NUTRIENT ALLOCATION TO EGG FORMATION OF LESSER SCAUP

by

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DEDICATION

In memory of my Aunt Judy, who inspired and exhibited a relentless, never give up and stop trying attitude throughout her life as she lived with cerebral palsy. I am extremely privileged to have had her as a role model, and will do my best to remember her crippling disabilities when life does not seem just.

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TABLE OF CONTENTS

TABLE OF CONTENTS- CONTINUED

LIST OF TABLES

Table Page

LIST OF FIGURES

ABSTRACT

Lesser Scaup (*Aythya affinis*) populations have declined for nearly three-decades. Recent evidence suggests that decreases in habitat quality and availability of spring staging areas may have resulted in a decline of recruitment. Recently, stable isotopes analysis has emerged as a powerful ecological tool to measure the degree of cross-seasonal effects of birds. In 2006 through 2008 in southwestern Montana, I used carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes to assess how red blood cells (RBC), which is a proxy for stored body reserves (endogenous), change over time as local foods (exogenous) are consumed, and to estimate the relative contributions of endogenous reserves and exogenous foods for egg formation.

From arrival through the egg laying period, $\delta^{15}N$ values of RBC decreased while $\delta^{13}C$ values became more stable, a pattern consistent with expectations of endogenous tissues equilibrating with local dietary sources. In 2006 and 2008, isotopic values for egg albumen and yolk protein were similar to those expected from local dietary sources, which indicated that most protein used for producing egg albumen and yolk protein was obtained on the breeding grounds (exogenous sources). In 2007, endogenous reserves contributed on average 26% and 10% more for producing albumen and yolk protein, respectively, than in 2006 and 2008 combined.

Due to small differences in $\delta^{13}C$ values between female endogenous lipids upon arrival to the breeding grounds and those of local invertebrate lipids, it was not possible to separately estimate the contributions of endogenous and invertebrate lipids to egg lipid formation. My results suggest that local invertebrates and endogenous lipid reserves contributed on average 51% (SE = 7%) to egg lipid production. The remaining contributions to eggs were derived from local seed sources.

Despite recent findings of reduced endogenous reserves during spring migration, results from the females in this study suggest that the amount of time that females spend on the breeding grounds prior to nest initiation may be adequate in some years to allow them to attain adequate exogenous foods for reproduction. Future isotopic research is now needed across latitudinal gradients, allowing length of prebreeding season to vary, while separating out the contribution from endogenous versus exogenous sources.

INTRODUCTION

The continental population of lesser and greater scaup (*Aythya affinis* and *A. marila*) reached an all-time low in 2008, 37% below the 1955-2005 long-term average (U.S. Fish and Wildlife Service 2008) and more than 3 million birds below the North American Waterfowl Management Plan goal of 6.3 million scaup. Two reviews of long-term databases have provided important insights into the continental decline of scaup (Allen et al. 1999, Afton and Anderson 2001). Both reviews noted a decrease in the sex and age ratios (number of females relative to males and number of immatures relative to adults, respectively) of lesser scaup in the U.S. harvest (Allen et al. 1999, Afton and Anderson 2001). These results indicate that recruitment and female survival of lesser scaup have declined during this period. One potential explanation for the observed patterns in the continental lesser scaup population is provided by the spring condition hypothesis (Anteau and Afton 2004). The spring condition hypothesis suggests that declines in the quantity and quality of winter and spring habitats have resulted in females arriving on breeding areas in poorer body condition than they did historically, which in turn has resulted in females having reduced reproductive success and breeding-season survival rates (Anteau and Afton 2004).

Metabolic demands of breeding are great for waterfowl (King 1973), and waterfowl are reliant on endogenous reserves to breed successfully (Alisauskas and Ankney 1992). Scaup have been shown to use lipid reserves, which have been assumed to have been acquired while on spring-staging areas, for clutch formation, but rely less on these reserves for incubation than do similar-sized waterfowl (Afton and Ankney 1991,

Esler et al. 2001). However, because scaup spend long periods at breeding areas prior to laying (Afton 1984), it is unknown where endogenous reserves are acquired. If scaup females are arriving on breeding grounds in poorer body condition than they did historically, they may need to spend additional time on the breeding grounds acquiring the reserves necessary to fuel breeding activities before they can begin egg laying. Therefore, reduced spring condition could result in later nest initiation and declines in recruitment (Dawson and Clark 2000). Moreover, if females are unable to overcome nutrient reserve deficits after arrival on the breeding grounds they may lay fewer eggs or not breed at all.

In this thesis, I am investigating the link between food resources acquired at spring migration habitats versus foods acquired on the breeding grounds and later used for egg formation in nesting lesser scaup. This study was conducted at Red Rock Lakes National Wildlife Refuge in the Centennial Valley of southwest Montana. Red Rock Lakes National Wildlife Refuge (hereafter, the Refuge) provides a unique opportunity to test where egg macronutrients were acquired because of the narrow window of breeding opportunity for scaup that is similar in length to the western Canadian boreal forest (WCBF), an area where 70% of North American scaup breed. The 10,000 ha Refuge has an average elevation of 2,330 m and similar number of growing degree days of scaup in the WCBF (Gurney et al. *in preparation*). Recent band recoveries indicate that scaup marked on the Refuge winter in California (i.e. San Francisco Bay and Salton Sea) and the Snake River Valley in Idaho, representing one of the shortest migrations for North American lesser scaup (250 to 1100 km, USFWS unpublished data 2010). This short-

distance migration offers a feasible opportunity to study migratory connectivity between spring migration and breeding area habitats because of the lower total amount of macronutrients (i.e. protein and lipid) needed to fuel migration which could increase the importance of cross-seasonal effects.

My overall objective for this thesis was to identify the relative contribution of endogenous reserves, acquired during spring migration, to egg protein (Chapter 2) and egg lipid (Chapter 3) formation in female lesser scaup. In recent years, stable isotopes have been used to track macronutrient acquisition by birds (Hobson 2006). In the work supporting this thesis, red blood cells, a body tissue that is a proxy for past dietary information for approximately 5 weeks, were collected to gain insights into seasonal patterns of use of stored endogenous reserves after arrival on the breeding areas. Modeling the seasonal pattern for this tissue enabled me to gain information regarding when endogenous protein reserves were acquired for egg formation (Chapter 2). It is vital that wildlife managers understand the exact geographic site and timing of nutrient investment in order to prioritize management activities to that particular area where nutrients may be limiting. Intra-specific comparisons were also made by collecting body tissues from the same birds to assess individual nutrient allocation strategies for egg formation. It was expected that if foods found on the breeding grounds were used in egg formation, isotopic variability of egg constituents would be less variable than body tissues because foods with similar isotopic values were consumed for egg production (Chapter 2). Finally, models designed to help explain differences among nutrient allocation strategies of females for egg formation were assessed (Chapter 3).

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ENDOGENOUS CONTRIBUTIONS TO EGG PROTEIN FORMATION IN LESSER SCAUP

Introduction

Migratory birds store macronutrients as proteins and lipids during winter, spring migration, and breeding grounds and differentially use these nutrients for egg formation (Drent et al. 2007). The timing of breeding likely dictates the location where nutrients are acquired (Klaassen et al. 2006). For example, arrival date to the breeding ground and the amount of time elapsed between arrival and the commencement of egg laying influence the amount of endogenous reserves used for egg formation (Drent 2006, Drent et al. 2007, Klaassen et al. 2006). During spring migration, birds optimize body stores to efficiently travel to the breeding grounds. If body stores are reduced prior to spring migration, they must be regained during spring staging or at the breeding grounds due to the high energy demands of migration. Birds that increase body condition (i.e. body mass) after arrival to the breeding areas are thought to optimize efficiency by reducing body mass during spring migration (Drent 2006).

The location(s) used for nutrient acquisition for egg laying in waterfowl has long been debated (Ankney and MacInnes 1978, Bond et al. 2007, Davidson and Evans 1988, Ebbinge et al. 1982). Knowledge of where macronutrients for egg formation are derived is useful when considering where food limitations for breeding productivity may occur. Traditionally, nutrient dynamic studies have focused on a continuum to describe nutrient allocation to breeding activities. Income breeders are at one end of the continuum relying solely on local dietary sources found at the breeding grounds for breeding activities.

Conversely, capital breeders rely on stored body tissues acquired prior to arrival on the breeding area (Drent and Daan 1980). Recent studies have addressed where capital was acquired for breeding purposes in migratory birds that travel to breed (Klassen et al. 2006).

Conventional methods for measuring the amount of endogenous reserves used in egg formation have relied on correlating mass loss of a female to gains to her clutch (Hobson et al. 2004, Esler et al. 2001, Afton and Ankey 1991). For example, if endogenous proteins decline with an increase of protein to the clutch, then endogenous reserves would have presumably been used for egg formation. Unfortunately, this technique is unable to identify the geographic location(s) where capital resources were acquired, does not account for female body maintenance, and assumes 100% conversion efficiency in transferring endogenous nutrients to eggs (Hobson 2006), which seems unlikely to be true. Thus, previous studies have likely overestimated the role of endogenous reserves in clutch formation. Finally, due to the destructive nature of this technique, which entails killing several individuals through time, it cannot be used to study nutrient dynamics of bird populations of conservation status.

In recent years, stable isotopes have been used to track macronutrient acquisition by birds (Hobson 2006). Stable isotopes can be used to determine if endogenous reserves were acquired from either spring migration or local breeding area habitats (Hobson 2008). For instance, red blood cells of birds can be analyzed and their stable isotope values will reflect diet or geographic location during a prior time and place due to the slow turnover of this body tissue. Isotopic turnover occurs when an animal is introduced

to a new isotopically distinct diet. For example, naturally occurring stable isotopes in a consumer's tissues can be related ultimately to those in its diet, which provides a time-integrated estimate of assimilated foods (Hobson and Clark 1993). By knowing potential habitats selected during spring migration, and by collecting body tissues with a slow turnover rate upon arrival on the breeding grounds, the isotopic values in eggs can be used to determine the contributions of distinct habitats to egg formation. Examples of different isotopic regions or isoscapes include (1) agricultural vs. non-agricultural landscapes where the $\delta^{15}N$ values tends to be more positive in agricultural landscapes (Evans Ogden et al. 2006, Herbert and Wassenaar 2001, Yerkes et al. 2008) and (2) marine vs. freshwater systems with $\delta^{13}C$ and $\delta^{15}N$ values tending to be more positive in marine systems (Hobson et al. 2005, MacAvoy et al. 2000, Cree et al. 1999). Knowledge of these differences provides the opportunity to track the transfer of nutrients to eggs from one particular habitat to the next along a migration route. Furthermore, the carry-over of nutrients from spring migration to breeding areas habitats have been shown to effect population growth rates of birds (Calvert et al. 2009).

Continental population of scaup (*Aythya affinis* and *A. marila*), a migratory diving duck, reached an all-time low in 2008, 37% below the 1955-2005 long-term average (U.S. Fish and Wildlife Service 2008), and more than 3 million birds below the North American Waterfowl Management Plan goal of 6.3 million birds. Scaup exhibit one of the most protracted springtime migrations of any North American waterfowl species (Austin et. al 1998) and are one of the latest ducks to nest. Recent studies have reported that sites used during spring migration provide important amounts of nutrition for

reproduction in scaup (Anteau and Afton 2004, Badzinski and Petrie 2006, Anteau and Afton 2009). However, scaup arrive on breeding sites approximately 4-6 weeks before nest initiation in southerly latitudes (Afton 1984), and approximately 4 weeks in far northerly latitudes (Belrose 1980, Austin 1998, Martin 2007). Given this large amount of time, it seems possible that resources obtained on breeding sites may also play an important role in fueling nutritional demands of egg laying and incubation. After arrival on the breeding grounds, scaup increase their overall body mass (Martin 2007, Afton 1984, unpublished data USFWS 2009). Based on results obtained from conventional methods, lesser scaup have been shown to use small amounts (~25%) of endogenous protein reserves for clutch formation in sub-Arctic breeding populations, whereas midcontinent lesser and greater scaup do not use any (Esler et al. 2001, Afton and Ankey 1991, Gorman et al. 2008)

Currently, the contribution of nutrients acquired during spring migration or on the breeding grounds to egg protein formation in scaup is unknown. Therefore, I collected body tissues upon arrival and local dietary food items found at the breeding grounds and compared these isotopic values to those in eggs to determine the relative contribution of endogenous protein reserves acquired prior to arrival to the breeding grounds, and the relative contribution of local dietary sources. Knowing the site of nutrient acquisition for clutch formation is a crucial component to understand which season most limits clutch formation in scaup.

Objectives

My objectives were to (1) assess seasonal patterns in isotope values of red blood cells (RBC), a proxy for stored protein reserves acquired on spring migration areas, after arrival on the breeding grounds, (2) determine the percent contribution of endogenous and exogenous sources to egg formation in lesser scaup and to assess annual variations between the 3 years of the study, and (3) assess intra-specific nutrient strategies by comparing different time-integrated body tissues to assess where nutrients were derived for egg formation.

Band recoveries indicate that lesser scaup at my study site inhabit both marine and freshwater ecosystems and agricultural and non-agricultural sites during late-winter. These environments are expected to differ greatly in $\delta^{13}C$ and $\delta^{15}N$ values. Marine environments tend to be enriched (more positive) in both isotopes than freshwater or terrestrial environments, whereas agricultural landscapes tend to be enriched in ^{15}N compared to non-agricultural sites (Yerkes et al. 2008). Therefore, $\delta^{13}C$ (and potentially $\delta^{15}N$) values in red blood cells of scaup upon arrival to the breeding grounds were expected to vary considerably among individuals if different individuals used different winter habitats (i.e. marine and freshwater sites). Using arrival RBC isotopic values, marine and freshwater habitats were distinguished using a demarcation value for $\delta^{13}C$ values (see Methods, Yerkes et al. 2008). This allowed segregation of two habitats that scaup could use during spring migration for nutrient acquisition. In general, and accounting for isotopic discrimination (see Methods), if endogenous reserves were used for egg protein formation, then egg proteins were expected to have similar $\delta^{13}C$ and $\delta^{15}N$

values as RBC upon arrival on the breeding area. If local dietary sources were used for egg protein formation, then egg proteins were expected to have similar $\delta^{13}C$ and $\delta^{15}N$ values to those of local dietary sources found on the breeding grounds.

Because female scaup spend up to 35 days on the breeding areas prior to egg formation and are one of the latest nesting ducks in North America, I predicted that egg isotope values would indicate that local exogenous dietary sources were more important than stored endogenous reserves in nutrient allocation for egg protein formation. Furthermore, I predicted that relative contributions of endogenous reserves would remain constant at a population level over the course of the three-year study since variation in local conditions could be mediated by the long pre-breeding period at my site.

Study Area

Red Rock Lakes National Wildlife Refuge (hereafter Refuge) is located in the Centennial Valley of southwest Montana. The Refuge encompasses approximately 10,000 ha of natural and created montane wetlands at an elevation of 2,015 m, providing reproductive and migratory habitat for a diverse waterbird community. The study was conducted on Lower Red Rock Lake and the River Marsh (Figure 1), a 2,332 ha montane wetland complex located within the Refuge. The complex is comprised of nearly equal areas of shallow (< 2 m) open-water and palustrine emergent vegetation habitats. The southwestern portion of the complex is predominantly open-water habitat with interspersed islands of hardstem bulrush (*Schoenoplectus acutus*). The north and east extent of the complex is palustrine emergent vegetation dominated by sedge (*Carex* spp.) with typically small (< 2 ha), scattered open-water areas. The average annual

precipitation is 49.5 cm, with 27% occurring during May and June. The mean annual temperature is 1.8º C. The high elevation of the Centennial Valley provides a narrow window of breeding opportunity for scaup that is similar in duration to that found in the western boreal forest farther north. For example, the mean temperature for May and June, 1971-2000, are equivalent for Yellowknife, Northwest Territories ($x = 9.6^{o}$ C), and the Centennial Valley, Montana ($x = 9.3^{o}$ C, http:climate.weatheroffice.ec.gc.ca, http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?mtlake). Furthermore, Lower Red Rock Lake supports one of the highest density populations of breeding lesser scaup in North America (> 20 pairs/mi^2), exceeded only by areas such as Yukon and Old Crow Flats (30 and 34 pairs/mi^2, respectively) in Alaska and Yukon, respectively (Belrose 1980, USFWS unpublished data 2009). Due to the close proximity of Lower Red Rock Lake to their wintering areas, and lower total energy needed to fuel migration, offers a feasible opportunity to study cross-seasonal effects for egg formation in lesser scaup.

Methods

Endogenous (body) tissues were collected from female scaup upon arrival to the study area and through the egg laying period to assess how isotope values in endogenous tissues equilibrate to local foods. Local food items were collected to determine the isotopic value of the study site. Nest searches were conducted, and eggs were collected from discovered nests. Stable isotope measurements were then made to estimate the percent contribution of body tissues from spring migration and local dietary sources to egg formation. Spring migration was defined as the period between departure from the wintering grounds and arrival on the breeding areas. Pre-breeding was defined as the

period between a female's arrival on the breeding area and nest-initiation (Stephens et al. 2009).

Field Methods

Adult female lesser scaup were captured via night lighting on wetlands during the prebreeding and egg laying periods in May and June of 2006 -2008 (Lindmeier and Jessen 1961). Each female was banded with a U.S. Geological Survey aluminum leg band. Claw tissue, plasma, and red blood cells were collected from females for stable isotope analysis.

The distal portion of claws represent a time period corresponding with spring migration (Bearhop et al. 2003, Cutting et al. in preparation). Claws were collected from females in 2006 and 2007 to serve as a proxy for endogenous reserves for egg protein production (Bearhop et al. 2003). The distal ~1 mm of claw was collected on each of the middle and inside toes of each foot using forceps and scissors. Blood was collected from females in 2007 and 2008 to provide an isotopic endpoint for endogenous protein. Up to 3ml of blood was collected from each female for stable isotope analysis. Blood was extracted by bleeding the foot, brachia or jugular vein. Blood was centrifuged and transferred to individual vials, and stored frozen at -20^{o}C until stable isotope analysis was performed.

To obtain egg samples, nests were located using radio telemetry, trained dogs, foot searches, and behavioral observations of female scaup made while searching in sedge-dominated habitats. Nest searches began in early-June and continued through mid-July, while egg collection occurred throughout the nesting season. One to two eggs per

nest were collected at random. Eggs were hardboiled to easily separate yolk and albumen from each other, and samples were stored frozen until stable isotope analysis was conducted (Gloutney and Hobson 1998).

In 2007, a sample of female scaup were implanted with a VHF radio transmitters with percutaneous antennae to aid in finding nests and to assess possible individual variation in nutrient-allocation strategies during egg formation. Vehicle-mounted null-array telemetry systems were used to record daily locations of radio-marked females. If a female was present in the same location for 3 consecutive mornings, she was suspected of nesting and so I went to that location with a handheld antenna.

Because lesser scaup eat invertebrates (Rogers and Korschgen 1966) and emergent wetland seeds (Smith 2007, Strand et al. 2007, Afton and Hier 1991), both plant and animal material were collected as possible scaup food items during the prebreeding and egg laying periods. Invertebrates were collected at the study area during the breeding seasons of 2006-2009 via sweep sampling using a D-shaped dip net (1,200 µm mesh, 0.072 m^2 opening, WARD's Natural Science, Rochester, New York). Collected invertebrates included amphipods (*Gammarus*), leeches (*Hirudinea*), snails (*Gastropoda)* and water boatman (*Hemiptera*). Hardstem bulrush (*Schoenoplectus acutus*) and sedge seeds (*Carex* spp.) were also collected at the study area during the pre-breeding and egg laying periods of 2006 and 2007 for stable isotope analysis by finding intact seed heads that were from the previous year's growth.

Weather Measurements

Precipitation and temperature data were used to assess annual variations collected by the Natural Resource Conservation Service (NRCS) at a nearby Snotel site at an elevation of 2428 m and ~2 km south of the study site (http://www.wcc.nrcs.usda .gov/snotel/snotel.pl?sitenum=568&state=mt). Climatic data were collected every 3 hours. All Snotel precipitation and temperature data are available in the NRCS archival dataset. Water levels at the western outflow of Lower Red Rock Lake were measured throughout the nesting season and mean water level was summarized between the first to third quartile of the nest initiation period for a given year.

Isotopic Analysis

Red blood cell isotope values upon arrival on the breeding area serve as a proxy for macronutrients stored as endogenous reserves during spring migration. Based on body mass (g) of captured scaup during this study, estimated half-life incorporation rate of RBC was 22.7 ± 0.1 SE days (Carleton and Martinez del Rio 2005). Red blood cells were freeze dried, homogenized with a mortar and pestle, weighted (~1 mg), and encapsulated into tin capsules for stable isotope analysis via mass spectroscopy.

The $\delta^{13}C$ value of keratinized proteins (i.e. claws) tends to be more positive than metabolically active tissues (i.e. blood) (Tieszen et al. 1983). Since RBC was not collected in 2006, claw $\delta^{13}C$ values were adjusted using the mean difference of $\delta^{13}C$ between claws and RBC of females categorized as originating from a freshwater or marine environment in 2008. Claws were cleaned with a 2:1 chloroform/methanol

solution (to rid surface oils), freeze dried, chopped into small pieces, weighed (~1 mg) and encapsulated into tin capsules for stable isotope analysis.

Invertebrate samples were washed with distilled water, freeze dried and then powdered. Lipids were removed using a 2:1 chloroform/methanol solution and dried in a fume hood. Several droplets of 0.1N HCL solution were applied to lipid-free invertebrate samples without rinsing to remove carbonates. Egg and invertebrate samples were weighed (~1 mg), encapsulated in tin capsules, and analyzed for stable-carbon ($\delta^{13}C$) and stable-nitrogen ($\delta^{15}N$) isotopes using continuous flow isotope-ratio mass spectrometry (Hobson 2005). Stable isotope values were reported in parts per thousand (‰) relative to the standards Vienna PeeDee Belemnite for $\delta^{13}C$ and atmospheric (AIR) nitrogen for $\delta^{15}N$. Estimated analytical error for $\delta^{13}C$ and $\delta^{15}N$ was ±0.1‰ and ±0.3‰, respectively based on replicate within-run measurements of standards.

An aliquot of yolk and albumen was collected from the center concentric ring and the end opposite to that of the air cell from each egg, respectively. Samples were freeze dried and lipids removed from the yolk using a 2:1 chloroform:methanol rinse (Hobson et al. 2005). Lipids were removed from egg yolks twice to ensure that most lipids had been removed (Ricca et al. 2006) since lipids are depleted in $\delta^{13}C$.

Due to high initial variability of $\delta^{13}C$ and $\delta^{15}N$ in RBC, females were categorized as originating from different habitats using arrival RBC isotopic values. Marine and freshwater habitats were distinguished based on a demarcation value of -20‰ for $\delta^{13}C$. Values greater than -20‰ $\delta^{13}C$ were classified as originating from a marine habitat while

values less than -20‰ $\delta^{13}C$ were classified as originating from a freshwater habitat (Yerkes et al. 2008).

Stable isotope values in eggs differ in a predictable manner from those in the female's diet and endogenous reserves through the process of isotopic discrimination (Hobson and Clark 1992, Hobson 1995). Accordingly, discrimination values were applied to diet and endogenous body tissues to provide an estimate of egg protein values that are expected if each source is solely relied upon for fueling egg protein formation. This provided isotopic endpoints for the mixing model. Discrimination values have been experimentally determined from diet to egg (Hobson 1995) but not for endogenous reserves to egg. To estimate this, previous researchers have assumed that the mobilization of proteins to eggs from endogenous reserves involves similar isotopic discrimination as found for the carnivore model in Hobson (1995) (Gauthier et al. 2003, Schmutz et al. 2006, Bond 2007). To account for discrimination between diet and endogenous reserves to yolk protein formation, a value of 3.4‰ for $\delta^{15}N$ was used. Discrimination value for $\delta^{13}C$ in albumen production from lipid-free invertebrates and lipid-free endogenous reserves was 0.9‰; whereas discrimination between albumen and wetland seed was 1.5‰. A value of 0.0‰ was used to account for $\delta^{13}C$ discrimination between lipid-free diet or body tissues and lipid-free yolk proteins.

Data Analysis

Seasonal variation in $\delta^{13}C$ and $\delta^{15}N$ of RBC was modeled in light of the fact that isotope turnover in avian blood after a diet switch approximately follows an exponential function (Dietz et al. 2010, Oppel and Powell 2010). To investigate seasonal isotopic

trends, an exponential model was used to describe the change of $\delta^{13}C$ and $\delta^{15}N$ values through time. To account for non-constant variance of isotope residuals through time, the day covariate was added in the standard deviation. It was assumed that the standard deviation was a linear function of the data (standard deviation= y+ x*date). This improved the fit over the constant variance model and more importantly reduced the equilibrium value and increased the growth rate parameter estimates in the model. The exponential model was optimized using maximum likelihood.

A concentration-dependent, three-endpoint, two-isotope mixing model (IsoConc) was used to estimate relative contribution of RBC, local invertebrates and wetland seeds to egg formation in female lesser scaup (Phillips and Gregg 2001; Phillips and Koch 2002). This model accounts for differences when elemental concentrations of sources vary substantially (Phillips and Koch 2002). Concentrations are expressed as the proportion of the total weight of the sample during isotopic analyses (RBC: [C] = 0.448 ± 0.014, mean ± 1 SE; [N] = 0.140 ± 0.014; invertebrates: [C] = 0.353 ± 0.021, [N] = 0.085 ± 0.007; seeds: [C] = 0.416 ± 0.018, [N] = 0.013 ± 0.002). The assimilation of carbon and nitrogen in plant matter of a consumer's diet varies depending on the digestibility of the plant tissue. For example, nitrogen in plants is readily assimilated by birds, whereas carbon is less able to be assimilated due to the bird's inability to digest plant fibers (Robbins et al. 2002). Therefore, I followed Gauthier et al. 2003 and Manseau and Gauthier 1993 and assumed that scaup could assimilate 80% of total nitrogen of wetland seeds and assumed that carbon assimilation was equal to 35%. The concentration of assimilated N for wetland seeds became 0.029 (i.e., $[N]_{\text{wetland seeds}}$ x 0.80/0.35).

A one-way ANOVA was used to assess annual variation in arrival RBC and claw material in May on the breeding grounds and eggs from 2006 to 2008. A Tukey-Kramer Honestly Significant Difference (hsd) test was used to determine the significance of annual differences in stored endogenous reserves upon arrival on the breeding grounds and to evaluate whether the amount of endogenous reserves used in egg formation varied annually (Sokal and Rohlf 1995). A *t*-test was used to compare arrival body tissues to local dietary sources. All statistical analyses were conducted using R 2.8.1 (R Development Core Team 2009). Prior to analysis the data were tested for homogeneity of variance and normality of the residuals and confirmed to meet the assumptions of ANOVA. An alpha level of 0.05 was used to determine a statistical difference while an alpha level of 0.1 was used to determine a marginal difference throughout all analyses.

Results

A total of 69 females were captured during the 3-year study (20 in 2006, 6 in 2007, and 24 in 2008) after arrival on the breeding grounds and sampled to obtain estimates of the endogenous isotopic values of scaup during spring migration. A total of 54 eggs were collected during the three year study (20 in 2006, 6 in 2007, and 28 in 2008). The local dietary food isotopic endpoints corresponding to invertebrates and seeds was estimated from 27 invertebrate samples in 4 genera (e.g. amphipods (*Gammarus*), leeches (*Hirudinea*), snails (*Gastropoda)* and water boatman (*Hemiptera*)) and 5 wetland seed samples in 2 genera (e.g. Hardstem bulrush (*Schoenoplectus acutus*) and sedge seeds (*Carex* spp.)) that were collected during the three year study.

Mean temperature and precipitation from April-June varied among years with the warmest and driest period occurring in 2007. While 2006 and 2008 were "average" years, 2007 was the warmest and driest since 1991 at 8º C and 10.4 cm, respectively. The average precipitation from April-June in 2006 and 2008 was 25.4 cm and 23.1 cm, respectively. The average temperature April-June in 2006 and 2008 was 7.3º C and 4.7º C, respectively. The difference in mean water level of Lower Red Rock Lake during the first and third quartile of the nesting season for scaup in 2007 (SE = 0.03) was lower than the mean water level in 2006 (0.66 ± 0.03m SE) and 2008 (0.39 ± 0.01m SE). As a consequence of the dry year in 2007, most ponds surrounding the study area were dry by mid-June. Conversely, in 2006 and 2008 all of the ponds surrounding the study area were full of water throughout the prebreeding and egg laying periods.

Seasonal Patterns of $\delta^{13}C$ and
$\delta^{15}N$ Isotopes in Red Blood Cells

Female isotopic RBC values suggested that both freshwater and marine habitats had been used to store macronutrients as endogenous reserves prior to arrival on the breeding grounds: $\delta^{15}N$ values in RBC decreased in the freshwater group from 9.9 ± 0.7‰ SE to 6.2 ± 0.2‰ SE between 8-10 May and 2 July, while $\delta^{15}N$ values in the marine group decreased from 11.5 ± 2.0‰ SE to 4.7 ± 0.1‰ SE between 9-10 May and 2-3 July, 2008. In contrast, $\delta^{13}C$ values in RBC increased in the freshwater group from -22.4 ± 0.5‰ SE to -20.7 ± 0.1‰ SE between 8-10 May and 2 July, while $\delta^{13}C$ values in RBC decreased for the marine group from -13.9 ± 1.0‰ SE to -19.5 ± 0.04‰ SE between 9-10 May and 2-3 July, 2008.

Seasonal patterns of $\delta^{13}C$ and $\delta^{15}N$ values in RBC were fitted with an exponential function (Figure 2, Table 2). Less of the variation for $\delta^{13}C$ values in RBC was explained for the freshwater group than for the marine group (Freshwater: $r^2 = 0.11$, Marine: $r^2 = 0.63$). The $\delta^{13}C$ rate of change for RBC per day in the freshwater and marine group was 0.02‰ and 0.20‰, respectively. $\delta^{15}N$ values for RBC for both the freshwater and marine group showed a declining trend through the breeding season. Less variation of $\delta^{15}N$ values in RBC of the freshwater group was explained compared to $\delta^{15}N$ values in RBC of the marine group (Freshwater: $r^2 = 0.32$, Marine: $r^2 = 0.66$). The $\delta^{15}N$ rate of change in RBC per day of the freshwater and marine group was 0.02‰ and 0.04‰, respectively. Isotope values of RBC showed replacement of stored endogenous reserves, acquired on spring staging areas, with local food sources found on the breeding grounds.

Stable-Isotope Ratios of Arrival
Endogenous Tissues, Egg Components, and Diet Items:

The $\delta^{15}N$ values of arrival RBC (i.e. endogenous reserves) varied annually, but not for $\delta^{13}C$ ($\delta^{13}C$: $F_{2,47}$=1.88; p=0.29; $\delta^{15}N$: $F_{2,47}$=5.38; P<0.001; Table 2 and Figure 3). The $\delta^{15}N$ values in RBC for 2007 were significantly different than those for 2006 (P=0.005), whereas the difference between values for 2007 and 2008 was marginally significant (P=0.06). The arrival $\delta^{15}N$ values in RBC from 2006 and 2008 were not significantly different (P=0.31).

Egg albumen and lipid-free yolk protein $\delta^{13}C$ values varied by year (albumen; $F_{2,49} = 3.50$, $P = 0.04$, yolk protein; $F_{2,50} = 10.29$, $P < 0.001$, Table 3). The $\delta^{13}C$ value in albumen in 2008 was significantly different from the albumen value in 2006 ($P = 0.05$,

Table 3). The $\delta^{13}C$ value in albumen from 2007 was not significantly different than in 2006 ($P = 0.11$) and 2008 ($P = 0.82$). The $\delta^{13}C$ value of yolk protein in 2006 was significantly different than in 2007 ($P = 0.002$) and 2008 ($P < 0.001$), while the $\delta^{13}C$ value in yolk protein was not significantly different between 2007 and 2008 ($P = 0.48$).

Egg albumen and yolk protein $\delta^{15}N$ values also differed among years (albumen; $F_{2,49} = 17.37$, $P < 0.001$, yolk protein; $F_{2,50} = 35.98$, $P < 0.001$, Table 3). The $\delta^{15}N$ value in egg albumen was significantly different between 2007 and 2006 ($P < 0.001$) and $\delta^{15}N$ value in egg albumen was significantly different between 2008 and 2007 ($P < 0.001$). Egg albumen $\delta^{15}N$ values were not significantly different in 2008 and 2006 ($P < 0.001$). The $\delta^{15}N$ value in yolk proteins was significantly different between 2007and 2006 ($P < 0.001$), 2008 and 2006 ($P = 0.009$), 2008 and 2007 ($P < 0.001$).

The $\delta^{13}C$ and $\delta^{15}N$ values of lipid-free invertebrate samples did not differ among years ($F_{2,24} = 2.70$; $P = 0.09$ and $F_{2,24} = 2.64$; $P = 0.09$, respectively). Therefore, invertebrates were pooled to create a bulk isotopic endpoint for the local invertebrate diet during the 3-year study. The overall 3-year averages for $\delta^{13}C$ and $\delta^{15}N$ in lipid-free invertebrates were -21.7 ± 0.4‰ and 2.4 ± 0.5‰, respectively (Table 1, Figure 3). The mean values for $\delta^{13}C$ and $\delta^{15}N$ in local emergent wetland seeds collected in 2008 were -26.8 ± 0.3‰ SE, and 1.9 ± 0.6‰ SE, respectively.

Relative Contribution of Female
Body Tissue, Dietary Invertebrates
and Seeds to Egg Protein Formation:

It was hypothesized that endogenous reserves would contribute little to egg protein formation primarily due to the protracted time spent on the breeding grounds

prior to nesting. The contribution of endogenous reserves varied annually with endogenous reserves from freshwater and marine habitats contributing relatively equally to yolk protein formation (Figure 4, Table 4). The percent contribution of endogenous reserves to yolk protein formation from the freshwater and marine groups were similar in 2006 (Freshwater: 2% ± 6% SE; Marine: 3% ± 5% SE) and 2008 (Freshwater: 9% ± 6% SE; Marine: 3% ± 5% SE). In 2007, a higher contribution of endogenous reserves to yolk protein and albumen production was observed for both the freshwater (Yolk Protein: 26% ± 10% SE; Albumen: 11% ± 9% SE) and marine (Yolk Protein: 35% ± 10% SE; Albumen: 9% ± 9% SE) groups. The remaining contributions to egg protein formation were derived from local exogenous sources (Figure 4, Table 4).

Intraspecific Nutrient Strategies of Different Time-Integrated Body Tissues Based on Telemetered Birds:

Body tissues with different turnover rates were used to explore intraspecific nutrient allocation strategies in nesting lesser scaup. Results indicate a reduction in variation among body tissues of individuals through time, indicating a similar $\delta^{13}C$ source had been used for egg formation (Figure 5, Claws 3.6 SD, RBC 2.0 SD, Plasma 1.9 SD, Yolk Protein 1.1 SD, Albumen 0.5 SD). This represents a similar nutrient strategy for egg albumen production with some evidence that endogenous reserves were used for yolk protein formation.

Discussion

Upon arrival on the breeding grounds, lesser scaup had stored endogenous protein reserves that were acquired during spring migration. Those reserves were presumably

used for body maintenance throughout most of the prebreeding and egg-laying periods. Based on RBC isotope values upon arrival on the breeding grounds that differed from those expected from local diets, contributions from endogenous protein stores were greater during the earliest part of the egg laying period. Red blood cell isotope values declined precipitously until endogenous protein stores were completely replenished by local dietary sources by the end of the egg-laying period. In two of the three years of the study, little endogenous protein was available by the time egg-laying occurred. Instead, local dietary sources contributed most of the macronutrients to egg protein production. Two of the three years from this study were consistent with the findings from southern Manitoba by Afton and Ankney (1991). This study did not report evidence that endogenous protein was used for clutch formation in lesser scaup which indicates the breeding grounds are a primary source of nutrients for egg protein production.

Interactions between breeding and non-breeding events are likely amplified in populations with strong migratory connectivity (Martin et al. 2007, Calvert et al. 2009). Isotopic values of RBC in this study showed considerable variation upon arrival on the breeding grounds, which may suggest that cross-seasonal effects may not be as important for egg production. Throughout the breeding season, $\delta^{15}N$ values of RBC decreased while $\delta^{13}C$ values became more stable by the end of the egg-laying period. This convergence of both $\delta^{15}N$ and $\delta^{13}C$ values shows a replacement of stored reserves from spring migration areas with those from the breeding area. The high variability in $\delta^{15}N$ and $\delta^{13}C$ values in body tissues upon arrival supports the notion that multiple locations were used for staging during spring migration.

As shown in several previous studies, isotopic turnover in blood of birds tends to follow an exponential change following a switch to a new isotopic diet (Oppel and Powell 2010, Pearson et. al 2004, Morrison and Hobson 2004). Both $\delta^{15}N$ and $\delta^{13}C$ of RBC were described by an exponential model. Less variation was explained in the seasonal change of $\delta^{13}C$ values of RBC from the freshwater group than $\delta^{13}C$ values of RBC from the marine group. This might be due to the protracted nature of spring migration in scaup. For example, scaup likely selected freshwater habitats more than marine habitats during spring migration due to close proximity of freshwater habitats to the local breeding environment. Straight-line distance of marine habitats is >1100km from the local breeding grounds. Conversely, key freshwater spring stopover sites, such as the Snake River Valley in Idaho or the Great Salt Lake, are used for refueling purposes and are <500km from the local breeding environment. Since scaup have a notoriously slow migration pattern, arrival times on the breeding grounds likely differ among individuals. This asynchronous migration pattern may be supported by the high variation in isotopic values of RBC upon arrival. The higher proportion of females using freshwater sites was confirmed by a comparison of multiple body tissues at an intraspecific level which revealed a higher proportion of scaup using freshwater habitats during spring migration (see below).

In year 2007, scaup had the highest endogenous reserve contribution to yolk protein formation, and the earliest peak nest-initiation date. These findings are consistent with what has been reported in several other studies that have shown a higher contribution of endogenous reserves to egg formation earlier in the nesting season

(Morrison and Hobson 2004, Hobson et al. 2005). Furthermore, 2007 was an extreme drought year during which overall productivity may have been reduced (Rogers 1964), possibly leaving only older, and more experienced females in sufficient body condition for breeding. Females in better body condition upon arrival are capable of storing higher amounts of endogenous reserves than females in poor body condition (Ankney and MacInnes 1978). This was confirmed by comparing body mass of females captured on 10-12 May from 2007 and 2008. In 2007, body mass of female scaup was significantly higher than female body mass from 2008 (2007 - 95% CI: 683 to 727 g; 2008 - 95% CI: 635 to 680 g). Furthermore, during the drought year of 2007, temporary emigration was observed based on lower detection probability and a high rate of emigration of radio-marked females from the study site (USFWS unpublished data 2009).

Inter-annual variability existed with respect to the proportion of endogenous reserves used for yolk protein and albumen production. These results are the first published estimates showing inter-annual variability in how lesser scaup allocate macronutrients for egg production. In an earlier study by Afton and Ankney (1991), they collected female scaup over a 5-year period, but they did not present results on possible interannual variability. Previous nutrient dynamic study of scaup from southern Canada (Afton and Ankney 1991, Gorman et al. 2008) that used traditional techniques (i.e. regressing somatic tissues against reproductive tissues during the breeding season) found little evidence that endogenous proteins were used in egg protein formation. In 2006 and 2008, macronutrients for egg protein production were mostly allocated from local dietary sources (overall mean of local food sources for yolk protein and albumen production; 91-

100%). In 2007 endogenous reserves contributed significantly more (range for endogenous reserves used in yolk protein and albumen production 9-35%), which is similar to the results of nutrient dynamic's in scaup from interior-Alaska (Esler et al. 2001). These results highlight a flexible strategy in how macronutrients are used for egg protein formation. Alternatively, these results also indicate that when local resources are not available (dry years) there is an increase in contribution of stored endogenous reserves to egg formation.

Female scaup used at least two habitats during spring migration for storing endogenous proteins prior to arrival on the breeding grounds. The first habitat appears to be more common where six of the seven individuals had similar isotopic values to that of a freshwater environment. The second strategy is one where a significant amount of time was spent foraging in enriched marine waters during late winter and early spring migration. The egg protein constituents for all individuals were less variable than body tissues and relatively similar to one another, suggesting a similar nutrient allocation strategy where local breeding area food sources were used for egg formation. These results are supported by several band recoveries from the wintering period that show scaup using distinct geographic wintering locations from coastal southern-Mexico, Great Salt Lake, Utah, and Snake River Valley, Idaho. In 2009, eight satellite transmitters were deployed in female scaup to assess migration routes to their wintering areas (unpublished data USFWS 2009). From these data, several wintering areas were identified which includes southern Mexico, to coastal and interior Texas, and the Snake River Valley of

Idaho were identified as geographic locations scaup used to store nutrients prior to migrating to their breeding grounds in Montana.

Given the scaup's intermediate body size, high metabolism compared to other large bodied waterfowl (Nager 2006), increased mass gain after arrival to my study site (unpublished data USFWS 2009), and prolonged time spent on the breeding grounds prior to nesting in southern latitudes (Belrose 1980), support the notion that conditions on breeding areas influence nutrient demands for egg protein formation. Results from this study, at this southern latitude breeding site, are consistent with past nutrient dynamic studies in which egg proteins were obtained on the breeding grounds for female lesser scaup nesting in the Prairie Parklands of Canada and interior Alaska (Afton and Ankney 1991, Esler et al. 2001), and for greater scaup on the Yukon-Kuskokwim Delta, Alaska (Gorman et al. 2008). This study addressed differences between endogenous reserves acquired during spring migration and endogenous acquired on the breeding grounds. Furthermore, these differences likely require drastically different management strategies in order to ensure the habitat quality suitable for breeding scaup. Wildlife managers in southern latitudes of the breeding range of lesser scaup should not underestimate the importance of breeding habitats as a primary source of nutrients for clutch formation. Finally, future isotopic research is now needed across latitudinal gradients, allowing length of prebreeding season to vary, while separating out the contribution from endogenous versus exogenous sources. This will provide information into where and when scaup use either exogenous or endogenous sources for reproduction.

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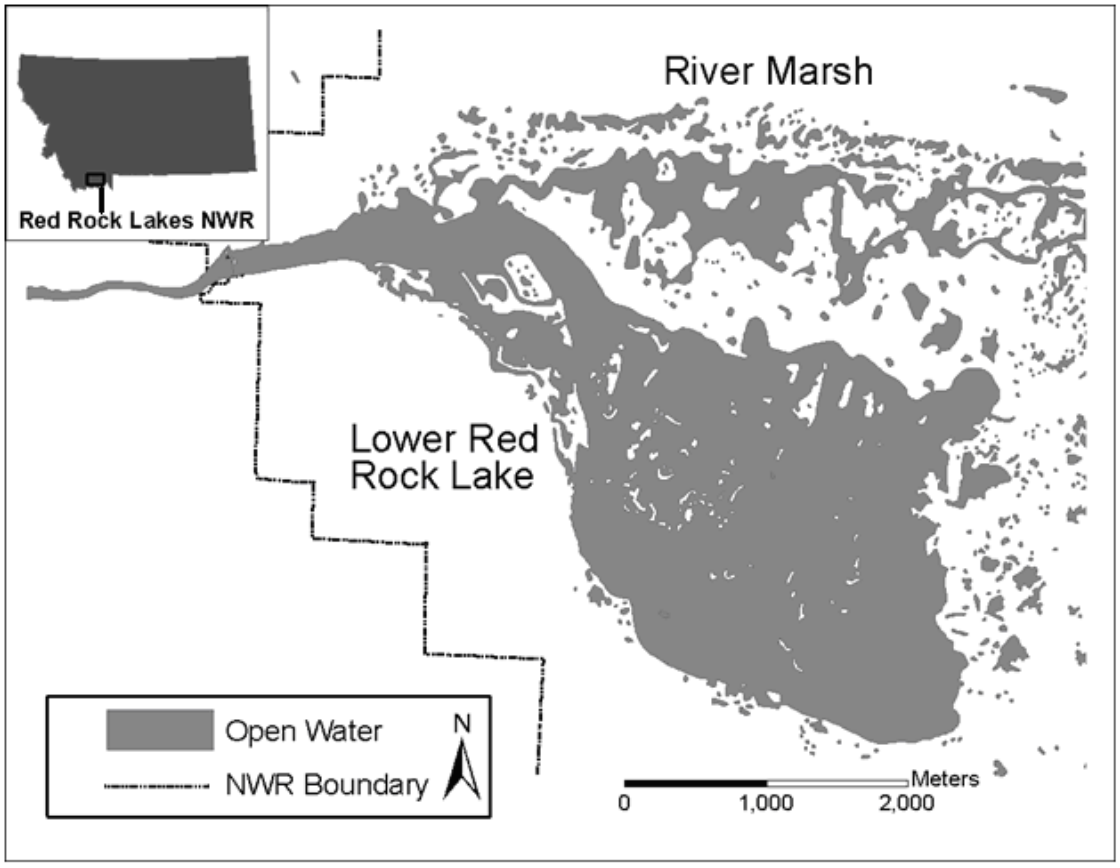

Figure 1. Location of the study area on Lower Red Rock Lake and River Marsh within Red Rock Lakes National Wildlife Refuge, Montana, USA. Inset shows the location of the study area in Montana, USA.

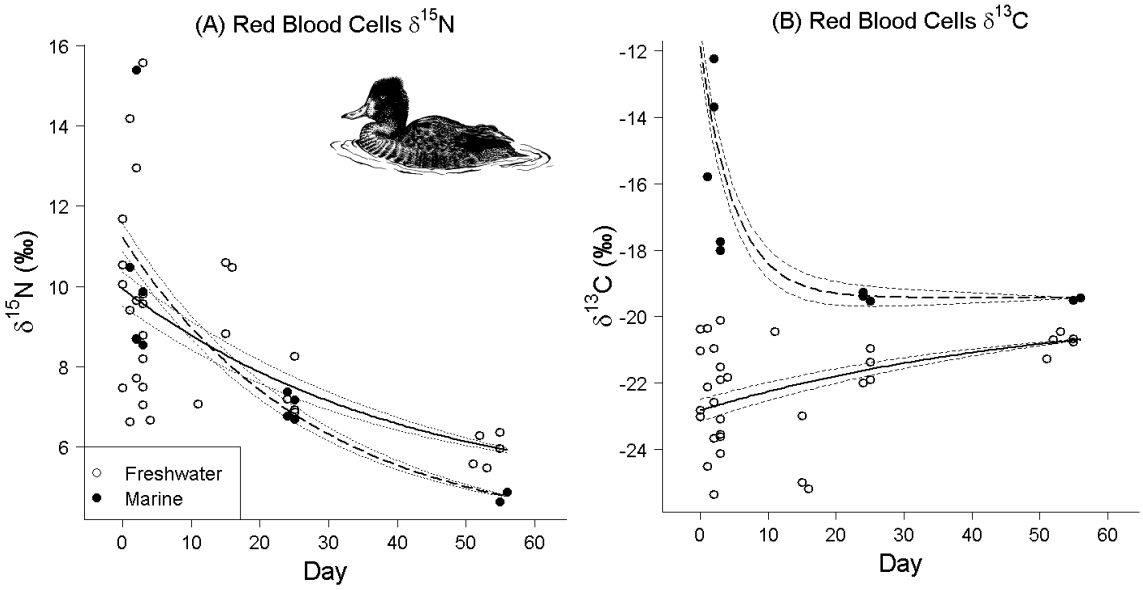

Figure 2. Seasonal patterns of $\delta^{15}N$ (A) and $\delta^{13}C$ (B) values in red blood cells of female lesser scaup (*Aythya affinis*) captured on their breeding grounds from arrival through the prebreeding and egg laying periods from 8 May (Day 0) to 3 July (Date 55), Red Rock Lakes National Wildlife Refuge, Montana, USA. Red blood cells from captured female scaup were categorized as originating from a marine ($\delta^{13}C > -20‰$) and freshwater ($\delta^{13}C \leq -20‰$) habitats (Yerkes et al. 2008). Exponential functions are as follows: (A) Freshwater: $\delta^{15}N = 4.493 + 5.442 * \exp(-0.024 \text{ x day})$; $r^2 = 0.32$; Marine: $\delta^{15}N = 3.751 + 7.120 * \exp(-0.036 \text{ x day})$; $r^2 = 0.66$; (B) Freshwater: $\delta^{13}C = -22.824 + 3.207 * (1 - \exp(-0.02 \text{ x day}))$; $r^2 = 0.11$; Marine: $\delta^{13}C = -19.440 + 6.207 * \exp(-0.199 \text{ x day})$; $r^2 = 0.63$. The dashed line indicates a 1 standard deviation around the average rate of change in isotopic values of red blood cells.

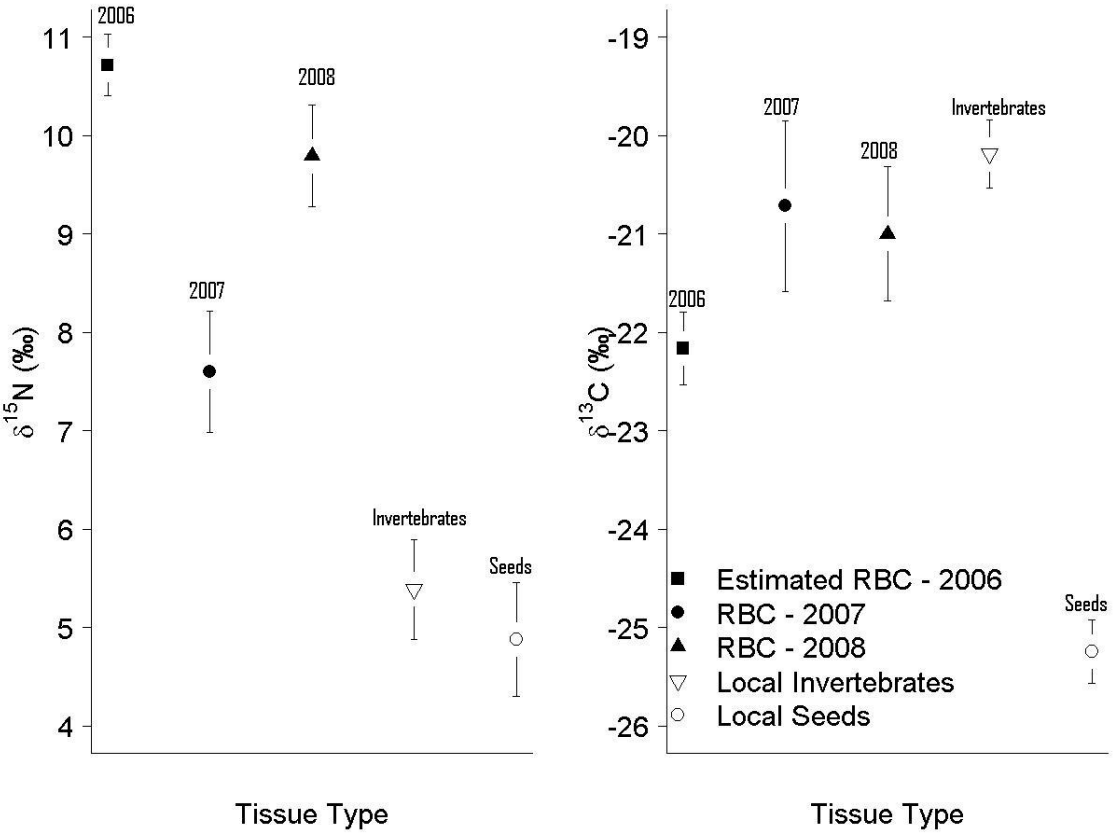

Figure 3. Stable isotope values (mean ± 1SE) of arrival red blood cells (RBC) in lesser scaup, and dietary lipid-free invertebrates and seeds during the prebreeding and egg-laying periods, 2006-2008, Red Rock Lakes National Wildlife Refuge, Montana, USA. Local invertebrates and wetland seeds were adjusted for isotopic discrimination ($\delta^{13}C$: 1.5 and $\delta^{15}N$: 3.0, Haramis et al. 2001). In 2006, $\delta^{13}C$ values of claws were normalized to RBC using the mean difference in $\delta^{13}C$ between claws and RBC of females from freshwater and marine environments in 2008.

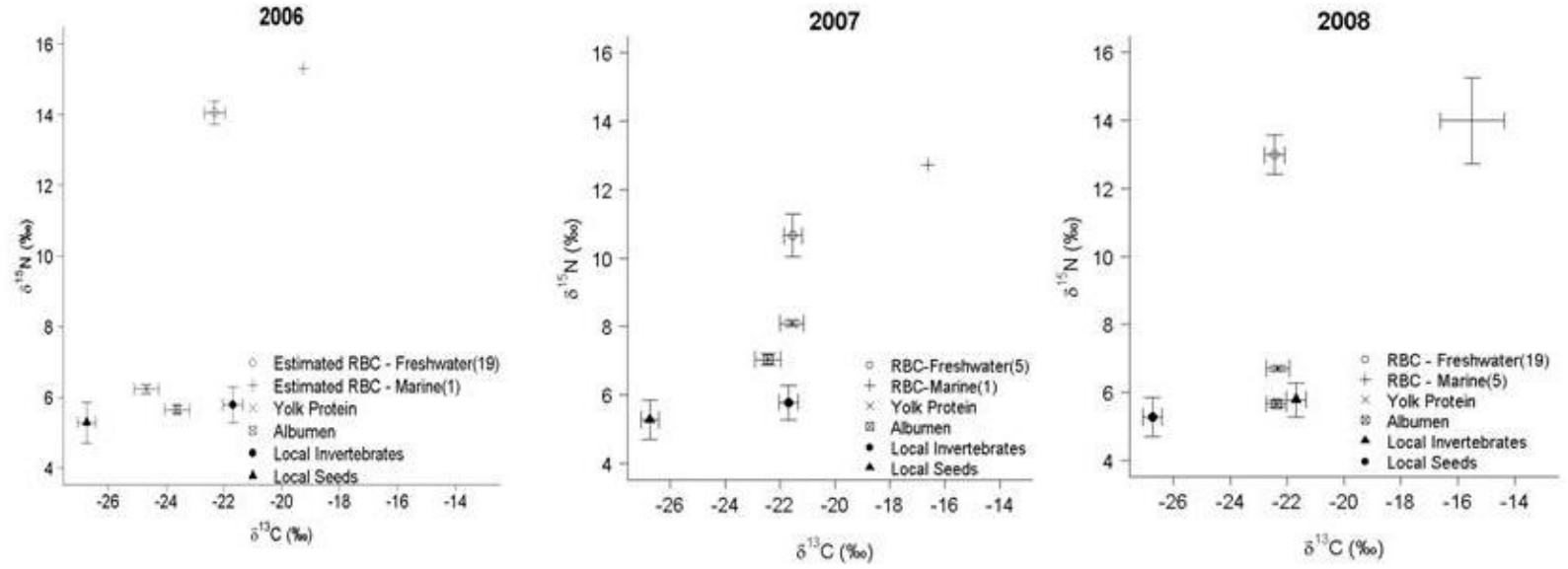

Figure 4. Predicted $\delta^{15}N$ and $\delta^{13}C$ values (mean $\pm$ 1SE) of red blood cells (RBC), aquatic invertebrates and wetland seeds if egg proteins (albumen and yolk protein) of female lesser scaup were derived entirely from any of these sources during the prebreeding and egg laying periods, 2006-2008, Red Rock Lakes National Wildlife Refuge, Montana, USA. Tissues were adjusted for discrimination (Hobson 1995). Since discrimination values for albumen differs between the herbivore and carnivore models, the discrimination value was averaged (-1.2‰) for only depiction purposes. Claws were collected in 2006 and were adjusted by the difference between claws and red blood cells from individuals captured in 2008 to create an estimated arrival RBC endpoint.

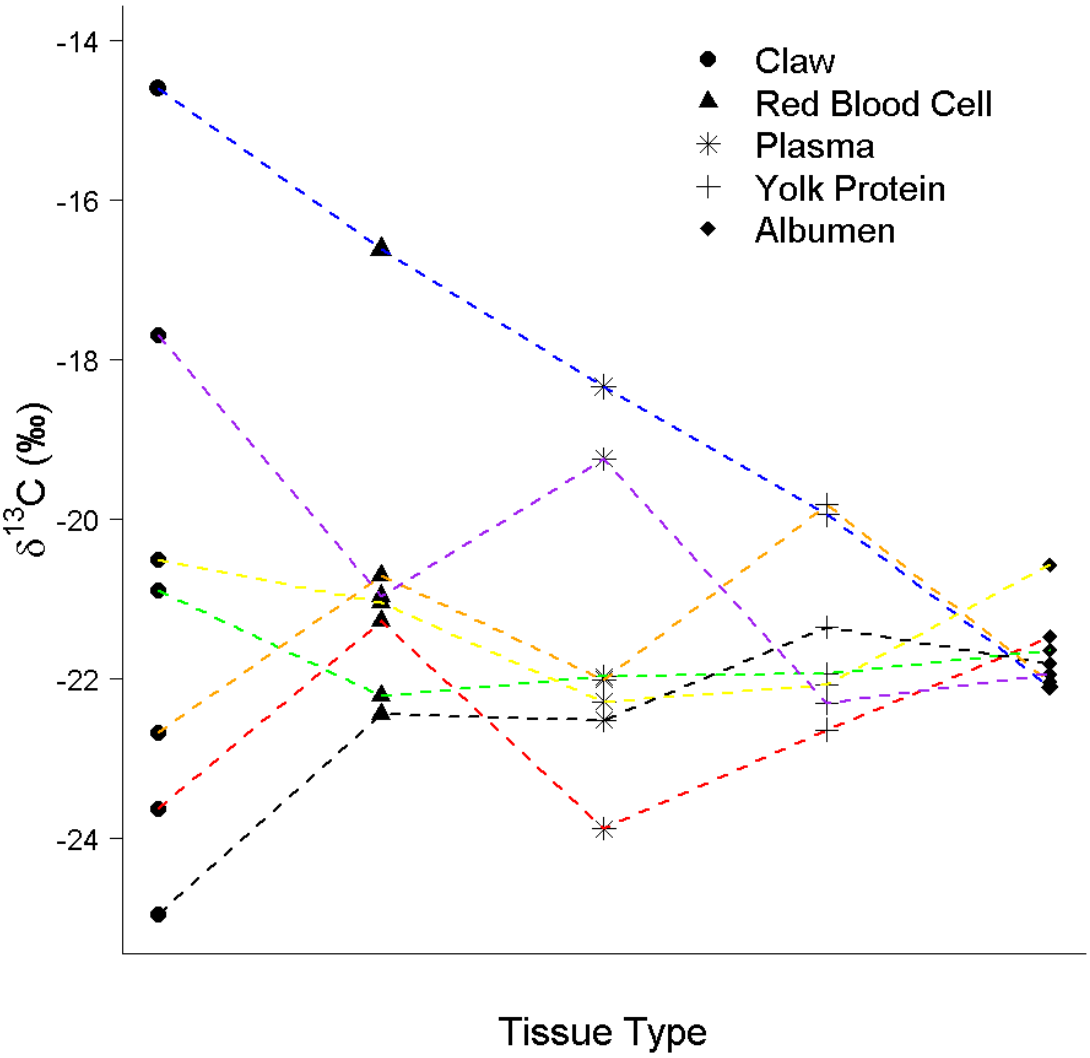

Figure 5. Intraspecific nutrient strategies for egg production in 7 female lesser scaup, Red Rock Lakes National Wildlife Refuge, Montana, USA. Stable-carbon isotope ($\delta^{13}C$) values of body tissues with different turnover rates and eggs collected from the same individuals reveal different strategies during late-winter and spring migration. The egg protein constituents for all individuals were less variable than body tissues and relatively similar to one another, indicating a similar nutrient source for egg formation. $\delta^{13}C$ values for claws were normalized to red blood cells using the mean difference between the two tissues from the 7 individuals.

Table 1. Stable-isotope values (means ± SD [*n*]) for wetland invertebrate and seed resources available to lesser scaup on the breeding grounds. All material was collected at Red Rock Lakes National Wildlife Refuge, U.S.A.

Organism[a]	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
	Invertebrate	
Amphipods	1.4 ± 2.5 (17)	-22.3 ± 1.4 (17)
Bivalve	1.5 (1)	-20.5 (1)
Leech	5.4 ± 0.9 (6)	-21.5 ± 1.9 (6)
Waterboatman	2.4 ± 0.2 (3)	-19.1 ± 2.1 (3)
All invertebrate groups	**2.4 ± 2.6 (27)**	**-21.7 ± 1.8 (27)**
	Seed	
Sedge	1.5 ± 1.1 (4)	-26.7 ± 0.8 (4)
Bulrush	3.4 (1)	-26.9 (1)
All seed groups	**1.9 ± 1.3 (5)**	**-26.8 ± 0.7 (5)**

[a]Scientific names: amphipods *Gammarus* spp., snails Gastropoda, leech Hirudinea, water boatman Hemiptera, sedge *Carex* spp., bulrush *Schoenoplectus acutus*.

Table 2. $\delta^{13}C$ and $\delta^{15}N$ isotope values (mean ± SE) of lesser scaup female body tissues, 2006-2008, Red Rock Lakes National Wildlife Refuge, Montana, USA.

Year	**Tissue**	**Date**	***n***	**$\delta^{15}N$**	**$\delta^{13}C$**
2006	*Claws*	28 May – 1 June	20	10.7 ± 0.3	-19.2 ± 0.7
2007	*Red Blood Cells*	13 – 16 May	6	7.6 ± 0.6	-20.7 ± 0.9
2008	*Red Blood Cells*	8 – 27 May	28	9.7 ± 0.3	-21.3 ± 0.3
		28 May – 16 June	7	7.2 ± 0.3	-20.4 ± 0.4
		17 June – 3 July	8	5.8 ± 0.3	-20.5 ± 0.3

Table 3. $\delta^{13}C$ and $\delta^{15}N$ isotope values (mean ± SE) in egg constituents (albumen and yolk protein) of Lesser Scaup, 2006-2008, Red Rock Lakes National Wildlife Refuge, Montana, USA.

Year	**Tissue**	***n***	**$\delta^{15}N$**	**$\delta^{13}C$**
2006	*Albumen*	19	5.7 ± 0.1	-23.3 ± 0.4
	Yolk Protein	20	6.2 ± 0.1	-24.7 ± 0.4
2007	*Albumen*	6	7.0 ± 0.2	-20.7 ± 0.2
	Yolk Protein	6	8.3 ± 0.2	-21.3 ± 0.5
2008	*Albumen*	28	5.7 ± 0.1	-21.2 ± 0.4
	Yolk Protein	28	6.7 ± 0.5	-22.3 ± 0.4

Table 4. Estimated mean and standard error contribution of egg nutrients allocated from endogenous body reserves (acquired prior to arrival to the breeding grounds), local invertebrates and local wetland seeds by year, determined by stable isotope analysis of lesser scaup, accounting for discrimination values from Hobson (1995). Red blood cell samples were grouped upon arrival to the breeding grounds as either migrating from marine or freshwater habitats, and subsequent relative contributions were calculated for each habitat.

Freshwater - Egg nutrients	**2006**		**2007**		**2008**	
and sources	Mean	1 SE	Mean	1 SE	Mean	1 SE
Yolk Protein						
Endogenous Reserves	0.02	0.06	0.26	0.10	0.09	0.06
Invertebrates	0.43	0.11	0.74	0.21	0.81	0.12
Seeds	0.55	0.09	0	0.14	0.10	0.10
Albumen						
Endogenous Reserves	0	0.07	0.11	0.09	0	0.07
Invertebrates	0.41	0.13	0.89	0.10	0.68	0.11
Seeds	0.59	0.11	0	0.15	0.32	0.14

Marine - Egg nutrients	**2006**		**2007**		**2008**	
and sources	Mean	1 SE	Mean	1 SE	Mean	1 SE
Yolk Protein						
Endogenous Reserves	0.03	0.05	0.35	0.10	0.06	0.05
Invertebrates	0.41	0.11	0.57	0.21	0.75	0.16
Seeds	0.56	0.10	0.08	0.14	0.19	0.13
Albumen						
Endogenous Reserves	0	0.04	0.09	0.09	0	0.05
Invertebrates	0.41	0.12	0.80	0.10	0.69	0.17
Seeds	0.59	0.10	0.11	0.15	0.31	0.13

CONTRIBUTIONS OF ENDOGENOUS LIPIDS TO EGGS OF LESSER SCAUP

Introduction

Bird migration is one of the most energetically demanding activities a bird faces (Witter and Cuthill 1993). Higher energy costs are associated with carrying larger fuel loads. Energy cost per unit of flight distance increases sharply with an increase in fuel load (Kullberg et al. 2005). The wintering area for many bird species is often several thousand kilometers from their breeding grounds so lipid stores destined for reproduction may be better acquired closer to the breeding grounds (Klassen et al. 2006). Thus, nutrient reserves acquired from foods consumed on the spring staging areas can influence reproductive performance of birds (Guillemette 2001). Therefore, birds must meet these challenges by balancing the need to fuel migration and the high costs of reproduction.

Traditionally, nutrient dynamic studies have focused on a continuum to describe where nutrients were derived for breeding activities (Drent and Daan 1980). Income breeders, like shorebirds and songbirds, are at one end of the continuum relying primarily on local dietary sources found at the breeding grounds for breeding activities (Klaassen et al. 2001, Langin et al. 2006). Capital breeders, like emperor geese (*Anser canagicus*), are at the other end of the spectrum and rely heavily on stored body tissues acquired prior to arrival on the breeding area (Schmutz et al. 2006).

Birds that acquire lipid stores during spring migration (capital breeders) likely have a different strategy to fuel reproduction than do birds that acquire lipids stores on the breeding grounds (income breeders). In some waterfowl species, a body condition

threshold is likely necessary prior to breeding (Reynolds 1972). Waterfowl individuals in good body condition are capable of having larger clutches, nesting earlier, greater reproductive effort, and rely on larger endogenous reserves than do females in poorer body condition (Rotella et al. 2003, Arnold et al. 2002). If a female has lost substantial endogenous reserves as a result of migration, she will have to select certain food items, either high in protein, lipid, or carbohydrate, while on the breeding grounds in order to regain lost body reserves to insure they initiate egg-laying early in the nesting season (Drent 2006).

Egg formation is an energetically demanding process (Nager 2006). The total mass of a females clutch can be a significant proportion (and in some cases even exceed) the total body mass of the laying female (Perrins 1996, and Nager 2006). Alisauskas and Ankney (1992) estimated the average daily energy expenditure for North American waterfowl during egg-laying is 160% of basal metabolic rate.

Lesser scaup (*Aythya affinis*, hereafter scaup), a species that has been declining for nearly three decades (U.S. Fish and Wildlife Service 2008), feeds on invertebrates (Rogers and Korschgen 1966) and emergent wetland seeds (Smith 2007, Strand et al. 2007, Afton and Hier 1991) and is believed to be limited by foods high in lipid content, especially during the prebreeding season (Gray 1980, Woodin and Swanson 1989). Upon arrival on the breeding area in the springtime, protein-rich invertebrates may sometimes be scarce. In order to regain lost lipid reserves from migration that are necessary for egg-laying, it may be advantageous for scaup to select foods high in carbohydrates, such as wetland seeds, which provide better substrates for lipid synthesis than invertebrates

(Drobney 1991). Because female scaup likely need to exceed a body-condition threshold before they can undergo rapid follicular growth (Esler et al. 2001, DeVink 2008), and in some years confront challenges such as density dependence, food limitation, or severe weather, scaup may need to select foods high in either lipid or carbohydrate content which can be easily converted and used as energy for egg lipid formation.

Scaup exhibit one of the most protracted spring migrations of any North American duck species (Austin et. al 1998). Badzinski and Petrie (2006) showed that scaup increase lipid reserves during spring migration by an average of 1.2 g/day (although no change in body lipid was observed at one of the collection sites) of endogenous lipids while staging on the Great Lake. Esler et al. (2001) and Afton and Ankney (1991) reported that lesser scaup use two-thirds endogenous lipid reserves for clutch formation, which was assumed to have been acquired from spring staging areas. However, due to the protracted period scaup spend on the breeding ground prior to nesting in southerly latitudes (Afton 1984, USFWS 2009 unpublished data), scaup may also rely on local dietary sources to fuel egg lipid formation.

In recent years, stable isotopes have been used to track macronutrient acquisition by birds (Hobson 2006). Stable isotopes can be used to determine if endogenous reserves were acquired on spring stopovers, or local breeding area habitats if these regions differ isotopically in food web values (Hobson 2008). In such cases, isotopic measurements of abdominal lipid samples from birds upon arrival on the breeding grounds and from local dietary sources can be used to determine the contributions of habitats prior to arrival versus local dietary sources to egg formation.

Currently, the relative contributions of (a) endogenous reserves, acquired prior to the arrival on the breeding grounds, and of (b) local dietary sources, consumed while on the breeding grounds, are unknown for egg lipid formation in scaup. Therefore, for birds breeding in southwest Montana, I collected abdominal lipid samples from females when they arrived on the breeding grounds, local dietary food items found at the breeding grounds, and lipids from scaup egg yolks. Following the application of expected isotopic discrimination factors relating lipid sources to egg lipids, $\delta^{13}C$ values were then compared of female lipids and foods with those from yolk lipids to estimate the relative contributions of endogenous lipid reserves (acquired prior to arrival to the breeding grounds) and local dietary lipid sources.

Study Area

Red Rock Lakes National Wildlife Refuge (hereafter Refuge) is located in the Centennial Valley of southwest Montana. The Refuge encompasses approximately 10,000 ha of natural and created montane wetlands at an elevation of 2,015 m, providing reproductive and migratory habitat for a diverse waterbird community. The study was conducted on Lower Red Rock Lake and the River Marsh (Figure 6), a 2,332 ha montane wetland complex located within the Refuge. The complex is comprised of nearly equal areas of shallow (< 2 m) open-water and palustrine emergent vegetation habitats. The southwestern portion of the complex is predominantly open-water habitat with interspersed islands of hardstem bulrush (*Schoenoplectus acutus*). The north and east extent of the complex is palustrine emergent vegetation dominated by sedge (*Carex* spp.) with typically small (< 2 ha), scattered open-water areas. The average annual

precipitation is 49.5 cm, with 27% occurring during May and June. The mean annual temperature is 1.8º C. The high elevation of the Centennial Valley provides a narrow window of breeding opportunity for scaup that is similar in duration to that found in the western boreal forest. For example, the mean temperature for May and June, 1971-2000, are equivalent for Yellowknife, Northwest Territories ($x = 9.6^{o}$ C), and the Centennial Valley, Montana ($x = 9.3^{o}$ C, http:climate.weatheroffice.ec.gc.ca, http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?mtlake). Furthermore, Lower Red Rock Lake supports one of the highest density populations of breeding lesser scaup in North America (> 20 pairs/mi^2), exceeded only by areas such as Yukon and Old Crow Flats (30 and 34 pairs/mi^2, respectively) in Alaska and Yukon, respectively (Bellrose 1980). Due to the close proximity of Lower Red Rock Lake to their wintering areas, and lower total energy needed to fuel migration, offers a feasible opportunity to study cross-seasonal effects for egg formation in lesser scaup.

Methods

Female scaup were captured and abdominal lipid samples were collected (*n*=12) using biopsy shortly after they arrived on the study area to determine the isotopic values of lipids obtained on spring migration areas. Local dietary items (*n*=25) were collected (throughout the study area during the pre-breeding and egg laying periods until early-July) to determine isotopic values of scaup foods on the local study site. Once nest initiation occurred, nest searches were conducted and eggs (*n*=53) were collected from discovered nests. After tissue samples were collected, stable-carbon (δ^{13}C) isotope signatures of each type of material collected were determined and analyzed to estimate

the percent contribution of body tissues from spring migration and local dietary sources to egg lipid formation. Spring migration was defined as the period between departure from the wintering grounds and arrival on the breeding areas. Pre-breeding was defined as the period between a female's arrival on the breeding area and nest-initiation (Stephens et al. 2009).

Field Methods

Adult female lesser scaup were captured via night lighting on wetlands during the pre-breeding period in May of 2008 (Lindmeier and Jessen 1961). Each female was banded with a U.S. Geological Survey aluminum leg band. Abdominal lipid biopsies were collected from females for $\delta^{13}C$ analysis and considered as representative of endogenous lipid values from spring migration areas. Lipid biopsies were collected by parting feathers using isopropyl alcohol in the abdominal region where lipid depots were found. The site was than prepped with Novasan® Disinfectant solution. Using sterilized (via autoclave) surgical instruments the skin was lifted with forceps and a small (< 10 mm) incision in the skin was made with a surgical scalpel. A quantity of approximately 30mg of lipid in the region of the incision was removed with forceps. Bleeding was stemmed with direct pressure with sterile gauze and the incision site was sealed with suture and vet grade adhesive (VetBond). Lipid samples were stored frozen until stable isotope analysis.

Endogenous lipids were sampled from females only in 2008. Therefore, I made the assumption that these lipid samples were representative of the endogenous isotope values among years. Females (n=12) were captured immediately after open water

became available on the study site during 9-13 May, 2008 to ensure samples represented lipid reserves accumulated prior to arriving on the breeding area. Abdominal fat samples were stored frozen until stable isotope analysis could be conducted. Two lipid samples (1 muscle and 1 abdominal) collected from a radio-marked female who was recovered dead, on 16 June 2008 were also analyzed opportunistically to assess the endogenous isotope values for $\delta^{13}C$ after foraging on local food sources for approximately 1 month. Capture and sampling protocols were conducted under approved federal, state, and animal welfare permits.

To obtain egg samples, nests were located using radio telemetry (in 2007; see Chapter 2), trained dogs, foot searches, and behavioral observations of female scaup by searching in sedge-dominated habitats. One egg was collected at random from each nest found and nest initiation date was estimated by field-candling eggs (Weller 1956). Eggs were hardboiled to easily separate yolk and albumen from each other, and samples were stored frozen until stable isotope analysis was conducted (Gloutney and Hobson 1998).

Because lesser scaup eat invertebrates (Rogers and Korschgen 1966) and emergent wetland seeds (Smith 2007, Strand et al. 2007), both plant and animal material were collected as possible scaup food items during the pre-breeding and egg laying periods. Invertebrates were collected at the study area during the pre-breeding and egg laying periods of 2007 and 2008 via sweep sampling using a D-shaped dip net (1,200 µm mesh, 0.072 m^2 opening, WARD's Natural Science, Rochester, New York). Collected invertebrates included: amphipods (*Gammarus*), leeches (*Hirudinea*), snails (*Gastropoda)* and water boatman (*Hemiptera*). Hardstem bulrush (*Schoenoplectus*

acutus) and sedge seeds (*Carex* spp.) were also collected at the study area during the pre-breeding and egg laying periods for stable isotope analysis by finding intact seed heads that were from the previous year's growth. Invertebrates and wetland seeds were stored frozen until stable isotope analysis was conducted.

Weather Measurements

Precipitation and temperature data were used to assess annual variations collected by the Natural Resource Conservation Service (NRCS) at a nearby Snotel site at an elevation of 2428 m and ~2 km south of the study site (http://www.wcc.nrcs.usda .gov/snotel/snotel.pl?sitenum=568&state=mt). Climatic data were collected every 3 hours. All Snotel precipitation and temperature data are available in the NRCS archival dataset. Water levels at the western outflow of Lower Red Rock Lake were measured throughout the nesting season and mean water level was summarized between the first to third quartile of the nest initiation period for a given year.

Isotopic Analysis

Abdominal lipids were weighted (~1 mg), and encapsulated into tin capsules for stable isotope analysis via mass spectroscopy. Invertebrate samples were washed with distilled water and freeze dried. Samples were then powdered. Lipids were removed using a 2:1 chloroform/methanol solution and dried in a fume hood. Stable isotope values were reported in parts per thousand (‰) relative to the standards Vienna PeeDee Belemnite for $\delta^{13}C$. Measurement precision of within run standards for $\delta^{13}C$ was ± 0.1‰.

Yolk lipid $\delta^{13}C$ values were determined to quantify the contributions of endogenous and local dietary sources. An aliquot of yolk lipid was collected from each egg from the center concentric ring of the yolk. Egg yolks were lipid extracted twice using a 2:1 chloroform/methanol solution to ensure that most lipids had been removed (Ricca et al. 2007). Egg, invertebrate, and seed samples were weighed (~1 mg), encapsulated in tin capsules, and analyzed for stable-carbon ($\delta^{13}C$) isotope using continuous flow isotope-ratio mass spectrometry as described by Hobson (2005).

Stable isotope values in eggs differ in a predictable manner from the isotope values in the female's diet and endogenous reserves, a result of so-called isotopic discrimination. Accordingly, discrimination values that correct for this difference were used to adjust values obtained for diet and endogenous body tissues. Discrimination values from diet to egg have been experimentally determined (Hobson 1995), but values have not been worked out yet for the endogenous reserves to egg transition. Researchers instead have used isotopic discrimination factors associated with the carnivore model of Hobson (1995) as a proxy for the transfer of endogenous reserves to eggs (Gauthier et al. 2003, Schmutz et al. 2006, and Bond et al. 2007). Discrimination between invertebrate and endogenous lipids to yolk lipid formation was assumed to be 0.0‰ for $\delta^{13}C$. For the $\delta^{13}C$ discrimination value associated with conversion of plant carbohydrates to yolk lipid I used the -2.6‰ value of the herbivore model from Hobson (1995)

Data Analysis

Body tissues, wetland seeds, and invertebrates were considered as source endpoints in an isotope mixing model in order to calculate contributions of these sources

to egg lipid formation. Annual variation of the estimated contribution from each source to egg formation was tested by comparing confidence intervals (90 and 95% CIs were both evaluated). Later, model selection was used to further assess relationships that could help explain variation in $\delta^{13}C$ values in egg lipids. It was assumed that scaup could assimilate either wetland seed or invertebrate tissues after arrival on the breeding grounds and prior to nest initiation. Since endogenous and invertebrate lipid tissues were similar in $\delta^{13}C$ (Endogenous reserves; $\delta^{13}C$ value -22.8 ± 0.7‰ (SE), and invertebrate lipid; $\delta^{13}C$ value -22.7 ± 0.4‰ (SE)), the contribution of each source could not be distinguished to egg lipid formation. Therefore, endogenous and invertebrate lipid tissue $\delta^{13}C$ signatures were combined as one source in the isotope mixing model. This resulted in a two-source one-isotope mixing model (Phillips and Gregg 2001). This mixing model accounts for variation in isotopic signatures of endogenous tissues, local dietary items, and egg lipid signatures. Mean $\delta^{13}C$ ratios were calculated for abdominal lipids, egg lipids, and local invertebrate lipids and wetland seeds.

Linear regression was used to evaluate competing models that contained various combinations of nest initiation date, clutch size, and year (excluding 2007) to attempt to explain variation in egg lipid $\delta^{13}C$ values. A set of models were evaluated with combinations of main effects and 2-way interactions. Data from 2007 was excluded because of a small sample size (n = 6) and because all samples were collected during the first quarter of the nesting season (11-16 June). A correlation was tested between full clutch and Julian date prior to analysis to detect possible multicollinearity among variables.

Akaike's Information Criterion adjusted for sample size (AICc, Akaike 1973, Burnham & Anderson 1998) was used to evaluate the amount of support that the data provided for each model in my *a priori* model set. All statistical analyses were conducted using R 2.8.1 (R Development Core Team 2009). Prior to analysis the data were tested for homogeneity of variance and normality of the residuals and confirmed to meet the assumptions of linear regression.

Results

Mean temperature and precipitation from April-June varied among years with the warmest and driest year occurring in 2007. While 2006 and 2008 were "average" years, 2007 was the warmest since 1991 (when temperature data were first recorded at the nearby Snotel site) and driest since 1985 (April-June precipitation = 6.6 cm) at 8º C and 10.4 cm, respectively. Average precipitation from April-June in 2006 and 2008 was 25.4 cm and 23.1 cm, respectively. Average temperature from April-June in 2006 and 2008 was 7.3º C and 4.7º C, respectively. The difference in mean water level of Lower Red Rock Lake during the first and third quartile of the nesting season for scaup in 2007 (SE = 0.03) was lower than the mean water level in 2006 (0.66 ± 0.03m SE) and 2008 (0.39 ± 0.01m SE). As a consequence of the dry year in 2007, most ponds surrounding the study area were dry by mid-June. Conversely, in 2006 and 2008 all of the ponds surrounding the study area were full of water throughout the prebreeding and egg laying periods.

A total of 12 abdominal lipid samples were collected from female lesser scaup in 2008 after arrival on the breeding grounds. The isotopic endpoints of the local food web was estimated from 25 invertebrate lipid samples (4 genera) in 2007 and 2008 on 21

locations, and 5 wetland seed samples (2 genera) in 2006 and 2007 on 5 locations (Figure 6). A total of 53 eggs from 53 nests were collected during the 3-year study (20 in 2006, 6 in 2007, and 27 in 2008). A subset of 40 eggs from 2006 and 2008 (excluding 2007) was used to examine the relative importance of full clutch, nest initiation date, and year. The subset of eggs was from nests where full clutch size was determined (some nests were destroyed or abandoned prior to full clutch size determination). A correlation between full clutch and Julian date was determined to be slight (r = -0.02).

Stable-Isotope Ratios ($\delta^{13}C$) in Arrival Abdominal Lipids, Egg Yolk Lipids, and Local Dietary Invertebrates and Wetland Seeds

Endogenous lipids of female scaup upon arrival on the breeding area had a mean $\delta^{13}C$ value of -22.8 ± 0.7‰ (SE) in 2008. Invertebrate lipid values were almost identical to those for endogenous lipids and invertebrate lipids did not differ between years (P = 0.56; -22.5 ± 0.5‰ SE in 2007, and –23.0 ± 0.8‰ SE in 2008). In contrast, the overall mean $\delta^{13}C$ value of wetland seeds (-29.4 ± 0.3‰, when adjusted for discrimination by -2.6‰ which allows the prediction of egg lipid values formed from seeds) was significantly more negative than endogenous and invertebrate lipid signatures (P < 0.001). The egg lipid $\delta^{13}C$ values for the three years of the study fell between values for (1) endogenous and invertebrate lipids, and (2) wetland seeds (Figure 7, Table 5 and 6). Mean value for $\delta^{13}C$ in egg lipids was -26.2 ± 0.3‰ SE across the three years (-26.9 ± 0.4‰ SE in 2006, -25.2 ± 0.4‰ SE in 2007, and -25.9 ± 0.4‰ SE in 2008).

Relative Contribution of Female Abdominal Lipids
and Dietary Invertebrates and Seeds to Egg Lipid Formation

Since endogenous and invertebrate lipids were collected in 2008, and 2007 and 2008, respectively, it was assumed the $\delta^{13}C$ values of these tissues were representative among years when tissues were not collected. Based on my results from the isotope mixing model, there was evidence of annual variation in source contributions to egg lipid formation among years (Figure 7, Table 7). However, because of uncertainty about the estimates, strong conclusions about annual variation cannot be made. For each of the sources used in egg lipid formation, 95% confidence interval limits on the estimated contributions from each source overlapped in all three years (Table 7). However, when 90% confidence intervals were examined, there was evidence of annual variation between sources used in egg lipid formation. In 2006, wetland seeds contributed slightly more than did the contribution of the combined endogenous and invertebrate lipid source (90% CI = 51 to 75%, and 25 to 49%, respectively). In 2007, the opposite effect was observed where wetland seeds contributed less to egg lipid formation than did the combined endogenous and invertebrate lipid source (90% CI = 24 to 50%, and 50 to 76%, respectively).

Regardless of possible annual variation, the results indicate that each source played a relatively important role in egg lipid formation as each source contributed at least 21% (lowest value of all 95% lower confidence limits) of the total macronutrients used for egg lipid formation in each year (Table 7).

Examining the Relative Importance of Nest Initiation Date, Clutch Size, and Year to Egg Lipid Formation

Covariates were analyzed that could explain variation in $\delta^{13}C$ of egg lipid values. The $\delta^{13}C$ values from 40 eggs collected from 40 nests in 2 different years (2006 and 2008) came from full clutches that ranged in size from 5 to 11 eggs (mean = 7.7, SE = 0.3) and that were initiated from 21 May through 7 July (mean = 20 June , SE = 1.6 days). Among the models that attempted to explain variation in $\delta^{13}C$ values of egg lipids, a model that allowed $\delta^{13}C$ to vary by year but not by clutch size or initiation date was the top-ranked model (Table 8). The most parsimonious model estimated the egg lipid $\delta^{13}C$ values for 2006 and 2008 as -26.9 ± 0.5‰ SE and -25.7 ± 0.4‰ SE, respectively. The 95% confidence intervals for annual signature estimates from the top model slightly overlapped (95% CI = -26.0‰ to -27.8‰ in 2006, and -24.8‰ to -26.5‰ in 2008). The second best-supported model was an intercept-only model (mean of the $\delta^{13}C$ egg lipid signature for both years = -26.2 ± 0.3‰ SE), which received more support ($\Delta AICc$ = 1.78) from the data than did other models containing full clutch, Julian date, additive, or interactive models ($\Delta AIC_c \geq 2.42$). In the best models containing full clutch or Julian date, the estimated coefficients overlapped zero (0.15 ± 0.19 SE and 0.19 ± 0.33 SE, respectively), and the proportion of variation explained by these models was only modestly better than that of the top model (Table 8).

Discussion

This study was the first to show evidence of possible inter-annual variability in nutrient allocations of female lesser scaup. Inter-annual variability was observed at the

90% confidence interval for the role of endogenous and invertebrate lipids to egg lipid formation. In this study endogenous and invertebrate lipids contributed on average between 37 - 63% of the total macronutrients used for egg lipid production. If the $\delta^{13}C$ values in abdominal and muscle lipid sample collected from 1 female during peak nest initiation was compared to the $\delta^{13}C$ values in egg lipids among the years of the study, this would indicate that almost all egg lipids were derived from local dietary lipid sources (assuming the endogenous lipid from this female represented the local dietary lipid isotope value because of foraging on local foods for approximately 1 month). Since arrival lipids had higher $\delta^{13}C$ values compared with $\delta^{13}C$values in egg lipids, local dietary sources were consumed in order to have created the observed lower $\delta^{13}C$ egg lipid values.

There was evidence of plasticity in the way macronutrients are allocated for egg lipid formation between years. The top model contained year which best described $\delta^{13}C$ values in egg yolk lipids. Therefore, scaup may be able to adjust their breeding strategy based on certain variables they encounter between years. The amount of time lapsed after arrival to the breeding grounds until nest initiation likely dictates the degree to which endogenous reserves, acquired at stopovers during spring migration, are used for egg lipid formation (Klaassen et al. 2006). Although I do not have exact arrival dates of female scaup to the study area for every year, arrival dates likely varied among years, which may help explain variation in nutrient allocation strategies. The first unmarked scaup observed during the pre-breeding season in 2006 and 2007 was in early April (2006; 6 April, and 2007; 7 April) (J. Warren, U.S. Fish and Wildlife Service, personal communication). Although arrival dates of resident breeding scaup were likely later in

spring. Six radio-marked scaup marked in 2007 arrived at my study site between 6 and 7 May, 2008. Therefore, scaup in 2008 spent almost 5 weeks consuming local dietary carbohydrates and lipids before the first nest was discovered on 11 June.

A significant relationship was not found between $\delta^{13}C$ values in yolk lipids and clutch size. Although clutch sizes of waterfowl have been shown to decline during the breeding season which is correlated to the reduction in endogenous reserves within the female's body (Alisauskas and Ankney 1992). Furthermore, Esler et al. (2001) showed a consistent proportion of endogenous reserves used in egg formation throughout the breeding season. Results from the current study corroborate this finding in which there was no evidence for a decline in endogenous lipid contribution to egg lipid production throughout the breeding season. Although the contribution of endogenous and invertebrate lipid to egg lipid formation was confounded, a change in the contribution of endogenous reserves to egg formation throughout the nesting season cannot be ruled out.

Lower estimates were found of endogenous reserves to egg lipid formation compared to the studies of Esler et al. (2001) and Afton and Ankney (1991). In the two previous studies, scaup allocated approximately two-thirds endogenous lipids to egg lipid formation. I found that scaup mobilize on average between 37-63% combined endogenous and invertebrate lipid to egg formation. Recent studies have shown the importance of wetland seeds in diets of lesser scaup during spring migration and pre-breeding season (Afton and Hier 1991, Smith 2007, and Strand et al. 2007). Wetland seeds that are high in carbohydrates were acquired during pre-breeding and egg laying and were subsequently used for egg lipid formation. Scaup relied on seeds, which are an

energy-rich food source, during the course of the three-year study. These results may have significant implications to the current understanding of how scaup allocate energy for egg lipid production. Furthermore, these results appear to be an otherwise un-described strategy for scaup that shifted, from an assumed protein-rich invertebrate diet, to a diet based on wetland seeds in order to augment their ability to synthesize carbohydrates more efficiently for the use in egg lipid production.

Possible mechanisms that could explain this shift in diet include: food quality and availability, weather, density dependence, and high elevation of my study site. Gurney et al. (in review) found that clutch size and the number of growing degree days at my study site were the smallest and shortest (average of 1.3 eggs less per clutch, and average of 77 growing degree days less), respectively, than seven other breeding areas spanning across North America. Consequently, due to one of the highest breeding densities of scaup in North America and high elevation of my study site (Bellrose 1980, USFWS unpublished data 2009), may increase competition during prebreeding and reduce food availability which may provide some basic understanding into why this population used local plant carbohydrates for egg lipid formation. The use of carbohydrate-rich wetland seeds to increase the rate of lipid accumulation for egg lipid production is evidence of a flexible strategy that scaup used to alter their otherwise carnivorous behavior during a period after arrival on the breeding grounds when either protein-rich invertebrates or food items high in lipid content may still be relatively scarce (Swanson et al. 1974, Gray 1980, Woodin and Swanson 1989).

This study was largely based on nutrient-allocation strategies at a population level. Although individuals may allocate nutrients differently for egg lipid formation, sample size were too small to test for differences at an intra-specific level. The primary objective of this study was to address geographic differences between endogenous lipids acquired during spring migration versus endogenous lipids acquired while on the breeding grounds that were subsequently used for egg lipid formation. I found evidence of plasticity for nutrient allocation strategies between these two sources for egg lipid formation.

Nutrient allocation strategies of lesser scaup may differ depending on latitude of the breeding area. The mean growing season length is generally shorter in northerly latitudes than in southerly latitudes (Smith et al. 2004), likely influencing spring phenology of food availability consumed by lesser scaup. Scaup may be limited by fewer constants in southerly latitudes due to a more constant environment than scaup in northerly latitudes. Therefore, the availability of wetland seeds on breeding habitats in southerly latitudes could be a reliable source of nutrients for egg production that may not otherwise be available for breeding scaup in northerly latitudes. By understanding which season most limits nutrient acquisition for egg lipid production (e.g. spring migration or breeding area habitats), managers can focus their efforts to that particular habitat in order to maximize habitat quality for breeding female scaup (Hobson et al. 2005). Furthermore, management strategies aimed at conserving or enhancing wetland habitats will differ depending on sites of investment of endogenous reserves used in egg lipid formation. Wildlife managers in southern latitudes of the breeding range of lesser scaup

should not underestimate the importance of breeding habitats as a primary source of nutrients for clutch formation. Finally, future comparative studies of lesser scaup are encouraged that allow the role of variation in arrival dates on the breeding grounds and the lengths of the pre-breeding season, in turn mediated by latitude, can be investigated.

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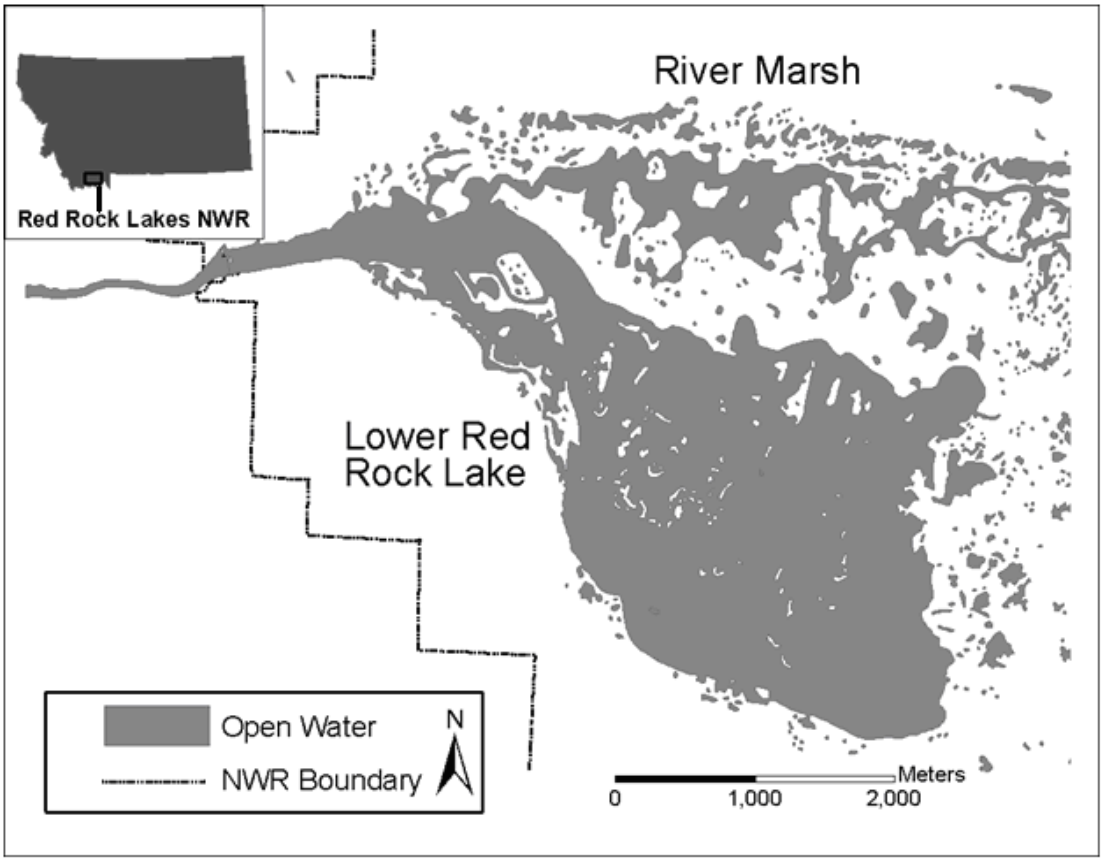

Figure 6. Location of the study area on Lower Red Rock Lake and River Marsh within Red Rock Lakes National Wildlife Refuge, Montana, USA. Inset shows the location of the study area in Montana, USA.

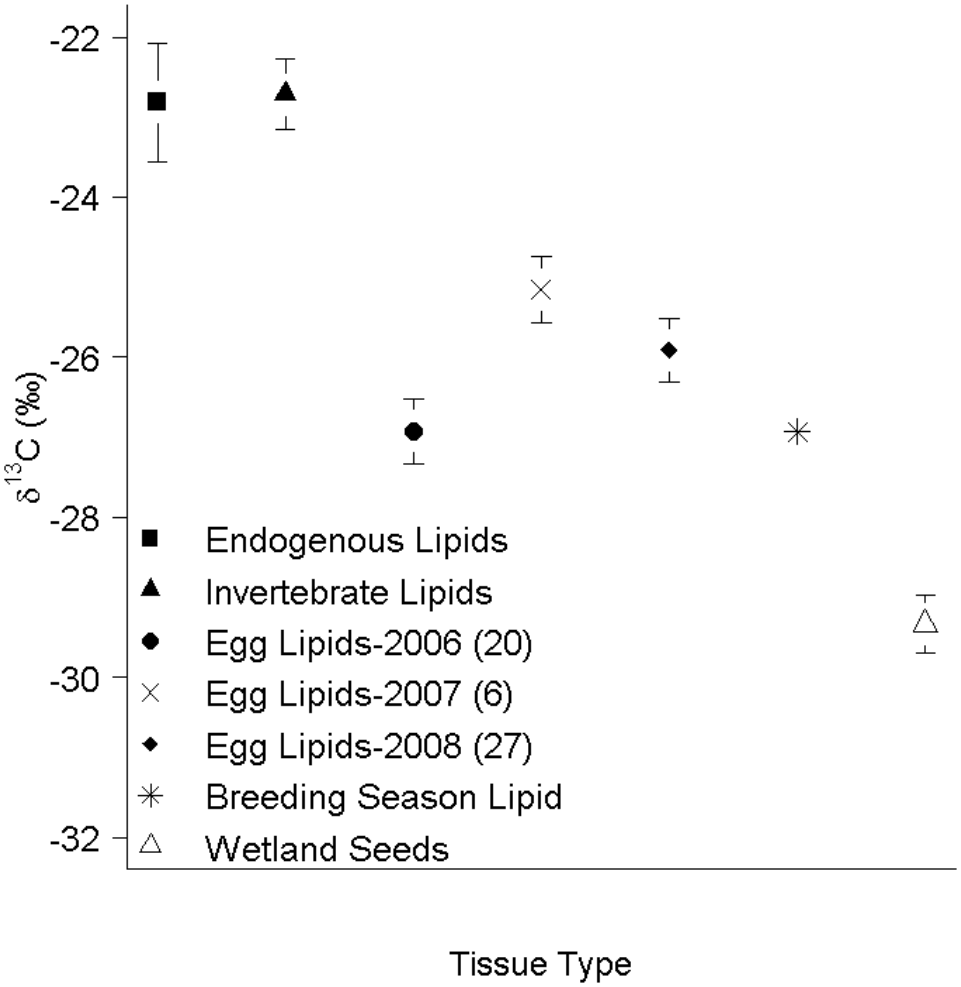

Figure 7. Predicted $\delta^{13}C$ values (mean $\pm$ 1SE) of arrival body lipids, invertebrates and wetland seeds if egg lipids of lesser scaup were derived entirely from any of these sources during the pre-breeding and egg-laying periods, 2006-2008, Red Rock Lakes National Wildlife Refuge, Montana, USA. Breeding season lipid was from 1 female located dead on 16 June, 2008. Sources were adjusted for discrimination such that $\delta^{13}C$ values of wetland seed were adjusted by -2.6‰ while $\delta^{13}C$ values of endogenous and invertebrate lipid were adjusted for discrimination by 0.0‰ (Gauthier et al. 2003).

Table 5. Stable-isotope values (means ± SD [*n*]) for wetland invertebrate lipid and seed resources available to lesser scaup on the breeding grounds. All material collected at Red Rock Lakes National Wildlife Refuge, U.S.A.

Organism[a]	$\delta^{13}C$ (‰)	Year of collection
	Invertebrate	
Amphipods	-23.4 ± 2.0 (13)	2007-2008
Snails	-22.7 ± 0.6 (4)	2007
Leech	-23.1 ± 3.9 (3)	2007-2008
Waterboatman	-20.8 ± 1.9 (5)	2007-2008
All invertebrate groups	**-22.7 ± 2.6 (25)**	
	Seed	
Sedge	-26.7 ± 0.8 (4)	2007-2008
Bulrush	-26.9 (1)	2008
All seed groups	**-26.8 ± 0.7 (5)**	

[a]Scientific names: amphipods *Gammarus* spp., snails Gastropoda, leech Hirudinea, water boatman Hemiptera, sedge *Carex* spp., bulrush *Schoenoplectus acutus*..

Table 6. $\delta^{13}C$ values for egg yolk lipids from lesser scaup, Red Rock Lakes National Wildlife Refuge, Montana, USA, 2006-2008.

Year	**Tissue**	***n***	**$\delta^{13}C$**	**SE**
2006	Yolk Lipid	20	-26.9	0.4
2007	Yolk Lipid	6	-25.2	0.4
2008	Yolk Lipid	27	-25.9	0.4

Table 7. The relative contribution of lesser scaup endogenous and invertebrate lipids versus the contribution of wetland seeds to egg lipid production determined by $\delta^{13}C$ isotope analysis. Discrimination values were applied to endogenous and invertebrate lipids and wetland seeds (Gauthier et al. 2003). Calculations were based on using one isotope ($\delta^{13}C$) from a linear mixing model of Phillips and Gregg 2001.

	Endogenous and Invertebrate Lipids					**Local Wetland Seeds**				
Year	**mean (se)**	**95%lcl**	**90%lcl**	**90%ucl**	**95%ucl**	**mean (se)**	**95%lcl**	**90%lcl**	**90%ucl**	**95%ucl**
2006	0.37 (0.07)	0.23	0.25	0.49	0.51	0.63 (0.07)	0.49	0.51	0.75	0.77
2007	0.63 (0.08)	0.47	0.50	0.76	0.79	0.37 (0.08)	0.21	0.24	0.50	0.53
2008	0.52 (0.07)	0.38	0.40	0.64	0.66	0.48 (0.07)	0.34	0.36	0.60	0.62

Table 8. Model-selection results for a candidate model set attempting to explain variation in $\delta^{13}C$ values of egg yolk lipid of female lesser scaup (*Aythya affinis*), in 2006 and 2008 at Red Rock Lakes National Wildlife Refuge, Montana, USA. The model with the smallest AIC_c value is considered best (Burnham and Anderson 1998). AIC_c (the model selection criteria), Δ AIC_c (differences in AIC_c between the top model and each subsequent model), w_i ("weight of evidence" in favor of each model), K (number of parameters estimated), and r^2 for each model. Combination of variables that were used to model $\delta^{13}C$ values in egg yolk lipids include: full clutch (total number of egg in clutch when incubation occurs), year (2006 and 2008), and Julian date (when nest initiation occurred).

Covariates in Model	K	AIC_c	Δ AIC_c	w_i	r^2
Year	3	166.77	0.00	0.41	0.10
Null	2	168.55	1.78	0.17	-
Full Clutch + Year	4	169.19	2.42	0.12	0.10
Julian date + Year	4	169.22	2.45	0.12	0.10
Full Clutch	3	170.24	3.47	0.07	0.02
Julian date	3	170.52	3.75	0.06	0.01
Julian date * Year	5	171.63	4.85	0.04	0.10
Julian date + Full Clutch	4	172.20	5.42	0.03	0.03
Julian date * Full Clutch	5	173.90	7.12	0.01	0.05

CONCLUSION

Historically, techniques used to assess nutrient allocation strategies in waterfowl have been unable to distinguish endogenous reserves acquired on spring staging areas from exogenous foods acquired while on the breeding grounds (Afton and Ankney 1991, Esler et al. 2001). This has led to the overestimation of the true role of endogenous reserves in egg formation (Hobson 2006). The overall objective was to determine the geographic origin of nutrients used for egg formation. Here, seasonal, population level, patterns of $\delta^{13}C$ and $\delta^{15}N$ values in red blood cells (RBC) of lesser scaup were modeled to determine how these values responded to a dietary shift after consuming foods found on the breeding grounds (Chapter 2). I presented results assessing nutrient allocation strategies in egg protein (Chapter 2) and lipid formation (Chapter 3). The importance of nest initiation date, clutch size, and year (excluding 2007) was also examined in models designed to explain variation in $\delta^{13}C$ values of egg lipids (Chapter 3). Finally, I compared intraspecific nutrient strategies to assess if individuals used a similar nutrient allocation strategy for egg formation (Chapter 2).

From arrival through the egg laying period, $\delta^{15}N$ values of RBC decreased while $\delta^{13}C$ values increased and became less variable, a pattern consistent with expectations of endogenous tissues equilibrating with local dietary sources (Chapter 2). In agreement with my predictions, in 2006 and 2008, isotopic values for egg albumen and yolk protein were similar to those expected from local dietary sources, which indicated that most protein used for producing egg albumen and yolk protein were obtained on the breeding grounds (exogenous sources). The relative contribution of endogenous reserves for egg

protein formation at my study site was between 2% (SE = 6%) to 35% (SE = 10%) for yolk protein formation and 0% (SE = 7%) to 11% (SE = 9%) for albumen production (Chapter 1). The role of endogenous reserves for egg protein formation corroborate past nutrient dynamic studies of lesser scaup (Afton and Ankney 1991 and Esler et al. 2001).

Due to small differences in $\delta^{13}C$ values between female abdominal lipids upon arrival to the breeding grounds and those of local invertebrate lipids, it was not possible to separately estimate the contributions of endogenous and exogenous (invertebrate) lipids. My results suggest that local invertebrates and/or endogenous lipid reserves contributed on average 51% (SE = 7%) to egg lipid production and that the importance of these two sources may have varied by year (90% Confidence Interval = 25 to 49% in 2006, 50 to 76% in 2007, and 40 to 64% in 2008). The remaining contributions to eggs were derived from local seed sources (-29.4 ± 0.7‰ SD $\delta^{13}C$ value). A model that allowed $\delta^{13}C$ values to vary by year, but not by clutch size or initiation date was the top-ranked model. Based on estimates from my top-ranked model, $\delta^{13}C$ egg lipid values from 2006 and 2008 were -26.9 ± 0.5‰ (SE) and -25.7 ± 0.4‰, respectively. However to thoroughly assess annual variation in sources to egg lipid formation, it would be good to have a larger sample size from more years and to have samples of eggs, body tissues, and foods coming from each year so that more thorough analyses could be done.

Finally, body tissues with differing turnover rates were used to explore intraspecific nutrient allocation strategies in female lesser scaup. Results show that female scaup used at least two habitats during spring migration for storing endogenous proteins prior to arrival on the breeding grounds (Chapter 2).

This was the first study of lesser scaup to: 1) assess nutrient allocation strategies using stable isotope techniques, 2) address the exact geographic location of macronutrients acquisition, and 3) assess, to a limited extent, inter-annual nutrient strategies. Despite recent findings of reduced body reserves during spring migration, the amount of time spent on the breeding grounds prior to nest initiation across North America may be long enough to allow female scaup to regain lost body reserves from migration, assuming ample food resources are present. My study highlights the flexibility on nutrient allocation strategies to breeding by scaup and the importance of breeding area food sources as a primary source for macronutrients in egg formation. Finally, this study addressed differences between endogenous body reserves acquired during spring migration and endogenous acquired on the breeding grounds.

Future nutrient dynamic studies may want to consider the use of additional techniques to complement the use of stable isotopes. For example, fatty acid analysis may provide an alternative way to track nutrients for egg production (McWilliams et al. 2004). Furthermore, plasma metabolites could be used to assess fat deposition, fat catabolism, and protein catabolism of scaup on spring staging and the breeding grounds (Smith and McWilliams 2010, Anteau and Afton 2008, Williams et al. 2007). A combination of techniques may provide a comprehensive measure of habitat quality and productivity on staging and/or breeding grounds.

Nutrient allocation strategies of lesser scaup may differ depending on latitude of the breeding area. The mean growing season length is shorter in northerly latitudes than in southerly latitudes (Smith et al. 2004), likely influencing spring phenology of food

availability consumed by lesser scaup. Scaup may be limited by fewer constants in southerly latitudes due to a more constant environment than scaup in northerly latitudes. Therefore, the availability of wetland seeds on breeding habitats in southerly latitudes could be a source of nutrients for egg production that may not be available for breeding scaup in northerly latitudes. By understanding which season most limits nutrient acquisition for egg lipid production (e.g. spring migration or breeding area habitats), managers can focus their efforts to that particular habitat in order to maximize habitat quality for breeding female scaup (Hobson et al. 2005). Wildlife managers in southern latitudes of the breeding range of lesser scaup should not underestimate the importance of breeding habitats as a primary source of nutrients for clutch formation. Future comparative studies of lesser scaup are encouraged to allow the role of variation in arrival dates on the breeding grounds and the lengths of the pre-breeding season, in turn mediated by latitude, can be investigated.

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