INTRASPECIFIC VARIATION IN NUTRIENT RESERVE USE DURING CLUTCH FORMATION BY LESSER SCAUP

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Abstract. We studied nutrient reserve dynamics of female Lesser Scaup (*Aythya affinis*) to identify sources of intraspecific variation in strategies of nutrient acquisition for meeting the high nutritional and energetic costs of egg formation. We collected data from interior Alaska and combined these with data for Lesser Scaup from midcontinent breeding areas (Afton and Ankney 1991), allowing a rangewide analysis for the species. We found little evidence that nutrient reserve use differed between Alaskan and midcontinent Lesser Scaup, except that subarctic birds used a small amount of protein reserves when forming eggs, whereas midcontinent birds did not. Mineral reserves contributed relatively little to the clutch, but endogenous lipid accounted for approximately two-thirds of the lipid in the clutch. Levels of endogenous lipid and protein at initiation of clutch formation declined with date of initiation. Also, absolute amounts of lipid and protein reserves used declined through the season, corresponding to smaller clutch sizes. Our data are consistent with a seasonally variable threshold of lipid reserves for initiation of clutch formation and considerable reliance on lipid reserves, suggestive of lipid control of productivity via effects on clutch size and initiation dates. However, our data cannot refute the hypothesis that clutch size or initiation dates are set by other factors that in turn dictate the amount of lipid reserves that are stored and used. Despite uncertainty regarding the role of nutrient limitations on productivity, maintenance of adequate food resources on winter, migration, and breeding areas should be a management concern, given the high costs of clutch formation by Lesser Scaup, evidence of recent population declines, and potential links between nutrition and productivity.

Key words: Aythya affinis, breeding, clutch, egg formation, intraspecific variation, Lesser Scaup, nutrient reserves.

Variación Intraespecífica en el Uso de las Reservas de Nutrientes durante la Formación de Huevos en *Aythya affinis*

Resumen. Estudiamos la dinámica en la reserva nutricional de hembras de *Aythya affinis* para identificar fuentes de variación intraespecífica en las estrategias de adquisición de nutrientes. Estos nutrientes permiten afrontar los altos costos nutricionales y energéticos que demanda la producción de huevos. Colectamos datos en el interior de Alaska y los combinamos con información sobre *A. affinis* para áreas de cría del centro del continente (Afton y Ankney 1991), permitiendo un análisis para una extensa área de distribución de la especie. Encontramos escasa evidencia sobre variaciones en el uso de nutrientes de reserva entre *A. affinis* de Alaska y del centro del continente. Como excepción, las aves subárticas usaron una pequeña cantidad de las reservas proteicas cuando produjeron los huevos, en contraposición con las aves del centro del continente que no las usaron. Las reservas minerales contribuyeron relativamente poco a la formación de huevos, pero los lípidos endógenos representaron casi dos-tercios de los lípidos presentes en los huevos. Los niveles de lípidos endógenos y de proteínas al comienzo de la producción de huevos disminuyeron en relación con la fecha de inicio. Además, las cantidades absolutas de reserva de lípidos y proteínas usadas disminuyeron a lo largo de la estación, correspondiéndose con nidadas más pequeñas. Nuestros datos son consistentes con la existencia de un umbral estacional variable en las reservas de lípidos que determina el inicio de la formación de huevos, y con la seguridad relativa que ofrecen las reservas de lípidos. Estos resultados sugieren que los lípidos controlan la productividad de las aves a través de efectos sobre el tamaño de la nidada y la

fecha de inicio. Sin embargo, nuestros datos no pueden refutar la hipótesis que el tamaño de la nidada o la fecha de inicio estén determinados por otros factores que a su vez determinen la cantidad de reservas de lípidos que son almacenadas y usadas. A pesar de las dudas sobre el rol que juega la limitación de nutrientes sobre la productividad, las estrategias de manejo deberían considerar el mantenimiento de reservas alimenticias adecuadas en áreas de invernada, migración y reproducción. Esto se justifica dado el alto costo que representa para *A. affinis* la producción de huevos, la evidencia sobre recientes disminuciones poblacionales, y los vínculos potenciales entre nutrición y productividad.

INTRODUCTION

Energetic and nutritional costs of egg formation by waterfowl are high relative to most other birds (King 1973). Considerable research has been directed at understanding strategies that waterfowl employ to meet these costs, with particular emphasis on studies of nutrient reserve dynamics of females (Alisauskas and Ankney 1992). Nutrient reserve studies evaluate the relative importance of foods and stored reserves as sources of nutrients and energy for clutch formation (reviewed by Ankney and Alisauskas 1991a, Alisauskas and Ankney 1992). These studies have provided valuable insights into selective pressures affecting waterfowl reproduction and, further, offer implications for conservation efforts to maintain or improve waterfowl productivity. Alisauskas and Ankney (1992) identified research directions for enhancing understanding of strategies that waterfowl use to meet costs of clutch formation, and they recommended studies of intraspecific variation.

Several factors have been examined with respect to intraspecific variation in nutrient reserve use by female waterfowl during clutch formation. First, reliance on reserves may be seasonally variable due to a dynamic threshold of nutrient reserves required for clutch initiation (Reynolds 1972). Under this hypothesis, individuals that are able to accumulate relatively large nutrient reserves breed earlier in the season, have larger clutches, and rely more heavily on endogenous nutrients for clutch formation. Results from several studies are consistent with these predictions (Krapu 1981, Alisauskas and Ankney 1994, Esler and Grand 1994), although most studies of nutrient reserve dynamics have not considered date of initiation of clutch formation as a potential source of intraspecific variation. Second, nutrient reserve use varies with nest attempt within a breeding season; renesting females of several species do not rely on reserves for formation of second clutches (Krapu 1981, Rohwer 1992, Esler and Grand 1994). Fi-

nally, variation in sources of nutrients used for clutch formation has been assessed by location and related to habitat (Young 1993, Esler and Grand 1994) or latitude (MacCluskie and Sedinger 2000), although this important aspect of nutritional ecology of breeding waterfowl has received little study.

We studied nutrient reserve dynamics of female Lesser Scaup (*Aythya affinis*) during clutch formation to identify sources of intraspecific variation in nutrient acquisition. We combined new data from birds collected in interior Alaska with data from midcontinent scaup (Manitoba, Canada; Afton and Ankney 1991) to provide an evaluation across the species' range. Our objectives were (1) to define nutritional and energetic costs of clutch formation, (2) to determine relative contributions of foods and stored reserves for meeting clutch formation costs, (3) to assess intraspecific variation in nutrient reserve dynamics related to nesting date and nest attempt, and (4) to contrast strategies of nutrient acquisition between subarctic Alaska and midcontinent areas. We also evaluated potential nutritional or energetic mechanisms that could lead to lowered productivity, which has been hypothesized as an important factor affecting recent population declines (Austin et al. 2000, Afton and Anderson 2001).

METHODS

Data collection for Lesser Scaup from southwestern Manitoba was described by Afton and Ankney (1991). We collected 60 female Lesser Scaup at Yukon Flats National Wildlife Refuge, Alaska (64°25′N, 149°59′W) from 8 May to 30 June 1991, providing a data set appropriate for comparing nutrient reserve use to midcontinent scaup and for evaluating factors affecting nutrient reserve use across the species' range. Yukon Flats is in interior Alaska and is characterized by boreal forest interspersed with wetlands and associated meadows. In 1991, approximately 260 000 Lesser Scaup were surveyed on Yukon

Flats, making them the most abundant duck species on the area (Conant and Dau 1991).

Breeding status of collected females was assigned based on ovary characteristics (Esler 1994) and brood patch attributes. We defined breeding status categories as *nondeveloped*: dry weight of the largest ovarian follicle ≤0.15 g; *rapid follicle growth* (RFG): largest follicle >0.15 g dry weight; *laying*: ≥1 postovulatory follicle and a preovulatory follicle of sufficient size to be ovulated within 24 hr (>7.27 g dry mass) or presence of an oviducal egg; *renesting-RFG*: a series of postovulatory follicles, the largest of which could not have ovulated recently (<5 mm diameter) or a brood patch, and the largest follicle >0.15 g dry weight; *renesting-laying*: a series of regressed postovulatory follicles or a regrowing brood patch, ≥1 recent postovulatory follicle, and ≥1 preovulatory follicle to ovulate within 24 hr or presence of an oviducal egg; *incubating*: a series of postovulatory follicles and a brood patch; *early incubating*: within the first day after conclusion of laying (largest postovulatory follicle diameter >9.48 mm). Dates of initiation of RFG were estimated based on models of ovarian follicle growth (Esler 1994), number of postovulatory follicles, and a laying rate of one egg per day (Austin et al. 1998, Esler 1999). Examination of eye color (Trauger 1974) suggested that nearly all Lesser Scaup collected in Alaska were older than second year, and thus we did not test for age effects.

Following field dissection to remove reproductive organs (see below) and upper digestive tract contents, carcasses were double-wrapped in plastic and frozen. In the laboratory, carcasses were thawed and plucked, and remaining digestive tract contents were removed. The gizzard was dried, weighed, and discarded assuming that it was all protein. Abdominal fat was weighed and discarded under the assumption that it contained little water. Also, a sample of fat-free heart muscle was removed in the field for genetic analysis and weighed to the nearest 0.1 g. Protein content of the removed heart pieces was estimated by multiplying wet weight by 0.2457, the average proportional dry weight of samples ($n = 60$) of fat-free heart pieces.

We determined nutrient contents of muscles of the right breast and leg, liver, and the remaining carcass based on standard procedures of lipid extraction using petroleum ether in a modified Soxhlet apparatus (Dobush et al. 1985) and combustion in a muffle furnace to determine proportions of protein and mineral (Ankney and Afton 1988). We estimated total somatic nutrients (FAT, PROTEIN, ASH) for each bird by adding component values.

In the field, the ovary and oviduct (if it contained a developing egg) were removed and preserved in 10% formalin. Reproductive nutrients (R-NUTRIENT), i.e., fat, protein, and ash committed to the oviduct, ovary, oviducal egg, and eggs laid (R-FAT, R-PROTEIN, R-ASH), were estimated according to methods in Esler and Grand (1994). Estimates of damaged follicle contents were calculated by first describing relationships between dry weight of follicles and the dry weight of the next smallest follicle, from samples of ovaries with intact follicles: Dry follicle = 0.14 + (2.94 × dry smaller follicle)−(0.41 × dry smaller follicle^2) + (0.03 × dry smaller follicle^3), $n = 126$, $r^2 = 0.99$.

Using this model, we first estimated dry weights of damaged follicles. Estimated dry weight then was multiplied by proportions of fat and protein from intact ovaries, resulting in estimates of fat and protein for each damaged follicle. Average (± SE) lipid content of 14 oviducal egg yolks and 3 laid eggs was 5.82 ± 0.22 g, average protein content of 6 intact oviducal eggs and 3 laid eggs was 8.88 ± 0.25 g, and average mineral content (dry shell) of 3 laid eggs was 4.38 ± 0.19 g. Egg components that could be estimated for individuals were used in determining their R-NUTRIENT values; otherwise, average egg nutrient values were used. For example, for birds with oviducal eggs, yolk lipid and protein were used and albumen protein was estimated if the egg was not complete. Using an individual's egg components is advantageous because variation in egg size and composition within clutches is generally less than that among females (Duncan 1987), although not always (Flint and Grand 1999).

To allow correction for potential variation in somatic PROTEIN and ASH measurements related to body size (Sedinger et al. 1997), we conducted principal components analysis on morphometric variables measured on all females from both areas (wing chord, tarsus, culmen, and keel). The first principal component described positive correlation with loadings ranging from 0.43 to 0.55 and explained 42% of the total original variance. We used scores from the first prin-

cipal component (PC_1) as an index of body size and included PC_1 as a potential main effect during model selection.

STATISTICAL ANALYSES

We estimated daily nutritional and energetic costs of clutch formation using models of ovarian follicle growth (Esler 1994) to estimate daily increases in follicle dry mass, proportions of lipid and protein in dry follicles, average albumen dry mass, and estimates of energy densities of 39.5 kJ g^{-1} and 23.6 kJ g^{-1} for egg lipid and protein, respectively (Alisauskas and Ankney 1992, Robbins 1993). We also contrasted daily energy contributions to eggs relative to basal metabolic rate (BMR) of Lesser Scaup, which was estimated to be 226.1 kJ day^{-1} (Alisauskas and Ankney 1992).

We used standard least-squares general linear models to examine sources of variation in nutrient reserve dynamics of Lesser Scaup using a combined data set of midcontinent and Alaskan birds. Our analyses of nutrient reserve dynamics explored the relationship between endogenous reserves (FAT, PROTEIN, and ASH) and the corresponding investment of those nutrients (R-FAT, R-PROTEIN, and R-ASH) in the clutch (Alisauskas and Ankney 1992). R-NUTRIENT values with negative regression coefficients indicate the decline in nutrient reserves in grams for each gram committed to reproduction and can be interpreted as the maximum proportion of egg nutrients derived from nutrient reserves. We also included other explanatory variables to evaluate their effects on nutrient reserves, including date of initiation of RFG (RFGINIT), area (midcontinent vs. Alaska), nest attempt (first nests vs. renests), and body size (PC_1 for PROTEIN and ASH models). RFGINIT for each bird was adjusted relative to the earliest initiation (16 May), which was set at 0; average RFG initiation dates ($\pm$ SE) of collected birds were similar between subarctic (5 June $\pm$ 1.4 days) and midcontinent females (8 June $\pm$ 2.2 days). Area and nest attempt were included as indicator variables (Ramsey and Schafer 1997), with midcontinent and first nests set as reference levels. We also included terms for interactions of R-NUTRIENT $\times$ RFGINIT, area, and nest attempt. Main effects of explanatory variables were interpreted as effects on amounts of reserves (intercept), and interaction terms indicated effects of explanatory variables on rates of reserve use.

Comparisons of protein reserve dynamics were somewhat constrained because carcass analysis of midcontinent birds included feathers (Afton and Ankney 1991), whereas Alaska birds were plucked prior to analysis. Because of these different laboratory techniques, we had an *a priori* expectation of area differences in total protein (i.e., total protein estimates higher for midcontinent birds than Alaskan), but were unable to determine if there was an absolute difference in size of protein reserves excluding feathers. However, our primary interest was to compare rates of protein use as they related to other variables, including area. Because feather protein is not available for catabolism for clutch formation, data on rates of protein reserve use were directly comparable between our study areas.

We used information-theoretic methods to direct model selection and parameter estimation (Burnham and Anderson 1998). This approach uses the principle of parsimony to determine which model or models provide the best fit to the data (Hilborn and Mangel 1997), and avoids assumptions and biases of traditional stepping (i.e., forward, backward, and stepwise) model selection procedures (Flack and Chang 1987, Anderson et al. 1994). Using these methods, model uncertainty can be incorporated when evaluating importance of model parameters and deriving parameter estimates. Use of the information-theoretic approach to data analysis deviates from traditional methods based on significance testing, but is supported by a growing body of literature describing the pitfalls of statistical hypothesis tests (Johnson 1999, Anderson et al. 2000), particularly for observational studies.

For model selection, we considered models with all possible combinations of explanatory variables, with the exception of constraining potential PROTEIN models to include only those with an area main effect (see above). This resulted in 127 candidate models for FAT, 128 for PROTEIN, and 255 for ASH. We recognize the value of limiting numbers of candidate models (Burnham and Anderson 1998, Anderson et al. 2000); however, in this situation, we concluded that all parameter combinations were biologically plausible and interpretable and that there was no *a priori* justification for not considering any of the combinations of effects. Because of this approach, we based our inference primarily on

relative support for parameters across all models and model-averaged parameter estimates, rather than basing inference on a particular model or set of models.

For each model, we calculated Akaike's Information Criterion including a small-sample-size correction (AIC_c), which measures the fit of the model to the data while balancing the loss of precision associated with overfitted models (i.e., including too many model parameters). Smaller AIC_c values indicate more parsimonious, and thus better-approximating, models. We compared candidate models using ΔAIC_c, which is the difference between the AIC_c for each respective model and the lowest observed AIC_c (Burnham and Anderson 1998). Models with $\Delta AIC_c \leq 2$ are considered to have strong support from the data (Burnham and Anderson 1998).

Support for model parameters was evaluated by summing AIC_c weights for all models that included each parameter. An AIC_c weight indicates the likelihood that a particular model is the best of the candidate models, given the data (Burnham and Anderson 1998). Therefore, parameters that occur in most or all of the models best fit by the data will have high summed weights (close to 1) and thus are well-supported for drawing inference.

To estimate parameters, we calculated model-averaged parameter estimates based on AIC_c weights (Burnham and Anderson 1998) for all candidate models. We calculated SE for parameter estimates based on unconditional variances calculated across the same models.

RESULTS

Of the 60 female Lesser Scaup collected at Yukon Flats, Alaska, 29 were first nesters (16 in RFG, 12 laying, and 1 early incubator that was included in all analyses). We excluded one bird originally classified as first-nest RFG from analyses because the estimate of RFG initiation was 27 June, 10 days later than any other first nest. We believed that this bird may have been a misidentified renester, as postovulatory follicles regress rapidly (Esler 1994), and thought that inclusion with first nesters could result in inappropriate model fitting in regions with very sparse data. Fourteen renesters were collected, including 8 in RFG and 6 laying. Remaining collected birds included 13 classified as nondeveloped, and 3 incubating. The sample of midcontinent Lesser Scaup included 6 in RFG, 12 laying, and 1 early incubator (Afton and Ankney 1991).

As a result of the 5-day duration of RFG (Esler 1994), contributions of nutrients and energy to eggs peaked on the sixth day of RFG. For a 9-egg clutch, the median in Alaska, clutch formation costs were maximized for 4 days at the energy equivalent of one egg. The estimates reported here represent the energy and nutrients of egg constituents and do not incorporate costs of acquisition or synthesis; thus costs are absolute minima. Energy equivalents in egg constituents during peak clutch formation, approximately 450 kJ, were estimated to be 199% of BMR, comparable to the estimate of 187% for Lesser Scaup by Alisauskas and Ankney (1992).

LIPID RESERVE USE

In the best-fitting model, lipid reserves of Lesser Scaup declined with commitment of R-FAT and were smaller in females that initiated clutch formation later in the season (Table 1). These parameters were included in all models with $\Delta AIC_c \leq 2$. Also, summed AIC_c weights for these parameters were high (Table 2), which was strong evidence that they explained important variation in the data. The weighted estimate for R-FAT was −0.68, indicating that as much as 68% of the lipid in the clutch was derived from lipid reserves. Lipid reserves at initiation of RFG averaged 1.43 g smaller with each day that females delayed initiation. This result is consistent with a seasonally declining lipid threshold for initiation of clutch formation. No other explanatory variables were supported strongly by the data (Table 2), suggesting that lipid reserve dynamics did not differ substantially between midcontinent and subarctic Lesser Scaup and that first nesters and renesters employed similar strategies after accounting for the effect of differences in RFG initiation dates.

To estimate the absolute amount of lipid reserves used during clutch formation through the nesting season, we first estimated the linear relationship between clutch size and nest initiation date for a sample of nests found during incubation (J. B. Grand, unpubl. data):

$$\text{Clutch size} = 30.40 - 0.13 \times \text{nest initiation date}$$
$$(n = 11, \ r^2 = 0.73),$$

where nest initiation dates were coded as Julian dates. We accounted for the five-day difference between RFG initiation and nest initiation (Esler

TABLE 1. General linear models describing variation in nutrient reserves of female Lesser Scaup during clutch formation. The models best fit by our data, and those with substantial support ($\Delta AIC_c \leq 2$), are presented.

Response variable	Model	Explanatory variables[a]	Number of parameters	ΔAIC_c	AIC_c weight	R^2
FAT	1	R-FAT, RFGINIT	3	0.00	0.16	0.45
	2	R-FAT, RFGINIT, RFAT × RFGINIT	4	0.98	0.10	0.46
	3	R-FAT, RFGINIT, R-FAT × AREA	4	1.38	0.08	0.46
	4	R-FAT, RFGINIT, R-FAT × NEST	4	1.74	0.07	0.45
	5	R-FAT, RFGINIT, AREA	4	2.00	0.06	0.45
PROTEIN	1	PC₁, RFGINIT, AREA, AREA × R-PROTEIN	5	0.00	0.17	0.90
	2	PC₁, R-PROTEIN, RFGINIT, AREA, AREA × R-PROTEIN	6	1.89	0.07	0.90
ASH	1	PC₁, R-ASH × RFGINIT	3	0.00	0.08	0.20
	2	PC₁, AREA, R-ASH × RFGINIT	4	1.41	0.04	0.21
	3	PC₁, AREA, R-ASH × RFGINIT, R-ASH × AREA	5	1.64	0.04	0.24
	4	PC₁, NEST, R-ASH × RFGINIT	4	1.76	0.03	0.21
	5	PC₁, RFGINIT, R-ASH × RFGINIT	4	1.80	0.03	0.21

[a] Explanatory variable abbreviations: R-FAT, R-PROTEIN, R-ASH = fat, protein, and ash, respectively, committed to reproduction; RFGINIT = date of initiation of rapid follicle growth; AREA = study area (Alaska vs. midcontinent); NEST = nest attempt (first nest vs. renest), PC₁ = principal components score indicating body size.

1994) to estimate clutch size by RFG initiation date. Based on this relationship and the parameter estimates for RFG initiation date and R-FAT reported above, we estimated that approximately 27 to 47 g of endogenous lipid were used during clutch formation (Fig. 1), far exceeding amounts of other nutrient reserves used.

PROTEIN RESERVE USE

Two models of protein reserve dynamics had $\Delta AIC_c \leq 2$ (Table 1). These both included terms for body size (PC_1), date of RFG initiation, and the area × R-PROTEIN interaction. As all models were constrained to include an area term, this also appeared in both models. Inclusion of the PC_1 term was strongly supported by the data (Table 2), and the positive parameter estimate predictably indicated that endogenous protein increased with body size. The summed AIC_c weight also was high for date of RFG initiation. Females that initiated RFG later in the season had smaller protein reserves; protein reserves were approximately 0.29 g lower for each day delay in RFG initiation (Table 2). A negative parameter estimate for the area × R-PROTEIN interaction was moderately supported (Table 2), suggesting that rate of protein reserve use differed by area, with Alaska birds deriving up to 7% of their clutch protein from endogenous sources. Absolute amount of endogenous protein used by Alaska birds through the season was relatively small (Fig. 1). The estimate of an approximately 40-g difference between areas presumably was due to inclusion of feathers in lab analyses of midcontinent birds. The main effect for R-PROTEIN was included in Model 2 (Table 1) and estimated the rate of reserve use for midcontinent birds. However, this parameter was not well supported by the data (Table 2) and had a small, positive parameter estimate; thus we conclude that there is little evidence that midcontinent scaup relied on protein reserves during clutch formation. The weight of evidence suggested that nest attempt did not affect protein reserves.

MINERAL RESERVE USE

Five models describing variation in ASH had $\Delta AIC_c \leq 2$ (Table 1); however, nearly 80% of the variation in the data was left unexplained by these models. PC_1 and the interaction of R-ASH × date of RFG initiation were included in the five best-fitting models and also were reasonably

TABLE 2.   Parameter estimates from general linear model analyses describing variation in Lesser Scaup nutrient reserves during clutch formation. Parameter likelihoods are AIC_c weights summed across all considered models that included a given variable, and indicate the importance of that variable. Parameter estimates are weighted averages (based on model AIC_c weights) from all candidate models and SE are based on unconditional variances. Explanatory variable abbreviations are defined in Table 1.

Response variable	Explanatory variable	Parameter likelihood	Parameter estimate ± SE
FAT	Intercept		101.31 ± 9.23
	R-FAT	0.97	–0.68 ± 0.25
	RFGINIT	0.99	–1.43 ± 0.38
	AREA[a]	0.26	–0.21 ± 2.10
	NEST[b]	0.29	1.74 ± 2.98
	R-FAT × RFGINIT	0.40	0.01 ± 0.01
	R-FAT × AREA[a]	0.31	–0.06 ± 0.08
	R-FAT × NEST[b]	0.31	–0.11 ± 0.14
PROTEIN	Intercept		169.29 ± 4.26
	PC_1	0.97	2.71 ± 0.91
	R-PROTEIN	0.33	0.01 ± 0.03
	RFGINIT	0.89	–0.29 ± 0.11
	AREA[a]	1.00	–39.69 ± 3.56
	NEST[b]	0.27	–0.22 ± 1.04
	R-PROTEIN × RFGINIT	0.32	0.00 ± 0.00
	R-PROTEIN × AREA[a]	0.61	–0.07 ± 0.05
	R-PROTEIN × NEST[b]	0.28	–0.02 ± 0.03
ASH	Intercept		27.92 ± 1.11
	PC_1	0.88	0.71 ± 0.29
	R-ASH	0.40	–0.02 ± 0.03
	RFGINIT	0.35	–0.02 ± 0.02
	AREA[a]	0.43	–0.62 ± 0.59
	NEST[b]	0.27	–0.10 ± 0.30
	R-ASH × RFGINIT	0.71	–0.003 ± 0.002
	R-ASH × AREA[a]	0.36	0.02 ± 0.03
	R-ASH × NEST[b]	0.24	0.01 ± 0.03

[a] AREA is a categorical variable with midcontinent as the reference value; parameter is interpreted as the effect for Alaska birds.

[b] NEST is a categorical variable with first nests as the reference value; parameter is interpreted as the effect for renesting birds.

well supported by the data (Table 2), given the set of candidate models. As with protein, the positive parameter estimate for PC_1 simply accounted for larger-bodied individuals having more endogenous mineral. The negative parameter estimate for the R-ASH × date of RFG initiation interaction (Table 2) indicated that the rate of mineral reserve use increased over the course of the nesting season, although the total amount used was slight throughout (Fig. 1). Even when maximized at the latest date of RFG initiation, use of ash reserves corresponded to about 5% of the mineral in the clutch. There was no evidence that mineral use differed between midcontinent and subarctic areas or between first nests and renests.

INITIATION THRESHOLDS

Differences in body composition between females with nondeveloped ovaries and those in RFG have been interpreted to indicate that minimum or target levels of nutrient reserves are required to initiate clutch formation, i.e., there are initiation thresholds (Ankney and Alisauskas 1991b). Because we found that both lipid and protein reserves of Lesser Scaup females at the beginning of clutch formation declined with advancing initiation dates, we examined our data for evidence of a declining initiation threshold as the season progressed. For each nondeveloped bird collected in Alaska, we calculated deviations in their reserve levels (adjusted for body size for protein) from the estimate of average nutrient reserve levels at initiation of RFG for the date upon which the nondeveloped bird was collected, based on parameter estimates in Table 2. We found that lipid reserves of nondeveloped birds averaged 22.1 ± 9.0 g (SE) below the average lipid level at RFG initiation. Protein re-
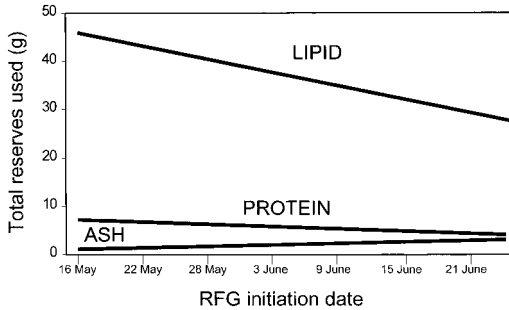
FIGURE 1. Estimates of average nutrient reserves used during clutch formation by Lesser Scaup through the nesting season. Protein use is based on use by birds from Alaska only, as midcontinent birds did not use protein reserves; lipid and ash use represent Alaska and midcontinent birds combined. RFG = rapid follicle growth.

serves of nondeveloped birds averaged 6.5 ± 2.8 g lower than estimated levels at RFG initiation.

DISCUSSION

Like other waterfowl, Lesser Scaup had high costs of clutch formation, both in terms of nutrients and energy. Across their breeding range, Lesser Scaup met these costs with lipid reserves for much of the clutch lipid, and acquisition of exogenous sources of protein and mineral for most of these nutrients in the clutch (Fig. 1).

SOURCES OF INTRASPECIFIC VARIATION

Initiation date. We found that the strategy by which female Lesser Scaup met clutch formation costs included lower average lipid and protein reserves at initiation of RFG as the breeding season progressed (Table 2). Also, Lesser Scaup used lower absolute amounts of lipid and protein reserves with advancing initiation dates, reflecting seasonally declining clutch sizes (Fig. 1). Also, we documented that females with nondeveloped ovaries had lipid and protein reserves lower than the average estimated at RFG initiation for the date of collection. Our results are consistent with a hypothesis of a seasonally declining body-mass threshold for initiation of egg-laying (Reynolds 1972), in which heavy females initiate early, have large clutches, and rely on large endogenous reserves, with delays in initiation, smaller clutch sizes, and less use of reserves for females with lower body masses and presumably smaller nutrient stores. Other nutrient reserve studies also have documented these patterns. Mallards (*Anas platyrhynchos*) nesting

early in the season had both the largest lipid reserves and largest clutches (Krapu 1981). Early-nesting Ruddy Ducks (*Oxyura jamaicensis*) had higher nutrient reserve thresholds for clutch initiation than later nesting birds, which corresponded to declining clutch size with time (Alisauskas and Ankney 1994); rates of nutrient use were either similar (lipid) or higher (protein and mineral) for early nesters relative to late nesters. Similarly, lipid reserves at the beginning of clutch formation and the rate of lipid reserve use both declined with date of initiation of clutch formation of Northern Pintails (*Anas acuta*), which in turn corresponded to declining clutch size (Esler and Grand 1994).

While Lesser Scaup nutrient reserve use generally followed the predictions of Reynolds (1972), our data did not strongly support a shift in proportional reliance on stored reserves versus foods, which would be indicated by interaction terms of R-NUTRIENTs × date of RFG initiation. In other words, the proportion of the clutch derived from nutrient reserves did not vary over the season. This is in marked contrast to some other ducks, particularly those that nest early in the breeding season. For example, rate of lipid reserve use during clutch formation by Northern Pintails varied from more than three times the amount of lipid being invested in the clutch at the beginning of the season to no use of lipid reserves by season's end (Esler and Grand 1994). We speculate that these interspecific differences in nutrient reserve use reflect differences in dynamics of food availability. Northern Pintails initiated first nests immediately after spring thaw, when food availability presumably was low and thus endogenous reserves were the primary source of nutrients and energy; as the season progressed, food availability presumably increased, corresponding to decreasing reliance on reserves. Lesser Scaup nest relatively late, and variation in exogenous food availability over the range of initiation dates likely is less dramatic; hence proportional reliance on reserves is constant, although absolute amount of reserve use changes as a result of seasonally declining clutch size.

Despite the relationship between initiation date and nutrient reserve use in many, although not all (Ankney and Alisauskas 1991b, Gammonley 1995), nutrient reserve studies, many investigators have not analyzed their data including this factor. We recommend that investigators

routinely incorporate initiation date as a potential explanatory variable, both to account for more of the variation in their data sets and to lend insight into the strategies of waterfowl nutrient acquisition and allocation for clutch formation.

The ecological significance of initiation date, reserve use, and clutch size interactions is debatable. Data fitting the paradigm described by Reynolds (1972), including our own, are consistent with a hypothesis that females strive to maximize reserves before clutch formation, based on fitness advantages of larger clutch sizes and early initiation dates. This hypothesis rests on the premise that reserves are the proximate factor responsible for initiation dates and clutch sizes. However, there are other explanations for declining clutch size over the course of the nesting season (Rohwer 1992). One could argue that some other factor dictates optimum timing of laying or clutch size and that birds store and use only the reserves necessary to meet the nutritional requirements corresponding to their optimum strategy (Arnold and Rohwer 1991, but see Ankney et al. 1991). We cannot refute this alternative explanation based on our data.

Nest attempt. For Lesser Scaup, there was little evidence that the strategy of reserve use by renesters differed from first nesters after accounting for date effects. In previous studies, renesting females have not relied on endogenous reserves for formation of second clutches (Krapu 1981, Rohwer 1992, Esler and Grand 1994), in contrast to first nesters. Lesser Scaup may be able to achieve the lipid threshold for clutch initiation for renests by either not exhausting reserves for their first clutch or by quickly rebuilding reserves before renest initiation. Lesser Scaup can potentially renest quickly, as the duration of RFG is estimated to be 5 days (Esler 1994).

Geographical differences. Comparison of nutrient reserve use during clutch formation across habitats and latitudes is critical for understanding flexibility in nutrient acquisition strategies within a species (Ankney and Alisauskas 1991a, Alisauskas and Ankney 1992), under the premise that geographical differences in nutrient availability might elicit different responses in nutrient reserve use. In particular, comparisons between boreal forest and midcontinent habitats have been recommended (Ankney and Alisauskas 1991a), under the assumption that the latter

have greater exogenous nutrient availability. If exogenous nutrients are limiting in boreal forests, one would predict that Alaskan Lesser Scaup would rely more on endogenous reserves. Our results are not consistent with that prediction because the strategy of nutrient reserve use was similar between subarctic and midcontinent areas, with the exception of a relatively small amount of protein reserve use by Alaskan birds. We concur with MacCluskie and Sedinger (2000) that macroinvertebrate abundance may be similar between boreal and temperate areas during the waterfowl breeding season and suggest that studies of prey phenology, abundance, and quality in both areas would be a worthwhile research direction.

Few studies have examined geographical differences in nutrient reserve use. Use of nutrient reserves by Northern Pintails did not differ between boreal forest and tundra at similar latitudes in subarctic Alaska (Esler and Grand 1994). Young (1993) detected variability in lipid reserve use of female Mallards among study sites in Saskatchewan. However, Young (1993) did not consider the potential interaction of initiation date and reserve use, which may have confounded interpretation of habitat effects. On one of Young's (1993) study sites, collected Mallard hens initiated with less lipid and used lipid at a slower rate; however, average nest initiation of those birds also was later and, thus, is in agreement with patterns demonstrated in other species, irrespective of habitat (Esler and Grand 1994). Only one prior study has examined the effect of latitude on intraspecific variation in nutrient reserve use. MacCluskie and Sedinger (2000) reported that Northern Shovelers (*Anas clypeata*) in subarctic Alaska relied less on nutrient reserves than those in midcontinent areas (Ankney and Afton 1988), and suggested that high wetland productivity and longer days for foraging in subarctic Alaska allowed clutch formation entirely from exogenous sources of nutrients.

NUTRIENT RESERVES AND CONTROL OF CLUTCH SIZE

Observational field studies, such as ours, are limited in their ability to determine whether clutch size is proximately limited by the size of nutrient reserves. Improvements in analysis methods (Sedinger et al. 1997) can strengthen the approach for describing correlations between

nutrient reserves and termination of clutch formation. However, determining cause and effect within the relationship of clutch size and nutrient reserve use is difficult (Arnold and Rohwer 1991). Further, the factors that limit clutch size may vary among individuals; MacCluskie and Sedinger (2000) speculated that nutrient availability may regulate clutch size of females with smaller nutrient reserves or those in poorer habitat, whereas clutch size of other individuals may be dictated by ultimate controls. This ''mixed model'' would be difficult to detect under field conditions.

As demonstrated by controlled experiments, nutrition can influence egg production (Eldridge and Krapu 1988), and thus nutritional limitation of clutch formation under natural conditions is plausible. Use of nutrient reserves is widely acknowledged as a prerequisite for nutrient limitation of clutch size (Sedinger et al. 1997). Therefore, we can conclude that protein and mineral available to Lesser Scaup likely do not limit clutch size, given the relatively small use of these reserves. Our data are consistent with the potential for lipid limitation of clutch size. Nutritional regulation of clutch size is an important research topic for understanding variation in waterfowl productivity; manipulative experiments designed to address this question would be exceedingly valuable.

IMPLICATIONS FOR LESSER SCAUP
CONSERVATION

Comparisons of waterfowl nutrient reserve use during clutch formation reveal considerable interspecific variation (Ankney and Alisauskas 1991a, Alisauskas and Ankney 1992). For example, some Arctic nesting geese rely almost entirely on endogenous reserves (Ryder 1970, Ankney and MacInnes 1978, Raveling 1979). Breeding ducks are variable in their use of reserves (Ankney and Alisauskas 1991a), ranging from heavy reserve use (Krapu 1981, Esler and Grand 1994) to less use (Afton and Ankney 1991) to no use (MacCluskie and Sedinger 2000). Lesser Scaup employ an intermediate strategy for egg production, relying on stored lipid reserves, accumulated during winter, spring, or on breeding areas before laying, and also exogenous protein, mineral, and some lipid from breeding areas.

Despite some uncertainty about the role of nutrients as a proximate control of clutch size under natural conditions, clutch formation by Lesser Scaup clearly requires a large amount of energy and nutrients, which must be derived from either nutrient reserves or exogenous foods. Also, it is clear that, under conditions of nutrient restrictions, individual nutritional status can be related to productivity (Eldridge and Krapu 1988), through effects on clutch size, timing of nesting, or breeding propensity. Therefore, it is quite possible that nutrient limitations constrain Lesser Scaup productivity and that maintenance of adequate food resources during periods of nutrient reserve storage and egg formation is an appropriate management goal, particularly given that reduced productivity has been hypothesized as a factor in Lesser Scaup population declines (Austin et al. 2000, Afton and Anderson 2001). We recommend studies that address the timing and location of acquisition of lipid reserves and evaluation of factors that might constrain lipid accumulation. Also, factors that affect availability or quality of exogenous nutrients on breeding areas should be assessed.

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