Body Mass and Clutch Size May Modulate Prolactin and Corticosterone Levels in Eiders

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ABSTRACT

Altered body condition, increased incubation costs, and egg loss are important proximate factors modulating bird parental behavior, since they inform the adult about its remaining chances of survival or about the expected current reproductive success. Hormonal changes should reflect internal or external stimuli, since corticosterone levels (inducing nest abandonment) are known to increase while body condition deteriorates, and prolactin levels (stimulating incubation) decrease following egg predation. However, in a capital incubator that based its investment on available body reserves and naturally lost about half of its body mass during incubation, corticosterone should be maintained at a low threshold to avoid protein mobilization for energy supply. This study focused on the regulation of corticosterone and prolactin release in such birds during incubation, when facing egg manipulation (control, reduced, or increased) or a stressful event. Blood samples were taken before and after clutch manipulation and at hatching. Corticosterone levels were determined before and after 30 min of captivity. Female eiders exhibited a high hypothalamic-pituitary-adrenal sensitivity, plasma concentration of corticosterone being increased by four- to fivefold following 30 min of captivity. The adrenocortical response was not modified by body mass loss but was higher in birds for which clutch size was increased. In the same way, females did not show different prolactin levels among the experimental groups. However, when incubation started, prolactin levels were correlated to body mass, suggesting that nest attendance is programmed in relation to the female initial body condition. Moreover, due to an artifactual impact of bird manipulation, increased baseline corticosterone was associated with a prolactin decrease in the control group. These data suggest that, in eiders, body mass and clutch size modification can modulate prolactin and corticosterone levels, which cross-regulate each other in order to finely control incubation behavior.

Introduction

Costs of reproduction are traditionally defined as a fitness reduction induced by current reproductive investments. This concept leads to the hypothesis of shared energy stores between current and future reproduction (Stearns 1992). How animals regulate their current reproductive effort and their parental fitness is thus a central question of life-history theory. Behavioral decisions during reproduction reflect the physiological state of the adult animal (Drent and Daan 1980) and have been described as state-dependent decisions (MacNamara and Houston 1996; Gauthier-Clerc et al. 2001). For example, capital breeders like the common eider depend on endogenous reserves for egg production while fasting during incubation (Korschgen 1977; Drent and Daan 1980; Gabrielsen et al. 1991; Criscuolo et al. 2000). They adjust their parental effort during incubation according to the depletion of their body reserves and might desert their clutches once they reach a critical lower threshold (Chaurand and Weimerskirch 1994; Ancel et al. 1998; Criscuolo et al. 2002b). Studies on hormonal mechanisms underlying parental decisions have pointed out the role of prolactin that facilitates incubation behavior in birds (Buntin 1996). Prolactin is maintained by egg stimuli (Hall 1987). On the contrary, increased levels of corticosterone, the principal glucocorticoid secreted by the hypothalamic-pituitary-adrenal axis (HPA) in birds (Siegel 1980), redirect behavioral and metabolic processes from high-energy-demanding to emergency activities that potentiate survival (i.e., from reproduction to foraging; Silverin 1986; Wingfield et al. 1998). Because glucocorticoids can suppress reproduction, they play an important role in Darwinian

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fitness (Romero 2002). Recently, magnitudes of both unstressed and stressed glucocorticoid release have been suggested to change depending on the time of year, for example, being commonly elevated during the breeding season in birds (Romero 2002). However, in a fasting bird, uncontrolled elevation of corticosterone plasma concentration may lead to protein catabolism and therefore to the disturbance of lipid use and of the overall energetic balance (Cherel et al. 1988). Consequently, a trade-off must be set up between the need for corticosterone release for reproductive control and modulating corticosterone secretion to avoid fasting disturbance. Whether the adrenocortical response is related to body mass, body condition, or the amount of fat reserves in nonfasting birds (Astheimer et al. 1992; Hood et al. 1998; Long and Holberton 2004), it is still undefined how the degradation of body condition while fasting or the modification of incubation costs (e.g., clutch size; Criscuolo et al. 2003) may affect the ability to react to a potential stressor in a bird that naturally loses 40% of its initial body mass during reproduction. Moreover, an experimental increase in corticosterone is able to induce a decrease in prolactin levels (Criscuolo et al. 2005), giving support to a hypothetical balanced regulation of the respective levels of these two hormones and, therefore, of eider incubation behavior.

This study focused on how baseline or stress-induced corticosterone release are controlled in fasting eiders. Since eiders are fasting and breeding in an unpredictable arctic environment, we expected a low HPA sensibility to handling stress. Moreover, any change in corticosterone should be mirrored in an opposite way for prolactin in the hypothesis of reciprocal control. These changes would be induced by internal (body mass) or external factors (clutch size manipulation). To test this hypothesis, we experimentally modified clutch sizes and incubation costs, and we predicted that birds that suffered enlarged clutch sizes would exhibit increased baseline or stressed levels of corticosterone, as well as mirrored-decreased levels of prolactin.

Methods

The study was conducted in Kongsfjorden in June 1999 and 2001 on the western coast of the Svalbard Archipelago (78°55′N), Norway, in a colony of common eiders nesting on the mainland and on the small island of Prins Heinrich (about 200 nests). A total of 113 females were studied in this experiment.

Corticosterone Levels during the Breeding Cycle

In order to determine baseline corticosterone levels of females over the breeding season, birds were caught in 1999 either with a net when resting on the beach (preincubating females accompanied by a male) or on the nest using a bamboo pole with a nylon snare (incubating birds). Blood samples (1 mL) were obtained within 5 min of capture ($n = 67$). Eiders were divided into groups of preincubating females ($n = 6$), incu-

bating females (days 0–3, $n = 5$; days 4–10, $n = 6$; days 11–20, $n = 18$; days 21–24, $n = 9$), and females at hatching with at least one piping egg ($n = 23$).

How Are Baseline Levels of Corticosterone and Prolactin Modified by Clutch Size Manipulation and Body Mass Loss?

In 2001, we tested the impact of clutch size manipulation and body mass loss on hormonal levels of three groups of incubating females. All birds started incubation within the same 10-d period (June 10–20). Birds were caught on the nest after incubating for 5–7 d. Baseline corticosterone and prolactin concentrations were determined from the analysis of blood samples collected within 5 min of capture. Initial clutch size and body mass (± 2 g) were recorded. Females were separated into (1) a control group where eggs were exchanged but clutch size was not modified ($n = 10$), (2) a group with a clutch reduced to two eggs (the smallest natural clutch size; $n = 18$), and (3) a clutch-enlarged group with a postmanipulated clutch size of six eggs (the largest natural clutch size; $n = 14$). Birds for which clutch size was modified had all laid four eggs. For all experiments, the blood was collected from a wing vein and transferred to 5-mL tubes containing heparin (for prolactin measurements) or EDTA and kept on ice during transport to the laboratory. The samples were then centrifuged at 6,000 rpm for 5–10 min at 4°C. Finally, aliquots of plasma (200 μL) were put into several 1.5-mL microtubes and stored in a -20°C freezer until analysis. Corticosterone concentrations were measured by radioimmunoassay analysis, using an ^{125}I RIA double antibody kit from ICN Biomedicals (as described in Criscuolo et al. 2005). The radioimmunoassay for the prolactin concentrations were performed and validated at the Centre d'Ecologie et Physiologie Energétiques (CEBC), as already reported (Lormée et al. 2000; Criscuolo et al. 2002a).

One week after clutch manipulation (days 10–12), the birds still incubating were caught again, and a blood sample was collected within 5 min to determine baseline corticosterone and prolactin. Only six females of the control group, 10 of the clutch-reduced group, and 10 of the enlarged group could be caught a second time.

Is HPA-Axis Sensitivity Modulated by Clutch Size Manipulation and Body Mass Loss?

One week after clutch size reduction, females that were sampled within 5 min for the determination of the baseline levels of corticosterone and prolactin were also used to determine the corticosterone response to capture. To measure the adrenocortical-stimulated level of corticosterone, birds were thereafter kept in a black bag, and an additional blood sample was collected 30 min after capture. Birds were then weighed (± 2 g) and released. To complete the data collected on the influence of body mass on individual variations of adrenocortical re-

sponse, we measured in two additional groups of females the adrenocortical response to capture of female eiders: (1) during the prelaying period ($n = 5$), when females have the largest body mass, and (2) at the end of incubation ($n = 5$), when they had fasted for 24–26 d and lost 30%–40% of their initial body mass (Korschgen 1977).

Relationship between Corticosterone and Prolactin Changes

To look deeper into the relationship that might exist between corticosterone and prolactin levels in eiders, we looked for any correlation between baseline or poststress corticosterone and prolactin levels in birds used in 2001, both before and after clutch size manipulation. Analyses were also done by using changes in hormonal levels, defined as a magnitude ratio of the plasma levels measured both before-after egg manipulation and before-after 30 min of handling. For each individual, baseline corticosterone value before egg manipulation was substract or divided (ratio) by baseline corticosterone after manipulation. The same procedure was run for prolactin levels. All regressions were tested.

Statistical Analysis

After testing for normality (Shapiro-Wilk, $P > 0.01$), data were compared using t-tests (HPA response and body mass comparisons of pre- and late-incubating females), an ANOVA for repeated measurements (basal corticosterone levels) for the nonbreeding and breeding groups of females in 1999, and a two-factor ANOVA for repeated measurements (clutch groups and treatment) for the clutch manipulated females in 2001. Relationships among corticosterone levels, body mass, clutch size, prolactin levels, and sampling time were studied with a Pearson regression analysis. Values are means ± SE.

Results

Corticosterone during the Breeding Cycle

Baseline corticosterone did not differ significantly from the preincubating stage (fed females with a body mass of 2,168 ± 119 g) to hatching (after more than 3 wk of fasting, with a body mass of 1,364 ± 29 g), with an overall mean plasma concentration of 25.2 ± 1.4 ng/mL ($F_{5,62} = 0.22$, $P = 0.22$, data not shown).

How Are Baseline Levels of Corticosterone and Prolactin Modified by Clutch Size Manipulation and Body Mass Loss?

Plasma corticosterone concentrations did not change significantly with handling duration when females were sampled within 5 min of capture ($r = 0.039$, $F = 0.033$, $P = 0.8576$, $n = 42$) and were thus used as baseline corticosterone levels (before capture). Baseline levels of corticosterone in incubating females in the three groups were not significantly different before or after clutch manipulation (control [C] 10.3 ± 3.3, re-

duced clutches [R] 14.3 ± 2.3, enlarged clutches [E] 17.1 ± 3.7 ng/mL, $F_{2,21} = 0.12$, $P = 0.889$; Fig. 1). However, all groups exhibited a significant increase in their baseline levels of corticosterone 1 wk after clutch manipulation (C 20.9 ± 5.4, R 22.2 ± 4.4, E 25.1 ± 4.9 ng/mL, $F_{1,21} = 15.882$, $P = 0.001$). Indeed, baseline levels of corticosterone before and after clutch manipulation were correlated ($r = 0.614$, $y = 0.8814x + 10.977$, $P = 0.001$). We did not find any relationship between the baseline corticosterone level and body mass ($r = 0.053$, $P = 0.804$) or clutch size ($r = 0.085$, $P = 0.694$) before or after clutch manipulation ($r = 0.077$, $P = 0.722$; $r = 0.189$, $P = 0.378$, respectively).

Prolactin levels were correlated to initial body mass in the clutch manipulated groups, heavier birds having the higher prolactin concentrations (before egg manipulation, $r = 0.489$, $P = 0.013$; Fig. 2A). In contrast, prolactin levels were not correlated to clutch size before or after egg manipulation ($r = 0.104$, $P = 0.622$; $r = 0.005$, $P = 0.98$, respectively). Plasma prolactin levels of incubating female eiders were similar before (C 50.0 ± 1.1, R 49.8 ± 0.9, E 49.8 ± 0.5 ng/mL) and after clutch size manipulation (C 46.9 ± 1.3, R 43.5 ± 1.5, E 45.1 ± 1.4 ng/mL, $F_{2,21} = 0.104$, $P = 0.902$; Fig. 2B). Prolactin concentrations were decreased in all groups 1 wk after the clutch size manipulation ($F_{1,21} = 8.038$, $P = 0.01$). However, decreasing or enlarging clutch sizes cannot be associated with a higher decrease in plasma prolactin concentration ($F_{2,21} = 0.123$, $P = 0.885$).

Is HPA-Axis Sensitivity Modulated by Body Mass or Clutch Size Modification?

In incubating female eiders, there is no influence of body mass (initial or final) on the adrenocortical response after 30 min of handling, as shown by the regression analysis conducted on the three experimental groups ($r = -0.106$, $P = 0.613$; $r =$
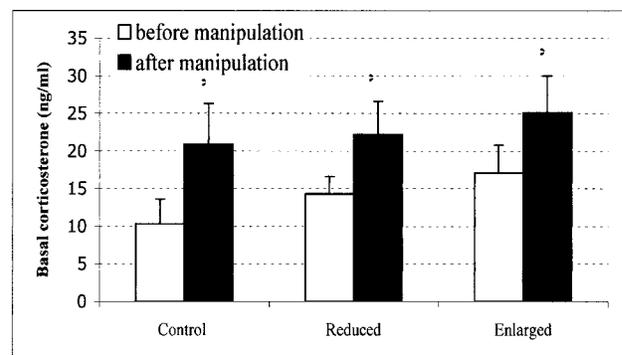

Figure 1. Baseline plasma levels of corticosterone (females sampled within 5 min of capture) before and 1 wk after egg manipulation. Values are means ± SE. An asterisk indicates intragroup significant difference. No groups differed from each other.
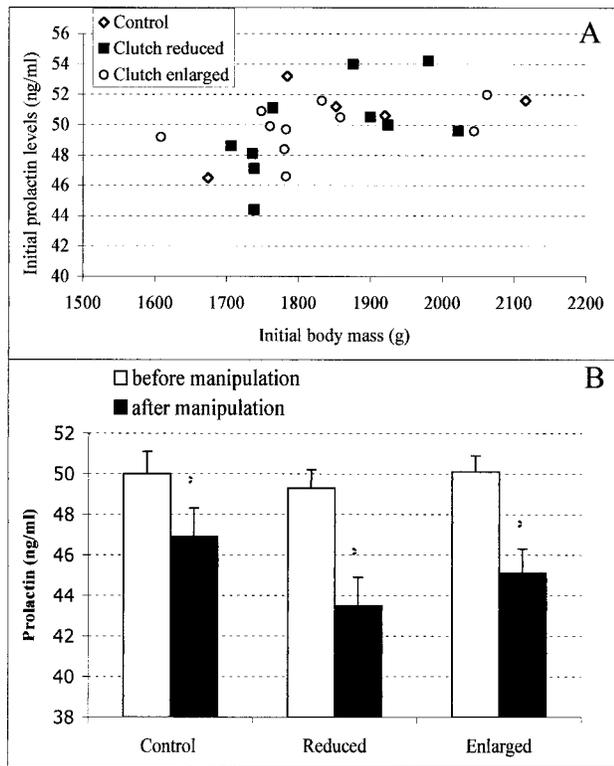
Figure 2. *A*, Plasma levels of prolactin (in the three experimental groups) in relation to the initial body mass. Pearson's correlation: $y = 0.09x + 33.8$, $r = 0.49$, $P = 0.013$. *B*, Plasma levels of prolactin (females sampled within 5 min of capture) before and 1 wk after egg manipulation. Values are means $\pm$ SE. An asterisk indicates intragroup significant difference. No groups differed from each other.

-0.245, $P = 0.238$, respectively; Fig. 3*A*). Similarly, despite preincubating females being significantly heavier than late-incubating females, that is to say, females closed to hatching (2,236 $\pm$ 87 vs. 1,408 $\pm$ 38 g, respectively, *t*-test, $t = 6.06$, df $= 8$, $P = 0.009$), these females showed comparable baseline corticosterone (20.7 $\pm$ 3.7, 19.9 $\pm$ 3.9 ng/mL, respectively, $t = 0, 14$, df $= 8$, $P = 0.45$) and a similar adrenocortical response to capture and handling (54.4 $\pm$ 11, 67.5 $\pm$ 12.2 ng/mL, respectively, $t = -0, 74$, df $= 8$, $P = 0.24$). However, corticosterone levels after handling were significantly higher in both groups ($t = -2, 91$, df $= 8$, $P = 0.022$; $t = -3, 71$; df $= 8$, $P = 0.0029$).

All three clutch-manipulated groups exhibited a strong response to handling after 30 min, their plasma concentrations of corticosterone being around fourfold higher after capture ($F_{1,22} = 222.168$, $P < 0.001$). The rate of increase in corticosterone levels after 30 min reached 267% $\pm$ 89% (*C*), 487.6% $\pm$ 127.6% (*R*), and 627.4% $\pm$ 288.1% (*E*) of their respective basal values. When comparing the acute adrenocortical response of the three experimental groups, we found that the females incubating an enlarged clutch exhibited higher 30-min corticoste-

rone levels than the control groups (*C* 76.6 $\pm$ 5.6, *R* 89.2 $\pm$ 6.2, *E* 108.0 $\pm$ 7.5 ng/mL, $F_{2,22} = 4.42$, $P = 0.024$; Fig. 3*B*). Similarly, a bigger clutch was correlated to a higher corticosterone level after 30 min of handling ($r = 0.445$, $P = 0.026$; Fig. 3*C*).

The Relationship between Corticosterone and Prolactin Changes

We found a significant negative relationship between differences, calculated as before-after egg manipulation plasma levels for each
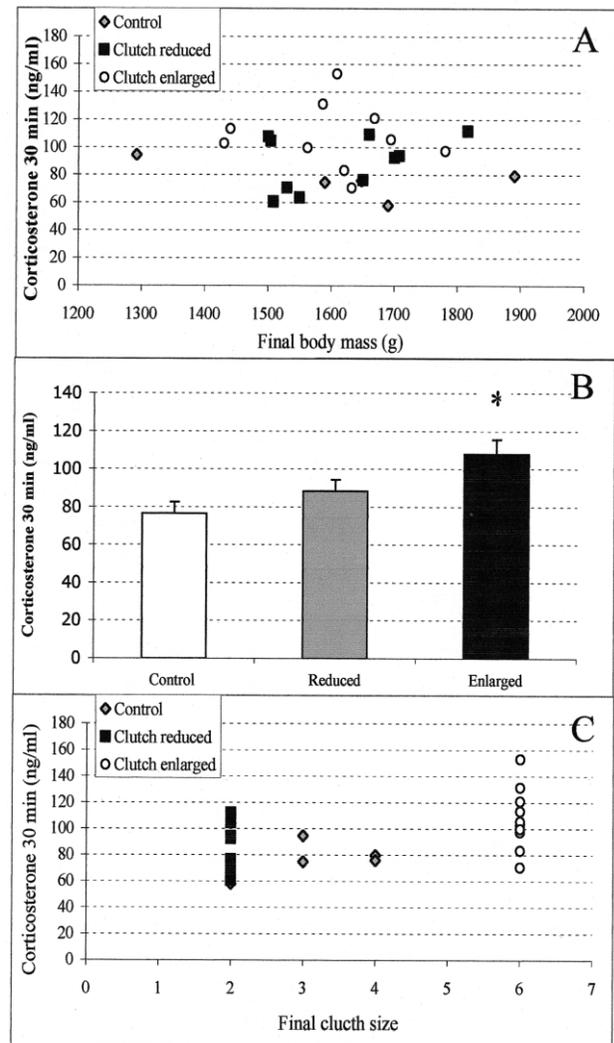

Figure 3. *A*, Plasma levels of corticosterone after 30 min of capture (in the three experimental groups) in relation to the female's body mass 1 wk after clutch manipulation. Pearson's correlation: $y = -0.075x + 106.3$, $r = -0.25$, $P = 0.238$. *B*, Plasma levels of corticosterone after 30 min of capture, 1 wk after egg manipulation. Values are means $\pm$ SE. An asterisk indicates intergroup significant difference. *C*, Plasma levels of corticosterone after 30 min of capture (in the three experimental groups) in relation to the clutch size 1 wk after clutch manipulation. Pearson's correlation: $y = 5.46x + 73.2$, $r = 0.45$, $P = 0.026$.

hormone, in baseline corticosterone and prolactin levels only in the control group ($y = -0.111x - 2.1405$, $r = -0.94$, $P = 0.029$), whereas the regression conducted after pooling the values of the three groups was not significant ($r = 0.091$, $P = 0.696$). However, all groups pooled, prolactin levels before egg manipulation were negatively related to the ratio of 30-min corticosterone increase after handling ($r = -0.446$, $P = 0.029$). The entire regression analysis is presented in Table 1.

Discussion

Our study shows that (1) body mass changes (within the mass range recorded here) have no impact on corticosterone levels of incubating eiders, either baseline or after exposure to handling stress; (2) baseline levels of corticosterone were higher during the second compared with during the first blood sample, traducing an artifactual impact of bird manipulation on baseline corticosterone measurements; (3) adrenocortical reaction was influenced by clutch size enlargement, as females incubating an increased clutch showed higher 30-min corticosterone levels; (4) prolactin levels did not change after clutch manipulation and were not correlated to body mass loss; (5) prolactin levels before egg manipulation were positively correlated to body mass, and females that had high prolactin levels before clutch size manipulation were also those that showed the lowest corticosterone levels after 30 min of handling; and (6) the artifactual rise in corticosterone induced by bird manipulation is correlated with a decrease in prolactin levels in control females.

Prolactin and Corticosterone Interaction

Clutch size reduction or egg addition did not have a specific impact on prolactin levels, which does not agree with the observation that prolactin secretion is proximately controlled by stimuli resulting from the number of eggs present in the nest (Hall 1987). Surprisingly, we found that prolactin levels had decreased in all groups 1 wk after clutch manipulation. Concomitant to this prolactin decrease, birds also showed higher baseline levels of corticosterone when caught a second time. Therefore, because of this unexpected artifactual stress induced by the capture method, higher baseline corticosterone levels could be responsible for the downregulation of plasma prolactin concentrations (as observed in the control group). After experimental corticosterone treatment, female eiders showed decreased prolactin levels (Criscuolo et al. 2005). Moreover, we found that birds having the highest prolactin levels before egg manipulation showed the lowest rise of corticosterone after stress exposure. Consequently, our fortunate capture artifact supports the idea that corticosterone and prolactin plasma levels are linked by closely related interactions that may influence parental behavior. The lack of this hormonal relationship (increase in baseline corticosterone/decrease in prolactin) in the clutch manipulated group is likely to be due to the fact that other factors, like egg manipulation, are also able to modulate the endocrine status of the birds.

Table 1: Regression analysis of prolactin and corticosterone changes before and after egg manipulation of incubating female eiders ($n = 26$), all experimental groups pooled

	PRL before EM	PRL after EM	Basal C before EM	Basal C after EM	30-min C	C Difference	C Rate of Change	PRL Difference
PRL after EM	**.501**							
	.013							
Basal C before EM	.099	−.183						
	.644	.403						
Basal C after EM	.174	−.189	**.614**					
	.406	.376	**.001**					
30-min C	.074	−.098	.258	.276				
	.727	.618	.223	.181				
C difference	−.056	−.053	−.300	**−.690**	−.059			
	.805	.815	.174	**.000**	.794			
C rate of change	**−.446**	−.028	**−.552**	**−.764**	−.194	**−.456**		
	.029	.808	**.006**	**.000**	.364	**.038**		
PRL difference	.016	**.900**	**−.425**	−.242	−.103	.091	−.121	
	.945	**.000**	**.050**	.290	.656	.696	.610	
PRL rate of change	.330	**.942**	−.242	−.281	−.050	−.107	.128	**.938**
	.133	**.000**	.278	.205	.827	.637	.581	**.000**

Note. The table gives the Pearson correlation value and the P value (bilateral) for each regression. Significant relationships are in bold. PRL = prolactin plasma concentration; EM = egg manipulation; C = corticosterone plasma concentration; 30-min C = corticosterone level after 30 min of handling; C difference = difference between basal corticosterone concentration before-after egg manipulation; C rate of change = ratio of basal corticosterone levels before-after egg manipulation; PRL difference = difference between prolactin levels before-after egg manipulation; and PRL rate of change = ratio of prolactin levels before-after egg manipulation.

*Internal and Environmental Factors That Modulate
Hormonal Status*

As we suggested in previous work (Criscuolo et al. 2002*a*), internal factors such as reduced body reserves, energy status, and metabolic flux could be involved in the modulation of prolactin levels and, thus, parental care in a capital incubator. Accordingly, we found a relationship between initial body mass and prolactin; the heavier the females were, the higher the prolactin levels. Heavier females have previously been shown to be more attentive to their nests in eiders (Erikstad and Tveraa 1995), thereby giving support to the hypothesis that investment in incubation and parental behavior may be programmed at the beginning of incubation, according to the amount of body reserves available. Consequently, the determination of other hormonal links between body condition and the central nervous system (and thus prolactin secretion) could be of interest. In this regard, the measurement of leptin secreted by adipose tissue known to modulate prolactin secretion in mammals (Watanobe et al. 1999) could provide new information. Fasting birds may enter in a critical phase of fasting that has been associated with reproductive abandonment (phase 3; Le Maho et al. 1981) and with a decrease in plasma prolactin concentrations (Cherel et al. 1994). Thus, there may be a lower threshold in prolactin that directly induces nest abandonment, as shown by an experimental dramatic decrease using bromocriptin (F. Criscuolo et al., unpublished data).

In contrast to other studies (Astheimer et al. 1992; Hood et al. 1998), eiders do not show a concomitant rise in corticosterone with the decrease in body mass while fasting. When looking at fasting physiology, it is primordial that birds avoid high levels of corticosterone and the associated enhanced protein catabolism (Cherel et al. 1988). During fasting, corticosterone is implicated in reproduction failure only when a low threshold in body lipid reserves is reached (Cherel et al. 1988; Pereyra and Wingfield 2003). Indeed, none of our birds was in a critical energetic situation as described by Criscuolo et al. 2002*b*), and there was no change in adrenocortical response with the body mass loss in eiders that mainly corresponds to body fat use (Korschgen 1977). These data apparently contrast with what is observed in fasting migratory birds, in which the adrenocortical response to stress is increased in birds that have sufficient body fat reserves left (Jenni et al. 2000). However, it seems that what Jenni et al. called emaciated birds were birds that had already entered in phase 3 of fasting, a state of emergency in which an increase of corticosterone levels would have no great interest. Therefore, we would conclude that body mass loss during fasting does not modulate adrenocortical response to stress, because fasting is a normal state of the annual cycle of those birds. According to our observation of the relationship with initial body mass, prolactin could lock corticosterone secretion, HPA sensitivity, and the incubation behavior until the critical lipid threshold is eventually reached (and corticosterone

rise for gluconeogenesis). This is further illustrated by the fact that females having the higher prolactin levels before egg manipulation also had the lowest ratio of corticosterone increase after 30 min of handling. Such a negative relationship has recently been illustrated in failed breeders of kittiwakes (*Rissa tridactyla*), where stress-induced corticosterone and prolactin levels are inversely correlated (Chastel et al. 2005). Few data are available about the mechanisms linking corticosterone and prolactin interactions. In mammals, prolactin potentiates corticosterone secretion (Lo and Wang 2003). Interestingly, oxytocin seems to inhibit prolactin release while stimulating corticosterone secretion (Muir and Pfister 1988). Controlling for such a regulation by avian oxytocin (mesotocin), known to have behavioral effects in birds (Jonaidi et al. 2003), would be a first step in the understanding of prolactin and corticosterone control of reproduction.

Adrenocortical response in birds can vary with latitude, but whether the adrenocortical response is lower in Arctic-living birds compared with temperate species is still a subject of debate (Astheimer et al. 1995; Silverin et al. 1997; Silverin and Wingfield 1998). Given that female eiders breeding in the high Arctic showed corticosterone levels increased by four- to fivefold (Fig. 3*B*), eiders face two options. First, they can be unaffected by stressful events and continue incubation no matter what the breeding conditions are. The continuation of reproductive behavior despite a robust adrenocortical response to capture has previously been illustrated in the tree sparrow (*Spizella arborea*) breeding in Alaska (Astheimer et al. 2000). However, the tree sparrow is a short-lived bird for which global fitness depends more on seasonal fecundity than on adult survival. Thus, and second, eiders should have a high adrenocortical sensitivity in order to promote adult survival when breeding conditions deteriorate, because the common eider is a long-lived species (20 yr; Korschgen 1977). Favoring adult survival, and thereby future reproduction, rather than a costly breeding attempt is in accordance with life-history predictions (Stearns 1992). Interestingly, all birds but two finished incubation after the capture and handling experiment. This suggests that despite reaching high plasma corticosterone concentrations, a rapid return to baseline levels allows the female to resume incubation. This could be explained by the corticosteroid-binding globulins, which buffer high corticosterone levels and reduce chances of nest desertion during stress (Love et al. 2004; but see Romero 2002).

Increased adrenocortical responses were found in eiders that incubated enlarged clutch size. This observation may illustrate the lower reproductive value of increased clutch sizes in eiders, as higher corticosterone levels, in theory, increase the risk of nest desertion (Silverin 1986). The common eider is a determinate layer, and clutch size is already fixed before the first egg is laid (Svennen et al. 1993). Intraspecific nest parasitism is quite common in eiders (Robertson et al. 1992), and egg predation is known to be higher in parasitized than in nonparasitized nests (Bjørn and Erikstad 1994). This suggests a higher cost for the

adult eider to incubate more eggs than initially programmed, as observed in passerines (Siikamäki 1995) or in Wilson's phalarope (*Phalaropus tricolor*; Delahanty and Oring 1993). Thus, our results are in line with previous data showing that egg addition in eider nest increases triiodothyronine levels, suggesting a concomitant rise in metabolic rate, adult heat production, and incubation costs that may endanger adult survival (Criscuolo et al. 2003).

In conclusion, we did not observe a change in reaction to capture and handling during incubation while eiders lost body mass, reaction to handling being strong. Such species breeding in less-predictable environments should show a high amplitude of corticosterone response (Holberton and Able 2000), but HPA sensibility may also depend on the degree of parental investment by the sexes (Wilson and Holberton 2005) and may vary when the risk of brood loss is important (i.e., when fat reserves are exhausted). Therefore, whether low basal corticosterone may be attributed to fasting adaptation and to the low rate of protein mobilization, reaction to stress may be differently controlled before and after entering in phase 3 of fasting. At this stage, females must balance the demands of breeding with the demands of immediate survival, and we know that basal corticosterone is then at its highest level (Criscuolo et al. 2002*b*). In this context, a large adrenocortical reaction to stress to warn the female about the urgency of starting to forage may not be necessary, and HPA sensibility is consequently reduced. However, comparisons with temperate-zone breeding eiders remain to be done. Moreover, and contrary to what is suggested for most of the bird species (Romero 2002), eiders seem to have no seasonal differences in glucocorticoid release (as shown by the prelaying birds). Whether wintering or molting eiders react differently to a stressor than breeding eiders remains to be established. We found several links relating body mass and prolactin, or clutch size and corticosterone, which is in accordance with Pettifor et al.'s suggestion (1988). Also, our study illustrated that the birds lay the size of clutch for which they are prepared to incubate, from a physiological and endocrine point, and that any change in egg number may reduce reproduction success.

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