



## Characterizing the nutritional strategy of incubating king eiders *Somateria spectabilis* in northern Alaska

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We measured plasma concentrations of variables associated with lipid metabolism (free fatty acids, glycerol, triglyceride, and  $\beta$ -hydroxybutyrate), protein metabolism (uric acid), and baseline corticosterone to characterize the nutritional state of incubating king eiders *Somateria spectabilis* and relate this to incubation constancy at two sites, Kuparuk and Teshekpuk, in northern Alaska. King eiders at both sites appeared to employ a partial-income incubation strategy, relying on both endogenous and exogenous energy resources. Females maintained high invariant levels of free fatty acids,  $\beta$ -hydroxybutyrate, and glycerol throughout incubation, indicating that fat reserves were a major energy source, and not completely depleted during incubation. Similarly, uric acid did not increase, suggesting effective protein sparing or protein ingestion and adequate lipid reserves throughout incubation. Baseline corticosterone and triglyceride levels increased during incubation, indicative of an increase in foraging during late stages of incubation. Incubating females at Kuparuk had higher triglyceride concentrations but also had higher  $\beta$ -hydroxybutyrate concentrations than females at Teshekpuk. This dichotomy may reflect a short-term signal of feeding overlaying the longer-term signal of reliance on endogenous lipid reserves due to higher food intake yet higher metabolic costs at Kuparuk because of its colder environment. Incubation constancy was not correlated with plasma concentrations of lipid or protein metabolites.

Female waterfowl rely to a varying degree on stored reserves to meet their maintenance needs during incubation, ranging from total reliance on endogenous reserves to near total reliance on exogenous resources (Afton and Paulus 1992). The importance of endogenous reserves for successful incubation has been demonstrated in several arctic-nesting waterfowl, including lesser snow geese *Chen caerulescens caerulescens* (Ankney and MacInnes 1978), and common eiders *Somateria mollissima* (Parker and Holm 1990). Afton and Paulus (1992) asserted that incubation behavior is strongly correlated with body size; larger species are able to accumulate more endogenous reserves and therefore can maintain higher incubation constancy (i.e. the percentage of time spent incubating eggs). This may also be seen at the individual level; females with greater body reserves can afford to feed less, resulting in higher incubation constancies. Females can benefit from increased incubation constancy through increased egg-hatchability (Afton and Paulus 1992), shortened incubation periods (Zicus et al. 1995), and increased egg survival as the majority of egg predation likely occurs during incubation recesses (Swennen et al. 1993). Thus, higher incubation constancy is positively related to nest success.

King eiders *S. spectabilis* are near the extremes of the waterfowl continuum in severity of climate at the nesting grounds and reliance on nutrient reserves for reproduction (Kellett 1999), although little is known about their breeding strategies. They are close in body size, mass loss during incubation, and incubation constancy to common eiders (Parker and Holm 1990, Kellett and Alisauskas 2000, Bolduc and Guillemette 2003), which arrive on the breeding grounds with large endogenous stores and fast throughout egg laying and incubation (Bolduc and Guillemette 2003). King eiders have been classified as relying primarily on endogenous reserves during incubation (Lawson 2006). However, we have observed feeding during incubation, but the degree to which they rely on exogenous resources is unknown. Feeding during incubation is likely a mechanism to slow the rate of mass loss, not prevent it entirely, and may allow females to complete incubation within a limited body mass safety margin (Mallory and Weatherhead 1993, Criscuolo et al. 2002). Waterfowl breeding strategies vary with the individual, and additionally appear to be spatially and temporally flexible at the population level (Klaassen et al. 2006). King eiders may differ in nutritional strategies between population segments

for example, incubation constancy varies between sites in northern Alaska while mass loss does not (R. L. Bentzen unpubl. data). This suggests that food intake rates are variable between sites and that king eiders utilize a mixed strategy of income and capital incubation.

Several studies have suggested that measurement of plasma metabolite concentrations (including triglycerides, glycerol, free fatty acids,  $\beta$ -hydroxybutyrate, and uric acid) provide useful information on physiological state (e.g. feeding vs. fasting; Williams et al. 1999, Jenni and Schwilch 2001) and patterns of fuel utilization (Jenni-Eiermann et al. 2002), and that baseline corticosterone (CORT) can be a reliable hormonal indicator of nutritional condition in various avian species (Love et al. 2005). Plasma triglycerides are the main form in which lipids are transported to the adipose tissues. Mobilization of triglycerides releases glycerol and free (non-esterified) fatty acids. Free fatty acid concentrations are usually regarded as proportional to fatty-acid release and oxidation, and plasma glycerol levels to glycerol turnover (cf. Jenni-Eiermann and Jenni 1996).  $\beta$ -hydroxybutyrate is primarily synthesized during fasting, replacing part of the glucose, especially in the brain (Robinson and Williamson 1980) and is an indicator of lipid catabolism, glucose shortage and fasting (Jenni-Eiermann and Jenni 1996). Uric acid is the main end product of nitrogen excretion in birds and as such can be used as an index of protein catabolism (Boismenu et al. 1992). CORT is known to play a key role in promoting gluconeogenesis, especially from protein (Chester-Jones et al. 1972), and is an important mediator regulating the use of endogenous energy stores (Robin et al. 1988, Groscolas and Robin 2001) and stimulating foraging (Astheimer et al. 1992). As incubation progresses, females may rely on exogenous resources, endogenous stores (lipid and protein), or a combination of the two. Females relying on endogenous stores may begin mobilizing protein stores when lipid reserves are depleted.

We evaluated the nutritional condition of incubating female king eiders by measuring levels of circulating corticosterone, and lipid and protein metabolites across incubation at two sites, Kuparuk and Teshekpuk, on the coastal plain of northern Alaska. Additionally we investigated the relationship between the nutritional state of incubating females and incubation constancy. We hypothesized that king eiders rely primarily on fat reserves during incubation, but also feed during incubation recesses. We predicted that, as body stores are depleted during incubation: (1) CORT would increase to redirect behavioral and metabolic processes from reproduction to foraging and promote gluconeogenesis (Chester-Jones et al. 1972), (2) triglyceride, free fatty acids, and uric acid levels would remain constant, reflecting constant lipid transport to the tissues, protein intake, and adequate fat reserves, and (3) glycerol and  $\beta$ -hydroxybutyrate would increase as lipids are mobilized from the tissues (Jenni-Eiermann and Jenni 1996, Hollmén et al. 2000).

Second, we hypothesized that females nesting at Kuparuk must have similar food intake rates in order to maintain similar mass loss rates, despite higher incubation constancy rates. We predicted that plasma metabolite levels would not differ between sites. Third, we hypothesized that at the individual level and assuming that food availability

remains the same, as incubation constancy decreases, food intake rates would increase and lipid mobilization would decrease, due to a trade-off between incubation constancy and time spent foraging for exogenous resources. We predicted that as incubation constancy decreases, triglyceride and uric acid levels would increase, and that glycerol, free fatty acids and  $\beta$ -hydroxybutyrate levels would decrease.

## Methods

### Study area and nest searches

We studied king eiders nesting at two sites on the North Slope of Alaska, one near Teshekpuk Lake ( $153^{\circ} 07'W$ ,  $70^{\circ} 25'N$ ), and another within the Kuparuk oilfields ( $149^{\circ} 41'W$ ,  $70^{\circ} 27'N$ ) in 2005 and 2006. The Teshekpuk study site was approximately 10 km inland from the southeast shore of Teshekpuk Lake and has experienced minimal human impact. The Kuparuk study site was in an area between the Colville and Kuparuk river deltas developed for oil production. Temperatures at Kuparuk were cooler during the years of the study (minimum daily temperature averaged  $1.5^{\circ} C$  lower; R. L. Bentzen unpubl. data). We systematically searched wetland basins on foot for king eider nests, beginning in mid-June at both sites. We calculated nest-initiation dates from estimated incubation stages as determined by candling eggs (Weller 1956), assuming a laying interval of one egg per day and an incubation length of 23 d (Lamothe 1973).

### Field methods

We opportunistically placed data loggers (HOBO-TEMP, Onset Computer Corporation) in nests at Teshekpuk and Kuparuk, and programmed them to record nest temperature every 2 min ( $n = 14$ ) in 2005. We used blown chicken eggs, dyed an olive green and attached to a bolt, to hold the thermistor probes. The bolt was driven into the ground under the nest, anchoring the probe and dummy egg in the nest (cf. Quakenbush et al. 2004). This allowed for a quick response to any change in temperature because the probe had only an eggshell between it and the incubating female. We defined incubation constancy as the percentage of time the female was on her nest over the week prior to blood sampling.

We trapped female king eiders on the nest using a drop or bow net between mid-incubation (8 d after initiation of incubation), and hatch (23 d after initiation) at both sites in 2005 and at Kuparuk in 2006. Blood samples ( $\sim 500 \mu l$ ) were collected via jugular venipuncture and transferred to microcentrifuge tubes; syringes and microcentrifuge tubes were treated with heparin prior to use. We kept blood samples on ice and centrifuged within 3 h of collection to separate plasma from red blood cells, and froze plasma immediately after separation. We took samples within 3 min of capture to determine baseline CORT and corticosterone-binding globulin (CBG) levels at Kuparuk in 2005 ( $n_{Kuparuk} = 20$ ) and 2006 ( $n_{Kuparuk} = 7$ ), and within 10 min of capture to determine fat metabolite levels in 2005 at both sites ( $n_{Kuparuk} = 22$ ;  $n_{Teshekpuk} = 13$ ). Blood

samples were collected once per bird; if blood sampling took longer than 3 min, we did not assay for CORT. We captured four individuals at Kuparuk in both mid and late incubation in 2005; all other individuals were captured only once in a season. We banded (USFWS aluminum bands) and weighed all captured birds.

### Metabolite determinations

We assayed plasma samples for five metabolites: free glycerol, triglyceride,  $\beta$ -hydroxybutyrate, free fatty acids, and uric acid (following Guglielmo et al. 2002 and Seaman et al. 2005). Metabolite concentrations were determined at Simon Fraser University using a Powerwave 340  $\times$  microplate spectrophotometer (BioTec Instruments, USA). We ran assays in 400  $\mu$ l flat-bottom, 96-well polystyrene microtitre plates (NUNC, Denmark), and measured free glycerol and triglycerides sequentially by endpoint assay (Sigma; 5  $\mu$ l plasma, 240  $\mu$ l reagent A, 60  $\mu$ l reagent B); free fatty acids were measured by endpoint assay (WAKO Diagnostics, Richmond, VA; 5  $\mu$ l sample, 100  $\mu$ l reagent A, 200  $\mu$ l reagent B, read at 550 nm). We measured uric acid using a Quantichrom endpoint assay kit (BioAssay Systems, Hayward, CA; 5  $\mu$ l sample, 200  $\mu$ l working reagent, read at 590 nm) and  $\beta$ -hydroxybutyrate by kinetic assay (Megazyme; D3-HBA reagent; 10  $\mu$ l sample, 272  $\mu$ l working reagent, 2  $\mu$ l enzyme, read at 492 nm). Intra-assay coefficients of variation (CV%) were 3.6% ( $n=10$ ) for glycerol, 3.3% ( $n=10$ ) for triglyceride, 4.0% ( $n=12$ ) for  $\beta$ -hydroxybutyrate, 4.2% ( $n=15$ ) for free fatty acids, and 5.7% ( $n=9$ ) for uric acid. Inter-assay CV% was 6.0% and 5.6% respectively ( $n=17$ ; eider samples were assayed with other samples). All samples for  $\beta$ -hydroxybutyrate, free fatty acids, and uric acid were assayed in only two plates per metabolite and mean metabolite concentrations for a Sigma hen pool were 2.21 and 2.09 mmol/l, 0.616 and 0.599  $\mu$ mol/l, and 0.550 and 0.548 mmol/l, respectively, for the two plates.

### Corticosterone determinations

We determined total plasma levels of CORT with a radioimmunoassay (Wingfield et al. 1992) at the University of Alaska, Fairbanks. CORT concentrations were measured in duplicate for each plasma sample after extraction in dichloromethane. We used recovery values (87-99%) following extractions to adjust assayed concentrations of CORT. We processed all samples collected in 2005 in one assay and samples collected in 2006 in a second assay. Intra- and inter-assay CV% were  $<2\%$ .

We used radioligand binding methods for measuring corticosterone binding globulin (CBG) based on those described in Deviche et al. (2001). We optimized incubation time (2 h), plasma dilution (1:198), and tritiated CORT concentration (6nM  $^3\text{H}$ ) for king eiders. Affinity (Kd) estimates were 9.2nM. The intra-assay CV% was 1%. We estimated free CORT concentrations using the equation of Barsano and Baumann (1989), outlined in Deviche et al. (2001).

### Statistical analysis

We examined variation in body mass in relation to day of incubation using analysis of covariance (ANCOVA) with site as a factor and day of incubation as a covariate (SAS Institute 1990). To investigate patterns of fuel utilization across incubation and between sites, we examined relationships among plasma chemistry variables and age of the nest (day of incubation) at Kuparuk and Teshekpuk using ANCOVA. Additionally, we examined variation in plasma levels of baseline total and free CORT and CBG binding capacity between years (2005-2006) and age of the nest using ANCOVA. We adjusted P-values using the Benjamini-Hochberg false discovery rate (Benjamini and Hochberg 1995). We examined residuals for normality (Shapiro-Wilk,  $P > 0.05$ ), outliers and influential observations, and homoscedasticity. Uric acid and free CORT concentrations were log transformed to meet the assumptions of ANCOVA. We included first order interactions between factors and covariates and removed nonsignificant interactions from the final models based on type III mean square errors.

We used the nonparametric Mardia-Watson-Wheeler test for more than two samples (Zar 1999) to determine if the distribution of timing of trapping females differed between sites. This analysis was conducted using Oriana (Kovach Computing Services 2005). Given the non-von Mises distribution of trap times (Watson's  $U^2$ -test,  $U^2 = 0.12$ ,  $P = 0.03$ ), and low concentrations, we used a linear nonparametric approach to correlate timing of trapping with metabolite level (Spearman's  $R$ ) and examined the graphs for nonlinear relationships. We adjusted P-values from the five correlations using the Benjamini-Hochberg false discovery rate (Benjamini and Hochberg 1995).

Lastly, we investigated the hypothesis that, at the individual level, as incubation constancy decreases, food intake rates increase and lipid mobilization decreases. We used general linear models (PROC GLM; SAS 1990) to evaluate the relationship between plasma concentrations of triglyceride, free fatty acid, uric acid, body mass and site on incubation constancy the week prior to blood sampling, while controlling for age at capture, using 6 *a priori* candidate models ( $n = 14$ ). We did not include CORT in the models as it severely limited the dataset. We excluded plasma concentrations of  $\beta$ -hydroxybutyrate and glycerol from the model set to reduce the number of models investigated. We did not include any nonlinear or interaction terms due to the limited dataset. Constancy was arcsin square root transformed to improve normality (Shapiro-Wilk,  $P > 0.05$ ). We used Akaike's information criterion adjusted for small sample size (AIC<sub>c</sub>) to select the best approximating models as it allowed us to evaluate a number of competing nested models without violating the rules of multiple comparisons and error rates (Burnham and Anderson 2002). Values are reported as means  $\pm$  SE (or circular SD).

### Results

We captured a total of 48 female eiders during days 8-23 of incubation at Teshekpuk and Kuparuk, Alaska, 2005

and 2006, however, levels of corticosterone, triglyceride, glycerol,  $\beta$ -hydroxybutyrate, free fatty acids and uric acid were not all measured in all females. During this period of incubation females lost an average of  $18.4 (\pm 3.6; F_{1,45} = 26.31, P < 0.05)$  g day<sup>-1</sup>, and weight loss was not different between sites ( $F_{1,45} = 0.65, P > 0.05$ ). The four females trapped during both mid- and late-incubation lost an average of  $19.1 (\pm 3.0)$  g day<sup>-1</sup>. On average females weighed  $1214.6 (\pm 21.9)$  g at the end of incubation (>18 days incubation).

Triglyceride levels increased with nest age ( $F_{1,33} = 4.84, P < 0.05$ ) while  $\beta$ -hydroxybutyrate ( $F_{1,29} = 0.61, P > 0.05$ ), glycerol ( $F_{1,33} = 0.81, P > 0.05$ ), free fatty acids ( $F_{1,31} = 0.06, P > 0.05$ ), and uric acid did not vary with nest age ( $F_{1,30} = 3.59, P > 0.05$ ; Fig. 1). Triglyceride levels were higher at Kuparuk ( $0.86 \pm 0.07$  mmol/l,  $n = 23$ ) than Teshekpuk ( $0.52 \pm 0.09$  mmol/l,  $n = 13$ ;  $F_{1,33} = 9.19, P < 0.05$ ), as was  $\beta$ -hydroxybutyrate (Kuparuk  $3.08 \pm 0.22$  mmol/l,  $n = 22$ , Teshekpuk  $2.05 \pm 0.31$  mmol/l,  $n = 10$ ;  $F_{1,29} = 6.76, P < 0.05$ ). Glycerol was lower at Kuparuk than Teshekpuk (Kuparuk  $0.28 \pm 0.02$  mmol/l,  $n = 23$ , Teshekpuk  $0.39 \pm 0.05$  mmol/l,  $n = 13$ ;  $F_{1,33} = 5.45, P < 0.05$ ), while free fatty acids ( $0.66 \pm 0.06$  mmol/l,  $n = 34$ ) and uric

acid ( $0.27 \pm 0.03$  mmol/l,  $n = 33$ ) did not vary between sites (FFA,  $F_{1,31} = 0.41, P > 0.05$ ; uric  $F_{1,30} = 1.61, P > 0.05$ ). Females were trapped between 09.26 and 21.50 h (local time) at Kuparuk and 09.27 and 19.33 (local time) at Teshekpuk and the distribution of trap times varied between Kuparuk ( $14.29 \text{ h} \pm 286 \text{ min}$ ) and Teshekpuk ( $12.52 \text{ h} \pm 96 \text{ min}$ ;  $W = 7.06, P = 0.03$ ). However, plasma levels of the five metabolites were not significantly correlated with trap time (Spearman's  $R < |0.03|, P > 0.05$ ).

Further analysis indicated that total and free CORT increased with nest age ( $F_{\text{total}, 1,23} = 7.58, P < 0.05$ ;  $F_{\text{free}, 1,23} = 11.83, P < 0.05$ ; Fig. 2) while CBG binding capacity did not ( $F_{1,23} = 1.56, P > 0.05$ ). Free CORT and CBG binding capacity did not vary between years ( $F_{1,23} < 2.3, P > 0.05$ ), while total CORT was higher in 2006 than 2005 ( $F_{1,23} = 5.32, P < 0.05$ ). Total CORT was not correlated with any of the metabolites in 2005 ( $R < 0.34, n = 18, P > 0.05$ ). Plasma metabolite levels were not determined in 2006.

King eiders had very high incubation constancy ( $0.98 \pm 0.01, \%$  time on nest). Females took an average of  $0.61 (\pm 0.08)$  recesses day<sup>-1</sup>, with an average length of  $31 (\pm 8)$  min. We did not find any correlation between

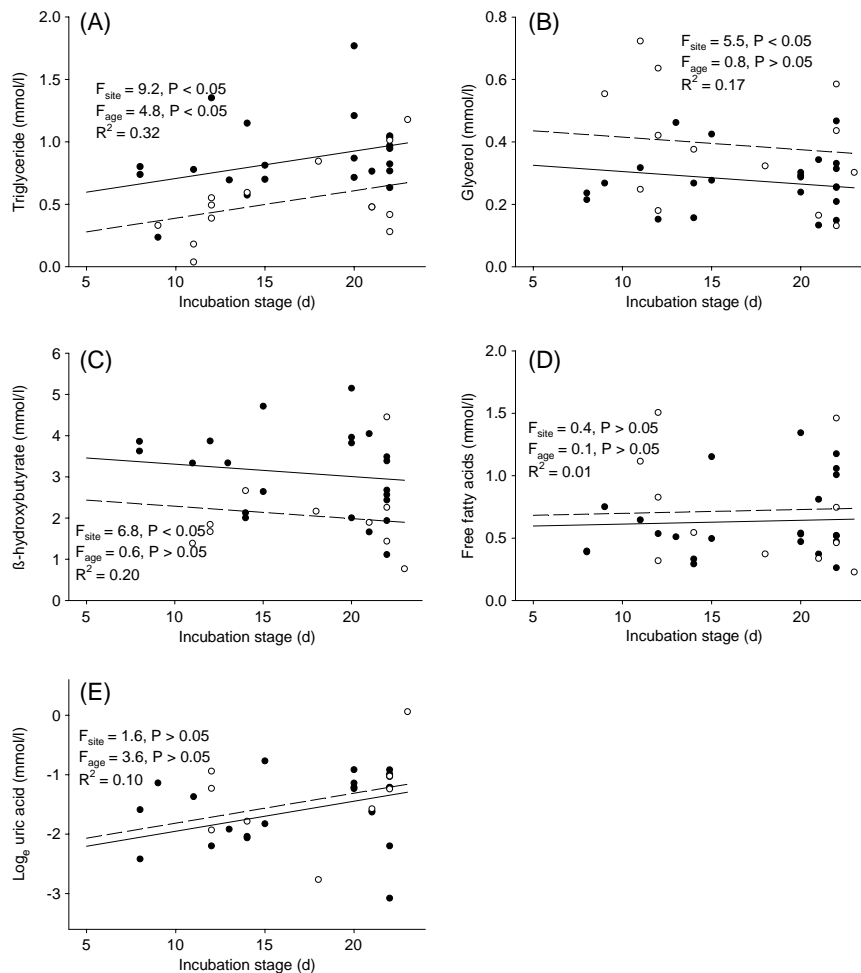


Figure 1. Plasma concentrations of triglyceride (A), glycerol (B),  $\beta$ -hydroxybutyrate (C), free fatty acids (D) and uric acid (E), and model predictions from analysis of covariance (ANCOVA; all interactions insignificant), relating incubation stage (age) to metabolite level (with site as a factor) in king eider *Somateria spectabilis* females at Kuparuk (filled circles, solid line) and Teshekpuk (open circles, dashed line),

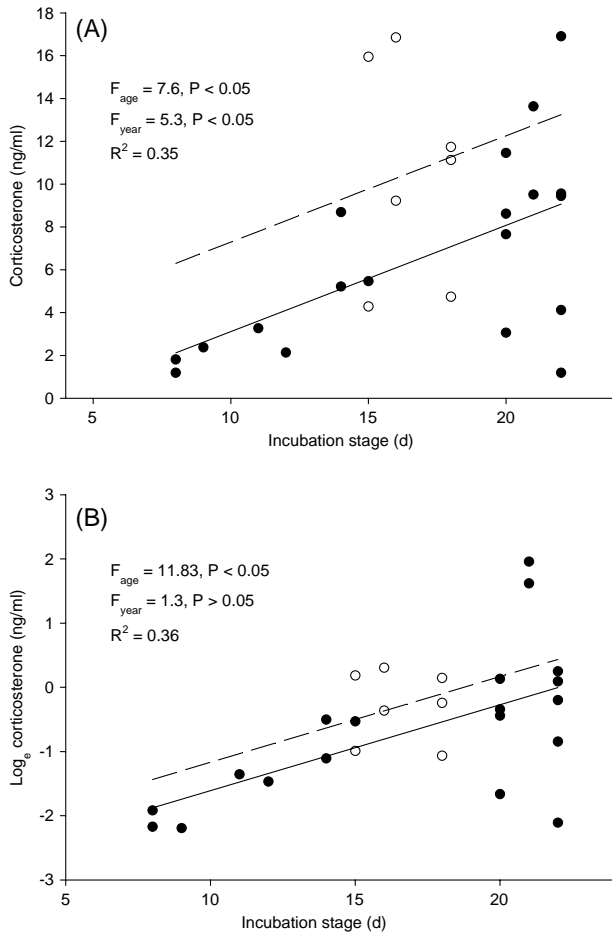


Figure 2. Baseline total (A) and free (B) plasma levels of corticosterone during incubation and model predictions from analysis of covariance (ANCOVA; all interactions insignificant), relating incubation stage (age) to corticosterone level (with year as a factor) in king eider *Somateria spectabilis* females at Kuparuk, Alaska, 2005 (filled circles, solid line), and 2006 (open circles, dashed line).

incubation constancy the week prior to blood sampling and plasma concentrations of triglycerides ( $\beta_{\text{triglyceride}} = -0.004$ , 95% CI =  $-0.106, 0.099$ ), uric acid ( $\beta_{\text{uric}} = -0.210$ , 95% CI =  $-0.605, 0.185$ ), or free fatty acids ( $\beta_{\text{FFA}} = > 0.000$ , 95% CI =  $-0.104, 0.105$ ; Table 1). The top model was 8.5  $AIC_c$  units from the next best model and supported an effect of body mass and nest age at

capture on incubation constancy. Incubation constancy the week prior to trapping increased with body mass ( $\beta_{\text{mass}} = 0.001$ , 95% CI =  $0.000, 0.001$ ), and nest age ( $\beta_{\text{age}} = 0.009$ , 95% CI =  $0.002, 0.017$ ), although effect sizes are small.

## Discussion

Our data suggest that king eider females nesting on Alaska's North Slope relied on both endogenous and exogenous energy resources during incubation, as hypothesized. However, levels of plasma metabolites across incubation differed from our predictions in some cases. Constant levels of free fatty acids,  $\beta$ -hydroxybutyrate, and glycerol were maintained throughout incubation indicating that fat reserves were not, in general, completely depleted during incubation (Le Maho et al. 1981, Groscolas 1986, Hollmén et al. 2000). Contrary to our predictions, plasma triglycerides actually increased over incubation, suggesting that king eiders were feeding throughout incubation and that total food intake increased as incubation progressed. Plasma uric acid concentrations did not increase in king eiders during incubation, indicating either effective protein sparing and adequate lipid reserves remaining through the end of incubation, or feeding rates high enough to prevent endogenous protein utilization and providing a constant intake of exogenous protein.

In fasting birds uncontrolled elevation of CORT concentration may lead to protein catabolism and therefore to the disturbance of lipid use and of the overall energetic balance (Cherel et al. 1988). Accordingly, common eiders (a capital breeder) maintain low thresholds of CORT throughout incubation; at least until body lipids are depleted, potentially to reduce body protein mobilization for energy supply (Criscuolo et al. 2006). However, in an income-breeder that feeds to some extent during incubation, CORT may increase as body reserves are depleted redirecting behavioral and metabolic processes from reproduction to foraging (Astheimer et al. 1992). King eiders showed both an increase in CORT and lipid transport to the tissues over the incubation period, indicating that they utilized a partial-income incubation strategy. It is likely that increased CORT stimulated foraging, thus increasing triglyceride levels and preventing fat reserve depletion (i.e. maintaining constant levels of lipid transport from the tissues). Potentially the increase in CORT allowed effective fat and protein sparing and thus the constant levels

Table 1. General linear models of incubation constancy the week prior to blood sampling for female king eiders *Somateria spectabilis* (n = 14) nesting at Teshekpuk and Kuparuk, Alaska, 2005. Models are sorted by  $AIC_c$  and incorporated parameters of site, nest age at trapping (age), body mass at trapping, and plasma concentrations of triglyceride, glycerol, free fatty acids,  $\beta$ -hydroxybutyrate, and uric acid.

Incubation constancy model	$K^a$	$AIC_c^b$	$\Delta AIC_c^c$	$w_i^d$	$R^2$
Age, mass	4	-74.33	0.00	0.98	0.60
Age	3	-65.82	8.51	0.01	0.01
Age, uric acid	4	-63.09	11.24	0.00	0.10
Age, site	4	-61.79	12.55	0.00	0.02
Age, triglyceride	4	-61.78	12.55	0.00	0.02
Age, free fatty acid	4	-61.77	12.56	0.00	0.01

<sup>a</sup> Number of parameters in the model. <sup>b</sup> Akaike information criterion adjusted for small sample size. <sup>c</sup> Difference between model  $AIC_c$  and  $AIC_c$  value of the best model. <sup>d</sup>  $AIC_c$  weights.

of  $\beta$ -hydroxybutyrate, glycerol, and free fatty acids across incubation; however, we found no correlation between CORT and triglyceride levels. This may be due to the small sample size of females where both CORT and triglyceride levels were measured. Interestingly, we detected an increase in both free and total CORT during incubation. There appeared to be no buffering of the 'active' portion of the hormone titer by CBGs, indicating that the increase of total CORT was biologically relevant.

A partial-income strategy does not necessarily imply primary reliance on exogenous resources. King eiders lose  $\sim 30\%$  of their pre-incubation body mass during incubation (this study, Kellett and Alisauskas 2000) and are certainly relying heavily on lipid reserves. Published concentrations of glycerol (mean  $\pm$  SD;  $288 \pm 124$   $\mu$ mol/l),  $\beta$ -hydroxybutyrate ( $3.20 \pm 1.92$  mmol/l), and triglyceride ( $1.02 \pm 0.37$  mmol/l) in common eiders (Hollmén et al. 2000) during late incubation were very similar to those found in king eiders, suggesting they have similar incubation strategies, both relying primarily on endogenous reserves. However, Hollmén et al. (2000) showed that, in some years, common eiders might transition to utilization of body proteins as a primary energy source after depleting their lipid reserves. We found no evidence of this in king eiders, however, we only examined one year and patterns of resource utilization may vary among years. King eiders may be able to prevent the depletion of lipid reserves through foraging throughout incubation and thus may not need to transition to body protein use in late incubation.

We predicted that eiders nesting at Kuparuk, which exhibited higher incubation constancy yet maintained similar weight loss to those at Teshekpuk, would show similar lipid transport to the tissues, indicating similar food intake rates, and similar rates of lipid mobilization from the tissues, reflecting similar mass loss. However, our results were more complex; higher triglyceride concentrations at Kuparuk indicated that females fed more relative to Teshekpuk (see Jenni-Eiermann and Jenni 1996), but higher  $\beta$ -hydroxybutyrate at Kuparuk indicated a higher rate of lipid mobilization in birds there (Le Maho et al. 1981, Groscolas 1986). This pattern may be reflective of short-term feeding during incubation breaks generating a transient "feeding" signal in terms of elevated triglyceride which overlays the longer-term "fasting" signal of elevated  $\beta$ -hydroxybutyrate over the course of incubation with different rates of metabolic response to feeding among the metabolites. There is some evidence for different rates of metabolic responses to feeding, but only over a short time scale and in passerines (Jenni-Eiermann and Jenni 1996, Zajac et al. 2006). The disjunct in king eiders on the coastal plain of Alaska could reflect site quality or ambient temperature differences. Temperatures at Kuparuk were cooler during the years of our study potentially leading to a greater cost of incubation (Fast et al. 2007). In addition, if females must expend more energy incubating, but also have access to higher quality food, we could see this pattern of higher food intake and higher levels of lipid mobilization. It would be expected that CORT levels would also vary between sites given the difference in triglyceride levels,

however, we were unfortunately unable to pursue this due to sample size limitations.

Incubating king eider females generally take one break per day, in the afternoon, (R. L. Bentzen unpubl. data) and have been observed feeding during these times. Since triglyceride and  $\beta$ -hydroxybutyrate levels can respond within 10-20 min to an increase in feeding rate (Zajac et al. 2006), timing of trapping in relation to daily feeding bouts could affect the results and could potentially explain the observed site differences. However, we do not feel that this can explain the observed site differences, as there were no correlations between trap time and metabolite level.

In conclusion, king eider females nesting on Alaska's North Slope relied on both endogenous and exogenous energy resources during incubation. Fat reserves were not, in general, completely depleted during incubation, potentially because females were feeding and total food intake increased as incubation progressed. Increased CORT across incubation may have stimulated this foraging, increasing triglyceride levels and preventing fat reserve depletion (i.e. maintaining constant levels of lipid transport from the tissues) and may have allowed effective fat and protein sparing. Females at Kuparuk had higher food intake rates relative to Teshekpuk but also higher rates of lipid mobilization. This dichotomy may reflect a short-term signal of feeding overlaying the longer-term signal of reliance on endogenous lipid reserves. It may indicate that females expended more energy incubating (cooler ambient temperatures), but also had access to higher quality food at Kuparuk. Food resources on the breeding grounds evidently play an important role in king eider reproduction.

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