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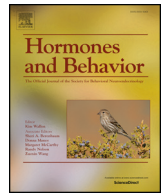
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The stress response is attenuated during inclement weather in parental, but not in pre-parental, Lapland longspurs (*Calcarius lapponicus*) breeding in the Low Arctic



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ABSTRACT

Birds breeding at high latitudes can be faced with extreme weather events throughout the breeding season. In response to environmental perturbations, vertebrates activate the hypothalamic-pituitary-adrenal (HPA) axis and synthesize corticosterone, which promotes changes in behavior and physiology to help the animal survive. The parental care hypothesis suggests that the HPA axis activity should be downregulated during the parental stage of breeding to prevent nest abandonment. However, it is unknown what happens to HPA axis activity in response to severe weather at the transition from the pre-parental to parental stages of breeding. We sampled baseline corticosterone levels and the time course of corticosterone elevation over 60 min of restraint stress and assessed body condition and fat stores in Lapland longspurs (*Calcarius lapponicus*) breeding in the Low Arctic in the presence and absence of snowstorms. The results showed that during the pre-parental stage, HPA axis activity was up-regulated in response to snowstorms, with corticosterone levels continuing to increase through 60 min of restraint. However, once birds were parental, HPA axis activity was unaffected by snowstorms and levels peaked at 10 min. Fat levels and body condition did not change in response to snowstorms but fat levels declined in males during the pre-parental stage. These data suggest that the parental care hypothesis can be applied to severe storm events; parental birds restrained the activity of the HPA axis, likely to focus on the reproductive effort that is already underway, while pre-parental birds greatly upregulated HPA axis activity in response to snowstorms to maximize self-preservation.

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1. Introduction

Arctic breeding birds are faced with a short breeding season in which they have a single opportunity to raise a brood. To optimize physiology and behavior, wild animals rely upon the expression of multiple life history stages and sub-stages throughout the annual cycle (Jacobs and Wingfield, 2000, Ricklefs and Wikelski, 2002). In the absence of environmental perturbation, each life history stage occurs sequentially in an unaltered fashion (e.g. migration, breeding, molt; (Wingfield, 2008). However, in response to perturbations such as food shortages, predation attempts, social instability, or storms, the current life history stage is abandoned for the emergency life history stage favoring self-

preservation (Wingfield et al., 1998, Wingfield and Sapolsky, 2003). The stress hormone corticosterone has been proposed as a key mediator of the emergency life history stage and is synthesized following the activation of the hypothalamic-pituitary-adrenal (HPA) axis in response to metabolic and psychological stressors.

The activity of the HPA axis, as measured through corticosterone synthesis in response to stress handling, changes dramatically according to life history stage in arctic-breeding birds with synthesis being maximal during breeding and minimal during molt (Astheimer et al., 1995, Reneerkens et al., 2002, Holberton and Wingfield, 2003, Meddle et al., 2003, Krause et al., 2015c, Walker et al., 2015). The degree to which the activity of the HPA axis changes across the breeding life history stage is thought to be influenced by parental status, as corticosterone concentrations that are elevated beyond a certain threshold may be incompatible with chick rearing (Wingfield et al., 1995, Angelier and Chastel, 2009). Generally both at the individual and population level,

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exposure to acute restraint stress evoke similar elevations in corticosterone within a life history stage in the absence of severe perturbations (Cockrem and Silverin, 2002, Ouyang et al., 2011, Cockrem, 2013). However, multiple species, including Lapland longspurs (*Calcarius lapponicus*), can modify HPA axis activity within a life history stage in response to severe weather as shown through increases in baseline concentrations of corticosterone (Wingfield et al., 1983, Rogers et al., 1993, Smith et al., 1994, Astheimer et al., 1995, Romero et al., 2000, Raouf et al., 2006, Spée et al., 2010). Storms can restrict an animal's ability to forage and increase thermoregulatory costs, as increases in baseline and/or stress-induced levels of corticosterone have been observed in response to experimental manipulations of food availability (Kitaysky et al., 1999, Lynn et al., 2003, Lendvai et al., 2014), temperature (de Bruijn and Romero, 2011), and precipitation (de Bruijn and Romero, 2013). Corticosterone can promote the mobilization of energy stores, increase foraging, promote movement, and modulate the immune system (Sapolsky et al., 2000). To date there are very few published studies that describe stress-induced levels of corticosterone during inclement weather (Smith et al., 1994). The Lapland Longspur is an ideal study species as it breeds in the Arctic and so is exposed to inclement weather and it has been subject of many endocrine studies over the last 2 decades.

The threshold for triggering the emergency life history stage is thought to fluctuate across life history stages due to associated costs (Wingfield et al., 1998). For instance, during breeding when birds are parental (presence of eggs or chicks), activation of the emergency life history stage may be resisted due to trade-offs between current reproduction and future reproductive potential (Wingfield and Kitaysky, 2002, Angelier and Chastel, 2009, Bókony et al., 2009). To predict how an individual will respond to an environmental stressor, several classifications have been proposed including “take it” (cope with local conditions and attempt to breed), “leave it” (abandon breeding), or “take it and then leave it” (attempt to cope with conditions but forced to abandon) (Wingfield and Kitaysky, 2002). Each strategy has associated changes in physiology and morphology that are important for promoting survival. The “leave it” strategy can include abandonment of territories to seek refuge at a different site offering greater food resources and better weather as corticosterone levels rapidly rise in response to a disturbance (Breuner and Hahn, 2003, Boyle et al., 2010). The “take it” strategy in response to food shortages may include changes in body condition (fat and mass) while maintaining low levels of corticosterone (Kitaysky et al., 1999, Harding et al., 2011). However, the “take it and then leave it” approach occurs when an individual attempts to endure a food shortage but then must abandon as metabolic resources are depleted and corticosterone levels rise (Wingfield et al., 1983, Wingfield, 1985, Astheimer et al., 1995, Kitaysky et al., 1999, Spée et al., 2010, Ouyang et al., 2012, Thierry et al., 2013). Each of these strategies is intimately tied to current life history stage, residual reproductive success, breeding strategy, and energetics, and although corticosterone levels can provide important insights regarding an animal's energetic state and physiological condition, corticosterone alone is not sufficient to determine the strategy adopted by a particular individual (Wingfield and Kitaysky, 2002, Bókony et al., 2009).

To understand the strategy utilized by arctic-breeding Lapland longspurs in the face of environmental stressors, birds were caught during 2–3 day snowstorms at both the pre-parental stage (prior to egg lay) and parental stage (during egg laying or onset of incubation) to assess body condition and HPA axis activity - both at baseline level and in response to acute restraint stress. The parental care hypothesis, put forward by Wingfield et al. (1995), predicts that HPA axis activity, especially peak-induced concentrations, should be down-regulated as birds transition from the pre-parental to parental stage so that the likelihood of nest abandonment is decreased; but, this hypothesis has never been tested in relation to snowstorms. We hypothesized that regardless of parental status, baseline corticosterone levels would be elevated in response to snowstorms. We predicted that parental birds would

adopt a “take it” strategy, i.e., with decreased HPA activity and lower body condition since they have invested time and resources into offspring production, while pre-parental birds should adopt the “leave it” strategy, with increased HPA activity and higher body condition as activation of the emergency life history stage devotes all resources towards self-preservation.

2. Methods

2.1. Study site and species

Lapland longspurs (*Calcarius lapponicus*) were caught within 2 km of Toolik Field Station on the North Slope of Alaska (N 69° 38', W 149° 36') USA, during the summer breeding seasons of 2012 and 2013. Lapland longspurs are circumpolar breeding long-distance migrants that can be found from the Low to High Arctic (Custer and Pitelka, 1977, Walker et al., 2015). They are socially monogamous and display biparental care (Lyon and Montgomerie, 1987). Due to the shortness of the arctic summers, Lapland longspurs have a limited period in which to breed but can re-nest until approximately June 9th in the vicinity of Toolik Lake, Alaska, or June 29th near Barrow, Alaska, if the first clutch is lost to a storm or to predation (Custer and Pitelka, 1977, Hunt and Wingfield, 2004). Females lay 4–6 eggs and incubate without assistance from the male (Custer and Pitelka, 1977, Hunt and Wingfield, 2004). During the sampling period the diet of Lapland longspurs is largely composed of seeds and berries, while later in the season during the nestling phase arthropod intake greatly increases (Custer and Pitelka, 1978, Wingfield et al., 2004).

2.2. Environmental conditions

Meteorological data were collected by Toolik Field Station Environmental Data Center (EDC) (2014). Time lapse photography was employed to assess snow cover by collecting images at 0600, 1200, and 1800. Percent snow cover captured by each image was quantified using ImageJ (NIH, Bethesda, MD). The threshold tool was used by converting the image to 8 bit color, then white pixels were converted to red pixels, and the number of red pixels in the image was quantified to produce % snow-covered ground. Snow cover values are reported as the daily mean of the three photos.

2.3. Capture and blood sampling

A total of 163 males and 25 females were captured in 2012 and 2013 using seed-baited potter traps and Japanese mist nets with playback. In order to determine parental status for females, birds were predominantly classified using brood patch development (percent defeathered, vascularization, and presence of edema) (Bailey 1952). Since males do not develop a brood patch, a combination of mean clutch initiation date (Mean \pm S.D.) for the site: May 30 \pm 3.02 in 2012 and June 4 \pm 2.3 in 2013 and/or known clutch initiation for an individual ($N = 1$ pre-parental, $N = 3$ parental stage). Males from unknown nests were assigned to pre-parental (2012: May 15 \pm 1.92; 2013: May 22 \pm 4.36) or parental (2013: June 7 \pm 2.48) if their capture date exceeded mean clutch initiation by one or more standard deviations.

An acute restraint handling protocol was used to measure HPA activity in response to restraint stress (Astheimer et al., 1992, Wingfield et al., 1992). Blood samples were collected from a subset of the birds, during the pre-parental stage during storms (Male = 1, Female $N = 5$), on storm-free days (Male $N = 15$; Female $N = 12$) and during the parental stage during storms (Male = 4; Female = 3) and on storm free days (Male = 8; Female = 2). A baseline blood sample was collected within 3 min of capture by puncturing the alar vein with a 26 gauge needle and collecting the blood into a microcapillary tube. The mean time to sample was 113 \pm 39 s (S.D.) which is sufficient for corticosterone samples at baseline or near baseline in this species (Romero and Reed, 2005).

Birds were placed within an opaque cotton bag and additional blood samples collected at 10, 30 and 60 min post-capture. Prior to release, each bird was banded with an aluminum USGS band and given a unique set of color bands for later identification in the field. Morphometrics of wing chord, tarsus, and beak were measured to the nearest 0.1 mm using calipers. Body mass was measured to the nearest 0.1 g using a Pesola spring scale. Fat was scored on a scale from 0 (Lean) to 5 (Fat) as previously described (Kaiser, 1993). Blood samples were stored on ice until processing in the laboratory. To separate the plasma from erythrocytes, samples were centrifuged at 10,000 rpm for 5 min. Plasma was aspirated with a Hamilton syringe, placed into a microcentrifuge tube, and stored at -35°C until hormone quantification. All procedures were conducted with prior approval from UC Davis Institutional Animal Care and Use Committee (IACUC) under protocol #17812.

2.4. Hormone assays

Corticosterone levels were quantified using a radioimmunoassay as previously described in detail by Wingfield et al. (1992). In brief, 10 μL of plasma was measured and combined with 2000 CPM of tritiated corticosterone to monitor percent-recoveries to determine individual extraction efficiency. Next, 4 mL of redistilled dichloromethane was added to each sample to extract the steroids from the plasma. Extracts were dried under a stream of nitrogen in a water bath at 35°C and then reconstituted using 550 μL of phosphate-buffered saline with gelatine (PBSG). A 100 μL aliquot was added to a scintillation vial and combined with scintillation fluid to determine percent recoveries. Duplicate 200 μL aliquots were assayed by adding 100 μL ($\sim 10^4$ CPM) of tritiated label (Perkin Elmer NET399250UC) and 100 μL of antibody (Esoterix Inc. B3–163). Unbound steroid was stripped from solution by the addition of 500 μL of dextran coated charcoal followed by centrifugation at 3000 RPM. The supernatant was decanted and combined with scintillation fluid (Perkin Elmer Ultima Gold: 6013329) and counted for 6 min or within 2% accuracy on a Beckman 6500 liquid scintillation counter. Final hormone values were corrected using the individual recovery for each sample. Mean recoveries were 86.56% and intra- (calculated using C.V. between duplicates) and inter-assay variations were 8.57% and 10.22%, respectively. The mean \pm detection limits of the assays was 8.98 ± 0.39 pg per tube.

3. Statistical analyses

Statistical analyses were performed using JMP 11 Pro (SAS Institute Inc., Cary, NC, 1989–2007). All variables were checked for normality using the Shapiro-Wilks test by plotting the residuals against the predicted value. Due to the low sample size for hormonal data we lacked sufficient sample sizes to explore the effect of sex or year in the model. However, no sex based differences were detected in either in the presence or absence of snowstorms in a larger data set in which the reproductive status of all of individuals was not definitively known (Krause et al. in prep). A mixed effects model was tested using a residual covariance structure in which individual was included as a random effect to test how hormone levels and body condition were affected by parental status, stress, storm and their interactions. All post hoc analyses were performed using Tukey's Honestly Significant Difference (HSD) test and the associated t-tests are reported. Effect sizes were estimated for all post hoc tests using Cohen's D and are reported throughout (Cohen, 1992). Since a greater number of individuals were captured than sampled for blood, we were able to explore sex based differences for both fat and body condition metrics; although we could not explore effect of year. Fat levels were compared using an ordinal logistic model with the main effects of parental status, storm, and their interaction. Body condition was calculated by first conducting a principal components analysis using wing chord, tarsus, and beak lengths and then saving the PC1 scores. Next body mass was regressed against PC1 and then the residuals were saved to generate a body condition index.

4. Results

4.1. Snowstorms

Snowstorms occurred in both years of the study and each time the snow cover was below 20% prior to the storm. In 2012, a 3 day snowstorm commenced on the evening of May 25 and terminated the evening of the 27th, while in 2013 a snowstorm began on June 4 and lasted until June 6th. In both years, temperatures declined and snow fell continuously resulting in the ground being completely covered with snow. Breaks in the storm typically occurred for 2–4 h in the afternoon on each day, which allowed some of the snow to melt so that snow cover measured 60–70% (Fig. 1a&b).

Snowstorms in the two years differed in timing relative to reproductive schedule of Lapland longspurs. In 2012 a subset of the population had initiated egg laying when the snowstorm occurred while others were not yet breeding. In 2013 snow melt was unusually late because of early season snowstorms and colder than average temperatures which ultimately delayed clutch initiation (Fig. 1a&b). At the time of the snowstorms in 2013, some birds were not breeding yet, some were laying eggs and some had just initiated incubation.

During the snowstorms, Lapland longspurs were observed in mixed flocks of approximately 20–50 individuals, with other species such as horned larks (*Eremophila alpestris*), pectoral sandpipers (*Calidris melanotos*), white-crowned sparrows (*Zonotrichia leucophrys gambelii*), long-billed dowitchers (*Limnodromus scolopaceus*) and savannah sparrows (*Passerculus sandwichensis*) foraging on available bare patches of ground soon after the storm began. By the second day of the storm, flocks of all species began to enlarge.

4.2. Model results

Corticosterone levels increased in response to acute restraint stress (Table 1). There were significant effects of storm, parental status and storm interaction, and a three-way interaction of parental status, storm, and stress (Table 1; Fig. 2).

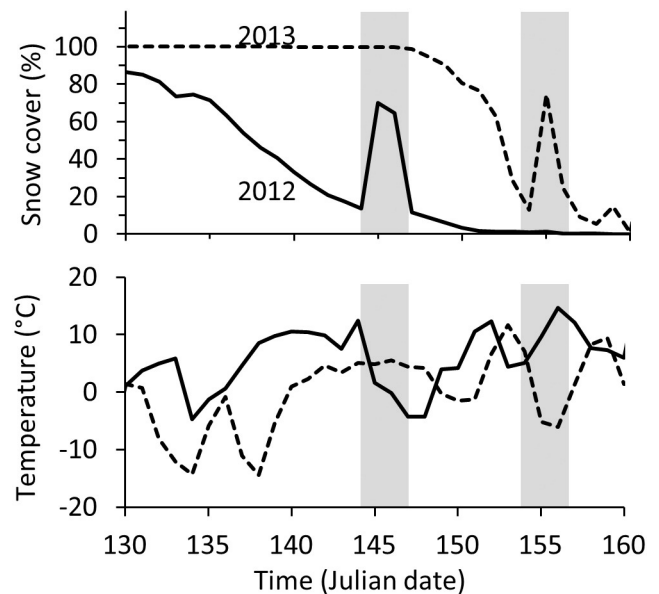


Fig. 1. Snow cover and temperatures experienced by Lapland longspurs during late May through early June in 2012 (solid line) and 2013 (dashed line). The snowstorms are indicated by the shaded regions (grey) and coincide with rapid increases in snow cover. Snowstorms occurred on Julian days 145–147 (May 25–27) in 2012 and Julian days 154–156 (June 4–6) in 2013.

Table 1

The effects of parental status (parental), handling time (stress), and snowstorms on corticosterone levels in response to acute stress in Lapland longspurs. Corticosterone levels were analyzed using a linear mixed effects model with individual included as a random. Asterisks and bold text indicate statistical significance.

Independent variable	Corticosterone		
	D.F.	F	P
Parental	1,182	0.15	0.71
Storm	1,182.8	5.57	0.04*
Stress	3, 182.8	85.96	<0.001*
Parental*storm	1, 182.8	6.39	0.01*
Parental*stress	3,182.8	0.71	0.57
Storm*stress	3,182.8	0.05	0.98
Parental*storm*stress	3, 182.8	4.44	0.005*

4.3. Stress profiles in response to acute restraint stress

Tukey’s post hoc analyses indicated that on storm-free days corticosterone in response to acute restraint stress significantly increased from baseline to the 10 min time point in both pre-parental ($t = 6.66, P < 0.001, d = 3.50$) and parental ($t = 3.14, P = 0.05, d = 2.45$) birds but did not significantly change through the 30 and 60 min time points (Tukey’s HSD $P > 0.05$).

In response to acute restraint stress during a snowstorm, corticosterone at 10 min increased for both pre-parental ($t = 5.60, P < 0.001, d = 2.44$) and parental ($t = 5.43, P < 0.001, d = 5.55$) birds from baseline. Pre-parental birds had higher corticosterone levels at 60 min compared to 10 ($t = 5.60, P < 0.001, d = 1.80$) and 30 ($t = 3.82, P = 0.004, d = 0.97$) minutes (Fig. 2). In parental birds, corticosterone was higher at 10 compared to the 60 min time point ($t = 2.91, P = 0.04, d = 2.24$; Fig. 2).

4.4. The effect of snowstorm on the stress response within either pre-parental or parental birds

In pre-parental birds, corticosterone levels during snowstorms compared to storm-free days were not different at the baseline ($t = 0.38, P = 0.99, d = 0.62$) or 10 ($t = 0.20, P = 0.99, d = 0.10$) min; however levels were higher at 30 ($t = 3.03, P = 0.05, d = 0.91$) and 60 ($t = 7.62, P < 0.001, d = 1.84$) min (Fig. 2).

Parental birds during snowstorms compared to storm-free days had higher corticosterone levels at 10 min ($t = 3.40, P = 0.004, d = 1.71$). No significant differences were found at the other time points (Tukey’s HSD $P > 0.05$; Fig. 2).

4.5. The effect of snowstorms on the stress response across pre-parental and parental stages

During snowstorms, pre-parental birds had lower corticosterone levels at 10 min ($t = 3.07, P = 0.01, d = 1.71$) than parental birds but higher levels at 60 min ($t = 8.93, P < 0.001, d = 2.05$). On storm-free days, the stress response was significantly lower in pre-parental birds compared to parental birds at 30 ($t = 3.22, P = 0.03, d = 1.00$) and 60 min ($t = 4.47, P < 0.001, d = 1.03$; Fig. 2).

4.6. The effect of parental status and snowstorms on total fat and body condition

The main effects of sex and the interaction of storm and sex were significant for fat levels (Table 2, Fig. 3). Females had higher fat levels than males regardless of parental status or snowstorms (Table 2). Female fat levels were not affected by parental status ($\chi^2 = 0.57, P = 0.44$), storms ($\chi^2 = 3.65, P = 0.06$), or the interaction of storm and parental status ($\chi^2 = 0.05, P = 0.81$). In males, fat levels were affected by the interaction of storm and parental status ($\chi^2 = 4.03, P = 0.04$). Fat levels in males were highest on storm-free days during the pre-parental stage.

The main effect of parental status and the interaction of parental status and sex significantly predicted body condition (Table 2; Fig. 3). Tukey’s post hoc comparisons indicated that male body condition was not affected by parental status while females were in higher body condition during the parental stage. No differences in body condition were detected between the two sexes (Tukey HSD $P > 0.05$).

5. Discussion

Severe weather events can occur at any time throughout the year but offer greater energetic challenges within certain life history stages such as breeding. Breeding birds in the Arctic can face severe environmental conditions during the early season prior to snow melt out and again later in the season when storms can completely cover the vegetation with snow. In this study, during snowstorms, vegetation was nearly completely snow covered throughout most of the day except for brief periods of snow melt in the afternoon. Snowstorms likely limited access to food resources such as berries, seeds, and arthropods; and individuals were seen congregating in mixed flocks at open patches of ground to forage. In response to these severe weather events, we predicted based on the parental care hypothesis that pre-parental birds should adopt the “leave it” strategy in which adrenal responsiveness is enhanced while parental birds should adopt the “take it” strategy and reduce adrenal responsiveness. Once parents have devoted resources towards a current reproductive attempt, HPA axis activity should be downregulated according the parental care hypothesis even in the

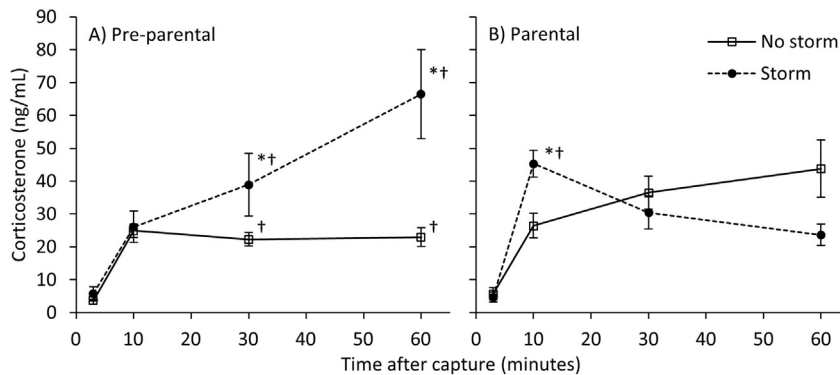


Fig. 2. Effects of parental status and snowstorms on corticosterone levels in response to acute restraint stress in both male and female Lapland longspurs. Blood samples were collected from birds during the (A) pre-parental stage during storms (Male N = 1, Female N = 5) and on storm-free days (Male N = 15, Female N = 12) and during the (B) parental stage during storms (Male N = 4, Female N = 3) and on storm-free days (Male N = 8, Female N = 2). Asterisks denote significant differences between time points in response to snowstorms within a breeding stage. Daggers denote significant differences across stages of breeding. Values represent means ± S.E.M.

Table 2

The effects of parental status (parental), handling time (stress), and snowstorms on body condition and fat stores in Lapland longspurs. Corticosterone and body condition were analyzed using linear mixed effects models and fat stores using an ordinal logistic model. Asterisks and bold text indicate statistical significance.

Independent variable	Body condition			Fat stores	
	D.F.	F	P	χ^2	P
Parental	1, 59.9	5.76	0.02*	0.15	0.70
Storm	1, 50.5	3.31	0.07	0.04	0.84
Sex	1, 135.7	0.40	0.53	14.48	<0.001*
Sex*storm	1, 50.5	0.10	0.75	6.99	0.008*
Parental*sex	1, 59.9	5.79	0.02*	1.70	0.19
Parental*storm	1, 48.1	0.25	0.62	2.07	0.15
Parental*sex*storm	1, 48.1	0.06	0.81	2.98	0.08

face of environmental stressors to prevent nest abandonment. Our results partially supported our hypothesis and predictions. In response to snowstorms compared to storm-free conditions, pre-parental Lapland longspurs allowed for greater increases in HPA axis activity up to 60 min, while parental birds allowed rapid increases at 10 min and then marked reduction thereafter. This relationship would suggest a more rapid onset of negative feedback in parental compared to pre-parental birds during snow storms. In the absence of snowstorms, HPA activity was higher in parental birds compared to pre-parental birds which is counter to the parental care hypothesis.

The magnitude of the HPA axis response to stress was upregulated in pre-parental birds during snowstorms when compared to storm-free days. In other words, the pre-parental birds adopted the “leave it” strategy in response to the snowstorm. At this stage of breeding, birds have not invested energy into reproduction and should, in theory, terminate the current life history stage and enter the emergency life history stage to maximize fitness (Wingfield et al., 1995). Thus the individual with the “leave it strategy” can delay reproduction until the storm passes and increase the likelihood of a successful breeding attempt (Lattin et al., 2016). The increase in HPA activity during the pre-parental stage under stormy conditions may suggest a more rapid reallocation of resources towards self-maintenance and entry into the emergency life history stage (Boonstra, 2004, Angelier and Wingfield, 2013). Conversely, in parental birds, HPA axis activity was restrained so that corticosterone levels were similar during snowstorm days as compared to

storm-free days. Corticosterone levels were higher at 10 min on stormy days compared to storm free days at this same stage but then levels quickly declined towards the 60 min time point. Elevated corticosterone levels in parental birds are linked to reductions in parental investment and increased nest abandonment (Silverin, 1986, Bonier et al., 2009, Spée et al., 2010, Spée et al., 2011, Ouyang et al., 2012, Thierry et al., 2013) which is exacerbated by severe weather (Astheimer et al., 1995, Thierry et al., 2013). Taken together, rapid inhibition of the stress response in parental birds, likely through enhanced negative feedback mechanisms, may be essential for reducing long-term exposure to elevated levels of corticosterone which can trigger nest abandonment. The reduction in HPA axis activity during snowstorms conforms to the predictions of the parental care hypothesis.

HPA axis activity is highly plastic as it adapts to demands associated with each life-history stage of the annual cycle which has been observed across taxonomic groups (Romero, 2002, Angelier and Wingfield, 2013). In the absence of snowstorms, HPA axis activity was measurably higher in the parental stage compared to the pre-parental stage of breeding with measureable differences occurring at the 30 and 60 min time points. Other than in Lapland longspurs breeding in the High Arctic in Greenland, this is the first time that a difference in stress physiology has been detected between these two particular stages of breeding and this difference is opposite of that predicted by the parental care hypothesis; most studies in the past report values during either breeding or molt (Wingfield et al., 1994b, Astheimer et al., 1995, Romero et al., 1998, Romero et al., 2000). The elevations in stress-induced levels of corticosterone were dominated by male samples at this comparison which is the period in which females were incubating. As a consequence it is hard to associate this increase in HPA activity to nestling provisioning rates, which has been previously demonstrated in other birds species (Lendvai et al., 2007, Ouyang et al., 2013), but may be attributed to increased competition between males for fertile females. A significant decline in HPA axis activity has been demonstrated from arrival to breeding in Lapland longspurs breeding in Greenland (Walker et al., 2015) while southern populations show a decline from breeding to molt (Astheimer et al., 1995, Romero et al., 2000). However the degree of modulation is much lower than other songbirds breeding in the same region of Alaska, which have been classically described to follow the parental care hypothesis (Wingfield et al., 1994a, Holberton and Wingfield, 2003, Meddle et al., 2003, Krause et al., 2015a).

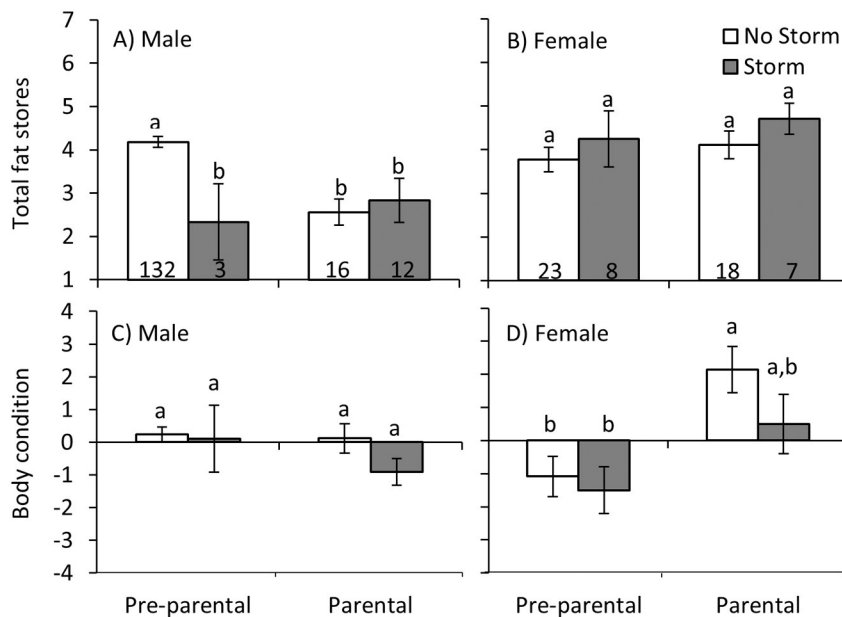


Fig. 3. Effects of parental status and snowstorms on total fat levels and body condition in (A, C) male and (B, D) female Lapland longspurs. Letters that are not the same indicate significant difference between groups. Numbers in the bar graph indicate sample sizes. Values represent means \pm S.E.M.

Differences in HPA axis activity would suggest that peak-induced levels of corticosterone are regulated based on life history stage and environmental parameters. Previous research on breeding Lapland longspurs in Barrow, Alaska, suggests that HPA axis activity is maximal and the adrenal gland is the limiting factor in corticosterone synthesis, as increases in exogenous adrenocorticotrophic hormone (ACTH), arginine vasotocin (AVT), and corticotropin releasing factor (CRF) were unable to further increase circulating levels of corticosterone when birds were parental (Romero et al., 1998). A potential mechanism for augmenting the adrenal gland responsiveness to ACTH is through increased sympathetic drive as denervation of the splanchnic nerve results in depressed corticosterone synthesis (Jasper and Engeland, 1994, Ulrich-Lai et al., 2006). Additionally, other points of regulation cannot be discounted, such as glucocorticoid (GR) or mineralocorticoid (MR) receptor or 11 β -hydroxysteroid dehydrogenase (11 β -HSD) expression may allow for rapid changes in peak-induced levels of corticosterone through negative feedback mechanisms as well as modify effector tissue sensitivity to hormones (Breuner and Orchinik, 2001, Harris et al., 2001, Dickens et al., 2009, Lattin et al., 2012, Liebl and Martin, 2013, Krause et al., 2015b, Lattin and Romero, 2015). What is unknown is how quickly receptor or steroid metabolizing enzyme expression can change in response to severe weather that can allow for rapid changes in peak-induced levels of corticosterone through negative feedback mechanisms. Further work is needed to understand the point of regulation in response to severe weather events.

Baseline corticosterone levels were not significantly different between pre-parental and parental birds sampled either in the presence or absence of snowstorms despite increased thermoregulatory costs and observed changes in foraging behavior. This concurs with a study by Astheimer et al. (1992) in which they also failed to detect differences in baseline levels of corticosterone in Lapland longspurs across a 3 day snowstorm. Corticosterone has often been used as a proxy for metabolic status and even individual quality. Previous research has shown that in the “take it” strategy, corticosterone levels are regulated at a low level until metabolic resources become depleted (Kitaysky et al., 1999). While prolonged fasting can result in elevated levels of corticosterone both at baseline and stress-induced levels suggesting that body condition influences the activity of the HPA axis (Kitaysky et al., 2001, Angelier et al., 2015). Interestingly, we found no difference in fat levels between storm-free and storm days during the pre-parental stage or parental stage in females. Fat stores in pre-parental males declined during snowstorms but we view this difference with caution due to the low sample size in this particular group. In addition, body condition was not affected by snowstorms for either sex. These results are in alignment with the “take it” approach as birds were able to maintain body condition and, for the most part, fat stores and therefore keep baseline levels of corticosterone low. We cannot rule out that most birds were able to maintain their body condition due to the presence of additional food used for potter traps. Birds may have been able to modify behavior by increasing foraging rates in preparation for inclement weather. However, evidence from captive white-crowned sparrows and zebra finch (*Taeniopygia guttata*), indicated that baseline levels of corticosterone peak within 9 h of a food shortage and return to baseline levels 23 h later, although reductions in corticosterone binding globulin resulted in elevated levels of free corticosterone (Lynn et al., 2003, Lynn et al., 2010). It could be that our sample timing may have missed a transient peak in baseline corticosterone levels. A failure to detect differences in baseline corticosterone does not disprove potential effects on feeding rates.

6. Conclusions

The activity of the HPA axis is plastic depending upon life history stage, parental status and environmental conditions. This study demonstrates that in response to snowstorms, Lapland longspurs conform to the parental care hypothesis such that the stress response was

restrained in birds that had already initiated clutches compared to birds that had not. This is likely an adaptive mechanism promoting parental behavior to maximize fitness (Kitaysky et al., 1999, Spée et al., 2010, Ouyang et al., 2012, Thierry et al., 2013, Vitousek et al., 2014). This study provides new insights into how the HPA axis is modulated in the face of severe environmental conditions. Further studies are required to address the effects of this modulation on reproductive success and the mechanisms by which plasticity of the HPA axis is regulated via changes in negative feedback.

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References

- Angelier, F., Chastel, O., 2009. Stress, prolactin and parental investment in birds: a review. *Gen. Comp. Endocrinol.* 163, 142–148.
- Angelier, F., Wingfield, J.C., 2013. Importance of the glucocorticoid stress response in a changing world: theory, hypotheses and perspectives. *Gen. Comp. Endocrinol.* 190, 118–128.
- Angelier, F., Wingfield, J.C., Parenteau, C., Pellé, M., Chastel, O., 2015. Does short-term fasting lead to stressed-out parents? A study of incubation commitment and the hormonal stress responses and recoveries in snow petrels. *Horm. Behav.* 67, 28–37.
- Astheimer, L.B., Buttemer, W.A., Wingfield, J.C., 1992. Interactions of corticosterone with feeding, activity and metabolism in passerine birds. *Ornis Scand.* 23, 355–365.
- Astheimer, L.B., Buttemer, W.A., Wingfield, J.C., 1995. Seasonal and acute changes in adrenocortical responsiveness in an arctic-breeding bird. *Horm. Behav.* 29, 442–457.
- Bailey, R.E., 1952. The incubation patch of passerine birds. *The Condor.* 54, pp. 121–136.
- Bókony, V., Lendvai, Á.Z., Liker, A., Angelier, F., Wingfield, J.C., Chastel, O., 2009. Stress response and the value of reproduction: are birds prudent parents? *Am. Nat.* 173, 589–598.
- Bonier, F., Moore, I., Martin, P., Robertson, R., 2009. The relationship between fitness and baseline glucocorticoids in a passerine bird. *Gen. Comp. Endocrinol.* 163.
- Boonstra, R., 2004. Coping with changing northern environments: the role of the stress axis in birds and mammals. *Integr. Comp. Biol.* 44, 95–108.
- Boyle, W.A., Norris, D.R., Guglielmo, C.G., 2010. Storms drive altitudinal migration in a tropical bird. *Proceedings of the Royal Society of London B: Biological Sciences.*
- Breuner, C.W., Hahn, T.P., 2003. Integrating stress physiology, environmental change, and behavior in free-living sparrows. *Horm. Behav.* 43, 115–123.
- Breuner, C.W., Orchinik, M., 2001. Seasonal regulation of membrane and intracellular corticosteroid receptors in the house sparrow brain. *J. Neuroendocrinol.* 13, 412–420.
- Cockrem, J.F., 2013. Corticosterone responses and personality in birds: individual variation and the ability to cope with environmental changes due to climate change. *Gen. Comp. Endocrinol.* 190, 156–163.
- Cockrem, J.F., Silverin, B., 2002. Variation within and between birds in corticosterone responses of great tits (*Parus major*). *Gen. Comp. Endocrinol.* 125, 197–206.
- Cohen, J., 1992. A power primer. *Psychol. Bull.* 112, 155.
- Custer, T.W., Pitelka, F.A., 1977. Demographic features of a Lapland longspur population near Barrow, Alaska. *Auk* 94, 505–525.
- Custer, T.W., Pitelka, F.A., 1978. Seasonal trends in summer diet of the Lapland longspur near Barrow, Alaska. *Condor* 80, 295–301.
- de Bruijn, R., Romero, L.M., 2011. Behavioral and physiological responses of wild-caught European starlings (*Sturnus vulgaris*) to a minor, rapid change in ambient temperature. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 160, 260–266.
- de Bruijn, R., Romero, L.M., 2013. Artificial rain and cold wind act as stressors to captive molting and non-molting European starlings (*Sturnus vulgaris*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 164, 512–519.
- Dickens, M., Romero, L.M., Cyr, N.E., Dunn, I.C., Meddle, S.L., 2009. Chronic stress alters glucocorticoid receptor and mineralocorticoid receptor mRNA expression in the European starling (*Sturnus vulgaris*) brain. *J. Neuroendocrinol.* 21, 832–840.
- Environmental Data Center, 2014. Meteorological monitoring program at Toolik, Alaska. Toolik Field Station, Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775 http://toolik.alaska.edu/edc/abiotic_monitoring/data_query.php (Retrieved March 13, 2013).
- Harding, A.A., Welcker, J., Steen, H., Hamer, K., Kitaysky, A., Fort, J., Talbot, S., Cornick, L., Karnovsky, N., Gabrielsen, G., Grémillet, D., 2011. Adverse foraging conditions may impact body mass and survival of a high Arctic seabird. *Oecologia* 167, 49–59.

- Harris, H.J., Kotelevtsev, Y., Mullins, J.J., Seckl, J.R., Holmes, M.C., 2001. Intracellular regeneration of glucocorticoids by 11 β -hydroxysteroid dehydrogenase (11 β -HSD)-1 plays a key role in regulation of the hypothalamic-pituitary-adrenal Axis: analysis of 11 β -HSD-1-deficient mice. *Endocrinology* 142, 114–120.
- Holberton, R.L., Wingfield, J.C., 2003. Modulating the corticosterone stress response: a mechanism for balancing individual risk and reproductive success in arc-breeding sparrows? *Auk* 120, 1140–1150.
- Hunt, K.E., Wingfield, J.C., 2004. Effect of estradiol implants on reproductive behavior of female Lapland longspurs (*Calcarius lapponicus*). *Gen. Comp. Endocrinol.* 137, 248–262.
- Jacobs, J.D., Wingfield, J.C., 2000. Endocrine control of life-cycle stages: a constraint on response to the environment? *Condor* 102, 35–51.
- Jasper, M.S., Engeland, W.C., 1994. Splanchnic neural activity modulates ultradian and circadian rhythms in adrenocortical secretion in awake rats. *Neuroendocrinology* 59, 97–109.
- Kaiser, A., 1993. A new multi-category classification of subcutaneous fat deposits of songbirds (Una Nueva Clasificación, con Multi-categorías, para los Depósitos de Grasa en Aves Canoras). *J. Field Ornithol.* 64, 246–255.
- Kitaysky, A.S., Kitaiskaia, E.V., Wingfield, J.C., Piatt, J.F., 2001. Dietary restriction causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks. *J. Comp. Physiol. B.* 171, 701–709.
- Kitaysky, A., Wingfield, J., Piatt, J., 1999. Dynamics of food availability, body condition and physiological stress response in breeding black-legged kittiwakes. *Funct. Ecol.* 13, 577–584.
- Krause, J.S., Chmura, H.E., Pérez, J.H., Quach, L.N., Asmus, A., Word, K.R., McGuigan, M.A., Sweet, S.K., Meddle, S.L., Gough, L., Boelman, N., Wingfield, J., 2015a. Breeding on the leading edge of a northward range expansion: differences in morphology and the stress response in the arctic Gambel's white-crowned sparrow. *Oecologia* 1–12.
- Krause, J.S., McGuigan, M.A., Bishop, V.R., Wingfield, J.C., Meddle, S.L., 2015b. Decreases in mineralocorticoid but not glucocorticoid receptor mRNA expression during the short Arctic breeding season in free-living Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *J. Neuroendocrinol.* 27, 66–75.
- Krause, J.S., Meddle, S.L., Wingfield, J.C., 2015c. 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*). *Physiol. Biochem. Zool.* 88, 589–598.
- Lattin, C.R., Romero, L.M., 2015. Seasonal variation in glucocorticoid and mineralocorticoid receptors in metabolic tissues of the house sparrow (*Passer domesticus*). *Gen. Comp. Endocrinol.* 214, 95–102.
- Lattin, C.R., Breuner, C.W., Romero, L.M., 2016. Does corticosterone regulate the onset of breeding in free-living birds?: the CORT-flexibility hypothesis and six potential mechanisms for priming corticosteroid function. *Horm. Behav.* 78, 107–120.
- Lattin, C.R., Waldron-Francis, K., Richardson, J.W., de Bruijn, R., Bauer, C.M., Breuner, C.W., Michael Romero, L., 2012. Pharmacological characterization of intracellular glucocorticoid receptors in nine tissues from house sparrow (*Passer domesticus*). *Gen. Comp. Endocrinol.* 179, 214–220.
- Lendvai, Á.Z., Giraudeau, M., Chastel, O., 2007. Reproduction and modulation of the stress response: an experimental test in the house sparrow. *Proc. R. Soc. B Biol. Sci.* 274, 391–397.
- Lendvai, Á.Z., Ouyang, J.Q., Schoenle, L.A., Fasanello, V., Haussmann, M.F., Bonier, F., Moore, I.T., 2014. Experimental food restriction reveals individual differences in corticosterone reaction norms with no oxidative costs. *PLoS One* 9, e110564.
- Liebl, A.L., Martin, L.B., 2013. Stress hormone receptors change as range expansion progresses in house sparrows. *Biol. Lett.* 9.
- Lynn, S.E., Breuner, C.W., Wingfield, J.C., 2003. Short-term fasting affects locomotor activity, corticosterone, and corticosterone binding globulin in a migratory songbird. *Horm. Behav.* 43, 150–157.
- Lynn, S.E., Stamps, T.B., Barrington, W.T., Weida, N., Hudak, C.A., 2010. Food, stress, and reproduction: short-term fasting alters endocrine physiology and reproductive behavior in the zebra finch. *Horm. Behav.* 58, 214–222.
- Lyon, B.E., Montgomerie, R.D., 1987. Ecological correlates of incubation feeding: a comparative study of high arctic finches. *Ecology* 68, 713–722.
- Meddle, S.L., Owen-Ashley, N.T., Richardson, M.I., Wingfield, J.C., 2003. Modulation of the hypothalamic-pituitary-adrenal axis of an Arctic-breeding polygynandrous songbird, the Smith's longspur, *Calcarius pictus*. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270, 1849–1856.
- Ouyang, J.Q., Hau, M., Bonier, F., 2011. Within seasons and among years: when are corticosterone levels repeatable? *Horm. Behav.* 60, 559–564.
- Ouyang, J.Q., Muturi, M., Quetting, M., Hau, M., 2013. Small increases in corticosterone before the breeding season increase parental investment but not fitness in a wild passerine bird. *Horm. Behav.* 63.
- Ouyang, J.Q., Quetting, M., Hau, M., 2012. Corticosterone and brood abandonment in a passerine bird. *Anim. Behav.* 84, 261–268.
- Raouf, S.A., Smith, L.C., Brown, M.B., Wingfield, J.C., Brown, C.R., 2006. Glucocorticoid hormone levels increase with group size and parasite load in cliff swallows. *Anim. Behav.* 71, 39–48.
- Reneerkens, J., Morrison, G., Ramenofsky, M., Piersma, T., Wingfield, J.C., 2002. Baseline and stress-induced levels of corticosterone during different life cycle substages in a shorebird on the high arctic breeding grounds. *Physiol. Biochem. Zool.* 75, 200–208.
- Ricklefs, R.E., Wikelski, M., 2002. The physiology/life-history nexus. *Trends in ecology & Evolution* 17, 462–468.
- Rogers, C.M., Ramenofsky, M., Ketterson, E.D., Nolan Jr., V., Wingfield, J.C., 1993. Plasma corticosterone, adrenal mass, winter weather, and season in nonbreeding populations of dark-eyed juncos (*Junco hyemalis hyemalis*). *Auk* 110, 279–285.
- Romero, M.L., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *Gen. Comp. Endocrinol.* 128, 1–24.
- Romero, L.M., Reed, J.M., 2005. Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 140, 73–79.
- Romero, L.M., Reed, J.M., Wingfield, J.C., 2000. Effects of weather on corticosterone responses in wild free-living passerine birds. *Gen. Comp. Endocrinol.* 118, 113–122.
- Romero, L.M., Soma, K.K., Wingfield, J.C., 1998. Hypothalamic-Pituitary-Adrenal Axis Changes Allow Seasonal Modulation of Corticosterone in a Bird.
- Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* 21, 55–89.
- Silverin, B., 1986. Corticosterone-binding proteins and behavioral effects of high plasma levels of corticosterone during the breeding period in the pied flycatcher. *Gen. Comp. Endocrinol.* 64.
- Smith, G.T., Wingfield, J.C., Veit, R.R., 1994. Adrenocortical response to stress in the common diving petrel, *Pelecanoides urinatrix*. *Physiol. Zool.* 67, 526–537.
- Spée, M., Beaulieu, M., Dervaux, A., Chastel, O., Le Maho, Y., Raclot, T., 2010. Should I stay or should I go? Hormonal control of nest abandonment in a long-lived bird, the Adélie penguin. *Horm. Behav.* 58, 762–768.
- Spée, M., Marchal, L., Lazin, D., Maho, Y., Chastel, O., Beaulieu, M., Raclot, T., 2011. Exogenous corticosterone and nest abandonment: a study in a long-lived bird, the Adélie penguin. *Horm. Behav.* 60.
- Thierry, A.-M., Massemin, S., Handrich, Y., Raclot, T., 2013. Elevated corticosterone levels and severe weather conditions decrease parental investment of incubating Adélie penguins. *Horm. Behav.* 63, 475–483.
- Ulrich-Lai, Y.M., Arnold, M.M., Engeland, W.C., 2006. Adrenal Splanchnic Innervation Contributes to the Diurnal Rhythm of Plasma Corticosterone in Rats by Modulating Adrenal Sensitivity to ACTH.
- Vitousek, M.N., Jenkins, B.R., Safran, R.J., 2014. Stress and success: individual differences in the glucocorticoid stress response predict behavior and reproductive success under high predation risk. *Horm. Behav.* 66, 812–819.
- Walker, B.G., Meddle, S.L., Romero, L.M., Landys, M.M., Reneerkens, J., Wingfield, J.C., 2015. Breeding on the extreme edge: modulation of the adrenocortical response to acute stress in two high arctic passerines. *J. Exp. Zool. A Ecol. Genet. Physiol.* 323, 266–275.
- Wingfield, J.C., 1985. Influences of weather on reproductive function in female song sparrows, *Melospiza melodia*. *J. Zool.* 205, 545–558.
- Wingfield, J.C., 2008. Comparative endocrinology, environment and global change. *Gen. Comp. Endocrinol.* 157, 207–216.
- Wingfield, J.C., Kitaysky, A.S., 2002. Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? *Integr. Comp. Biol.* 42, 600–609.
- Wingfield, J.C., Sapolsky, R.M., 2003. Reproduction and resistance to stress: when and how. *J. Neuroendocrinol.* 15, 711–724.
- Wingfield, J.C., Deviche, P., Sharbaugh, S., Astheimer, L.B., Holberton, R., Suydam, R., Hunt, K., 1994a. Seasonal changes of the adrenocortical responses to stress in redpolls, *Acanthis flammea*, in Alaska. *J. Exp. Zool.* 270, 372–380.
- Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M., Richardson, R.D., 1998. Ecological bases of hormone-behavior interactions: the “emergency life history stage”. *Am. Zool.* 38, 191–206.
- Wingfield, J.C., Moore, M.C., Farner, D.S., 1983. Endocrine responses to inclement weather in naturally breeding populations of white-crowned sparrows (*Zonotrichia leucophrys pugetensis*). *The Auk*, pp. 56–62.
- Wingfield, J.C., O'Reilly, K.M., Astheimer, L.B., 1995. Modulation of the adrenocortical responses to acute stress in Arctic birds: a possible ecological basis. *Am. Zool.* 35, 285–294.
- Wingfield, J.C., Owen-Ashley, N., Benowitz-Fredericks, Z.M., Lynn, S., Hahn, T., Wada, H., Breuner, C., Meddle, S., Romero, L.M., 2004. Arctic spring: the arrival biology of migrant birds. *Acta Zool. Sin.* 50, 948–960.
- Wingfield, J.C., Suydam, R., Hunt, K., 1994b. The adrenocortical responses to stress in snow buntings (*Plectrophenax nivalis*) and Lapland longspurs (*Calcarius lapponicus*) at barrow, Alaska. *Comp. Biochem. Physiol. C: Pharmacol. Toxicol. Endocrinol.* 108, 299–306.
- Wingfield, J.C., Vleck, C.M., Moore, M.C., 1992. Seasonal changes of the adrenocortical response to stress in birds of the Sonoran desert. *J. Exp. Zool.* 264, 419–428.