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Physiological response to etho-ecological stressors in male Alpine chamois: timescale matters!

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Abstract

From a life history perspective, glucocorticoids secreted by the neuroendocrine system, integrating different sources of stress through an adaptive feedback mechanism, may have important consequences on individual fitness. Although stress responses have been the object of several investigations, few studies have explored the role of proximate mechanisms responsible for the potential trade-offs between physiological stress and life history traits integrating social and environmental stressors. In 2011 and 2012 we collected data on faecal cortisol metabolites (FCM) in a marked male population of Alpine chamois, within the Gran Paradiso National Park (Italy). Using a model selection approach we analysed the effect of potential etho-ecological stressors such as age, social status (territorial vs. non-territorial males), minimum temperature, snow depth and precipitation on FCM variation. To correctly interpret environmentally- and socially-induced stress responses, we conducted model selections over multiple temporal scales defined *a priori*: year, cold months, spring, warm months, mating season. Over the year, FCM levels showed a negative relationship with minimum temperature, but altogether climatic stressors had negligible effects on glucocorticoid secretion, possibly owing to good adaptations of chamois to severe weather conditions. Age was negatively related to FCM during the rut, possibly due to greater experience of older males in agonistic contests. Social status was an important determinant of FCM excretion: while both the 'stress of subordination' and the 'stress of domination' hypotheses received some support in spring and during the mating season, respectively, previous data suggest that only the latter may have detrimental fitness consequences on male chamois.

Introduction

The concentration of glucocorticoids (or their metabolites), determined as a parameter of adrenocortical activity, has been widely used as a physiological indicator of stress in a number of species (Möstl and Palme 2002; Sheriff et al. 2011). Through an adaptive feedback mechanism, the secretion of glucocorticoids by the neuroendocrine system integrates environmental changes and social and physiological traits such as reproduction, growth, digestion, immunization, or energy mobilization, thus enabling animals to cope with stress events (Sapolsky et al. 2000). Long-term production of high levels of glucocorticoids, however, may reduce fitness by impacting on survival and reproductive success, for example through the suppression of the immune function (Sapolsky 1992; Möstl and Palme 2002). From a life history perspective, it is therefore important to identify the etho-ecological mechanisms responsible for the potential trade-offs between stress response and different life history traits.

Several predictable or unpredictable ecological challenges may trigger physiological stress responses in wild animals. Individuals living in temperate environments – especially those dwelling on mountains – for example must typically cope with strong seasonal changes in weather conditions (Boonstra 2004). A number of studies found a clear seasonal pattern of glucocorticoid secretion in temperate mammals, with higher concentrations occurring during colder periods (e.g. Bubenik et al. 1983: white-tailed deer *Odocoileus virginianus*; Saltz and White 1991: mule deer *Odocoileus hemionus*; Konjevic et al. 2011: fallow deer *Dama dama*), but in mountain hare *Lepus timidus*, Rehnus et al. (2010) did not find similar patterns. Major determinants of stress responses may be represented by climatic parameters such as minimum ambient temperature and snow depth, which were negatively correlated with glucocorticoid metabolite levels in red deer *Cervus elaphus* (Huber et al. 2003a). Less predictable events such as flooding (Corlatti et al. 2011 for red deer) tourist disturbance (Cederna and Lovari 1985; Zwijacz-Kozica et al. 2013 for chamois *Rupicapra* spp.; Rehnus et al. 2014 for mountain hare) and hunting (Bateson and Bradshaw 1997: red deer) may as well act as environmental stressors for wild animals.

Increased concentration of glucocorticoids may also reflect social and behavioural traits such as mating competition: in bison *Bison bison* bull, Mooring et al. (2006) found a drastic increase of glucocorticoid metabolite levels by the time of the rut and similar results were found in male Alpine chamois *Rupicapra rupicapra rupicapra* (Corlatti et al. 2012). Furthermore, hormonal regulation normally plays a role in the expression of alternative male mating tactics (AMTs, i.e. discrete variations in mating behaviour for access to females: Taborsky et al. 2008): increasing evidence suggests that, during the mating season, dominant individuals show higher glucocorticoid concentration than do subordinates (Creel 2001; Mooring et al. 2006; Fichtel et al. 2007: sifakas *Propithecus verreauxi*). In turn, different hormonal levels may help to explain the maintenance of AMTs within populations through a condition-dependent mechanism (Corlatti et al. 2012).

Although a number of studies have explored the variation of physiological stress responses combining social and environmental stressors in different *taxa*, e.g. Cavigelli (1999) in lemurs *Lemur catta*, Foley et al. (2001) in elephants *Loxodonta africana*, Mashburn and Atkinson (2007) in Steller sea lion *Eumetopias jubatus*, Rubenstein (2007) in superb starling *Lamprotornis superbus* and Nováková et al. (2008) in spiny mice *Acomys cahirinus*, to our knowledge similar approaches are rare in wild ungulates (Garcia Pereira et al. 2006 in Pampas deer stags *Ozotoceros bezoarticus*). Methodologically, the correct interpretation of environmentally- and socially-induced stress response may critically depend on the definition of appropriate temporal scales. If, on the one hand, the influence of environmental stressors, such as weather conditions, on glucocorticoid concentration is more likely to be detected over large timescales, e.g. year – owing to large variations in climatic values, the acute effect of seasonal ethological stressors such as mating dominance may not be detectable over the same timescale, because of a dilution effect (i.e. the variance explained by mating behaviour would be reduced, when including periods without social tension). Consequently, the use of multiple temporal scales appears crucial for the correct biological interpretation of different determinants of stress response.

The chamois, *Rupicapra* spp., is the most abundant mountain-dwelling ungulate of Europe and the Near East. The sexes are nearly monomorphic: males weigh 30-40% more than females only at the start of the rut (Garel et al. 2009; Rughetti and Festa-Bianchet 2011), but this dimorphism rapidly declines to about 6% from November to January (Garel et al. 2009). While female chamois and their kids live in herds, grown-up males tend to live solitary for most of the year and clump together during the rut (November, in Europe; Krämer 1969). Spatial and behavioural observations showed that, during the rut, some dominant males (i.e. territorials) patrol a relatively small area in which they try to keep females and chase away intruders, whereas other males (i.e. non-territorials) display following behaviour and territory intrusions (Krämer 1969; von Hardenberg et al. 2000; Corlatti et al. 2012). The onset of territoriality in chamois, however, may occur well before the mating season, as early as springtime (von Hardenberg et al. 2000; Corlatti L., unpublished data).

Taking advantage of 2 years of data on marked individuals, we investigated the monthly pattern of glucocorticoid metabolite excretion and the variation in the effect of potential etho-ecological stressors (age, social status – territorial vs. non-territorial males, minimum ambient temperature, precipitation, snow depth) on glucocorticoid metabolite excretion in male chamois over multiple temporal scales. Specifically, we hypothesised that:

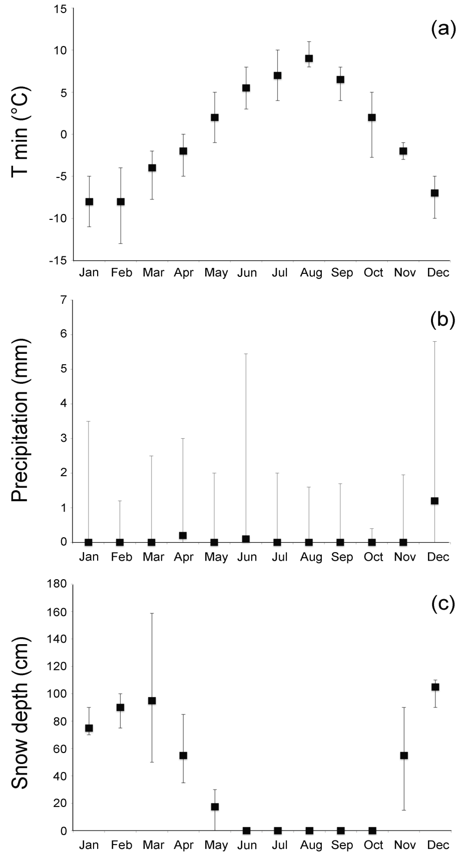
1) male chamois would show a strong seasonal pattern in glucocorticoid metabolite concentration, with higher levels during the cold months (Dalmau et al. 2007), and a peak during the mating season (Mooring et al. 2006);
2) the effect of etho-ecological stressors would change over different timescales: over the year, we would expect minimum ambient temperature be the main determinant of glucocorticoid metabolite excretion (inverse relationship: Dalmau et al. 2007; Huber et al. 2003a); in the cold months, the large variation in snow depth should be the main determinant of glucocorticoid metabolite excretion (inverse relationship: Huber et al. 2003a); in spring, when the onset of territoriality occurs, social status should be the main determinant of glucocorticoid metabolite excretion with territorial males showing higher concentrations than non-territorial males (Mooring et al. 2006); in the warm months, the mild climatic conditions should not act as stressors for male chamois while an effect of social status, due to the maintenance of territories (von Hardenberg et al. 2000) might be expected; during the rut, according to the ‘stress of domination’ hypothesis (i.e. increased glucocorticoid levels as a cost of dominance, Creel 2001), we would expect territorial males to show higher concentrations of glucocorticoid metabolites than non-territorial males (Mooring et al. 2006; Corlatti et al. 2012).

Materials and methods

Study area and population

The ‘upper Orco Valley’ study site, within the Gran Paradiso National Park (hereafter GPNP, Western Italian Alps, 45°26’30” N, 7°08’30” E), extends over ca. 10 km^2 between 1,800 and 3,000 m a.s.l., oriented west to east. Meadows of coloured fescue *Festuca varia* dominate the south-facing slope, whereas the north-facing slope is characterised by the presence of woods of larch *Larix decidua* and patches of alder shrubs *Alnus viridis*. In 2011 and 2012 the study area was characterised by daily mean precipitation rates between 2.8 mm in winter and 4.4 mm in autumn, and by daily minimum temperatures averaging between -7.0 °C in winter and 7.4 °C in summer (own data, Fig. 1).

Figure 1: Daily values of a) minimum ambient temperature (in °C), b) precipitation (in mm) and c) snow depth (in cm) collected from Lago Serrù weather station (2,275 m a.s.l.), in the upper Orco Valley (GPNP) in 2011 and 2012. Values are medians and interquartile ranges.



The chamois population in the GPNP has been protected since 1922, and the male chamois population size in the study site, estimated through mark-resight method, shows densities of ca. 9 individuals/km^2 (Corlatti L., unpublished data). Chamois survival in the Park is limited mainly by winter starvation, whose severity may change according to sex, age and winter harshness (Rughetti et al. 2011).

Twenty-two adult male chamois were darted by the personnel of the Park, between February 2010 and November 2012, with a mixture of xylazine and ketamine. Each male was equipped with an individually recognizable GSM-GPS Pro-Light collar (Vectronic Aerospace GmbH, Berlin) with VHF beacon device. We assumed that chamois behaviour was not affected by radio-collars, as shown by Nussberger and Ingold (2006). The sedative effect was reversed by an injection of atipamezole (Dematteis et al. 2009). The entire procedure usually took less than 40 minutes and was always performed with the assistance of a veterinarian. These methods are in line with Italian law.

Sampling design and laboratory analyses

While the concentration of glucocorticoids (or their metabolites) can be obtained by blood sampling, in the last decades non-invasive procedures have been increasingly used (Sheriff et al. 2011). In particular, the analysis of faecal cortisol metabolites (FCM) offers the advantage that samples can be easily obtained and the procedure is feedback free (Möstl and Palme 2002; Huber et al. 2003b).

Between January 2011 and December 2012, one of us (LC) collected 1 fresh faecal sample/animal on a monthly basis, for as many marked chamois as possible within a given month, depending on their detectability. Marked males were first detected by means of GPS data location and/or with the aid of VHF telemetry. Spotted animals were observed until defecation: faeces were collected soon afterwards, to avoid degradation or washing out effects (Rehnus et al. 2009), and put into separate plastic bags. Within 10 h from sampling, each scat was frozen at -20°C until analysis. Over the 2 years, we collected a total of 393 scats and sampling effort was evenly distributed throughout each month (Fig. 2).

A total of 0.5 g of each well-homogenised faecal sample was extracted with 5 ml aqueous methanol (80%; Palme et al. 2013) and all samples were analysed in duplicate. FCM levels were measured with an 11-oxoaetiocholanolone enzyme immunoassay (EIA, detecting FCM with a 5ß-3α-hydroxy-11-oxo structure) whose details were first described by Möstl et al. (2002). The sensitivity of the method was 3 pg/well and the intra- and inter-assay coefficients of variation were 9.7 and 12.5%. This method proved suited in several ruminant species, including Alpine chamois (e.g.: Möstl et al. 2002; Huber et al. 2003a; Hoby et al. 2006; Kleinsasser et al. 2010).

Etho-ecological correlates

Variation in FCM levels was investigated as a function of several ethological (social status, age) and ecological (minimum temperature, precipitation, snow depth) variables. From the behavioural standpoint, we distinguished between territorial and non-territorial males. For the rutting season 2011 and 2012, we assumed that territorial males would: a) have a higher site fidelity, i.e. smaller home ranges, estimated as 90% Fixed Kernel; b) win more intra-sexual interactions – calculated as the proportion between number of interactions won and total number of interactions, collected during hourly sessions of observation *ad libitum* (Altmann 1974) – than non-territorial males. These two parameters were combined in a data matrix to perform multivariate hierarchical clustering. In 2011 nine males were classified as territorials and 10 as non-territorials; these animals maintained the same tactic in the 2012 rut. In 2012, three other males were observed and sampled: one was classified as territorial, 2 were classified as non-territorial. This approach is based on the framework developed by Corlatti et al. (2012) to which we refer the readers for more details. The age of marked individuals (in years) was estimated by counting horn rings (Schröder and von Elsner-Schack 1985) during captures.

Minimum ambient temperature (in °C), precipitation (in mm) and snow depth (in cm) data were collected from a weather station in the westernmost part of the study site (Lago Serrù, 2,275 m a.s.l.). Assuming an approximate excretion time lag of 18 h (Huber et al. 2003a), we included in our analysis the weather data registered the day prior to faecal sample collection.

Temporal scales

To investigate the temporal variation in the effect exerted by the etho-ecological variables on FCM levels, we defined the following temporal scales, based on weather (Fig. 1) and/or behavioural features:

- year: large variations in minimum ambient temperature, precipitation and snow depth;
- December-March: relatively large variations in precipitation and snow depth; lack of social tension among males;
- April-May: relatively large variations in minimum ambient temperature, precipitation and snow depth; increase of social tension among males following the onset of territoriality (Corlatti L., unpublished data);
- June-October: relatively large variations in minimum ambient temperature and precipitation; possible social tension among males for the maintenance of territories (von Hardenberg et al. 2000);
- November: relatively large variations in snow depth; peak of the social tension among males due to the rutting period (von Hardenberg et al. 2000; Corlatti et al. 2013).

Statistical analyses

Prior to analysis, FCM concentrations were $\log_{10}$-transformed and the assumption of normality was confirmed using the Pearson chi-square test. To analyse the monthly variation of FCM, we first fitted a linear mixed model setting FCM as the response variable, month as a fixed factor and animal identity as a random effect to account for pseudoreplication. We then ran a *post-hoc* Tukey test to compare each month with every other month in a pairwise manner.

To analyse the effect of etho-ecological variables on FCM levels at different temporal scales, for each time-period (year, December-March, April-May, June-October, November) we first fitted a set of linear mixed models, from the simplest (i.e. with only one fixed factor) to the most complex one (i.e. including all 5 fixed factors, without interactions). For each model, FCM was set as the response variable; age, social status (territorial vs. non-territorial males), minimum temperature, precipitation and snow depth were considered as fixed factors. In all mixed models, we accounted for variability of sampling period by setting the interaction year:month as a random effect; animal identity was also set as a random effect to account for pseudoreplication. The variance inflation factor gave no evidence of multicollinearity issues (always < 2 for every variable in all global models). For each time period, we then ran a model selection based on the Akaike Information Criterion corrected for small samples (AICc) (Burnham and Anderson 2002). Following Richards et al. (2011), we provisionally selected models with ΔAICc value < 6: to avoid retention of overly complex models, we then removed models from the candidate set if they were more complex versions of models with a lower AICc value. To address the issue of model selection uncertainty, we averaged the models retained in the candidate set. For each model in the candidate set, we also computed the R^2 statistics recently developed by Nakagawa and Schielzeth (2013).

For all statistical analyses, we used R 3.0.1 (R Development Core Team 2013) in RStudio 0.98.501 (RStudio 2013). Linear mixed effect models were built with the function `lmer` ('lme4' library: Bates et al. 2014). Tukey post-hoc tests were performed using the function `glht` ('multcomp' package: Hothorn et al. 2008). Model selection and averaging, as well as the calculation of the R^2 statistics were performed using the 'MuMIn' library (Bartoń 2013). The goodness of fit of every global model (homoscedasticity, normality of errors and independence) was checked through visual inspection of residuals.

Results

Faecal cortisol metabolite levels showed a variation over the year, with higher values in the cold months, lower values in the warm months, and a peak during the November rut (Fig. 2). Four models, with R^2 statistics between 38-40%, were retained to explain the variation of FCM levels over the year (Table 1), which was negatively influenced by minimum ambient temperature, although the estimate (Table 2, Fig.2) suggests a weak effect. No effect of age, behaviour and precipitation was detected (Table 2, Fig. 3). The same models (FCM as a function of the individual effects of minimum temperature, precipitation, age or social status) competed to explain the variation of FCM levels in December-March (Table 1), and despite the relatively higher values of R^2 statistics (45-46%) we did not find any significant relationship with the selected explanatory variables. In April-May only one model (FCM as a function of social status, Table 1) was retained in the candidate set – despite its relatively low value of R^2 (26%; Table 2) – with non-territorial males showing significantly higher FCM levels than territorial males (Table 2, Fig. 4a). Four models that included the individual effects of age, social status, minimum ambient temperature and snow depth, competed to explain the variation of FCM levels in June-October (Table 1), yet we found no significant relationships and relatively low values of R^2 statistics (34-35%; Table 2). During the November rut, social status and age competed to explain variation in FCM levels (Table 1): both models achieved fairly high values of R^2 statistics (75% and 69%, respectively; Table 2).

Figure 2: Monthly pattern of concentrations of faecal cortisol metabolites (FCM, in ng/g faeces) of male chamois *Rupicapra rupicapra* in the Gran Paradiso National Park in 2011 and 2012. Values are medians and interquartile ranges. On top, a compact letter display indicates $P < 0.05$ by post hoc Tukey test (i.e. significantly different treatments have no letters in common: Piepho 2004); on bottom, numbers in parentheses indicate monthly sample size.

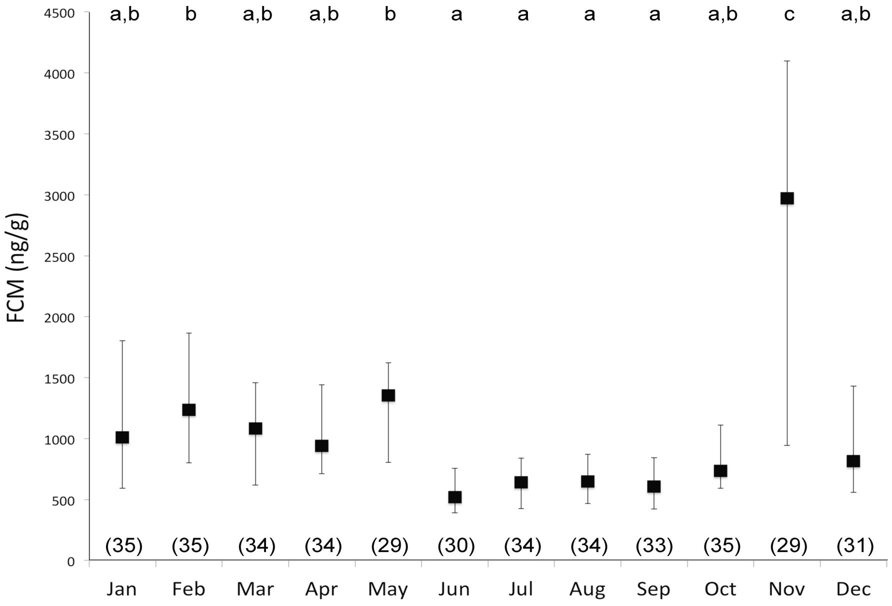
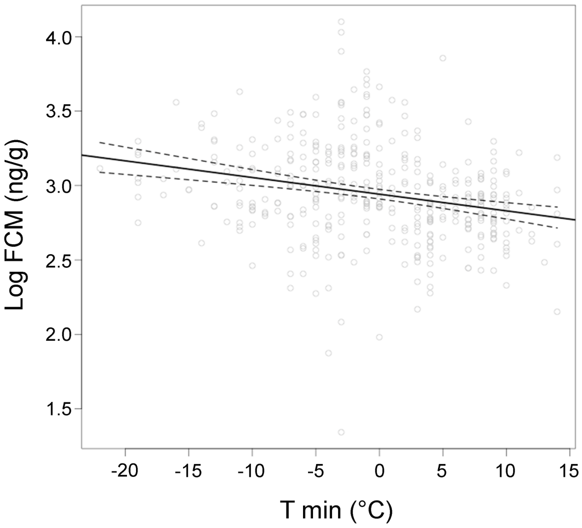


Figure 3: Yearly variation of concentration of faecal cortisol metabolites (FCM, log-transformed, in ng/g faeces) as a function of minimum ambient temperature (T min, in °C) for male chamois in the Gran Paradiso National Park in 2011 and 2012. Linear regression line is reported with 95% Confidence Interval.



Even though social status did not show a significant effect, the confidence interval of the estimate suggests that territorial males had much higher FCM values than non-territorial males (Table 2) – as confirmed also by Fig. 4b – and the lack of significance was likely due to the small sample size (Fig. 2). Age, on the other hand, showed a significant negative relationship with FCM (Table 2, Fig. 5).

Figure 4: Levels of faecal cortisol metabolites (FCM, log-transformed, ng/g faeces) of territorial and non-territorial male chamois in the Gran Paradiso National Park in a) April-May and b) November of 2011 and 2012. Values are medians and interquartile ranges.

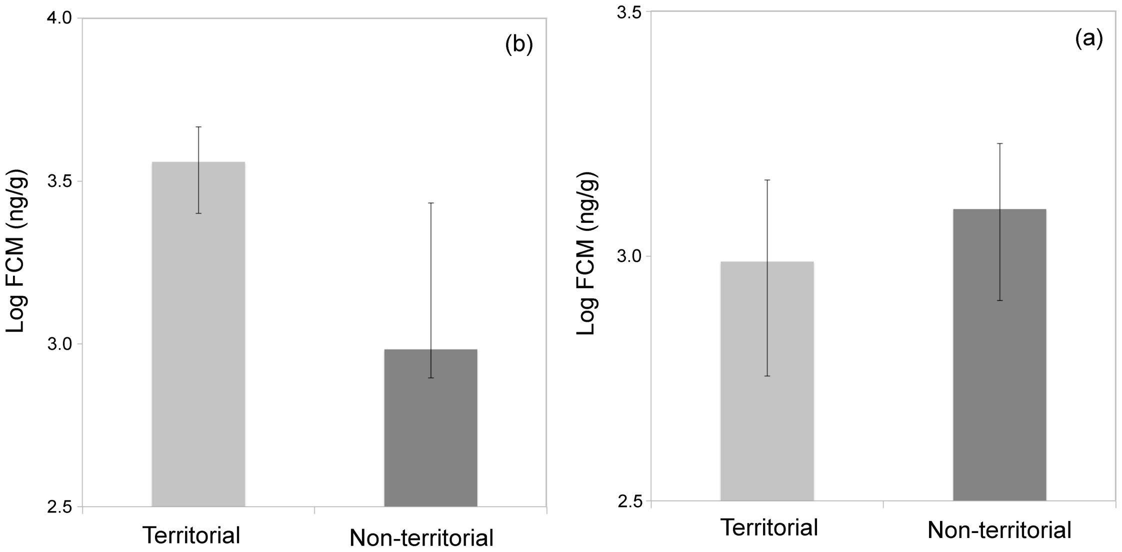


Figure 5: Variation in concentration of faecal cortisol metabolites (FCM, log-transformed, in ng/g faeces) as a function of age (in years) for male chamois in the Gran Paradiso National Park in November of 2011 and 2012. Linear regression line is reported with 95% Confidence Interval.

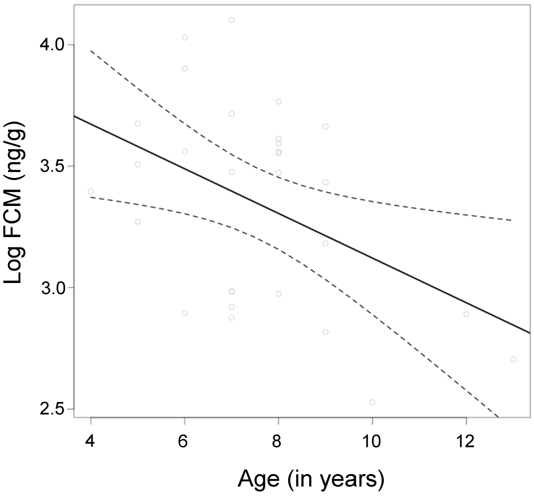

Log FCM (ng/g)
4.0
3.5
3.0
2.5
4
6
8
10
12
Age (in years)

Table 1: Model selection for the effects of age, social status (territorial and non-territorial males), minimum temperature (T min), precipitation and snow depth on the level of faecal cortisol metabolites of male chamois *Rupicapra rupicapra* in the Gran Paradiso National Park in 2011 and 2012, at different temporal scales (year, December-March, April-May, June-October, November). The 'x' indicates terms included in the model. The table reports degrees of freedom (df), differences in Akaike's Information Criterion corrected for small sample size (ΔAICc) between each model and the model with the lowest AICc, the Akaike's weights (Weight) and the R^2 of each model. Only models with ΔAICc < 6 are reported.

Age	Social status	T min	Precipitation	Snow	df	ΔAICc	Weight	R^2
YEAR								
	x				5	0.00	0.514	40%
		x			5	1.05	0.304	38%
x					5	3.06	0.111	40%
			x		5	5.43	0.034	40%
DEC-MAR								
	x				5	0.00	0.712	46%
x					5	3.01	0.158	46%
		x			5	5.10	0.056	45%
			x		5	5.78	0.039	45%
APR-MAY								
	x				5	0.00	0.913	26%
JUN-OCT								
	x				5	0.00	0.440	35%
x					5	0.88	0.284	35%
				x	5	2.63	0.118	34%
		x			5	3.99	0.060	34%
NOV								
	x				5	0.00	0.596	75%
x					5	1.86	0.235	69%
x	x				6	3.23	0.119	71%

Table 2: Average parameter estimates from models with ΔAICc < 6, after removing overly complex models (see details in the text), for the levels of faecal cortisol metabolites (FCM) of male chamois *Rupicapra rupicapra* in the Gran Paradiso National Park in 2011 and 2012, at different temporal scales (year, December-March, April-May, June-October, November). FCM was a function of age, social status (territorial [T] vs. non-territorial [NT] males), minimum temperature (T min), precipitation and snow depth. Significant results are shown in bold. CI=confidence interval.

	Estimate	Standard Error	z value	Lower CI	Upper CI
YEAR					
(Intercept)	2.955	0.057	51.989	2.883	3.107
social status [T]	-0.020	0.048	0.416	-0.114	0.074
T min	**-0.007**	**0.003**	**2.135**	**-0.013**	**-0.001**
age	0.003	0.011	0.250	-0.019	0.025
precipitation	0.002	0.002	0.935	-0.002	0.006
DEC-MAR					
(Intercept)	2.997	0.104	28.615	2.793	3.201
social status [T]	-0.038	0.085	0.440	-0.205	0.129
age	0.009	0.018	0.485	-0.026	0.044
T min	-0.003	0.006	0.466	-0.015	0.009
precipitation	-0.001	0.005	0.284	-0.011	0.009
APR-MAY					
(Intercept)	3.128	0.082	37.563	2.967	3.289
social status [T]	**-0.172**	**0.077**	**2.196**	**-0.323**	**-0.021**
JUN-OCT					
(Intercept)	2.775	0.105	26.387	2.569	2.981
social status [T]	-0.033	0.051	0.646	-0.133	0.067
age	0.020	0.013	1.518	-0.005	0.045
snow	0.009	0.013	0.701	-0.016	0.034
T min	-0.005	0.005	1.111	-0.014	-0.004
NOV					
(Intercept)	3.406	0.445	7.575	2.534	4.278
social status [T]	0.350	0.182	1.823	-0.007	0.707
age	**-0.089**	**0.039**	**2.198**	**-0.165**	**-0.013**

Discussion

Our results confirmed the importance of analysing the effects of different etho-ecological variables over multiple temporal scales, to investigate the sources of potential trade-offs between stress responses and life history traits.

As we hypothesised, over the year faecal cortisol metabolites (FCM) showed a tendency for higher values in the colder months and lower values in the warmer months, in line with other studies on ungulates (Huber et al. 2003a; Dalmau et al. 2007). Several physiological processes may concur to explain the observed seasonal pattern. On the one hand, in the Alpine environment colder months are typically characterised by scarcity of food resources: in turn a decline in nutritional intake may possibly trigger a shift from an anabolic metabolism in summer to a catabolic metabolism in winter, reflected by an increase in glucocorticoid secretion (Foster and McGarry 1988). On the other hand, several studies suggested that increased levels of glucocorticoids may reflect an adaptation to harsh climatic conditions (Yousef et al. 1971; Dantzer and Mormede 1983; Bubenik et al. 1998) and in the closely related Pyrenean subspecies (Southern chamois), Dalmau et al. (2007) indeed found a negative relationship between FCM and minimum ambient temperature. Although our results are in line with those of Dalmau et al. (2007), our estimate suggests a much weaker effect of minimum ambient temperature on FCM excretion, possibly due to an evolutionary divergence towards better adaptations to cold climates in Northern chamois. While the dark colour of winter coat, typical of Northern chamois, should favour the absorption of sunlight, the paler Southern chamois' winter coat – with large isabelline patches on the throat, neck, shoulders and flanks – may rather reflect an adaptation to relatively warmer climates, as this species inhabits lower latitudes and elevations (Lovari and Scala 1980). Furthermore, recent data also suggest that Alpine chamois may have evolved adaptations to harsh winter conditions, such as reduced metabolic rates and a thermoneutral zone at about -10 °C (Haymerle 2013). Because this temperature seldom occurred in the study site over the year, it appears unlikely that our marked males experienced severe and prolonged thermal challenges.

On the seasonal level, minimum temperature did not exert a noteworthy influence on glucocorticoid secretion, possibly owing to the good adaptation of chamois to the Alpine winter climate, and to the milder temperatures registered in the remaining months, which are unlikely to have acted as environmental stressors. Furthermore, the abundance of food resources between spring and autumn precluded declines in nutritional intake and reductions of metabolic rate, hence the increase in glucocorticoid secretion. Similarly, no significant relationships were found with other weather parameters (i.e. precipitation and snow depth), either at the yearly or at the seasonal level. While snow depth negatively influenced FCM excretion in red deer (Huber et al. 2003a), it did not influence glucocorticoid secretion in Alpine chamois, possibly due to better morphological adaptations to snowy environments in the latter than in the former.

Ethological variables, as expected, greatly influenced FCM excretion in male chamois over different time frames. While the lack of social tension among males may explain the absence of statistically significant relationship between social status and FCM in winter, and the scattered distribution of adult males in the warm months reduces their encounter probability and in turn limits the potential for an increase of glucocorticoid levels, social status was the main determinant of FCM excretion in spring, when the onset of territoriality occurs (von Hardenberg et al. 2000; Corlatti L., unpublished data). Contrary to our expectations, however, non-territorial males showed higher levels of glucocorticoids than territorial males. For the same population, von Hardenberg et al. (2000) showed that males are likely to maintain the territory occupied in the previous year: territorial males may benefit from a better knowledge of the area or, alternatively, their 'prior residence advantage' may have evolved as a 'bourgeois strategy', i.e. a conventional rule to settle contests (Maynard-Smith 1982). If so, the higher levels of FCM in non-territorial males during spring may suggest the occurrence of a 'stress of subordination' following greater investment in defensive behaviours (Blanchard et al. 1993). It is worth noticing, however, that overall stress levels in spring do not show a significant increase compared to FCM levels registered in the colder months, and remain well below the levels registered during the rutting season.

The seasonal pattern, in fact, confirmed the expected peak in FCM excretion during the November rut. Although several studies on ungulates did not find a clear increase in glucocorticoid secretion in the mating season (e.g. Bubenik et al. 1983: white-tailed deer; Chapple et al. 1991: axis deer *Axis axis*; Ingram et al. 1999: red deer), our data support the results of Mooring et al. (2006) on bison bulls. Male-male agonistic encounters and courtship behaviours peak during the rut: because they are energetically demanding, they represent physiological stressors that increase FCM excretion (Sapolsky 2002; Sands and Creel 2004). Alternatively, reduced faecal output – following the severe reduction of nutritional intake commonly observed in rutting male ungulates (Brivio et al. 2010) – might have caused an artificial increase in FCM measurements, but Cavigelli (1999), Foley et al. (2001) and Lepschy et al. (2010) did not find any clear relationship between these parameters. Furthermore, potential bias in FCM measurement due to cross-reactions with androgen metabolites (Ganswindt et al. 2003), that also showed increased values during the rut, are unlikely to have occurred: an earlier study on male African elephants (Ganswindt et al. 2010) indicated that the same 11-oxoaetiocholanolone EIA used in this study did not show cross-reactivity with excreted androgen metabolites. In turn, this suggests that the used EIA provides an acceptable degree of specificity for reliable assessment of glucocorticoid output from faeces.

Despite the lack of statistical significance, the effect size suggests higher levels of FCM in territorial males during the rut. Territorial males over the mating season invest more time in aggressive contests and in courtship behaviours (Corlatti et al. 2013), and incur in greater reduction of food intake (Corlatti and Bassano 2014) than non-territorial males. As a result, our data support the 'stress of domination' hypothesis, according to which higher glucocorticoid levels can be expected in dominants than subordinates when the former must fight more than the latter (Creel 2001). This result is in sharp contrast with the pattern observed in spring, which supported the 'stress of subordination' hypothesis (Creel 2001). Owing to the greater costs of territoriality during the rut, however, in the long-term dominant males may incur a life history trade-off between mating effort and reduction in parasite resistance, mediated by high levels of hormone metabolites (Corlatti et al. 2012), while in spring higher FCM levels do not translate into a greater parasite load in non-territorial males (Corlatti L., unpublished data).

Finally, even though Mooring et al. (2006) found a positive correlation between age and FCM levels, with older, dominant males showing higher glucocorticoid secretion, our data suggest that during the rut age had a negative effect on FCM excretion. Because dominant behaviour in adult male chamois is not related to age (Corlatti et al. 2012), our results may be explained by the experience gained over the years in dealing with agonistic contests: if so, younger, less-experienced animals may have been more stressed than older animals.

Conclusion

Our work emphasises the importance of choosing the appropriate temporal scales when evaluating the effects of etho-ecological correlates of glucocorticoid secretion. We confirm a seasonal pattern in FCM excretion but, possibly owing to good morphological and physiological adaptations to harsh weather conditions, altogether climatic stressors appear to exert a weak effect on glucocorticoid levels in Alpine chamois. Social status was an important determinant of FCM excretion in male chamois, especially during the rut. While both the 'stress of subordination' and the 'stress of domination' hypotheses were supported in different times of the year, previous data on the same population suggest that only the latter may have detrimental fitness consequences on male chamois, possibly leading to immunosuppression and shorter life span (Folstad and Karter 1992; Creel 2001).

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