

Endogenous contributions to egg protein formation in lesser scaup *Aythya affinis*

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Lesser scaup *Aythya affinis* populations have declined throughout the North American continent for the last three decades. It has been hypothesized that the loss and degradation of staging habitats has resulted in reduced female body condition on the breeding grounds and a concomitant decline in productivity. We explored the importance of body (endogenous) reserves obtained prior to arrival on the breeding ground in egg protein formation in southwestern Montana during 2006–2008 using stable-carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope analyses of scaup egg components, female tissue, and local prey items. From arrival on the breeding grounds through the egg-laying period, $\delta^{15}\text{N}$ values of scaup red blood cells decreased while $\delta^{13}\text{C}$ values became less variable; a pattern consistent with endogenous tissues equilibrating with local (freshwater) dietary sources. In 2006 and 2008, isotopic values for egg albumen and yolk protein indicated that most (>90%) protein used to produce these components was obtained on the breeding grounds. However, in 2007, a year with an exceptionally warm and dry spring, endogenous reserves contributed on average 41% of yolk and 29% of albumen. Results from this study suggest that female scaup can meet the protein needs of egg production largely from local dietary food sources. This highlights the importance of providing high-quality breeding habitats for scaup. Whether this pattern holds in areas with similar breeding season lengths but longer migration routes, such as those found in the western boreal forest, should be investigated.

Locations where waterfowl acquire protein and lipid for egg laying has long been debated (Ankney and MacInnes 1978, Ebbinge et al. 1982, Davidson and Evans 1988, Bond et al. 2007). 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 theoretical underpinnings of where capital is acquired for breeding purposes in migratory birds that travel to breed (Klaassen et al. 2006) but empirical data are lacking.

Macronutrients used for egg formation are stored by migratory birds as proteins and lipids during the winter, spring migration, and while on the breeding grounds (Drent et al. 2007). The timing of breeding likely dictates where macronutrients are acquired (Klaassen et al. 2006). For example, arrival date on 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, Klaassen et al. 2006, Drent et al. 2007). 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 (body mass) after arrival to the breeding areas are thought to optimize efficiency by lowering body mass during spring migration (Drent 2006).

Conventional methods for measuring the amount of endogenous reserves used in egg formation have relied on correlating mass loss of nutrients (lipid, protein, and mineral) in females with mass gains in those nutrients allocated to their clutch (Afton and Ankey 1991, Esler et al. 2001). 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, the geographic location where capital resources were acquired is not identified with this technique, it does not account for female body maintenance, and assumes 100% conversion efficiency in transferring endogenous reserves to eggs which seems unlikely (Hobson et al. 2004, Hobson 2006). Thus, previous studies that applied this technique have likely overestimated the contribution of endogenous reserves in clutch formation. Finally, due to its

destructive nature, which entails killing multiple individuals through time, it is not an ideal method for studying nutrient dynamics of bird populations of conservation concern.

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). By knowing potential habitats (marine, freshwater and agricultural) used during spring migration and by selecting a body tissue with a slow turnover rate (Yohannes et al. 2010), the isotopic values in eggs can be used to determine the contributions of distinct habitats to egg formation.

The North American continental population of lesser scaup and greater scaup *A. marila* have declined for almost three decades but appear to have stabilized at a lower level by 2008 (U.S. Fish and Wildlife Service 2008). This lower level is 37% below the 1955–2005 long-term average (U.S. Fish and Wildlife Service 2008), and >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 highlighted the importance of spring staging habitats for acquiring body reserves for reproduction. The spring condition hypothesis suggests that loss and degradation of staging habitats has resulted in reduced female body condition during spring migration and on the breeding ground and concomitant declines in adult survival or productivity (Anteau and Afton 2009). However, scaup arrive on breeding sites 4–6 weeks before nest initiation depending on latitude (Bellrose 1980, Afton 1984, Austin et al. 1998, Martin 2007). Resources obtained on breeding sites could play an important role in fueling nutritional demands of egg laying and incubation. Further, scaup females have been reported to increase their overall body mass by 57 g on the breeding grounds (Afton 1984).

On the basis of results obtained from conventional methods, lesser and greater scaup have been shown to use either small amounts ($\sim <25\%$) or no endogenous protein reserves for clutch formation in sub-Arctic breeding populations (Esler et al. 2001, Gorman et al. 2008), and midcontinent lesser scaup reportedly do not use any endogenous reserves (Afton and Ankey 1991). However, scaup at both the sub-Arctic and midcontinent populations reportedly use up to 66% endogenous lipids for clutch lipid formation (Afton and Ankey 1991, Esler et al. 2001). Yet, knowing the site of macronutrient acquisition for clutch formation is a crucial component to understand which season most limits clutch formation in any migratory bird. Therefore, we employed stable isotope techniques to achieve the following objectives: 1) assess patterns in breeding season isotope values of red blood cells (RBC), a proxy for stored protein reserves acquired on spring migration areas, 2) estimate the percent contribution of, and annual variation in, endogenous and exogenous protein sources to egg formation in lesser scaup, and 3) assess intra-specific protein strategies of females by comparing different time-integrated body tissues from radio-marked individuals to assess where protein were derived for egg formation.

Because female scaup are one of the latest nesting ducks in North America and spend up to 6 weeks on the breeding

areas in the southern extent of their breeding range prior to egg formation, we predicted that exogenous dietary sources would provide a greater proportion of protein for egg formation than would stored endogenous reserves. Furthermore, we predicted that individuals would use multiple spring staging habitats for fueling spring migration. Finally, we predicted that the relative contributions of endogenous reserves would remain constant over the course of the three-year study because variation in local conditions could be mediated by the long pre-breeding period at our site.

Methods

Study area and field collections

Our study site was located at Red Rock Lakes National Wildlife Refuge (hereafter Refuge) in the Centennial Valley of southwest Montana. The high elevation (2033 m) of the Centennial Valley provides a narrow growing season for scaup that is shorter in duration to that of more northerly breeding areas where the majority of lesser scaup breed (i.e. the western boreal forest). For example, the mean growing season length at the Refuge (latitude = 44°) was on average 16–73 d shorter than for sites in the Northwest Territories (latitude = 63°) and Alaska (latitude = 61°) recently used to compare intra-specific initiation dates and clutch size in lesser scaup across North America (Gurney et al. 2011). Furthermore, arrival dates for 4 adult female scaup in 2008 that were radio marked in 2007 was 7 May, whereas arrival dates for scaup on the Old Crow, Yukon (latitude = 63°), was 15 May in 1960. Mean nest initiation for 247 scaup nests discovered on our study site from 2006–2009 was 21 June (± 9.0 d SD), whereas nest initiation in Yellowknife, Northwest Territories was 19 June, 1994–2000 (± 9.4 d SD).

Adult female lesser scaup were captured via night lighting (Lindmeier and Jessen 1961) on wetlands during the pre-breeding (from arrival on the breeding area until nest initiation) and egg laying periods in May and June of 2006–2008 ($n = 20, 6,$ and 43 in 2006, 2007, and 2008, respectively). Each female received a U.S. Geological Survey aluminum leg band. Claw and blood samples were collected from each female for stable isotope analysis. Claws were collected from females in 2006 and 2007 to serve as an endpoint for endogenous reserves for egg protein production since they would represent protein acquired on the wintering grounds prior to arrival at the breeding site (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. Up to 3 ml of blood was collected from females in May of 2007 and 2008 to provide an isotopic endpoint for endogenous protein. Blood was extracted by bleeding the foot, brachia or jugular vein. Blood samples were centrifuged to separate plasma and red blood cells. Each fraction was then transferred to individual vials and stored frozen at -20°C until stable isotope analysis was performed.

To obtain egg samples, nests were located using trained dogs, foot searches, and behavioral observations of female scaup made while searching in sedge-dominated habitats in 2006 ($n = 20$) and 2008 ($n = 28$). Furthermore, a sample of seven eggs from seven females were collected from radio-marked females in 2007. In order to relate individual females

to specific nests, radio telemetry was the only method used to collect egg samples in 2007. This resulted in a smaller sample size than was achieved in the other two years of the study. Females were marked on the breeding grounds with a radio transmitter on 15 May, 2007, and intensively tracked throughout the pre-breeding season until their nests were discovered between 12 and 26 June (Cutting 2010). One of the seven females hatched a successful nest while predators destroyed the other nests. Nest searches for unmarked females 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, however only one egg per nest was later used for stable isotope analysis. Eggs were hardboiled to separate yolk and albumen, and samples were stored frozen until stable isotope analysis was conducted (Gloutney and Hobson 1998). Proteins make up approximately 40% of the weight of egg yolks in ducks (Ricklefs 1977).

Because lesser scaup eat invertebrates (Rogers and Korschgen 1966) and emergent wetland seeds (Afton and Hier 1991, Smith 2007, Strand et al. 2007), both plant ($n = 5$) from 2 genera and animal material ($n = 27$) from 4 genera were collected during the three-year study as possible scaup food items during the pre-breeding and egg laying periods (Table 1). Invertebrates were collected at the study area during the breeding seasons of 2006–2009 via sweep sampling using a D-shaped dip net (1200 μm mesh, 0.072 m^2 opening, WARD's Natural Science, Rochester, NY). Collected invertebrates included amphipods (Amphipoda), leeches (Hirudinea), snails (Gastropoda) and water boatman (Corixidae). Hardstem bulrush *Schoenoplectus* spp. and sedge seeds *Carex* spp. were also collected at the study area during the pre-breeding and egg laying periods of 2006 and 2007 by finding intact seed heads that were from the previous year's growth.

Weather measurements

Precipitation and temperature data, collected at a nearby Natural Resource Conservation Service snow telemetry (Snotel) site, were used to assess annual variations in weather. The weather station is at an elevation of 223 m above and ~ 2 km south of the study site and continuously collects data

Table 1. Stable-isotope values (means \pm 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, USA.

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

^aScientific names: amphipods *Gammarus* spp., snails Gastropoda, leech Hirudinea, water boatman Hemiptera, sedge *Carex* spp., bulrush *Schoenoplectus acutus*.

every 3 h (www.wcc.nrcs.usda.gov/snotel/snotel.pl?sitenum=568&state=m). Water levels (± 2.0 cm) at the western outflow of Lower Red Rock Lake were recorded hourly throughout the nesting season, and mean water level was calculated each year from the first to third quartile date of nest initiation.

Stable-isotope analysis

Red blood cell samples were freeze-dried and powdered with a mortar and pestle. Invertebrate samples were washed with distilled water, freeze-dried and then powdered. Several droplets of 0.1N HCL solution were applied to the invertebrates without rinsing to remove carbonates. Claws were cleaned with a 2:1 chloroform-methanol solution to rid surface oils and chopped into small pieces. An aliquot of yolk and albumen was collected from each egg. Lipids were removed from the egg yolk and invertebrates using a 2:1 chloroform-methanol rinse which was later evaporated in a fume hood (Hobson et al. 2005). Samples were weighed (~ 1 mg), and encapsulated into tin capsules.

Samples were analyzed for stable-carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes using continuous flow isotope-ratio mass spectrometry (Hobson et al. 2005). Samples from 2006 were analyzed at Northern Plateau Stable Isotope Laboratory, Univ. of Northern Arizona; samples from 2007 and 2008 were analyzed at the Univ. of California-Davis Stable Isotope Facility. Stable isotope values were reported in parts per thousand (‰) relative to the international standards Vienna PeeDee Belemnite for $\delta^{13}\text{C}$ and atmospheric (AIR) nitrogen for $\delta^{15}\text{N}$. Estimated analytical error for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was ± 0.1 ‰ and ± 0.3 ‰, respectively based on replicate within-run measurements of within-laboratory organic standards.

There was high variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in RBC samples from females captured shortly after arrival on the breeding grounds. Therefore, we categorized females as originating from marine or freshwater habitats using arrival RBC isotopic values based on a demarcation value of -20 ‰ for $\delta^{13}\text{C}$. Values greater than -20 ‰ $\delta^{13}\text{C}$ were classified as originating from a marine habitat while values less than -20 ‰ $\delta^{13}\text{C}$ were classified as originating from a freshwater habitat (Yerkes et al. 2008).

Due to differences in processes influencing isotopic discrimination, for a given diet, $\delta^{13}\text{C}$ values of keratinized proteins (i.e. claws) tend to be more positive than metabolically active tissues (i.e. blood, Tieszen et al. 1983). Since RBC was not collected in 2006, claw $\delta^{13}\text{C}$ values were adjusted to blood equivalents using the mean difference of $\delta^{13}\text{C}$ between claws and RBC of arriving females categorized as originating from a freshwater or marine environment in 2008.

Isotopic discrimination values were applied to diet and endogenous body tissue to provide an estimate of egg protein values that are expected if each source is solely relied upon for fueling egg protein formation (Hobson 1995). This provided isotopic endpoints for the mixing model. Discrimination values have been experimentally determined from diet to egg (i.e. representing exogenous allocations, Hobson 1995) but not for endogenous reserves to egg. To estimate discrimination values for endogenous reserves to

egg formation, previous researchers (Gauthier et al. 2003, Schmutz et al. 2006, Bond et al. 2007, Hobson et al. 2011) 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). Based on Hobson (1995), discrimination between diet and endogenous reserves to yolk protein formation was assumed to be 3.4‰ for $\delta^{15}\text{N}$. Discrimination value for $\delta^{13}\text{C}$ in albumen production from lipid-free invertebrates and lipid-free endogenous reserves was assumed to be 0.9‰; whereas discrimination between albumen and wetland seed was assumed to be 1.5‰ (Hobson 1995). No $\delta^{13}\text{C}$ discrimination between lipid-free diet or body tissues and lipid-free yolk proteins was assumed (Hobson 1995).

Data analysis

To investigate seasonal isotopic trends, an exponential model was used to describe the change in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values through time (Dietz et al. 2010, Oppel and Powell 2010). To account for non-constant variance of isotope residuals through time, the covariate day was added in the standard deviation. It was assumed that the standard deviation was a linear function of the data (standard deviation = $y + x \times \text{date}$). The exponential model was fit using maximum likelihood estimation.

Female scaup were assigned to have originated from a freshwater or a marine/estuarine biome based on arrival RBC $\delta^{13}\text{C}$ values following Yerkes et al. (2008). Based on 68 band recoveries and 7 satellite-marked females, both of these environments were utilized by female scaup during the winter and spring-staging periods. Yerkes et al. (2008) surveyed published isotope values of feathers, claws, muscle, blood, liver and hair from avian and mammalian species using known biomes in North America to create delineations between marine/estuarine and freshwater origins for northern pintail *Anas acuta* and other waterfowl.

A concentration-dependent, three-endpoint, two-isotope Bayesian mixing model (SIAR) without informative priors was used to estimate relative contribution of endogenous protein reserves, local invertebrates and wetland seeds to egg formation in female lesser scaup (Parnell et al. 2010). Elemental concentrations are expressed as the proportion of the total weight of the sample and were derived from the elemental analyser 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). We followed Manseau and Gauthier (1993) and Gauthier et al. (2003) in assuming scaup could assimilate 80% of total N and 35% of C from wetland seeds. The concentration of assimilated N for wetland seeds was estimated as 0.029 (i.e. $[\text{N}]_{\text{wetland seeds}} \times 0.80/0.35$). Because we had no way of linking the body reserve isotopes of an unmarked female to her eggs, we ran our Bayesian mixing model based on two scenarios. First, we assumed that all eggs came from females arriving with a terrestrial or freshwater body reserve signature based on the criteria in Yerkes et al. (2008). Secondly, we ran the model assuming all females arrived with body reserve signatures representing a marine habitat. In each year, we used actual RBC or adjusted claw data from the cohort of

females sampled in that year to provide year-specific estimates of the isotopic endpoints for reserves. We reasoned that actual nutrient allocations would be intermediate between these extremes.

A one-way ANOVA was used to assess annual variation from 2006 to 2008 in RBC and claws when birds arrived in May on the breeding grounds and in eggs. 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. Means are presented with ± 1 SE, unless stated otherwise. All statistical analyses were conducted using R 2.8.1 (R Development Core Team). Prior to analysis, the data were tested for homogeneity of variance and normality of the residuals and confirmed to meet the assumptions of ANOVA.

Results

Mean temperature and precipitation from April to June varied among years with 2007 being the warmest and driest recorded since 1991 with a mean temperature of 8°C and mean precipitation of 10.4 cm. Weather during April–June of 2006 and 2008 were more similar to long-term patterns; mean temperature was 7.3°C and 4.7°C and precipitation was 25.4 cm and 23.1 cm, respectively. The difference in mean water level of Lower Red Rock Lake during the nesting season in 2007 was 0.52 ± 0.14 m lower than the mean water levels of 2006 and 2008.

Seasonal patterns of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in red blood cells

Values of $\delta^{15}\text{N}$ in RBC decreased in the freshwater group from 9.9 ± 0.7 ‰ to 6.2 ± 0.2 ‰ between 8–10 May and 2 July, while $\delta^{15}\text{N}$ values in the marine group decreased from 11.5 ± 2.0 ‰ to 4.7 ± 0.1 ‰ between 9–10 May and 2–3 July, 2008. In contrast, $\delta^{13}\text{C}$ values in RBC increased in the freshwater group from -22.4 ± 0.5 ‰ to -20.7 ± 0.1 ‰ between 8–10 May and 2 July, while $\delta^{13}\text{C}$ values in RBC decreased for the marine group from -13.9 ± 1.0 ‰ to -19.5 ± 0.0 ‰ between 9–10 May and 2–3 July, 2008 (Fig. 1).

Less of the variation for $\delta^{13}\text{C}$ values in RBC was explained for the freshwater group than for the marine group (freshwater: $r^2 = 0.11$, $p = 0.07$; marine: $r^2 = 0.63$, $p = 0.004$; Fig. 1). The daily $\delta^{13}\text{C}$ rate of change for RBC in the freshwater and marine group was 0.02 ± 0.001 ‰ and 0.20 ± 0.003 ‰, respectively. $\delta^{15}\text{N}$ values for RBC for both the freshwater and marine group showed a declining trend through the breeding season. Less variation in $\delta^{15}\text{N}$ values in RBC of the freshwater group was explained compared to $\delta^{15}\text{N}$ values in RBC of the marine group (freshwater: $r^2 = 0.32$, $p < 0.001$; marine: $r^2 = 0.66$, $p = 0.003$). The daily rate of change in RBC $\delta^{15}\text{N}$ of the freshwater and marine group was 0.02 ± 0.001 ‰ and 0.04 ± 0.002 ‰, respectively.

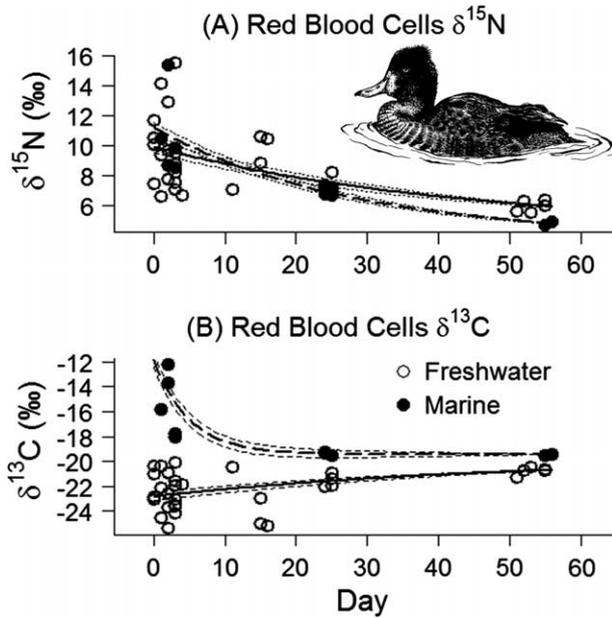


Figure 1. Seasonal patterns of $\delta^{15}\text{N}$ (A) and $\delta^{13}\text{C}$ (B) values in red blood cells of female lesser scaup *Aythya affinis* captured on their breeding grounds from arrival through the prebreeding and egg laying periods from 8 May (day 0) to 3 July (day 55), Red Rock Lakes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

Stable-isotopes of endogenous tissues, eggs, and dietary items

The $\delta^{15}\text{N}$ values of arrival RBC varied annually, while $\delta^{13}\text{C}$ did not ($\delta^{15}\text{N}$ $F_{2,47} = 5.4$, $p < 0.01$; $\delta^{13}\text{C}$ $F_{2,47} = 1.9$, $p = 0.29$; Table 2, Fig. 2). Egg albumen and yolk protein $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values varied by year ($\delta^{13}\text{C}$ albumen $F_{2,49} = 3.5$, $p = 0.04$; yolk protein $F_{2,50} = 10.3$, $p < 0.01$; $\delta^{15}\text{N}$ albumen; $F_{2,49} = 17.4$, $p < 0.01$, yolk protein; $F_{2,50} = 36.0$, $p < 0.01$, Table 2).

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

	Tissue type	2006	2007	2008	F value	DF	p
$\delta^{13}\text{C}$	Body tissue	-20.2 ± 0.7^A	-20.7 ± 0.9^A	-21.0 ± 0.3^A	1.9	2, 47	0.29
	Albumen	-22.4 ± 0.4^{AB}	-20.7 ± 0.2^B	-21.2 ± 0.4^B	3.5	2, 49	0.04
	Yolk protein	-24.7 ± 0.4^A	-21.3 ± 0.5^B	-22.3 ± 0.4^B	10.3	2, 50	<0.01
$\delta^{15}\text{N}$	Body tissue	10.8 ± 0.3^A	7.6 ± 0.6^B	9.8 ± 0.3^A	5.4	2, 47	<0.01
	Albumen	5.7 ± 0.1^A	7.0 ± 0.2^B	5.7 ± 0.1^A	17.4	2, 49	<0.01
	Yolk protein	6.2 ± 0.4^A	8.3 ± 0.2^B	6.7 ± 0.5^C	36	2, 50	<0.01

Note: F and p values are for non-factor ANOVA. Within a row, values that do not share the same superscript are significantly different from one another ($p < 0.05$).

Annual variation for $\delta^{13}\text{C}$ (2006; $-22.4 \pm 0.3\text{‰}$, 2007; $-22.2 \pm 0.5\text{‰}$, and 2008; $-20.8 \pm 0.7\text{‰}$) and $\delta^{15}\text{N}$ (2006; $1.3 \pm 1.0\text{‰}$, 2007; $4.4 \pm 0.8\text{‰}$, and 2008; $2.5 \pm 0.6\text{‰}$) values of lipid-free invertebrate samples did not differ ($F_{2,24} = 2.7$, $p = 0.09$ and $F_{2,24} = 2.6$, $p = 0.09$, respectively). Therefore, invertebrates were pooled to create a bulk isotopic endpoint for the local invertebrate diet during the 3-yr study. The overall 3-yr averages for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in lipid-free invertebrates were $-21.7 \pm 0.4\text{‰}$ and $2.4 \pm 0.5\text{‰}$, respectively (Table 1, Fig. 2). The mean values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in local emergent wetland seeds collected in 2006 and 2007 were $-26.8 \pm 0.3\text{‰}$, and $1.9 \pm 0.6\text{‰}$, respectively.

Relative contributions of endogenous and exogenous sources to egg protein formation

The contribution of endogenous reserves varied annually with those from wintering freshwater and marine habitats contributing equally to yolk protein formation (Fig. 2, Table 3). The mean percent contribution of endogenous reserves to yolk protein formation of the freshwater (range 4–10%) and marine (range 5–9%) group was similar in 2006 and 2008. In 2007, a higher contribution of endogenous reserves to yolk protein (range 26–41%) and albumen (range 18–29%) production was observed for both the freshwater and marine groups, respectively. The remaining contributions to egg protein formation were derived from local exogenous sources (Fig. 2, Table 3).

Intraspecific protein strategies of different time-integrated body tissues based on radio-marked birds

A reduction in $\delta^{13}\text{C}$ variation was found among body tissues of individuals through time (Fig. 3). Adjusted claw values varied considerably among the 7 radio-marked females at arrival on the study site ($\delta^{13}\text{C}$ range: -14.6 to -25.0‰). Whereas variation of $\delta^{13}\text{C}$ values in yolk protein ranged only from -19.8 to -22.6‰ . Egg albumen protein $\delta^{13}\text{C}$ values from the same females ranged from -20.6 to -22.1‰ .

Discussion

Isotope values of RBC in female scaup declined precipitously following arrival reflecting the pattern of replacement

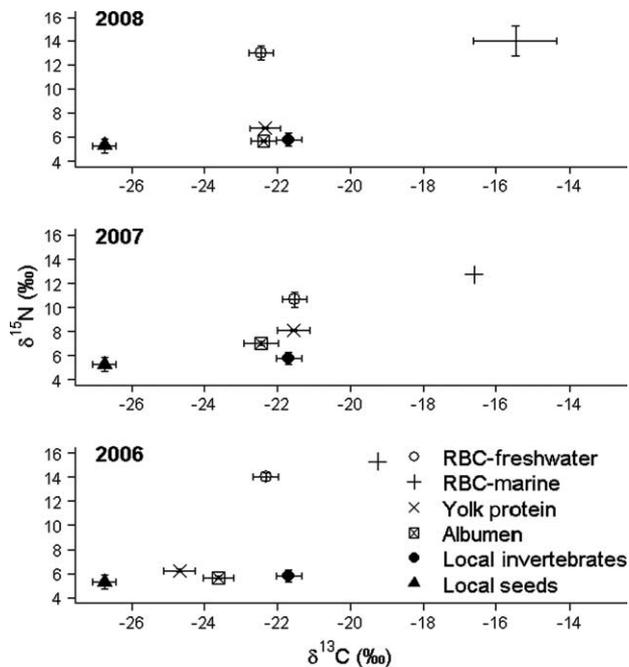


Figure 2. Predicted $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (mean \pm 1SE) of red blood cells (RBC), aquatic invertebrates and wetland seeds if egg proteins (albumen and yolk protein) of female lesser scaup were derived entirely from any of these sources during the prebreeding and egg 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.

of stored endogenous proteins with 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 for egg

production by the time egg laying occurred. Instead, local dietary sources contributed most of the macronutrients to egg protein production. Results from those two years were consistent with findings on lesser scaup from southern Manitoba (Afton and Ankney 1991). Those authors reported endogenous protein was not used for clutch formation, which would indicate that the breeding grounds are a primary source of protein for egg production.

Patterns of isotopic change in red blood cells

Interactions between breeding and non-breeding events are most readily detected in populations with strong migratory connectivity in which individuals from the same breeding area overlap in their nonbreeding distribution (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 suggests that birds came from several wintering biomes. For example, band recoveries of scaup harvested off our study site indicate they are wintering and migrating through habitats found in the Pacific, Central, and Mississippi Flyways (USFWS unpubl.). Throughout the breeding season, $\delta^{15}\text{N}$ values of RBC decreased while $\delta^{13}\text{C}$ values became less variable by the end of the egg-laying period. This convergence of both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values likely shows a replacement of stored reserves from spring migration stop-over locations with those from the breeding area location. The high initial variability in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of body tissues upon arrival thus became less variable through time; this is a pattern consistent with expectations of endogenous tissues equilibrating with local dietary sources.

Blood isotope values of birds tend to turn over in an exponential fashion following a switch to an isotopically different diet (Pearson et al. 2003, Morrison and Hobson 2004, Opper and Powell 2010). We found strong evidence for this in females that utilized marine habitats prior to arriving on the study site. In contrast, the exponential

Table 3. Mean (95% confidence interval) proportions of protein derived from body reserves, local invertebrates and local seeds to eggs of lesser scaup breeding at Red Rock Lakes National Wildlife Refuge, Montana, 2006 to 2008. Values are derived from the Bayesian mixing model SIAR without informative priors. Results from two models are shown. The freshwater model assumed all eggs were derived from females wintering in freshwater habitats ($\delta^{13}\text{C} < -20\text{‰}$) and were based on year-specific red blood cell isotope data from arriving birds. The marine model assumed all eggs were derived from females wintering in marine habitats ($\delta^{13}\text{C} > -20\text{‰}$).

Protein source	2006		2007		2008	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Freshwater model Yolk						
Reserves	0.04	0.008 to 0.065	0.41	0.238 to 0.568	0.10	0.048 to 0.156
Inverts	0.29	0.109 to 0.473	0.47	0.273 to 0.669	0.66	0.469 to 0.842
Seeds	0.68	0.483 to 0.862	0.12	0.000 to 0.307	0.24	0.051 to 0.444
Albumen						
Reserves	0.03	0.000 to 0.054	0.29	0.105 to 0.477	0.05	0.002 to 0.093
Inverts	0.44	0.154 to 0.678	0.55	0.336 to 0.775	0.80	0.645 to 0.945
Seeds	0.53	0.288 to 0.829	0.16	0.000 to 0.318	0.15	0.008 to 0.298
Marine model Yolk						
Reserves	0.05	0.015 to 0.092	0.26	0.158 to 0.356	0.09	0.048 to 0.124
Inverts	0.27	0.102 to 0.437	0.47	0.273 to 0.681	0.56	0.373 to 0.736
Seeds	0.68	0.504 to 0.851	0.28	0.087 to 0.451	0.36	0.179 to 0.546
Albumen						
Reserves	0.04	0.000 to 0.081	0.18	0.079 to 0.300	0.04	0.004 to 0.079
Inverts	0.43	0.152 to 0.644	0.52	0.302 to 0.752	0.74	0.564 to 0.920
Seeds	0.54	0.311 to 0.821	0.29	0.114 to 0.467	0.21	0.049 to 0.383

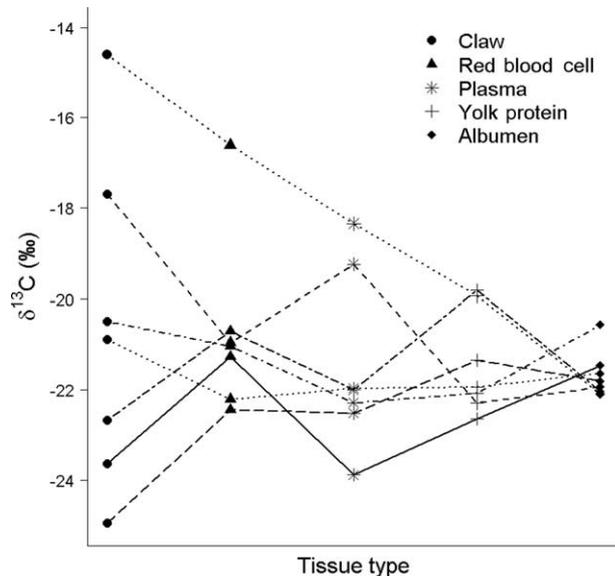


Figure 3. Intraspecific nutrient strategies for egg production in 7 female lesser scaup, Red Rock Lakes National Wildlife Refuge, Montana, USA. Stable-carbon isotope ($\delta^{13}\text{C}$) values of body tissues with different turnover rates and eggs collected from the same individuals reveal different strategies during late-winter and spring migration. Body and egg tissues from individual birds can be traced with the uniquely identifiable lines.

model provided a poor fit to the blood $\delta^{13}\text{C}$ turnover data for females that utilized freshwater habitats since freshwater stopover sites had similar $\delta^{13}\text{C}$ values to the local (C3) breeding environment.

Sources of protein for eggs

In 2007, scaup had the highest endogenous reserve contribution to yolk protein formation, and the earliest peak nest-initiation date in our study. These findings are consistent with reports from 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. This idea is supported by the fact that during the drought of 2007, body mass of female scaup in mid May was heavier than it was in 2008 (2007: 705 ± 11 g SE; 2008: 657 ± 11 g). During a drought year, Rogers (1964) showed the weights of ovaries of scaup were significantly lighter than were ovaries during the subsequent breeding season with high water conditions. In our case, either females anticipated drought conditions and arrived in better body condition, or only heavier females initiated breeding that year.

Our results are the first published estimates showing inter-annual variability in how lesser scaup allocate macronutrients for egg production. In the earlier 5-yr study by Afton and Ankney (1991), results on inter-annual variability were not considered. However, previous nutrient dynamic studies 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. During the drought year of 2007, females on the Refuge incorporated significantly more endogenous reserves into eggs, similar to scaup from interior Alaska that used endogenous protein reserves for egg formation (Esler et al. 2001). These results highlight a flexible strategy in how macronutrients are used for egg protein formation, with females varying the level of endogenous reserves used in egg formation dependent upon local conditions. When local resources are not available (dry years), there was an increase in contribution of stored endogenous reserves to egg formation.

Intraspecific comparison of radio-marked females

Based on females tracked through the breeding season, scaup more commonly relied on freshwater habitats than they did on marine habitats for acquiring endogenous protein reserves prior to arrival on the breeding grounds. However, some scaup undoubtedly lost some of their endogenous marine-derived reserves due to turnover during the protracted spring migration period foraging in freshwater areas. Several band recoveries and satellite transmitter data from the wintering period show a considerable proportion of females wintering in coastal areas (author's unpubl.). Regardless, few endogenous marine resources were transported into their eggs based on the depleted $\delta^{13}\text{C}$ values which were indicative of terrestrial or freshwater ecosystems. The egg protein isotope values for all individuals were less variable than were their body tissues and relatively similar to one another, which again suggests that females used a similar protein allocation strategy for egg formation based largely on similar local diets.

To nest, scaup can import resources as endogenous reserves, derive local sources through exogenous intake, or use a mixture of both sources. Given the scaup's intermediate body size, high metabolic rate compared to that of larger-bodied waterfowl (Nager 2006), and prolonged time spent on the breeding grounds prior to nesting in southern latitudes (Bellrose 1980), conditions on breeding areas may influence protein demands for egg formation more than other waterfowl.

Our results emphasize the importance of breeding ground conditions in two of the three years of the study in providing lesser scaup with the resources necessary for clutch formation. This is not to suggest that spring staging areas and wintering areas are not important for other aspects of scaup life history. Rather, it simply reveals the importance of breeding habitats in meeting nutritional demands of clutch formation, especially in non-drought years. Our finding is reinforced by recent isotope studies that have shown that birds historically viewed as capital breeders actually use considerable local dietary resource for breeding purposes (Gauthier et al. 2003). However, in future, we expect the frequency of dry years to increase at our study site in particular (U.S. Fish and Wildlife Service 2009) and in the western boreal Forest in general

(Austin et al. 2000). This drying trend will potentially make endogenous reserves and body condition of arriving scaup more important in the future. Further isotopic research across latitudinal gradients would be valuable to make the distinction between distant capital, local capital, and income sources for reproduction in this and other potentially vulnerable species (Klaassen et al. 2006).

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