or significant difference in the proportion of food consumed by females and males among treatments. Birds were checked on a daily basis and food provided accordingly. During

checks, birds were observed for apparent poor health; individuals showing abnormal behaviour (lethargic or lack of aversion to personnel) were removed from the experiment, examined by a veterinarian and euthanized if appropriate. During routine checks, I also monitored nests for eggs, which were removed, individually marked by date and pen number, and immediately frozen. Initially, I replaced scaup eggs with medium size white chicken eggs to simulate a hen's clutch, but found that females avoided chicken eggs and moved to the other nesting structure to continue laying eggs. When this practice was ceased, females continued laying in the same nest. Therefore, I was unable to compare clutch size among treatments or laying periodicity because of the possible effect of egg removal. If more than one initial egg was found in the pen, the clutch initiation date (CID) was adjusted accordingly based on one egg laid per day (Austin et al. 1998), and the eggs were given a mean date for use in Se analyses (e.g., if the first two eggs of a nest were detected on 20 May, the CID was recorded as 19 May, and both eggs were given laying dates of May 19.5 for egg Se analyses).

Birds were weighed to the nearest 10 g on a weekly basis using a 1000 g Pesola spring balance. I took 1.0 to 1.5 ml blood samples using 5 cc syringes with 18 gauge non-heparinized needles on a weekly basis for three weeks (4 samples) beginning on the day birds were removed from the treatment diets (18 May) in order to model Se loss from blood.

When the experiment ended, birds were euthanized by inhalation of vaporized isofluorane, weighed and frozen whole. Birds were then sent to the University of Saskatchewan for dissection and necropsy.

4.2.3 Se Determination

Total Se in liver, blood and eggs (yolk and albumen) was analyzed at the National Wildlife Research Centre (NWRC) of Environment Canada in Ottawa, Ontario. I analysed Se from all treatment bird eggs (n = 32, 15 ppm; n = 31, 7.5 ppm) , and 18 randomly selected control eggs following methods in Neugebauer et al. (2003). Briefly, egg and liver samples were homogenized and all tissues were weighed into preweighed, acid-washed test tubes, freeze-dried, and their dry masses recorded. Deionized H_2O (0.5 ml) and HNO_3 (0.5 ml to 1.5 ml, depending on sample dry weight) were added to each

test tube and allowed to sit overnight. The following day they were heated at 90°C in dry baths for 6 h. Samples were allowed to cool overnight, and volumes were adjusted to 4.0 or 5.0 ml, depending on initial amount of HNO_3 added, with deionized H_2O. Se was analyzed by graphite furnace atomic absorption spectroscopy by the method of standard additions using the Perkin-Elmer 3030b equipped with a deuterium background corrector, HGA-300 graphite furnace, and AS-40 autosampler.

4.2.4 Quality Assurance

Three standard reference materials (SRM) (dogfish muscle [NRCC DORM-2], dogfish liver [NRCC DOLT-3] and oyster tissue [NIST OT1566b]), five random duplicate samples, and two blank reagents were analysed for each set of approximately 30 liver and egg samples to assess quality assurance. Recovery of analytes from SRMs ranged from 78.0 to 120.0% and averaged 101.9 ± 13.4% (n = 10). Recoveries were all within the certified confidence intervals. Analytical precision, determined as percent relative standard deviation (RSD) of true duplicate samples, ranged from 1.3 to 5.2% and averaged 3.6 ± 1.1% (n = 8). Samples were not adjusted for blanks as Se concentrations were extremely low (0.0001 – 0.0038 μg/mL) in acid blanks. Detection limit for Se using this method was 0.5 μg/g.

Blood was analysed separately from liver and egg samples. I used the same three SRMs as above but also included bovine blood (IAEA A13). Recoveries of Se from SRMs averaging 96.5 ± 5.8% (n = 9) with the exception of bovine blood, which averaged 126 ± 71% (n = 3). The poorer results using bovine blood is likely due to its expected concentration (0.24 μg/g), which was close to the detection limit for Se. True duplicates averaged 10.3 ± 4.7 % RSD (n = 7), and ranged from 1 to 16% RSD.

4.2.5 Statistical Analyses

I used a χ^2 test to compare the proportion of females that initiated egg-laying between treatments. To compare clutch initiation date (CID) among treatments, I used a one-way ANOVA. The above analyses were performed using SPSS v. 13.0 (Norušis 1990). I calculated two-parameter exponential decay models to determine the depuration

rates in blood and eggs using the Regression Wizard function of Sigmaplot v. 8.02. Blood half-life was calculated using the following equation:

T(1/2)=ln(0.5)/ln((1-(x2/x1)^1/t)

Where: x1 = initial Se concentration, x2 = final Se concentration, t = time interval in days.

I used a linear regression of the residuals from these non-linear egg Se models over the laying sequence to test for intraclutch variability in egg Se levels. Finally, I ran Proc GLM in SAS v. 9.1 to compare mean Se concentrations in blood among treatments over the four sampling periods and also to compare mean female body mass among treatments over the nine repeated measurements (SAS Institute Inc. 1989).

4.3 RESULTS

One control female was found dead of an apparent aspergillosis infection on 15 June, without prior indication of distress (behaviour or mass loss; Dr. Glenn Olsen, Veterinarian, USGS, Laurel, pers. comm.). Control birds consumed no more food on average (10.2 ± 0.6 [SE] scoops) than did the 7.5 or 15 ppm treatments (9.0 ± 0.9 and 8.2 ± 0.7 scoops, respectively; ANOVA; $F_{2,20} = 1.849$; $P = 0.183$) during the treatment phase.

The number of females laying eggs by treatment were 5 of 7 controls, 4 of 8 in the 7.5 ppm group and 6 of 8 in the 15 ppm group, with no significant difference among groups (χ^2 test; $\chi^2 < 2.00$, $P > 0.157$). There was a large range of CID among females (20 May to 4 July), but mean CID (Controls – 8 June [SD = 8.3]; 7.5 ppm – 5 June [14.7]; 15ppm – 10 June [15.5]) did not differ among treatments (ANOVA; $F_{2,12} = 0.181$, $P = 0.837$). I also found no effect of treatment ($F_{2,19} = 2.06$, $P = 0.15$) or time*treatment interaction ($F_{16,152} = 0.92$, $P = 0.54$) on fresh body mass of females. However, variation among sampling periods and within treatments was high (Fig. 4.1). There were 79 ± 18(SE) and 64 ± 32 g declines in mean body mass of females in the 15 ppm and control groups during the treatment phase, but birds appeared to recover one week later and it did not appear to influence breeding probability or CID (Fig. 4.1).

The two-parameter exponential decay functions fitted for eggs explained most of the variation in egg selenium in both the 7.5 ppm ($R^2 = 0.98$; $F_{2,30} = 693.80$, $P < 0.001$) and 15 ppm ($R^2 = 0.94$; $F_{2,31} = 251.42$, $P < 0.001$) groups (Fig. 4.2). Based on the egg Se regression equations, it took 12 and 8 days post-treatment for the eggs of females from the 15 and 7.5 ppm treatments, respectively, to drop below the avian toxicity threshold concentration of 9 µg/g based on my observed moisture content of 66% (Fig. 4.2). As it takes approximately 7 days for a female to produce an egg – 6 days in rapid follicular growth and 1 day for albumen deposition (Alisauskas and Ankney 1992) - nutrients used to produce eggs may be below threshold concentrations even before this time. However, the Se concentrations in late-laid eggs for the 15 ppm and 7.5 ppm treatments (4.94 and 2.85 µg/g, respectively) were greater than the mean concentration in control eggs (1.28 µg/g) (Fig. 4.2). I found no evidence of intraclutch variation in Se deposition into eggs. Linear regressions of residual egg Se and laying sequence was non-significant in both the 15 ppm ($F_{1,32} = 0.25$, $P = 0.62$, $r^2 < 0.01$) and 7.5 ppm ($F_{1,31} = 0.68$, $P = 0.42$, $r^2 = 0.02$) treatments.

Again, the two-parameter exponential decay functions for blood were also significant, but explained less variation in both the 7.5 ppm ($R^2 = 0.75$, $F_{2,28} = 42.135$, $P < 0.001$) and 15 ppm ($R^2 = 0.56$, $F_{2,29} = 18.628$, $P < 0.001$) groups than was observed for eggs (Fig. 4.3). Using a repeated-measures MANOVA, I found a significant effect of sampling period ($F_{3,16} = 32.21$, $P < 0.001$), treatment ($F_{3,54} = 16.82$, $P < 0.001$), and time*treatment interaction ($F_{6,54} = 6.47$, $P < 0.001$) on blood Se concentration. Pairwise comparisons indicate that mean levels were significantly different between treatments in all four sampling periods (Table 4.1). The calculated half-life of blood Se in captive scaup was 16 and 22 days for the 15 and 7.5 ppm treatments, respectively, when treated birds were returned to control diets (Fig. 4.3).

Forty-three days after returning treatment birds to control diets, mean liver Se concentrations in females from the 15 ppm treatment (7.4 ± 0.9 SE µg/g) were significantly higher than controls (3.76 ± 0.5 µg/g). Females from the 7.5 ppm were intermediate (5.58 ± 0.5 µg/g), but did not differ from either of the two other groups.

Table 4.1. Geometric mean blood Se concentrations in control and Se-treated females following cessation of Se treatment.

		Sample Period (days post-treatment)			
Treatment		1	8	14	21
Control	Geo. Mean	1.60C	1.58C	1.59C	1.47C
	SE	0.09	0.06	0.14	0.03
	n	7	7	7	6
7.5 ppm	Geo. Mean	16.34B	11.72B	11.32B	8.01B
	SE	0.75	0.29	0.52	0.68
	n	8	8	7	8
15 ppm	Geo. Mean	30.85A	22.85A	18.72A	12.58A
	SE	3.8	1.57	1.47	1.37
	n	8	8	8	8

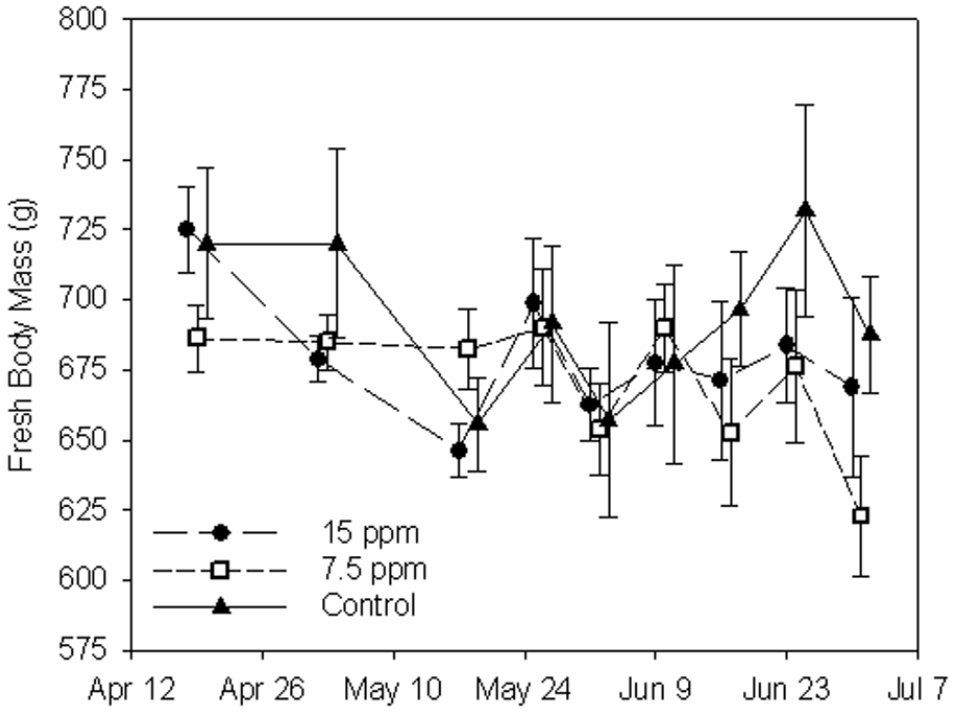

Figure 4.1. Mean (±SE) mass of females from control, 7.5 and 15 ppm treatments. All females were returned to control diets on May 18th after 4 weeks on their respective treatment.

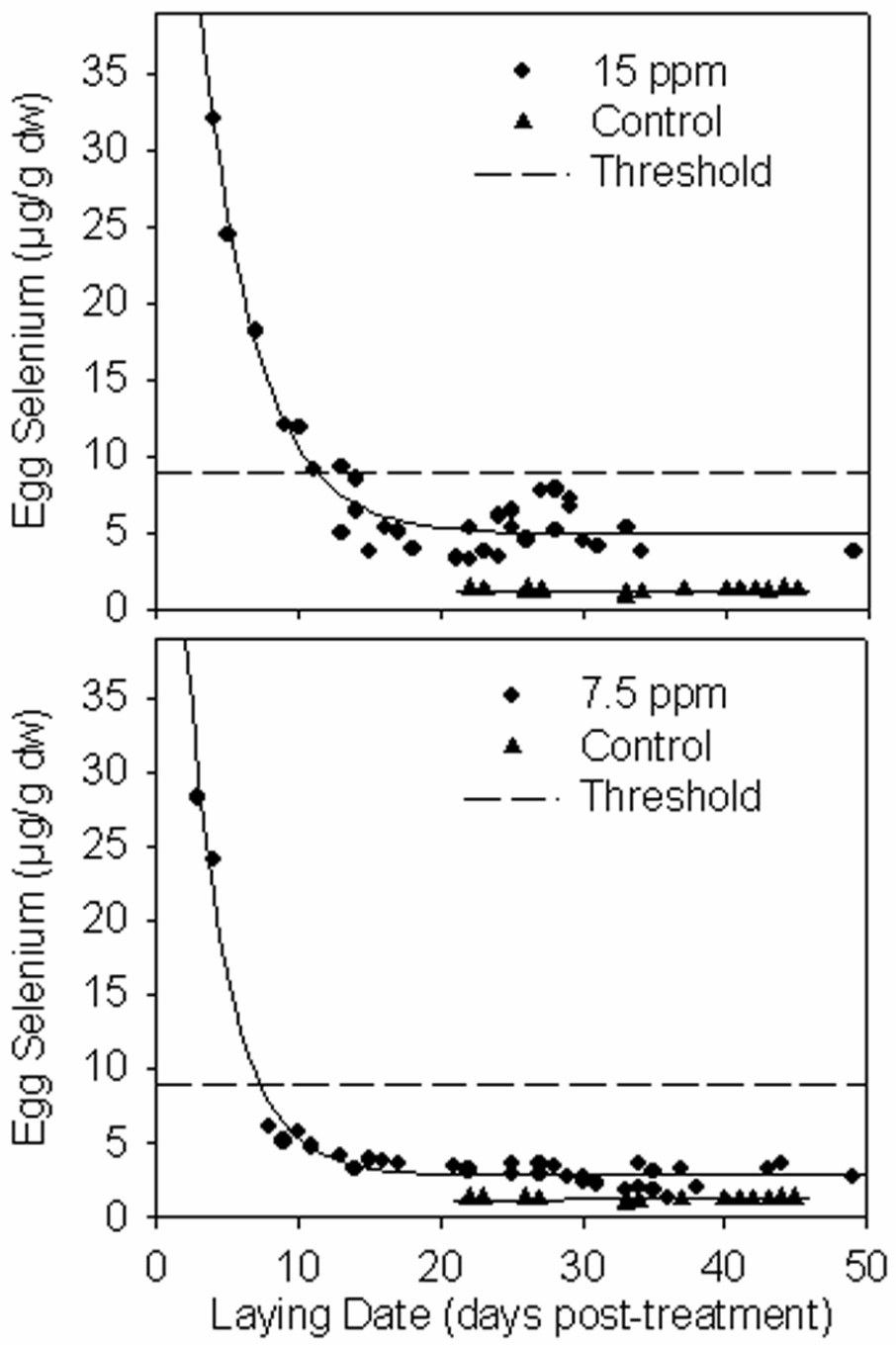

Figure 4.2. Selenium concentrations (µg/g dw) of fresh eggs collected from nests of females fed 15 ppm and 7.5 ppm compared to those of females from a control diet. The 9 µg/g threshold (dashed line) represents the suggested threshold for egg teratogenesis in birds (Heinz 1996). Fitted regression equations are: $y = 4.936 + 76.681\exp^{-0.262x}$ for the 15 ppm eggs, and $y = 2.852 + 73.945\exp^{-0.340x}$ for the 7.5 ppm eggs.

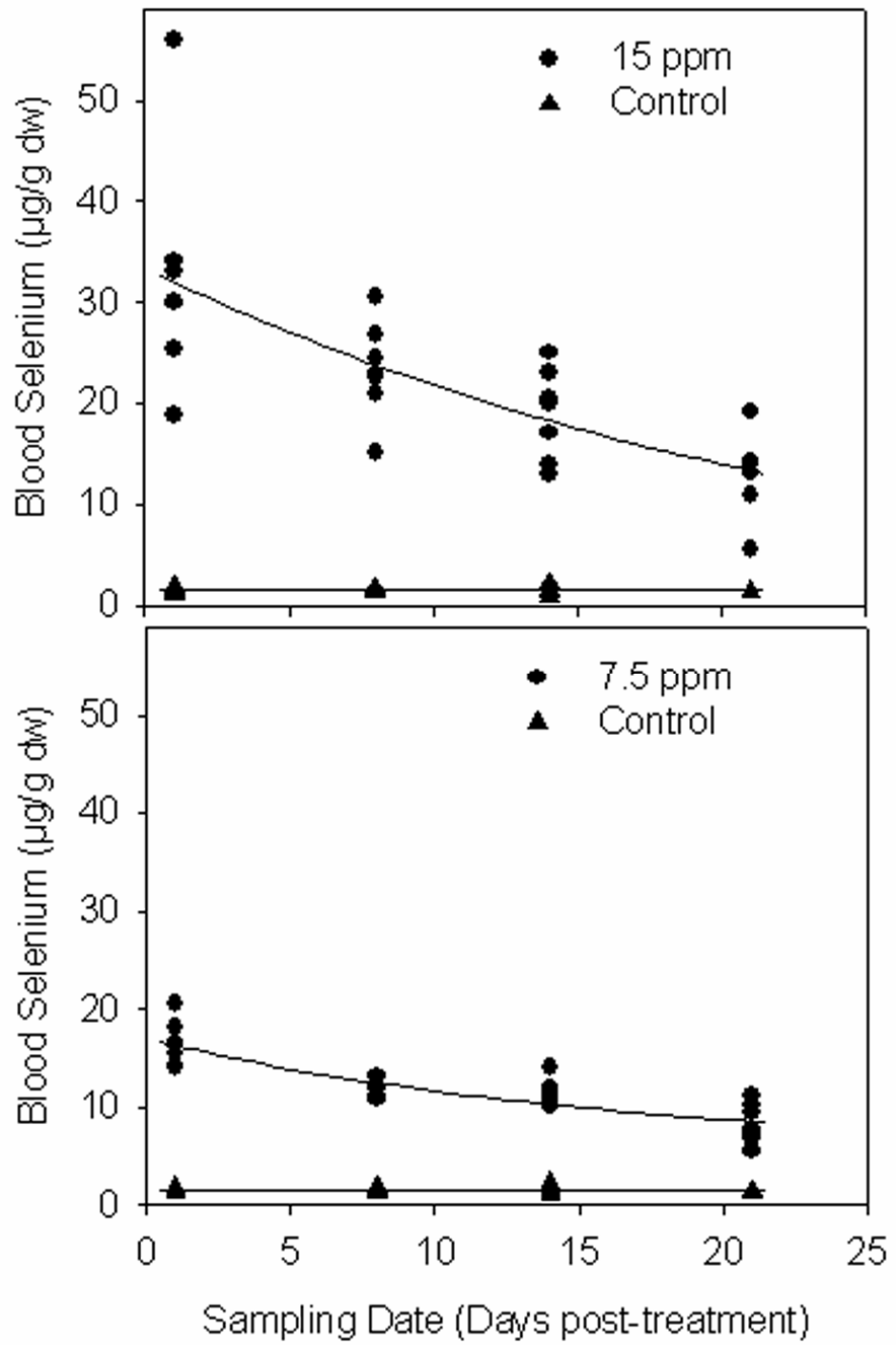

Figure 4.3. Selenium concentrations (µg/g dw) of whole blood sampled on a weekly basis for three weeks post-treatment from females fed diets containing 15 ppm and 7.5 ppm selenium compared to control females. Fitted regression equations are: $y = -2.141 + 35.552\exp^{-0.039x}$ for the 15 ppm group and $y = 5.370 + 1.610\exp^{-0.061x}$ for the 7.5 ppm group.

4.4 DISCUSSION

Despite high Se burdens in scaup on some staging and wintering areas, the significance of these reports to continental population declines is unclear. Avian reproduction is a Se-sensitive process (Heinz 1996). For example, Heinz and Fitzgerald (1993) observed that hatching success in mallards fed 15 ppm Se declined but returned to normal 14 days after hens were returned to a control diet. Furthermore, all surviving treatment hens bred, although on average 6.6 days later than control hens (Heinz and Fitzgerald 1993). Therefore, collectively Heinz and Fitzgerald's (Heinz and Fitzgerald 1993) results suggest that high dietary Se intake may not impair egg viability and breeding propensity of mallards if birds are removed from the source of Se sufficiently long enough before breeding.

Fox et al. (2005) did not detect high levels of Se in livers and eggs of breeding female scaup taken from several boreal nesting locations. Devink et al. (2007b) found that few (10%, $n = 71$) scaup arriving on boreal breeding grounds contained liver Se levels ≥11 μg/g, and none exceeded 33 μg/g. These data from the breeding grounds suggest that either contaminated birds are leaving the breeding population or Se is being depurated and not deposited into eggs.

I attempted to simulate Se exposure of scaup staging on the Great Lakes or other Se-enriched staging areas. Neither of my dietary treatments affected the proportion of females that laid at least one egg, and the group with the greatest proportion of egg-layers (6/8) was exposed to the highest concentration of Se (15 ppm). There was also no treatment effect on mean CID. It is unlikely that Se directly affects female breeding propensity, but studies suggest a nutrient threshold for females to attempt breeding (Afton and Ankney 1991, Esler et al. 2001); thus I expected a change in body mass would be required to produce a difference in the proportion of breeders among treatments. However, my repeated-measures MANOVA indicated no statistical effect of treatment or time*treatment interaction on body mass during the experiment ($P \geq 0.15$), although there were significant mass losses in both the 15 ppm and control groups during the treatment phase. Heinz and Fitzgerald conducted a similar experiment, but exposed captive mallards to 15 ppm selenomethionine for 21 weeks (vs. 4 weeks in this study). They observed that treatment birds lost mass compared to controls, and delayed clutch

initiation, but there was no difference in the proportion of females that laid eggs, or the number of ducklings produced. But, it is also possible that the difference between these studies was the duration of exposure to high Se concentrations, or an interspecific difference between scaup and mallards with respect to Se tolerance. Interspecies differences have been observed in other studies (Ohlendorf et al. 1986, Skorupa 1998), and recently, Anteau et al. (Anteau et al. 2007) reported a positive correlation between liver Se concentration and lipid reserves in lesser scaup during spring migration. Anteau et al.'s (2007) results directly contradict the predictions of the contaminants hypothesis as it relates to Se in scaup. I did observe a large variation in mean female body mass during the experiment (Fig. 4.1). The maximum absolute change in mean mass observed in each treatment was 44, 57, and 53 g/week for the control, 7.5, and 15 ppm treatments, respectively. This variability in body mass could be due to egg development (in the case of increased mass) or egg laying (in the case of decreased mass) as mean scaup egg mass = 48 grams (Austin et al. 1998). I was unable to account for changes in egg and ovarian development because of the uncertainty about possible follicular atresia and actual mass of reproductive organs. It is possible females developed follicles or resorbed follicles undetected.

Selenium transfer into eggs declined rapidly following cessation of dietary Se treatment; after only 12 and 8 days post-treatment in the 15 ppm and 7.5 ppm groups respectively, Se concentrations in eggs were below the level that Heinz (1996) proposed as the threshold for teratogenic effects in birds (3 µg/g wet weight ≈ 9 µg/g dw based on my observed 66% moisture content). My results were very similar to those of Heinz and Fitzgerald (1993) who observed that it took approximately 14 days for eggs of mallards exposed to 15 ppm selenomethionine to return to a level of hatching success equal to that of controls. I removed scaup from treatments on 18 May, which is late compared to the timing of scaup migration through Se-contaminated staging areas. Most scaup are already on breeding areas by mid-April to Mid-May (Bellrose 1980). Furthermore, CIDs of my captive scaup were on average approximately 10 days earlier than observed CIDs from scaup in the prairie-parklands and boreal forest (Toft et al. 1984, Koons and Rotella 2003). This difference in timing of CIDs would provide additional time for wild birds to eliminate endogenous Se. However, despite the relatively short period of time between

exposure to Se and egg-laying, only 9 of 65 eggs laid in the two treatment groups were above the 9 µg/g toxicity threshold (Heinz et al. 1989). Given that scaup eat primarily invertebrates (Austin et al. 1998), they are most likely to be exposed to Se in the form of selenomethionine or other seleno-amino acids (Heinz 1996, Ohlendorf 2002). As scaup use both exogenous and endogenous protein for egg formation (Esler et al. 2001), it appears that egg Se levels in this species will likely be reflective of a mix of these two protein sources. This is likely why I observed a rapid reduction in egg Se (Fig. 4.2) compared to blood levels (Fig. 4.3), with egg Se concentrations from later laid eggs being greatest in the 15 ppm followed by the 7.5 ppm and control groups. Similar to Bryan et al. (2003) I found no effect of laying sequence on residual egg Se concentrations, which was expected given that there is no evidence in birds of eggs as an excretory route for excess Se.

I calculated half-lives of 16 and 22 days for blood Se in my 15 and 7.5 ppm treatment groups, respectively, a slower depuration rate than the 9.8 day half-life reported for blood of captive mallards by Heinz et al. (1990). This discrepancy may be due to slightly higher initial blood concentration (10 to 15 µg/g wet weight) in mallards which would likely result in a faster half-life when the concentration gradient between tissue and diet is greater. This could be calculated with a two compartment model, similar to the liver model used in Heinz et al. (1990). Alternatively, this difference could be due to a lower Se concentration in the control diet used by Heinz et al. (< 0.05 ppm) compared to our control diet (0.65 ppm).

4.5 CONCLUSIONS

The region reporting the highest concentration of Se in mussels, according to the Center for Coastal Monitoring and Assessment, is the Great Lakes region. Waterfowl use-days by lesser and greater scaup combined on Long Point Bay, Ontario, increased from 38,500 in 1986, just before the zebra mussel colonisation of the area, to 3.5 million in 1997 (Petrie and Knapton 1999). Wormington and Leach (1992) also report that diving duck populations increased dramatically after the invasion of zebra mussels around Point Pelee National Park. I investigated the effects of Se on the physiology and

reproduction of captive scaup exposed to a dose similar to the maximum reported concentrations (7.4 μg/g) of Se in zebra mussels from 14 sites along the St. Lawrence river (Kwan et al. 2003) and an environmentally extreme dose (15ppm) greater than the maximum reported concentration (11.5 μg/g) in zebra mussels from the Great Lakes (Center for Coastal Monitoring and Assessment's Mussel Watch). However, both of these doses are above the mean (SE) concentrations in zebra mussels (5.0 ± 0.16 μg/g, n = 152) sampled from 25 sites on the Great Lakes between 1992 and 2003 (Center for Coastal Monitoring and Assessment's Mussel Watch). Our exposure duration simulated extended stopovers, but was not meant to simulate scaup overwintering on contaminated sites. I recognize numerous factors (other contaminants, differences in metabolic rates, external stressors such as predators, food availability, disease, etc.) that complicate extrapolating results from captive studies to wild populations. However, overall, I found no significant effect of dietary Se on survival, body mass, proportion of females attempting to breed, or the timing of clutch initiation consistent with results from wild birds (Devink et al. 2007b). I also demonstrated that soon after switching to a diet containing background levels of Se, females produced eggs that were below the threshold for teratogenicity suggested by Heinz (1996). Therefore, assuming that my study is applicable to wild scaup, I found no evidence to suggest that exposure to Se at concentrations greater than those that occur in the Great Lakes and for a duration of time likely experienced by scaup during spring migration through the Great Lakes negatively affected either their body mass or breeding probability. It is also important to note that most wild scaup would have been on breeding grounds by the time I removed my captive birds from their treatment diets (Austin et al. 1998). Therefore, they would have had a greater delay between departing Se-contaminated staging areas and nesting than my birds. Heinz and Fitzgerald (1993) suggested that wild waterfowl that winter in Se-contaminated areas, but migrate to uncontaminated areas to breed will likely not experience reproductive impairment from Se given the ability of females to depurate and low Se concentrations in exogenous nutrients. Furthermore, given that egg and follicle Se concentrations from scaup collected on boreal breeding grounds are within background concentrations (Fox et al. 2005, DeVink et al. 2007b), and that egg hatchability around Yellowknife has not changed since 1969 (Fournier and Hines 2001), there is no evidence to date suggesting that Se is

negatively affecting breeding scaup. However, I advocate for the continued monitoring of ecosystems that are susceptible to anthropogenic enrichment of Se, and for more research on the effects of Se on waterbirds wintering and breeding on the Great Lakes and associated wetlands where they may experience prolonged exposure to diets elevated in Se.

5. SELENIUM DYNAMICS IN WHITE-WINGED SCOTER EGGS: INSIGHTS FROM REPRODUCTIVE ENERGETICS

5.1 INTRODUCTION

Egg nutrients (lipids, protein, minerals) can come from two sources: exogenous (dietary) or endogenous (somatic tissues) (Drent and Daan 1980). In birds, there is a gradient of reproductive energetic strategies between capital breeders, which rely entirely on endogenous nutrients for egg formation, versus income breeders, which use entirely dietary nutrients (Meijer and Drent 1999). In oviparous species, reproductive impairment has been linked to maternal transfer of contaminants into eggs of fish (Miller 1993; Fisk et al. 1998), reptiles (Hopkins et al. 2004; Nagle et al. 2004) and birds (Heinz et al. 1989; Barger et al. 2001; Bryan et al. 2004), where contaminants are often deposited in association with egg nutrients.

Selenium (Se) is a component of the selenoamino-acids selenocysteine and selenomethionine, which are essential for normal embryonic development, but selenomethionine can become highly toxic to adults or embryos at elevated concentrations (Ohlendorf 2003). Egg Se concentrations > 9 $\mu g \cdot g^{-1}$ dry wt are thought to increase the probability of embryonic malformation or death in mallard eggs (Heinz 1996), though threshold concentrations can vary widely among species and studies (Fairbrother et al. 1999, Adams et al. 2003). In captive mallards fed Se during egg-laying, liver associated above 11 $\mu g \cdot g^{-1}$ were correlated with egg concentrations above the 9 $\mu g \cdot g^{-1}$ teratogenic threshold (Heinz et al. 1989). However, when females were removed from a high Se diet just prior to egg laying, egg concentrations declined rapidly, while somatic levels remained elevated for a longer duration (Heinz 1993, Chapter 4). Devink et al. (2007b) proposed the "Dilution Hypothesis" whereby this pattern of low egg and high somatic Se concentrations may be due to the use of exogenous protein with low Se concentrations for egg formation. Here, I test this hypothesis using the white-winged scoter (*Melanitta fusca;* hereafter scoter), a species of seaduck.

Scoters and other seaducks sampled on the breeding grounds are frequently reported having hepatic Se concentrations exceeding 33 $\mu g \cdot g^{-1}$ that are likely accumulated

from marine wintering areas (Henny et al. 1995, Wayland et al. 2003, Devink et al. 2007a). Meanwhile, food items (dietary protein source) from freshwater systems where scoters breed have much lower concentrations of Se (0.3 – 2.0 mg/kg dw; Jorgelina Muscatello, University of Saskatchewan, unpubl. data). Moreover, Henny et al. (1995) and Devink et al. (2007b) observed scoter egg and follicle concentrations far below the 9 $\mu g \cdot g^{-1}$ egg teratogenicity threshold despite high liver burdens. These characteristics make scoters a model species to test the dilution hypothesis. Scoters may arrive on the breeding grounds with high somatic Se burdens, but if they allocate exogenous nutrients acquired from breeding habitats with low Se to egg formation, egg Se concentrations would become diluted and may help to reduce reproductive impairment.

If the Dilution Hypothesis explains low scoter egg Se concentrations, I predict that: a) small yellow follicles (SYF), which contain mainly protein deposited over the previous several months, likely while birds are still at sea (Johnson 2000), will have significantly higher Se concentrations than follicles undergoing rapid-follicular growth (RFG) which occurs after birds return to freshwater breeding areas; b) RFG follicle Se concentrations will have a significant negative relationship with follicle size; c) RFG follicle Se will be positively related to liver Se (an index of SYF follicle concentration); and; d) follicle Se will be positively related to the proportion of endogenous protein used in egg formation of each follicle. I also assess the relationship between follicle selenium and follicle rank in the development order to assess whether there is systematic intraclutch variation in egg Se concentrations. Females could deposit large quantities of Se in first eggs with lower concentrations in subsequent eggs; intraclutch variation could cause first eggs to be above and late-forming eggs to be below teratogenic thresholds (Bryan et al. 2003).

5.2 METHODS

Scoters were collected according to methods described in Chapter 3. Methods used to determine follicle mass, nutrient content as well as liver, rapid follicular growth (RFG) and SYF follicle Se are also described in Chapter 3. Stable-carbon and nitrogen

isotope analysis of scoter follicles and methods used to determine the percent endogenous protein contents of follicles are described in Chapter 8.

All statistical analyses were conducted using SPSS v13.0 (Norušis 1990). I used *t*-test to compare mean log-transformed selenium concentrations between RFG and SYF follicles. I used ANCOVA to model the effects of collection (early vs. late), liver Se, follicle weight, follicle number, and percent endogenous protein (PerEndo, determined using the Isoconc stable-isotope mixing-model; Phillips and Koch 2002) on follicle Se concentrations. Isoconc provides a measure of the weight of contribution of each source item to the value in question. Here I define PerEndo as the percentage of endogenous protein contribution to follicle protein. Due to sample size concerns, I ran a power (Φ) analysis on the ANCOVA model parameters.

5.3 RESULTS

Se concentrations in SYF ranged from 5.1 to 40.7 $\mu g \cdot g^{-1}$ (geometric mean = 16.9; CI = 11.5-24.9, $n = 11$) and were significantly higher than concentrations in RFG follicles (*t*-test; $t = 5.32$, $P < 0.01$), which ranged from 0.85 to 6.2 $\mu g \cdot g^{-1}$ (geometric mean = 2.2; CI = 1.9-3.6, $n = 18$; Fig. 5.1).

An initial ANCOVA, which included time of collection as a 2-way factor (early and late), showed that collection period and year did not contribute significantly to the model ($Fs \leq 1.01$, $Ps \geq 0.33$). Therefore, I subsequently excluded collection period and ran the model using liver Se, follicle weight, follicle number, and PerEndo as covariates. The overall reduced model was highly significant ($r^2 = 0.91$, $F = 28.18$, $P < 0.001$; Table 5.1). As predicted, follicle weight and liver Se were highly significant model parameters ($Ps < 0.001$, $\Phi > 0.994$), but PerEndo was marginal as a predictor of RFG follicle Se ($P = 0.07$, $\Phi = 0.60$). Directions of the parameter estimates were consistent with the dilution hypothesis, where follicle Se was positively correlated with liver Se and negatively correlated with follicle mass (Table 5.1). Isoconc mixing-models estimated that scoters used between 0% and 36% endogenous protein for follicle formation but, contrary to predictions, there was a weak inverse relationship between PerEndo and follicle Se (Table 5.1). Follicle number was not a significant predictor in either model ($Ps > 0.76$).

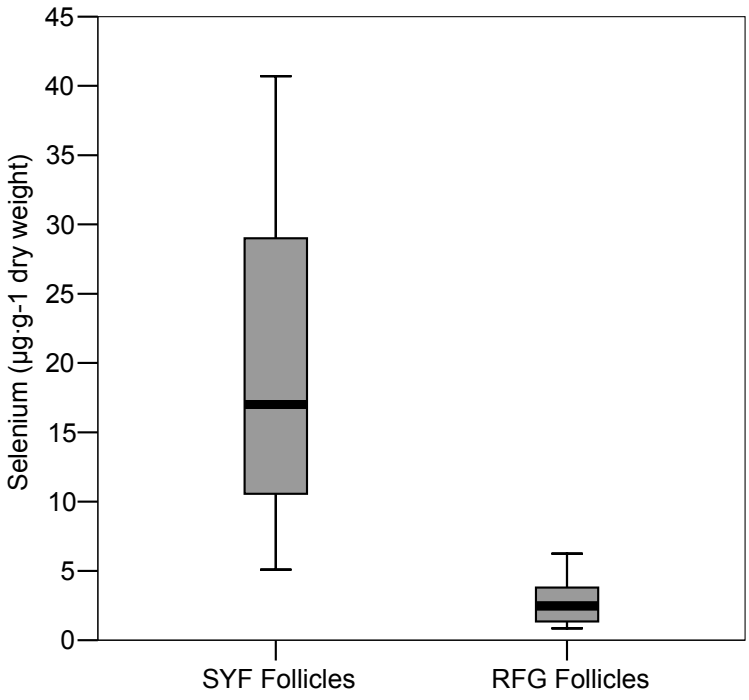

Figure 5.1. Boxplot of selenium concentrations in small yellow follicles (SYF) versus follicles undergoing rapid follicular growth (RFG). Thick lines, box margins and whiskers indicate median, quartiles and extreme values, respectively.

Table 5.1. ANCOVA testing for the effect of liver selenium, follicle mass, percent endogenous protein, and follicle number on selenium concentrations of follicles undergoing rapid follicular growth.

Parameter	β[a]	SE	F	P	Φ[b]
Intercept	3.070	0.747	16.87	<0.001	0.986
Liver Se	0.048	0.009	27.47	<0.001	0.999
Follicle mass	-0.093	0.020	21.23	<0.001	0.996
PerEndo	-0.022	0.011	4.06	0.07	0.596
Follicle number	-0.013	0.124	0.01	0.92	0.102

[a] – parameter estimate

[b] – power computed using alpha = 0.1

$r^2 = 0.91$, reduced model

5.4 DISCUSSION

Birds wintering on coastal marine areas often are exposed to higher concentrations of Se than in freshwater systems (Goede et al. 1989; Ohlendorf, 2003). Scoters probably accumulate endogenous burdens in their somatic tissues (i.e., liver, SYF follicles) during normal tissue turnover and while accumulating nutrients on marine areas for subsequent migration and reproduction. However, once on breeding grounds, their diet contains much lower Se concentrations and exogenous protein is used during reproduction (see chapter 8). The difference in Se concentrations between SYF (mean = 16.9 $\mu g \cdot g^{-1}$) and RFG follicles (mean Se = 2.2 $\mu g \cdot g^{-1}$; $P < 0.001$; Fig. 5.1) and the negative relationship between follicle size and Se concentrations (Table 5.1) are consistent with the dilution hypothesis that exogenous protein low in Se is used during RFG. At the onset of RFG, SYF follicles contain high concentrations of Se, but as follicles enter RFG they become diluted with dietary protein containing low Se concentrations that are typical of most freshwater breeding areas, thus preventing

potentially harmful egg Se concentrations. Meanwhile, somatic Se burdens decrease slowly with normal metabolic turnover as observed by Heinz (1993) (see also Chapter 4), which likely leads to some endogenous protein being incorporated into developing follicles (mean = 11% endogenous protein). Because of concern about the pattern of Se deposition into different follicles within a clutch, I also tested for an effect of follicle number on follicle Se. Based on my analysis of intraclutch variation in Se concentrations and the low egg Se concentrations compared to somatic burdens, it is evident that females do not preferentially deposit Se into eggs.

The relationship between endogenous protein use and follicle Se was weak, and my analysis revealed a lack of power to determine a significant relationship. However, this lack of power was likely due to the limited use of endogenous protein by females (mean = 11%, range = 0 – 36%), which is consistent with my prediction that exogenous protein is used for rapid follicular development. A larger range of endogenous protein use, assuming this occurs naturally, would have allowed for a stronger test of this hypothesis.

Trace metals have been more extensively studied in breeding common eiders (*Somateria mollissima*), a species of sea duck that generally breeds on marine islands (Goudie et al. 2000), than in breeding scoters. Across the Canadian Arctic, liver and egg Se concentrations (10-48 and 1-3 $\mu g \cdot g^{-1}$ dw, respectively; Mallory et al. 2004) were comparable to levels observed in birds I examined. However, eiders use somatic nutrients for egg formation to a greater degree than scoters (Parker and Holmes 1990). I would expect to see higher concentrations of egg Se in eiders. However, Mallory et al. (2004) also reported low concentrations of Se in muscle tissue (2-6 $\mu g \cdot g^{-1}$), which could result in a relatively low Se source of egg protein. Grand et al. (2002) noted high concentrations of Se in common and spectacled eider (*S. fischeri*) blood samples but low levels in eggs of females nesting on the Yukon-Kuskokwim Delta, AK. Given these results, and reports that eiders (Grand et al. 2002; Wayland et al. 2002, 2003) and scoters (Devink et al. 2007b) appear to be in good body condition despite high concentrations of Se, sea ducks have likely evolved energetic strategies or physiological means of dealing with higher Se exposure.

Several important assumptions in my test of the dilution hypothesis must be considered. First, my analysis was conducted using SYF and RFG follicles, which I assumed could be extrapolated to whole eggs. While Heinz (1993) showed significantly more Se in albumen than in yolk of eggs laid while females were on the Se treatment, once removed from the Se treatment concentrations in yolk and albumen were similar. Extrapolating these results, the scoters I collected would have been in the post-treatment phase and I would expect concentrations to be similar in yolk and albumen. While both components were not analysed separately, Devink et al. (2007b) did find that Se concentrations in whole oviducal eggs (yolk and albumen) were similar to concentrations in the next largest follicle (yolk only). This observation supports my assumption. Second, I assumed that most of endogenous Se in white-winged scoters is bioavailable and in an amino-acid form. Selenium is capable of binding to other elements (e.g., Hg, Cd, As) and forming biologically inert compounds (Scheuhammer et al. 1998, Ikemoto et al. 2004). If much of the Se in scoters is bound and unavailable, then the low follicle concentrations reported could be due to this process. Reports indicate that concentrations of potential interacting elements such as mercury are much lower than Se in scoters (Henny et al. 1995, Devink et al. 2007b), suggesting that most of the Se is probably bioavailable. This conjecture could be directly tested by speciating selenium in scoter livers. Third, I did not have direct measurements of Se in scoter food items from breeding grounds where these birds were collected, and assumed these foods were lower in Se than were those in marine wintering areas used by these scoters. Marine environments are naturally more enriched in selenium than most unpolluted freshwater aquatic systems (Haygarth, 1994; Ohlendorf, 2003), and Jorgelina Muscatello (University of Saskatchewan, unpubl. data) found invertebrates from four boreal wetlands in northern Saskatchewan, Canada, had concentrations between 0.3 and 2.0 mg/kg; therefore I believe this assumption is valid. Finally, I assumed that high liver Se concentrations were indicative of high muscle Se levels. Heinz et al. (1990) observed that muscle and liver tissue Se concentrations were similar in mallards fed a high Se diet for 6 weeks, and when birds were removed from the treatment the rate of elimination was slower in muscle than in liver. As the overwintering period of scoters, when they are likely exposed to higher levels of Se (see Chapter 6), exceeds 6 weeks, it is likely that concentrations in scoter muscle and liver would likely

have reached similar levels on wintering grounds (Heinz et al. 1990). Therefore, I assumed scoter muscle tissue would have Se concentrations similar to or greater than those in the liver at the time of sampling. However, given the relatively low Se concentrations in eider muscle compared to liver concentrations (Mallory et al. 2004), this assumption may be false. Regardless, this finding does not negate the conclusion that the source of egg nutrients is an important determinant of egg contaminant concentrations.

Maternal transfer of Se and other contaminants to eggs is the primary source of embryonic exposure to most contaminants in oviparous species. However, Se deposition into eggs may depend on the source (exogenous or endogenous) of egg nutrients. Females with low Se burdens may breed in Se contaminated environments and use exogenous nutrients for egg formation resulting in harmful egg Se levels. Conversely, females may carry high Se burdens during reproduction, yet nutrients used in the production of eggs may not be contaminated if they are acquired from sources with low Se concentrations. The latter scenario is the basis for my "dilution hypothesis", and is one plausible explanation for the apparent discrepancy between low egg concentrations and high somatic Se burdens in scoters as supported by my analyses. Thus, it is crucial to understand a species' nutrient acquisition patterns when studying egg Se dynamics in oviparous species. It is possible that Se exposure on wintering grounds affects reproduction of birds after migration if somatic tissues are used as a source of reproductive nutrients. However, reproductive energetic strategies and Se concentrations vary among individuals, which underscore the need to use techniques that provide information about individual reproductive energetic strategies. This study further demonstrates the potential for stable-isotope analysis to assist ecologists and toxicologists in better understanding nutrient allocation strategies and cross-seasonal effects of contaminant exposure in migratory wildlife (Hobson et al. 1997, Chapter 6).

6. CROSS-SEASONAL ASSOCIATION BETWEEN WINTER TROPHIC STATUS AND BREEDING GROUND SELENIUM LEVELS IN BOREAL WHITE-WINGED SCOTERS

6.1 INTRODUCTION

In migratory species, survival and reproductive success can be strongly affected by events occurring away from the breeding grounds (Heitmeyer and Fredrickson 1981, Webster and Marra 2005). Use of poor quality habitat on wintering and staging areas has been linked to reduced fitness in poor quality individuals (Gunnarsson et al. 2005). Contaminants acquired on wintering and staging areas may also be retained and carried to relatively pristine breeding grounds where they may affect reproductive success (Blais et al. 2005). Therefore, understanding whether there are carry-over effects from sources of variation in contaminant exposure may help to quantify the potential effect on fitness of non-breeding habitat change and to develop appropriate conservation strategies.

Selenium (Se) is an essential micro-nutrient required in small quantities for normal biological function, but is toxic to vertebrates at concentrations slightly over essential levels which are thought to range from 4 to 10 $\mu g \cdot g^{-1}$ (Heinz et al. 1989, Ohlendorf 2003). Selenium is also known to bioaccumulate with increasing trophic level within a food chain (Dobbs et al. 1996, Stuart et al. 2004), and concentrations are generally higher in marine than in freshwater environments (Haygarth 1994, Ohlendorf, 2003). While Se can be toxic to adult birds, developing embryos are considered far more sensitive and ecotoxicologists should be primarily be concerned with potential Se induced reproductive impairment (Heinz 1996). Embryos are exposed to Se by maternal transfer of organoselenium accumulated by the female through her diet and hatching failure has been observed at dietary concentrations that are only slightly greater than background levels (Heinz et al. 1989, Stanley et al. 1994). But, Se is also eliminated from the body through natural metabolic processes and excretion, though the rate of elimination depends on tissue metabolism (Heinz et al. 1990, Ohlendorf 2003). Therefore, variation in Se exposure within a species on the wintering grounds may not be detected on the breeding grounds if Se is eliminated rapidly.

Stable-isotopes analysis is an emerging tool for use in the study of cross-seasonal processes (Hobson 2005). Stable-nitrogen isotopes reflect relative trophic status of organisms within a food web, where a difference of *ca.* 3‰ for $\delta^{15}N$ normally represents one trophic level (DeNiro and Epstein 1981, Kelly 2000). This bioindicator has been used to explain variation in contaminant levels within food chains (Kidd et al. 1995, Quinn et al. 2003). However, to date no studies have reported on cross-seasonal sources of variation in Se concentrations in breeding birds. Metabolically inert tissues, such as feathers or claws, represent the assimilated diet of an organism at the time those tissues were formed (Bearhop et al. 2003, Hobson et al. 2006). Due to their slow growth, the distal portion of claws is thought to represent growth that occurred 2 to 5 months before sampling (Bearhop et al. 2003, Hobson et al. 2006, Robert Clark, Environment Canada, unpubl. data). Therefore, we examined whether there was a significant relationship between hepatic Se concentration and claw tip $\delta^{15}N$ values in female White-winged Scoters (*Melanitta fusca*; hereafter scoters) collected from boreal breeding grounds. Scoters winter in marine habitats yet breed in freshwater ecosystems in the boreal forest (Brown and Fredrickson 1997). Given that Se bioaccumulates with increasing trophic level, we tested the hypothesis that variation in Se levels in scoters on breeding grounds can be attributed to wintering ground trophic status. Specifically, we predicted that hapatic Se concentration of scoters on the breeding grounds will be positively related to claw tip $\delta^{15}N$ values.

6.2 METHODS

We collected 49 female scoters near Inuvik, NT (67^0N, 133^0W), over a two week period in 2003 (n = 14) and 2004 (n = 35). Birds were approached from shore or by canoe and when possible shot on the water to avoid excessive damage from shot. We did not use decoys to avoid condition bias (Pace and Afton 1999). Birds were individually bagged and stored under snow cover or in permafrost for up to 6 d before being frozen at -18 ^{0}C and transported to the Prairie and Northern Wildlife Research Center, Saskatoon, Saskatchewan, for dissection and analysis.

Claws from the third toe of the right foot were removed from each bird and placed individually in 20 ml scintillation vials. Claws were soaked in a 2:1 choroform:methanol solution for a minimum 24 h, then drained and rinsed with new solution to remove surface contamination. Claws were air dried again for 24 h before a 0.95-1.05 mg sample (~3mm from a claw 8-10mm in length) from the claw tip was removed for nitrogen isotope analysis. Samples were combusted using pyrolytic continuous-flow isotope-ratio mass spectrometry (CFIRMS) to determine carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios at the University of Saskatchewan, Saskatoon, Saskatchewan, Canada. All stable isotope ratio results are reported in delta notation (δ), in units of per mil (‰) and normalized to international standards ($\delta^{13}C$ – PeeDee Belemnite; $\delta^{15}N$ – air). Measurement error (95% CI) based on results from reference materials (egg albumen) analyzed every eight samples was ± 0.35‰. Due to variation in baseline $\delta^{15}N$ among food webs, interpretation of this biomarker as relative trophic level should only be done for a food web within an ecosystem (Cabana and Rasmussen 1996). Yet, scoters overwinter on both the East and West coasts, and occasionally in freshwater habitats (Brown and Fredrickson 1997). Therefore, we used discriminant function analysis based on $\delta^{13}C$ and $\delta^{15}N$ from scoters wintering on the Canadian Atlantic and Pacific coasts to identify wintering origin of our collected birds (Swoboda 2007); all birds were identified as wintering in marine areas along the Pacific coast.

We also analyzed developing follicles for $\delta^{15}N$ to test whether claw samples or hepatic Se were derived from the breeding grounds, as scoters use dietary protein for egg formation (Dobush 1986, JMD unpubl. data). Large yolky follicles were oven dried at 80^0C to constant weight. Then, they were soaked, rinsed and dried using the above method to remove lipids. Lean dry follicles were sampled and analysed for $\delta^{15}N$ using the above methods. A lack of relationship between hepatic Se and follicle protein $\delta^{15}N$ would indicate that liver Se did not originate from the breeding grounds. Likewise, a lack of relationship between claw $\delta^{15}N$ and follicle protein $\delta^{15}N$ would be consistent with the view that claw tissues we sampled were not produced from nutrients acquired on the breeding ground.

Liver was removed from carcasses, placed individually in acid-washed glassware and sent to the National Wildlife Research Center in Ottawa, ON, for Se analysis. Briefly,

tissue samples were homogenized and approximately 0.5 g placed into preweighed, acid-washed test tubes, freeze-dried, and their dry masses recorded. Deionzed H_2O (0.5 ml) and HNO_3 (either 0.5 ml or 1.0 ml) were added to each test tube and samples were allowed to sit overnight at room temperature. The following day they were heated at 100°C in dry baths for 6 h. Samples were allowed to cool overnight, volumes were then adjusted to 4.0 ml with deionized H_2O. Se was analyzed by graphite furnace atomic absorption spectrometry using the Perkin-Elmer 3030b equipped with a deuterium background corrector, HGA-300 graphite furnace, and AS-40 autosampler. All concentrations are reported on a dry weight basis. Standard reference materials (Tort-2, Dorm-2 and Dolt-2 from the National Research Council, Ottawa) were analyzed for quality assurance and all samples were within certified limits. Five true duplicates were also analyzed and sample recoveries ranged from 0.3 to 11.3 % Relative Standard Deviation.

We used ANCOVA to test for effects of collection day, year and claw $\delta^{15}N$ and year*collection day interaction on hepatic Se. In the absence of a significant year or year interaction effect, we removed the covariate year to reduce model complexity and used Multiple Regression Analysis (Norušis 1990). We used ANCOVA to test for a relationship between liver Se concentration and follicle protein $\delta^{15}N$ levels while controlling for effects of collection date, and linear regression to test for a relation between claw and follicle protein $\delta^{15}N$ values.

6.3 RESULTS

Hepatic Se concentration (ANCOVA; $F_{16} = 0.026$, $P = 0.83$) and claw $\delta^{15}N$ (linear regression; $F_{16} = 0.08$, $P = 0.78$) were not associated with follicle protein $\delta^{15}N$. There was no effect of year or year*collection day on liver Se (ANCOVA; $F_{1,44} < 0.12$, Ps > 0.73), so we reduced our model to include only claw $\delta^{15}N$ and collection date as predictors of liver Se concentrations. Claw $\delta^{15}N$ values ranged from 12.9 to 17.0‰, spanning almost two trophic levels (DeNiro and Epstein 1981, Kelly, 2000). Se concentrations varied from 3.9 to 75.1 $\mu g \cdot g^{-1}$ and 31 of the 49 females had concentrations in excess of 33 μg·g-1, the threshold for physiological impairment for captive adult

mallards (Heinz 1996). After controlling for collection date, there was a significant positive relationship between claw $\delta^{15}N$ and liver Se (multiple regression; $\beta_{clawN} = 3.9 \pm 1.7$ SE; $t_{1,46} = 2.258$, $P = 0.029$, $r = 0.32$; Fig. 6.1). There was a negative relationship ($\beta_{colldate} = -1.12 \pm 0.39$; $t_{1,46} = -2.844$, $P = 0.007$, $r = -0.39$) between liver Se and collection date.

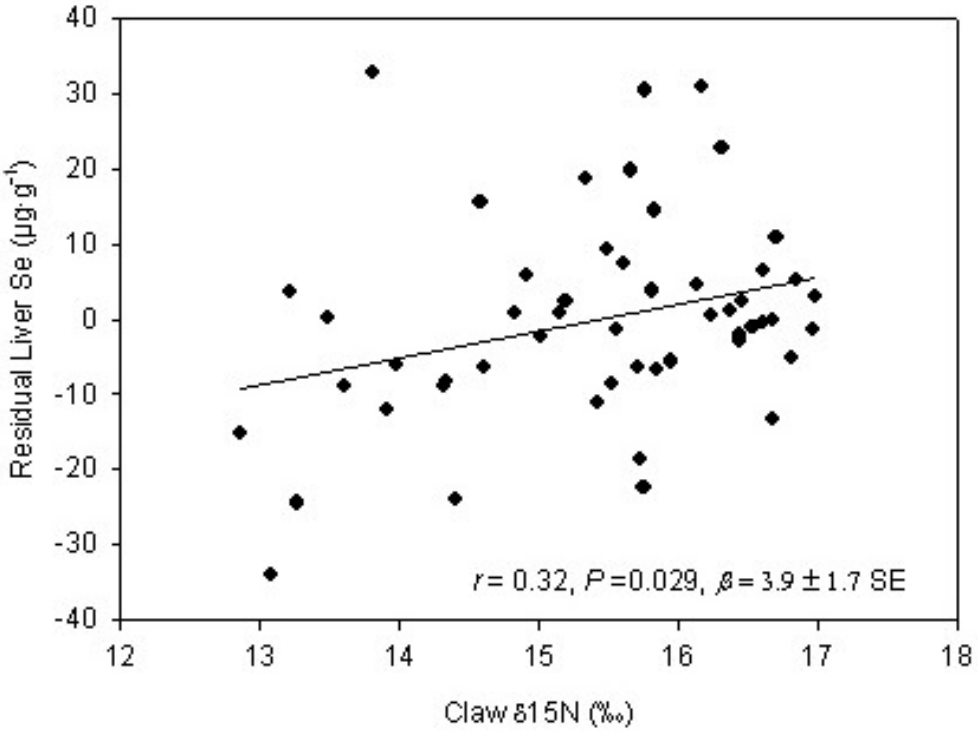

Figure 6.1. Plot of claw tip $\delta^{15}N$ versus residuals of liver Se concentration, corrected for effects of collection date, and partial correlation statistics. Higher claw tip $\delta^{15}N$ values are interpreted as higher trophic position.

6.4 DISCUSSION

The level of variation in both claw $\delta^{15}N$ and liver Se provided adequate variation to detect an effect of winter trophic position on breeding ground liver Se concentrations. Based on this parameter estimate for claw $\delta^{15}N$, females feeding at approximately one trophic level (Δ3‰ $\delta^{15}N$) higher on wintering grounds had 12 ± 5 μg·g-1 more liver Se at the time of collection (Fig. 6.1). The negative relationship between liver Se and collection date suggests that Se was likely depurated over time since leaving the marine wintering grounds (see also DeVink et al. 2007b), but not to an extent that prevented the detection of a correlation between apparent winter trophic level and liver Se concentrations. This demonstrates that in migratory species, differences in individual exposure to Se on wintering and staging areas can carry over onto breeding grounds. These results are in agreement with those of other studies that concluded that Se in tissues of eider ducks during the breeding period was mainly derived from their diet while at sea during late winter and early spring (Grand et al. 2002, Wilson et al. 2004). High dietary exposure to Se in marine wintering and staging areas could pose a risk to species that rely on endogenous protein for egg formation, because embryonic development is a sensitive endpoint for Se toxicity in birds and Se deposition into eggs is likely in the form of amino-acids (Heinz 1996). Fortunately, scoters use mostly exogenous protein to form eggs thus avoiding the deposition of potentially toxic levels of Se into eggs (Dobush 1986, JMD unpubl. data). However, in species that use endogenous nutrients high in Se content, transfer to eggs and subsequent reproductive failure could impact populations if exposure was widespread.

One potential source of uncontrolled variation in Se exposure is the uncertainty about wintering population origins along the Pacific coast. Though we identified all our birds as wintering on the West coast, it is possible they fed in locations with different baseline $\delta^{15}N$ values. Though this would cause differences in claw $\delta^{15}N$ (Cabana and Rasmussen 1996), it would not likely cause the relationship we observed between claw

$\delta^{15}N$ and liver Se. Indeed, scoters use protein acquired from dietary sources on breeding grounds to produce eggs (Dobush 1986, Chapter 8). Therefore, the lack of relationship between follicle protein $\delta^{15}N$ and either claw $\delta^{15}N$ or liver Se validated our assumption that liver Se was not solely acquired on the breeding grounds, and that the portion of claw we sampled did not represent a breeding ground diet signal.

There is interspecific variation in Se tolerance among birds, and species have likely adapted to different levels of Se exposure at the natural concentration in their habitat (Skorupa 1998). The cross-seasonal relationship we observed in somatic Se may have greater implications for species that winter or stage in habitats anthropogenically enriched in Se (e.g., the Great Lakes), where exposure may have increased above historical levels, and particularly for those species that use endogenous nutrients for egg formation. These birds may then accumulate higher Se burdens than normally experienced and increased transfer to eggs may lead to reproductive failure through teratogenesis (Heinz et al. 1989) and subsequently to population level changes (Skorupa 1998).

Cross-seasonal effects of habitat quality on the wintering grounds may influence fitness through effects on timing of departure from wintering grounds (Marra and Holmes 2001), body condition upon arrival (Heitmeyer and Fredrickson 1981, Gunnarsson et al. 2005), or contaminant levels (this study). Connecting sources of variation in factors that can impact reproductive success across seasons has been very difficult in the past. However, tools such as stable-isotope analysis are becoming increasingly useful to ecologists interested in these seasonal interactions (Hobson 2005).

7. STABLE ISOTOPES OF LESSER SCAUP AND RING-NECKED DUCK CLAWS: CLAW ISOTOPE RELATIONSHIPS WITH SPRING NUTRIENT LEVELS AND SELENIUM CONCENTRATIONS

7.1 INTRODUCTION

Habitat quality and selection through the annual cycle can influence breeding success and survival across seasons (Heitmeyer and Fredrickson 1981, Gunnarson et al. 2005, Webster and Marra 2005). In migratory birds, nutrients used to fuel migration and breeding may be acquired on wintering and staging habitat months in advance (Heitmeyer and Fredrickson 1981, Bond and Esler 2006). The importance of winter and spring food availability to subsequent reproduction is particularly important in species that depend largely on somatic nutrients during reproduction (capital breeding strategy; Drent and Daan 1980). While acquiring essential nutrients, birds also may accumulate harmful levels of contaminants. At sublethal doses, contaminants may result in reduced breeding success (eggshell thinning, Bowerman et al. 1995; embryonic mortality, Ohlendorf et al. 1990). Therefore, discerning links between variation in avian body condition or contaminant levels on breeding grounds and patterns of wintering ground distribution and migration strategies will improve our understanding of where and how non-breeding habitat affects reproductive fitness.

Continental scaup (lesser (*Aythya affinis*) and greater (*A. marila*) combined) breeding numbers has declined by an estimated 150,000 birds per year between 1978 and 1997 (Afton and Anderson 2001), with a continued decline and lack of recovery over the last decade (Wilkins and Otto 2006). Two explanations for this decline are the spring condition (Austin et al. 2000; Anteau and Afton 2004, 2006) and contaminant (Austin et al. 2000; DeVink et al. 2007b) hypotheses. These hypotheses purport that restricted spring nutrient availability and increased contaminant acquisition, particularly Selenium (Se), respectively, on wintering and staging areas are reducing scaup productivity. Ring-necked ducks, on the other hand, are closely related to scaup and have similar life-histories, yet are becoming more abundant in the boreal forest (Wilkins and Otto 2006). Devink et al. (2007b, 2008) compared body condition and contaminant levels in the two

species and found that scaup had similar body condition (more lipid, but slightly less protein) and slightly higher Se levels than ringnecks, on average, but that in general levels of both indices varied more within than between species. Differences in arrival body condition and contaminant levels are likely linked to wintering and early staging diet and location (e.g., Chapter 6), which can be delineated at large scales using naturally occurring stable-isotopes (Hobson 1999).

Stable-isotope ratios are known to vary with geographic location and among organisms due to known biogeochemical processes (Hobson 1999). Stable-nitrogen isotopes ($\delta^{15}N$) reflect relative trophic status of organisms within a food web, where a difference of *ca.* 3‰ (2-5‰) for $\delta^{15}N$ normally represents one trophic level (DeNiro and Epstein, 1981; Kelly, 2000). Soil samples taken throughout North America indicate that there is a pattern of decreasing $\delta^{15}N$ with increasing latitude (Fig. 7.1; Amundson et al. 2003), patterns that may be complicated by anthropogenic inputs of fertilizers and pollutants (e.g., Hebert and Wassenaar 2001). Furthermore, it is uncertain if this pattern is applicable to aquatic systems, though Clark et al. (2006) found a positive correlation (r = 0.64) between δD and $\delta^{15}N$ in duckling feathers that suggests a slight latitudinal effect. Stable-carbon isotopes ($\delta^{13}C$) are generally more enriched in marine as opposed to freshwater systems (Hobson 1999). In terrestrial systems, $\delta^{13}C$ varies on the landscape depending primarily on the photosynthetic pathways of the plants (CAM, C4 or C3) (Fig. 7.2; Suits et al. 2005). The latitudinal pattern of variation in stable-hydrogen isotopes (δD) has been used to determine migration patterns and link large scale breeding, wintering and staging areas in migratory species (Hobson and Wassenaar 1997, Hobson et al. 1999). In North America, δD in precipitation generally becomes more depleted with increasing latitude (Fig. 7.3). The isotopic signal of assimilated food items is incorporated into growing tissues; the period of time represented by the isotopic signal of a tissue depends on its metabolic rate. Metabolically inert tissues, such as feathers or claws, represent the assimilated diet of the organism at the time those tissues were formed (Bearhop et al., 2003). Due to their slow growth, the distal portion of claws is thought to represent growth that occurred 2 to 5 months prior to sampling (Bearhop et al., 2003, Hobson et al. 2006). This tool is also increasingly used to help understand when in the

annual cycle contaminants are acquired (Hobson et al. 1997, Sanpera et al. 2007, DeVink et al. 2007a).

Here, I compare claw tip $\delta^{15}N$, $\delta^{13}C$, and δD between scaup and ring-necked ducks to compare isotope values with nutrient (lipid and protein) content and Se levels of females on breeding grounds to assess possible cross-seasonal relationships. I also examine interspecific differences in wintering and staging habitats, and trophic–level.

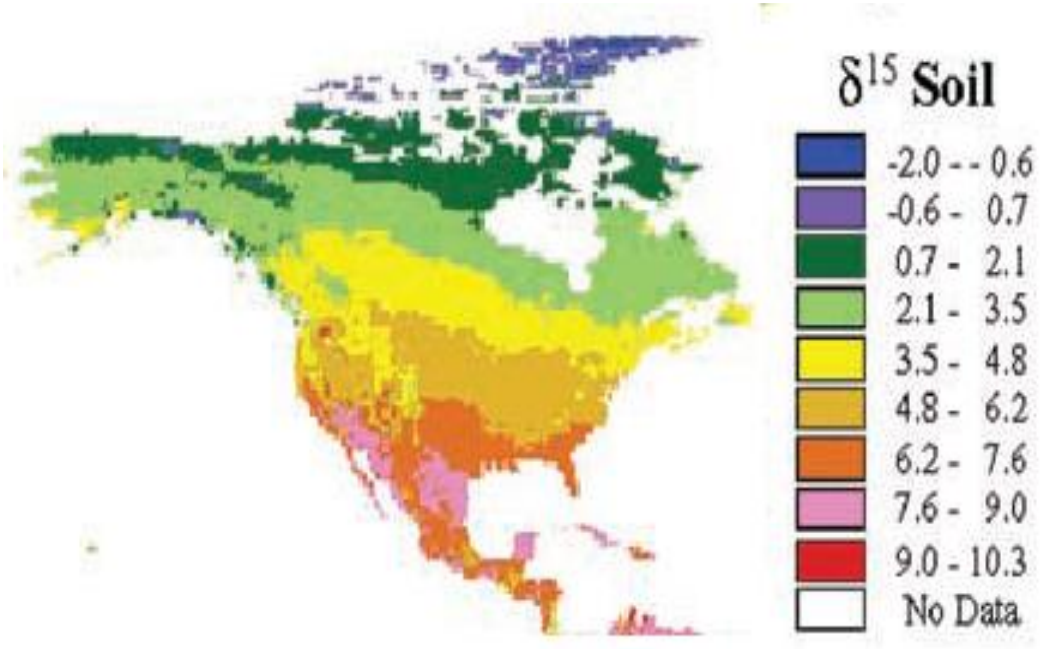

Figure 7.1. North American pattern of soil $\delta^{15}N$ values (adapted from Amundson et al. 2003).

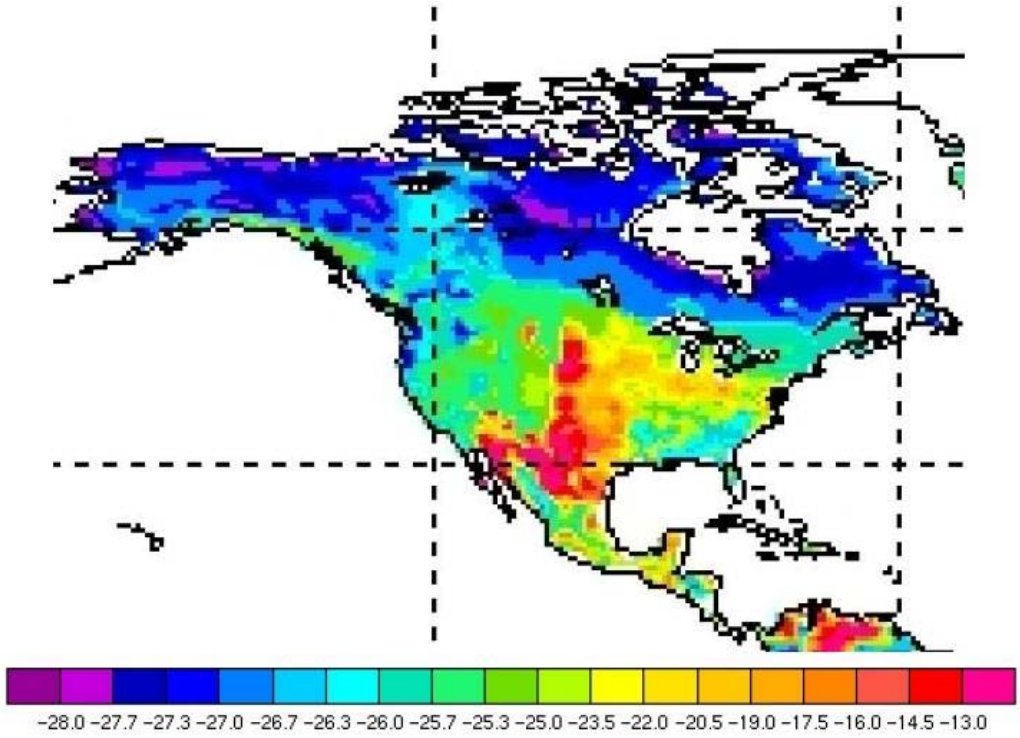

Figure 7.2. North American pattern of $\delta^{13}C$ values in terrestrial plants (adapted from Suits et al. 2005).

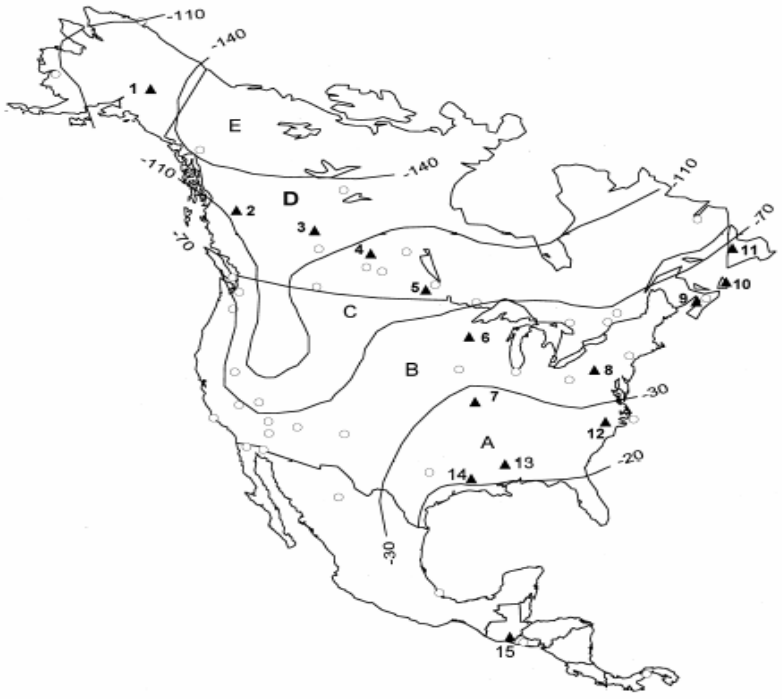

Figure 7.3. North American patterns of δD concentrations. *Circles* represent precipitation sampling sites and *triangles* indicate locations where breeding forest passerines were sampled (Adapted from Hobson and Wassenaar 1997).

7.2 METHODS

For detailed methods about field collections, see Chapter 2. Birds were also collected from a site located in the Saskatchewan River Delta (SK) near the Saskatchewan and Manitoba border, and ringnecks were not collected from the Inuvik (IN) site. Methods used to assess body condition and contaminants were described in Chapter 3. For methods used to determine $\delta^{13}C$ and $\delta^{15}N$ in claws, refer to methods in Chapter 6.

To analyze δD in claws, 0.3 mg (0.33 – 0.37 mg) of clean, dry, claw tip (approximately 1 mm of distal portion of claw) was weighed in silver capsules (see Chapter 6 for cleaning procedure). Sample analysis was conducted at the National Hydrology Research Institute, Saskatoon, Saskatchewan, Canada. Samples were combusted using online continuous-flow isotope ratio mass spectrometry (CFIRMS)

performed on a Micromass Optima dual-inlet isotope ratio spectrometer (Micromass UK, Manchester, UK) as described in Wassenaar and Hobson (2003). Deuterium concentration are expressed in delta notation (δ), in units per mil (‰), and normalized on the VSMOW-SLAP standard scale. Laboratory standard reference material (whale baleen) were also analysed after every 8 claw samples were run. Reproducibility of CFIRMS conducted in this laboratory and based on reference materials is ≤ 2‰ (Wassenaar and Hobson 2003).

7.2.1 Statistical Analysis

All statistical analyses were conducted using SPSS v.13 (Norušis 1990). I tested for an effect of year, collection date, location and year*location and year*collection date interactions on each stable-isotope within species using ANCOVA to identify spatio-temporal relationships between breeding ground collection sites and claw isotope values. In the absence of a year or year interaction effect, I removed these model parameters to reduce model complexity. I compared least-squares mean isotopic values between species by locations while controlling for collection date using ANCOVA. Intraspecific relationships between isotopic values and Se, somatic lipid and protein, controlling for collection date, also were evaluated using ANCOVA to determine whether variation in liver Se and nutrient levels were related to patterns of stable-isotope distribution. I used bivariate correlation analysis to determine the correlation between stable-isotope and the residual of Se, lipid and protein after controlling for collection date.

7.3 RESULTS

Year or year interaction effects were not detected in either species (*P*s > 0.10). Therefore, I removed these parameters to reduce model complexity.

Claw tip values of $\delta^{13}C$ ranged from -28.5 to -19.3‰ in scaup and -27.1 to -15.4‰ in ringnecks. After controlling for collection date, $\delta^{13}C$ became more enriched in claws of scaup collected at sites with increasing latitude, though only the AB sample was significantly more depleted, and $\delta^{13}C$ became more depleted in claws of ringnecks collected at sites with increasing latitude, though the differences were non-significant

(Fig. 7.4). Ringneck samples were more enriched in $\delta^{13}C$ than in scaup in AB and SK samples, but not in YK. In scaup, $\delta^{15}N$ values ranged from 6.1 to 17.1‰ and were more enriched at higher latitudes. This pattern was not observed in ringnecks, where both AB and YK were more enriched than SK, ringneck values ranged from 6.4 to 14.3‰. Between species comparisons indicated that scaup were more enriched in $\delta^{15}N$ at all three locations, though the difference between scaup and ringnecks in AB (~0.6‰) was much less than in SK or YK (~2‰) (Fig. 7.4). There was a large range of δD values observed in both scaup (-42 to -136‰) and ringnecks (-32 to -121‰). Deuterium values were very similar in scaup among locations, but increased with increasing latitude in ringnecks, though differences in ringnecks were non-significant. Differences between species were only significant at the YK location (Fig. 7.4).

The relationships between all three isotopes and Se, lipid and Protein indices for both species are summarized in Table 7.1. In both species, Se was positively related to claw tip $\delta^{15}N$ values (Scaup, $\beta = 1.45 \pm 0.41$ SE, $r = 0.48$; Ringneck, $\beta = 0.37 \pm 0.17$ SE, $r = 0.38$; Fig 7.5), and in ringnecks Se also was weakly related to δD ($\beta = 0.03 \pm 0.02$ SE, $r = 0.33$; Table 7.1). Nutrient indices showed very little relationship with any of the isotope values (Table 7.1), although protein was weakly inversely related to $\delta^{15}N$ values in scaup ($\beta = -0.37 \pm 0.20$ SE, $r = -0.18$).

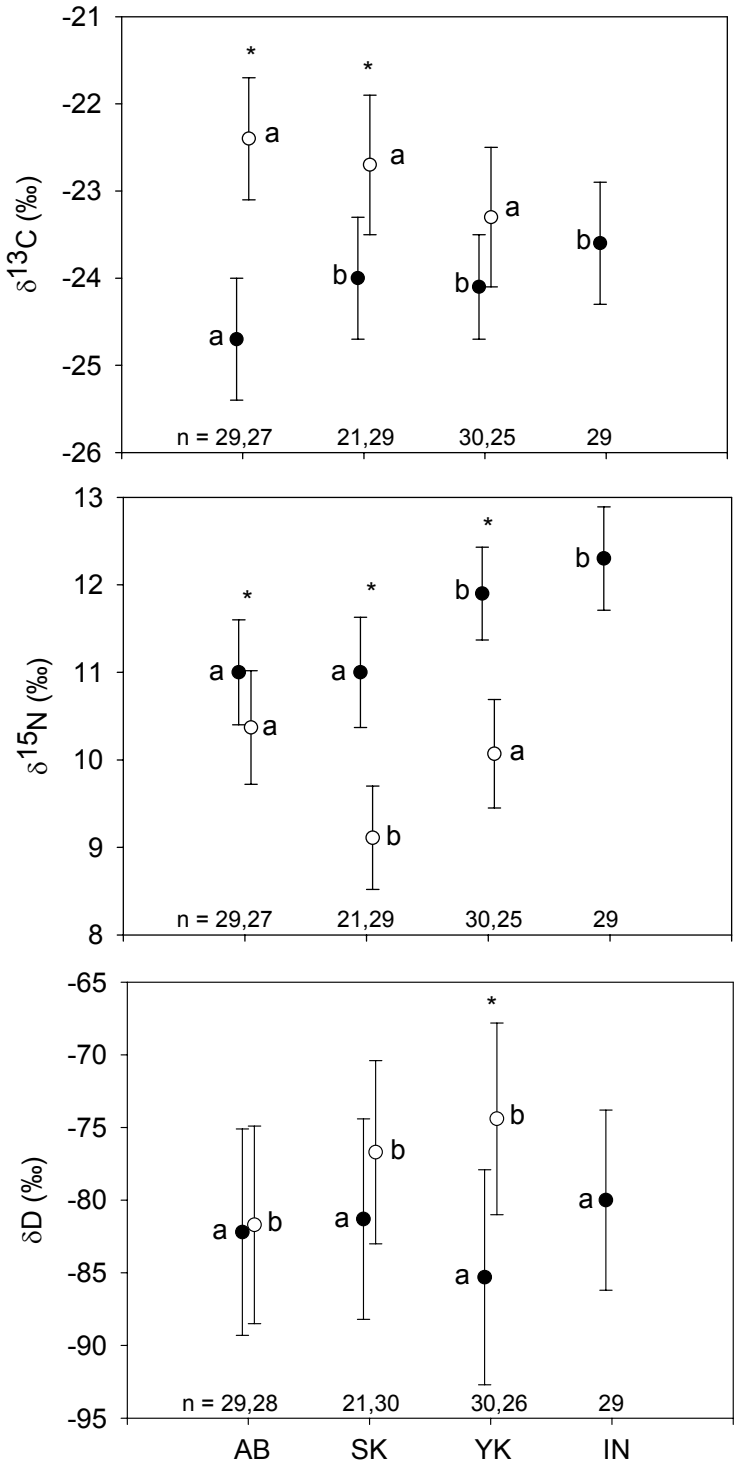

Figure 7.4. Mean ± 95%CI stable-carbon, -nitrogen, and -deuterium values of Lesser Scaup (closed circles) and Ring-necked Duck (open circles) claw tip from each collection site (Alberta – AB; Saskatchewan – SK; Yellowknife – YK; and Inuvik – IN). All values are corrected for collection date. Different letters beside means indicate significant intraspecific differences among locations; Asterisks above means indicate significant interspecific differences within a location. $\alpha = 0.05$.

Table 7.1. ANCOVA results examining the relationship between δD, $\delta^{15}N$, and $\delta^{13}C$ and liver selenium, somatic lipid and protein estimates in lesser scaup and ring-necked ducks collected from four boreal sites in 2003 and 2004. All models controlled for date of collection.

		Selenium			Lipid			Protein		
		N[a]	D[a]	*P*	N[a]	D[a]	*P*	N[a]	D[b]	*P*
LESC	δD	1	40	0.64	1	106	0.24	1	106	0.92
	$\delta^{15}N$	1	40	0.001	1	106	0.331	1	106	0.06
	$\delta^{13}C$	1	40	0.87	1	106	0.44	1	106	0.11
RNDU	δD	1	27	0.08	1	81	0.34	1	81	0.89
	$\delta^{15}N$	1	26	0.045	1	78	0.36	1	78	0.33
	$\delta^{13}C$	1	26	0.65	1	78	0.85	1	78	0.68

a – degrees of freedom

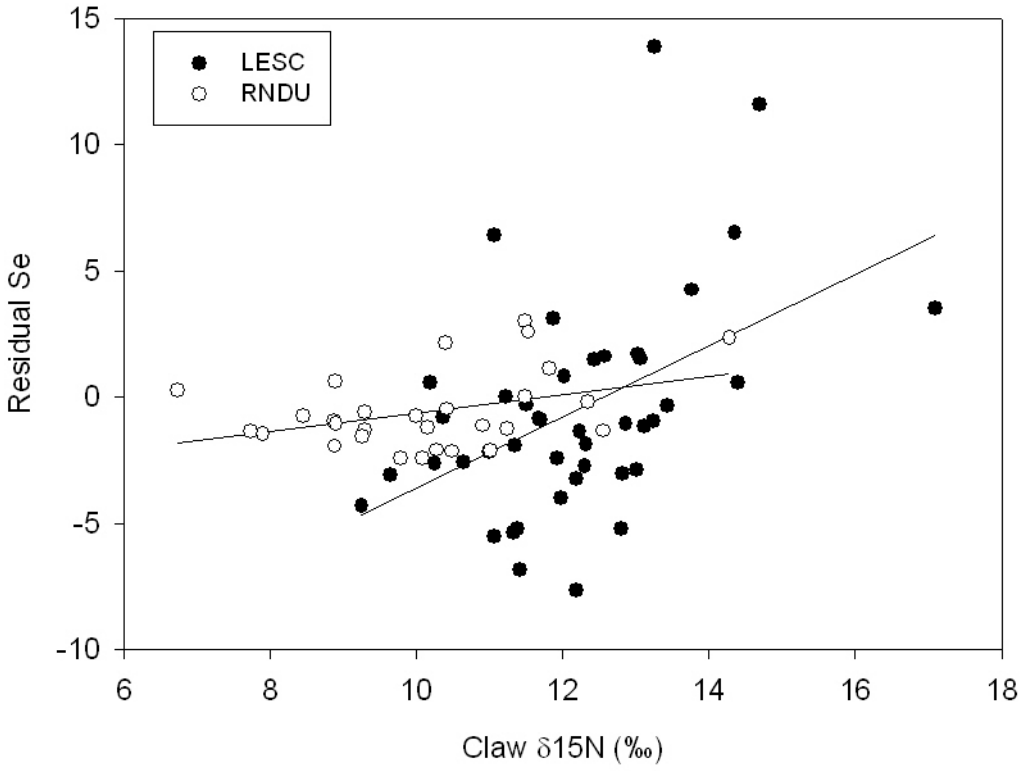

Figure 7.5. Correlation between the residual of liver selenium ($\mu g \cdot g^{-1}$ dry weight) corrected for date of collection and claw tip $\delta^{15}N$ in both lesser scaup and ring-necked ducks collected from four Canadian boreal forest locations in 2003 and 2004.

7.4 DISCUSSION

7.4.1 Deuterium

Deuterium values in claws have recently been determined to provide an index of latitudinal origin of the nutrients used to form the tissue analyzed (Hobson and Wassenaar 1997). After correcting for collection date, δD values were highly variable in both species, but there was an apparent positive correlation between collection sites and δD in ringnecks (Fig. 7.4). This indicates that birds collected farther north either winter farther south or departed the migration areas later than birds collected at lower latitudes in a leap-frog type migration pattern (Alerstam and Högstedt 1980, Zink 2002). This pattern is consistent with the mean timing of nest initiation in this species, which is generally earlier at southern latitudes than at northern latitudes. I did not observe a similar

pattern in scaup; claw tip δD values were similar on average among locations, with the exception of YK which was slightly more depleted. This pattern also is consistent with the mean timing of nest initiation in scaup where mean NID is less variable across latitudes (DeVink et al. 2008).

After adjusting date corrected values for a discrimination factor of approximately -27.8‰ from precipitation to claw δD (Clark et al. 2006, Hobson et al. 2006), values for scaup (mean = -59‰) and ringnecks (mean = -54‰) would place both species in zone B in Figure 7.3. Based on known migration corridors of both species (Bellrose 1980), the mean δD values would likely place birds in the vicinity of the U.S. mid-west, Great Lakes and adjacent states. However, the range of values in both species indicates that the sampled populations were possibly staging throughout the United States and southern Canada.

Excess Se is normally eliminated from an organism once its dietary exposure is reduced (Chapter 4). Boreal forest aquatic systems are likely more depleted in Se than agricultural and industrialized landscapes of North America (Eisler 1985). The observed patterns of δD across latitudes in both species also are consistent with the positive relationship between Se and δD in ringnecks, and the lack of a relationship between Se and δD in scaup (Table 7.1). Higher δD values in claw would indicate that at the time of claw formation, the bird was likely at lower latitude and that there would be a greater amount of time spent in low Se locations of the boreal before collection. A lack of relationship between δD and lipid or protein suggests that there was no relationship between nutrient stores upon arrival on breeding grounds and the location of birds at the time the claw tip was grown (2-5 months prior).

7.4.2 Nitrogen

Nitrogen isotope ratios are frequently used to compare trophic levels within a food web. Differences between trophic levels range from 2-5‰ and average 3‰ (DeNiro and Epstein, 1981; Kelly, 2000). However, the difficulty in using this tracer is that differences in baseline values among ecosystems and food webs can confound the interpretation of N values among systems (Cabana and Rasmussen, 1996). Given that at the time of claw growth, sampled scaup and ringnecks would likely have been feeding in

myriad different wetlands, I compared claw $\delta^{15}N$ values among species. Wintering and early migrant ringnecks generally include more vegetation in their diet than do scaup (Hohman and Eberhardt 1998, Austin et al. 1998). Therefore, one would expect that scaup would have higher $\delta^{15}N$ values than ringnecks, which was the case at the SK and YK sites where scaup were approximately 2‰ more enriched than ringnecks, or slightly less than one trophic level higher than ringnecks (Fig. 7.4). While scaup from the AB site were more enriched than ringnecks, the difference was not as pronounced and may be the result of differences in stopover locations or local forage.

Se is known to bioaccumulate in food chains (Dobbs et al. 1996, Stuart et al. 2004), and the significant positive relationship between Se and $\delta^{15}N$ in both scaup and ringnecks (Table 7.1; Fig. 7.5) are consistent with these findings. The difference in apparent trophic position between the two species may also explain the higher Se levels measured in scaup compared to ringnecks (Devink et al. 2007b). Alternatively, this relationship may be the result of some birds using marine and estuarine areas with higher $\delta^{15}N$ and Se values (Haygarth 1994, Ohlendorf 2003). The marginally significant negative relationship between protein and N in scaup could be the result of protein stress during migration. Recycling of amino acids could result in an enrichment of tissue $\delta^{15}N$ values (Hobson et al. 1993). However, Williams et al. (2007) found that captive birds fed *ad-libidum* had enriched $\delta^{15}N$ compared to birds fed a limited diet to meet only metabolic requirements. Therefore, the relationship I observed between protein and $\delta^{15}N$ is difficult to interpret.

7.4.3 Carbon

Marine and freshwater aquatic systems are known to vary in $\delta^{13}C$ where the marine environment is more enriched (Hobson 1999). In terrestrial systems within North America, there are large scale spatial patterns of $\delta^{13}C$ which are attributable mainly to plants with different photosynthetic pathways (Cam, C4 and C3) (Fig. 7.2; Suits et al. 2005). However, it is not known if this terrestrial distribution pattern can be applied to delineate origins of waterbirds. For the sake of interpreting my data, I assumed that this spatial distribution applied to waterbirds as well.

I observed opposite patterns of $\delta^{13}C$ between species among sites; scaup became more enriched in $\delta^{13}C$ at sites farther North and ringnecks became more depleted, though differences among sites were not significant in ringnecks (Fig. 7.4). At the AB and SK sites, scaup claws were significantly more depleted in $\delta^{13}C$ than ringnecks. This difference was small compared to the range of $\delta^{13}C$ across the landscape (Fig. 7.2). Given the virtually identical δD values of scaup and ringnecks at the AB site, it is likely that this was due to differences in habitat preferences and not due to dietary differences.

I found no significant relationship between $\delta^{13}C$ and either lipid, protein or liver Se levels (Table 7.1). As Se and $\delta^{13}C$ values are both more enriched in marine environments (Hobson 1999, Haygarth 1994), the lack of a relationship between these two variables is an indication that claw was probably grown during migration. This is supported by δD values (mean = -75 to -85‰) indicating a mid-continent location after correcting for fractionation.

7.5 CONCLUSIONS

Given that I controlled for collection date and that there was fairly consistent variation among samples within species, the variation in isotopic signal among locations in each species suggests that birds differed in location at the time the claw sample was grown. Because numerical declines in scaup have occurred across most of their range, this suggests that if the cause of the decline originates on the staging areas then it would not likely be restricted to one geographical location but widespread across the staging habitats. However, the interaction of species and location on mean isotope values (Fig. 7.1) suggests some level of segregation between scaup and ringnecks on the staging areas. This is consistent with reported differences in habitat use of the two species during early migration (Austin et al. 1998, Hohman and Eberhardt 1998). Given the differences in population trends, it is possible that changes to scaup migration habitats are affecting this species, though there was no relationship with nutrient stores (see below).

I found no significant relationship between isotopic variation in claw tip samples and either lipid or protein levels in females collected at boreal breeding sites with the exception of a weak relationship between protein and $\delta^{15}N$ in scaup. These results suggest that arrival body condition is not related to the pattern of distribution in those isotopic

signals. One possible interpretation of these results is that body condition of scaup and ringnecks is not related to staging areas used during migration at the time the claw sample analysed was grown (approximately two to five months prior to collection). It is possible that the adaptability and plasticity in waterfowl to acquire and deposit nutrients (Barboza and Jorde 2002) permits females to obtain nutrients throughout migration and upon arrival on breeding grounds. This would be inconsistent with the predictions of Anteau (2006) that habitat degradation in the upper mid-west affects body condition of females upon arrival, unless the birds I collected do not use that area for staging.

While scaup are reported to have a little more Se than ringnecks (Devink et al. 2007b), this difference is likely driven by differences in winter and spring trophic status as was observed in white-winged scoters (Devink et al. 2007a). Custer and Hohman (1994) reported hepatic Se concentrations of 3.3-4.4 $\mu g \cdot g^{-1}$ (dry weight) in wintering canvasbacks (*Aythya valisineria*) – levels very similar to those I observed in ringnecks (Chapter 3: Table 3.1). This is consistent with my observation of a trophic relationship to Se levels as ringnecks and canvasbacks both feed primarily on vegetation during the winter and early spring migration. As Se is positively related to trophic level within food webs (Dobbs et al., 1996; Stuart et al., 2004) this may increase the risk of Se contamination in scaup and species feeding at higher trophic levels. However, to date there is no evidence that observed Se concentrations in scaup have had a negative impact on adult physiology or reproduction (Fox et al. 2005, Devink et al. 2007b).

8. BODY COMPOSITION AND STABLE ISOTOPE ANALYSIS REVEAL PLASTICITY IN SCOTER REPRODUCTIVE ENERGETIC STRATEGIES

8.1 INTRODUCTION

Animals must balance the acquisition and allocation of nutrients (lipids, protein and minerals) to optimize reproductive fitness (Zera and Harshman 2001). During reproduction, birds' energetic demands can greatly exceed basal metabolic requirements (e.g., 280% higher than BMR in ruddy ducks *Oxyura jamaicensis*; Alisauskas and Ankney 1994). Female birds that draw entirely from somatic nutrient stores (muscle tissues, lipid deposits and bone minerals) to provide reproductive nutrients are termed, "capital breeders", whereas those that meet reproductive nutrient requirements through diet are known as, "income breeders" (Drent and Daan 1980). However, there is a continuum between the capital and income breeding strategies where some species or individuals rely on a mixture of dietary and somatic nutrients for reproduction (Thomas 1988).

The high nutrient (lipid, protein and mineral) demands of reproduction in waterfowl may influence timing of nest initiation (Krapu 1981; Alisauskas and Ankney 1992; Esler and Grand 1994), clutch size (Ankney and Afton 1988; Ankney et al. 1991; Esler and Grand 1994; but see Arnold and Rohwer 1991), and incubation constancy (Hohman 1986; Mallory and Weatherhead 1993; but see Manlove and Hepp 2000). In the absence of adequate nutrients, birds may abandon an ongoing reproductive effort (Mallory and Weatherhead 1993; Bustnes et al. 2002), or abstain from breeding (Ankney and Alisauskas 1991). Thus, determining how and when waterfowl acquire nutrients for reproduction is important to understanding their life-history strategies (Zera and Harshman 2001); improved understanding of nutrient acquisition and allocation patterns is also important for conservation of habitats that supply essential nutrients at the appropriate times of the annual cycle.

Theoretically, obligate capital or semi-capital breeders must surpass a threshold level of body nutrients to breed. However, as the degree of somatic nutrient use may vary within a species, Reynolds (1972) proposed the hypothesis of a seasonally-variable

nutrient reserve threshold. This hypothesis predicts that the threshold declines over the breeding season, an idea that has some support (Krapu 1981; Rohwer 1992; Esler et al. 2001). This may represent an adaptation to accommodate seasonally-variable local food availability, with early breeders having limited access to dietary nutrients, or differences in the optimal value that varies due to changes in the tradeoff between nutrients stores and time remaining to attempt to breed.

Body composition analysis (BCA) is a technique used regularly for assessing nutritional strategies of breeding birds where nutrients stored in somatic tissue of females are estimated and regressed against estimates of reproductive tissues (Alisauskas and Ankney 1985; Dobush 1986; Ankney and Afton 1988; Afton and Ankney 1991; Mann and Sedinger 1993; Esler and Grand 1994; Choinière and Gauthier 1995; Esler et al. 2001). If somatic stores remain unchanged with increasing reproductive investment, nutrients in the eggs and oviduct are assumed to have come directly from diet (income breeding). Likewise, a capital breeding strategy is inferred when somatic stores decline at a rate equal to that invested in reproductive tissues. Limitations of this technique have been previously identified (Alisauskas and Ankney 1992; Mann and Sedinger 1993; Sedinger et al. 1997; Meijer and Drent 1999) and are reviewed in the discussion.

Recently, stable-isotope analysis (SIA) has been used to examine energetic strategies in breeding waterbirds (Hobson et al. 2000), arctic-nesting geese (Gauthier et al. 2003), and seaducks (Hobson et al. 2005, Bond et al. 2007). This technique relies on known distribution patterns of naturally occurring stable isotopes of several elements in the environment (reviewed in Hobson 1999; Kelly 2000). In species that migrate between areas with isotopically distinct signals, it is possible to identify nutrients assimilated into tissues from one location after the bird has moved to another location. Therefore, in many situations it is possible to chemically trace and estimate the relative contributions of somatic or dietary nutrients to eggs (Hobson et al. 1997). This technique is more powerful with the use of multiple isotopic tracers (Chamberlain et al. 1997; Hobson et al. 1999).

Gauthier et al. (2003) applied a SIA approach to evaluate reproductive energetics in greater snow geese (*Chen caerulescens atlantica*), where energetics had previously been evaluated using BCA (Choinière and Gauthier 1995); conclusions were comparable.

However, to date no study has attempted to simultaneously use both techniques to contrast interpretations of the data obtained from the same birds, which is important as individual strategies may differ. Therefore, my objective was to use data from the same individual white-winged scoters (*Melanitta fusca*; hereafter scoters) to compare interpretations of reproductive allocation strategies based on both BCA and SIA techniques described above. I also compare the strategy of birds collected at the northern extent of their range with that of birds collected at the southern extent (Dobush 1986). This species is a good model for this comparison because scoters winter in marine environments but breed in freshwater habitats which differ in stable-isotopes of carbon ($\delta^{13}C$) and possibly nitrogen ($\delta^{15}N$) (Hobson 1999; Hobson et al. 2000). I also review advantages and limitations of each technique, and argue for using these methods simultaneously to provide a more robust assessment of species' and individuals' energetic strategies.

8.2 METHODS

8.2.1 Field Collections

In 2004, I collected female scoters soon after they arrived on the breeding grounds, according to animal care approval (University of Saskatchewan, UCACS protocol # 20030008) by shooting birds with a shotgun and without the use of decoys, in the vicinity of Inuvik, Northwest Territories ($67^0$38'N, $133^0$24'W). Females were collected between 4-7 June and again between 15-18 June and peak nest initiation (SD), as determined by back dating scoter broods, was 19 June (± 7 d; Stuart Slattery, IWWR, unpubl. data). Birds were immediately weighed (nearest 10 g using a 1000 g Pesola spring balance), and then frozen for several months prior to dissection and sample analysis. Due to the high latitude and timing of collections, I assumed all females had not previously nested that year. Amphipods (*Gammarus spp*., n = 53 individuals) and other aquatic invertebrates (n = 27 individuals) were collected opportunistically by hand within 30 cm of the surface near shoreline locations were most birds were observed feeding and where most nektonic invertebrates were observed. Samples were assumed to include the most common invertebrates available to scoters at the time they were observed feeding,

although benthic invertebrates, if consumed, were probably underrepresented. Amphipods were the most abundant invertebrate (by biomass) in most wetlands near the site (Lisette Ross, IWWR, unpubl. data); as well, they comprised the most important food source to scoters (JMD, unpubl. data.). Amphipods and other invertebrates belonged to the Class Hirudinea as well as to the following Orders: Trichoptera, Diptera, Hemiptera, Ephemeroptera, and Limnophila, all of which were important prey of scoters (JMD, unpubl. data.), and were preserved separately in 70% ethanol.

8.2.2 Carcass Analysis

Birds were thawed overnight and reweighed (nearest g using a digital scale) before taking measurements of total body length (nearest 1 mm with a ruler), wing chord (nearest mm with a 300 mm wing ruler), tarsus length (nearest 0.1 mm with digital calipers), total head length (0.1 mm with digital calipers), and keel length (nearest 0.1 mm with digital calipers). I excised and weighed (nearest 0.01 g with a digital scale) muscles of one breast, one leg, the empty gizzard, and the liver. Each was dried to constant mass in a drying oven at 80^0C and reweighed (Alisauskas et al. 1990). Gizzard and leg muscles were assumed to be largely protein and discarded. The dry breast muscle was reserved for isotope analyses. The remaining carcass, less reproductive organs (oviduct and ovaries/testes) and ingesta, was homogenized using a Hobart industrial bowl chopper and meat grinder with 5 mm plates. A 100 g subsample of homogenate was dried to constant mass and reweighed. Dried homogenate and livers were individually ground using an electric coffee mill, washed for 6 hr using petroleum ether in a modified Soxhlet apparatus to remove triglycerides, and then lean samples were dried and reweighed to estimate percent lipid in liver and homogenate subsample (Alisauskas et al. 1990). These values were used to estimate total carcass lipid mass. I used the sum of dry breast muscle mass (two times excised breast), dry leg muscle mass (two times excised leg), lipid-free dry liver mass and dry gizzard mass as an index of protein content as these are the major stores of metabolically active protein.

8.2.3 Gonad Analysis

I used follicle lipid and protein data from scoters collected in 2003 at the same study area to produce predictive regression equations to estimate reproductive nutrients of follicles for birds collected in 2004, which were used for my comparative analyses. Follicles from females collected in 2003 were used for contaminant analysis and stored in an improper manner for SIA. Each developing ovarian follicle from 2003 birds was removed and weighed (nearest 0.01 g using a digital scale). Follicles were dried to constant mass at 80^0C and reweighed. Each follicle was then washed with petroleum ether to remove lipids, dried and reweighed to determine lipid content and lean dry mass (LDM), which I assumed was largely protein (Alisauskas et al. 1990). Follicles were then combusted in a muffle furnace at 500^0C for 6 hr, cooled and reweighed to determine ash and protein content by subtraction. I calculated the following regression equations and used them to predict follicle lipid and protein content of 2004 follicles used for SIA:

$$Follicle\ lipid\ mass\ (g) = 0.305 * follicle\ wet\ mass\ (n = 74, r^2 = 0.994, P < 0.001)$$

$$Follicle\ protein\ mass\ (g) = 0.247 * follicle\ wet\ mass\ (n = 74, r^2 = 0.996, P < 0.001)$$

From 2004 birds, I removed and weighed (nearest 0.01 g with a digital scale) reproductive organs during dissections. Ovaries were examined for signs of follicles undergoing rapid follicular growth (RFG), post-ovulatory follicles (POF) and damaged follicles. All yolky follicles and oviducal eggs were removed, weighed individually, and dried to constant mass as above. The remaining non-developing follicles and oviduct were individually weighed, dried and re-weighed. The dry oviduct was considered 100% protein and discarded (Alisauskas et al. 1990). The ovaries less developing follicles were ground with a coffee grinder, washed with petroleum ether to extract lipids, and subsequently dried and reweighed to estimate lipid and determine LDM, which I assumed was protein (Alisauskas et al. 1990). In laying females, I counted the number of eggs laid based on the number of POFs observed and assumed that each laid egg consisted of 13.7 g of lipid and 14.3 g of protein in scoters (based on regression equations in Alisauskas and Ankney 1992). As 19 of the 77 follicles undergoing RFG were damaged during collection, I estimated their rank in the series based on size of the damaged follicle's membrane and size of undamaged follicles. Using the following least-squares quadratic regressions of adjacent RFG follicles (where $f_{lipid+1}$ and f_{LDM+1} are the follicle lipid mass

and LDM, respectively, of the damaged follicle and f_{lipid} and f_{LDM} are the lipid mass and LDM, respectively, of the next-smallest follicle), I estimated follicle lipid and LDM of damaged follicles:

$$f_{lipid+1} = 0.732 + 1.966 * f_{lipid} - 0.120 * f_{lipid}^{2} \ (n = 41, r^2 = 0.95, P < 0.001) \quad (8.1)$$

$$f_{LDM+1} = 0.546 + 2.019 * f_{LDM} - 0.165 * f_{LDM}^{2} \ (n = 41, r^2 = 0.94, P < 0.001) \quad (8.2)$$

To limit analyses to females producing eggs, breeding status was determined by the largest follicle mass. Alisauskas et al. (1990) used a threshold of 0.1 g dry mass for assigning ring-necked ducks to the RFG phase. Scoters produce larger egg yolks than ringnecks, but I used the same threshold as follicles appeared to fall well below (< 0.05 g) or above (> 1.2 g) this mass. Laying females were characterized as having at least one POF. I also estimated first egg date (FED) by calculating the date of first egg laying based on follicular development and a laying rate of 0.67 eggs/d (e.g., date of collection ± follicle status ∗ laying rate; Alisauskas and Ankney 1992).

8.2.4 Stable-isotope Analysis

Samples of dry breast muscle, dry follicle and dry abdominal fat were soaked for 48 hr in a 2:1 solution of chloroform:methanol (by volume). Breast muscle was drained, rinsed in new solution, and allowed to dry for a minimum 24 hr to produce lipid-free breast samples. The solutions from follicle and abdominal fat samples were drained into clean scintillation vials and allowed to evaporate until dry under a fume hood to produce follicle lipid and somatic lipid samples, respectively. Follicles were again rinsed in new solution and allowed to dry for a minimum 24 hr under a fume hood to produce lipid-free follicle samples. Processed abdominal fat samples were discarded. Pooled amphipods and other invertebrate samples were processed separately to provide two distinct isotopic signatures. After evaporating the preserving methanol under a fume hood, I dried and powdered the samples. Lipids were removed using the same technique as above and retrieved by evaporation under a fume hood. I dried the remaining lipid-free powdered samples and soaked them in 0.1N HCl solution without rinsing to remove carbonates (Hobson et al. 1995).

A 0.95-1.05 mg sample of each tissue was used for carbon and nitrogen isotope analysis. Samples were combusted using pyrolytic continuous-flow isotope-ratio mass spectrometry (CFIRMS) to determine carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios at the University of Saskatchewan, Saskatoon, Saskatchewan, Canada. All stable isotope ratio results are reported in delta notation (δ), in units of per mil (‰) and normalized to their respective international (IAEA) standards: carbon – Pee Dee Belemnite, and nitrogen – air. Measurement error was ±0.1‰ and ±0.2‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

I assumed that scoter spring breeding ground diets were composed entirely of invertebrates as other materials (plant, fish, etc.) were not detected in ingesta (JMD, unpubl. data). I also assumed that there was no difference in the digestibility of invertebrate C and N among invertebrate species collected, and therefore did not correct measures of invertebrate C and N concentrations in mixing models (Phillips and Koch 2002). I used the carnivore model from Hobson (1995) for diet to egg fractionation values (protein C-13 and N-15 fractionation of 0.0‰ and +3.5‰, respectively; lipid C-13 fractionation of -3.5‰). Given that fractionation of somatic to egg nutrient has not yet been determined, I used the same values from the carnivore model for protein but assumed no fractionation between somatic lipid and egg lipid (Gauthier et al. 2003; Hobson et al. 2005); the validity of this assumption is discussed below.

8.2.5 Statistical Analysis

All statistical analyses were conducted using SPSS version 13.0 (Norušis 1990). Statistical significance was evaluated against an $\alpha = 0.05$. I used principal component analysis of the correlation matrix of morphological measurements to produce an index of body size (first principal component, PC1), which was used to adjust protein estimates (Alisauskas and Ankney 1990). I used linear regression models to determine the relationship between somatic nutrients and corresponding reproductive nutrient investment by females. The slope of this relationship was used as an estimate of the maximum proportion of reproductive tissue produced with somatic nutrients. The Isoconc mixing model was used to determine proportions of source nutrients for my protein model where I had three sources (i.e., breast, amphipods, and other invertebrates) and two

isotopes (Phillips and Koch 2002). Isoconc models could not be used for lipids because low N content of lipids prevents accurate determination of $\delta^{15}N$; I could not use a two source linear mixing model because my follicle values fell outside the somatic-diet range of values. I used *t*-tests to compare mean lipid isotope values. If follicles lipids were derived from somatic tissues, I used ANCOVA to test for a relationship between somatic lipid and FED while controlling for reproductive lipids.

8.3 RESULTS

Of the 40 scoter females I collected, 20 each in the first and second collection periods. Of 25 females undergoing RFG or laying, I was only able to use 20 (6 from the first and 14 from the second collections) due to the small size of follicles from females just initiating follicular development, which prohibited sampling for stable-isotopes.

PC1 loadings for the 5 morphological measurements were positive and ranged from 0.58 to 0.86; PC1 explained 61% of the total variation in data of size measurements and was used as an index of structural size. I found a positive relationship between protein estimates and PC1 (regression; $F_{1,37} = 17.04$, $P < 0.001$, $r^2 = 0.32$); therefore, I adjusted protein estimates by adding the regression residual of somatic protein against size to the mean protein estimate and used this adjusted value in subsequent models. I did not adjust lipid estimates as there was no relationship between lipid and body size (GLM; $F_{1,37} = 0.90$, $P = 0.35$, $r^2 = 0.02$; see Sedinger et al. 1997; Esler et al. 2001). Somatic protein did not decline with investment in reproductive protein, but somatic lipids decreased with increased lipid investment (Fig. 8.1); somatic lipid stores decreased by 1.27 ± 0.40 g for each g of lipid invested in reproductive tissues. ANOVA showed that $\delta^{13}C$ values of both breast ($F_{1,16} = 4.92$, $P = 0.041$, Fig. 8.2) and abdominal fat ($F_{1,13} = 26.723$, $P < 0.001$, Fig. 8.3) had declined significantly from the first to second collections.

Isoconc mixing models predicted that females from early collections used on average 20% somatic protein for follicle formation (range = 0-35%) and females from my second collection used only 7% (range = 0-36%) (Fig. 8.2). Despite the range (0-36%) in apparent somatic protein use for follicle production, I found no relationship between estimates of somatic protein and percent somatic signal (regression; $F_{1,16} = 0.52$, $P =$

0.48, $r^2 = 0.03$). Most dietary protein in follicles was derived from amphipods, compared to other invertebrates, in the first (56% amphipod) and second (71% amphipod) collection periods.

After correcting dietary lipids for fractionation (-3.5‰; Table 2 in Hobson 1995), mean $\delta^{13}C$ of somatic lipids in first and second samples of scoters were lower by 2.5‰ and 4.8‰, respectively, than dietary lipids (*t*-tests; *P*s < 0.04, Fig. 8.3). Female somatic lipids were more enriched in $\delta^{13}C$ than follicle lipids in the first (2.4 ± 0.7‰ SE; *t*-test, n = 6, $P = 0.011$) and second (2.6 ± 1.0‰; *t*-test, $n = 9$, $P = 0.046$) collections (Fig. 8.3). The overall mean ± SE difference between somatic and follicle lipid $\delta^{13}C$ was 2.5 ± 0.6‰ (n = 15). Given a similar difference between somatic and follicle lipid $\delta^{13}C$ values in both collection periods and these values were ranked diet > somatic > follicles, I conclude that follicle lipids were derived entirely from somatic stores. I then tested to see if there was a relationship between FED (range = 8-19 June, mean = 14 June) and somatic lipid estimates; after controlling for investment in reproductive lipids, I found no relationship (ANCOVA; $F_{1,15} = 0.13$, $P = 0.73$).

Table 8.1. Variation in reported $\delta^{13}C$ of aquatic invertebrates from within and among forest wetlands.

Study	Location (Lat/Long)	Invertebrates (Inv.) Sampled	$\delta^{13}C$ min	$\delta^{13}C$ max
Within Wetlands				
Beaudoin et al. 2001	$55^0$12' / $111^0$38'	Pelagic	-27‰*	-22‰*
		Benthic	-28‰*	-13‰*
Pszkowski et al. 2004	$54^0$39' / $113^0$38'	Carnivorous	-26±0.7‰	-23±0.4‰
		Herbivorous	-24.5±0.4‰	-27±1.2‰
Among Wetlands				
France 2000	$48^0$30' / $98^0$13' (approx.)	*Macrodella decora*	-24‰*	-31‰*
		Percymoorensis marmoratis	-23‰*	-29‰*
France and Schlaepfer 2000	$49^0$22' / $91^0$32' (approx.)	Odonates	-32±3‰	-26±2‰
		Dipterans	-27±2‰	-33±2‰

* Indicates data were derived from figures, all other values were provided in the text or tables.

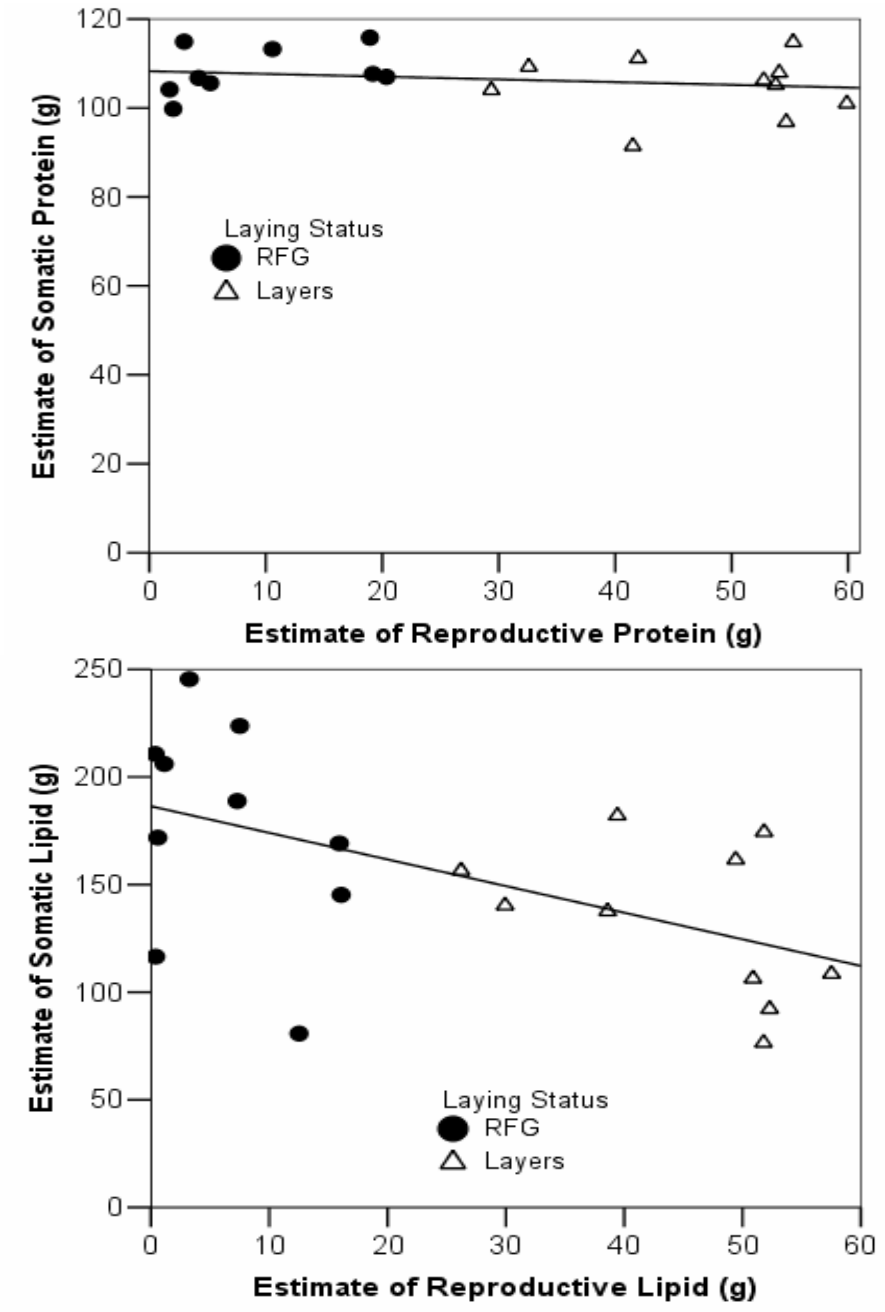

Figure 8.1. Regression of somatic protein (y = -0.06x + 108.15; $F = 0.79$, $r^2 = 0.04$, $P = 0.39$, df = 18) and lipid (y = -1.27x + 166.75; $r^2 = 0.31$, $F = 7.90$, $P = 0.012$, df = 19) over the respective reproductive nutrient in female white-winged scoters undergoing rapid-follicular growth (RFG) and laying eggs. Birds were collected from the Inuvik, NT, area in June 2004.

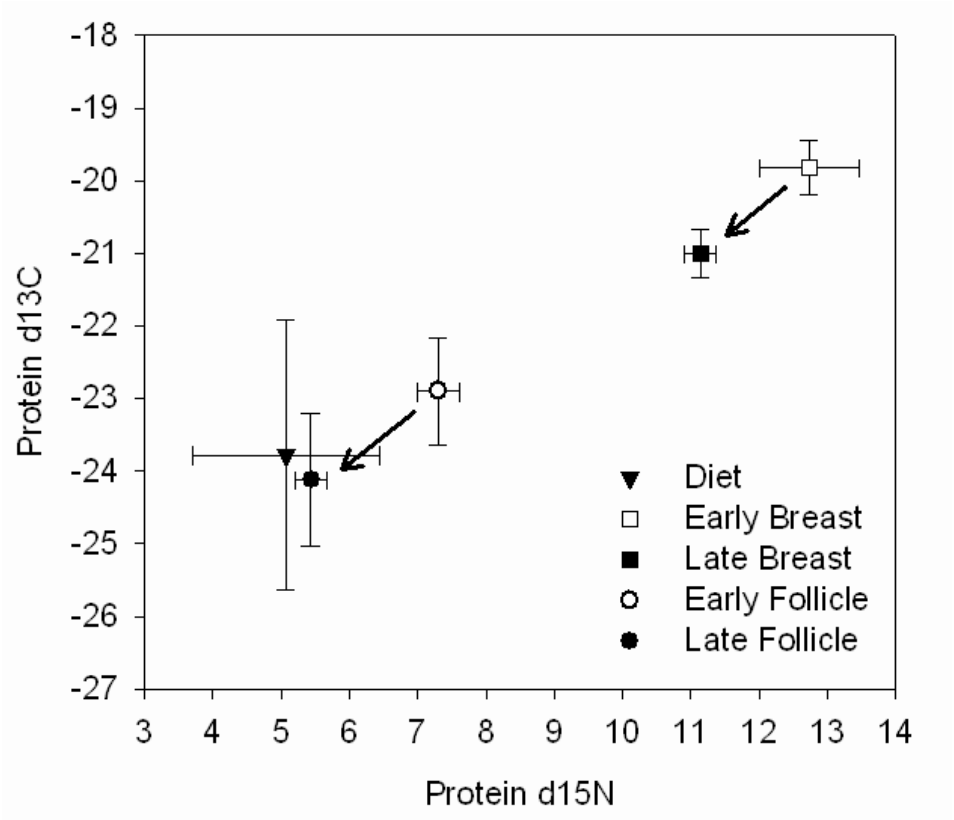

Figure 8.2. Mean (± SE) protein $\delta^{13}C$ and $\delta^{15}N$ values from breeding ground diet, breast and follicle samples of female white-winged scoters collected between 4 and 7 June (early) and between 15 and 18 June (late), 2004, in the Inuvik, NT, area. Arrows indicate differences between early and late samples of breast and follicle samples.

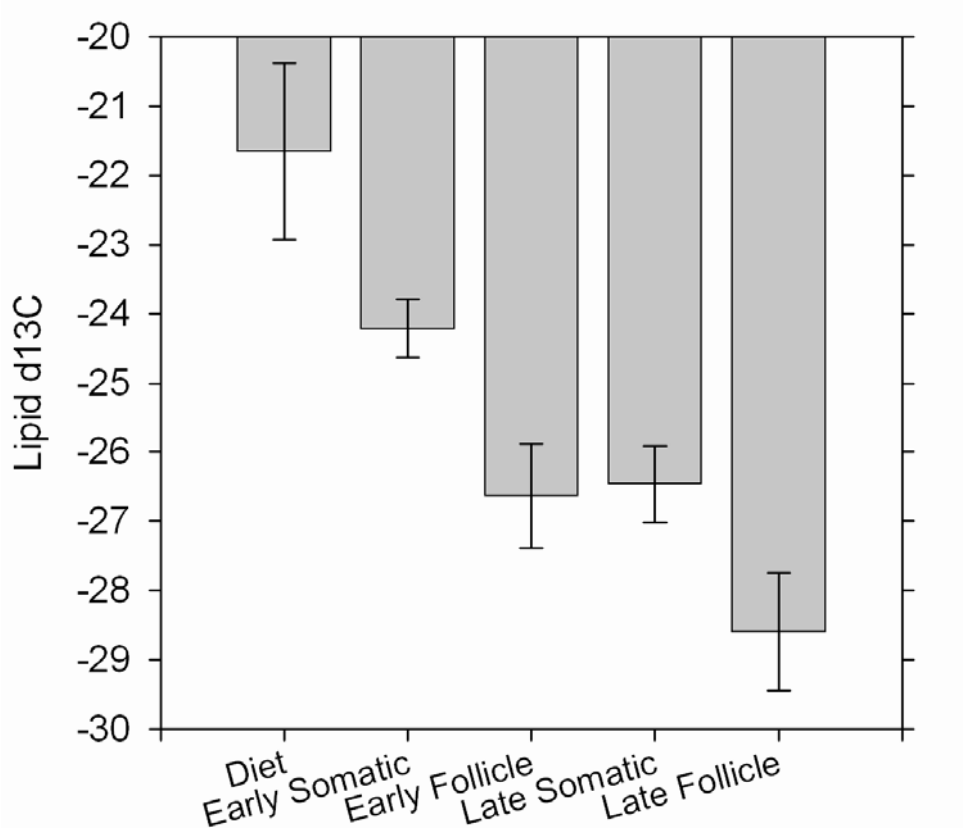

Figure 8.3. Mean (± SE) lipid $\delta^{13}C$ values from breeding ground diet, somatic lipid, and follicle lipid samples from female white-winged scoters collected between 4 and 7 June (early) and between 15 and 18 June (late), 2004, in the Inuvik, NT, area.

8.4 DISCUSSION

8.4.1 Body Composition vs. Stable-isotope Analysis

My BCA results suggest that female white-winged scoters do not invest somatic protein into reproductive tissues, but that all reproductive lipids could be derived from somatic tissues (Fig. 8.1). However, BCA has three potential flaws that could limit the interpretation of results. First, an inability to account for variation in true clutch size of the females could bias the interpretation (Mann and Sedinger 1993), though some circumstances allow for correction of clutch size (Sedinger et al. 1997). Second, BCA provides only a generalized or average strategy (i.e., regression parameter estimates) for birds used in the analysis. The destructive nature of BCA obviates a temporally repeated

sampling of an individual, which would be required to determine an individual's energetic strategy (Alisauskas and Ankney 1992). Third, use of lipids for clutch formation versus metabolic requirements cannot be differentiated (Meijer and Drent 1999).

SIA results for protein $\delta^{13}C$ and $\delta^{15}N$ were consistent with results from my BCA, whereby scoters derived most reproductive protein from dietary sources (Fig. 8.1 and 8.2). Heterogeneity in relative use of dietary vs. somatic (0-36% somatic) protein by individuals inferred from the stable-isotope approach was not detected using body composition data. Depletion in breast $\delta^{13}C$ between my first and second collections suggested that there was turnover in somatic protein carbon during reproduction, with marine-derived protein carbon being replaced by protein carbon from local freshwater sources. This leads to the first limitation of the SIA approach. For eggs to acquire a signal that is consistent with dietary sources and indicate an income strategy for egg formation, no somatic tissue should be transferred to the egg. However, captive studies suggest that about 1/3 of protein requirements during egg production in chickens and other Galliformes is for somatic tissue maintenance (Robbins 1981). Therefore, if maintenance protein metabolism results in somatic protein deposition into eggs, yet protein stores do not decrease in mass, as my body composition suggests, I question whether it is possible to detect a true 'income' strategy for egg formation using SIA. Such metabolic turnover could produce the somatic signal I observed in the scoter follicle protein.

To determine the relative contribution of somatic and dietary lipids to follicle lipids using a two source, one isotope linear model, my follicle values should have fallen between dietary and somatic values after correcting for tissue fractionation. However, in both collections, I observed that follicle lipids were equally more depleted (more negative) in $\delta^{13}C$ than somatic lipids, both tissues being more depleted than diet (Fig. 8.3). I draw two conclusions from this observation. First, follicle lipids may have been derived entirely from somatic lipids, which is consistent with BCA results. Second, my assumption that no fractionation occurs during transfer of somatic to follicle lipids, following Gauthier et al. (2003) and Hobson et al. (2005), may be wrong. Differences observed between somatic and follicle lipids in the first and second collections were nearly identical, 2.4 and 2.6‰ respectively, suggesting that there is a fractionation of

approximately -2.5‰ between somatic and follicle lipids in scoters (Fig. 8.3). This fractionation value is similar to the -3.5‰ value reported for a carnivore model by Hobson (1995). A fractionation of lipid $\delta^{13}C$ might occur during vitellogenesis in the liver. Vitellogenin is the phospholipid precursor of yolk phosphoproteins, lipovitellin, and phosvitin (Deeley et al. 1975). Thus, experiments are needed to better understand fractionation rates between somatic and egg nutrients (Gannes et al. 1997), and to clarify conclusions presented by Gauthier et al. (2003) and Hobson et al. (2005). Few natural systems would provide the opportunity to determine these rates, although Emperor Penguins produce eggs entirely from somatic tissues while fasting (Kirkwood and Robertson 1997). A third source of uncertainty when using SIA for diet or energetics studies is proper representation of dietary items. Isotopic signatures of invertebrates vary among species both within and among wetlands (Table 8.1). This source of isotopic heterogeneity emphasizes the importance of adequately sampling a variety of dietary sources, particularly when studying mobile organisms such as birds. I pooled invertebrate samples to estimate isotope ratios assumed to represent composite diets because scoters consume large quantities of invertebrates (JMD, unpubl. data), and because I could not determine which wetlands an individual female used when foraging.

For organisms that exhibit distinct stable isotope signals in somatic and diet nutrients, using SIA simultaneously with body composition analysis provides a more robust assessment of reproductive strategies. Consistent results from body composition analysis and population-averaged SIA results may serve as independent validation of conclusions about the energetic strategy at the population level. However, inconsistent results may justify testing of assumptions, particularly if fractionation coefficients are borrowed from other systems or processes.

Simultaneous use of both techniques can also help to determine the relative allocation of nutrients for production (i.e., egg formation) and maintenance (energy and protein metabolism). I observed that follicle lipids were possibly derived entirely from somatic lipids using SIA (Fig. 8.3), and BCA indicated that for every 1 g of reproductive lipid females catabolized 1.27 g of somatic lipids (β = -1.27±0.41 SE; Fig 8.1). Using data (Alisauskas and Ankney 1992: Table 2-2) about basal metabolic requirements (80 kcal/d) and egg energy requirements (210 kcal/egg $*$ 0.67 egg/d = 141 kcal/d) to evaluate

whether somatic lipids could provide all energy for egg production. I would expect a β of about -1.57 ((80 + 141 kcal) / 141 kcal = 1.57) when regressing somatic lipid over reproductive lipids if all energy requirements were met by catabolism of somatic lipids. The portion of the β that is represented by metabolic requirements is -0.57. Therefore, I can estimate that the contribution of somatic lipid catabolized by my scoters to metabolic energy requirements (0.27 / 0.57 = 0.47) is roughly 47%. However, my reproductive lipid regression model only explained 31% of the variance in somatic lipid stores, implying that there may be substantial individual heterogeneity in initial lipid stores and/or rate of depletion among individual female scoters. Therefore, when possible, I recommend using both SIA and BCA for studying reproductive energetics. Additionally, greater insights may be gained by including the use of other techniques, such as the analysis of fatty acid signatures to better understand specific dietary preferences (Hooker et al. 2001).

8.4.2 Scoter Reproductive Energetic Strategies

My results suggest that scoters acquire nearly all protein for follicle synthesis from dietary sources, yet females catabolize lipids at a rate greater than deposited into follicles. This finding is consistent with the nutrient limitation hypothesis of egg synthesis for scoters where females with inadequate lipid stores may forgo breeding (Ankney and Alisauskas 1992). However, I did not observe differences in somatic lipid mass across first egg dates, which fails to support the seasonally-variable nutrient limitation hypothesis (Reynolds 1972; Esler et al. 2001). This difference between use of protein and lipid reserves for follicle synthesis may be related to the protein:lipid ratio in the diet. Based on Table 2 in Krapu (1979), Ankney and Afton (1988) determined that aquatic invertebrates have a 14:1 ratio of protein: lipid. This heavily protein skewed ratio is consistent with the possibility that predators of aquatic invertebrate are more likely to be lipid-limited than protein-limited.

Dobush (1986) used BCA to examine the reproductive energetics of scoters at Redberry Lake, SK, located 1650 km south of my study site, and obtained similar results for protein; somatic mass did not decline with investment in reproductive tissues (β = -0.08, $F_{1,39}$ = 1.13, P > 0.05). However, there was also no depletion of somatic lipids with investment into reproductive tissues in these Redberry scoters (β = 0.06, $F_{1,39}$ = 0.08, P >

0.05). Assuming that there is no date effect between these studies, this suggests that somatic lipid use for reproduction in scoters could vary with latitude, and that lipid-limitation in scoters may increase with latitude. The mechanism for this pattern may be the timing of nest initiation in this species. At both sites, scoters nest on average in mid-June (Brown and Fredrickson 1997, Stuart Slattery, IWWR, unpubl. data); however, the timing of nest initiation relative to the chronology of local food items would be much earlier at northern latitudes where many lakes are still frozen during arrival and early nest initiation. More importantly, though, this comparison indicates that there is high intraspecific plasticity in the energetic strategies of scoters. If scoters are capable of utilizing somatic nutrients to meet reproductive demand, why then do they not utilize somatic tissue to nest earlier at southern latitudes such as at Redberry Lake? This question deserves further investigation.

9. SYNTHESIS

Reproduction in birds – waterfowl in particular – is a costly activity. In migratory birds, nutrients used in reproduction can be acquired during the winter, en route to or while on the breeding grounds, and a lack of sufficient nutrients before reproduction may cause some individuals to delay or forgo breeding. Throughout North America, waterfowl habitats are presently under constant threat of degradation (e.g., through pollution) or loss (e.g., through drainage) from industrialization of the landscape. While foraging to acquire reproductive nutrients, birds can ingest contaminants that may also lead to reduced health and fitness of individuals.

Breeding populations of scaup and scoters are currently at their lowest levels since breeding waterfowl surveys began in 1955 (Wilkins and Otto 2006), and their population declines are highly correlated (R = 0.89, Stuart Slattery, IWWR, unpubl. data). Both species are primarily boreal breeding waterfowl, with some of the highest densities occurring at the northernmost extent of the boreal forest (Austin et al. 1998, Brown and Fredrickson 1997). Of the two species, scaup have received the greatest attention due likely to their popularity among waterfowl hunters. Concern about the declining scaup population has been present for over a decade, but was formalized during the first scaup workshop held in 1998 where issues and hypotheses were presented (Austin et al. 2000). Ring-necked ducks are also primarily a boreal breeding waterfowl species, are closely related to scaup, but have an increasing population trend through the western boreal forest (Figure 1.3). Two frequently advocated hypotheses regarding the population declines of scaup are the SCH and contaminants hypotheses (Austin et al. 2000, Anteau and Afton 2004, Anteau and Afton 2006). However, very little research on scaup has been conducted in the boreal forest. This knowledge gap in scaup and scoter breeding ecology and the two primary hypotheses about scaup declines led to the main objectives of this thesis: to test the SCH and contaminants hypothesis on boreal breeding scaup, while comparing these results with simultaneously collected ring-necked ducks and scoters. The ring-necked duck was chosen as a "comparison" species given that it is the closest congener to scaup (other than greater scaup) and is also most abundant in boreal forest.

9.1 REPRODUCTIVE ENERGETICS

The cost of clutch formation in waterfowl is among the highest in birds (Alisauskas and Ankney 1992). Most waterfowl species are migratory and frequently invest some amount of endogenous nutrients to meet the nutritional demand of clutch formation, incubation and maintenance. These nutrients may be acquired at various times and locations during the pre-nesting period. If habitat where females acquire these nutrients are lost or degraded, birds may not be able to achieve a threshold body condition required to attempt reproduction. Also, this could lead to delayed reproduction, smaller clutch size or more frequent feeding breaks during incubation to compensate for sub-optimal nutrient stores. This is the basis for the SCH about scaup declines. The steady loss of wetlands in the UMW region and degradation of many more has led to concerns that these important scaup staging areas no longer provide the necessary nutrients for reproduction in scaup (Anteau and Afton 2006). Anteau and Afton (2004) reported that scaup collected in 2000-2001 were much lighter and had fewer nutrient stores than scaup collected two decades earlier from the same locations in Minnesota and Manitoba. Subsequent study of the food availability in the UMW wetlands and nutrient dynamics of scaup staging in this region also supported the SCH because of a measured decrease in quality and quantity of key scaup foods (Anteau 2006; Anteau and Afton 2006). In chapter 2, I compared the body condition of boreal scaup to ringnecks, and the body mass of scaup to those collected from the late 1960s. Overall, I found that scaup carried slightly less protein but more lipid than ringnecks after controlling for size. These species are more likely lipid-limited than protein-limited during clutch formation, given their greater use of endogenous lipid for clutch formation (Esler et al. 2001) Thus, I concluded that, relative to ringnecks, an average female scaup had adequate body condition for reproduction. Scaup also had very similar body mass to those of the 1960s; and I found that mass explained 40% of the variance in somatic lipids. Lipids are particularly important because of the carnivorous diets of scaup, ringnecks and scoters are heavily skewed to protein content. These results failed to support the SCH. The SCH may explain declines in scoter numbers. It is possible that a lack of food availability on wintering or staging areas of scoters is having a parallel cross-seasonal nutrient limitation effect to the proposed limitation in scaup due to shifts or declines in scoter pre-breeding

food availability. Currently, there is concern about food availability on marine scoter staging areas (Rodway et al. 2003, Lewis et al. 2007). Seaducks are known to forage heavily on herring spawn in spring prior to migration and reduced herring populations may be limiting food availability for staging scoters (Rodway et al. 2003, Lewis et al. 2007). This decline in herring spawn could reduce overall spring condition of scoters, which could parallel effects of scaup habitat loss and degradation in the UMW. However, it is also quite probable that the cause of both declines is located on their sympatric breeding grounds. These questions are currently being investigated (Eric Anderson et al., personal communication).

Reproductive strategies in birds have been the subject of myriad papers and books. The idea that conditions on wintering and staging areas outside the breeding grounds can influence an individual's fitness has also been understood for decades (Heitmeyer and Fredrickson 1981), but has received little investigation. This cross-seasonal effect is a fundamental premise in the argument for the SCH. However, what is unknown is the ability of females to compensate for periods of food deficiency during the migration and pre-breeding period by selecting other food items, migrating to alternate locations, or acquiring additional nutrients at a later date. This knowledge gap begs the question about when a female must surpass its nutritional threshold before reproduction is affected, or at what stage(s) does a female decide that she cannot attempt to reproduce? In seasonal breeders, the annual cycle begins when short days break the photorefractory phase when reproductive organs are inactive (Cockrem 1995). Females are then capable of being stimulated by increasing day length and physiological processes begin preparing an individual for reproduction. These changes are triggered by changes in photoperiod (Cockrem 1995). In late winter, females begin depositing small quantities of protein into some dormant follicles turning them into small yolky follicles (SYF), a preparatory stage before rapid follicular growth (Johnson 2000). This likely occurs in every female that is sexually mature, and is supported by my observation of SYF in every female scaup and ringneck collected, as these species are sexually mature in their second summer, but do not always breed (Austin et al. 1998, Hohman and Eberhardt 1998). However, SYF were not present in 3 female scoters, which have a more delayed sexual maturity – typically 3rd summer - than scaup or ringnecks (Brown and Frederickson 1997). It is unknown to what

degree nutritional deficiency in late winter may influence this SYF development stage, though I suspect it does not have much influence on this stage. However, given the ability of female waterfowl to rapidly deposit large nutrient stores when provided with adequate food, it is illogical that a female would forgo breeding due to nutritional deficiency long before the period of greatest nutritional demand. I found no relationship between claw tip isotope values and lipid levels in scaup or ringnecks (Chapter 7). One interpretation of this is that geographic and dietary differences at the time of claw growth do not influence arrival body condition in the boreal. Bond and Esler (2006) reported that harlequin ducks acquire pre-migration nutrients at different times depending on their diet. This suggests that at least during spring staging, some species of waterfowl exhibit plasticity in timing and method of nutrient acquisition. Moreover, one would expect that attaining a nutritional threshold would not be required until just before egg formation as waterfowl are capable of rapidly depositing nutrient stores (Barboza and Jorde 2002; Chapter 2). This is an area of research that should be further explored to better evaluate the SCH and to further our knowledge about physiological constraints in avian reproductive energetics.

Due to the potential plasticity in nutrient acquisition, I believe it would be too difficult in a captive setting to properly simulate food shortage and energetic requirements for migration and reproduction in scaup. However, the remaining uncertainty about interactions of body condition, survival and reproduction in scaup should be addressed. As the concern about body condition and lipid catabolism in scaup is focused primarily on the Upper Mid-West (UMW) United States (Anteau and Afton 2004, Anteau 2006), tracking birds from various wintering sites through migration and to the breeding grounds should be conducted to compare survival and breeding propensity in birds that migrate through the UMW versus other staging areas.

9.2 SELENIUM ECOTOXICOLOGY

The contaminants hypothesis has also been frequently advocated as a contributing factor to scaup declines as contaminants have been linked to population declines in other avian species (Keith 1996, Peakall 1996). Several studies (Hothem et al. 1998, Custer and Custer 2000, Custer et al. 2003) have screened scaup for a host of possible contaminants and found that only one, Se, was consistently reported above levels thought to be harmful

to waterfowl. Selenium (Se) is enriched in the environment from several anthropogenic activities: ore smelting, fossil fuel combustion, electronics manufacturing, among others (Barceloux, 1999; Haygarth, 1994; Ohlendorf, 2003). This contaminant has the potential to impact both species, scaup and scoters, given their affinity for molluscs and other prey items that may accumulate high concentrations of Se. Selenium is naturally elevated in marine systems where scoters overwinter, and industrial activity around the Great Lakes has led to an enrichment of Se in these waterbodies as well. With the introduction and rampant spread of zebra mussels into the Great Lakes, there has been an aggregation type numerical response by scaup to this new food source (Petrie and Knapton 1999). Therefore, concern about the effects of Se exposure to reproduction and physiology of scaup has led to numerous studies (Custer and Custer 2000, Custer et al. 2003, Fox et al. 2005, this thesis). In chapter 3, I compared Se levels in all three species and found that scaup and ringnecks had similar levels, though scaup again had slightly more, but both were many fold lower than levels observed in scoters. While many scoters had concentrations above levels thought to have physiological effects on adult mallards, I found evidence suggesting the contrary: scoters with higher Se levels were in better body condition and more likely to be developing eggs (Chapter 3). This relationship was likely the result of breeding scoters accumulating nutrients on marine staging areas before migration and simultaneously acquiring high Se burdens. Although the relative importance of those nutrients to migration and breeding must be further investigated. To better understand how Se accumulated on wintering and staging areas might affect scaup, I conducted a captive dosing experiment (Chapter 4) that examined the effects on reproduction and physiology. There was no apparent effect on breeding propensity in females, and egg Se concentrations declined rapidly once females were removed from treatment diets despite a more delayed response in somatic tissues (i.e., blood). This suggested that these captive scaup were utilizing exogenous protein to a large degree for clutch formation. In my collected scoters, I also observed that oviducal eggs and follicles had very low levels of Se despite elevated somatic burdens. After determining that scoters used primarily exogenous protein for egg formation (Chapter 8), I evaluated the relationship between reproductive energetic strategies and egg Se levels with the dilution hypothesis (Chapter 5). Here I concluded that low egg Se concentrations in scoters was in

fact due to their energetic strategy of allocating exogenous protein into developing eggs. Given that Se is particularly toxic to embryos, the fact that scoters utilize exogenous protein for egg formation may have partly been an adaptation preventing these birds from depositing harmful levels of Se from endogenous protein. It is also possible that their ability to overwinter in marine areas and to deposit nutrients from marine areas before migration and reproduction was facilitated by their income breeding strategy for protein.

Finally, I used stable-isotope analysis of claw tissue to elucidate links between known isotopic patterns and Se in scoters (Chapter 6) and between Se, lipid and protein levels in scaup and ringnecks (Chapter 7). In all three species Se was linked to apparent trophic status from the wintering or staging areas. Though, it is uncertain if use of estuarine systems may have resulted in this pattern. Despite the relatively low levels of Se in scaup and scoter eggs, this observation emphasizes the potential cross-seasonal importance of wintering grounds as sources of contaminants that are potentially hazardous to reproduction in migratory birds.

Selenium is a very complex environmental contaminant. Differences in sensitivity among species, the potential for interactions with other metals, variation in toxicity of the different chemical species of Se, and the ability of animals to eliminate this element through natural metabolic processes have led to many debates about toxicity thresholds. While I did not find evidence of a negative effect in birds collected from the western boreal forest, there may be the potential for an effect on birds breeding closer to environments where the element has been anthropogenically enriched (i.e., the Great Lakes). Further research into the effects of Se in scaup should be directed at birds that overwinter in high Se locations and that nest nearby. Also, a better understanding of the species of selenium ingested by scaup would help to interpret the exposure levels. The most common forms of Se in water are inorganic Selenite or Selenate (Ohlendorf 2003). However, once incorporated into plants and insects, Se becomes incorporated into seleno-amino acids (selenomethionine, selenocysteine, etc) (Ohlendorf 2003). As species vary in toxicity, greater knowledge about the species of Se ingested by scaup and other vertebrates would help to understand and establish potential threshold concentrations. Additionally, understanding the species of Se within scaup, scoters and other waterfowl tissues would also provide insight into why certain species of birds appear to vary in

sensitivity to Se. It is possible that some Se is bound to other metals or is in a form less toxic than seleno-proteins.

While I focused my contaminant analysis on Se and also tested for Hg, based on information available during the study, there are possibly other contaminants that should be examined in these species. Anteau et al. (2007) found that cadmium (Cd) had a weak negative correlation with lipid ($F_{1,73} = 6.92$, $P = 0.010$, partial $r^2 = 0.09$). Though concentrations observed from the Upper Mid-West (Anteau et al. 2007), as well as from California (Hothem et al. 1998) and the Great Lakes (Custer and Custer 2000) were all well below the generalized 133 $\mu g \cdot g^{-1}$ dry weight threshold for cadmium poisoning in birds (Furness 1996).

Screening studies for a variety of organic and inorganic contaminants in scaup have been conducted (Hothem et al. 1998, Custer et al. 2000), and there have been no contaminants that are consistently reported to be elevated. Certain new and emerging contaminants, such as polybrominated diphenyl ethers (PBDE), which are suspected to have similar effects as PCBs and DDT due to their similar structures (Rahman et al. 2001), could be accumulating in these species due to their higher winter trophic level than other waterfowl.

9.3 ALTERNATE HYPOTHESES

From the perspective of population declines in scaup and scoters, I found no evidence that either the contaminants hypothesis or the SCH likely caused numerical declines in these species. With a lack of support for these two hypotheses, I was left examining and comparing the life-history strategies of the three species I studied to identify patterns that may lead to new hypotheses that could simultaneously account for the declines of scaup and scoters. I focused my attention on the breeding grounds because these two species have significant range overlap only during this period. Furthermore, there has been no recovery of scaup despite efforts to reduce adult mortality through increased harvest restrictions. This is additional evidence that population trends are being driven by a lack of productivity, assuming there hasn't been compensatory mortality during the period of harvest restriction.

One salient difference when comparing ringnecks with scaup and scoters is the timing of nest initiation. Nest initiation in scaup and scoters is later than in ringnecks, and appears to vary less with latitude. Birds nesting at the southern portion of their respective ranges have a much longer delay between arrival (late April – early May) and nest initiation (mid-June) than birds at the northern edge of their range which arrive much later (early June) and nest almost immediately following arrival (mid-June). This reproductive strategy presumably evolved in response to a selective pressure at some period in their breeding season. While avian reproduction is very energetically demanding, Lepage et al. (1998) suggested that the period of greatest demand is actually during the growth and development in young. Dawson and Clark (1996) proposed that late nesting in scaup evolved in response to duckling food requirements relative to availability. The phenology of invertebrates, the main food items of ducklings, is temperature dependent. For females to time their nesting efforts optimally, many migratory species rely on photoperiodic thresholds. However, with rapid and fluctuating spring chronology due to climate change, a lack of flexibility may lead to a mismatch in the timing of food requirements and availability (mismatch hypothesis; Cushing 1974, Thomas et al. 2001, Crick 2004). Offspring of late nesting species, such as scaup and scoters, may hatch at a time that is no longer synchronous with peak food availability. This could lead to slower development and reduced survival of both ducklings and hens that also must moult and regain nutrient stores before fall migration. Earlier peak food availability could also explain how ringnecks are expanding their range farther north and increasing in population through the western boreal forest. Ringnecks generally nest earlier than scaup (Chapter 2), and earlier peak food availability could now be optimally timed for this species at Northern Boreal latitudes. MacInnes et al. (1990) found that arctic nesting geese advanced their nesting dates by approximately 30 days since the 1950s, which was likely due to climate change. These species also must time their nesting to coincide with food availability for their offspring. Since the 1960s there has been no apparent change in scaup NIDs (Chapter 2), though some suggest that nest initiation occurs later now (Anteau 2006). This mismatch hypothesis has been shown to affect bird populations directly (Visser et al. 1998) and indirectly (Winder and Schindler 2004), and

should be explored as a large scale phenomenon capable of having population scale impacts in birds, particularly in northern adapted species.

Shorter, milder winters also could have reduced constraints on predators. Some mammalian predators, such as the raccoon (*Procyon lotor*), and avian predators, such as the magpie (*Pica pica*) are expanding their range northward and colonizing new territories (McCarty 2001, Larivière 2004). Endemic predators may also have higher annual survival due to climate change, which could impact productivity of scaup and scoters. Brook et al. (2005) found a positive correlation between small mammal densities and nest success of scaup in the boreal forest. This suggests that predator populations and alternate prey availability influence nest success in scaup and likely other waterfowl. Estimates of nest success in greater scaup (0.01-0.61% nest survival; Walker et al. 2005) and lesser scaup (12.3% nest survival; Corcoran et al. 2007) in Alaska were generally low and may not be sufficient to sustain local populations. However, Walker et al. (2005) reported that daily nest success rates for scaup did not differ from other ducks studied, and were similar to ducks nesting in the mid-continent. Koons and Rotella (2003) did find that nesting habits of scaup and ringnecks differed in Prairie-Parkland Manitoba where scaup preferred upland nest sites and had lower nest success than ringnecks. However, Murdy (1964) indicated that near Yellowknife, NT, scaup and ringnecks were both wetland-associated nesters sharing a similar nest site preference for floating sedge-mats, which may indicate similar nest success rates between these species in this biome. Analysis of predator population trends and trapping data could provide some information about potential changes in predator communities and numbers that have impacted scaup.

In the face of their population declines, scaup hunting and harvest restrictions are a very contentious issue among waterfowlers, managers, and biologists. Between 1961 and 1994 the average annual harvest was approximately 341,000 birds (range: 93,000 to 687,000; Austin et al. 1998). Due to concern over the contribution of harvest to declines in scaup, beginning in 1986 and until 1994, the scaup harvest rate was reduced to approximately 0.03 (Boomer and Johnson 2007), whereas before this period it fluctuated to as high as > 0.08. However, this decade of heavily restricted harvest appears to have had little effect on the population trend. Recently, Boomer and Johnson (2007) estimated maximum sustainable yields (MSY) for scaup populations using stochastic population

models and calculated that recent harvest levels were above the MSY for current population estimates. This prompted a further reduction of the daily bag limit in the US to 1 scaup/person for 2007. It is difficult to determine the contribution of scaup harvest to the population declines. In a situation where the scaup population has declined due to a decrease in the carrying capacity of their habitat, harvest may act as a source of compensatory mortality and further restrictions would have no impact. However, Boomer and Johnson (2007) estimated the posterior carrying capacity of scaup to be 8.236 million birds. If correct, this could indicate an additive effect of harvest on scaup mortality. Indeed, this issue will continue to be debated around campfires and in meetings.

10. LITERATURE CITED

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APPENDIX

A. FLOCK SIZE OF LATE-SPRING BOREAL LESSER SCAUP (*AYTHYA AFFINIS*) AND RING-NECKED DUCKS (*A. COLLARIS*).

A.1 INTRODUCTION

Scaup are often observed migrating in large flocks (Austin et al. 1998). Because I was interested in collecting local birds for my study and avoiding potential migrants, I collected scaup and ringnecks from groups of greater than 8 birds after the peak migration had moved out of the collection sites. However, because of uncertainty about the size of groups found at each site, I wanted to know what proportion of the population this excluded at the time of collection. Also, I was interested in comparing the flock size of scaup and ringnecks in the boreal at the time of my collections to determine whether there were marked differences in flock size composition of the two species. If significant differences in flock size are observed, this could indicate bias for or against breeding females.

A.2 METHODS

In order to evaluate whether the birds I collected in the spring were representative of the general population, I analysed Ducks Unlimited Canada's spring boreal waterfowl surveys. All observations of scaup and ring-necked ducks group size from the Sahtu ($65^0$75'N, $130^0$00'W), Peace-Athabasca Delta ($58^0$60'N, $111^0$50'W), and Utikima Lake ($55^0$80'N, $115^0$50'W) regions were analysed. Surveys were conducted twice per spring (1^{st} and 2^{nd}) at each of three locations: Sahtu (21 May – 2 June, and 9 June – 19 June), Peace-Athabasca Delta (4 – 9 May, and 26 – 30 May), and Utikima (5 – 14 May, and 2 – 7 June) (Table A.1).Observations for each species were sorted into one of the following arbitrarily chosen group size categories (Lone males, Pairs, 3-4, 5-8, 9-20, 21-99, and >99). I calculated the percentage of total birds observed in each group size category averaged over all the survey years.

I used K-S tests to compare the distribution of group sizes between species within a location and survey (Norušis 1990).

A.3 RESULTS AND DISCUSSION

During the Sahtu region surveys, in both surveys 100% of the ringnecks (n = 78 and 97) were observed in groups of 8 or fewer birds, while 59% and 89% of scaup in the first (n = 930) and second (n = 428) surveys, respectively were found in groups of 8 or fewer birds (Fig. A.1). In the Peace-Athabasca region 72% (n = 257) and 80% (n = 546) of ringnecks and 47% (n = 416) and 63% (n = 294) of scaup were found in groups of 8 or fewer during the first and second surveys respectively (Fig. A.2). Finally, in the Utikima region 43% (n = 682) and 84% (n = 902) of ringnecks and 19% (n = 895) and 83% (n = 375) of scaup were found in groups of 8 or fewer during the first and second surveys, respectively (Fig. A.3). Given that my collection dates (see Table 2.1) were intermediate between the DU surveys, the proportion of the population by group size at the time of my collections was likely intermediate between those observed for the first and second surveys, assuming a linear change in flock size distributions. Therefore, during my collections the majority of scaup and ringnecks were likely found in groups of 8 or fewer. However, there were still a large number of birds in groups > 8. During the second surveys, there were no groups of > 99 birds observed in any region, which could indicate that most migrants had passed before for the second survey. Therefore, it is likely that my sample does represent a large proportion of the populations of both species despite the fact that during some of the DU surveys there were large flocks observed, though these occurred mainly in the first of the Utikima surveys and approximately 10 days prior to my collections.

Only in the second survey of the Utikima (K-S test; $D_{902,375}$ = 0.096, P = 0.015) and first survey of the Sahtu ($D_{78,920}$ = 0.165, P = 0.040) regions were there significant differences in the group size distribution of scaup and ringnecks (Table A.1). The significant difference in the distribution of the second survey of the Utikima region was driven by a larger percent of lone males observed (Fig. A.3). This is likely the result of ringnecks breeding earlier than scaup (See Fig. 2.2). Therefore, it is very unlikely that my collections were biased towards breeders or non-breeders in either species based on their flock size distribution in the survey areas.

Table A.1. Results from the K-S test of the distribution in group size between scaup and ring-necked ducks from three Ducks Unlimited boreal survey regions.

		Survey 1		Survey 2	
Region		RNDU	LESC	RNDU	LESC
Utikima	*n*	576	895	902	375
	Dates	125-134		153-158	
	D	0.046		0.096	
	P	0.456		0.015	
	Basins	225		250	
Athabasca	*n*	257	416	546	294
	Dates	124-129		146-150	
	D	0.056		0.017	
	P	0.703		1.000	
	Basins	39		42	
Sahtu	*n*	78	920	97	428
	Dates	141-153		161-171	
	D	0.165		0.116	
	P	0.040		0.234	
	Basins	206		155	

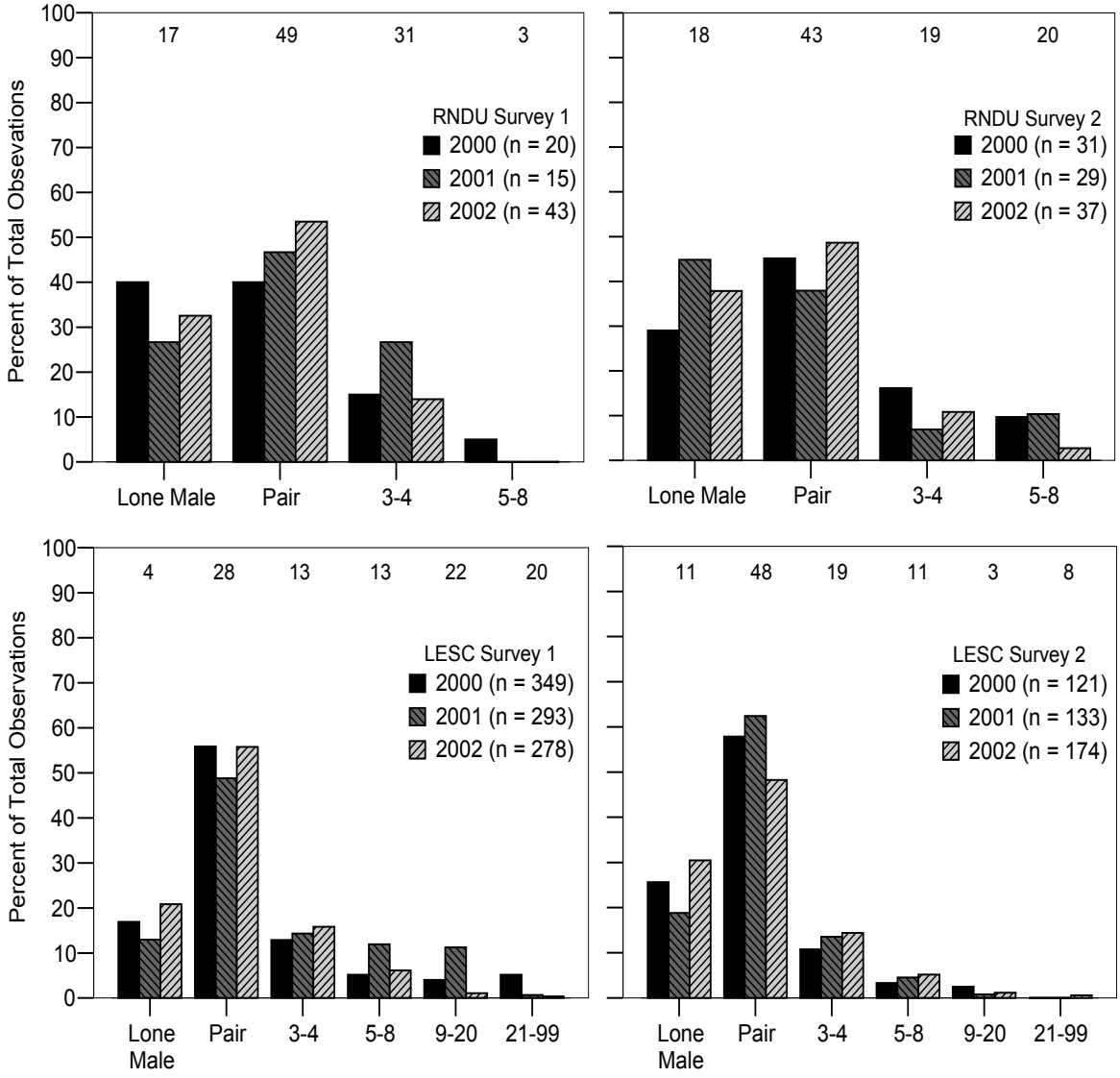

Figure A.1. Percent of RNDU and LESC group size observed on 206 and 155 wetland basins in the first and second surveys, respectively, in the Sahtu region. The first and second surveys were conducted between day 141-153 and 161-171, respectively, in 2000-2002. Numbers above each group size indicate the percent of all scaup observed over the three years combined. No groups of >99 scaup were observed.

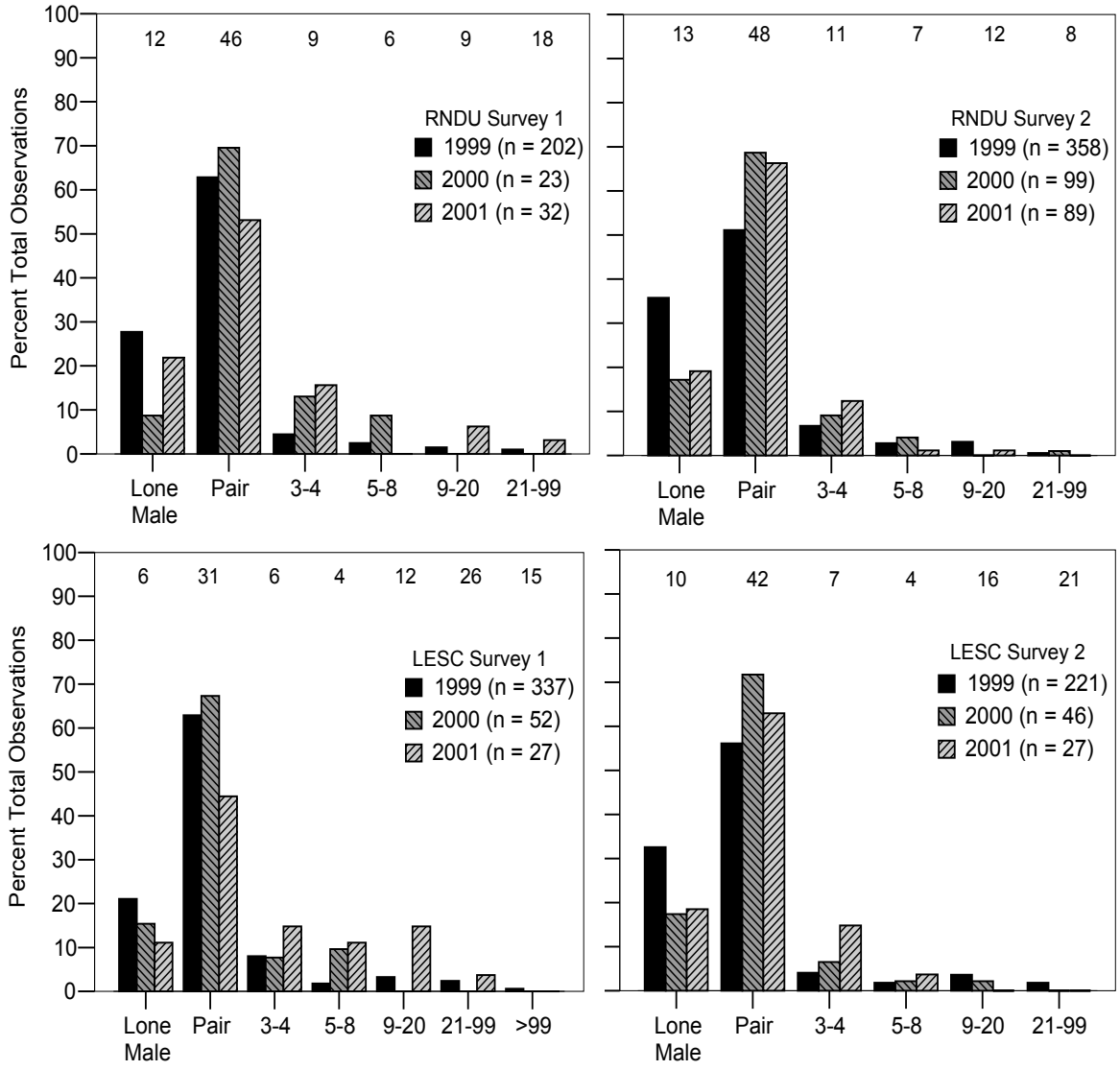

Figure A.2. Percent of RNDU and LESC group size observed on 39 and 42 wetland basins in the first and second surveys, respectively, in the Peace-Athabasca region. The first and second surveys were conducted between day 124-129 and 146-150, respectively, in 1999-2001. Numbers above each group size indicate the percent of all scaup observed over the three years combined.

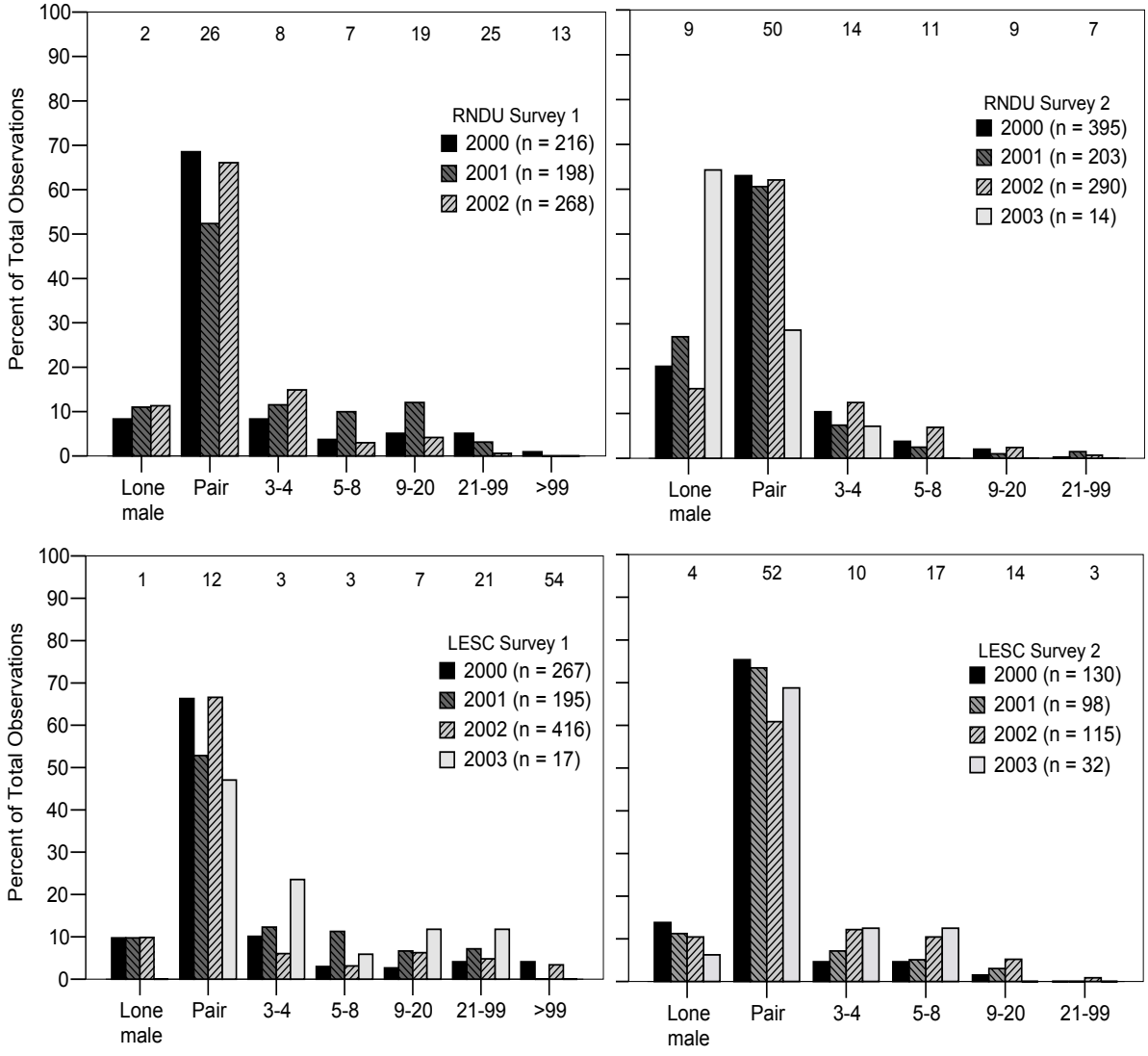

Figure A.3. Percent of RNDU and LESC group size observed on 225 and 250 wetland basins in the first and second surveys, respectively, in the Utikima region. The first and second surveys were conducted between day 125-134 and 153-158, respectively, in 2000-2003. Numbers above each group size indicate the percent of all scaup observed over the three years combined.