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SHORT NOTE

Increased hormonal stress reactions induced in an Alpine Black Grouse (*Tetrao tetrix*) population by winter sports

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Abstract In the Italian Alps the intensification of winter sports represents an increasing threat for Black Grouse (*Tetrao tetrix*). During the winters 2010/2011 and 2011/2012, characterised by different amounts of snowfall, 58 droppings from as many snow burrows were collected in three areas with different human disturbance to evaluate its effects on Black Grouse stress responses. Subjects in highly disturbed area showed higher concentrations of corticosterone metabolites during the winter with high snow cover highlighting a relationship between animals' stress and regular winter sports. As human-induced stress may contribute to population decline, tourism management should be planned to support Grouse conservation.

Keywords Endangered species · Alpine Galliformes · Conservation threat · Winter sports · Faecal corticosterone metabolites · Free-ranging populations

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Zusammenfassung

Durch Wintersport ausgelöste, hormonale Stressreaktionen bei einer alpinen Population von Birkhühnern (*Tetrao tetrix*)

In den italienischen Alpen stellen zunehmende Wintersportaktivitäten eine wachsende Bedrohung der Birkhühner (*Tetrao tetrix*) dar. In den Wintern 2010/2011 und 2011/2012, charakterisiert durch verschiedene Mengen an Schnee, wurden 58 Kotproben von ebenso vielen Schneehöhlen in drei durch Skitouristen unterschiedlich frequentierten Gebieten gesammelt. In den Kotproben wurden Stresshormonmetaboliten gemessen um den Einfluss der Störung durch den Menschen auf die Birkhühner zu erheben. Vögel im stark gestörten Gebiet wiesen höhere Konzentrationen an Kortikosteronmetaboliten im Winter mit hoher Schneelage auf. Dies legt einen Zusammenhang zwischen Stressreaktionen und regulärem Wintersport nahe. Da zusätzliche Belastungen zum Populationsrückgang beitragen können, sollten beim Tourismusmanagement Aspekte zum Schutz des Birkhuhnes berücksichtigt werden.

Introduction

Alpine Black Grouse (*Tetrao tetrix*) populations have dramatically decreased during the last decades, particularly since 1970s, and in many areas these animals are endangered or even extinct (Storch 2007). They have therefore been included in the European Directive 2009/147/EEC on the conservation of wild birds and in the IUCN Red List of Threatened Species.

Among the limiting factors that affect these populations, human outdoor leisure activities represent an increasing

threat. Human disturbance can indeed impact on individual fitness by frightening and driving off animals (Baines and Richardson 2007; Rolando et al. 2007; Patthey et al. 2008; Thiel et al. 2011) mainly during winter when energetic demands are higher and food resources are limited (Arlettaz et al. 2007; Rehnus et al. 2014). In this regard the intensification of winter touristic activities recorded in Italian Alps during the last decades seriously affects Black Grouse considering that winter sport infrastructures are mostly installed around their suitable habitat provoking its degradation and fragmentation (Arlettaz et al. 2007). Moreover skiers, snowboarders, and snowshoers can accidentally flush animals from their snow burrows, where Grouse spend most of their winter time, inducing an increase in their circulating glucocorticoids. This process, if repeated, may lead to a chronic stress state that can play a serious role in populations' decline (Baltic et al. 2005).

Here we used a non-invasive method (Sheriff et al. 2011) to investigate retrospectively the physiological stress response of a free-ranging Black Grouse population induced by snow sports in relation to different human disturbed areas. In particular, two sampling winters characterised by different amounts of snowfalls and snow cover were compared in order to evaluate the effects of contrasting levels of human disturbance on Black Grouse hormonal stress responses.

Materials and methods

Study area

The study area lies in the Veglia-Devero Natural park (North-West Italian Alps 443079.90 m E, 5129326.36 m N) at an altitude between 2,000 and 2,200 m a.s.l.. Three macro-areas with different human disturbance intensity were identified (Fig. 1):

- High disturbance: characterised by a ski resort with 24.72 ha, 2.042 km of ski lifts, ski runs and trails for freestyle skiing, ski mountaineering and snowshoeing; Black Grouse have to burrow their igloos close (less than 100–200 m) to the ski resort or among ski lifts;
- Moderate disturbance: close to high disturbed area and characterised by ski mountaineering trails; the distance between Grouse snow burrows and ski resort is around 500 m;

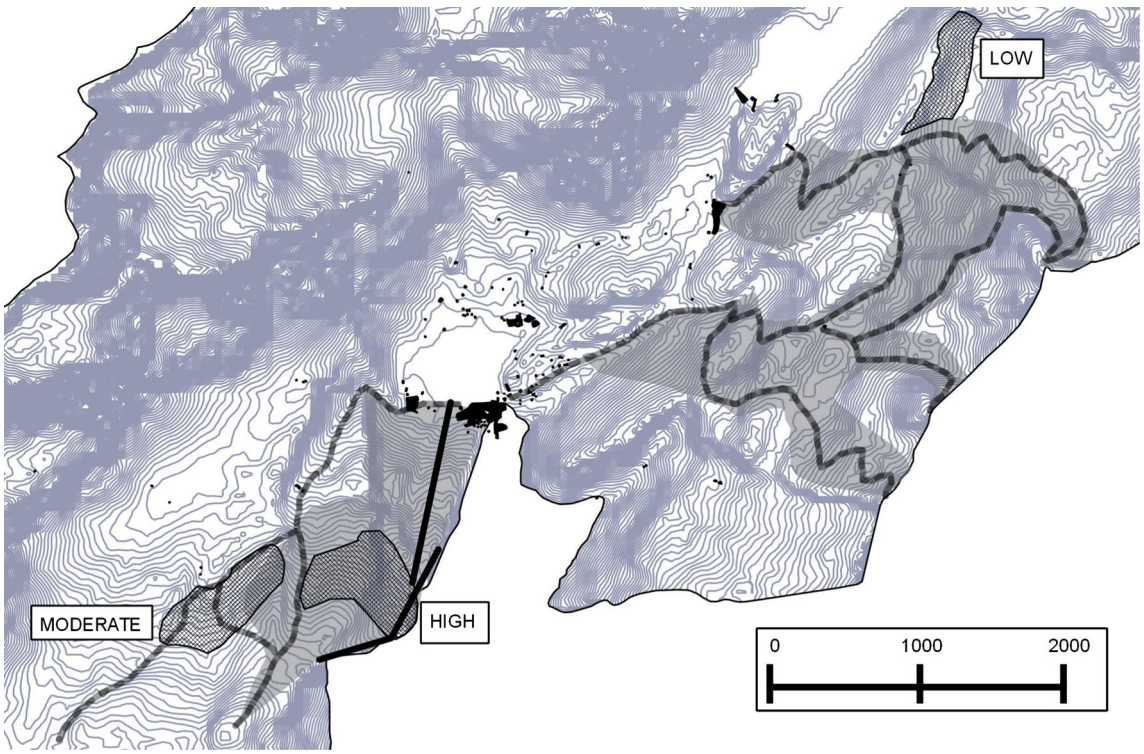

Fig. 1 Map of the three study areas (*dark grey*) characterised by different human disturbance, high, moderate and low, *black thick lines* represent the ski resort, *dotted lines* ski mountaineering and snowshoeing trails, *light grey areas* passage of tourists

- Low disturbance: far away and isolated but accessible to a few ski mountaineers and snowshoers. Here animals burrow igloos at a distance greater than 500 m from the stress sources.

Meteorological trend

Field sampling took place during two consecutive winters characterised by different meteorological trends. Using data registered by the weather station within the park, we distinguished the sampling years in relation to their different snow cover conditions:

- High snow cover (2010/2011): regular and abundant snowfalls from the second half of October till Spring providing a high snow cover during these months.
- Low snow cover (2011/2012): scarce snowfalls in January and February; in March a few abundant snowfalls occurred, but the following increasing temperatures and strong winds rapidly melted the snow cover.

Sampling of droppings

Black Grouse droppings were sampled by inspecting snow borrows after birds had flown away for natural reasons (e.g., they were not induced by the observer) avoiding any interference with the studied population. Sampling took place in January, February, and March. The droppings were collected the day after snowfalls from freshly opened snow burrows (which are clearly recognizable because the exit holes on the fresh snow are small and there is no ice at their bottom) or by inspecting the burrows immediately after the birds had flown away for natural reasons. This procedure enabled us to collect samples reflecting the hormone levels from the previous 8–12 h. A total of 58 droppings were collected from as many snow burrows and kept cold with silica-gel during the transport until the storage at −20 °C.

Analysis of glucocorticoid metabolites

Droppings were analysed through an enzyme immunoassay (Rettenbacher et al. 2004), validated for Black Grouse (Baltic et al. 2005) that quantified corticosterone metabolites (CM). Faecal concentration of CM reflects the hormone level in the plasma and can therefore be used to biomonitor the endocrine status (Touma and Palme 2005).

All the droppings per each snow burrow ($n = 5$–25) were homogenized to obtain a mean concentration of these metabolites over the last 8–12 h. An aliquot of 0.5 g of each sample was taken and extracted with 5 ml of 60 % methanol. Then 0.5 ml of the supernatant was dried down (3 h at 70 °C), transported to the Vetmeduni Vienna and redissolved in 60 % methanol (Palme et al. 2013). These aliquots were diluted 1:10 in assay buffer (pH 7.5) and analysed in the enzyme immunoassay.

Statistical analysis

Faecal CM concentrations were log transformed and modelled with a Generalized Linear Model with normal distribution. The model considered as explanatory variables area, year, and their interaction. Post hoc analysis (Sidak test) was performed if statistically significant factors or interactions were detected. The analyses were performed using SPSS Statistic 17.0® software; values were significant when $p < 0.05$.

Results

Concentrations of corticosterone metabolites were significantly influenced by area and by its interaction with year, while no difference was recorded between sampling years (Table 1; Fig. 2). During the high snow cover winter, CM values in highly disturbed areas were significantly higher than those from the moderate (pairwise comparison $p = <0.001$) and low disturbed (pairwise comparison $p = 0.007$) ones, while no difference was found between the two latter areas. During low snow cover winter, no differences were found between the three areas.

Discussion

The present study aimed at examining the impact of the interaction between snow cover and human disturbance on Black Grouse stress hormone levels. We found higher concentrations of faecal corticosterone metabolites during the winter with high snow cover and high snow sport disturbance, while during the winter with low snow cover, no differences between areas were found.

The higher CM levels in the high disturbed area during the winter with high snow cover indicate a stressful condition in Black Grouse. Although, because of the small

Table 1 Minimal linear model of factors affecting Black Grouse faecal corticosterone metabolites

	Wald Chi Square	*df*	*p* value
(Intercept)	1,652.545	1	<0.001
Year	1.064	1	0.302
Area	9.776	2	0.008
Year:Area	12.130	2	0.002

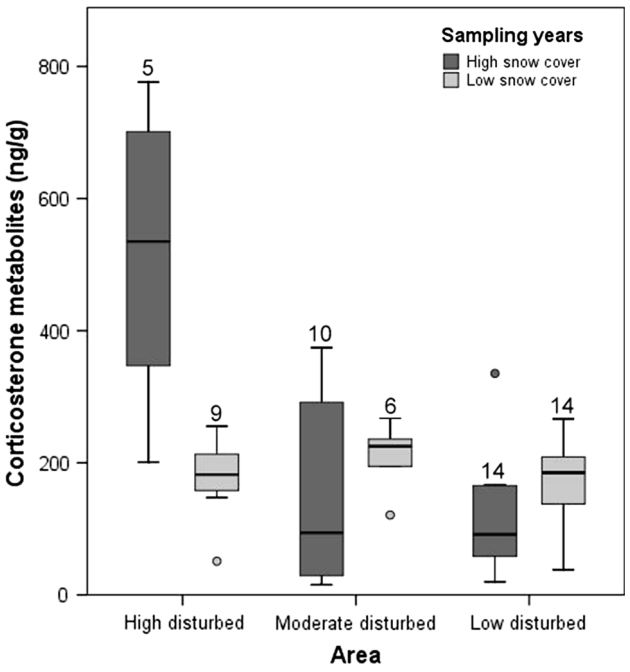

Fig. 2 Boxplots of concentrations of faecal corticosterone metabolites in Black Grouse in the three sampling areas (High, Moderate and Low disturbed) during the two sampling winters, High (2010/2011) and Low (2011/2012) snow cover. The 25th and 75th percentile (*box limits*), the median and outliers (*full circles*) are shown. Sample size is displayed above whiskers

sample size, this finding could be the result of the presence of just a few influential individuals, the regular snowfalls during this sampling year had indeed favoured the presence of visitors in the Grouse habitat. Considering that animals have to burrow igloos among ski lifts, the increase of adrenocortical activity emphasizes that the frequent attendance of tourists could negatively affect the birds' survival. Besides, in moderate and low disturbed areas, Black Grouse showed significantly lower stress hormone levels reflecting the less regular or sporadic public access to these areas. Moreover the much greater distance (about or more than 500 meters) between Grouse habitat and visitors' paths and infrastructures could contribute to a lower anthropogenic disturbance, as found by Thiel et al. (2011) for Capercaillie (*Tetrao urogallus*).

During the winter with low snow cover, the non-significant differences in CM values between the three areas indicate that Black Grouse were exposed to the same level of disturbance. The lack of snow during this winter has led to a drastic reduction of sport activities and public access to the areas, even in the high disturbed one, making the human disturbance levels uniform for animals of all three areas. Nevertheless CM values of moderate and low disturbed areas were higher compared to those of the winter with high snow cover. Considering the importance of snow conditions in Black Grouse winter roosting strategy (antipredator and energy-saving strategy of snow burrowing; Bocca et al. 2014), the scarce snowfalls and the related less appropriate snow conditions for excavating igloos appear to have evoked a rise of stress hormone levels in these subjects. Indeed the unusual climatic conditions of this winter seem to have imposed an allostatic load on the animals as reflected in the higher CM values (McEwen and Wingfield 2003).

Conclusions

Our results indicate a relationship between increased stress hormone levels in Black Grouse and the regular disturbance by snow sports. CM concentrations were significantly higher in the most disturbed area during winter characterised by high snow cover. As induced stress can alter animals' body conditions reducing their resistance to disease (Rehnus et al. 2014), human disturbance must be considered as a further serious threat for the fitness of this endangered species and may contribute to the population decline. This fact, together with the vulnerability of the population in the study area (Formenti et al. 2013), should encourage management plans to regulate tourists' access. Moreover these results should be considered in case of planning new snow infrastructures or new snow sports to prevent further threats for the Grouse population.

The lack of snow during the second sampling year appears to have evoked an increase of CM levels due to the additional allostatic load imposed on the animals by these unusual snow conditions. Considering expected climate changes in the future, further studies are needed to evaluate their effect on Black Grouse population.

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Conflict of interest The authors declare that they have no conflict of interest.

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