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Winter tourism increases stress hormone levels in the Capercaillie *Tetrao urogallus*

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Montane and alpine habitats in Europe remained relatively undisturbed until the beginning of the last century. Today, outdoor recreation activities are a major economic factor in alpine regions. Many tourism areas coincide with winter habitats of shy and endangered species. The Western Capercaillie *Tetrao urogallus* has suffered from rapid population declines during recent decades over much of its range. In central Europe, many Capercaillie are exposed to intensive human recreation activities in their habitats, which may contribute to this decline. However, little is known about their susceptibility to human recreation activities. This study assessed whether human recreation in winter evokes physiological stress responses in several populations of Capercaillie. During two winters, we sampled 1130 Capercaillie droppings in Germany and Switzerland of populations at various distances from winter recreation activities and measured concentrations of faecal corticosterone metabolites. Capercaillie in relatively dense and homogeneous mountain forests dominated by Norway Spruce *Picea abies* showed markedly increased stress hormone levels closer to locations with winter recreation activity. However, this physiological response to human recreation was not detectable in forests dominated by various pine species and a heterogeneous structure. Capercaillie may be particularly sensitive to recreation because any factor affecting their fine-tuned physiological and behavioural adaptations to survive under harsh winter conditions may lead to harmful fitness costs.

Keywords: corticosterone, grouse, human disturbance, recreation, stress ecology.

Outdoor recreation is a major concern for biodiversity conservation, causing population decline in endangered species (Venizelos 1991). It is one of the main causes for the decline of threatened and endangered species in the United States (Czech 2000). In Europe, montane and alpine habitats remained relatively undisturbed by tourism until the beginning of the last century. Today, skiing and other outdoor sports are among the most important economic factors in alpine regions (Elsasser & Messerli 2001). The increase in human recreation in these habitats is mainly due to the development of winter sport infrastructure such as buildings, car parks, hiking trails, ski-lifts, ski-runs and ski-tracks. During recent years, off-trail sport activities such as paragliding, snow-shoeing and free-riding snow sports have increased greatly and affect large areas in these hitherto rural and pristine habitats. These areas are also the habitats of rare and shy species, such as Black Grouse *Tetrao tetrix*, Western Capercaillie *Tetrao urogallus* (henceforth Capercaillie) and Chamois *Rupicapra rupicapra*. These species are most sensitive to human recreation activities in winter because this is energetically the most demanding season of the year in the mountains. In winter, tourism activities in these areas are much more intensive and more widespread (off-trail)

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than in summer, and thus have a high potential to act as a source of disturbance. It has already been shown that winter tourism negatively affects the species richness of alpine bird communities (Ronaldo *et al.* 2007), influences space use by Capercaillie (Summers *et al.* 2007, Thiel *et al.* 2008) and leads to physiological stress responses in both this species and Black Grouse (Arlettaz *et al.* 2007, Thiel *et al.* 2008).

The Capercaillie is expected to be particularly sensitive to human recreation activities (Storch 2000), and some case studies have already documented local population declines or even disappearance after an increase in human outdoor activities (Labigand & Munier 1989, Brenot *et al.* 1996). Increasing human recreation activities are therefore potentially a major cause of marked population declines during recent decades. Most of the remaining populations in central and western Europe today are confined to high-elevation coniferous mountain forests, to small populations (often < 200 individuals) and to fragmented habitat patches (Storch 2000, Storch *et al.* 2006). This has led to the listing of the Capercaillie in many national red-data books in central and western Europe as threatened or in danger of extinction (Storch 2000, Storch *et al.* 2006).

One reason why Capercaillie may be particularly sensitive to winter tourism is because it is restricted to feeding on conifer needles in winter (Klaus *et al.* 1989). Needles have a low nutrient content and are difficult to digest. This requires a long digestion time and results in a low rate of energy intake and a tight energy budget. Therefore, the potential of recreation activities to have negative effects is highest in winter. For example, additional energy expenditure could be caused by frequent escape flights of Capercaillie due to people. Flushing distances of Capercaillie as a response to an off-trail hiker in forests with high recreation intensity are longer than those with moderate or low recreation intensities (Thiel *et al.* 2007a). One physiological indicator of a bird's response to stressful events is the activation of the hypothalamo-pituitary-adrenal axis, resulting in the release of glucocorticoids (specifically corticosterone in birds). Glucocorticoids trigger adjustments in physiology and behaviour to help the organism to survive (Sapolsky 1987, Wingfield & Romero 2001). Plasma glucocorticoid concentrations are therefore widely used to diagnose a physiological stress response (e.g. Von Holst 1998, Möstl & Palme 2002). In free-living Capercaillie, it is impossible to sample blood without any feedback from capture and handling. However, the measurement of the concentration of corticosterone metabolites (CMs) excreted in droppings is a non-invasive method to quantify hormone production (Möstl & Palme 2002, Thiel *et al.* 2005b).

A repeated or lasting increase in corticosterone could impact on fitness, as chronically elevated levels of this stress hormone affect growth, body condition, immune-function, reproduction and survival (Wingfield *et al.* 1997). In an earlier study we demonstrated higher stress hormone levels in Capercaillie inhabiting forest patches with moderate and high than with low winter recreation intensities (cross-country and downhill skiing) in one focus population in southern Germany (Thiel *et al.* 2008). However, stress hormone studies across many populations at a large spatial scale and including many different types of recreation activities are still lacking.

In the present study we aimed to assess whether the physiological stress response to winter recreation is a general pattern across many Capercaillie populations over a large spatial scale. We therefore conducted an extensive sampling of Capercaillie droppings in populations across Germany and Switzerland to assess adrenocortical activity. We hypothesized that Capercaillie close to winter recreation activities or in habitats with high winter recreation intensity would show higher levels of CMs in droppings compared with Capercaillie far from human presence.

METHODS

Study areas

During two winters, 2003/2004 and 2004/2005, from 1 November to 31 March, we sampled fresh Capercaillie droppings in three study areas in central Europe (Fig. 1): the southern Black Forest in southwestern Germany (47°51′N, 7°58′E), the western Swiss Jura (46°33′N, 6°15′E) and the Swiss Alps (46°45′N, 9°04′E). In the Black Forest study area, we sampled at five sites totalling 11 km^2 between 900 and 1400 m asl. Forests consisted mainly of Norway Spruce *Picea abies* (49%), European Silver Fir *Abies alba* (19%) and Common Beech *Fagus sylvatica* (22%; Suchant *et al.* 2003). In the Swiss Jura, droppings originated from five sites with a total area of 4 km^2 at

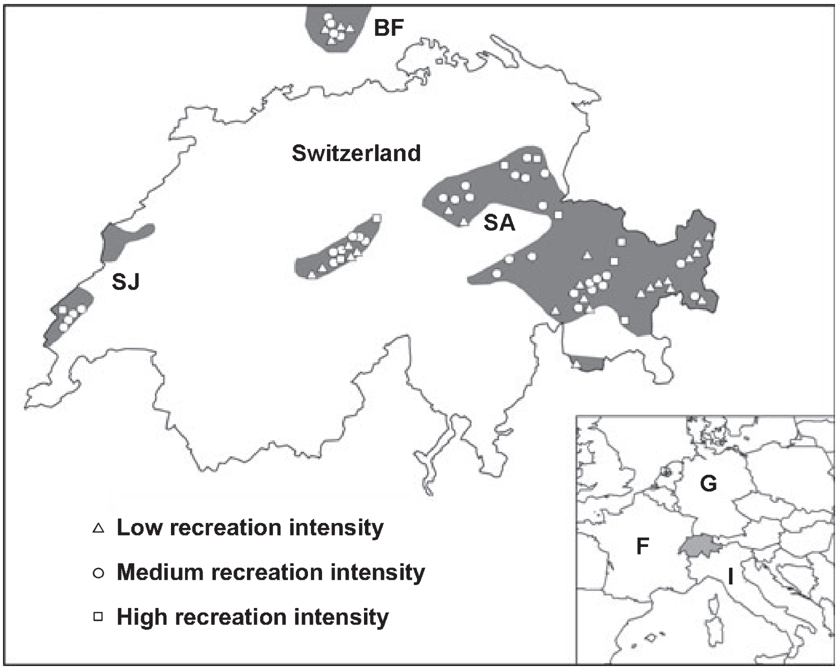


Figure 1. Location of the three study areas in Europe (inset; G = Germany; I = Italy; F = France): southern Black Forest (BF) in southwestern Germany, western Swiss Jura (SJ), and Swiss Alps (SA), where we collected 1130 droppings from Capercaillie at 71 sites in two winters (2003–2004 and 2004–2005) to analyse levels of stress hormone metabolites. The shaded area is the distribution range of the Capercaillie in Switzerland and the southern Black Forest. Sites are characterized according to the degree of human disturbance.

elevations ranging from 1300 to 1500 m asl. Forests were dominated by Norway Spruce (73%), European Silver Fir (12%) and Common Beech (10%; U. Ulmer pers. comm.). Pines were absent in the Black Forest and the Swiss Jura study sites. In the Swiss Alps, we collected droppings at 58 sites totalling 51 km^2 with a large variety of different forest types and elevations ranging from 1300 to 2200 m asl. Tree composition varied from pure to mixed coniferous forests with different dominant tree species (Norway Spruce, European Silver Fir, Scots Pine *Pinus sylvestris*, Mountain Pine *Pinus mugo*, Swiss Stone Pine *Pinus cembra*, Larch *Larix decidua*). However, tree composition was strongly dominated by either Norway Spruce or pines. Proportions of deciduous trees (Common Beech and Sycamore *Acer pseudoplatanus*) were < 10% at all sites of the Swiss Alps. Inter-site distances were > 1 km in all study areas, and sites were regarded as independent of each other, as Capercaillie space use in winter is restricted (Gjerde *et al.* 1985, Storch 1993). Snow covered the forest ground at all sites on all sampling days, and the majority of mean daily temperatures (November–January) were < 0 °C.

Winter recreation at our study sites was very diverse. In the southern Black Forest (see Thiel *et al.* 2008) and in the Swiss Jura it mainly consisted of cross-country skiing, downhill skiing and hiking, as well as some dog-sledding in the Swiss Jura. In the Swiss Alps, winter recreation strongly depended on site and included all types of skiing (on- and off-trail), snow-shoeing, hiking, sledding and dog-sledding. None of the study sites contained buildings or villages used year-round. At some study sites, only a single recreation activity was carried out; at other sites, several or none. The intensity of recreation ranged from sites without any human presence over the entire winter to heavily used skiing areas with several hundred skiers a day. There were no human activities other than winter recreation during our study period, except for some selective tree harvesting in early winter.

Estimated population size in the Black Forest study area exceeded 60 Capercaillie (Braunisch & Suchant 2006), in the Swiss Jura study sites about 56 (S. Sachot pers. comm.) and in all Swiss Alp sites combined at least 350 (K. Bollmann and P. Mollet pers. comm.).

Sampling procedure

Droppings were located on the snow surface by walking along contour lines in Capercaillie core areas (see methods in Thiel *et al.* 2007b). Capercaillies excrete two types of droppings. Caecal droppings are of a viscous, odorous and unshaped nature and were not sampled. Intestinal droppings in winter, with conifer needles as the only food, are uniform in shape (elongated cylinders of about 50 mm in length), colour and composition, and are not odorous. Because conifer needles are low in metabolizable energy, many droppings are excreted (about one every 12 min; Klaus *et al.* 1989) and many can be found on the snow surface. These include droppings excreted by birds feeding or roosting in trees. Because the male is about twice as heavy as the female, almost all droppings could be assigned to a sex by diameter (males > 10 mm, females < 8 mm) and length (males > 60 mm, females < 40 mm; K. Bollmann unpubl. data). Because stress levels may vary between day and night and when exposed to ground predators or not, we distinguished between droppings excreted on the ground (scattered occurrence) and those excreted from trees (in heaps or in a small area under a tree). Droppings excreted on the ground are all excreted during the day while feeding or roosting, because in all study areas, Capercaillie have their night roosts only in trees (Thiel *et al.* 2007b). Droppings excreted from trees are excreted during night or day while roosting or during the day while feeding on the tree. A further complication is introduced by the fact that there is a time delay of about 1–3 h between concentrations of plasma corticosterone and excreted CMs (Thiel *et al.* 2005b), thus CMs in some droppings on the ground may reflect the situation on the tree, or vice versa. However, Capercaillie roost most of the time in winter, and this may therefore be of minor importance. Home ranges of Capercaillie in winter are small (Storch 1993) and the daily activity budget is restricted to a few hours (Gjerde & Wegge 1987). Therefore, the droppings occurred in piles (e.g. below roosting or foraging trees) or were more scattered but spatially clumped within a few metres. From each such location, we sampled 5–15 individual droppings, which we designated a dropping sample. We only sampled fresh droppings and considered dropping samples from the same sex within a circle of 300 m (28.3 ha) as originating from the same individual, because, at low densities, Capercaillie only occasionally aggregate in flocks (Klaus *et al.* 1989). Thus, each dropping sample was assigned to a potential individual (INDIVIDUAL) to avoid pseudo-replication. For each dropping sample we determined the dominating FOREST TYPE (spruce or pine forest), SEX (male or female), minimum daily temperature from the next meteorological station corrected for the elevation by 0.6 °C per 100 m (TEMPMIN; MeteoSchweiz), DATE (transformed Julian day by numbering each day from 1 November to 31 March), type of DROPPING (with two types: excreted on a tree while night roosting or foraging, and excreted on the ground while day-roosting or walking; see methods in Thiel *et al.* 2007b), distance (in m) to the next location with frequent winter recreation activities (RECREATION; ski-run, ski-track, ski-lift, hiking trail, road, etc.), and study area (AREA; Black Forest, Swiss Alps, Swiss Jura). The potential disturbance sources were digitized in GIS (ArcGIS 9.1, ESRI, Redlands, CA, USA) and the distance from each dropping sample to the next winter recreation activity was calculated.

To test for the effect of winter recreation intensity, as opposed to distance to the next potential recreation activity as above, we defined three classes of recreation intensity. We assigned dropping samples in forests with no human presence during the winter (e.g. inaccessible areas or > 500 m from any recreation activities) to the category of low recreation intensity. Droppings found within 50 m of all regularly used tourism infrastructure in forests, such as permanent ski-tracks, hiking trails or other sport facilities, were assigned to the category high recreation intensity (90th percentile flushing distance of 50 m; Thiel *et al.* 2007a). All remaining Capercaillie habitat was classified as having moderate recreation intensity.

Analysis of CMs in droppings

Glucocorticoids are extensively metabolized before excretion (Rettenbacher *et al.* 2004) and the metabolism varies between species (Palme *et al.* 2005). Therefore, the method of analysis of the CMs has to be validated for each species (Möstl & Palme 2002, Touma & Palme 2005).

The concentration of CMs in Capercaillie droppings was measured with a cortisone enzyme immunoassay (EIA), which was developed for chicken (Rettenbacher *et al.* 2004) and successfully validated for Capercaillie (Thiel *et al.* 2005b). In short, the temporal excretion of CMs, the types of metabolites and their detection by an antibody were investigated with a radiometabolism study in captive Capercaillie, followed by a biological validation experiment. The excretion of labelled ^{3}H-corticosterone started 1–4 h after injection and showed in either one broad peak or two peaks. The first peak can be assigned to metabolites excreted via urine and the second to the excretion of metabolites via faeces (Thiel *et al.* 2005b, see also Rettenbacher *et al.* 2004). Metabolites of peak radioactivity samples were separated by reversed-phase high-pressure liquid chromatography (RP-HPLC) and revealed the presence of three to four major radioactive peaks, indicating that several CMs were excreted. The highest amount of immunoreactivity was detected with the cortisone EIA, measuring metabolites with a common 3,11-dione structure. The physiological validation experiment consisted of injecting ACTH (adrenocorticotropic hormone), which elicits an immediate release of corticosterone into the bloodstream. As expected, the cortisone assay showed a sharp increase of CMs in the droppings 1–3 h after injection.

The concentration of CMs in droppings may change with time after defecation and storage conditions. Because the exact time since excretion of droppings collected in the field is unknown, an experiment measuring the effect of different temperatures (−20, 8 and 21 °C, respectively) and storage times (1, 7 and 21 days) was performed (Thiel *et al.* 2005b). This study confirmed that CMs can be reliably measured if droppings are collected within 7 days after defecation and ambient temperature is < 9 °C, conditions that were fulfilled in our winter study.

The concentration of CMs varies between individual droppings excreted within a short time by the same individual (Baltic *et al.* 2005) because of pulsative excretion (Klasing 2005) or other factors. In accordance with Scheiber *et al.* (2005), we pooled and homogenized the 5–15 droppings of each dropping sample before lyophilization, extraction and analysis (Thiel *et al.* 2005b) to obtain an overall value of the concentration of CMs representative of a longer time span of several hours. This is in contrast to plasma samples, in which the concentration of corticosterone is measured at the time point of blood sampling. Therefore, a comparison of CM concentration in droppings and corticosterone levels in plasma is not possible.

Data analysis

With the same rate of CM excretion, the concentration of CMs in droppings depends on the quantity of droppings produced, which in turn primarily depends on food intake and the proportion of food absorbed by the digestive tract. For example, under low ambient temperature, energy needs and thus food intake are higher and the concentration of CMs is 'diluted' across a higher abundance of droppings. If one food type provides a higher proportion of metabolizable energy, and thus produces a lower amount of faeces for the same amount of energy extracted, the concentration of CMs should be higher than with a food-type providing a lower proportion of metabolizable energy. Extensive feeding experiments with captive Capercaillie demonstrated that needles from *Pinus* species (*Pinus sylvestris*, *Pinus mugo*, *Pinus cembra*) provided about 20% more metabolizable energy per unit dry mass than needles from *Picea abies* and *Abies alba* (Lieser *et al.* 2006). Because *Pinus* species are preferred over *Picea abies* and *Abies alba* (Schroth *et al.* 2005), we expected generally higher concentrations of CMs in droppings from pine forests than from spruce forests. Furthermore, pine and spruce forests differ in many respects such as habitat quality, food quality and predator composition (see Discussion for details). Therefore, we analysed our data separately for spruce forests sampled in all three study areas (290 dropping samples from the Black Forest, 101 from the Swiss Jura and 277 from the Swiss Alps) and for pine forests sampled only in the Swiss Alps study sites (462 dropping samples).

We used mixed model residual maximum likelihood (REML) analysis (Patterson & Thompson 1971) to identify factors affecting CM concentration in dropping samples with INDIVIDUAL fitted as a random effect. The model for spruce forests contained all six predictor variables as fixed effects. In the model for pine forests, the variable AREA was omitted because pine forests were only sampled in the Swiss Alps. The order of fitting of the predictor variables was the same in both models. Years were pooled. The three continuous predictor variables TEMPMIN, DATE and RECREATION were also included in the model as both linear and squared terms to test for non-linearity. In a first step, we included all two-way interaction terms, which we expected *a priori* to be of biological relevance. Non-significant interaction terms were omitted from the final model. We used GENSTAT (VSN International, Hemel Hempstead, UK) for Windows version 7.3 for this analysis.

RESULTS

In droppings sampled in spruce forests, all predictor variables except area were significantly related to CM concentration (Table 1). Males generally had higher CM levels than females, but this pattern was dependent on minimum daily temperature and sampling date (interaction terms SEX × TEMPMIN and SEX × DATE significant; Table 1). CM levels increased non-linearly with decreasing temperature (squared TEMPMIN significant) and this trend was more pronounced in males than in females (interaction term SEX × TEMPMIN significant; Fig. 2). CM levels of males increased non-linearly from November to March, whereas those of females did not change with date (Fig. 3). Males and females walking or day-roosting on the ground had higher CM levels than those foraging or night-roosting in trees, and this difference tended to become larger with progressing season (interaction DATE × DROPPING significant; Fig. 3). Distance to the next recreation activity significantly affected CM levels and the nearly significant squared term of RECREATION indicated a non-linear effect (Fig. 4). Within a distance of 500 m, CM levels of males and females increased with increasing closeness to recreation activities, whereas recreation activities further than 500 m from the birds did not affect CM levels. There were no differences in CM levels between the Black Forest, the Swiss Jura and the Swiss Alps.

When we ran this model with the categorical variable recreation intensity instead of distance to the next recreation activity, we obtained very similar results and a significant effect of recreation intensity (Wald: 14.65, $P < 0.001$; model not shown). Mean CM level increased from sites with low to those with moderate and to those with high recreation intensity (Fig. 5). The predicted means from the model were 40 ng/g dropping (se 1.5) for sites with low recreation intensity, 45 ng/g (se 1.2) for sites with moderate recreation intensity and 51 ng/g (se 1.7) for sites with high recreation intensity.

Table 1. Dependence of the concentration of corticosterone metabolites in Capercaillie droppings (n = 668) from spruce forests on various predictor variables analysed in a multivariate linear REML model with the individual bird INDIVIDUAL as random effect.

Variable	Effects ± se	Wald	df	P
SEX (female)	−6.819 ± 1.782	25.80	1	< 0.001
TEMPMIN	−0.245 ± 0.387	5.57	1	0.019
squTEMPMIN	0.041 ± 0.035	7.67	1	0.006
DATE	−0.008 ± 0.196	9.84	1	0.002
squDATE	0.001 ± 0.001	0.64	1	0.424
DROPPING (ground)	9.652 ± 1.803	32.64	1	< 0.001
RECREATION	−0.030 ± 0.009	12.20	1	< 0.001
squRECREATION	0.00002 ± 0.00001	3.07	1	0.080
AREA		0.33	2	0.721
(Swiss Alps)	−2.447 ± 2.096			
(Swiss Jura)	−0.592 ± 2.550			
SEX × TEMPMIN	−0.198 ± 0.596	56.58	1	0.011
SEX × squTEMPMIN	−0.033 ± 0.059	9.68	1	0.095
SEX × DATE	0.352 ± 0.264	8.38	1	0.004
SEX × squDATE	−0.003 ± 0.001	3.38	1	0.067
DATE × DROPPING (ground)	0.113 ± 0.048	5.75	1	0.017

The predictor variables included sex (SEX; the effect given for females refers to a reference value of zero for males), minimum daily temperature (TEMPMIN) and its quadratic term (squTEMPMIN), date (DATE) and its quadratic term (squDATE), type of dropping (DROPPING; the effect given for ground refers to a reference value of zero for tree), distance (in m) to the next location with frequent winter recreation activities (RECREATION) and its quadratic term (squRECREATION) and AREA (the effects given for Swiss Alps and Swiss Jura refer to a reference value of zero for Black Forest). For each variable are given the effects ± se, the Wald statistics, degrees of freedom and the P-value.

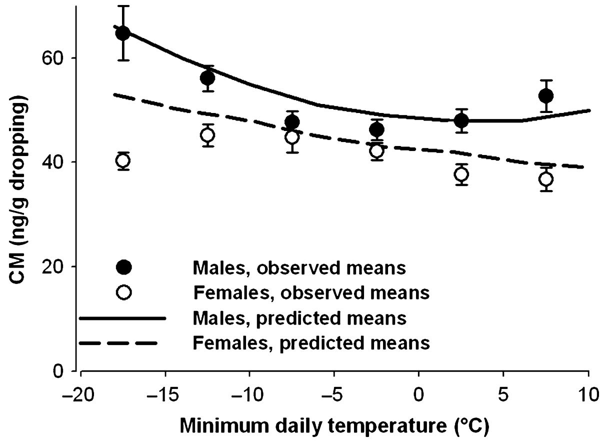
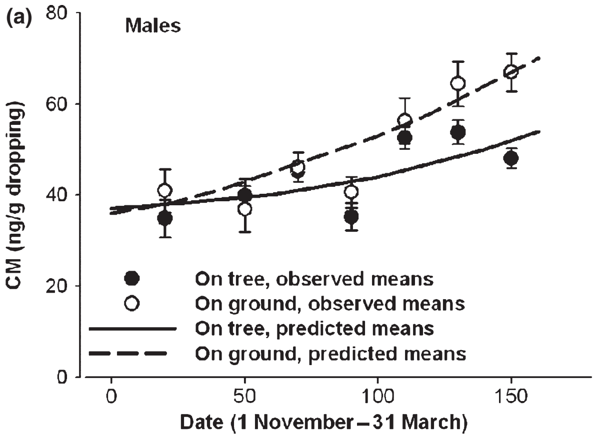


Figure 2. Observed means (± se) of concentrations of corticosterone metabolites (CMs) in Capercaillie droppings (n = 668) found in spruce forests for males and females grouped at 5 °C intervals of minimum daily temperature (TEMPMIN). The curvilinear dependence predicted from the REML model presented in Table 1 is indicated for males and females.



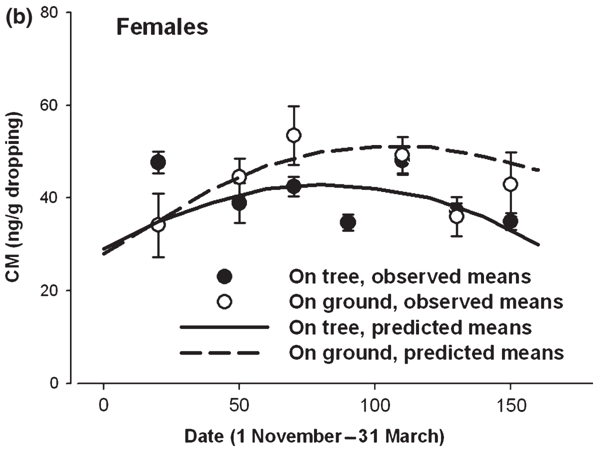


Figure 3. Observed means (± se) of concentrations of corticosterone metabolites (CMs) in Capercaillie droppings (n = 668) of males (a) and females (b) found in spruce forests for Capercaillie roosting or walking on the forest ground and Capercaillie night-roosting or foraging in trees grouped in 20-day periods (DATE = transformed Julian day by numbering each day from 1 November to 31 March). The curvilinear dependence predicted from the REML model presented in Table 1 is indicated for droppings excreted from trees and on the forest ground.

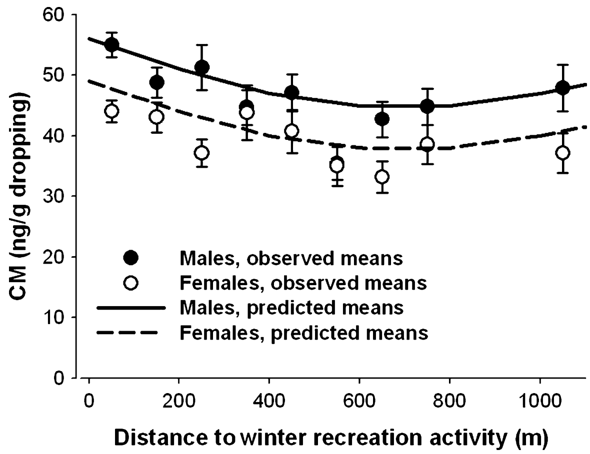


Figure 4. Observed means (± se) of concentrations of corticosterone metabolites (CMs) in Capercaillie droppings (n = 668) found in spruce forests for males and females grouped at 100-m intervals of the distance to the next winter recreation activity. The curvilinear dependence predicted from the REML model with the continuous variable RECREATION presented in Table 1 is indicated for males and females.

In droppings sampled in pine forests of the Swiss Alps, CM levels (mean ± se) were generally higher than in spruce forests (54 ± 0.9 ng/g dropping vs. 46 ± 0.8 ng/g; ANOVA df = 1130, F = 49.5, P < 0.001). The only predictor variables with a significant relation to CM concentration were sex (Wald: 8.63, P = 0.003) and temperature (squared TEMPMIN; Wald: 13.18, P < 0.001). Similarly to the analysis of droppings in spruce forests, CM levels of droppings from pine forests were higher in males than in females (56 ± 1.0 ng/g dropping vs. 50 ± 1.7 ng/g), and increased non-linearly with decreasing temperature. However, date, type of dropping and distance to the next recreation activity had no or only a weak significant effect on CM levels. The same held for the categorical variable recreation intensity, which had no significant effect on CM concentrations (Fig. 5).

DISCUSSION

Susceptibility to human winter recreation activities

This study showed that Capercaillie physiology is associated with human winter recreation activities in spruce forests across several populations and a wide variety of intensity of outdoor sport activities. Therefore the individual physiological response of Capercaillie to winter recreation activities may not be unique to the spatially restricted population in the Black Forest (Thiel *et al.* 2008), and seems to be a general pattern in central European populations, at least in spruce forests. The development and validation of the non-invasive technique to determine adrenocortical activity in Capercaillie by measuring CMs in their droppings has made such studies possible (Palme 2005, Thiel *et al.* 2005b, Touma & Palme 2005). By carefully accounting for factors affecting CM concentrations in droppings at the methodological level (storage temperature, time since excretion) and analytical level (inclusion of factors such as sex, temperature and main food in the statistical model), we are

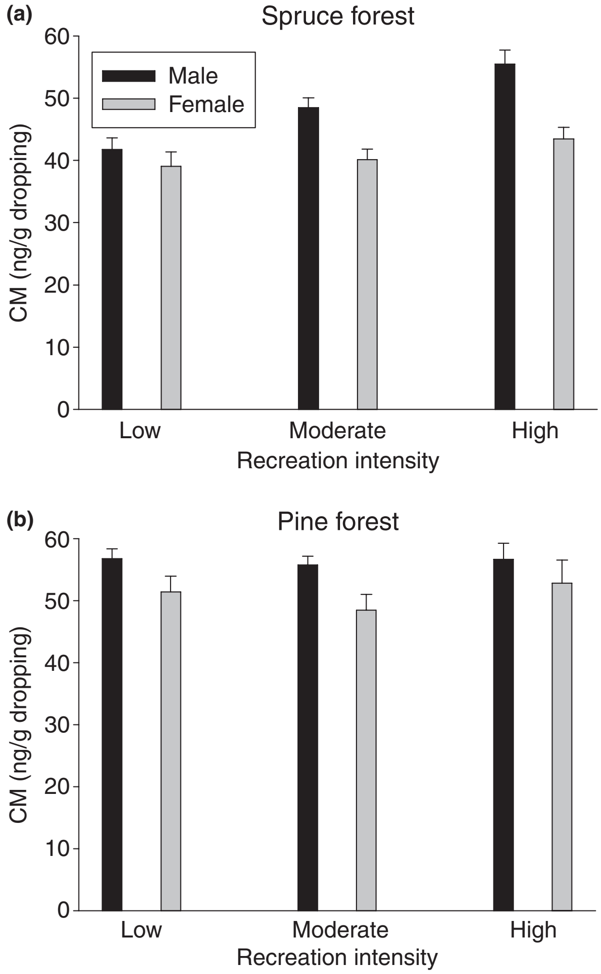


Figure 5. Observed means (± se) of concentrations of corticosterone metabolites (CMs) in Capercaillie droppings found in sites with low, moderate and high recreation intensity for spruce (a) and pine forests (b).

confident that CM levels are a good index of stress. This is confirmed by a flushing experiment in Black Grouse (Arlettaz *et al.* 2007).

This study adds to the very limited evidence base of individual physiological responses of grouse to human activity (Arlettaz *et al.* 2007, Thiel *et al.* 2008). In Black Grouse, experimental flushing of individuals resulted in an increase of CM levels and Black Grouse living in areas of winter recreation have higher CM levels than those living in control areas (Arlettaz *et al.* 2007). In one focus population in the Black Forest, Capercaillie and people used the same habitats and Capercaillie often could not avoid humans because of a lack of suitable alternative habitats (Thiel *et al.* 2008). In the Black Forest study, CM levels of Capercaillie were lower in areas with low compared with moderate or high recreation intensity and Capercaillie avoided disturbed parts of their home-ranges. In the present study we showed on a much wider scale that the adrenocortical activity increased when birds were closer than 500 m to human recreation activities, whereas birds further away were not affected. This pattern may have two causes. First, the susceptibility of Capercaillie to human recreation activities may extend over a much larger distance than the 50 m from which 90% of Capercaillie flush in response to a single off-trail hiker (Thiel *et al.* 2007a). Secondly, the observed pattern may be the result of a decreasing human presence with distance from the next location with frequent winter recreation activities (ski-run, ski-track, ski-lift, hiking trail, road), because we did not measure the actual disturbance distance. The two factors probably act together, because at many sites winter recreation activities do not extend substantially beyond the ski-trail or hiking trail. Hence, human winter recreation is likely to affect Capercaillie physiologically even beyond the flushing distance. Because winter recreation activities have greatly increased in all the central European mountain areas, this may represent a new threat to grouse species and possibly to many other species.

Free-ranging animals perceive humans as predators and respond to the occurrence of people with anti-predator behaviour (Beale & Monaghan 2004), including the activation of the hypothalamo-pituitary-adrenal axis with the release of glucocorticoids into the blood. This adjusts the behaviour and the physiology of animals to an acutely dangerous situation (Wingfield & Romero 2001). In our dropping samples, CM concentrations were in the range of those found in captive birds (30–60 ng/g dropping), but clearly lower than in ACTH-injected birds, which showed CM peaks 5–60 times higher (Thiel *et al.* 2005b). In our samples, corticosterone release was integrated over several hours. Therefore, it was not possible to decide whether the elevated CM concentrations in droppings near recreation facilities are due to one or a few acute stressors (the classical stress response, for example, to flushing) or a chronic elevation of baseline levels. However, the flushing experiment with Black Grouse demonstrated that one single flushing event increased CM levels in droppings excreted the next day (Arlettaz *et al.* 2007), suggesting a longer lasting elevation of CM levels. This is of importance because, if elevated corticosterone levels persist or a series of acute stressors initiate multiple consecutive stress responses, an animal becomes chronically stressed (Wingfield & Romero 2001). In a chronically stressed animal, elevated corticosterone does not exert the short-term physiological and/or behavioural changes crucial for alleviating or ameliorating an acute stressor, but instead becomes detrimental, potentially leading to pathological conditions (Wingfield *et al.* 1997). Human disturbance can cause chronic stress with such deleterious effects. Carolina Chickadees *Poecile carolinensis* from logged forests have higher faecal CM levels and a lower body mass than birds from undisturbed forests (Lucas *et al.* 2006). Juvenile Hoatzins *Opisthocomus hoazin* living at sites with ecotourism have a lower body mass and higher mortality, and show a stronger increase of corticosterone to experimental stress compared with individuals at undisturbed sites (Müllner *et al.* 2004). The increased adrenocortical activity in Capercaillie close to disturbance sources in spruce forests has therefore a high potential to reduce their fitness.

Sex differences

CM levels of males were higher than those of females in spruce and pine forests, and increased from early to late winter in males, but not in females. Higher CM levels in males were also reported in a previous study (Thiel *et al.* 2008), and most probably reflect sex-specific differences in circulating corticosterone levels. This would fit with the greater flushing distances of males (Thiel *et al.* 2007a). The increase of CM levels in males towards the end of winter, as found in captive birds (Hissa 1984), is probably caused by the upcoming lekking season. In contrast to females, males start lekking behaviour on warm winter days even in the absence of females (Klaus *et al.* 1989, Thiel *et al.* 2005a).

Environmental conditions

Minimum daily temperature strongly affected CM levels in Capercaillie droppings. CM levels increased with decreasing temperatures, especially at temperatures below −10 °C. This is most probably an adaptation to harsh environmental conditions. In winter, when Bilberry *Vaccinium myrtillus* and other important and nutrient-rich food sources on the ground are inaccessible, Capercaillie feed solely on conifer needles. Conifer needles represent a superabundant but low-quality food with high contents of cellulose and secondary plant compounds (Lindén 1984, Andreev 1988). Capercaillie are well adapted to this food, and decompose and slowly digest cellulose with the aid of bacteria in the large caeca (Moss & Hansson 1980). As a result, the rate of food and energy intake in winter is strongly limited. Therefore, Capercaillie reduce their spatial and daily activity in winter to reduce energy expenditure (Gjerde & Wegge 1987, Storch 1993). However, below the thermo-neutral zone (< −3 °C for males and < +9 °C for females; Rintamäki *et al.* 1984), metabolic rate needs to be increased. Corticosterone is well known to accelerate metabolism, and is suggested to initiate food-searching behaviour during periods of food deprivation (Astheimer *et al.* 1992). Elevated corticosterone levels also serve to mobilize energy stores via protein catabolism (Harvey *et al.* 1984). Therefore, the increased CM levels under low ambient temperatures are most probably an adaptation to harsh environmental conditions. Huber *et al.* (2003) found the same effect of minimum ambient temperature and snow on faecal glucocorticoid metabolites in Red Deer *Cervus elaphus*.

Predation risk may explain why Capercaillie on the ground (walking or day-roosting) showed higher CM levels than birds on trees (foraging or night roosting). Capercaillie on the ground are more conspicuous than in trees and are exposed to a higher risk of predation by abundant and ground-hunting predators such as the Red Fox *Vulpes vulpes* (Wegge *et al.* 1987). Therefore, the birds are probably more careful and feel less safe on the ground than in trees, especially in winter when camouflaging vegetation on the forest ground is rare. This explanation is supported by the finding that male Capercaillies on the ground in areas with high hunting pressure had longer flushing distances than those perching in trees (Thiel *et al.* 2007a).

As expected, CM levels in pine forests were generally higher than in spruce forests because needles of the various pine species provide about 20% more metabolizable energy than needles of Norway Spruce and European Silver Fir (Lieser *et al.* 2006, see Data analysis). Therefore, Capercaillie living in pine forests are probably not more stressed than those in spruce forests, despite their higher CM levels in droppings, because they benefit from a more energy-dense food. There may be three reasons why CM levels from pine forests were not significantly related to factors other than sex and ambient minimum temperatures. First, the higher variation of CM levels compared with spruce forests indicates that food composition may be variable (e.g. variation in the proportion of more energy-dense pine needles) or that factors related to habitat quality may vary more between pine than between spruce forests, given that habitat quality is known to affect corticosterone levels (Homan *et al.* 2003). Secondly, pine forests were much more heterogeneous than spruce forests in terms of tree species composition (several pine species mixed with a variety of other tree species), creating ecologically varying forests with different structures, canopy covers, food availability and possibly predator guilds and thus predation pressure. Thirdly, the data sample from pine forests was unbalanced in that we found only a few droppings close to recreation activities during warm days. This unequal distribution of samples over the distance to winter recreation and heterogeneity in pine forests of food composition and habitat quality may have masked effects of recreation intensity.

CONCLUSIONS

Physiological and behavioural adaptations allow Capercaillie to survive harsh winter conditions with low temperatures, deep snow and a limited rate of energy intake for several months a year. Any factor affecting this fine-tuned physiological and behavioural balance and any additional factor affecting the tight energy budget may lead to negative effects. This study suggests a physiological stress response to human recreation with potentially harmful consequences. Thiel *et al.* (2008) demonstrated that Capercaillie preferred undisturbed areas and avoided highly disturbed areas within their home-ranges. Longer flushing distances of Capercaillie in areas with higher recreation intensity could lead to more frequent flushing caused by humans (Thiel *et al.* 2007a). Recreation activities could also prevent the use of preferred feeding trees, and thus lead to higher energy costs. Moreover, wires and cables of ski-lifts can cause mortal collisions for grouse (Miquet 1990). Therefore, we believe that Capercaillie are especially sensitive to winter recreation, and the risk for negative effects is high. The access of people to undisturbed Capercaillie winter habitats should therefore be prevented. Recreation activities should be kept away from core Capercaillie wintering areas, especially during the physiologically most demanding winter days with temperatures far below 0 °C.

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