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CORTISOL AND CAMERA-TRAPPING AS USEFUL TOOLS TO EXPLORE ECOLOGICAL ASPECTS OF THE EUROPEAN WILDCAT *(FELIS SILVESTRIS SILVESTRIS)*

IL CORTISOLO E IL FOTORAPPOLAGGIO QUALI STRUMENTI UTILI AD APPROFONDIRE ASPETTI DELL'ECOLOGIA DEL GATTO SELVATICO EUROPEO (*FELIS SILVETRIS SILVESTRIS*)

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Abstract. The combined use of different monitoring techniques for wildlife studies (with particular attention dedicated to felids) assumes remarkable importance to the knowledge of all those ecological and physiological variables which may affect the welfare of the target species. The quantification of glucocorticoid metabolites in hair is a non-invasive tool that provides important information regarding the endocrine status and represents a valuable method for studying potential stressors that may affect carnivores under both natural and non-natural conditions. Cortisol is the main glucocorticoid hormone of the hypothalamic-pituitary-adrenal gland axis and is considered a standard stress indicator for animal welfare. Differences in hair cortisol concentration between subspecies such as wild and feral cats is related to either physiological and/or ecological conditions. Wildcats generally have to cope with ecological pressures (e.g., hunting for food, competition, anthropic disturbance) that may lead to an increasing allostatic load. Nevertheless, hair cortisol comparisons between felid species or subspecies in response to both natural and non-natural factors is still poorly considered. Therefore, further studies are strongly suggested. At the same time, camera-trapping, i.e., the use of remotely triggered cameras that automatically take images and/or videos of animals transiting in front of them, represents a useful tool to study species' population parameters, as well as the interactions among species within a specific area. Such data may be combined to cortisol analyses to provide further insights on species (e.g., wildcat) welfare within different wildlife communities. Therefore, this information might be used by conservationists to delineate adequate management plans aimed at fostering wildcat conservation.

Riassunto. *L'utilizzo combinato di differenti tecniche di monitoraggio per lo studio della fauna (con particolare riferimento ai felini) assume un'importanza rilevante ai fini della conoscenza di tutte quelle variabili di natura ecologica e fisiologica che possono influire sul benessere delle specie in esame. La misura dell'accumulo di glucocorticoidi all'interno del pelo rappresenta un metodo non invasivo che fornisce importanti informazioni in merito ai potenziali fattori stressogeni di origine naturale e artificiale che influenzano la sopravvivenza di varie specie di carnivori a livello globale. Il cortisolo rappresenta il principale ormone glucocorticoide prodotto dall'asse ipotalamo-ipofisi che viene riconosciuto come indicatore standard del benessere animale. Differenze in termini di concentrazioni di cortisolo accumulatosi nel pelo tra sottospecie sono dovute a differenti risposte metaboliche basali e pressioni ecologiche. I gatti selvatici generalmente sono sottoposti a pressioni ecologiche (e.g., caccia, competizione, disturbo antropico) che possono indurre un aumento del carico allostatico. Tuttavia, confronti fra livelli di cortisolo tra specie o sottospecie appartenenti alla Famiglia Felidae sono ancora ampiamente assenti. Di conseguenza, ulteriori studi sono fortemente consigliati. Parallelamente, il foto/video-trappolaggio, ovvero l'utilizzo di macchine fotografiche in grado di scattare foto e video ogni qualvolta un animale passa dinnanzi ad esse, rappresenta un'importante tecnica per lo studio sia dei parametri di popolazione delle specie, che delle interazioni tra esse in una data area. Questi dati potenzialmente possono essere combinati all'analisi del cortisolo per fornire ulteriori evidenze sullo stato di salute delle specie (es. il gatto selvatico) all'interno di diverse comunità animali. Tali informazioni possono quindi essere utilizzate a favore della conservazione del gatto selvatico.*

Introduction

The combined use of different monitoring techniques for wildlife conservation purposes assumes notable importance in the light of the potential effects that either ecological and/or physiological variables may exert on animal welfare. Abiotic and biotic changes are common within the environment, and animals may respond through temporal variation in their vital rate and/or an alteration in their physiological response (Darlingtont *et al*. 1990). Another common response used by wild species in response to a potential distress condition is the modification in terms of behavioural pattern (Melberg 2012; Centore *et al*. 2018). For instance, throughout the use of camera-trapping the effects derived from the competition among species could be highlighted. For instance, Melberg (2012) observed spatial and temporal segregation between roe deers (*Capreolus capreolus*) and wild boars (*Sus scrofa*). It was showed that roe deer fawns tended to be spatially segregated in relation to wild boar activity. Furthermore, roe deer displayed a non-random movement pattern towards wild boar rooting behaviour. In addition, a lower roe deer presence at rubbing trees was found, suggesting that adult individuals avoided sites often frequented by wild boar. Finally, a pattern of temporal avoidance was found between the two species in which both roe deer and wild boar were active during the same hours but with different frequency. Therefore, the combination of camera-trap surveys along with physiological analyses may provide further and detailed insights concerning the ecological interactions among species as well as in terms of the effect of potential distress conditions (Millspaugh *et al.* 2001; Zwijacz-Kozica *et al.* 2013; Ewacha *et al.* 2017).

Cortisol: the "stress hormone"

The ability of an organism to adapt to changes in environmental conditions has been receiving increased attention in recent years (Ruiz-Gomez *et al.* 2011; Montiglio *et al.* 2012). A *stressor* is defined as a stimulus which leads to the activation of the acute stress response system (Wingfield *et al.* 2011). A stimulus becomes a stressor when an individual cope with it with uncertainty due to the absence of information (Levine & Ursin 1991). The acute stress response consists in the temporary interruption of the normal functions to counteract the impact of the stressor and to re-establish the homeostasis (Sapolsky *et al.* 2000). As opposed to acute stress, chronic stress occurs when the prolonged exposition to a stressor leads to the breakdown of physiological systems (Sapolsky *et al.* 2000; Mcewen & Wingfield 2003; Romero *et al.* 2009). The most common physiological response toward a distress situation is the activation of the hypothalamic-pituitary-adrenal (HPA) gland axis which culminates in the secretion of glucocorticoids from the adrenal cortex (Sapolski *et al.* 2000). These hormones facilitate increased energy mobilization and suppression of several system processes (i.e., digestion, immunity, growth and reproduction) as well as a return to baseline conditions via negative feedback to the brain (Wingfield *et al.* 1998). Nevertheless, prolonged stimulation of the HPA axis may lead to chronic consequences such as muscle wastage, growth suppression, inhibition of reproduction, neuronal cell death, and suppression of the immune system (Sapolsky *et al.* 2000). Such chronic stimulation could be significant in animals living in human-altered habitats (Millspaugh *et al.* 2001; Zwijacz-Kozica *et al.* 2013; Ewacha *et al.* 2017) or during particular life stages (e.g., reproduction, pregnancy, lactation) (Reeder & Kramer 2005). Assessing the glucocorticoid levels may, thus, represent a useful tool to monitor the physiological status of a population under both natural and non-natural conditions. Cortisol is recognized as the first stress hormone involved in allostasis (an active process involved in maintaining and/or re-establishing homeostasis) and is frequently measured to assess adrenocortical activity in vertebrates (Mcewen 1998), but it is further involved in a wide range of metabolic functions such as metabolism regulation, growth rate, reproductive and immune system regulation (Hamilton *et al.* 2008). Moreover, within an organism that suffers from acute or chronic stress various other mechanisms spanning from molecular (e.g., heat shock proteins) to organismal (e.g., sympathetic nervous system activation) functions are activated. In this sense, considering cortisol as a simple stress hormone oversimplifies its function (Dickens & Romero 2013; Macdougall-Shackleton *et al.* 2019). Cortisol concentrations can be measured with invasive and non-invasive techniques in several matrices such as blood (Gardiner & Hall 1997), faeces (Millspaugh *et al.* 2001, 2004), urine (Rehbinder & Hau 2006), milk (Gygax *et al.* 2006), saliva (Negrão *et al.* 2004), eggs (Mingist *et al.* 2007), claws (Comin *et al.* 2014), feathers (Frongia *et al.* 2020), and hair (Prandi *et al.* 2018). However, compared to other matrices (i.e., blood) that provide a short-term measure of glucocorticoids concentration (< 3 min.), measurements of glucocorticoid metabolites in matrices such as hair represents a valuable method to trace an individual's 'stress history' in the long-term period, as cortisol concentration in hair is deposited over an extended time frame (variable from weeks to years, depending on the hair length) (Meyer & Novak 2012). Additionally, hair is a relatively stable matrix that does not decompose as rapidly as other body fluids or tissues do (Balíková 2005). Hair growth rate consists of three phases: anagen (active growth), catagen (transition) and telogen (resting) (Harkey 1993), and hormones can only be accumulated within the hair shaft during the anagen phase via passive diffusion from blood vessels (Meyer & Novak 2012). The accumulation occurs within the hair follicle located several millimetres below the skin surface (Harkey 1993). Therefore, there is a time delay between cortisol incorporation into the hair and the time spent by this hair section arriving at the skin surface (Stalder & Kirschbaum 2012; Montillo *et al.* 2019).

Measuring long-term physiological response in wild felids

Within the *Felidae* Family cortisol-level analysis have received little attention in the last decade (Naidenko *et al*. 2011, 2019; Fanson *et al*. 2012; Creel *et al*. 2013; Narayan *et al*. 2013; Bhattacharjee *et al*. 2015; Mondol *et al*. 2020) and as far as concerns the European wildcat (*Felis silvestris silvestris*) such analyses have been rarely treated and mostly on faecal matrices (Piñeiro *et al*. 2012, 2015). To the best of our knowledge, the only studies in which hair cortisol was analysed within the Felis silvestris subspecies was carried out by Franchini *et al*. (2019) and Filacorda *et al*. (2021), in which they compared hair cortisol concentrations (HCC) between wild and feral cats (*Felis silvestris catus*) living in different environmental conditions (Fig. 1), and the phisiological response of wildcats towards habitat fragmentation and interspecific competition (Fig. 2), respectively. Beyond the existence of species-specific differences in the secretion of metabolic hormones there may be several either environmental or ecological factors that may induce different cortisol accumulations between subspecies or individuals belonging to the same species but living in different environmental contexts. For instance, Naidenko *et al*. (2011) obtained a significant difference in terms of HPA gland axis activity between wild and captive Amur tigers (*Panthera tigris altaica*) where wild specimens, probably due to unfavourable environmental

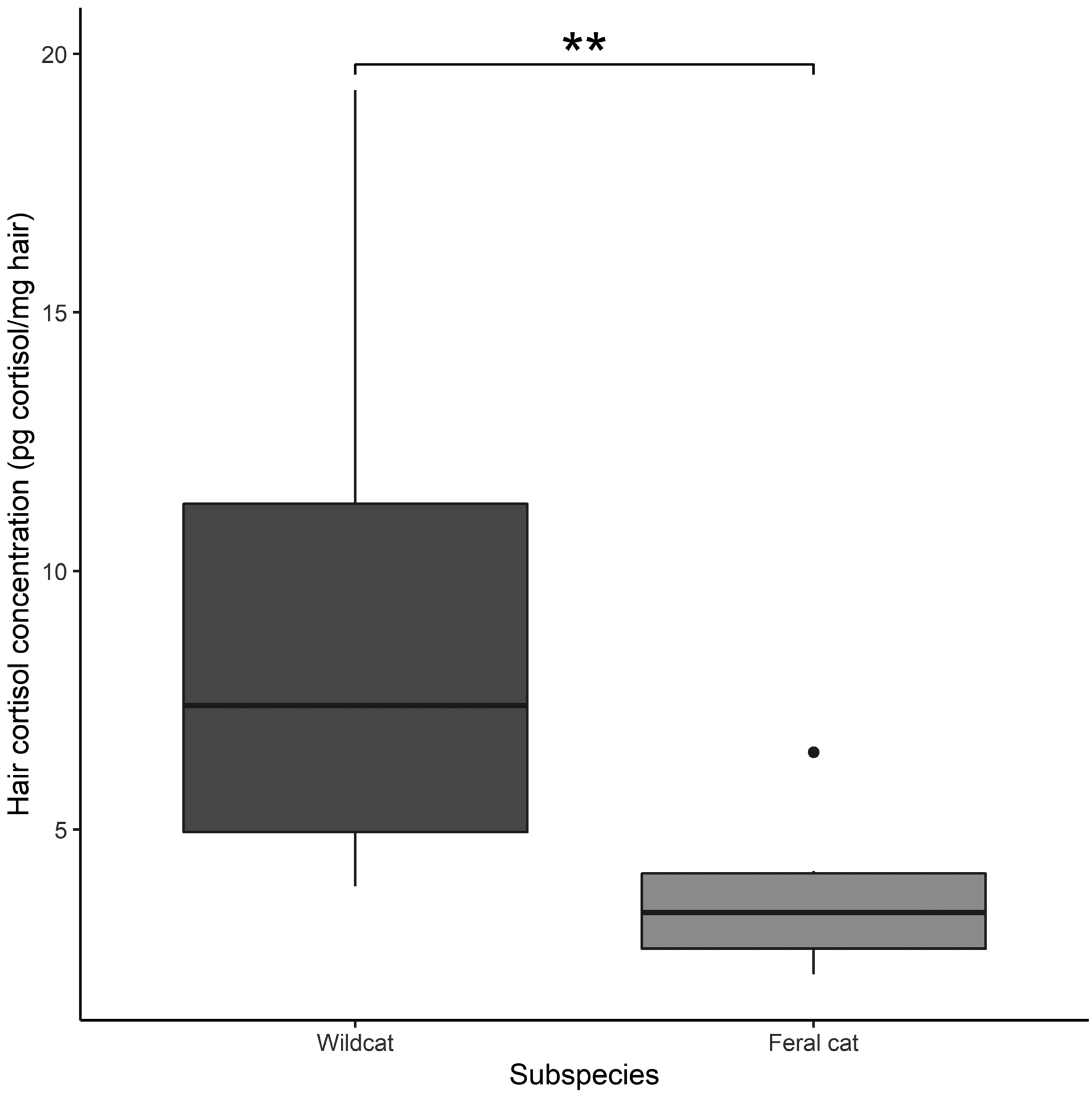

Fig. 1 - Boxplot showing the difference in terms of hair cortisol concentration (pg cortisol/mg hair) between subspecies.

conditions in which they lived, showed significantly higher cortisol levels compared with captive ones. In our case, the difference recorded in terms of HCCs between wild and feral cats may be related to various factors. The first explanation could be related to underlying differences in metabolism, diet, and/or energy regulation which may have affected steroid production (Romero *et al*. 2009).

A second explanation might be related to differing degrees of tolerance of each subspecies toward anthropic disturbance or towards other con(sub)specifics. Feral cats are known to live in close contact with humans (and each other), although only in relation to foraging behaviour (Natoli 1994). In this sense, they may be more tolerant toward humans and intraspecific presence than wild individuals which are instead forced to live in less suitable or unsuitable habitats. The effect of anthropic disturbance on cortisol accumulation has received considerable attention in recent years and has been studied in various species. For instance, Naidenko *et al*. (2019) compared faecal glucocorticoid levels between two tiger subspecies, the Amur tiger and Bengal tiger (*Panthera tigris tigris*) living in two extreme habitats. From the analysis, they recorded that faecal cortisol metabolites (FCMs) were significantly higher in Bengal tigers living in India than in Amur tigers living in the Russian Far East and, as explained by the authors, these reasons might be related to tiger density or anthropogenic disturbance.

A third explanation may be related to differences in terms of environmental pressures to which each subspecies was subjected. For instance, Naidenko *et al*. (2011) compared cortisol levels between wild and captive Amur tigers, showing that wild tigers had significantly higher cortisol concentrations compared with captive ones and that the reason might be related to the unfavourable influences of low temperatures and deep snow cover. As the feral cats lived in colonies in close contact with human beings who regularly supplied them with food, they were not subjected to stressors (i.e. hunting for food) which might have led to increased HPA gland axis activity. Moreover, factors such as inter-specific and intraspecific competition might have affected individual wildcats' welfare resulting in higher hair cortisol accumulations. In Friuli Venezia Giulia, the main medium-sized carnivores which may compete with the wildcat for territory and/or food resources are the beech marten (*Martes foina*), the pine marten (*Martes martes*), the red fox (*Vulpes vulpes*), and the golden jackal (*Canis aureus*). However, research focused on assessing the impact of inter-specific competition on wildcat welfare is still rather sparse. A research realized by Filacorda *et al*. (2021) has been revealing an increasing trend in terms of HCC measured in wildcats in response to increased golden jackals' density (Fig. 2). Following the mesopredator suppression theory (Soulé *et al*. 1988) the biggest carnivore living within the area behave as apex predator killing the smaller sized competitors for competition reduction and, occasionally, for feeding (Soto & Palomares 2013). The presence of domestic cats in the diet of jackals has been reported in other studies (Penezić & Ćirović 2015; Lanszki *et al*. 2016; Vlasseva *et al*. 2017). Moreover, interspecific competition between jackals and other wildcat species has been recorded as well (Petersen *et al*. 2019; Selvan *et al*. 2019). To our knowledge, to date, no bibliographic information is available regarding the interspecific killing between golden jackals and European wildcats. However, since both species present a similar trophic niche feeding mainly over small mammals and birds (Franchini *et al*. 2017; Vlasseva *et al*. 2017) the competition for trophic resources is very likely.

Camera-trapping as useful tool for wildcat presence and pupulation estimation

Monitoring wildlife populations is important for conservation management but is often difficult to achieve effectively, particularly for rare and cryptic species such as carnivores, which are often threatened and exist at low densities and in fragmented populations (Gese 2001). The use of camera trapping, i.e., remotely triggered cameras that automatically take images and/or videos of animals transiting in front of them (Rovero *et al*. 2013), as a technique to survey and monitor wildlife has increased dramatically in recent years (O'Connell *et al*. 2011). The technological advances in infrared sensors and digital photography have led to cost-effective, non-invasive means of generating reliable detection of elusive wildlife (Kucera & Barrett, 2011). Camera trap (CT) methodology now encompasses a wide range of equipment and ecological applications: CTs are now being used to assess wildlife distribution, abundance, behaviour and community structure (Rovero *et al*. 2013; Meek *et al*. 2014).

In this sense, despite the species is widely (but patchily) distributed (Africa, Asia, Europe) and globally categorized as "Least Concern" by the International Union for the Conservation of Nature (IUCN) (Yamaguchi *et al*. 2015) data on presence, abundance and distribution of European wildcats are extremely valuable because this species is subject to several global threats including local extinctions and population fragmentation, especially in Europe (Nowell & Jackson, 1996), Rovero *et al*. (2013) reviewed the sampling designs for different research aims using CTs. They suggest a grid arrangement of

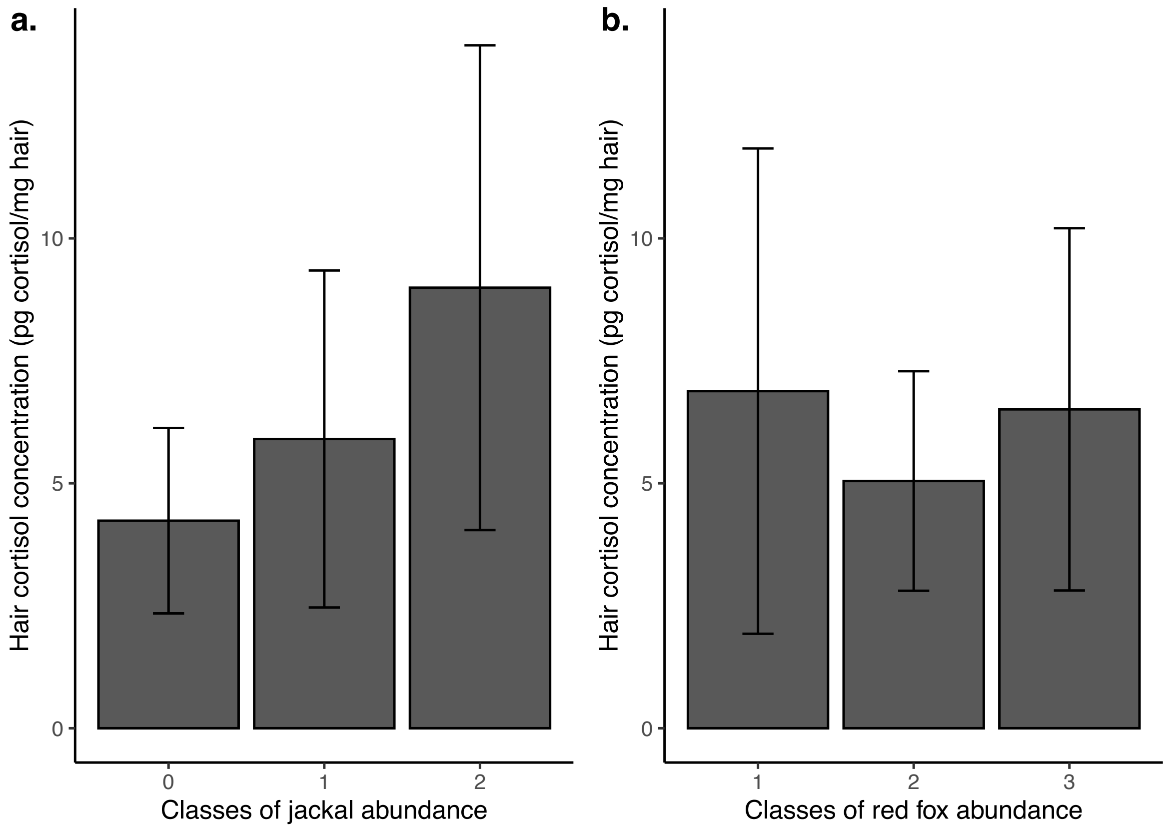

Fig. 2 - Hair cortisol level variations in response to (**a**) golden jackal, and (**b**) red fox subdivided into classes of abundance (golden jackal: class 0 = absence of the species, class 1 = hunters/technician reports or collection of carcasses during one year, class 2 = acoustic stimulation, hunters/technician reports and/or collection of carcasses during two years; red fox: class 0 = 0–0.99 ind./100 ha, class 1 = 1–1.99 ind./100 ha, class 2 = more than 2 ind./100 ha). The only significan effect was recorded for the golden jackal.

CTs as good for species inventories and occupancy studies. In the latter case, occupancy can be used as a surrogate of abundance for species with relatively small home ranges, such as wildcats (Rovero *et al*. 2013). Furthermore, a 'capture-mark-recapture' (CMR) approach, which relies on individual recognition of members of the studied population based on coat pattern, allows population abundance estimates (Rovero *et al*. 2013) and can be used for wildcats (Anile et al, 2010; Kilshaw *et al*. 2015). In these ways, wildcat presence, density and abundance have been determined from camera-trapping studies (Monterroso *et al*. 2005; Sarmento *et al*. 2009; Anile *et al*. 2010; Cafariello *et al*. 2012; Kilshaw *et al*. 2015). Indeed, a grid arrangement for CTs was used (Monterroso *et al*. 2005; Sarmento *et al*. 2009; Kilshaw *et al*. 2015) and/or they were deployed in order to obtain the same density of CTs in the sample area for wildcat density estimations (Anile *et al*. 2010; Kilshaw *et al*. 2015). Although in the occupancy studies camera placement should be ideally random, in other sampling designs CTs should be deployed in order to maximize species detection such as along trails, near drinking sites, etc. This is particularly true for CMR approaches (Rovero *et al*. 2013). For this reason, preliminary explorations of the study area should be carried out in order to find good places where CTs can be placed (Anile *et al*. 2010; Kilshaw *et al*. 2015). Furthermntroversial results (Kèry *et al*. 2011; Anile *et al*. 2012; Steyer *et al*. 2013; Kilshaw *et al*. 2015; Velli *et al*. 2015), and this may have genetic bases. In fact, it has been shown that felids' response to catnip is genetically determined (Bradshaw 1992). Therefore, assuming that even in the case of valerian attractants the response is driven by genetic factors, the lack of attractiveness in small populations, such as the Sicilian (Anile *et al*. 2012) and the Scottish one (Kilshaw *et al*. 2015), compared to the others (Velli *et al*. 2015) may be attributable to genetic bases.

A future prospective: integrating camera-trapping to cortisol analyses

Further information that CTs provide is the date and time at which each photograph is taken, allowing analysis of activity patterns and niche partitioning (Ridout & Linkie, 2009). Due to the niche differentiation that occurs among sympatric species, understanding the coexistence mechanisms of species will contribute to the conservation and management of ecological communities (cited in Zhao *et al.* 2020). This is particularly true for medium-sized carnivores at intermediate trophic levels (Prugh *et al.* 2009) which generally show high species richness, resource partitioning and different habitat use (Roemer *et al.* 2009). The aggressive interactions among medium-sized carnivores normally influence their activity patterns and are expected to be stronger for species with high dietary overlap, (Schoener 1974; Donadio & Buskirk, 2006; Ritchie & Johnson, 2009). Such studies have been carried out, focusing even on wildcat. For instance Monterroso *et al.* (2014) found that temporal segregation among wildcat, Iberian lynx (*Lynx pardinus*), red fox and pine marten may play an important role in facilitating medium-size coexistence, especially with increasing community complexity.

Moreover, not only interactions among different species should be considered, but also those within the same group, i.e., among wildcats, feral domestic cats and hybrids (BIRÒ *et al.* 2005). In Hungary, Birò and colleagues (2005) found similar wildcats' and hybrids' prey characteristics, as well as high trophic niche overlap (77-88%) among the above-cited cat groups. These findings led the authors to conclude that wildcat populations may be negatively affected by trophic relations with feral cats and hybrid cats, especially where high feral domestic cat densities or low wildcat densities occur.

On the other hand, even the presence and abundance of ungulates may affect wildcat welfare. Indeed, the negative effect on vegetation cover due to overgrazing produced by large ungulate species has been reported in other studies (Flowerdew & Ellwood 2001; Smit *et al.* 2001; Massei & Genov 2004; Lozano *et al.* 2007). Lozano *et al.* (2007) showed that in some areas overgrazing was so intense that have led to the jeopardization of wildcat populations by reducing rabbits' availability. Therefore, it is easy to understand that in highly complex communities stressful direct or indirect interactions may occur.

To the best of our knowledge few works were focused in assessing the relation between cortisol concentrations and both presence and densities of competitors, predators or other species (Monclús *et al.* 2009; Filacorda *et al.* (2021). The preliminary results presented by Filacorda *et al.* (2021) revealed that in those areas in which wildcats showed higher HCCs, data obtained from camera traps apparently showed lower wildcat densities and, on the contrary, higher densities of both wild boar and golden jackal. Populations parameters and activity patterns among species can be evaluated through camera-trapping (**Fig. 3**) (Rovero *et al.* 2013), and therefore their effect on wildlife (e.g., wildcat) welfare can be tested, providing useful information on species interactions and conservation management.

Conclusion

Hair cortisol analysis assumes remarkable importance in the assessment of the long-term physiological response of wild species, hence, contributing to lay the foundation for future works focused in assessing the various physiological and ecological factors affecting the HPA gland axis activity of those populations living under a range of environmental conditions and leading to the establishment of adequate conservation plans toward the most endangered species (or subspecies).

Furthermore, camera-trapping may represent a useful tool to investigate wildcat's population parameters, as well as the interactions that might occur among the species in a specific area.

Most importantly, through a multidisciplinary approach, cortisol and camera-trapping data may be combined to provide further insights on wildcat welfare within different wildlife communities. Such an information may represent an important scientific baseline for managers ad conservationists to delineate adequate management plans aimed at fostering the conservation of wildcat populations.

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Fig. 3 - Use of camera trap to monitor wildcat presence as well as the presence of competitors, potential predators, preys and other species within a specific area. (a) Red fox (*Vulpes vulpes*), (b) Common pheasant (*Phasianus colchicus*), (c) Roe deer (*Capreolus capreolus*), (d) and (e) Wildcat (*Felis silvestris silvestris*), and (f) Golden jackal (*Canis aureus*). Images a-d were recorded in a different site of images e-f

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