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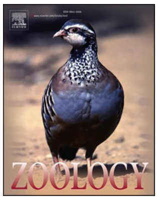

Maternal and social status affect hair cortisol concentrations in brown bears

Marcello Franchini [a,*,1], Ashlee J. Mikkelsen [b,2], Agnieszka Sergiel [c,3], Nuria Selva [d,c,4], David M. Janz [e,5], Jonas Kindberg [f,g,6], Andreas Zedrosser [b,h,**,7]

[a] *Department of Agrifood, Environmental and Animal Sciences, University of Udine, Via delle Scienze 206, Udine 33100, Italy*
[b] *Department of Natural Sciences and Environmental Health, University of South–Eastern Norway, Gullbringvegen 36, Bø 3800, Norway*
[c] *Institute of Nature Conservation, Polish Academy of Sciences, Adama Mickiewicza 33, Krakow 31120, Poland*
[d] *Estación Biológica de Doñana, Consejo Superior de Investigaciones Científicas, 41092 Sevilla, Spain*
[e] *Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada*
[f] *Norwegian Institute for Nature Research, Trondheim, Norway*
[g] *Department of Wildlife, Fish, and Environmental Studies, Swedish University of Agricultural Sciences, Umeå, Sweden*
[h] *Institute for Wildlife Biology and Game Management, University for Natural Resources and Life Sciences, Gregor–Mendel–Strasse 33, Vienna 1180, Austria*

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ABSTRACT

Hair cortisol concentration is affected by different biological factors, including age, sex, and reproductive status, and can provide important insights into fitness. Using cortisol concentrations of wild Scandinavian brown bears, *Ursus arctos*, the purpose of this study was to investigate cortisol variations among individuals in relation to age, sex, and reproductive status (solitary individuals vs individuals in a family group). Cortisol concentrations were measured in 448 hair samples of 303 brown bears (162 males and 141 females, from one to nine years of age) captured from 1990 to 2016. We found that members of family groups, i.e., mothers as well as their dependent offspring, had significantly higher cortisol concentrations compared to solitary individuals (males or females). Higher energetic costs in family groups are likely linked to growth of the offspring as well as maternal costs incurred by the rearing of offspring. Our findings provide valuable insights into the physiological effects of maternal care and reproductive status in mammals.

1. Introduction

Allostasis is the concept of maintaining a stable balance of energetic incomes and costs through both predictable life history events, like reproduction, and unpredictable perturbations (Wingfield, 2005). Energy expenditure is mediated partly through the hypothalamic–pituitary–adrenal (HPA) axis, which secretes glucocorticoids in response to metabolic demands (Sapolsky, 2000). At baseline concentrations, these hormones are involved in the immune response, water balance, and various metabolic pathways (Sapolsky, 2000; MacDougall-Shackleton et al., 2019). Circulating glucocorticoid concentration increases in response to noxious stimuli, activating secondary physiological pathways, known as the “vertebrate stress response” (Sapolsky, 2000) in which these hormones reallocate energy away from long–term fitness investments like immunity and reproduction (Sapolsky, 2000; Landys et al., 2006) and shift resources towards immediate survival (Wingfield and Kitaysky, 2002).

Life history traits, such as sex, or reproductive status, and age (Heimbürge et al., 2019) can results in differences in hormone concentrations. Some glucocorticoids, such as cortisol, may decline with age

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* Corresponding author.
** Corresponding author at: Department of Natural Sciences and Environmental Health, University of South–Eastern Norway, Gullbringvegen 36, Bø 3800, Norway.

E-mail addresses: marcello.franchini@uniud.it (M. Franchini), andreas.zedrosser@usn.no (A. Zedrosser).

[1] 0000-0003-3050-3859
[2] 0000-0003-1620-7711
[3] 0000-0002-3455-4218
[4] 0000-0003-3389-201X
[5] 0000-0002-1866-9787
[6] 0000-0003-1445-4524
[7] 0000-0003-4417-3037

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(Comin et al., 2012; Montillo et al., 2014) related to higher metabolic costs linked to growth and development (Comin et al., 2012; Montillo et al., 2014). Pregnant females generally show higher circulating cortisol levels as cortisol is important for foetal organ maturation and the induction of parturition (Heimbürge et al., 2019). After their offspring are born, females tend to have higher cortisol levels due to the energetic costs linked to rearing offspring (Mislan et al., 2016; Cattet et al., 2018), such as lactation (Derocher et al., 1993; Arnould and Ramsay, 1994).

Cortisol can be measured in different matrices such as blood or urine (Heimbürge et al., 2019). However, hair cortisol concentrations (HCCs) provide an estimate of circulating cortisol concentrations over a longer period and is more stable than other matrices (Macbeth et al., 2010; Prandi et al., 2018; Franchini et al., 2023). During the active hair growth phase, cortisol is accumulated in the hair shaft via passive diffusion from blood vessels (Meyer and Novak, 2012). Consequently, the individual HPA axis activity over weeks to even years can be measured in hairs (Macbeth et al., 2010; Meyer and Novak, 2012), and its long–term measurements across different age and reproductive classes can help to understand the physiological demands of a species.

Here we took the brown bear *Ursus arctos* as a model species to study the relationship between long–term measurements of cortisol in hairs and life history traits, i.e., solitary bears vs females with dependent offspring vs offspring accompanying mothers. Research on congeneric polar bears (*U. maritimus*) suggested effects of age (Mislan et al., 2016) and reproductive status (Macbeth et al., 2012; Mislan et al., 2016) on long–term HPA axis activity. In comparison, there may be a negative (Cattet et al., 2018) or no associations between HCC and age in brown bears (Macbeth et al., 2010). We hypothesize that rearing offspring is energetically costly and causes increased levels of HCCs (Heimbürge et al., 2019; Alekseeva et al., 2020). High energetic and/or nutritional costs linked to lactation can lead to higher cortisol concentrations in mothers (Derocher et al., 1993; Arnould and Ramsay, 1994; Cattet et al., 2018). We therefore predict 1) higher HCC concentrations in females with dependent offspring compared to solitary males and females. We further hypothesize that offspring accompanying mothers have higher energetic demands than solitary adults and ultimately higher cortisol levels due to the costs linked to rapid growth (Dahle et al., 2006). We therefore predict 2) higher HCC concentrations in offspring accompanying mothers compared to solitary males and females.

2. Material and methods

2.1. Study area

The study area was located in south–central Sweden (61°N, 15°E), covers approximately 13,000 km^2, and is characterized by bogs, lakes, and managed coniferous forests. The terrain is hilly, with elevations ranging from 200 to 850 m. The area is predominantly (~80 %) covered by intensively managed boreal forests of different ages 0–100 years (Swenson et al., 1999), and mainly composed of Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), and birch (*Betula* spp; Moe et al., 2007). Heather (*Calluna vulgaris*), grasses, and berry–producing shrubs dominate the understory vegetation, while bogs and lakes occupy the remaining area. Precipitation ranges between 350 and 450 mm during the vegetation period, and snow cover lasts from approximately November until the end of April/early May (Elfström et al., 2008).

Human density is low at 1–7 inhabitants/km^2, but the landscape is crisscrossed by gravel roads with a density of approximately 1 ± 0.50 km/km^2 (Martin et al., 2010; Ordiz et al., 2014). Human presence is highest during summer and autumn, primarily due to hunting, as well as berry and mushroom picking (Martin et al., 2010). The population density of brown bears in the study area is ~ 23 individuals per 1000 km^2 (Bischof et al., 2009).

2.2. Sample collection and laboratory analysis

Bears (only adults and sub–adults) were captured as part of an individual–based long–term monitoring project by remote drug delivery from a helicopter, shortly after leaving the hibernation den in April and early May (Arnemo and Evans, 2017). Guard hair of wild brown bears grows at a rate of approximately 0.7 mm/day from late May until late September (Jimbo et al., 2020). In our study, bears were captured in late April and early May, just after leaving the hibernation den. Therefore, our samples represent cortisol concentrations diffusing into the hair shaft during the previous year's hair growth period.

Offspring in family groups were captured for the first time as yearlings (Zedrosser et al., 2013; Arnemo and Evans, 2017). In many cases, bear age was known because offspring of radio–collared females were followed from the year of birth (Swenson et al., 2001; Zedrosser et al., 2013). In cases in which the year of birth was unknown, age was estimated by counting the cementum annuli of an extracted premolar (Matson et al., 1993). Captures were approved by the appropriate national authorities (Swedish Environmental Protection Agency, Stockholm: #NF–412–4762; Swedish Board of Agriculture: #35–846; Swedish Ethical Committee on Animal Research, Uppsala: #277, #40 and #C59).

Hair samples were collected during capture with pliers from the same body region (i.e., shoulder blades), then air–dried and stored in the dark at room temperature until further analysis (Sergiel et al., 2020). Prior to cortisol extraction, we removed as much underfur and debris from guard hairs as possible, documented the presence of follicles, and then weighed the sample to the nearest milligram. Only hairs samples with intact follicles were considered in the analysis (Sergiel et al., 2020). We followed the procedures in Macbeth et al. (2010) and Sergiel et al. (2020) by first washing each hair sample a total of three times in 40 μL HPLC grade methanol per mg hair for three minutes. After drying the hair for at least 24 h, we used a mixer mill (Retsch MM4000; Retsch GmbH, Germany) at 30 Hz to grind each hair sample to a fine powder. A 25 mg mass of powdered hair was placed in plastic tubes with 0.5 mL HPLC grade methanol and gently vortexed for 10 s. Samples were then placed on a rotator at low speed for 24 h and then centrifuged for 15 min at 2150 g (Sergiel et al., 2017). We collected the supernatant and transferred it to a 12 mm glass test tube, then used two replications of additional washings in 0.5 mL of fresh methanol, centrifuged for another 15 min at 2150 g and collected the resulting supernatant. The three supernatants were pooled and dried under nitrogen gas at 38 °C. Dried steroid hormones were reconstituted in 0.2 mL of phosphate buffer for 12 h at 4 °C. We vortexed reconstituted samples on a low setting for 40 s, transferred to 1.5 mL plastic vials, then centrifuged for 15 min at 2150 g. We then analysed 50 μL aliquots in duplicate with an enzyme linked immunoassay kit from Oxford Biomedical (Rochester Hills, MI, USA) and standardized extracted HCC to hair mass. Intra– and inter–assay coefficients of variation (SD/mean × 100 %) were 4.9 % and 5.1 %, respectively, and parallelism was observed between the standard curve and serially diluted bear hair extracts (Macbeth et al., 2010; Sergiel et al., 2020).

Hair cortisol concentrations have been measured throughout this monitoring effort, and in 2014 Oxford Biomedical changed the antibody they used in their commercial kits, resulting in differences in the detection sensitivity of the kits prior and after the antibody change (Wilson et al., 2021; Supplementary material). To account for and correct difference in cortisol values measured with each kit, we followed the protocols within Wilson et al. (2021) and used the published equations to correct cortisol values measured with the kits manufactured prior to 2014 (i.e., with the previous antibody; Supplementary material). We acknowledge that correcting cortisol concentrations is an imperfect solution and will introduce additional variation into our analysis. However, we are confident that it does not bias our inferences (Supplementary material).

2.3. Statistical analysis

HCC variations in relation to age and reproductive status were analysed using generalized linear mixed models (GLMMs) with a Gamma distribution and a log–link function to accommodate positive data with a left–hand skew. We used HCC as the response variable, age and reproductive status as fixed factors, and bear ID as random factor to account for the autocorrelation among repeated measurements of individuals among years (i.e., recaptures). Capture and handling can affect cortisol accumulation in the hairs of brown bears (Cattet et al., 2014). Our samples exhibited high variability, not only among individuals but also across different years for bears captured multiple times (Supplementary material). However, while individual variation accounted for a considerable proportion of the variance in our data (~ 0.14), no trends in this variation suggested potential biases in our models or inferences (Supplementary material). Therefore, we decided to statistically control for potential effects of multiple captures by including individual ID as a random effect in our models. We chose not to include body mass into the analyses because it is highly correlated with age in brown bears, and highly variable among years (Zedrosser et al., 2006; Swenson et al., 2007). We compared the model structure of an intercept–only model (i.e., null model) with the most complex model (i.e., the one containing the interaction between fixed factors) and another model in which only the additive effect between fixed factors was considered.

We tested for multicollinearity among fixed factors using the variance inflation factor (VIF) implemented in the ‘*car*’ package (Fox and Weisberg, 2019). VIFs ≥ 5 were considered as threshold values to define high correlation among predictors (Akinwande et al., 2015) and these factors were not included in the same model. The selection of the most parsimonious model was based on Akaike’s Information Criterion corrected for small sample sizes (AICc; Hurvich and Tsai, 1989). We used the ‘*blme*’ package (Chung et al., 2013) in the statistical software R 4.3.1 (R Core Team, 2023) for all statistical analyses.

3. Results

We used HCCs from 448 hair samples collected from 303 individuals, aged one to nine years old (Table 1). We had a total of 162 males and 141 different females; 71 were sampled as solitary females while 44 were sampled as females accompanied by offspring. Bears we recaptured between zero and eight times, but we did not account for the number of recaptures in our models (Supplementary material).

The GLMMs that best explained the variation in HCC included either the interaction between ‘age’ and ‘reproductive status’ or the additive effect of these fixed factors (Table 2). However, in both models, only ‘reproductive status’ showed a significant effect, while ‘age’ had no significant influence (Table 3). The highest HCCs were observed in dependent offspring (mean = 7.07 ± 3.26 (SD) pg cortisol/mg hair; median = 6.28 pg/mg; range = 3.32 – 15.59 pg/mg), followed by mothers with dependent offspring (mean = 4.87 ± 3.94 pg/mg; median = 4.87 pg/mg; range = 2.70 – 27.41 pg/mg), solitary females (mean = 4.81 ± 2.29 pg/mg; median = 4.69 pg/mg; range = 2.19 – 31.33 pg/mg), and solitary males (mean = 4.81 ± 2.29 pg/mg; median = 3.99 pg/mg; range = 1.76 – 11.22 pg/mg) (Fig. 1).

Table 1
Total number of hair samples collected from 303 captured brown bears in south–central Sweden, 1990–2016. Sample sizes are shown in relation to age, sex, and reproductive status.

Age	Reproductive status				**Total**
	Offspring accompanying mother	Solitary female	Female with dependent offspring	Solitary male	
1	190	–	–	–	**190**
2	17	13	–	8	**38**
3	–	19	–	19	**38**
4	–	35	–	12	**47**
5	–	21	–	13	**36**
6	–	13	11	12	**36**
7	–	6	5	9	**20**
8	–	13	3	9	**25**
9	–	5	2	13	**20**
Total	**207**	**109**	**37**	**95**	**448**

Table 2
Ranking of generalized linear mixed models used to evaluate the effect of life history traits (age, reproductive status) on hair cortisol concentration in brown bears in south–central Sweden. The sample size consisted of 448 hair samples collected from 303 individual bears captured during 1990–2016. Abbreviations: AICc = Akaike’s Information Criterion corrected for small sample sizes. ΔAICc = difference in AICc values. An interaction between explanatory variables is denoted by ‘:’.

Model	Fixed factor/s	Random factor	AICc	ΔAICc
1	Age:Reproductive status + Age + Reproductive status	Bear ID	1994.83	0
2	Age + Reproductive status		1994.83	0
Null	~1		2031.08	36.25

Table 3
Beta estimates ($\widehat{\beta}$), standard errors (SE), and lower and upper 95 % confidence intervals (LCI, UCI) from the top model used to evaluate the interaction effect of life history traits (age, reproductive status) on hair cortisol concentration of brown bears in south–central Sweden. The sample size consisted of 448 hair samples collected from 303 individual bears captured during 1990–2016. An interaction between explanatory variables is denoted by ‘:’.

Parameter	$\widehat{\beta}$	SE	LCI	UCI	*p–value*
Age	0.02	0.06	−0.10	0.14	0.72
Female with dependent offspring	1.79	0.47	0.87	2.71	< 0.001
Offspring accompanying mother	2.10	0.13	1.85	2.35	< 0.001
Solitary female	1.65	0.13	1.40	1.90	< 0.001
Solitary male	1.65	0.14	1.38	1.92	< 0.001
Age:Female with dependent offspring	0	0	0	0	–
Age:Offspring accompanying mother	−0.23	0.12	−0.47	0.01	0.07
Age:Solitary female	−0.01	0.07	−0.15	0.13	0.91
Age:Solitary male	−0.03	0.07	−0.17	0.11	0.71

4. Discussion

We found that mothers accompanied by dependent offspring had higher HCC compared to solitary males and females (support prediction 1). We also found that both yearling and two–year–old dependent offspring had higher cortisol levels compared to all other bear groups (support prediction 2) including females with dependent offspring.

The higher HCCs in mothers with dependent offspring are likely related to the high energetic demands for rearing offspring, mainly lactation (Farley and Robbins, 1995; López-Alfaro et al., 2013; Mislan et al., 2016). During peak lactation in brown bears (when offspring are ~150–250 days old) offspring consume around 1200 g milk per day or approximately 11,715 kJ/g per day (Farley and Robbins, 1995). This means that a mother of two is transferring over 20,920 kJ/g to her offspring each day, not including the energetic costs to produce milk. Within this study population, the average energy content of the “typical” bear diet is 13.57 kJ/g (Mikkelsen et al., 2023), so a mother nursing two offspring would need to eat over 1.5 kg of food each day just to support lactation demands. In our sample, females with dependent offspring weighed on average (± SD) 86 ± 11 kg while solitary females of the same age weighed 77 ± 17 kg. Nevertheless, we did not compare the ratio between percentages of fat and lean mass.

Differences in diet, specifically greater carnivory, are sometimes associated with greater metabolic demands in male brown bears due to their larger body sizes (Welch et al., 1997; Jacoby et al., 1999), but our

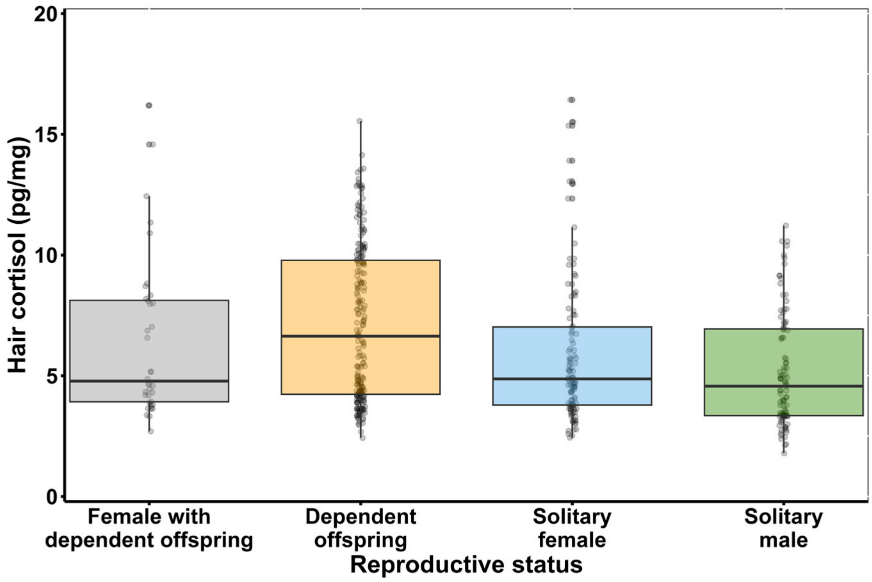

Fig. 1. Differences in hair cortisol concentrations (pg/mg) of brown bears in south–central Sweden in relation to an individual's reproductive status (offspring accompanying mother, mother accompanied by dependent offspring, solitary female, solitary male). The sample size consisted of 448 hair samples collected from 303 bears captured during 1990–2016.

results indicate that there is little difference in HCCs between solitary males and females, and solitary males had the lowest cortisol concentrations. Additional factors may explain the observed higher cortisol concentrations in females with dependent offspring, which had considerably higher cortisol concentrations than solitary males. If cortisol is an accurate proxy for metabolic demands (Romero, 2002), males in our sample had the lowest metabolic demands, even when accounting for their larger body size (Ohdachi et al., 1992; Cattet et al., 2014). Therefore, differences in diet between male and female brown bears may be driven by other factors rather than metabolic demands alone (Heimbürge et al., 2019), or, possibly, males may have physiological adaptations that keep biologically available free cortisol concentrations low, even under greater metabolic stress.

Stressors related to cub safety may contribute to higher cortisol concentrations in females with dependent offspring. Especially during the mating season, females with dependent offspring are under additional stress due to the risk of intraspecific killing of offspring by other bears (especially males – Swenson, 2003; Rode et al., 2006; Steyaert et al., 2016). Maternal strategies to avoid infanticide mirror standard anti–predator responses, which include temporal and spatial avoidance and increased vigilance. Aside from indicating overall metabolic demands, cortisol also acts as an appetite stimulant (Vera et al., 2017), thus higher cortisol concentrations in females with dependent offspring may be part of lactation which stimulates the female to forage more, given her higher energetic demands (Vera et al., 2017). Cortisol also plays important roles in water and mineral balance (Vera et al., 2017) as well as allocating proteins into milk during production and stimulating lactation (Motil et al., 1994; Hannan et al., 2023). Thus, high concentrations of cortisol in lactating females likely reflects a combination of the diverse roles that cortisol plays within the body.

The high HCCs recorded in dependent offspring and their mothers are in line with other studies in Ursids (Mislan et al., 2016; Cattet et al., 2018) as well as other mammals (e.g., vervet monkeys *Chlorocebus aethiops sabaeus* – Fairbanks et al., 2011; domestic pigs *Sus scrofa* – Bacci et al., 2014). Other factors might be involved in observing similar cortisol levels in dependent offspring: (*i*) similar physiological responses between mothers and dependent offspring to shared ecological stressors (Macbeth et al., 2012), (*ii*) genetic heritability (Macbeth et al., 2012), (*iii*) direct cortisol transfer during lactation (Macbeth et al., 2012), especially in females in poor body condition (Derocher and Stirling, 1998; Robbins et al., 2012; Mislan et al., 2016), or (*iv*) the combined effects of all these factors (Macbeth et al., 2012).

In domestic species (e.g., horses *Equus caballus* – Comin et al., 2012; Montillo et al., 2014) higher metabolic costs linked to growth, adaptation and learning in a new environment may contribute to higher cortisol in young individuals compared to adults. Young individuals are also a social class vulnerable to conspecific infanticide (Swenson, 2003; Rode et al., 2006; Steyaert et al., 2016) and constantly exposed to novel stimuli. Our results showed that social class is an important factor to consider when describing HCCs across ages, as physiological demands are likely to be different among different classes.

Our findings add important pieces of knowledge about the role that social class may have in shaping the physiological response in mammals, although this response is often species–specific (reviewed in Heimbürge et al., 2019). Hair cortisol is a suitable indicator of the long–term physiological response of wild species in response to both predictable and unpredictable changes in the environment and metabolic demands. However, with respect to potential long–term stressors we emphasize the importance of considering cortisol as more than a simple 'stress hormone' since this definition oversimplifies its important physiological roles in the life history of wildlife such as Ursids (Boonstra, 2013). Cortisol is involved in a wide range of physiological functions, e.g., growth and development, carbohydrate and lipid homeostasis, and regulation of reproductive and immune systems (Dickens and Romero, 2013, MacDougall-Shackleton et al., 2019). Findings related to cortisol concentrations in hairs should be compared to or complemented with findings based on cortisol concentrations obtained from other media, e.g., blood, to aid in a more comprehensive understanding of the role of reproductive status as a potential chronic stressor in brown bears, and, more broadly, in other wildlife species.

CRediT authorship contribution statement

Kindberg Jonas: Writing – review & editing, Visualization, Validation. **Zedrosser Andreas:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Franchini Marcello:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Selva Nuria:** Writing – review &

editing, Visualization, Validation, Supervision. **Janz David M.:** Writing – review & editing, Visualization, Validation. **Mikkelsen Ashlee J.:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Sergiel Agnieszka:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Data curation.

Declaration of Competing Interest

No competing interests are reported by the authors.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.zool.2025.126257.

Data availability

Data will be made available from the corresponding authors on reasonable request.

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