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Original Articles

The importance of intrinsic traits, environment and human activities in modulating stress levels in a wild ungulate

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ABSTRACT

Identifying the ecological and anthropogenic processes that affect wildlife physiology, and that may operate as chronic stressors, is of prime importance to implementing appropriate management and conservation strategies. Although advances have been made in understanding the physiological ecology of wild ungulates, little is known of how multiple intrinsic and ecological factors work, either independently or synergistically, to modulate their stress responses. By using faecal glucocorticoid metabolites (FGM) as indicators of stress, a set of environmental and human determinants affecting the stress physiology of wild red deer (*Cervus elaphus*) was examined in the Mediterranean ecosystems of south-western Europe, where this species is subjected to contrasting weather regimes and hunting management systems. Variation-partitioning techniques were also used to estimate the comparative influence of factors related to an individual's intrinsic characteristics, environmental conditions and management practices in shaping physiological stress levels. Our results showed that factors related to hunting management were the main drivers of FGM variation in red deer, followed by those related to the environmental conditions and individuals' traits, and their effects were closely associated to spatio-temporal variability. Holding massive hunting events involving the use of hounds, as well as high population densities, were related to more long-term stress levels in the populations studied. Evidence was also found that supplementary feeding practices may mitigate the negative effects of reduced food availability in overabundant deer populations. Weather conditions were also significant factors explaining variation in stress levels; accumulated rainfall and an increase in ambient temperatures during the coldest months were associated with a decrease in stress hormone levels. No differences in hormonal concentrations were found between males and females, but higher levels of hormone metabolites were detected in younger animals in both sexes. Our findings provide an integrated perspective of how multiple factors impact on stress physiology in large wild herbivores and highlight the importance of considering management practices, as well as spatio-temporal variation, when assessing stress-inducing factors in wild populations. Given the implications of this study regarding the impact of human activities on physiological stress levels in wild animals, it could be an important basis to support wildlife management decisions.

1. Introduction

During their evolutionary history, organisms have developed different adaptive physiological and behavioural mechanisms to cope with adverse conditions or stressors (Bijlsma and Loeschcke, 2005; Boonstra, 2005). However, persistent exposure to harmful stimuli can seriously affect their physiology, with additional consequences to their physical condition (Romero and Butler, 2007). Glucocorticoids (*i.e.*, cortisol and

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corticosterone) are steroid hormones that play a vital role in regulating a wide range of physiological processes, including the stress response (Randall et al., 2002). In mammals and other vertebrates, these hormones are synthesised and secreted by the adrenal cortex following activation of the hypothalamus-pituitary-adrenocortical (HPA) axis, a key element of the neuroendocrine system that regulates the body's reactions to internal and environmental challenges (Ulrich-Lai and Herman, 2009). Stress can be divided into acute or chronic, depending on the duration of the exposure to the stressors. While an acute stress response is limited in time and operates as an adaptive survival mechanism that allows organisms to restore physiological homeostasis, chronic stress elicits prolonged hypersecretion of glucocorticoids and can have detrimental effects on the animals' health, growth, reproductive performance (Boonstra, 2005; Reeder and Kramer, 2005; Wingfield and Sapolsky, 2003), and ultimately can compromise their survival (Pride, 2005). Due to its potentially negative effects on individuals' fitness, chronic stress has been proposed as having an important role in the dynamics of wild populations (*e.g.*, Bonier et al., 2009).

Recent advances in field endocrinology have made it possible to use new techniques for stress assessment in wildlife (reviewed by Sheriff et al., 2011). Among these, the quantification of glucocorticoid metabolites in faecal material has become a valuable tool for conservation and management issues (Millspaugh and Washburn, 2004; Wikelski and Cooke, 2006), as it permits the monitoring of the physiological status of both individuals and populations in a simple and non-invasive way (Keay et al., 2006). Moreover, because faecal glucocorticoid metabolite (FGM) levels represent pooled fractions of the amount of hormones that have been secreted and metabolised over a broad period of time (Palme et al., 2005; Wasser et al., 2000), they can provide a long-term and integrated profile of the animals' adrenocortical activity (Dantzer et al., 2014; Sheriff et al., 2010). Owing to its great potential, FGM analysis has been increasingly applied to monitoring stress responses in a wide variety of wild animals (Chinnadurai et al., 2009; Wasser et al., 2000; Young et al., 2004).

Several factors may act as important sources of stress for mammals. Human activities such as hunting (Bateson and Bradshaw, 1997; Burke et al., 2008), tourism (Rehnus et al., 2014; Zwijacz-Kozica et al., 2013), or other recreational events (Creel et al., 2002) can lead to increased stress levels in their populations. Land use practices and the degree of human intervention in natural habitats can also affect species' physiology (Navarro-Castilla et al., 2014; Rimbach et al., 2013). Apart from human-induced stressful situations, social factors (Creel et al., 2013), an elevated risk of predation (Sheriff et al., 2009) as well as seasonal variations in the ambient temperature or in the availability of food resources (Beehner and McCann, 2008; Dalmau et al., 2007) may also represent important environmental and ecological stressors. These extrinsic factors can affect, either independently or synergistically, individuals' physiological condition, and may also interact with intrinsic factors (*e.g.*, sex, life-history stage, reproductive status) in an integrated fashion (Crespi et al., 2013; Dantzer et al., 2014).

Understanding how species cope with environmental or anthropogenic stressors and the physiological impact of such factors, is an increasingly relevant topic in ecological research and has proven to be a very useful diagnostic tool for supporting wildlife management decisions. Wild ungulates have a wide global distribution and are important both ecologically and economically (Gordon et al., 2004). In Europe and North America, ungulate populations have expanded significantly over the last decades as a result of direct human intervention or by natural processes, which has led to increased concerns regarding their management (Apollonio et al., 2010; Gordon et al., 2004). Such expansion poses new challenges for ungulates, because they have to cope with different forms of anthropogenic pressure, such as changes in the landscape, hunting, and various other human-induced disturbances (Acevedo et al., 2011; Stankowich, 2008). Moreover, in a scenario of climate change, ungulates may have to adapt to new environmental conditions (Mysterud and Sæther, 2011). Studies investigating the additive and interactive effects of different intrinsic, ecological, and anthropogenic causes of variation in stress hormone levels in wild animals are still quite rare. Many studies focused on how a single or few factors affect hormonal levels (*e.g.*, Corlatti et al., 2011; Zwijacz-Kozica et al., 2013), but the contribution of multiple factors in the modulation of those levels has often been neglected (but see Sheriff et al., 2012). Here, data from a large-scale cross-sectional survey was used in order to examine the influence of a set of factors – intrinsic (sex and age class), environmental (weather conditions, seasonality, topography, and geographic position) and human (hunting pressure, supplementary feeding, deer population density, and landscape alteration) – that may potentially explain FGM concentrations in a wild highly sexually dimorphic ungulate. We used the red deer (*Cervus elaphus*) in the Mediterranean habitats of the Iberian Peninsula as a model. We also used an integrated approach to explore the main drivers of FGM variability in this species.

Based on current knowledge and the most comprehensive theoretical frameworks on the ecology of stress that have been presented to date (McEwen and Wingfield, 2003; Romero et al., 2009), we hypothesise that anthropogenic factors are the primary drivers of FGM variation, followed by environmental factors. Stress load is expected to be higher in those areas where the levels of disturbance caused by hunting activities are higher (Bateson and Bradshaw, 1997). We also predict increasing FGM levels with the increase of population density (density-dependent effects; *e.g.*, Creel et al., 2013), a situation that may lead animals to a chronic stress state (allostatic overload type I, *sensu* McEwen and Wingfield, 2003). Finally, the effects of the seasonal climate and adverse weather conditions on FGM concentrations are also predictable. Low ambient temperatures during the coldest months are expected to increase circulating glucocorticoids and, consequently, FGM levels (Huber et al., 2003a). On the other hand, since the accumulated rainfall during the autumn and winter is crucial for plant regeneration in Mediterranean areas after the summer drought, we expect a decrease in FGM concentrations with increasing food availability (Rodriguez-Hidalgo et al., 2010).

2. Materials and methods

2.1. Study areas

This study was performed at eight areas located in the Iberian Peninsula with contrasting environmental conditions and game management practices, where wild populations of red deer are present (Fig. 1; Table 1): *Lombada* National Hunting Area and *Sierra de la Culebra* Regional Hunting Reserve (LSC); *Lousã* Mountain (LOU); *Cubeira* Tourist Hunting Area (CUB); *Negrita Norte* Tourist Hunting Area (NEG); *Doñana* National Park (DN); *Quintos de Mora* (QM); *Montes Universales* Hunting Reserve (RCMU); *Caspe-Fraga* Social Hunting Area (CF). All study areas share a Mediterranean type climate, but present distinct temperature or precipitation regimes (Fig. 1). Management practices also differ among the populations studied and depend largely on the administrative authorities' or gamekeepers' objectives (for details see subsection 2.2.3). The characteristics of the study areas and red deer populations are summarised in Fig. 1 and Table 1.

2.2. Sampling and data collection

Sampling was carried out during three consecutive hunting seasons (September to February), from 2010–2011 to 2012–2013. Fresh faecal samples were collected directly from the rectum of 289 hunted red deer for FGM determination (Table 1). The collection, transportation and storage of samples were done according to Santos et al. (2014). The sex and age class of each sampled animal were also recorded. Age determination was performed from the observation of tooth eruption patterns in younger animals (Sáenz de Buruaga et al., 2001) or by counting incremental cementum layers on sectioned roots of the first