

Climatic cues and glucocorticoids in a free-ranging riparian population of red deer (*Cervus elaphus*)

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Abstract. We measured faecal cortisol metabolites of a free-ranging riparian population of red deer to investigate potential effects of season, ambient temperature, precipitations and water level on the annual secretion pattern. Individuals may cope with environmental challenges through the secretion of stress hormones (glucocorticoids) which allows the integration of environmental change and life history traits by means of an adaptive feedback mechanism. Adaptations regard cyclic day-to-day activities, short-term environmental stressors or long-term ecological pressures. We detected a clear seasonal pattern of glucocorticoid metabolites secretion, with higher levels in winter and lower levels in summer. The model relating glucocorticoids secretion to minimum ambient temperature was the best fit to our dataset, although the observed pattern might as well be due to declining nutritional intake and reduction of metabolic rate in the cold season. We observed an improvement of the fit when stochastic events (flash flood) were included in the model, and discussed their role as potential contingent environmental stressors.

Key words: climate, faecal cortisol metabolites, stress, ungulates

Introduction

Habitats are dynamic environments in which several situations, either predictable or stochastic, may trigger the adaptive response of animals through behavioural, morphological or physiological modifications. The production of glucocorticoids by the neuroendocrine system is a major pathway that integrates environmental change and life history traits such as reproduction, growth, digestion, immunization, or energy mobilization by means of an adaptive feedback mechanism (Sapolsky et al. 2000). Glucocorticoids have been used as physiological indicators of stress in different species (Möstl & Palme 2002). Although short term secretions of glucocorticoids are considered beneficial for the organism, as they enable animals to cope with

unpredictable stress events (Sapolsky et al. 2000) long term secretion may lead to overall reduced fitness by affecting survival and reproductive success (Sapolsky 1992, Möstl & Palme 2002).

For a captive red deer (*Cervus elaphus*) population, Huber et al. (2003a) found a clear seasonal pattern of glucocorticoid metabolites secretion, with higher level in winter and lower level in summer. The same variation has been reported by other studies on deer species in temperate climates, like white-tailed deer (*Odocoileus virginianus*, Bubenik et al. 1983) and mule deer (*Odocoileus hemionus*, Saltz & White 1991). However, some controversial results still persist (see studies on other Cervidae by Bubenik & Brown 1989, Monfort et al. 1993, Reyes et al. 1997, Bubenik et al. 1998).

We aimed to investigate seasonal variations in the

faecal cortisol metabolites (FCM) secretion of a free-ranging riparian population of red deer in response to potential sources of stress represented by different climatic factors such as ambient temperature and precipitation. We also included the effect exerted by the water level of the main river, because riparian regions might be particularly susceptible of drastic alterations in plant and animal communities following stochastic events such as flash floods.

Material and Methods

The study area (Lobau water forest, within the Danube-Auen National park) is a riparian habitat strongly influenced by the water level of the Danube, stretching over 2400 ha from the south-eastern side of the city of Vienna (Austria). The study population of red deer is endemic in the Lobau and currently shows a density of about 10 individuals/100 ha. Forestry activities are no longer carried out within the Park, and the main source of human disturbance is represented by tourism (Arnberger & Hinterberger 2003, Sterl et al. 2008).

Glucocorticoid metabolites can be measured as a parameter of adrenal activity in faecal samples, which offer the advantage of being easily collected and feedback free (Möstl & Palme 2002, Touma & Palme 2005). Between January and December 2007 we collected 12 fresh (to avoid degradation or washing out effects; Rehnus et al. 2009) faecal samples per month on an anonymous basis. The collections were evenly distributed throughout each month and always occurred within the borders of the Park. Faeces were put into separate plastic bags and frozen immediately at -20°C . Samples were extracted (80 % methanol) and analysed with an 11-oxoetiocholanolone enzyme immunoassay (EIA) as described by Möstl et al. (2002). The EIA has already been successfully validated and applied in red deer (Huber et al. 2003a, b). We obtained climatic data measured at “Großenzersdorf” meteorological station (minimum and maximum ambient temperature [in $^{\circ}\text{C}$], rainfall [in mm]), and at “Korneuburg” hydrological station (water level of the Danube [in cm]).

We run a logarithmic transformation of FCM values (FCM secretion typically follows an exponential trajectory) as our data were not normally distributed. To check for significant intra-annual differences in FCM level, we performed a comparison of the mean FCM values between months by means of a General Linear Model. We run the Tukey post-hoc test to compare each group mean with every other group mean in a pairwise manner. To check the relationship between abiotic factors and FCM level, we analyzed data by means of General Linear Models. We standardized the

four predictors (monthly mean minimum temperature; monthly mean maximum temperature; monthly overall precipitation; monthly mean water level) and used them to create a set of additive models (excluding those with minimum and maximum temperature or with precipitation and water level, as Fig. 1 suggested very high levels of covariation between these pairs of variables). Model selection was based on minimizing AICc (Akaike Information Criterion corrected for small sample size) (Akaike 1973, Burnham & Anderson 2002). A difference in the AICc of < 2 indicates competitive models. In addition, we used likelihood ratio tests (LRT) to test hypotheses between nested models. To quantify the independent correlation of each predictor variable with the response variable for the set of candidate models, we isolated the amounts of variance attributable to each predictor variable by means of a hierarchical partitioning analysis, which allows the identification of the predictors that explain most variance independently of the others, helping to solve the problems presented by multi-collinearity (Mac Nally 2002). Significance level was fixed at 0.05. All analyses have been performed with the statistic software R 2.8.1 (R Development Core Team 2009).

Results

The General Linear Model detected a significant variation in the seasonal level of FCM ($F_{11, 132} = 6.944$, $P < 0.0001$). The Tukey post-hoc test (see Fig. 1) gave a tendency for January and December as months reporting the highest concentrations of FCM, although they were not significantly different from March, April, May and September. Specifically, in September we observed a peak of FCM, whose level was significantly different from the one observed in August ($P = 0.005$); the difference with the level in October was not statistically significant, though the P -value (0.08) suggests a tendency.

As to the relationship between abiotic factors and FCM, two models were equally adequate to interpret our dataset, their Δ AICc being less than two (Table 1). We chose the model with only minimum temperature over the model with minimum temperature and water level, for the principle of parsimony and because the LRT showed that addition of the parameter “water level” did not significantly improve the fit (LRT: $\chi^2 = 3.359$, d.f. = 1, $P = 0.0668$).

Additionally, although the model relating FCM with minimum temperature and water level had the lowest absolute AICc, the result from the hierarchical partitioning analysis of $r^2 (= 0.111)$ from this model gave evidence for the minimum temperature as the

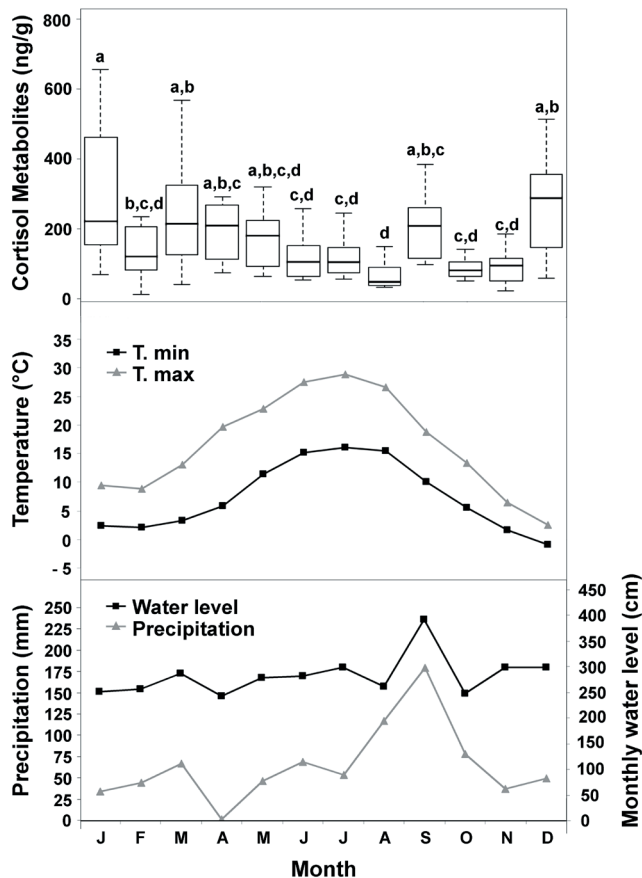


Fig. 1. Seasonal pattern of red deer faecal cortisol metabolites (in ng/g; values are medians and quartiles; different letters indicate $P < 0.05$ by post hoc Tukey test), ambient temperature (minimum and maximum, in °C), precipitation (in mm) and water level of the Danube (in cm).

concentrations in summer (Fig. 1). The data analysis suggests the minimum ambient temperature as the main factor to influence the physiological response of individuals in terms of glucocorticoids secretion, a result that is in line with what was previously found by Huber et al. (2003a). However, although the high level of secretion of glucocorticoids in winter might indeed reflect an adaptation to harsh environmental condition (Yousef et al. 1971, Dantzer & Mormede 1983, Huber et al. 2003b), the relatively mild minimum temperatures reported seem unlikely to represent a thermal challenge. The observed pattern of secretion might be due to declining nutritional intake and reduction of metabolic rate, typically higher during the cold season (Saltz & White 1991, DelGiudice et al. 1992, Tsuma et al. 1996). A annual FCM rhythm could also reflect the change from an anabolic metabolism during summer to a catabolic metabolism during winter. How and if the environmental condition (temperature in this case) impacted this rhythm still remains unclear. Fig. 1 also shows a peak in the production of FCM in September. As found in other species (Thiel et al. 2008) human disturbance (tourism) might be a potential source of stress for the deer population. However, Arnberger & Hinterberger (2003) did not detect any peak in tourists' presence in September (the busiest month being May). The biological cycle of red deer suggests that the onset of the rutting season might trigger the peak of FCM production in September. However, Huber et al. (2003a) analyzing faecal samples of "captive red deer living undisturbed

Table 1. Values of AICc, Δ AICc and Akaike's weights for each of the eight predictive models (candidate models highlighted in bold).

General linear model	AICc	Δ AICc	Akaike's weights
Temp.min.+Water level	839.22	0.00	0.52
Temp.min.	840.47	1.24	0.28
Temp.min.+Rain	842.53	3.30	0.10
Temp.max.	844.21	4.99	0.04
Temp.max.+Water level	844.44	5.22	0.04
Temp.max.+Rain	846.15	6.93	0.02
Rain	852.56	13.33	0.00
Water level	852.64	13.41	0.00

main factor that contributes to the explained variance in level of FCM (independent effect: minimum temperature 0.096; water level 0.015).

Discussion

We detected a clear pattern in the seasonal level of FCM, with higher concentrations in winter and lower

in a large enclosure, [whose] fecal cortisol metabolite levels are likely to represent basal cortisol production affected by season, sex, and reproduction" did not find any evidence for such a pattern. Ingram et al. (1999) also did not find any evidence for an increase in glucocorticoids during the rut in red deer, a result that is consistent with other studies on several ungulate species

such as axis deer (*Axis axis*, Chapple et al. 1991), white-tailed deer (Bubenik et al. 1983), and Pyrenean chamois (*Rupicapra pyrenaica*, Dalmau et al. 2007).

The adopted model selection approach, on the other hand, showed that the model with minimum temperature and water level had the lowest absolute AICc. Although the addition of the parameter “water level” did not significantly improve the fit of the model, the *P*-value of the LRT (= 0.0668) suggests a tendency that might help to explain the observed pattern. Fig. 1 actually shows a clear, sudden raise of the water level of the Danube in September. This event was associated with a flood of the surrounding lands, and in turn determined an augment in the level of the interconnected internal bodies of waters and, consequently, a diminution of the available habitat for red deer. In this scenario, we could speculate that deer individuals might have been “forced” to move and clump together in smaller areas or unsuitable habitats, with subsequent increase of intra-specific competition in terms of space and food (see recent findings by Li et al. 2007 and Christofolletti et al. 2010) or higher disturbance rate.

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Our results support the main conclusion of Huber et al. (2003a), indicating a clear seasonal pattern of glucocorticoid metabolites secretion, with higher level in winter and lower level in summer. Moreover, stochastic events such as flash floods might possibly play an important role as contingent environmental stressors.

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