Short communication

Snow cover and snowfall impact corticosterone and immunoglobulin A levels in a threatened steppe bird

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Abstract

Birds use both the corticosterone stress response and immune system to meet physiological challenges during exposure to adverse climatic conditions. To assess the stress level and immune response of the Asian Great Bustard during conditions of severe winter weather, we measured fecal corticosterone (CORT) and Immunoglobulin A (IgA) before and after snowfall in a low snow cover year (2014) and a high snow cover year (2015). A total of 239 fecal samples were gathered from individuals in Tumuji Nature Reserve, located in eastern Inner Mongolia, China. We observed high CORT levels that rose further after snowfall both in high and low snow cover years. IgA levels increased significantly after snowfall in the low snow cover year, but decreased after snowfall in the high snow cover year. These results suggest that overwintering Asian Great Bustards are subjected to climatic stress during severe winter weather, and the hypothalamic-pituitary-adrenal axis and immune system react to this challenge. Extreme levels of stress, such as snowfall in already prolonged and high snow cover conditions may decrease immune function. Supplemental feeding should be considered under severe winter weather conditions for this endangered subspecies.

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1. Introduction

Predicted changes in global climate include not only rising temperatures but also increasing frequency, intensity, duration, and unpredictability of extreme weather events (Coumou and Rahmstorf, 2012). The impacts of climate extremes on animal species are highly varied, including changes in rates of mortality, breeding success, and phenology (Barthe et al., 2015; Reichert et al., 2012). Heavy snowfall represents one type of extreme climate event, and many studies describe stress responses to snowfall in birds (Formenti et al., 2015; Krause et al., 2016), and resulting changes to their phenology (Gladalski et al., 2014; Martin et al., 2017). However, little information is available concerning the physiological effects of snow on steppe birds. As residents of a strongly seasonal climate, steppe birds may have better resources to cope with climate stress. However, on the other hand, steppe birds may be particularly vulnerable to extreme snowfall weather events, which elevate already high energy demands during long winters. Additionally, while birds in forest environments are able to shift their foraging strategy under conditions of snow cover (Doherty and Grubb, 2003; Nakamura and Shindo, 2001), in a grassland environment, snow cover makes major food resources more difficult to access.

Both endocrine and immunological techniques have been used to measure stress responses. Concentrations of cortisol (for most mammal species) or corticosterone (CORT, for rodent, bird, amphibian, and reptile species), are often used as measures of animals’ physiological response to stress (Cockrem, 2013). Another frequently used indicator is the level of secretory Immunoglobulin A (IgA), the most abundant class of mucosal antibody, which protects against respiratory pathogens, infectious agents and foreign proteins (Campos-Rodríguez et al., 2013; Chávez-Zichinelli et al., 2013). Stressors activate the hypothalamic pituitary adrenal (HPA) axis, as well as the sympathetic axis and brain-gut axis,

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which triggers changes in secretory IgA levels to face an acute stressor, and corticosterone-induced immune suppression when confronted with chronic stressors (Glaser and Kiecolt-Glaser, 2005; Gourkow et al., 2014). We are not aware of any study which has examined the effects of snow on CORT and IgA in steppe birds, mainly because of the difficulty of collecting samples in the field. Improvements in noninvasive sampling methods to quantify stress offer opportunities to more easily carry out repeated sampling as well as reduce stress to study organisms (Millspaugh and Washburn, 2004).

Our study species, the Great Bustard (Otis tarda) is listed as Vulnerable globally, with a population of approximately 50,000 individuals (BirdLife International 2016, A2cd+3cd+4cd, ver 3.1). Previous research on this species in Europe has found that inclement winter weather affects breeding phenology (Biczó and Péczely, 2007), and normally sedentary populations of the Great Bustard in Europe (O. t. tarda) are observed to undertake facultative migration under conditions of snow cover (Block, 1996; Streich et al., 2006).

We work specifically with the Asian subspecies (O. t. dybowskii, “Asian Great Bustard”), which is at much higher conservation risk, with a population size estimated at 1200–2200 individuals (Kessler et al., 2013). These birds inhabit a more continental climate, which includes breeding grounds in Mongolia, northeastern China and eastern Russian South Siberia. Asian Great Bustards breeding in northern Mongolia regularly undertake long-distance migrations, overwintering in more southerly areas of China (Kessler, 2015), likely to avoid severe winter weather and conditions of food shortage due to snow cover on breeding grounds (Kessler et al., 2013). While populations breeding in northeastern China were also previously migratory, in recent years some individuals have begun to overwinter in an area that is impacted by extreme snowfall events.

Our aim was to quantify the physiological stress response of wintering Asian Great Bustards to snowfall using a non-invasive methodology. We hypothesized that extreme snowfall would increase the stress response, as assessed by fecal CORT and IgA concentrations before and after snowstorms.

2. Materials and methods
2.1. Study area

This study was conducted in the Tumuji Nature Reserve (TNR), located in eastern Inner Mongolia, People’s Republic of China, which covers an area of 76,210 hm² (Fig. 1). TNR is located in the transition zone from temperate grassland to meadow steppe, and includes grassland, wetland and farmland. TNR falls within a warm temperate zone, with the highest temperature and lowest recorded temperatures 39 °C and −33.5 °C, respectively, and an annual average temperature of 4 °C. Prior to the 1990s, there were no Great Bustards overwintering in TNR. Since that time, the number of wintering Great Bustards increased yearly, reaching a stable number of around 100 individuals. Overwintering Great Bustards occupy agricultural fields and natural grassland in TNR, feeding primarily on corn, beans, and wild grass. No supplemental food was provided to the Great Bustards during the time of the study, and is only very rarely provided at other times.

2.2. Sampling and measuring

We non-invasively sampled Great Bustard feces during two consecutive winters characterized by different meteorological trends. Air temperature and snow data from December of each year, including snowfall in mm, snow cover in cm, and number of days with snow cover, were collected from the weather station within the reserve and the National Meteorological Station located in Jalaid Banner, Inner Mongolia (Table 1). A snow cover day was defined by the Meteorological Station as a day when snow covers > 50% of the land. Snow depth was defined as the greatest depth recorded during the month of December. These data allowed us to characterize the sampling years in relation to differing snow cover conditions: (1) low snow cover (23 snow cover days with maximum depth 4 cm, December 2014) and (2) high snow cover (31 snow cover days with maximum depth 7 cm, December 2015). During both winters, we collected feces 3 days before snowfall as predicted by weather forecasting, and again on the second day after snowfall.
We carried out this procedure for 7 snowfalls (all light snow (0.5–2 cm each)) in December 2014 and 6 snowfalls (1 extreme snow (7 cm), 1 heavy snow (5 cm) and 4 light snow (0.5–2 cm each)) in December 2015. Feces were randomly gathered from areas where Great Bustards had been observed, after birds had flown away for natural reasons, avoiding any human disturbance of the studied population. At TNR, Great Bustards are typically found in several flocks distributed separately across the reserve. To avoid re-collecting fecal samples from the same individual in a flock, only fresh feces (soft and not covered by snow) were collected, and samples were collected only once at each flock during each collection period, with a minimum distance of 2 m between samples. A total of 61 and 54 feces were collected before and after snowfall in 2014, and 66 and 58 in 2015. All fecal samples were stored in a cooler in the field, and transferred to a freezer for storage.

Fecal CORT was extracted as described by Wasser et al. (2000) with a minor modification (extracts were centrifuged at 1000 g for 20 min). Fecal IgA was extracted as described by Carlsson et al. (2007), also with a minor modification (0.5 g of fecal samples was diluted with 10 ml of PBS, 0.05% Tween 20). A sensitive enzyme-linked immunosorbent assay (ELISA) method was used to determine the amount of total CORT and IgA in the feces. This method, which uses commercial anti-chicken antibodies of CORT and IgA, has been validated in Crested Ibis (Nipponia nippon) (Zhang, 2012).

2.3. Statistical analysis

Non-normally distributed data (IgA, Kolmogorov-Smirnov test, P < 0.05) were square-root transformed to meet parametric assumptions. We tested for effects of snow cover (categorical variable – high or low) and snowfall (categorical factor – before or after snowfall) on CORT and IgA concentrations by constructing two Generalized Linear Models (GLM), using CORT and IgA as response variables. Snow cover, snowfall, and snow cover × snowfall were used as predictor variables in each model. The relationship between fecal CORT and IgA was analyzed using the Pearson correlation test. Statistical analyses were conducted using SPSS 17.0.2 (SPSS Inc., Chicago, IL, USA), with the criterion for significance set at p < 0.05 and adjusted with the Bonferroni correction where multiple comparisons were made.

3. Results

Weather data (Table 1) indicate that mean and low air temperatures were similar in the low snow cover year (2014) and the high snow cover year (2015). However, twice as much snow fell in December in 2015 as in 2014, and the snow was almost twice as deep. There were 25% more snow cover days in 2015 than in 2014.

Table 1 Air temperature, snowfall, snow depth, snowfall days and snow cover days before snowfall and after snowfall in December of the low snow cover year (2014) and high snow cover year (2015).

<table>
<thead>
<tr>
<th></th>
<th>Mean air temperature (°C)</th>
<th>Lowest air temperature (°C)</th>
<th>Heaviest snowfall (mm)</th>
<th>Highest snow depth (cm)</th>
<th>Snowfall days</th>
<th>Snow cover Days (&gt;50% of land covered)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low snow cover year (2014)</td>
<td>-14.8</td>
<td>-27.0</td>
<td>5.7</td>
<td>4</td>
<td>7</td>
<td>23</td>
</tr>
<tr>
<td>High snow cover year (2015)</td>
<td>-14.4</td>
<td>-25.5</td>
<td>11.3</td>
<td>7</td>
<td>6</td>
<td>31</td>
</tr>
</tbody>
</table>

Table 2 The effects of snow cover, snowfall and their interaction on the fecal corticosterone (CORT) and IgA of Asian Great Bustards.

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Predictor Variable</th>
<th>Wald Chi Square</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORT</td>
<td>Snow cover</td>
<td>9.519</td>
<td>1</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Snowfall</td>
<td>324.293</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Snow cover × Snowfall</td>
<td>15.141</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IgA</td>
<td>Snow cover</td>
<td>6.195</td>
<td>1</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>Snowfall</td>
<td>1.447</td>
<td>1</td>
<td>0.229</td>
</tr>
<tr>
<td></td>
<td>Snow cover × Snowfall</td>
<td>26.545</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Fig. 2. Pairwise comparison of effects of snow cover and snowfall on the fecal corticosterone (CORT, a) and immunoglobulin A (IgA, b) levels of Asian Great Bustards. Box limits represent the 25th and 75th percentiles. Horizontal lines through the box represent median values, and circles the mean value.
The interaction of snow cover and snowfall in our model was a significant predictor of CORT levels ($\chi^2 = 15.141$, $p < 0.001$; Table 2). Pairwise comparison revealed that CORT levels rose significantly after snowfall in both the low snow cover year (2014) ($p < 0.001$) and the high snow cover year (2015) ($p < 0.001$; Fig. 2a). Meanwhile, significantly higher levels of CORT were observed in the high snow cover year (2015), than in the low snow cover year (2014), both before snowfall ($p = 0.012$) and after snowfall ($p < 0.001$; Fig. 2a).

The interaction of snow cover and snowfall was also a significant predictor of IgA levels ($\chi^2 = 26.545$, $p < 0.001$; Table 2). In a pairwise comparison, IgA was significantly higher after snowfall than before snowfall in the low snow cover year (2014) ($p = 0.000 0 < 0.01$). There was also a significant difference between IgA levels before and after snowfall in the high snow cover year (2015), however, this was a decreasing trend ($p = 0.012 < 0.05$). The Pearson test indicated positive but no significant correlation between fecal CORT and IgA levels ($r = 0.013$, $p = 0.839$).

4. Discussion

Though repeat sampling of individuals is not possible under our non-invasive sampling methodology, the high level of variation observed (e.g. in IgA levels) makes it less likely that our findings were made with samples from only a limited number of individuals. We found that CORT levels rose after snowfall in both high and low snow cover conditions, and were higher overall during the high snow cover year. Our findings indicate that snowy conditions present challenges to Asian Great Bustards. In conditions of snow cover, the dormant vegetation and seeds which serve as winter diet for this species (Liu et al., 2017), would not be available, which may cause food shortage and nutrition stress. The Great Bustard also lacks a uropygial gland, which produces oils used to waterproof feathers (Jacob and Ziswiler, 1982). Great Bustards roosting on snow encounter cooling effects of the snow itself, as well as the thermal challenge of plumage dampened by any snow melted by body heat (Streich et al., 2006). We expect that these dietary and thermal challenges initiate a stress response during snowfall or periods of snow cover, activating the HPA axis and the release of CORT from the adrenal gland (Cockrem, 2007). Our findings concerning the response of this steppe bird, the Asian Great Bustard, are in line with other studies of birds, which have reported that snowfall was followed by an increase in circulating CORT levels (Rogers et al., 1993).

Elevated CORT levels have been observed in birds exposed to low ambient temperatures (Bize et al., 2010; Dorn et al., 2014; López-Jiménez et al., 2016), indicating that low temperatures might represent an alternative explanation for our findings. However, mean and low air temperatures varied little between the two winters of our study, which makes snowfall a more likely explanation for the significant changes in CORT we observed. Although a direct physiological response to snowfall is suggested in this study, alternative proximate factors (e.g. human disturbance, predation) may also play a role in the winter physiology of large steppe birds. Addressing these hypotheses will require additional research.

We observed an increase in IgA level after snowfall in the low snow cover year, but a decrease after snowfall in the high snow cover year. It is possible that when CORT levels, already elevated during the chronic stress of the high snow cover winter, were further elevated by the acute stress of snowfall, immune suppression resulted. Such a decrease in immune function following an episode of acute stress during a period of chronic stress could be analogous to the decrease in immunoglobulins observed after administration of corticosterone to Common Eiders (Somateria mollissima) experiencing the chronic stress of fasting during incubation (Bourgeon and Raclot, 2006). Thus, it appears as though the immune system reacted with an increase of IgA levels to face an acute stressor (snowfall during the low snow cover year) while during conditions of chronic climate stress in the high snow cover year, an additional stressor induced an immune depression. Our findings suggest that the severe climates inhabited by steppe birds such as the Great Bustard represent a source of chronic stress, and that the introduction of additional stressors may depress immune function.

5. Implications for conservation

Harsh winter weather conditions can cause population declines in a number of ways (Dantzer et al., 2014). Snow cover may directly limit food intake, and associated thermal challenges further challenge energy balance. Facultative migration triggered by winter weather may result in additional mortality due to energetic expenditures or threats on the migratory pathway. Poor wintering conditions may also affect population levels indirectly via carry-over effects on reproductive success in the subsequent breeding season (Järvištö et al., 2016). Climate change predictions indicate that severe winter weather events may become more common in the range of the Asian Great Bustard (Batıma et al., 2005; Dadvadorj et al., 2009), and snowfall in the mid-east area of Inner Mongolia has increased significantly in the past 20 years (Li et al., 2013). Our findings that snowfall and snow cover are associated with an endocrine stress response in Asian Great Bustards suggest an additional way in which extreme winter weather negatively impacts the species, as exaggerated or prolonged response to stress can suppress aspects of immune function, negatively affecting health, specifically, increasing susceptibility to infectious disease and delaying wound healing.

During severe winter weather, supplementary food is provided and snow cover is cleared to provide access to forage in Central Europe (Bankovics and Széll, 2014; Lóránt and Bankovics, 2013; Streich et al., 2006). This both decreases the chance that these birds will undertake facultative migration, which is associated with high mortality, and improves energy intake. Given the critical conservation status of the Asian Great Bustard, areas hosting wintering individuals should consider similar measures under severe weather conditions. Suitable wintering climates for the Asian Great Bustard are predicted to shift northwards and westward during the 21st century (Mi et al., 2016). This may result in additional scenarios similar to that at our field site, Tumiju Nature Reserve, where previously migratory breeding Asian Great Bustards have become sedentary. Managers of reserves hosting breeding populations should be prepared to provide suitable winter habitat conditions for these endangered birds, should those populations also shift from a migratory to a sedentary life history.

Competing interests

We have no competing interests

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ygcen.2018.02.014.

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