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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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A B S T R A C T

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 (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,
Corticosterone levels can provide important insights regarding an animal’s energetic state and parental stage (during egg laying or onset of incubation) to assess the strategy adopted by a particular individual (Wingfield et al., 2004). To understand the strategy utilized by arctic-breeding Lapland longspurs in response to food shortages, birds were caught during 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. To assess body condition and HPA axis activity influential during acute restraint handling, protocol was used to measure HPA activity in response to 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 ± 39 s (S.D.) which is sufficient for corticosterone samples at baseline or near baseline in this species (Romero and Reed, 2005).

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. To assess body condition and HPA axis activity influential during acute restraint handling, protocol was used to measure HPA activity in response to 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 ± 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 μL 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 gelatin (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 (−10^6 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 ± 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-Wilk’s 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 remained 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 gambelli), 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).

<table>
<thead>
<tr>
<th>Year</th>
<th>Snow Cover (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>80</td>
</tr>
<tr>
<td>2013</td>
<td>60</td>
</tr>
</tbody>
</table>

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.
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 (χ² = 0.57, P = 0.44), storms (χ² = 3.65, P = 0.06), or the interaction of storm and parental status (χ² = 0.05, P = 0.81). In males, fat levels were affected by the interaction of storm and parental status (χ² = 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 to the parental care hypothesis even in the

![Fig. 2](https://example.com/fig2.png)
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 snowstorms. 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 stormy 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, Spéé et al., 2010, Spéé 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 measurable 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 bird 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).

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.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Body condition</th>
<th>Fat stores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D.F.</td>
<td>F</td>
</tr>
<tr>
<td>Parental</td>
<td>1, 59.9</td>
<td>5.76</td>
</tr>
<tr>
<td>Storm</td>
<td>1, 50.5</td>
<td>3.31</td>
</tr>
<tr>
<td>Sex</td>
<td>1, 135.7</td>
<td>0.40</td>
</tr>
<tr>
<td>Sex*storm</td>
<td>1, 50.5</td>
<td>0.10</td>
</tr>
<tr>
<td>Parental*sex</td>
<td>1, 50.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Parental*storm</td>
<td>1, 48.1</td>
<td>0.25</td>
</tr>
<tr>
<td>Parental<em>sex</em>storm</td>
<td>1, 48.1</td>
<td>0.06</td>
</tr>
</tbody>
</table>

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 ± 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 adrenocorticotropic 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 splanchic nerve results in depressed corticosterone synthesis (Jasper and Engelard, 1994, Ulrich-Lai et al., 2006). Additionally, other points of regulation cannot be discounted, such as glucocorticoid (GR) or mineralocorticoid (MR) receptor or 11β-hydroxysteroid dehydrogenase (11β-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 though 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 activity and, for the most part, fat stores and therefore keep baseline levels of corticosterone unusually low. 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 confirms 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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