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The Effects of Acute Restraint Stress on Plasma Levels of Prolactin and Corticosterone across Life-History Stages in a Short-Lived Bird: Gambel's White-Crowned Sparrow (*Zonotrichia leucophrys gambelii*)

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ABSTRACT

q1 The general reproductive effort model attempts to predict the resources that will be allocated to a current reproductive bout or to future survival by aborting the current reproductive attempt. Life-history theory predicts that short-lived species should devote more resources toward a reproductive event because brood value is far greater compared with that of long-lived species that have multiple breeding opportunities. Previous bird studies have used patterns of hormone secretion to understand the regulation of parental investment in response to environmental challenges, such as stress. The two key hormones investigated have been prolactin, which promotes parental investment, and corticosterone, which can reduce parental investment. Research on long-lived seabirds showed that prolactin levels decrease in response to a stressor, but the magnitude of the decline was positively correlated with future reproductive potential. However, little is known about the role of prolactin in short-lived species. Here we present prolactin and corticosterone data from a short-lived Arctic breeding, migratory songbird—the white-crowned sparrow, *Zonotrichia leucophrys gambelii*—at multiple stages of the breeding and nonbreeding seasons following standardized acute restraint stress. These data show that both prolactin and corticosterone are modulated seasonally. Corticosterone levels increased significantly in response to acute restraint stress during the breeding season in both sexes, but prolactin levels did not change in response to acute restraint stress at any stage of the annual cycle. We found no relationship between corticosterone or prolactin at either baseline or peak induced levels during any stage of breeding.

Keywords: hypothalamic-pituitary-adrenal axis (HPA), stress, parental investment, life-history theory, brood value.

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Introduction

Life-history theory predicts that animals will adjust physiology, morphology, and behavior to maximize fitness at different periods of the annual cycle (Wingfield 2008). In terms of energetic demands, breeding is considered one of the most demanding stages within the annual cycle. Events leading up to or disturbances during the breeding season can influence whether an individual initiates breeding or continues with an ongoing breeding effort. The general reproductive effort model attempts to understand the costs of reproduction and the adaptive trade-offs that influence an individual's decision to breed (Williams 1966). Reproductive effort is highly influenced by the lifetime reproductive potential of an individual (Bókony et al. 2009). For instance, short-lived birds should devote more resources toward a reproductive event because lifetime residual reproductive potential is low, and as a consequence, brood value is far greater than that of long-lived birds (Williams 1966). In addition, the duration of the breeding season can influence reproductive effort when probability of successful renesting is low, such as in the Arctic, as described by the short season hypothesis (Wingfield et al. 1995; Holberton and Wingfield 2003).

Application of endocrine mechanisms to this problem contributes to understanding life-history trade-offs during the breeding season, since they may be intricately tied to the maintenance of energy balance (McEwen and Wingfield 2003; Romero et al. 2009). Harsh environmental conditions or accumulated wear and tear on an individual may lead to negative energy balance that will force the abandonment of the current life-history stage and entry into the emergency life-history stage (Wingfield et al. 1998; McEwen and Wingfield 2003; Romero et al. 2009). In response to environmental perturbations (e.g., predation, storms, food shortages), the hypothalamic-pituitary-adrenal (HPA) axis is activated, resulting in a rise in circulating corticosterone, which then mediates its effects through mineralocorticoid and glucocorticoid receptors (Sapolsky et al. 2000; Wingfield and Sapolsky 2003). In birds, corticosterone is the primary stress hormone associated with entry into the emergency life-history stage because it fa-

cilitates changes in physiology, morphology, and behavior to enhance self-preservation (Wingfield et al. 1998; Wingfield 2008).

Many birds and mammals display seasonal changes in their glucocorticoid response to acute restraint stress (Romero 2002), and numerous Arctic breeding songbird species exhibit a higher stress response at the beginning of the breeding season, when conditions tend to be poor and social instability is high (Holberton and Wingfield 2003; Meddle et al. 2003; Krause et al. 2015). Entry into the emergency life-history stage during the breeding season can have detrimental consequences on reproductive success. Therefore, the parental care hypothesis postulates that birds will decrease the magnitude of the stress response as they become parental in order to facilitate parental investment (Wingfield et al. 1995), with the greatest attenuation of the HPA axis occurring in the sex that provides the most parental care (O'Reilly and Wingfield 2001). In addition, when brood value is high, the stress response is further reduced to ensure successful fledging of young (Lendvai et al. 2007; Bókony et al. 2009). However, in the face of a severe environmental perturbation, nest abandonment will eventually occur because sustained elevations in corticosterone have been linked to reproductive failure as energy is devoted toward self-preservation (Wingfield et al. 1983; Astheimer et al. 1995; Spée et al. 2010).

Although much effort has focused on stress and the measurement of stress hormones in relation to nest abandonment, prolactin is reported to work in opposition to corticosterone (Angelier and Chastel 2009). Prolactin is a pituitary hormone that may prevent or delay entry into the emergency life-history stage because it promotes parental investment across taxa (Buntin 1996). Prolactin is thought to mediate the transition from the preparental to parental phase as levels increase at nesting onset and plateau during the parental phase (Wingfield and Goldsmith 1990). In addition, increased prolactin levels are thought to be involved with the degree of parental investment (Angelier et al. 2007a; Angelier and Chastel 2009; Miller et al. 2009), as experimental manipulation of prolactin levels resulted in increased brooding, feeding rates, and/or chick protective behaviors in **q2** ring-necked doves, *Streptopelia capicola* (Buntin et al. 1991; Koch et al. 2004); house finches, *Carpodacus mexicanus* (Badyaev and Duckworth 2005); and willow ptarmigan, *Lagopus lagopus* (Pedersen 1989). In mammals, stress increases circulating concentrations of prolactin (De Vlaming 1979), while data obtained from birds suggest that prolactin concentrations tend to decrease with acute restraint stress (Opel and Proudman 1986; Gratto-Trevor 1991; Schoech et al. 1996; Chastel et al. 2005; Angelier et al. 2007a, 2007b, 2009a, 2009b, 2013; Verreault et al. 2008; Miller et al. 2009; Heidinger et al. 2010; Schmid et al. 2011). In long-lived seabirds, it has been hypothesized that prolactin levels decline in older individuals to a lesser extent than in younger individuals because with age the potential for future reproductive opportunities declines and, in theory, brood value increases. As a result, rates of nest abandonment in this cohort decreased (Angelier et al. 2007a). These findings support the hypothesis that prolactin, in conjunction with corticosterone, plays an important role in stress response modulation in breeding individuals. Given this potential for interactions of these two

hormones, measurement of both prolactin and corticosterone in relation to the emergency life-history stage may be key to unraveling connections between stress, the environment, and reproduction.

Arctic breeding songbirds provide a unique study system to understand the importance of brood value and life-history trade-offs mediated through endocrine signaling mechanisms. Gambel's white-crowned sparrows, *Zonotrichia leucophrys gambelii*, are short lived, with an average life span of 2–5 yr (Chilton et al. 1995). Here we examine the seasonal responses of corticosterone and prolactin to acute restraint stress across the breeding and nonbreeding seasons. We hypothesized that circulating levels of prolactin and corticosterone undergo seasonal modulation and respond to stress. Since brood value is much higher in short-lived birds, we predicted that any decrease in prolactin in response to capture and restraint would be minimal and that a negative relationship would exist between prolactin and corticosterone at both basal and stress-induced levels, in order to minimize the effects of stress and promote parental investment. This is the first study to investigate the effects of stress on circulating levels of prolactin and corticosterone in a free-living Arctic breeding passerine that is constrained by a short breeding season.

Material and Methods

Study Site and Species

Gambel's white-crowned sparrows were captured on their breeding grounds in the vicinity of Toolik Lake Research Station on the North Slope of Alaska (69°38′N, 149°36′W); on their wintering grounds in Davis, California (38°33′N, 121°44′W); and during autumn migration at Tioga Pass, California (37°54′N, 119°15′W), during 2011 and 2012. Gambel's white-crowned sparrows are long-distance migrants that winter in the American southwest and breed from southern to northern Canada and in Alaska (Oakeson 1954; Chilton et al. 1995). Summers at high latitudes are short and allow time for only a single brood, but renesting can occur if depredation occurs early in the breeding season (Wingfield and Farner 1979). A typical clutch contains four to five eggs, and nest attentiveness increases as eggs are laid, but true incubation by the female is thought to occur at either the penultimate or ultimate egg (Norment 1995). This species is socially monogamous and shows biparental care of offspring (Oakeson 1954; Chilton et al. 1995). Previous studies at high-latitude sites in Alaska and in the Yukon Territory indicate that reproductive success was typically above 75%, and very few nests are lost to predation (Oakeson 1954; Norment 1992).

Birds were grouped by individual life-history stages and substages rather than Julian date. We tested for the influence of Julian date—while accounting for life-history stage—on hormone levels and found no significant relationships for prolactin ($F_{1,95} = 0.02$, $P = 0.88$) or corticosterone ($F_{1,95} = 0.13$, $P = 0.73$). Winter stage occurred from the time birds arrived on the wintering grounds until the onset of the prealternate molt, which typically commenced during late February and

lasted until late March or early April. The breeding season was divided into substages that included territory establishment, incubation, feeding young, and prebasic molt. All blood samples were collected in accordance with University of California Davis Institutional Animal Care and Use Committee approval under protocol 17812.

Capture and Blood Sampling

A standard restraint handling protocol was used to measure hormonal responses to acute restraint stress (Wingfield et al. 1992). A total of 88 birds were captured using primarily Japanese mist nets in conjunction with playback on the breeding grounds or seed-baited potter traps on the nonbreeding grounds. Birds were caught between 9 a.m. and 4 p.m. on the breeding grounds and between 8 a.m. and 11 a.m. on the nonbreeding grounds. We tested for time of day effects on hormone concentrations but found no relationship for prolactin ($F_{1,84} = 3.68$, $P = 0.058$) or corticosterone ($F_{1,85} = 0.44$, $P = 0.50$). The near positive relationship between prolactin and time of day was due to a positive relationship during molt ($F_{1,22} = 12.59$, $P = 0.001$) only. A baseline blood sample was collected following venipuncture with a 26-gauge needle and collected into heparin-coated microhematocrit tubes. The mean ($\pm$SD) time to sample was 124 ± 40 s during the breeding season and 143 ± 40 s during the nonbreeding season. Previous studies indicate that sample collection within 3 min of capture reflects baseline concentrations of both prolactin and corticosterone (Chastel et al. 2005; Romero and Reed 2005; Schmid et al. 2011), and we found no significant relationships between time to sample for prolactin ($F_{1,84} = 0.07$, $P = 0.77$) or corticosterone ($F_{1,85} = 0.04$, $P = 0.83$). The bird was then placed into an opaque cotton bag, and a second blood sample was collected 30 min from time of capture. Each bird received a U.S. Geological Survey aluminum band in conjunction with a unique set of color bands so that individuals could be later identified in the field. Measurements of wing chord, tarsus, beak, body mass, and fat score (scale from 0 [lean] to 5 [fat]; Wingfield and Farner 1978) were recorded before release. Blood samples were stored in plastic tubes on ice until later processing in the laboratory. Samples were centrifuged at 10,000 rpm for 5 min to separate the plasma from the red blood cells. Plasma was aspirated with a Hamilton syringe, placed into a microcentrifuge tube, and stored at $-35°C$ until hormone quantification.

Hormone Assays

Corticosterone

A radioimmunoassay was used to quantify plasma concentrations of corticosterone, as previously described (Wingfield et al. 1992). Tritiated corticosterone was purchased from Perkin Elmer (NET 399250UC) and corticosterone antibody from Esoterix (B3-163). Samples were combined with Ultima Gold scintillation fluid and counted for 10 min or within 2%

accuracy on a Beckman 6500 scintillation counter. Mean recoveries were 84.33%, and inter- and intra-assay coefficients of variation were 10.70% and 9.76%, respectively.

Prolactin

Plasma immunoreactive prolactin was determined using a radioimmunoassay for recombinant-derived European starling (*Sturnus vulgaris*) prolactin previously described by Bentley et al. (1997) and validated for use in white-crowned sparrows by Maney et al. (1999a). All samples were measured in duplicate and undiluted in a single assay. The serial dilution of pooled samples ran parallel along the standard curve within the dilution range assayed. The sensitivity of the assay was 0.3 ng/mL, and the intra-assay coefficient of variation was 7.6%.

Statistical Analyses

Statistical analyses were performed using JMP 11 (SAS Institute, Cary, NC, 1989–2007). All variables were checked for normality using the Shapiro-Wilks test by plotting the residuals against the predicted value. When prolactin was analyzed using the breeding life-history stages only, the assumptions of normality were met. When all life-history stages across the annual cycle were added into the model, the residuals did not meet assumptions of normality; however, this did not produce results that were different compared with the model that had only the breeding life-history stage. Corticosterone data met the assumptions of normality. A mixed-effects model with a repeated-measures design was tested using a residual covariance structure in which each individual was included as a random variable to test how the dependent variable of hormone level was affected by the independent variables of stress, sex, life-history stage, and their interactions. To control for factors that could have affected hormone levels, we included the covariables time to sample, time of day, day of year, and their interactions with life-history stage, but none of them were significant, and they were removed from the model. All post hoc analyses were performed using Tukey's honestly significant difference (HSD) test. The relationships between corticosterone and prolactin were investigated using Pearson's correlations at both baseline and 30-min time points for both sexes and individually. The relationship between basal corticosterone and prolactin was also investigated using linear mixed-effects models with individual as a random factor, in which one hormone was assigned as the dependent variable and the other hormone, life-history stage, and their interaction were assigned as independent variables. Last, to investigate the relationship between hormone concentrations and body condition, corticosterone or prolactin was assigned as the dependent variable, and the independent variables of fat or mass, prolactin or corticosterone, life-history stage, and their interactions were assigned as independent variables.

Results

Prolactin

Prolactin levels changed across life-history stage but not in response to acute restraint stress (table 1; fig. 1). The interaction between life-history stage and sex was significant. The following interactions were not significant: two-way interactions of stress and sex, stress and life-history stage; three-way interaction of stress, life-history stage, and sex. Post hoc analyses were performed to investigate the interaction of sex and life-history stage on prolactin levels. For both sexes, prolactin levels were higher while on the breeding grounds (territory establishment, incubation, feeding young, and prebasic molt) compared with when the birds were en route to or on their wintering grounds (autumn migration, winter, and prealternate molt). Within the breeding season, female prolactin levels during incubation were higher than during territory establishment ($t = 4.22, P = 0.004$) but were not different from when feeding young ($t = 3.25, P = 0.08$) or during prebasic molt ($t = 3.15, P = 0.11$). In males, prolactin levels did not change over the course of the breeding season (Tukey's HSD >0.05). There was no difference in prolactin levels at any stage during the nonbreeding season for either sex (Tukey's HSD >0.05). Comparison of prolactin between the sexes at each stage of breeding indicated that females had higher prolactin levels during incubation only ($t = 4.02, P = 0.009$).

Corticosterone

Corticosterone levels increased in response to acute restraint stress (table 1; fig. 2). The main effects of life-history stage and sex; the two-way interactions of sex and life-history stage, stress and life-history stage, and stress and sex; and the three-way interaction between life-history stage, stress, and sex on corticosterone were significant. To investigate the three-way interaction, we first analyzed the effects of life-history stage, stress, and their interaction for each sex. The interaction of stress and life-history stage was significant for both sexes (table 2). Next, we investigated the effects of sex, stress, and their interaction for each life-history stage. The interaction of

stress and sex was significant at all stages except for prebasic molt (table 3).

Post hoc analyses indicated that basal corticosterone levels were not significantly different across stages of breeding both within and between sexes (Tukey's HSD >0.05). In males, stress-induced corticosterone levels were higher at territory establishment compared with incubation ($t = 3.63, P = 0.01$), feeding young ($t = 2.46, P = 0.01$), and molt ($t = 2.80, P = 0.008$). Stress-induced corticosterone remained unchanged from incubation to feeding of young ($t = 0.36, P = 0.17$). During molt, stressed-induced corticosterone levels were lower than all other stages (incubation: $t = 3.96, P = 0.005$; feeding of young: $t = 2.64, P = 0.005$). In females, stress-induced corticosterone levels were higher at territory establishment compared with feeding young ($t = 3.52, P = 0.02$) and molt ($t = 4.35, P < 0.01$). Significant differences in stress-induced corticosterone levels were detected between the sexes at territory establishment ($t = 4.20, P < 0.01$) and incubation ($t = 2.98, P = 0.03$) but not when feeding young ($t = 2.74, P = 0.08$) or during the prebasic molt ($t = 1.96, P = 0.23$).

Relationships between Basal Corticosterone, Prolactin, Body Mass, and Total Body Fat

During the parental phase, including incubation and feeding of young, no relationships were found between prolactin and corticosterone in females ($r = 0.06, P = 0.74$) or males ($r = 0.39, P = 0.20$) at basal levels or in females ($r = 0.17, P = 0.57$) or males ($r = 0.11, P = 0.73$) at stress-induced levels. Using the linear mixed-effects model, there was no relationship between corticosterone and prolactin levels in males ($F_{1,32} = 1.14, P = 0.29$) or females ($F_{1,32} = 1.14, P = 0.29$). The interaction of corticosterone and life-history stage was not significant in males ($F_{3,32} = 1.32, P = 0.30$) or females ($F_{3,32} = 0.42, P = 0.74$).

There was no relationship between total body fat and prolactin (males: $F_{1,32} = 1.34, P = 0.26$; females: $F_{1,32} = 0.23, P = 0.63$) or total body fat and corticosterone (males: $F_{1,32} = 0.36, P = 0.55$; females: $F_{1,32} = 0.009, P = 0.92$). The interaction of total fat and life-history stage was not significant for prolactin

Table 1: Mixed-effects model with repeated measures for effects of life-history stage, sex, and stress on circulating levels of prolactin and corticosterone in Gambel's white-crowned sparrow

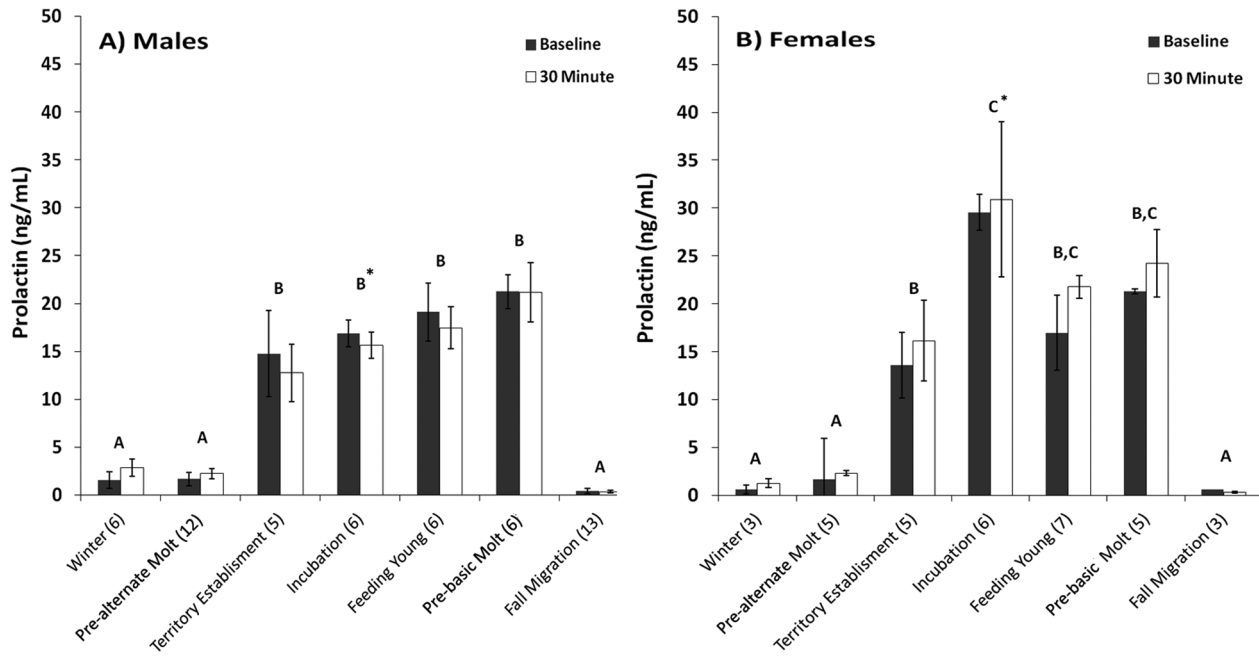| Variable	Corticosterone			Prolactin		
	df	F	P	df	F	P
Life-history stage	3, 39	16.44	<.001	6, 76	30.24	<.001
Stress	1, 39	276.92	<.001	1, 76	1.28	.26
Sex	1, 39	11.56	.002	1, 76	2.59	.11
Life-history stage × stress	3, 39	20.32	<.001	6, 76	.17	.99
Life-history stage × sex	3, 39	5.06	.005	6, 76	2.42	.03
Stress × sex	1, 39	13.50	.001	1, 76	3.32	.07
Life-history stage × stress × sex	3, 39	5.08	.005	6, 76	.73	.62

Figure 1. Effect of acute restraint stress on plasma levels of prolactin in male (*A*) and female (*B*) Gambel's white-crowned sparrows at different stages of the annual cycle. Acute stress had no effect on circulating prolactin levels in either sex at any stage. Baseline prolactin levels were significantly different across the annual cycle, with levels significantly elevated in the breeding compared with the nonbreeding season. Data points that share the same letter are not significantly different. Asterisks indicate sex-based differences at that life-history stage. Numbers in parentheses indicate sample sizes. Data are means ± SEM.
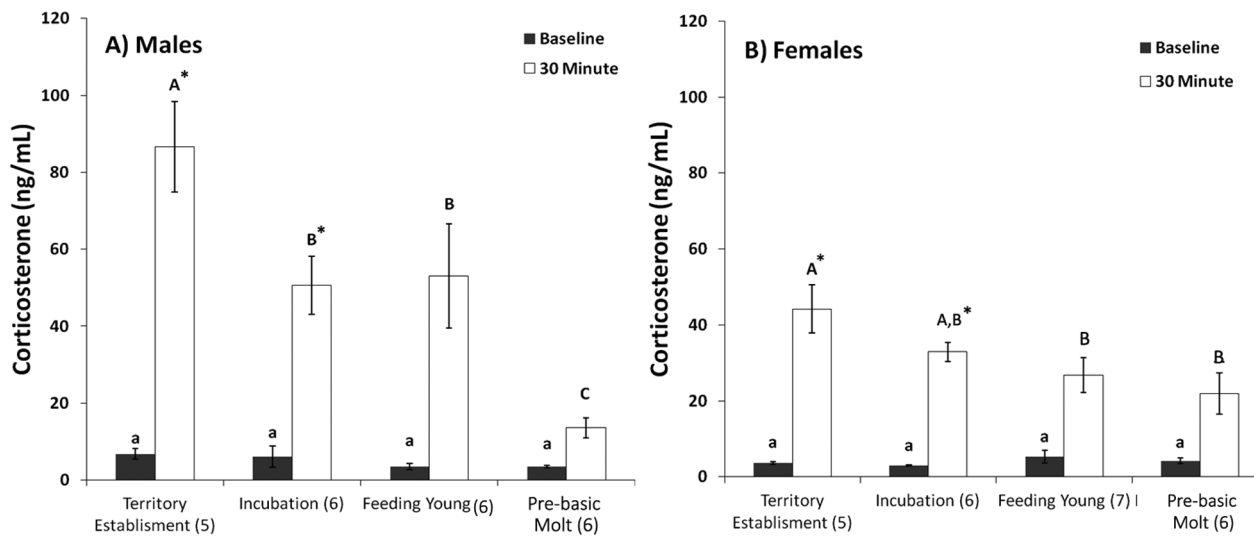

Figure 2. Effect of acute restraint stress on plasma levels of corticosterone in male (*A*) and female (*B*) Gambel's white-crowned sparrows at different stages of the breeding season. Baseline levels of corticosterone did not differ significantly over the breeding season in either sex. Corticosterone levels increased significantly in males and females in response to acute stress at all stages of breeding. In males, stressed-induced levels were highest at arrival, declined at incubation through feeding of young, and then declined again at molt. Females showed attenuated modulation of stress-induced corticosterone levels over the breeding season. Data points that share the same letter are not significantly different. Asterisks indicate sex-based differences at that life-history stage. Numbers in parentheses indicate sample sizes. Data are means ± SEM.

Table 2: Mixed-effects model with repeated measures for effects of life-history stage and stress on corticosterone levels by sex in Gambel's white-crowned sparrow

	Males			Females		
	df	F	P	df	F	P
Life-history stage	3, 19	14.20	<.001	3, 20	2.82	.07
Stress	1, 19	142.52	<.001	1, 20	143.09	<.001
Life-history stage × stress	3, 19	16.71	<.001	3, 20	4.53	.01

(males: $F_{3,32} = 1.62$, $P = 0.22$; females: $F_{3,32} = 1.02$, $P = 0.40$) or for corticosterone (males: $F_{3,32} = 0.48$, $P = 0.69$; females: $F_{3,32} = 0.50$, $P = 0.68$).

There was no relationship between body mass and prolactin (males: $F_{1,32} = 0.90$, $P = 0.35$; females: $F_{1,32} = 0.55$, $P = 0.31$) or body mass and corticosterone (males: $F_{1,32} = 0.90$, $P = 0.35$; females: $F_{1,32} = 0.55$, $P = 0.46$). The interaction of mass and life-history stage was not significant for prolactin (males: $F_{3,32} = 0.45$, $P = 0.71$; females: $F_{3,32} = 1.28$, $P = 0.31$) or corticosterone (males: $F_{3,32} = 0.45$, $P = 0.71$; females: $F_{3,32} = 1.28$, $P = 0.31$; fig. 3).

Discussion

Seasonal Responsiveness of Prolactin to Acute Stress

Prolactin levels did not change in response to acute restraint stress at any stage within the annual cycle of male and female Gambel's white-crowned sparrows. Studies in mammals indicate that prolactin levels increase in response to restraint handling stress, which is thought to be a part of the stress response (De Vlaming 1979). Evidence for prolactin modulation in response to stress in avian species is not as abundant, but previous studies in turkey, *Meleagris gallopavo* (Opel and Proudman 1986); semipalmated sandpiper, *Calidris pusilla* (Gratto-Trevor et al. 1991); black-legged kittiwakes, *Rissa tridactyla* (Chastel et al. 2005); mourning dove, *Zenaida macroura* (Miller et al. 2009); glaucous gulls, *Larus hyperboreus* (Verreault et al. 2008); snow petrels, *Pagodroma nivea* (Angelier et al. 2007*a*); black-browed albatross, *Thalassarche melanophris* (Angelier et al. 2007*b*); Florida scrub-jays (Schoech et al. 1996); Eurasian hoopoe, *Upupa epops* (Schmid et al. 2011); cape petrel, *Daptian capense* (Angelier et al. 2013); and common terns, *Sterna hirundo* (Heidinger et al. 2010), indicate that prolactin levels decrease in response to stress. Furthermore, in a previous captive study on Gambel's white-crowned sparrows, no effect of acute stress was found on circulating levels of prolactin after 30 min of handling (Maney et al. 1999*b*), which is in alignment with our findings. In long-lived birds, the magnitude of the decrease in prolactin levels in response to acute restraint stress is thought to be an important mediator of parental investment (Angelier and Chastel 2009). In each listed example, prolactin measurements were taken in either a long-lived species breeding toward the poles or a short-lived species breeding at latitudes lower than 58°N. Species breeding at lower latitudes with extended breeding seasons may have a greater propensity to abandon a brood and attempt to renest if conditions permit. Semipalmated sandpipers breeding at Hudson Bay, Canada, often have only one chance to raise a brood, but females frequently abandon the nest early, leaving males to provision the precocial young. Males also may abandon the young just before or postfledging (Gratto-Trevor 1991). Gambel's white-crowned sparrows breeding in the low Arctic, at 68°N, typically have one opportunity to raise a brood, with limited opportunities to renest if the clutch is lost early in the season (Wingfield and Farner 1979). Life-history theory predicts that short-lived species with a brief breeding season and, as a result, low lifetime reproductive potential should maximize reproductive effort at the cost of future survival. For long-lived seabirds, as future reproductive potential declines, prolactin levels in response to acute restraint stress decline at a reduced rate in old compared with young individuals. Thus, the maintenance of prolactin levels following a 30-min standardized restraint stress in Gambel's white-crowned sparrow may be an adaptive mechanism for maximizing parental investment in a harsh environment to ensure high reproductive output.

In our study, blood samples were collected immediately on capture and 30 min later, which should have been sufficient

Table 3: Mixed-effects model with repeated measures for effects of sex and stress on corticosterone levels by life-history stage in Gambel's white-crowned sparrow

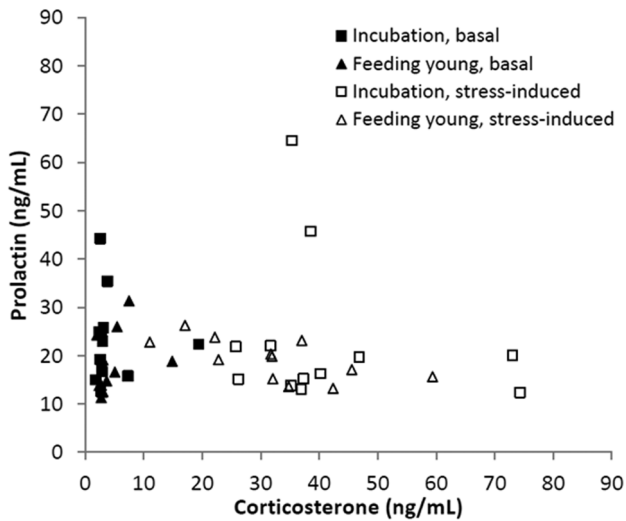|	Territory establishment			Incubation			Feeding young			Prebasic molt		
	df	F	P	df	F	P	df	F	P	df	F	P
Stress	1, 9	73.10	<.001	1, 10	152.37	<.001	1, 10	80.74	<.001	1, 10	25.76	<.001
Sex	1, 9	9.89	.01	1, 10	4.18	.07	1, 10	1.86	.20	1, 10	1.88	.20
Stress × sex	1, 9	7.81	.02	1, 10	5.79	.04	1, 10	5.98	.03	1, 10	1.99	.19

Figure 3. Pearson correlations between prolactin and corticosterone in Gambel's white-crowed sparrows using combined data from incubation (squares) and feeding of young (triangles). No significant relationship was found at baseline (filled symbols; $r = 0.06$, $P = 0.74$) or at stress-induced levels (open symbols; $r = 0.03$, $P = 0.87$).

to detect an effect of stress on plasma prolactin levels. Declines in prolactin can be detected in as little as 15 min, with most studies showing declines at 30 min (Gratto-Trevor et al. 1991; Schoech et al. 1996; Angelier et al. 2007a; Schmid et al. 2011). Because of limitations on the total amount of blood that could be collected, we were able to sample at only two time points. This means that there is an alternative possible explanation for not detecting a change in prolactin levels in response to stress handling due to our 30-min sampling interval. For instance, Chastel et al. (2005) showed that prolactin levels initially increased at 15 min, declined at 30 min, and continued to do so until the 60-min sampling point. Previous studies on captive Gambel's white-crowned sparrows also failed to detect a difference in prolactin levels between basal and the 30-min time point (Maney et al. 1999b). This would suggest that even in the absence of social and environmental cues, prolactin levels were unchanged in response to 30 min of acute restraint stress in this species. We cannot discount the possibility that prolactin levels may have changed at either an earlier or a later time point. Nonetheless, there is overwhelming evidence that for most species, prolactin levels decline at 30 min of acute stress, which would suggest that at least at this time point, prolactin is regulated differently.

Seasonal Modulation of Prolactin

In this study, prolactin levels were modulated over the course of the annual cycle, and the highest levels were observed during the breeding season. These findings are similar to those reported for Puget Sound white-crowned sparrows, *Zonotrichia leucophrys pugetensis*, and other bird species (Hiatt et al. 1987; Sharp et al. 1998). In captive Gambel's white-crowned sparrows,

increasing photoperiod triggered the secretion of prolactin, and in a second experiment, this was found to be under the stimulatory influence of vasoactive intestinal peptide (Maney et al. 1999a, 1999b). Increased circulating prolactin levels at the onset of the breeding season are thought to mediate the transition from the sexual to the parental phase (Sharp et al. 1998). Our data show that only females displayed modulation of prolactin within the breeding season, with levels increasing sharply at the transition from the period in which territories were being established to incubation. When the sexes were compared, prolactin levels were significantly different at incubation only. Previous studies have shown that if parental care is provided by just one sex, then prolactin levels are higher in those individuals compared with their mates (reviewed by Sharp et al. 1998). Because Gambel's white-crowned sparrows show biparental care (Norment 1995), it is not surprising that we did not observe sex-based differences in prolactin levels at other stages of breeding; however, the difference at incubation is likely to be partially explained by the fact that only the females incubate the eggs (Norment 1995). This tactile and visual cue at this stage may promote a greater release of prolactin in females when compared with males.

In our study, prolactin levels were elevated during the prebasic molt, but we emphasize that a majority of the samples were collected early in July at the onset of the prebasic molt. At this point, birds are likely still provisioning offspring, particularly fledglings that are not completely independent. The continued interactions with offspring may promote and maintain prolactin levels until molt intensity increases. Peak molt occurs during the last week in July to the first week in August (Morton et al. 1969). In Puget Sound white-crowned sparrows and song sparrows, *Melospiza melodia*, plasma prolactin levels tend to be highest during incubation and feeding of young and decline rapidly during molt, which was coincident with gonadal regression and photorefractoriness (Hiatt et al. 1987; Wingfield and Goldsmith 1990; Bentley et al. 1997).

Seasonal Modulation of Corticosterone to Stress

Basal levels of corticosterone did not differ over the course of the breeding season for either sex. However, stress-induced corticosterone levels in males and, to a lesser extent, in females were modulated over the course of the breeding season. Previous research on Gambel's white-crowned sparrows showed that corticosterone secretion in response to acute restraint stress was higher in males—but not females—during the preparental phase when compared with the parental phase of breeding (Holberton and Wingfield 2003). Corticosterone was higher in males when compared with females during territory establishment and incubation phases, and previous research has shown sex-based differences in corticosterone levels following acute restraint stress at the preparental stage (Astheimer et al. 1994; Holberton and Wingfield 2003). The parental care hypothesis predicts that the corticosterone stress response should be downregulated during the parental phase to promote parental investment. Prolonged elevations can lead to the activation of the emergency life-history

stage, in which resources are diverted away from reproduction and toward self-maintenance (Wingfield et al. 1995). Interannual variation in climate or food availability could result in either attenuation or strengthening of the stress response during the preparental phase, when conditions are more thermally challenging. Studies on white-crowned sparrows breeding in the vicinity of Fairbanks, Alaska, had no sex-based differences in the stress response at the onset of breeding, but these samples may have included individuals that were still migrating to their breeding grounds (Romero et al. 1997). It is thought that corticosterone secretion is reduced during prebasic molt to allow the growth of high-quality feathers (Romero and Wingfield 1999). In this study, both sexes showed reductions in acute restraint stress levels of corticosterone over the course of the breeding season; only males showed significant attenuation at molt compared with all other stages.

Relationship between Corticosterone and Prolactin

Corticosterone and prolactin may contribute to mediating life-history trade-offs through opposing actions. However, in this study, we found no relationship between corticosterone and prolactin at either baseline or stress-induced levels. A review by Angelier et al. (2013) indicated that most studies failed to find a correlation between prolactin and corticosterone. The authors hypothesized that significant correlations may occur only when conditions are challenging. Prolactin and corticosterone play differential roles in regulating physiology and behavior in response to environmental perturbations. Prolactin will modify parental behavior, while changes in corticosterone may be more important for regulating metabolic process, and each hormone has the ability to influence the other. Experimental elevations of prolactin increased circulating levels of corticosterone, which enhanced parental investment (Miller et al. 2009). Both chronic elevation and exogenous administration of corticosterone caused reductions in prolactin secretion in several bird species (Criscuolo et al. 2005; Angelier et al. 2009a; Spée et al. 2010). We found no significant relationships between body mass or fat stores and our hormone measures. This may suggest that conditions were not especially challenging such that corticosterone would increase the mobilization of fuel stores. The relationship between these two hormones is obviously complex, and knowledge of breeding ecology, current reproductive investment, and energetics are important to determining the relationship between these two hormones.

Summary and Conclusions

Environmental perturbations—such as predation, storms, and food shortages—are unavoidable in the wild, particularly for species breeding in the Arctic. Vertebrates utilize hormonal secretions in response to perturbations to mediate changes in physiology, morphology, and behavior to enhance fitness. The decision to abandon the nest during breeding in response to a perturbation is heavily influenced by prolactin and corticosterone in an antagonistic manner. Our results showed that in Arctic breeding white-crowned sparrows, prolactin secretion peaked during breeding and was found to be unresponsive to acute restraint stress, which may serve as an adaptive mechanism to ensure high reproductive output in a short breeding season. These results differ from long-lived birds breeding in similarly extreme environments: in response to acute restraint stress, older birds compared with younger birds maintained higher circulating levels of prolactin. Life-history theory predicts that short-lived individuals should maximize reproductive effort because of higher brood value. Further work is required to understand the physiological mechanisms that govern parental investment in short-lived birds breeding in harsh environments.

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