

Species-specific seasonal variation in faecal cortisol metabolites of four ungulate species in the Bieszczady Mountains (Poland)

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Abstract:

Nature-based tourism is often promoted as compatible with biodiversity conservation, yet its physiological correlates in wildlife remain incompletely understood. Protected areas can limit direct exploitation and habitat alteration without eliminating non-lethal human presence. We measured faecal cortisol metabolite (FCM) concentrations in four ungulate species across high- and low-tourism periods in Bieszczady National Park and managed forests. We examined seasonal differences and associations with daily visitor numbers, distance from forest roads or tourist trails, management designation, and sex. FCM concentrations were higher during the high-tourism season in European bison, wild boar, and roe deer (approximately 2.3-fold, 2.9-fold, and 2.0-fold, respectively). In red deer, FCM concentrations were approximately 27% lower, but this contrast was not statistically detectable. We detected no statistically detectable associations with visitor number, distance, or management designation, although the management comparison was not replicated at the landscape level. Because season also covaried with environmental conditions, reproductive stage, and hunting pressure, these seasonal associations cannot be attributed specifically to tourism. The results illustrate species-specific seasonal variation and support combining physiological indicators with conventional wildlife monitoring; they also motivate further studies integrating visitor exposure, environmental covariates, and management interventions.

Keywords: wildlife conservation, stress hormones, anthropogenic disturbance, nature-based tourism, human-wildlife interactions, mountainous areas.

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Nature-based tourism is often promoted as compatible with biodiversity conservation, yet its physiological correlates in wildlife remain incompletely understood. Protected areas can limit direct exploitation and habitat alteration without eliminating non-lethal human presence. We measured faecal cortisol metabolite (FCM) concentrations in four ungulate species across high- and low-tourism periods in Bieszczady National Park and managed forests. We examined seasonal differences and associations with daily visitor numbers, distance from forest roads or tourist trails, management designation, and sex. FCM concentrations were higher during the high-tourism season in European bison, wild boar, and roe deer (approximately 2.3-fold, 2.9-fold, and 2.0-fold, respectively). In red deer, FCM concentrations were approximately 27% lower, but this contrast was not statistically detectable. We detected no statistically detectable associations with visitor number, distance, or management designation, although the management comparison was not replicated at the landscape level. Because season also covaried with environmental conditions, reproductive stage, and hunting pressure, these seasonal associations cannot be attributed specifically to tourism. The results illustrate species-specific seasonal variation and support combining physiological indicators with conventional wildlife monitoring; they also motivate further studies integrating visitor exposure, environmental covariates, and management interventions.

Keywords

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25 25 1. Introduction

26 26 Nature-based tourism is widely promoted as a sustainable form of land use that supports
27 27 both economic development and biodiversity conservation (Silva et al. 2023). Because most
28 28 nature-based tourism destinations are located in protected areas (Eagles et al. 2002, Spenceley
29 29 2015), these areas have become focal points for recreational activity worldwide, with visitor
30 30 numbers rising rapidly in recent years, particularly after the COVID-19 pandemic (Spalding et
31 31 al. 2021, Calderón et al. 2022). Although protected areas are established to safeguard wildlife
32 32 populations and ecological processes, they are increasingly experiencing intensive human use,
33 33 which creates potential conflicts between conservation objectives and recreation (Tolvanen and
34 34 Kangas 2016, Wójcicki et al. 2023, Seguin et al. 2024).

35 35 Protected areas are commonly assumed to buffer wildlife from anthropogenic
36 36 disturbance and support viable populations (Ishwaran et al. 2008, DeFries et al. 2010). By
37 37 restricting direct exploitation and habitat alteration, they may reduce some pressures, but they
38 38 do not necessarily eliminate non-lethal human presence. Tourism has been associated with
39 39 changes in wildlife behaviour, energetics, and stress physiology (Ranaweerage et al. 2015), and
40 40 studies in protected areas have reported physiological responses associated with recreation
41 41 (Szott et al. 2020). However, a detected physiological difference does not, in itself, establish
42 42 chronic stress, adverse fitness effects, or a failure of a protected area to function as a refuge;
43 43 those interpretations require species-specific validation and appropriate comparisons.

44 44 The effects of human disturbance on wildlife have most often been assessed through
45 45 behavioural responses, such as changes in vigilance, habitat use, or activity patterns (Taylor
46 46 and Knight 2003, Stankowich 2008, Pęksa and Ciach 2015, Scholten et al. 2018, Wójcicki et
47 47 al. 2024, Gaynor et al. 2025). Behavioural observations are informative, but they do not always
48 48 reveal the population-level consequences of disturbance, and animals may remain in suboptimal
49 49 habitats because of ecological constraints (Gill et al. 2001, Griffin et al. 2007, Wilson et al.

2020). Physiological indicators can complement these measures by describing variation in adrenal activity, including glucocorticoid secretion (Sapolsky et al. 2000, Dantzer et al. 2014). Nevertheless, elevated glucocorticoid metabolites should not, on their own, be treated as proof of harmful chronic stress or reduced fitness. Physiological and behavioural responses may also differ between sexes, owing to variation in reproductive investment, habitat use, and sensitivity to perceived risk. Some ungulate studies report sex-specific spatial or behavioural responses to human or predator risk, including stronger avoidance by females in particular contexts (Ciuti et al. 2004, Ciuti and Apollonio 2008, Stankowich 2008, Gallotta et al. 2025, Nakamura et al. 2026). Gestation and lactation can also affect energetic requirements and faecal glucocorticoid metabolite concentrations in females (Pavitt et al. 2016; Grotta-Neto et al. 2024), so the expected direction of sex differences may depend on both reproductive state and local exposure to disturbance.

Previous studies have reported physiological responses in wildlife associated with tourism or recreation (Müllner et al. 2004, Thiel et al. 2008, Zwijacz-Kozica et al. 2013), but these responses are not uniform and may depend on species, exposure patterns, habitat, and other environmental conditions (Zwijacz-Kozica et al. 2013, Gaynor et al. 2025). This question is relevant in mountain regions, which are popular destinations for outdoor recreation and are experiencing growing pressure on sensitive ecosystems (Dax and Tamme 2023). Importantly, seasonal changes in visitor activity coincide with changes in temperature, snow cover, forage availability, reproductive state, and – in hunted species – hunting pressure. A seasonal comparison can therefore identify temporal patterns in physiological indicators, but cannot on its own isolate tourism as the cause. It remains important to describe such patterns across species and to identify what additional evidence is needed to assess the contribution of recreation.

In this study, we examined seasonal variation in faecal cortisol metabolite (FCM) concentrations in four free-ranging ungulate species in the Bieszczady Mountains of south-eastern Poland: European bison *Bison bonasus*, wild boar *Sus scrofa*, red deer *Cervus elaphus*, and roe deer *Capreolus capreolus*. Samples were collected during high-tourism and low-tourism periods in Bieszczady National Park and in managed forests. We formulated four predictions. First, FCM concentrations would differ between the two seasonal periods and, if factors associated with recreation contributed to this pattern, would be higher during the high-tourism period. This prediction addressed seasonal contrast and did not claim that tourism could be isolated from other seasonal influences. Second, the magnitude or direction of the seasonal contrast would differ among species. Third, FCM concentrations would be lower in the national park than in managed forests; because this comparison involved one national park and two forest districts, we treated it as a site-level comparison rather than a replicated test of management designation. Fourth, we tentatively predicted higher FCM concentrations in females, based on potential differences in risk avoidance and reproductive demands, while recognising that sex-specific exposure – including hunting pressure on males – could produce context-dependent or opposite patterns. We also examined whether daily visitor numbers and measured distances from tourist trails or forest roads were associated with FCM, predicting higher concentrations with more visitors and closer proximity.

2. Materials and Methods

2.1. Study area

This study was conducted in the Bieszczady Mountains, in Bieszczady National Park (protected area) (49°06'N, 22°38'E, 650 m a.s.l.) and in state forests (managed forests) in Baligród Forest District (49°19'N, 22°17'E, 447 m a.s.l.) and Komańcza Forest District (49°20'N, 22°04'E, 455 m a.s.l.) (Fig.1). The climate is temperate and predominantly

continental. The slopes and valleys are covered by Carpathian beech forests (the predominant species are European beech *Fagus sylvatica* L. and silver fir *Abies alba* Mill.). There is no forest management in Bieszczady National Park; only protective measures are undertaken (e.g., removing trees that threaten hiking trails or controlling pests). In state forests surrounding the national park, forest management is carried out (tree harvesting, tree planting, forest protection measures), although some areas are subject to restrictions on logging due to their natural value.

Red deer, roe deer and wild boar are the predominant ungulate species in the area. A population of European bison also occurs here (Tab. 1). Red deer, roe deer and wild boar are game species. The hunting season for red deer runs from 21 August to 28 February (bulls), from 1 October to 1 January (hinds), and from 1 January to 28 February (calves). The hunting season for roe deer runs from 11 May to 30 September (bucks) and from 1 October to 15 January (does and fawns). Wild boar can be hunted all year round, but the most intensive hunting occurs between October and March. European bison is not a game species in Poland, and hunting these animals is prohibited. Bison can only be culled in exceptional cases (e.g. for population regulation, when an individual is so ill that it has little chance of survival, or in the event of threats to human health and safety), and such cases are extremely rare and not dependent on the season. Hunting is prohibited in Bieszczady National Park. In state forests hunting is conducted either individually (the hunter hunts alone or with a guide, including stalking on foot or hunting from a stand) or collectively (groups of various sizes with beaters and dogs).

The high tourist season in the Bieszczady Mountains begins in May and ends in October. During the remaining months (November to April), visitor numbers are very low (Wasiak 2025). Visitors to Bieszczady National Park must follow designated hiking trails. Walking on the trails from dusk to dawn, as well as walking off the trails, is prohibited. Bicycles are permitted on the trails. The trails are also used by park rangers for vehicle transport. Forest roads in state forests are open to the public. Walking and cycling are permitted 24 hours a day,

but vehicles are prohibited. Vehicles operating on other forest roads include those used by foresters and companies engaged in logging and timber removal. In state forests it is permitted to walk off the roads and move around the forest stands.

2.2. Data collection

We collected faeces of European bison, wild boar, red deer and roe deer between 2023 and 2025 during the high tourist season (May–October) and the low tourist season (January–April, November–December). We established 500 m sections on each tourist trail and forest road and collected samples along these sections throughout the study period. We selected sections so that there were no terrain barriers (such as gullies or streams) and the slope did not exceed 10% up to a distance of 250 m from the tourist trail or forest road. We located the sections more than 300 m from public roads, car parks and tourist shelters. We collected samples at distances of up to 50 m and at 200–250 m from the tourist trails and forest roads. We chose these two distance ranges for two reasons: (1) within 50 m of trails and forest roads, we found rubbish left by people (food scraps, food and beverage packaging, tissues, etc.), which provided olfactory cues that could attract certain ungulates (Aguilar-Vega et al. 2024), while this proximity to roads and tourist trails might also have acted as an additional stressor due to the potential for encountering humans. In contrast, at a distance of approximately 200–250 m, the trails and forest roads were completely obscured by vegetation and terrain features within our study areas, a factor that could have influenced the animals' sense of security (Borowski et al. 2021). We did not, however, treat the 200–250 m distance bands as undisturbed zones, especially in state forests, where the presence of humans away from forest roads, within the forest stand, is generally acceptable. We collected only fresh faeces, because FCM concentrations may decrease rapidly from the first days after deposition (Donini et al. 2022). To avoid pseudoreplication, we visited each study plot only once during each field trip (no more than once every two months), and we surveyed each trail or forest road section only once along

the predefined distance. We took only one sample from each faecal deposit encountered. We did not identify individual animals; therefore, we treated each faecal sample as an independent observation representing a different, unknown individual. The collected faeces were then frozen, freeze-dried and stored at -20 degrees Celsius until laboratory analysis.

We quantified the number of visitors (people and vehicles) per day using camera traps placed near tourist trails and forest roads. We set the cameras to be triggered by a motion sensor, allowing the recording of all people walking and vehicles passing on each trail or road. Based on the video recordings, we calculated the average daily number of visitors on tourist trails and forest roads during the sample collection periods, which were usually 5 days.

2.3. Sample analysis

Quantification of faecal 11-oxoetiocholanolone (a faecal metabolite of cortisol) was performed using an ELISA assay (11-Oxoetiocholanolone ELISA Kit, Cayman Chemical) according to the manufacturer's protocol, with minor modifications to the sample preparation procedure. Faeces were lyophilised, and 50 mg of dried sample was homogenised in 500 μ l of distilled water. After adding 4 μ l of 1.74 M acetic acid, samples were extracted with 800 μ l of ethyl acetate, vortexed, and incubated at room temperature for 10 minutes, followed by centrifugation at $3000 \times g$ for 5 minutes. The organic phase was collected, and the extraction was repeated four times, combining the ethyl acetate fractions. The combined organic extracts were evaporated in a vacuum centrifuge. The precipitate was dissolved in 500 μ l of assay buffer, centrifuged to remove particulate matter, and the supernatant was used for further analysis. Each sample was diluted and measured in duplicate. All concentration values are expressed in nanograms per gram of dry matter of faeces.

To determine the sex of individuals, DNA was isolated from faecal samples using a dedicated kit for nucleic acid isolation from faeces – the Syngen Stool DNA Mini Kit (Syngen, Wrocław, Poland). The primer pair SRYBF - TGAACGCTTTCATTGTGTGGTC and SRYBF

- GCCAGTAGTCTCTGTGCCTCCT was used to amplify the SRY marker fragment (Ražanskè et al. 2021). The polymerase chain reaction (PCR) was carried out under the following conditions: initial denaturation at 95°C for 15 min, denaturation at 95°C for 30 s, primer annealing at 50°C for 20 s, extension at 72°C for 20 s, and final extension at 72°C for 10 min. The number of reaction cycles was 35. Each PCR reaction was performed in a volume of 20 µl and contained: 4 µl Silver Hot Start PCR Mix Load (Syngen, Wrocław, Poland), 0.5 µl (10 µM) of each primer, 13 µl PCR-grade water, and 2 µl of extracted DNA. The amplification products were visualised by electrophoresis on a 1.5% agarose gel with Green DNA Gel Stain (Syngen, Wrocław, Poland).

2.4. Statistical analysis

We performed all statistical analyses in R (R Core Team 2026) using RStudio (version 2026.05.1+225) as the integrated development environment. Data manipulation and visualisation were performed with the dplyr (Wickham et al. 2023) and ggplot2 (Wickham 2016) packages, and estimated marginal means were calculated using the emmeans package (Lenth 2025). We fitted generalised linear models (GLMs) with the glm() function in R. Separate GLMs were fitted for each study species to evaluate associations between faecal 11-oxo-etiocholanolone concentrations and seasonal and site-related predictors. Because concentrations were continuous, strictly positive, and right-skewed, we used a Gamma error distribution with a log-link function. The explanatory variables were tourist season (high-tourism vs low-tourism period), daily number of visitors using the tourist trail or forest road, range of distances from the trail or road (0-50 m vs 200-250 m), land-management regime (national park vs state forest), and sex. We treated tourist season, land-management regime, and sex as categorical predictors, and daily visitor number and measured distance (m) as continuous predictors. Under this parameterisation, the intercept corresponds to the low-tourism period, state forest, female individuals, zero recorded visitors, and distance 0-50 m;

coefficients for the continuous predictors describe their association with concentration per one-unit increase. Because faecal samples originated from unidentified individuals and each sample was treated as an independent observation, we considered fixed-effects models appropriate. To assess whether repeated sampling locations influenced the results, we also evaluated generalised linear mixed models including sampling location (Spot) as a random intercept. The variance explained by this random effect was negligible and model fit was not improved, so the results presented are based on GLMs. Statistical significance was assessed at $\alpha = 0.05$.

3. Results

A total of 367 faecal samples were collected, comprising 67 (37 females, 30 males) from European bison, 83 (51 females, 32 males) from wild boar, 113 (61 females, 52 males) from red deer, and 104 (66 females, 38 males) from roe deer.

3.1. European bison

FCM concentrations were higher during the high-tourism season in European bison ($\beta = 0.84 \pm 0.21$ SE, $t = 3.97$, $p < 0.001$; Tab. 2). Based on the fitted model, FCM concentrations increased from 276 ng/g (95% CI: 199–383 ng/g) during the low-tourism season to 640 ng/g (95% CI: 488–839 ng/g) during the high-tourism season (Fig. 2), corresponding to an approximately 132% increase. None of the remaining explanatory variables, including average daily number of visitors, distance from the tourist trail or forest road, land-management regime, or sex, significantly affected FCM concentrations (all $p > 0.05$; Tab. 2).

3.2. Wild boar

FCM concentrations were higher during the high-tourism season in wild boar ($\beta = 1.07 \pm 0.35$ SE, $t = 3.03$, $p = 0.003$; Tab. 2). Based on the fitted model, the concentration increased from 217 ng/g (95% CI: 122–384 ng/g) during the low-tourism season to 634 ng/g (95% CI: 427–941 ng/g) during the high-tourism season (Fig. 3), corresponding to an approximately 192% increase. None of the remaining explanatory variables, including average daily visitor

numbers, distance from the tourist trail or forest road, land management regime, or sex, significantly affected faecal FCM concentrations (all $p > 0.05$; Tab. 2).

3.3. Red deer

Tourist season was not a significant variable in red deer ($\beta = -0.32 \pm 0.22$ SE, $t = -1.41$, $p = 0.161$; Tab. 2). FCM concentrations averaged 509 ng/g (95% CI: 362–715 ng/g) during the low-tourism season and 371 ng/g (95% CI: 280–490 ng/g) during the high-tourism season (Fig. 4), corresponding to an approximately 27% decrease. Likewise, daily number of visitors, distance from the tourist trail, land-management regime, and sex did not significantly influence faecal FCM concentrations (all $p > 0.05$; Tab. 2).

3.4. Roe deer

FCM concentrations were higher during the high-tourism season in roe deer ($\beta = 0.71 \pm 0.22$ SE, $t = 3.17$, $p = 0.002$; Tab. 2). Based on the fitted model, the expected concentration increased from 209 ng/g (95% CI: 141–310 ng/g) during the low-tourism season to 424 ng/g (95% CI: 345–522 ng/g) during the high-tourism season (Fig. 5), corresponding to an approximately 103% increase. In addition, FCM concentrations were approximately 1.9-fold higher in males than in females ($\beta = 0.65 \pm 0.19$ SE, $t = 3.44$, $p < 0.001$; Tab. 2). Daily number of visitors, distance from the tourist trail, and land-management regime did not significantly affect hormone concentrations (all $p > 0.05$; Tab. 2).

4. Discussion

Our study identified pronounced species-specific seasonal patterns in faecal cortisol metabolites, but these patterns should be interpreted as seasonal associations rather than direct effects of tourism. FCM concentrations were approximately 2.3-fold higher in European bison, 2.9-fold higher in wild boar, and 2.0-fold higher in roe deer during the high-tourism season, whereas red deer showed an estimated 27% decrease that was not statistically detectable. The magnitude and direction of change therefore varied markedly among species. This interspecific

variation is the central ecological finding of the study and indicates that physiological responses to the same seasonal context are not uniform across ungulates. At the same time, the high-tourism and low-tourism periods differed in temperature, snow cover, forage and diet, reproductive stage, and hunting pressure. Because we did not include a tourism-free control period that was comparable in all other respects, the design cannot distinguish a tourism effect from a broader seasonal effect.

We detected no statistically significant association between FCM concentrations and management designation in any of the four species. This result should not be interpreted as evidence that the national park and managed forests provide equivalent conditions: a non-significant contrast is not an equivalence test, and management designation was not replicated at the landscape level. Our comparison included one national park and two managed forest districts, which limits inference about management categories. Previous studies have reported seasonal or recreation-associated physiological patterns in mountain ungulates and other wildlife (Pelletier 2006, Zwijacz-Kozica et al. 2013, Pęksa and Ciach 2015), while broader work has emphasised that behavioural observations alone may not reveal all consequences of disturbance (Gill et al. 2001, Dantzer et al. 2014). These studies provide context, but they do not demonstrate that tourism caused the seasonal contrasts observed here. We also lack validated species-specific thresholds linking FCM concentrations measured with this assay to adverse fitness or population-level consequences. Research on cortisol and other physiological measures in harvested red deer is relevant background, but it does not establish a harmful threshold for faecal FCM in these four species (Dziki-Michalska et al. 2024a). Interpretation also requires caution because faecal metabolite concentrations can be influenced by environmental conditions and time since deposition (Donini et al. 2022), and physiological state in ungulates may vary with food availability and hunting practices (Santos et al. 2018). Finally, the location of a faecal deposit is not a direct measure of where, when, or how intensively the

animal encountered people before defecation. These limitations make it inappropriate to infer refuge failure or biological harm from the present seasonal contrasts alone.

An increase in FCM indicates a seasonal difference in a physiological marker, but it does not, by itself, demonstrate chronic stress, incomplete recovery between disturbance events, or cumulative fitness costs (Sapolsky et al. 2000, Romero 2004). Work in conservation physiology shows why repeated exposure and glucocorticoid dynamics warrant investigation (Dantzer et al. 2014, Madliger et al. 2018), and studies in other systems have reported physiological responses associated with tourism or recreation (Müllner et al. 2004, Thiel et al. 2008). However, the relationship between glucocorticoids and fitness is context-dependent and not necessarily straightforward (Bonier et al. 2009, Madliger and Love 2016). In our study, the high-tourism period was also a distinct season in terms of environmental conditions, reproductive state, and hunting exposure; therefore, the observed increases are consistent with responses to one or more factors associated with that period, not specifically with tourism. Likewise, the absence of a statistically detectable seasonal contrast in red deer does not establish that the species was unaffected by, or adapted to, recreation. Differences among species and contexts have been reported in previous work on ungulate responses to tourism and hunting (Zwijacz-Kozica et al. 2013, Ensminger et al. 2020). Our data identify species-specific seasonal patterns but do not establish chronic stress, reduced fitness, or population-level consequences.

In roe deer, model-predicted FCM concentrations were approximately 2.0-fold higher in males than in females. This sex difference was not anticipated, although sex-specific endocrine patterns can reflect differences in behaviour, spatial ecology, and reproductive strategies (Bonier et al. 2009, Dantzer et al. 2014). While females may show stronger avoidance of perceived risk in some contexts (Gallotta et al. 2025), the high-tourism period overlaps almost completely with the roe deer buck hunting season, so greater hunting exposure may have contributed to the higher FCM concentrations in males (Dziki-Michalska et al. 2024b).

The seasonal pattern in red deer is also difficult to interpret in isolation: red deer show behavioural responses to natural predators and human activities (Jayakody et al. 2008, Kuijper et al. 2014), and hunting pressure can coincide with periods of high human activity in forests. Studies of hunted cervids have reported varied physiological responses, including both increases and decreases in glucocorticoid measures (Dziki-Michalska et al. 2024a, Ensminger et al. 2020). Other work suggests that proximity to anthropogenic activity and behavioural adjustments in space use or daily timing may also shape FCM patterns (Carbillet et al. 2020), while evolutionary or behavioural responses to hunting have been proposed as further possibilities (Potratz et al. 2024). The lack of an association with visitor numbers in alpine red deer provides another example of context-dependent responses (Anderwald et al. 2021).

Because we did not measure hunting intensity, reproductive state, the number of direct human encounters, or animal movements, these explanations remain hypotheses rather than conclusions. The male roe deer result cannot be attributed specifically to hunting, and the red deer result should not be interpreted as evidence of resistance to tourism or of equivalent physiological conditions in both seasons. Future studies combining FCM with direct measures of exposure, hunting, forage, reproductive stage, and movement could help distinguish these alternatives.

Behavioural measures such as vigilance, habitat use, and activity patterns remain important for assessing wildlife responses to recreation (Taylor and Knight 2003, Stankowich 2008). However, behavioural responses do not always predict population-level consequences, and animals may face ecological constraints on where or when they can move (Gill et al. 2001, Griffin et al. 2007). Physiological indicators may therefore add a complementary dimension to behavioural and distributional data (Dantzer et al. 2014, Madliger et al. 2018). The present study did not measure behaviour directly, and its results do not establish that animals experienced hidden fitness costs or that apparent behavioural tolerance occurred. Rather, the

species-specific seasonal patterns support the value of combining physiological sampling with direct observations of human encounters, behaviour, and space use when evaluating human–wildlife interactions.

This question is particularly relevant in mountain ecosystems, where recreational pressure can overlap with habitats important for wildlife (Selwood and Zimmer 2020). Although montane species are adapted to demanding environmental conditions, mountain ecosystems remain vulnerable to human-derived pressures (Schmeller et al. 2022). Spatial overlap between tourism hotspots and sensitive species has been identified as a potential challenge for conservation areas (Liu and Liu 2025), yet relatively few studies have assessed refuge function at scales directly relevant to land management (Selwood and Zimmer 2020). As visitation to protected areas continues to grow, management requires evidence that distinguishes seasonal patterns from effects attributable to recreation (Wójcicki et al. 2023).

Here, we treat the refuge concept as a broader management hypothesis rather than a formally validated physiological threshold. Our data cannot establish whether any observed FCM concentration represents a biologically harmful state or defines a threshold at which a protected area ceases to function as a refuge. Nonetheless, the results support evaluating recreation alongside other seasonal pressures when planning wildlife monitoring. If independent studies establish that particular recreation patterns are associated with adverse physiological outcomes, management could consider not only visitor numbers but also the spatial and temporal distribution of human presence. Trail and infrastructure planning, together with measures to prevent off-trail use, may help reduce overlap between people and wildlife (Taylor and Knight 2003, Wolf et al. 2019). Physiological measures such as glucocorticoid metabolites could complement monitoring of abundance, distribution, and behaviour, provided they are interpreted with appropriate species-specific context (Cooke and O'Connor 2010, Madliger et al. 2018). Management interventions intended to maintain low-disturbance areas

should be evaluated empirically, rather than assumed to improve physiological conditions or ensure refuge function.

Conclusions

Our study identified pronounced, species-specific seasonal variation in faecal cortisol metabolites in four ungulate species in the Bieszczady Mountains. FCM concentrations were higher during the high-tourism period in European bison, wild boar, and roe deer, whereas red deer showed an estimated decrease that was not statistically detectable. The direction and magnitude of the seasonal pattern therefore differed among species. Because tourism coincided with seasonal changes in environmental conditions, reproduction, and hunting pressure, the patterns cannot be attributed specifically to tourism. Nor do FCM concentrations alone establish chronic stress, biological harm, or failure of protected areas to provide refuges. These findings support using physiological indicators alongside conventional monitoring and emphasise the need for future studies that directly measure visitor exposure and seasonal covariates (Dantzer et al. 2014, Madliger et al. 2018).

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Statements and Declarations

Ethics statement

As this study involved no capturing, handling, or close approach, formal approval from an Animal Ethics Committee was not required. The study was conducted under permits from Bieszczady National Park for working in this protected area. All efforts were made to minimise disturbance to animals during the study. Sample collection was conducted using non-invasive methods, and care was taken to avoid causing additional stress or behavioural disruption to wildlife. Fieldwork procedures were designed to ensure that animals were not approached, handled or intentionally disturbed, and all observations were carried out in accordance with best practices for ethical wildlife research.

AI use statement

The authors used AI tools to assist with language editing, including improving grammar, spelling, clarity and overall fluency of the manuscript. No AI tools were used for data analysis, interpretation of results, or generation of scientific content. All scientific conclusions, interpretations and final decisions regarding the content of the manuscript are the sole responsibility of the authors.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interests

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Consent to publish declaration

Not applicable

Consent to participate declaration

Not applicable

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Tab.1. Wild ungulate densities (ind./km²) in the Bieszczady Mountains within the study sites (Pirga and Polakiewicz 2023, unpubl. data).

Species	Bieszczady	Komańcza FD	Baligród FD
National Park			
Red deer	0.44	2.8	2.08
Roe deer	0.31	2.64	1.95
Wild boar	0.2	2.31	1.63
European bison	0.54	0.3	0.95

Tab. 2. Results of generalized linear models (Gamma error distribution, log link) explaining variation in faecal 11-oxo-etiocholanolone concentrations in European bison, wild boar, red deer and roe deer in the Bieszczady Mountains.

Species	Predictor	Estimate	SE	t	p
European bison	Tourist season	0.841	0.212	3.97	<0.001
	Visitor/day	0.001	0.002	0.72	0.477
	Distance	-0.0001	0.0011	-0.10	0.923
	Management	-0.455	0.252	-1.81	0.076
	Sex	-0.098	0.170	-0.58	0.567
Wild boar	Tourist season	1.074	0.354	3.03	0.003
	Visitor/day	-0.001	0.002	-0.38	0.703
	Distance	0.002	0.002	0.90	0.372
	Management	0.009	0.352	0.03	0.979
	Sex	-0.205	0.325	-0.63	0.529
Red deer	Tourist season	-0.318	0.225	-1.41	0.161
	Visitor/day	-0.003	0.002	-1.36	0.178
	Distance	0.002	0.001	1.46	0.149
	Management	0.123	0.227	0.54	0.588
	Sex	0.256	0.212	1.21	0.230
Roe deer	Tourist season	0.707	0.223	3.17	0.002
	Visitor/day	-0.002	0.002	-1.05	0.297
	Distance	-0.0003	0.0012	-0.24	0.815
	Management	-0.349	0.184	-1.89	0.061
	Sex	0.654	0.190	3.44	<0.001

SE – standard error, t – t-value, p – p-value

Fig. 1. Locations of study plots within the study area in the Bieszczady Mountains in Poland.

Fig. 2. Faecal 11-oxo-etiocholanolone concentrations in European bison in relation to tourist season, land-management regime and sex. Dots represent individual faecal samples. Black circles indicate model-predicted marginal means estimated from the generalised linear model with a Gamma error distribution and log link, and error bars represent 95% confidence intervals. Total sample size for this species: $n = 67$.

Fig. 3. Faecal 11-oxo-etiocholanolone concentrations in wild boar in relation to tourist season, land-management regime and sex. Dots represent individual faecal samples. Black circles indicate model-predicted marginal means estimated from the generalised linear model with a Gamma error distribution and log link, and error bars represent 95% confidence intervals. Total sample size for this species: $n = 83$.

Fig. 4. Faecal 11-oxo-etiocholanolone concentrations in red deer in relation to tourist season, land-management regime and sex. Dots represent individual faecal samples. Black circles indicate model-predicted marginal means estimated from the generalised linear model with a Gamma error distribution and log link, and error bars represent 95% confidence intervals. Total sample size for this species: $n = 113$.

Fig. 5. Faecal 11-oxo-etiocholanolone concentrations in roe deer in relation to tourist season, land-management regime and sex. Dots represent individual faecal samples. Black circles indicate model-predicted marginal means estimated from the generalised linear model with a Gamma error distribution and log link, and error bars represent 95% confidence intervals. Total sample size for this species: $n = 104$.

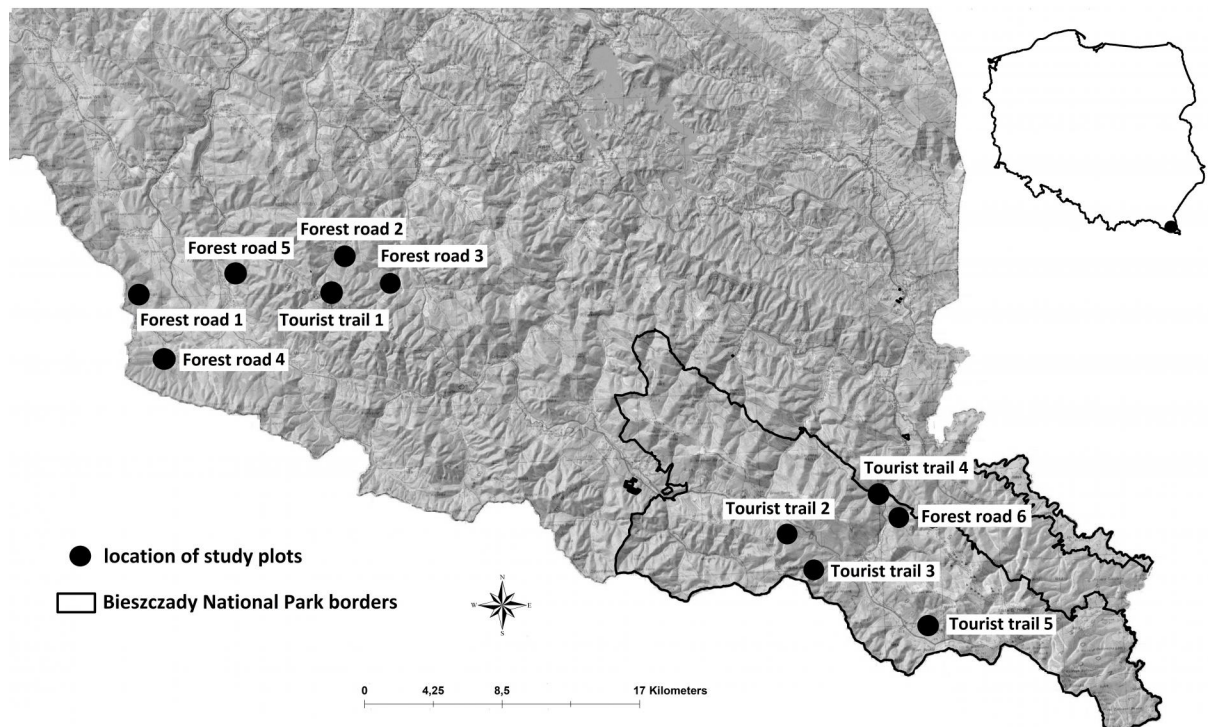


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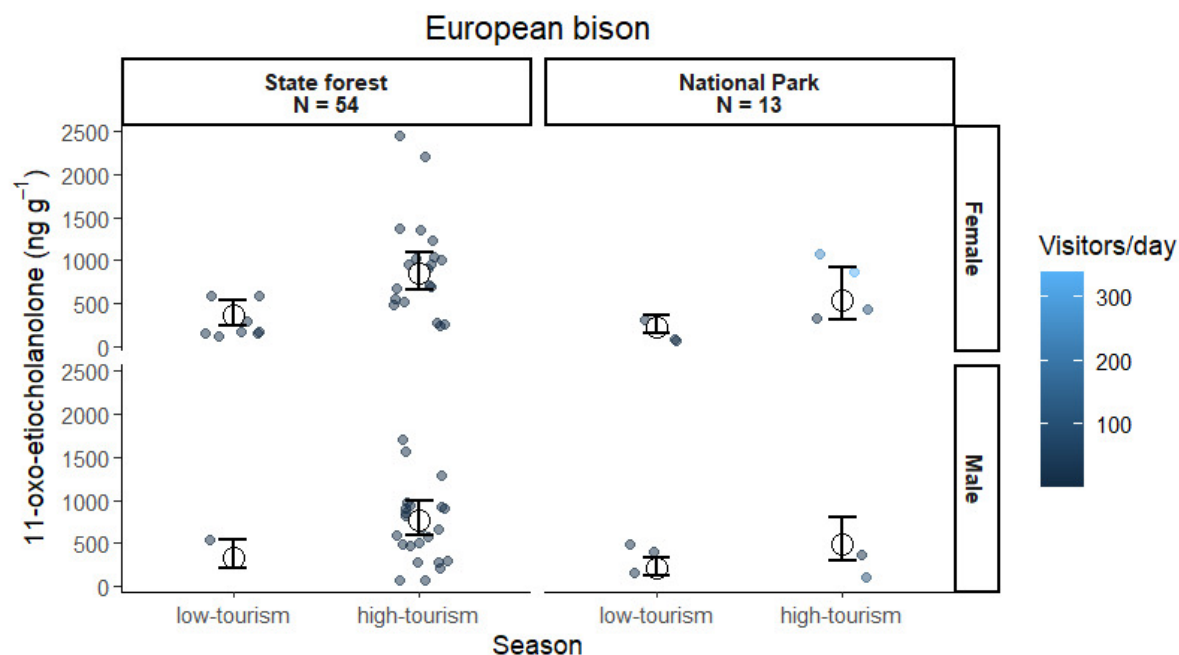


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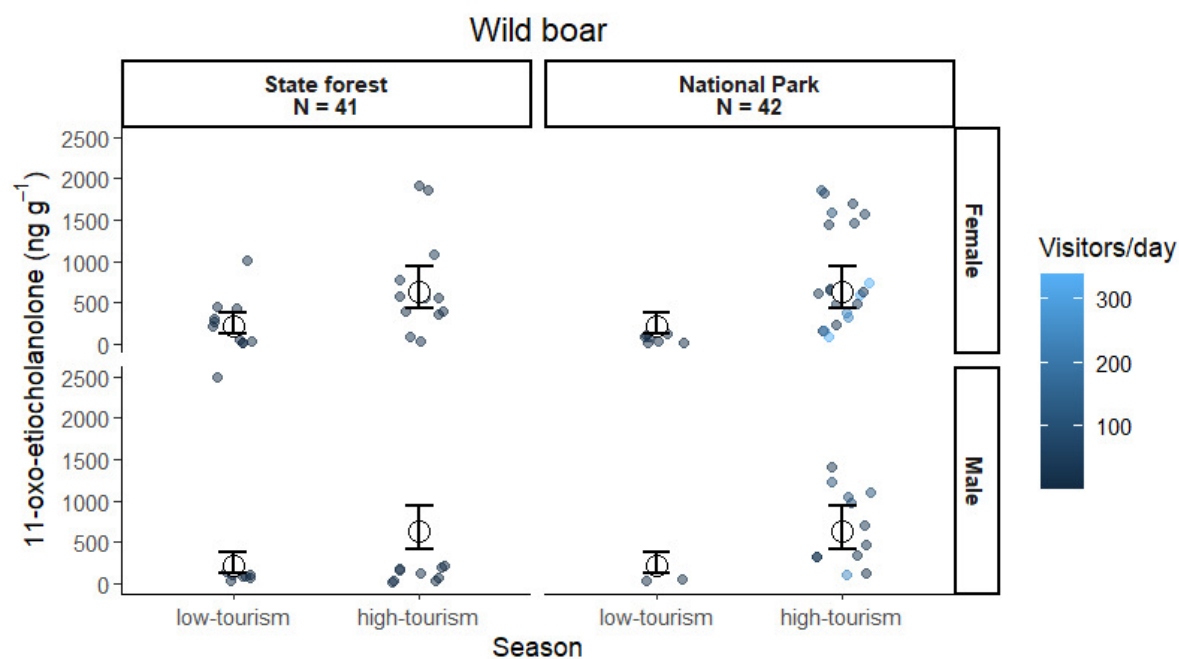


Fig. 3. Faecal 11-oxo-etiocholanolone concentrations in wild boar in relation to tourist season, land-management regime and sex. Dots represent individual faecal samples. Black circles indicate model-predicted marginal means estimated from the generalised linear model with a Gamma error distribution and log link, and error bars represent 95% confidence intervals. Total sample size for this species: $n = 83$.

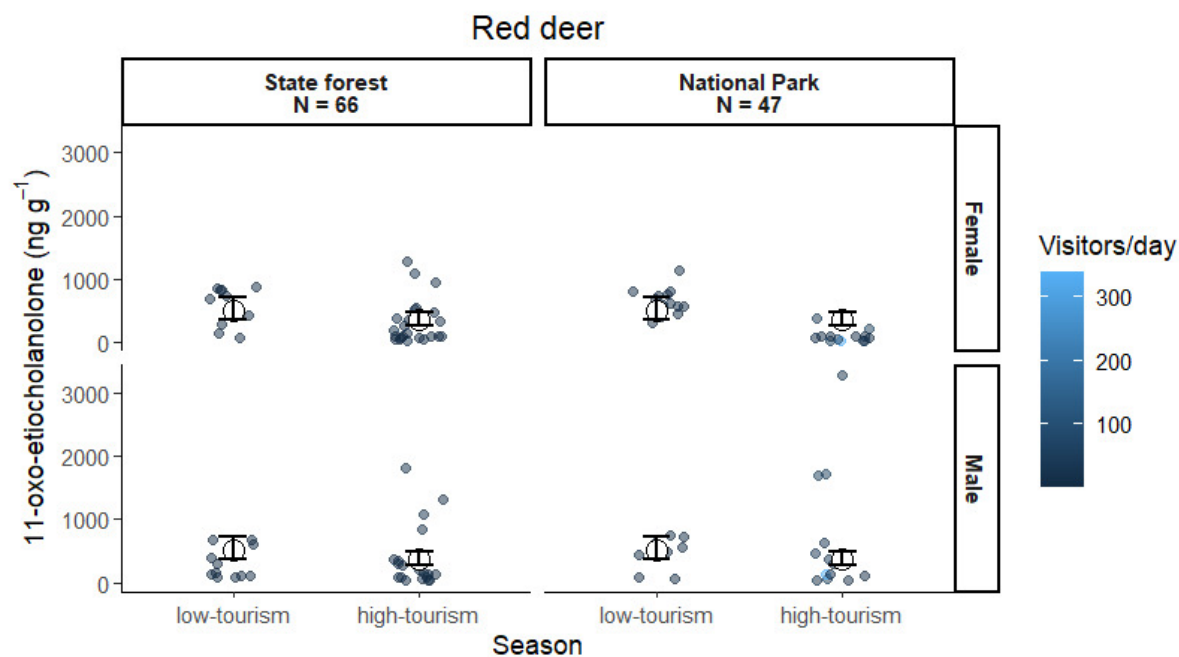


Fig. 4. Faecal 11-oxo-etiocholanolone concentrations in red deer in relation to tourist season, land-management regime and sex. Dots represent individual faecal samples. Black circles indicate model-predicted marginal means estimated from the generalised linear model with a Gamma error distribution and log link, and error bars represent 95% confidence intervals. Total sample size for this species: $n = 113$.

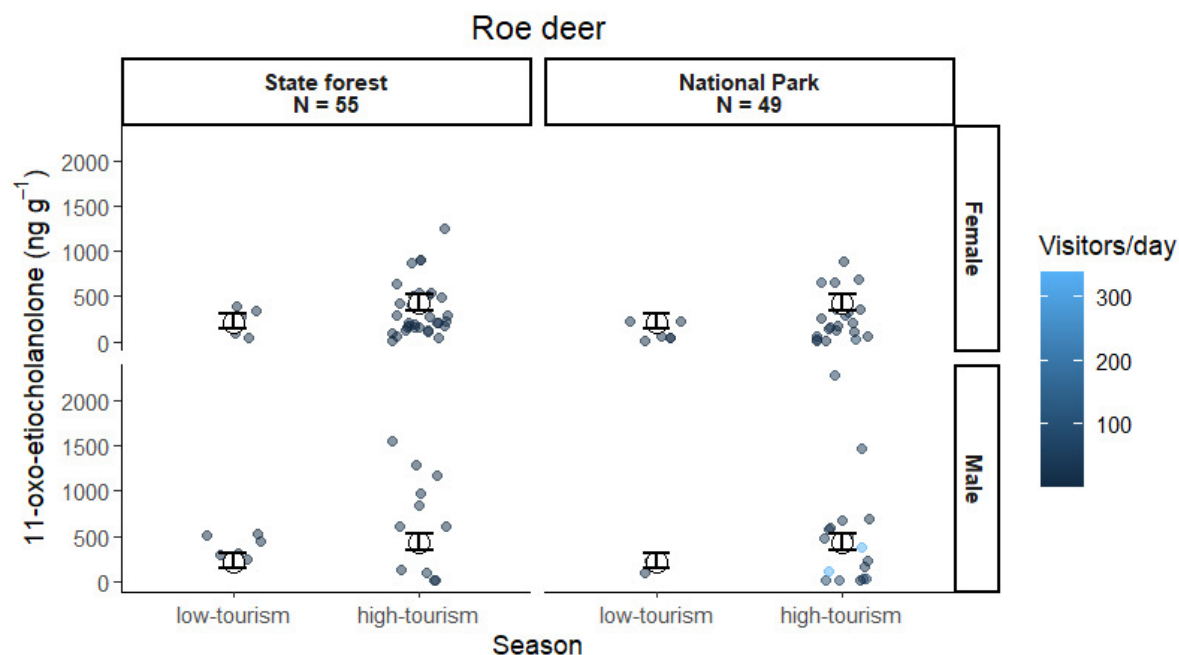


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Fig. 1. Locations of study plots within the study area in the Bieszczady Mountains in Poland.

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Fig. 4. Faecal 11-oxo-etiocholanolone concentrations in red deer in relation to tourist season, land-management regime and sex. Dots represent individual faecal samples. Black circles indicate model-predicted marginal means estimated from the generalised linear model with a Gamma error distribution and log link, and error bars represent 95% confidence intervals. Total sample size for this species: n = 113.

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