

THE ROLE OF NUTRIENT RESERVES FOR CLUTCH FORMATION BY NORTHERN PINTAILS IN ALASKA¹

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Abstract. We analyzed carcass composition of female Northern Pintails (*Anas acuta*) in Alaska to assess the importance of nutrient reserves for formation of first clutches ($n = 85$) and renests ($n = 39$). Habitat (tundra vs. boreal forest), hen age (yearling vs. adult), and year (1990 vs. 1991) did not affect nutrient reserve use. During formation of first clutches, Northern Pintail hens relied on lipid reserves more than any other duck species that has been studied. For much of the nesting season, lipid reserves were used to meet costs beyond those incurred by clutch formation. Date of initiation of rapid follicle growth was related to lipid reserve dynamics; lipid reserves at initiation and the rate of lipid use both declined through the season. Protein reserves declined slightly with commitment of protein to the clutch and with date of initiation of rapid follicle growth. Use of mineral reserves for first nests was negligible. Renesting females did not use nutrient reserves. Lipid reserve levels were positively related to the amount of lipid reserves needed to complete the clutch and clutch sizes predicted from a lipid dynamics model were consistent with known clutch sizes. Similar relationships did not exist for protein. We suggest that lipid reserve levels affect timing of nesting and proximately limit clutch size of Northern Pintails.

Key words: *Anas acuta*; clutch size; nest initiation; Northern Pintail; nutrient reserves; renest.

INTRODUCTION

Clutch formation by female waterfowl (Anatidae) requires relatively large amounts of lipid, protein, and mineral. The question of whether meeting these demands limits clutch size of ducks has received considerable attention (Ankney et al. 1991, Arnold and Rohwer 1991). Arnold et al. (1987) suggested that clutch size evolved in response to time limitations imposed by egg viability and nest predation, and that other factors, including nutrient limitation, acted only to fine tune clutch size (see also Arnold and Rohwer 1991). Others (e.g., Ankney et al. 1991) have hypothesized that the level of nutrients available to laying hens, in both food and nutrient reserves, was the primary factor influencing observed clutch sizes. Among proponents of this hypothesis there has been disagreement about whether lipid or protein requirements were generally limiting (Drobney and Fredrickson 1985, Drobney 1991, Ankney et al. 1991, Ankney and Alisauskas 1991a, Mann and Sedinger 1993).

One way to examine these issues has been to study dynamics of nutrient reserves through the breeding season. These studies can determine the

relative importance of nutrients acquired from exogenous (dietary) and endogenous (nutrient reserve) sources and provide insight into potential limiting effects of nutrients. Some arctic nesting geese (Ryder 1970, Ankney and MacInnes 1978, Raveling 1979) and Common Eiders (*Somateria mollissima*; Korschgen 1977) relied almost entirely on endogenous reserves for clutch formation. For those species, it has been generally accepted that size of their reserves dictated clutch size. Patterns of nutrient acquisition for clutch formation are not as clear within the Anatini and Aythyini (Krapu and Reinecke 1992). All species within these tribes that have been studied used lipid reserves to varying degrees (Ankney and Alisauskas 1991a). Use of protein reserves has been documented in ducks with highly herbivorous diets during clutch formation, e.g., Gadwall (*Anas strepera*; Ankney and Alisauskas 1991b), which apparently have difficulty obtaining exogenous protein. Mineral reserves were not used by most species (Ankney and Alisauskas 1991a).

Northern Pintails (*A. acuta*; hereafter pintails) nest early in the season, relative to other North American ducks. Availability of exogenous nutrients is presumably low in early spring, particularly at northern latitudes where some pintail nests are initiated during spring thaw. Also, clutch

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formation of ducks breeding in northern wetlands may be constrained by limits on season length and productivity not existent in mid-continent prairies and parklands (Calverley and Boag 1977) where most other studies of nutrient reserve use have been conducted. Thus, we assessed nutrient reserve dynamics of female pintails in Alaska, predicting that they would be subject to high demands on reserves during clutch formation and would be likely to experience nutrient limitation of clutch size, if such a mechanism existed. We also examined potential sources of intraspecific variation in nutrient reserve use including effects of initiation date, habitat, hen age, year, and nest attempt.

STUDY AREAS AND METHODS

These studies were conducted in 1990 and 1991 on two sites in subarctic Alaska. On Yukon Delta National Wildlife Refuge (NWR), pintail hens were collected in tundra habitat near the mouth of the Kashunuk River (61°26'N, 165°27'W), an area with high numbers of breeding pintails (Grand, unpubl. data). Pintails also were collected near Mallard Lake (64°24'N, 149°59'W) on Yukon Flats NWR, which is primarily boreal forest interspersed with numerous wetlands, the most productive of which are associated with graminoid meadows (Heglund 1988). Pintails were the most abundant duck on the Yukon Delta and were second in number to Lesser Scaup (*Aythya affinis*) on Yukon Flats (Conant and Dau 1991). These areas averaged 50.7% of the pintails in Alaska and 25.3% of the continental total during 1990 and 1991, based on standardized breeding pair surveys (Conant and Dau 1991).

Female pintails were collected by shooting from arrival through incubation and reneating. The ovary was removed and preserved in 10% formalin. If the oviduct contained a developing egg, it also was preserved.

Breeding status was assigned based on models of ovarian follicle dynamics (Esler, unpubl. ms.): *nondeveloped*—dry mass of the largest follicle ≤ 0.15 g; *rapid follicle growth (RFG)*—largest follicle > 0.15 g dry mass; *laying*— ≥ 1 postovulatory follicle and a preovulatory follicle of sufficient size to be ovulated within 24 hr (> 4.5 g dry mass) or an oviducal egg; *reneating-RFG*—a series of postovulatory follicles, the largest of which could not have been ovulated recently, or a brood patch, and the largest follicle > 0.15 g dry mass; *reneating-laying*—a series of regressed

postovulatory follicles or a regrowing brood patch, ≥ 1 recent postovulatory follicle, and ≥ 1 preovulatory follicle > 4.5 g dry mass or an oviducal egg. Wing covert and bursal characteristics were used to determine age (Esler and Grand, in press).

CARCASS ANALYSIS

In the laboratory, pintails were thawed and plucked, and digestive tract contents were removed. The gizzard was dried, weighed, and discarded with the assumption that it was all protein. Dry mass of a piece of heart tissue removed for genetics work was estimated by multiplying wet weight by 0.285, the average proportion dry mass of a sample of 24 fat-free heart pieces; this also was assumed to be protein. Abdominal fat was weighed and discarded because it contains little water and its exclusion improves precision of lipid estimates from extraction (C. D. Ankney, pers. commun.).

Nutrient contents of muscles of the right breast and leg, liver, and the remaining carcass were determined using standard procedures of lipid extraction using petroleum ether in a modified Soxhlet apparatus (Dobush et al. 1985) and ashing in a muffle furnace to determine proportions of protein and mineral (Ankney and Afton 1988). Total nutrient reserve levels (FAT, PROTEIN, ASH) were calculated for each bird by adding all component values.

REPRODUCTIVE TISSUE ANALYSIS

Analyses of nutrient reserve dynamics require measures of both nutrient reserves and reproductive nutrients, i.e., nutrients committed to the oviduct, ovary, oviducal egg, and all eggs laid (R-FAT, R-PROTEIN, R-ASH). The ovary was treated like the leg and liver. The oviduct was dried and weighed; the resulting dry mass was considered protein.

Reproductive nutrients were estimated indirectly when (1) ovarian follicles were damaged upon collection and (2) females had laid eggs. To estimate nutrient content of damaged follicles, simple linear regressions (Steel and Torrie 1980) were used to describe the relationship between nutrients in follicles and nutrients in the next smallest follicle for a sample of intact ovaries (Afton and Ankney 1991). The amount of nutrients in each follicle was estimated by multiplying dry mass of the follicle by % nutrient in the entire ovary. The resulting equations were used to estimate protein and lipid in damaged

follicles: Follicle lipid = $0.306 + 1.630$ smaller follicle lipid; $n = 120$, $r^2 = 0.96$, $P < 0.001$. Follicle protein = $0.360 + 1.619$ smaller follicle protein; $n = 120$, $r^2 = 0.96$, $P < 0.001$.

Nutrients in eggs already laid were estimated by multiplying the number of eggs laid (determined by examination of postovulatory follicles) by estimates of fat and protein in each egg. Estimates of egg fat and protein were either (1) the amount of those nutrients in the hen's completely developed oviducal egg, if present; or (2) the average amount of nutrients in pintail eggs from a sample of oviducal eggs and eggs collected from nests. The former was used, when possible, because egg size variation within clutches is less than that among females (Duncan 1987a). Eighteen unincubated eggs from three nests and 33 completely formed oviducal eggs were used to determine average nutrient content of pintail eggs. Eggs from nests were boiled to facilitate separation of yolk and albumen. Albumen was dried and weighed (it was considered all protein) and the yolk was treated like other samples that were lipid-extracted. Dry shell was determined only for eggs from nests and averaged 3.00 g. Average lipid and protein in pintail eggs was 4.50 g and 6.90 g, respectively.

Thus, reproductive nutrients were calculated:

$$\text{R-FAT} = \text{fat}_{\text{ovary}} + \text{fat}_{\text{oviducal egg yolk}} \\ + (\# \text{ eggs laid} \cdot \text{egg fat})$$

$$\text{R-PROTEIN} = \text{protein}_{\text{ovary}} + \text{dry oviduct} \\ + \text{oviducal egg albumen} \\ + \text{protein}_{\text{oviducal egg yolk}} \\ + (\# \text{ eggs laid} \cdot \text{egg protein})$$

$$\text{R-ASH} = \text{ash}_{\text{ovary}} + \text{oviducal egg shell} \\ + (\# \text{ eggs laid} \cdot 3.00)$$

ADJUSTMENTS FOR BODY SIZE

To remove variation in nutrient reserves due to body size, we used principal components analysis (see Ankney and Afton 1988) on five morphological characters, i.e., lengths of wing chord, tarsus, culmen, keel, and immovable spine (defined as the length from the posterior point on the last cervical vertebra to protruberances on the most anterior caudal vertebra). The resulting first principal component described positive correlation with loadings ranging from 0.37 to 0.49

and with an eigenvalue of 2.01, explaining 40.3% of the total original variance. We used scores from the first principal component (PC_1) as an indicator of body size and regressed nutrient reserves against PC_1 . Lipid reserves were not related to body size ($P = 0.8466$); however relationships were found for protein and mineral ($P < 0.001$). Residuals from these models were used to generate new protein and mineral values, corrected for body size (see Ankney and Alisauskas 1991b); corrected values were used in all analyses.

DATA ANALYSES

For comparison to previous studies, we used simple linear regressions to describe relationships between nutrient reserves and reproductive nutrients (Alisauskas and Ankney 1992). We also used analysis of covariance and regression models (PROC GLM of SAS, Freund and Littell 1981) to examine variation in nutrient reserves. For each nutrient, a separate model was run to determine effects of each categorical variable (habitat, age, and year) and their interactions with covariates (reproductive nutrients and date of initiation of RFG [RFGINIT]) by examining Type III sums of squares. Females of unknown age were not included in analyses of age effects. Nonsignificant ($P > 0.05$) effects of categorical variables were excluded from further analyses. Models including the continuous variables, their interaction, and any effects of categorical variables were run. If a model was significant ($P < 0.05$), nonsignificant interactions and variables were removed based on examination of Type III sums of squares.

To assess the role of each nutrient in limiting clutch size, we first calculated amounts of lipid, protein, and mineral that would still be put into eggs to complete total clutch commitment for a sample ($n = 18$) of late layers (<5 developing follicles) of known clutch size. To do so, we subtracted amounts of nutrients already committed to eggs from the total amounts of nutrients estimated to be in the completed clutch. We then estimated amounts of nutrient reserves that would be used while those nutrient requirements were being met, based on rates of nutrient reserve use from the general linear models for lipid and protein and from the simple linear model for mineral (see Results). For protein and mineral, rates of nutrient reserve use were constants, but use of lipid reserves varied by RFGINIT (see Results).

TABLE 1. Simple linear regressions describing the relationships between nutrient reserves (Y) and the amount of that nutrient committed to reproduction (X) for female pintails collected in subarctic Alaska, 1990–1991.

Nutrient	Intercept	Slope	r^2	P
First nests ($n = 85$)				
FAT	101.01	-2.58	0.36	<0.001
PROTEIN	134.42	-0.20	0.13	<0.001
MINERAL	27.05	-0.13	0.05	0.031
Renests ($n = 39$)				
FAT	31.09	-0.16	0.04	0.235
PROTEIN	125.88	-0.02	<0.01	0.622
MINERAL	26.12	0.06	0.03	0.279

Finally, we regressed nutrient reserves of these birds by the estimate of additional reserves they would use. Significant positive relationships suggest limitation, i.e., exhaustion of the nutrient reserve to a certain point results in termination of laying and, thus, regulates clutch size (Ankney and Afton 1988, Ankney and Alisauskas 1991b).

We estimated nutrient levels at the end of laying by calculating averages for early post-laying hens ($n = 12$), i.e., with a postovulatory follicle ≥ 4.0 mm diameter, which corresponds to <3 days after the last egg laid (Esler, unpubl. ms.). Using these as estimates of termination thresholds, we calculated predicted clutch sizes using the general linear models for lipid and protein, to determine if models of nutrient reserve dynamics had explanatory value for known clutch sizes.

RESULTS

Our sample consisted of 124 female pintails including 85 first nesters (35 RFG and 50 laying)

and 39 renesters (19 RFG and 20 laying). In 1990, 23 and 39 pintails were collected on Yukon Flats and Yukon Delta, respectively, and in 1991, 31 were collected at each site. Fifty-seven hens each were in after second year and second year age-classes, and 10 were of unknown age.

NUTRIENT RESERVE USE

Lipid, protein, and mineral reserves declined ($P < 0.05$) 2.58 g, 0.20 g, and 0.13 g, respectively, for every g of that nutrient committed to reproduction for first nests (Table 1). However, general linear models (Table 2) explained more variation in the data (see r^2 values in Tables 1 and 2). Habitat, age of the hen, and year did not affect any model.

Changes in lipid reserves were best described by a model including R-FAT, RFGINIT, and their interaction (Table 2). This analysis does not represent an extrapolation, as our data were well distributed (Fig. 1) across ranges of RFGINIT (0–41 days) and R-FAT (0.04–34.65 g). Lipid

TABLE 2. General linear models describing changes in nutrient reserves relative to the amount of that nutrient committed to reproduction (R-NUTRIENT) and date of initiation of rapid follicle growth (RFGINIT) for female pintails collected in subarctic Alaska, 1990–1991. Only significant ($P < 0.05$) effects are shown.

Nutrient	Model			Intercept	Variable	Estimate	F	P
	F	r^2	P					
First nests ($n = 85$)								
FAT	32.70	0.55	<0.001	148.25	R-FAT	-3.36	21.94	<0.001
					RFGINIT	-3.35	19.28	<0.001
					R-FAT \times RFGINIT	0.08	4.29	0.040
PROTEIN	18.93	0.32	<0.001	140.75	R-PROTEIN	-0.14	7.12	0.009
					RFGINIT	-0.45	22.16	<0.001
MINERAL	2.24	0.08	0.090					
Renests ($n = 39$)								
FAT	1.46	0.04	0.235					
PROTEIN	2.30	0.16	0.095					
MINERAL	1.28	0.10	0.298					

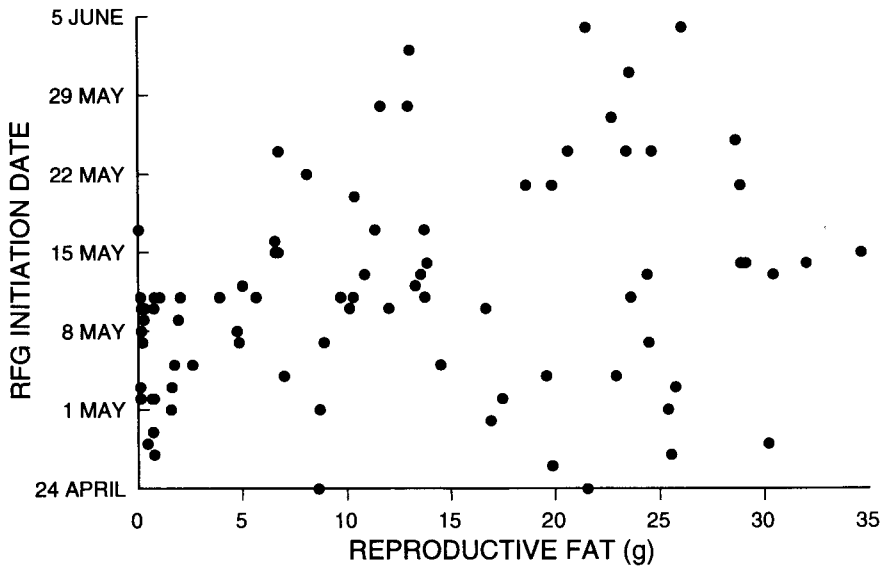


FIGURE 1. Distribution of data used to generate a model of lipid reserve dynamics of first-nesting pintails in Alaska.

reserves decreased with commitment to reproduction and later initiation dates (Fig. 2). The interaction term indicated that the rate of lipid commitment decreased with later RFGINIT. In other words, later nesting hens began clutch commitment with smaller lipid reserves and had a slower rate of lipid reserve use (and therefore used less total reserves). Commitment of lipid reserves for every g of R-FAT ranged from 3.35 g at the earliest date (24 April) to 0 g approximately 40 days later; the slope was approximately 1.0, i.e., reserve use was commensurate with demands of clutch formation, at 28 days after earliest initiation (22 May).

Levels of protein reserves were related to R-PROTEIN and RFGINIT (Table 2). Protein reserves declined approximately 0.14 g for every g committed to reproduction, irrespective of RFGINIT. Also, protein reserves declined an average of 0.45 g per day, over all levels of commitment to reproduction.

The general linear model for mineral reserves including R-ASH, RFGINIT, and their interaction was not significant ($P = 0.09$) (Table 2) and, therefore, simple linear regression (Table 1) best describes mineral reserve use. If use of mineral reserves occurred at the rate suggested by linear regression analysis, 3.2 g or approximately the equivalent of the shell of one egg of an eight-egg clutch would be derived from reserves.

We described the relationship between known clutch sizes and timing of nesting with a simple linear regression.

$$\text{Clutch size} = 8.49 - 0.04 \cdot \text{RFGINIT},$$

$n = 18$, $r^2 = 0.26$, $P = 0.032$. Using changing clutch size and rates of reserve use (Table 2), we estimated the average amount of lipid and protein reserves used during clutch formation throughout the breeding season (Fig. 3). Although reliance on lipid reserves declined through the season, the amount of lipid reserves used greatly exceeded that of protein reserves.

Simple linear and general linear models were not significant ($P > 0.05$) for any nutrient for renesting hens (Tables 1 and 2). Average (\pm SE) lipid, protein, and mineral reserves of renesters were 29.3 g (± 1.5), 125.4 g (± 0.9), and 26.4 g (± 0.4), respectively. These values are similar to estimates of nutrient levels at the end of laying for first nests (see below).

NUTRIENTS AND CONTROL OF CLUTCH SIZE

Estimates of the amount of reserves to be used during completion of clutch formation were related to endogenous reserves for lipid ($P = 0.002$, $r^2 = 0.45$) (Fig. 4) but not for protein ($P = 0.357$, $r^2 = 0.05$) or mineral ($P = 0.872$, $r^2 < 0.01$). The relationship for lipid was not evident if the effect of initiation date was not considered. For ex-

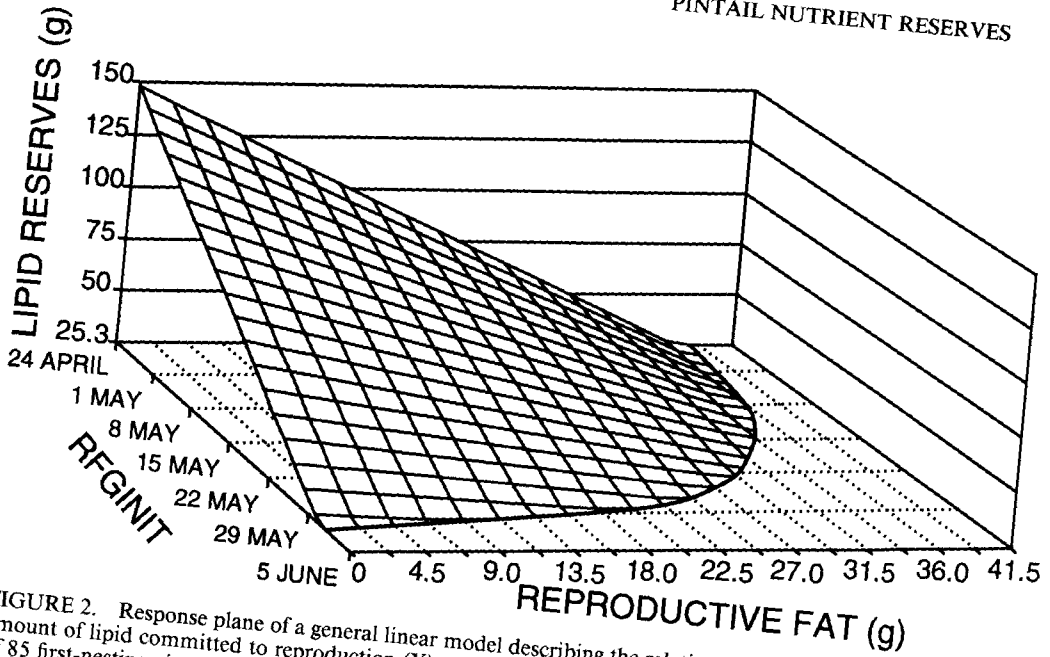


FIGURE 2. Response plane of a general linear model describing the relationship of lipid reserves (Z) with the amount of lipid committed to reproduction (X) and date of initiation of rapid follicle growth (Y) for a sample of 85 first-nesting pintails collected in subarctic Alaska, 1990–1991. An estimated lower lipid threshold of 25.3 g is the minimum value on the Z axis.

ample, there were no relationships between lipid reserves and less precise measures of future commitment such as the number of developing follicles or even the amount of lipid to be incorporated in the clutch ($P = 0.394$ and 0.356 , respectively). Similar measures for protein and mineral also were nonsignificant ($P > 0.15$). The intercept of the lipid model was 21.7 g, which

corresponds well to other estimates of lipid levels at the end of laying (see below). Mann and Sedingler (1993) suggested that these types of analyses could be flawed because a positive correlation could result from the relationships of both nutrient reserves and measures of future commitment with stage of laying (i.e., number of eggs already laid). Using analysis of variance, we found

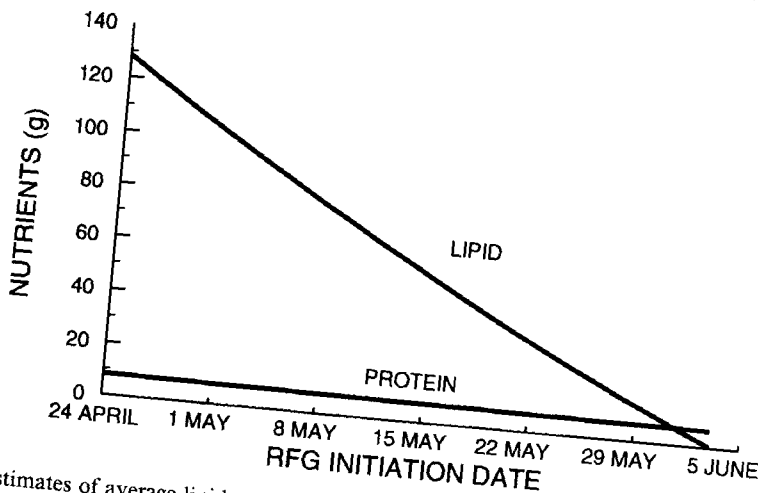


FIGURE 3. Estimates of average lipid and protein reserves used during clutch formation by female pintails in subarctic Alaska, through the nesting season.

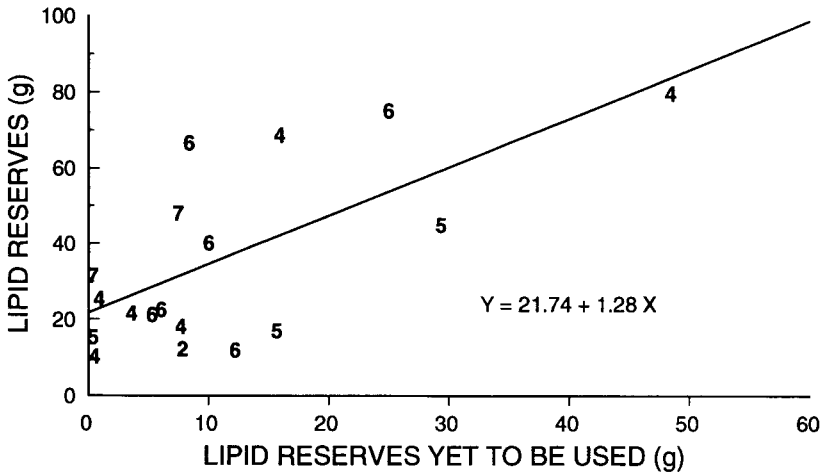


FIGURE 4. Relationship between lipid reserve levels (Y) and estimates of the amount of reserves that will be used during the remainder of clutch formation (X) for female pintails with known clutch sizes collected in subarctic Alaska, 1990–1991. Symbols are the number of ovulated follicles.

that the number of ovulated follicles was not related ($P = 0.532$) to lipid reserves (Fig. 4) and, thus, concluded that a spurious correlation was not acting on these data.

Average (\pm SE) levels of lipid, protein, and mineral of early incubators were 25.3 g (\pm 3.5), 124.4 g (\pm 2.7), and 25.0 g (\pm 1.1), respectively. Similarly, nutrient levels at termination estimated by applying slopes from models of nutrient reserve use to birds of known clutch size were 24.9 g (\pm 4.2), 122.0 g (\pm 1.9), and 23.9 g (\pm 0.6), respectively.

Estimated clutch size, using the lipid model and lower threshold, was similar to known clutch size (Fig. 5) until hens began relying on exogenous nutrients for clutch formation costs (late May). After that point, concordance between estimated and known clutch sizes dropped off as reliance on lipid reserves diminished. For the protein model, lack of agreement between estimated and known clutch sizes demonstrated primarily that there was not a consistent lower protein threshold.

DISCUSSION

NUTRIENT RESERVE DYNAMICS OF FIRST NESTS

Use of lipid reserves by female pintails supported our prediction of relatively high reliance on nutrient reserves. Comparing results of simple linear regressions (Table 1), rate of lipid reserve use

was similar to that of other Alaska pintails (Mann and Sedinger 1993) and averaged higher than that of Mallards (*Anas platyrhynchos*) (Ankney and Alisauskas 1991a, Young 1993) and Canvasbacks (*Aythya valisineria*) (Barzen and Serie 1990), which heretofore had shown the greatest reliance on lipid reserves among ducks. Lipid reserve use by pintails exceeded costs incurred by clutch formation for much of the season, i.e., lipid reserves also were used to meet maintenance or other costs. This represents a strategy intermediate to other ducks studied, which used lipid reserves equal to or less than their clutch requirements (Alisauskas and Ankney 1992), and northern nesting geese, which relied heavily or exclusively on endogenous reserves (Ankney and MacInnes 1978, Raveling 1979, Bromley and Jarvis 1993).

Reynolds (1972) hypothesized that body weight acted on the date of nest initiation and clutch size, proposing that heavier females initiated nests earlier and had larger clutches. Many studies have found that duck clutch sizes declined with nest initiation date (Rohwer 1992), including those of pintails (Duncan 1987b; Grand, unpubl. data), but relationships of nutrient reserves with clutch size and initiation date are not definitively known. Our data are the first to model Reynolds' (1972) premise regarding the relationship among all three factors. General linear models indicated that timing of first nests was related to the amount of lipid reserves at initiation as well as the rate

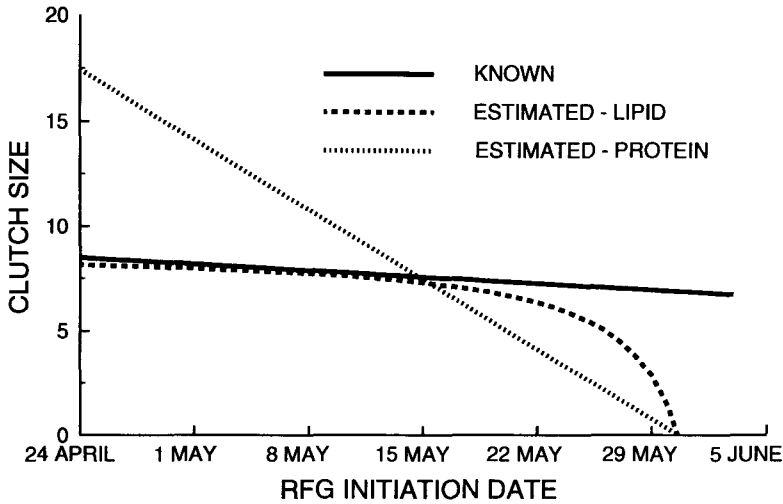


FIGURE 5. Comparisons of known clutch sizes of pintails with those estimated from models of nutrient reserve use and estimated termination thresholds.

at which lipid reserves were used (Table 2, Fig. 2). This implies a trade-off between advantages of early-nesting (Rohwer 1992) and higher costs of egg production. Females with larger reserves are able to nest early but those without adequate lipid reserves nest later when demands on reserves are reduced. Reynolds' (1972) hypothesis also is supported by agreement between seasonal declines in pintail clutch size with estimated clutch size declines based on lipid reserve dynamics (Fig. 5), and a lower threshold of lipid reserves that terminated clutch formation (Fig. 4) (see below for discussion of the limiting effect of lipid reserves).

Protein reserves declined slightly, approximately the amount contained in one egg, during formation of an average eight-egg clutch (Fig. 3, Table 2). A model of protein reserve use by laying pintails from another area in Alaska was not significant (Mann and Sedinger 1993), although the trend and estimated slope were similar to those we found. Protein levels of our Alaskan pintails declined at a rate less than that reported for American Wigeon (*Anas americana*; Ankney and Alisauskas 1991a) and similar to that for Gadwalls (Ankney and Alisauskas 1991b). Ring-necked Ducks (*Aythya collaris*) may have used protein during peak demands (Alisauskas et al. 1990). Other *Anas* and *Aythya* species maintained or gained protein through egg formation (Ankney and Alisauskas 1991a). Ankney and Alisauskas (1991a) considered use of protein re-

serves by American Wigeon, Gadwall, and Ring-necked Ducks a predictable outcome of their more herbivorous diets.

Use of protein reserves by Alaskan pintails could be necessary to meet a small protein deficit during clutch formation, because dietary protein may be in short supply, particularly early in the breeding season (Alisauskas and Ankney 1992). However, changes in protein levels are not necessarily caused by protein deficits (Alisauskas and Ankney 1992). Reductions in protein reserves of female pintails could result from muscle atrophy associated with the transition from migration to a more sedentary behavior regime. Also, gizzard dry mass declined 3.3 g during formation of an eight-egg clutch (Esler, unpubl. data), constituting nearly half of the estimated protein reserve reduction. This decline, and other changes in gut morphology, may be related to changes in diet (Kehoe et al. 1988) rather than costs of clutch formation.

Mineral reserves of female pintails declined by a small amount during clutch formation but we found no evidence that mineral acquisition was limiting. Other authors have found inter- and intra-specific variability in use of mineral reserves by breeding waterfowl (Ankney and Alisauskas 1991a, Young 1993).

NUTRIENT LIMITATION OF CLUTCH SIZE

Ultimate determination of clutch size in waterfowl has received considerable attention (Roh-

wer 1992). Arnold et al. (1987) proposed that clutch size of ducks is limited by a combination of egg viability and nest predation, and that nutrient acquisition has little effect. This does not appear to have explanatory value for variation in observed clutch sizes of Alaskan pintails. The optimal clutch size of 14 eggs (Arnold et al. 1987) would be extraordinary for pintails (Duncan 1987b; Grand, unpubl. data). Furthermore, our data suggest that nutrient reserves are necessary to produce the average pintail clutch. We believe that nutrient availability, specifically of lipid (see below), is the proximate limiting factor for most females, particularly those nesting early in the season. We agree that an upper limit on clutch size may exist, and the argument of Arnold et al. (1987) regarding the ultimate causes of this upper limit may be reasonable; our data do not address this. However, we suggest the ultimate upper limit would rarely act upon clutch size of pintails.

LIMITING NUTRIENT

Our results suggest that lipid requirements directly limit clutch size of pintail first nests, based on: (1) storage and relatively high use of lipid; (2) relationships among timing of nesting, lipid at initiation, and rate of lipid use; (3) a positive relationship between the size of lipid reserves and amount of lipid reserves yet to be committed by late layers; and (4) agreement between clutch sizes estimated by a model of lipid reserve dynamics and known clutch sizes.

The premise of the protein limitation hypothesis is that storage and use of lipid reserves occur to reduce the need for hens to spend time foraging for lipid-rich foods so that they can devote foraging efforts towards protein-rich invertebrates (Krapu 1981, Drobney and Fredrickson 1985, Drobney 1991). We agree that hens may not be able to forage for both protein and lipid components of eggs without a reduction in clutch size because of limitations on foraging, illustrated by the large number of invertebrates needed to meet protein demands of clutch formation (Drobney and Fredrickson 1985). However, we also agree with Ankney and Afton (1988) that the protein limitation hypothesis overemphasizes the role of protein and disregards other factors that likely influence lipid reserves including maintenance costs and the lipid component of the clutch. We conclude that lipid reserves impose a direct proximate limit on pintail clutch size, and that other

factors could indirectly, and secondarily, affect clutch size through their effects on lipid reserves.

Mann and Sedinger (1993) suggested that exhaustion of protein reserves directly limited clutch size of Alaskan pintails. However, we believe their conclusions were based on faulty methods and may not be valid. In particular, as a test for limiting nutrients, Mann and Sedinger (1993) regressed number of developing follicles against residuals from models of nutrient reserve use (i.e., regressions of nutrient reserves by reproductive nutrients). However, when applied across levels of reproductive nutrients, residuals do not indicate absolute amounts of remaining reserves, which are the critical values to compare to future clutch commitment when considering the existence of a lower threshold that terminates laying. Also, Mann and Sedinger (1993) presented a regression of protein reserves by proportion of clutch laid as evidence of limitation. However, that analysis was subject to the same confounding effects of stage of laying that they warn against.

We found no evidence that exhaustion of protein reserves limited clutch size of Alaska pintails, i.e., that protein had a direct proximate effect. Protein reserve use was slight and did not change through the season, and there was no relationship between protein reserves and protein needed to complete the clutch. Also, later nesting hens initiated RFG with smaller protein reserves than those of early nesters at the end of laying.

Lack of adequate dietary protein can affect reproductive performance (Krapu and Swanson 1975, Duncan 1987a, Eldridge and Krapu 1988). However, if clutch formation were generally limited by protein, we would expect pintails to use large amounts of endogenous protein, as do northern-nesting geese (Ankney and MacInnes 1978, Raveling 1979, Ankney 1984, Bromley and Jarvis 1993) and eiders (Korschgen 1977).

RENESTS

The strategy of nutrient acquisition by reneesting pintails is similar to that of late first nests. Their nutrient reserve dynamics and clutch size are that which would be expected for any bird nesting late in the season without large nutrient reserves.

Our results are similar to other studies that found reneesting females do not store and use nutrient reserves (Barzen and Serie 1990, Krapu 1981, Rohwer 1992). Arnold and Rohwer (1991) interpreted this as evidence that nutrient reserves

generally are not important for clutch formation. However, Ankney et al. (1991) suggested that this emphasizes the importance of nutrient reserves for most first nests and the role of nutrients in limiting clutch size. Renesters can rely on exogenous reserves during clutch formation because habitat quality and food availability may be improved from the time when the earliest nests are initiated; this may be particularly true for pintails. However, they still may be limited by their ability to forage for all of the egg components concurrently when daily costs of clutch formation are maximized, resulting in smaller clutch sizes in reneesting females.

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