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# Concentrations of cortisol and testosterone related to relationships between members of a bachelor group of red deer stags

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# STATEMENT

I hereby declare that I have completed this thesis by myself using the cited literature. I agree with utilization of included information on condition of the correct citation. This Ph.D. thesis, neither its substantial part, was not submitted to gain another or the same academic title.

In Prague: 27/01/2021 Bruno Esattore, M.Sc.

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## Foreword

In this thesis, we analyse and discuss the effects of inter-individual differences in aggregation patterns and social strategies on hormonal concentrations in the red deer (*Cervus elaphus*), presenting the methodological framework and results deriving from the data collected in a five-years timeframe and organized in three studies.

The *fil rouge* of this dissertation is the research of inter-individual differences in associative patterns and the verification of their consistency in time and across different contexts. In our first study (Study I), we go against the convention according to which the rank and the dominance are the main factors influencing the physiological dynamics in red deer, extending the perspective to the different typologies of inter-individual relationship and to how they could affect the hormonal concentrations during the period of antler growth (from April to August). We also pay attention to how individual males perceive their social position within a given group. By adapting and applying two well-known theories [i.e., "The Social buffering hypothesis" by Cohen and Wills (1985) and "The Challenge hypothesis" by Wingfield et al. (1990)], we assume that the deer will choose between two different strategies –one more "interaction-prone", with consequent privileged access to the resources, and one less "aggression-oriented", benefitting from all the advantages of non-hostile social company- testing the effects of this choices on both hormonal concentrations (testosterone and cortisol) and antler growth.

Presuming that the above-mentioned differences would reflect some characteristics of the individuals, in the second study presented in this thesis (Study II) we tested whether the behavioural characteristics would remain constant in time on varying of the social context or not. By grouping and separating the animals following similar behavioural strategies, we expect that the individuals will not change their more or less interaction-keen nature according to the changed social environment. In extension of the Study I, we checked the effect of the two different strategies on the concentrations of testosterone and cortisol.

Finally, presuming that the different proneness to fight, the perception of the one's own position in the hierarchy and the associative preferences represent some characteristics that are innate for each, different individual, in our third study (Study III) we apply the method of trait ratings assessed with questionnaires in an attempt to take the first steps into the investigation and the study of personality in captive red deer males.

The reader will find a general overview of the biology and ecology of the species (paragraph 1.1), in which both the sex segregation and the associative patterns of the group of males are described. Successively, we discuss the importance of the definition of a hierarchy in the bachelor group, analysing the different means of interaction (both physical and ritualized) apt to prove the individual valour of the stags and to create the hierarchy itself (paragraph 1.2). The following paragraphs present a review of the role played by two major hormones (i.e., testosterone and cortisol) in the biology of the deer, focusing also on their regulation via feedback mechanisms and their connection to the dominance (1.3); together with this, in the paragraph 1.4, we review the existing literature on the topic of neuroendocrine regulation of the antlers, summarizing the most relevant trends and points of discussion on the matter.

Finally, in the last part of the introduction we summarize the findings of the paper that has represented the reference point of this whole work [Bartoš et al. (2010), paragraph 1.5], focusing on the inter-individual differences in perception of the interactions. We expand the discussion on the topic by introducing a new approach to the matter, involving the "Challenge hypothesis" (Wingfield et al. 1990) and the "Social buffering hypothesis" (Cohen & Wills 1985) (paragraph 1.6) that will represent a key point of discussion in the Study I and Study II. Last, in the paragraph 1.7 we glimpse at the existing literature on animal personality, laying the foundations for what will be discussed in the Study III.

In the section 2 we present the general material and methods, describing the facility where our studies took place and the procedures that are in common to all the three studies, leaving the description of the peculiarities of each work to the homonym section of the single studies. Finally, we present the theoretical background, the hypotheses, analyses, main findings and discussion of the single studies (paragraph 3, 4 and 5), followed by a general discussion (paragraph 6) in which the main issues from the three works are highlighted and, at last, a paragraph summarizing the general conclusions (paragraph 7).

Finish the dissertation the references (paragraph 8), the acknowledgement (paragraph 9), a list of scientific contribution (paragraph 10) and the supplementary material in Appendix (paragraph 11).

# 1. General introduction

## 1.1 Biology of the red deer (*Cervus elaphus*)

The red deer is a large cervid native to much of Europe, Western Asia and part of Northern Africa and it shows one of the highest levels of sociality among the species of cervids currently existing (Mazzarone & Mattiello 2010).

Among ungulates, especially those showing high levels of sexual dimorphism, it is not uncommon to find segregation between sexes [e.g., the mule deer (*Odocoileus hemionus*) (Bowyer 1984), the bighorn sheep (*Ovis canadensis*) (Ruckstuhl 1998), the fallow deer (*Dama dama*) (Ciuti & Apollonio 2008), the markhor (*Capra falconeri*) (Ahmad et al. 2017), the Mediterranean mouflon (*Ovis aries*) (Bourgoin et al. 2018), and the Siberian ibex (*Capra sibirica*) (Wang et al. 2018)] and the red deer represents no exception.

Generally speaking, individuals of different sexes live in segregated groups for most of the year, eventually merging in the imminence of the rut. The factors affecting the segregation of the groups are surely various (Ruckstuhl & Neuhaus 2002; Loe et al. 2006; Ruckstuhl 2007) and reflect the different needs of the two sexes (Main 2008). Thus, hinds will most likely choose areas with better-quality food in order to get in good condition and be ready to deal with the pregnancy and the early calf survival (Illius & Gordon 1987; Conradt et al. 1999). Shortening the grass, the hinds indirectly influence the distribution of the stags, as the remaining food supply would not be enough for them. As a result, the stags move out to not-yet-eaten areas with more low-quality vegetation and are therefore more likely to be found in areas with less desirable food (Illius & Gordon 1987).

The social organization of the sex-segregated groups is different and complex (Clutton-Brock et al. 1982). Hinds tend to inhabit ranges and core areas overlapping those of their mothers (Clutton-Brock et al. 1982) and associate more with their own kin, rather than with unrelated individuals (Mitchell 1977; Gaudin et al. 2015). The social organization of the female groups is matriarchal, with a unit consisting of a matriarch, her mature daughters and their dependent offspring of both sexes (Lowe 1966; Knight 1970; Franklin et al. 1975; Mitchell 1977). Aggregations higher in number have to be considered sporadic and normally due to a common need (Knight 1970). Thus, social ties among hinds are irregular and, all considered, quite unstable and loose and may vary from hour to hour (Clutton-Brock et al. 1982 and references therein).

The situation is consistently different for the stags. As opposed to the hinds, stags disperse from their mothers' home ranges and associate with individuals not closely related to each other (Clutton-Brock et al. 1982; Gaudin et al. 2015) creating so called "bachelor groups". Associations of male deer are also known to be temporary and appear to be even looser (Clutton-Brock et al. 1986) and less well-defined than the females'. Analyses of the grouping of red deer males throughout the velvet period have shown that individuals tended to associate with others of similar rank or age (Clutton-Brock et al. 1982; Appleby 1983a; Bartoš & Perner 1985), with the younger stags remaining on the periphery of the group and the older animals spending more time in the central part of the area. The reason why similar individuals associate together can be found both looking to some social factors and considering that animals of different age classes have different needs. Thus, these requirements are most likely to be met occupying different parts of the whole area (Mitchell 1977). Aging stags are reportedly solitary (Clutton-Brock et al. 1982).

It is important to state that the majority of the above-described notions of biology and ecology of the red deer come from the studies performed in the unique framework of the Isle of Rum. For this reason, despite the extensive documentation, the conclusions regarding the stability of social bonding and the food preferences of the animals may more or less significantly diverge from the results obtained in more standard contexts for the species.

## 1.2 Dominance, threats and physical agonistic interactions

Studies on the red deer have often described the existence of a dominance hierarchy within the social group of stags (Lincoln 1972; Appleby 1980; Bartoš et al. 1987; Bartoš et al. 1988). Red deer males establish social hierarchies where the animals physically compete with one another by mean of elaborate forms of threat and aggression, both physical and ritualized (Bartoš 1982), to assess their rank and determine the diversified access to the resources (Johnson et al. 2007; Wilson et al. 2011; Kidjo et al. 2016; Michel et al. 2016).

Male deer establish their hierarchy during the velvet period [the time between antler casting and velvet shedding (Bubenik 1982a), i.e., usually between the end of February or March and the end of July or August when the growing antlers are covered in a richly vascularized tissue called "*velvet*" that has the function to carry nutrients]. During this time, the growing antlers are very fragile and would easily break, therefore they cannot be used in physical agonistic encounters, as it happens after antler cleaning -for instance, during the rut. For this reason, stags resort to an ample repertoire of other means of confrontation in order to validate or change the rank previously achieved in the hierarchy. These interactions tend to intensify among animals comparable in physical value and social rank (Bartoš 1986; Veiberg et al. 2004) as both the individuals may benefit from the optimization of their position in the hierarchy (Bernstein 1981).

Thanks to the high frequency of interaction among animals, in his paper Bartoš (1982) managed to have a complete picture of the social hierarchy of the bachelor group, offering a detailed collection of the different means of threat and attack used by the stags.

According to that study, the main means of expression in red deer of both sexes are: direction of the look, position of the ears, head and body posture, eye opening, and tail elevation. Different combinations of these elements offer a wide repertoire of expressions, ranging from the highest threat to the most submissive behaviour, passing through displays of high/low self-assurance.

The basic vehicle of expression is the head (Fig.1) and the "direct look" seems to be the most important display of dominance. Thus, it represents the highest level of self-assurance an animal can show. This mean of threat is used by dominant animals against conspecifics of consistently lower rank and it is considered to become less and less effective against animals of similar rank. The “direct look” can also be considered the triggering element of any encounter. A combination between “direct look” and “elevated ears” represents a clear statement of dominance and may instil either a submissive response or a counter-display that may lead to an attack. Conversely the “side look” would be the behaviour to display to signal a submissive position and avoid a physical encounter.

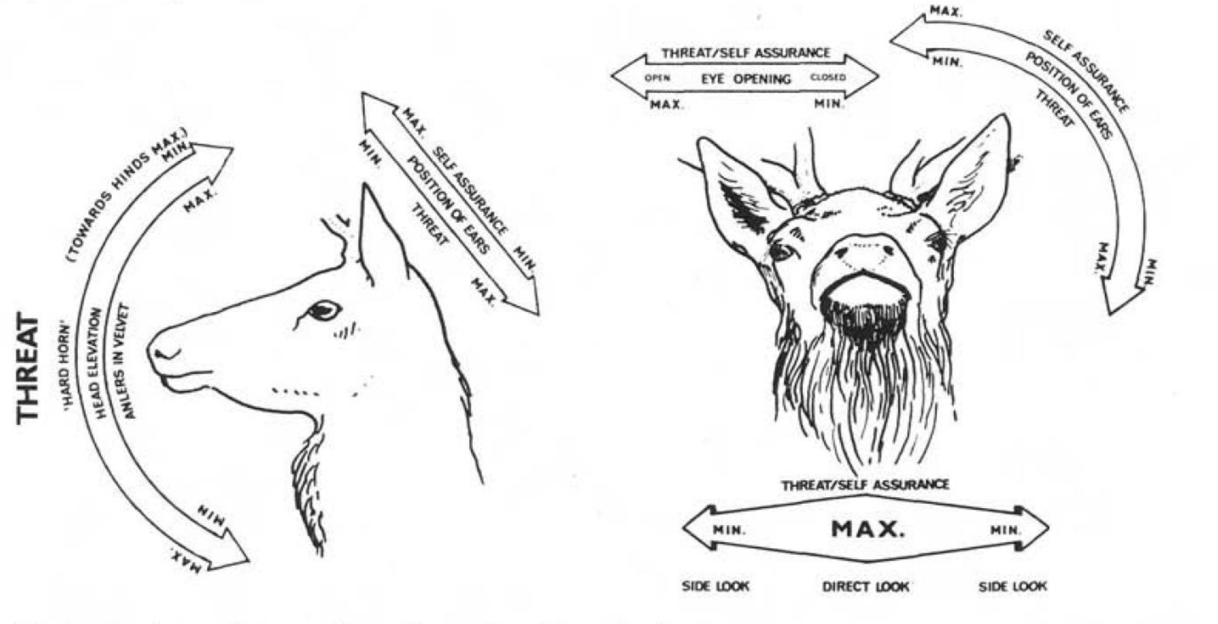

**Figure 1: Expressive positions of a red deer’s head (from Bartoš 1982)**

As it has already been mentioned above, also the position of the ears is an important indicator of the intents of the individual. An animal with the ears in an upright position is vigilant and likely tense, whereas the ears drawn back are a clear expression of threat that maximizes when the ears are pressed against the neck. Conversely, a submissive head position in combination with the ears drawn back has to be interpreted as a sign of diffidence and may occur when the result of an encounter is not clear in advance (e.g., when two unknown stags meet).

Another interesting element is the elevation of the head, as its meaning varies according to the state of the antler cycle. When the antlers are in velvet, to raise the head (the "high chin" threat described in Clutton-Brock et al. 1982) represents a powerful threat. Once the head has been raised, it is kept there until the threatened animal responds. The intensity of this kind of threat can be enhanced by the protrusion of the tongue from the side of the mouth, a hissing sound and the rolling of the eye and consequent displaying of their white part (Lincoln 1972).

Eye opening and tail elevation can be considered as accessory elements of a threat. Having the eyes half-closed is a sign of diffidence, whereas a gradual opening of the eyes conveys the opposite message. The elevation of the tail, instead, does not play any key role in the interactions and just an elevated position should be interpreted as a sign of diffidence and tension. Last, any expression of threat can be enhanced by the presentation of the opened pre-orbital glands (Bartoš 1983).

As it has been already explained before, these threats become almost completely set aside when the antlers are mineralized and cleaned. In fact, once the antlers are finally ready, the most effective displays will be performed lowering the head and showing the hard weapons.

Concerning the physical attacks, the red deer will display the following means of attack: “biting”, “kicking”, “chasing” and “head-butting” (mostly amongst females, for the already mentioned fragility of the antlers in velvet). Most of these do not need further details being the name of the interaction already self-explanatory. However, an exception should be done for the interaction labelled “kicking”.

“Kicking” can be performed by both the forelegs and the hind legs (being this very rare and typical of subordinate animals, likely in distress). The type of kick by a foreleg indicates the rank-relationship between the animals involved in the interaction. More dominant animals execute the kick like a touch, standing on the remaining three legs and in combination with the direct look towards the subordinate individual. When two animals are closer in rank the display gets rougher and can be performed with both the front legs.

Sometimes the “high chin” interaction between two animals of comparable rank leads to a “flail” (i.e., an exchange of kicks between animals standing on the hind limbs). The effort to maximize the threat expression results in the rise of one stag on the hind legs, immediately followed by the second deer. The animals attempt (and frequently manage) to kick each other swinging the front legs in the air until one of them loses the balance and backs off. This kind of interaction is very rare as it may result in serious injuries.

Generally speaking, among ungulates [especially polygynous species engaging in a restricted period of sexual activity (Santiago-Moreno et al. 2007; Willisch & Neuhaus 2010)], males invest much energy in attempt to gain a high rank that will ensure them a privileged access to the resources (i.e., food, mates) (Barroso et al. 2000; Favati et al. 2014). The establishment of a dominance hierarchy seems to be essential for coexisting in a social group (Lovari et al. 2015) and the hierarchy is built via mutual assessment through behaviours (e.g., direct and indirect aggressive interactions, appeasement and avoidance) (Lovari et al. 2015). The creation of a dominance hierarchy will not only benefit the dominant animals, as the male reproductive success is greatly skewed towards them [e.g., as in McElligott et al. (2001), Santiago-Moreno et al. (2007), Mainguy et al. (2008), and Willisch and Neuhaus (2010). For a different insight, compare with Røed et al. (2002) and DeYoung et al. (2006)] but will also prevent less valuable individuals from being injured in an unbalanced fight (Pelletier & Festa-Bianchet 2006; Willisch & Neuhaus 2010).

These miscellanea of typologies of both physical interactions and threats are therefore necessary to establish a social hierarchy in order to determine the access to the resources (with a privileged access for the dominant animals) and reduce the possibilities of being involved into dangerous fights during the rutting season.

Once the animals have had the chance to confront each other and the hierarchy has been defined, this will stay roughly constant for the whole year, unless the dominant deer suffers severe injuries or the presence of a “stranger deer” strikes up the band again. Even though some studies about deer observed in various natural and artificial conditions (e.g., Lincoln 1972; Mitchell 1977; Clutton-Brock et al. 1982; Suttie et al. 1985; Bartoš 1990) have described a linear hierarchy among the stags of the bachelor group, this possibility has to be considered, in fact, rare and dependent on the social situation within the population (Appleby 1983b; Bartoš & Bubenik 2011). Instead, it would probably be more accurate to imagine the social behaviour of the bachelor group as a complex net of inter-individual relations.

## 1.3 Hormones, HPG and HPA axes and social dominance

### 1.3.1 Hormones: Testosterone and Cortisol

Social behaviour has been linked to different hormones and the best established connections are to steroid hormones (Adkins-Regan 2005).

In this project, we decided to consider two steroid hormones that play a key role in the life or the red deer: testosterone (an androgen) and cortisol (a glucocorticoid).

Testosterone is one of the key hormones in the life of red deer stags and it regulates some of the most important and delicate aspects of their life. Being the hormone promoting the development of the secondary sex characteristics across many species of the animal kingdom (Ketterson & Nolan 1999; Hau 2007), its role in the antler growth and regulation has been widely discussed and, mostly, supported (Lincoln 1972; Bartoš 1980; Bubenik 1982b; Goss 1983; Suttie et al. 1984; Barrell et al. 1985; Bartoš 1990; Li et al. 2003; Bartoš et al. 2009; Gaspar-Lopez et al. 2010, but see paragraph 1.4 for a more complete discussion on the topic). Last, as it will be explained later in this paragraph, testosterone concentrations can be modified throughout behaviour due to a feedback mechanism.

Different is the case of cortisol. Generally speaking, glucocorticoids play an important role in metabolic responses, and their concentrations are increased in order to face a major energy mobilization and redistribution in the face of enhanced physical activity (Koolhaas et al. 2011). Moreover, the role of cortisol as a response to a stressful situation has been widely accepted (Monfort et al. 1993; Adkins-Regan 2005; Cockrem 2007; Bartoš et al. 2012).

However, it is obligatory to clarify the concept of stress, as its sense has been recently cleared up. During its first appearance in the literature of the late fifties of the 20th century, stress was originally defined as "*the non-specific response of the body to a noxious stimulus*" (Selye 1950, as in Koolhaas et al. 2011). Further developments of the research on the topic lead to the definition of "stressor" as "*a stimulus that threatens the homeostasis",* and "stress response" as "*the reaction of the organisms aimed to regain homeostasis"* (Chrousos 2009).

The response itself to a challenging situation does not represent a threat to the wellness of a healthy organisms being, conversely, adaptive. The situation changes, however, when the stimulus persists for a prolonged amount time. Thus, the exposure to a long-lasting challenging situation may exceed the regulating capacity of the organism and cause not only a state of exhaustion, but also lead to a depletion of the resources of the organism with adverse consequences for the general well-being of the individual. The exceedance of the natural regulatory capacity of the organism with impairing of the body is at the basis of the revisited concept of stress proposed by Koolhaas et al. (2011), which we will refer to in this dissertation.

### 1.3.2 HPG and HPA axes

Generally speaking, animals are inserted in a physical and social environment. For these reasons, hormones are required to respond appropriately to them. As it has been summarized by Adkins-Regan (2005), it is known that external stimuli are detected by sensory organs and that, consequently, the signal is conveyed to the brain which, in case of neuro-hormones, immediately coordinates the answer. In case of gonadal and adrenocortical steroids, they are regulated by other hormones produced by the anterior pituitary. The anterior pituitary is a non-neural tissue; therefore, it is necessary to find a way to translate the information coming from the outside world into the endocrinology of the anterior pituitary, in order to initiate its regulatory function. The Hypothalamic-Hypophyseal portal system carries out this function, connecting the hypothalamus to the anterior pituitary. The hypothalamic neurosecretory cells produce a set of peptides having either excitatory or inhibitory nature. Once these peptides are released in the system, they reach the cells of the anterior pituitary and regulate their hormone production.

The Gonadotropin-releasing-hormone (GnRH) is a hypothalamic releasing peptide that increases the level of Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH), the gonadotrophic hormones that stimulate the increase of the level of gonadal steroids. LH is released in a pulsatile manner, resulting from the rhythmic activity of a set of hypothalamic neurons called GnRH pulse generator. A set of negative feedback between gonadal hormones, anterior pituitary and hypothalamus does so that the latter will maintain the hormones within a certain range. If, for example, testosterone levels get too high, less GnRH and LH are produced, which decreases the production of testosterone. If testosterone gets too low, GnRH and LH levels are increased and, consequently, testosterone levels rise. This system is called Hypothalamic-pituitary-gonadal axis (HPG).

On the other hand, a parallel system, analogous to the HPG, exists.

The Hypothalamic-pituitary-adrenal axis connects the hypothalamus to the anterior pituitary and the adrenal cortex, creating the pathway for the steroid hormone response to a stressful situation. When a stressful situation occurs in the environment surrounding the organism, peptides in the paraventricular nuclei of the hypothalamus [e.g., Corticotropin-releasing-hormone (CRH)] are released in the portal system. After the CRH reaches the anterior pituitary, it stimulates the release in the circulation of Adrenocorticotropic hormone (ACTH) and other hormones. This stimulates, in turn, the production of glucocorticoids from the adrenal cortex. Negative feedback loops (meaning that the challenging situation is over) bring the hormones back to the baseline level. Conversely, a positive feedback keeps fomenting the mechanism (see Fig.2 for a summarizing and explicative graph).

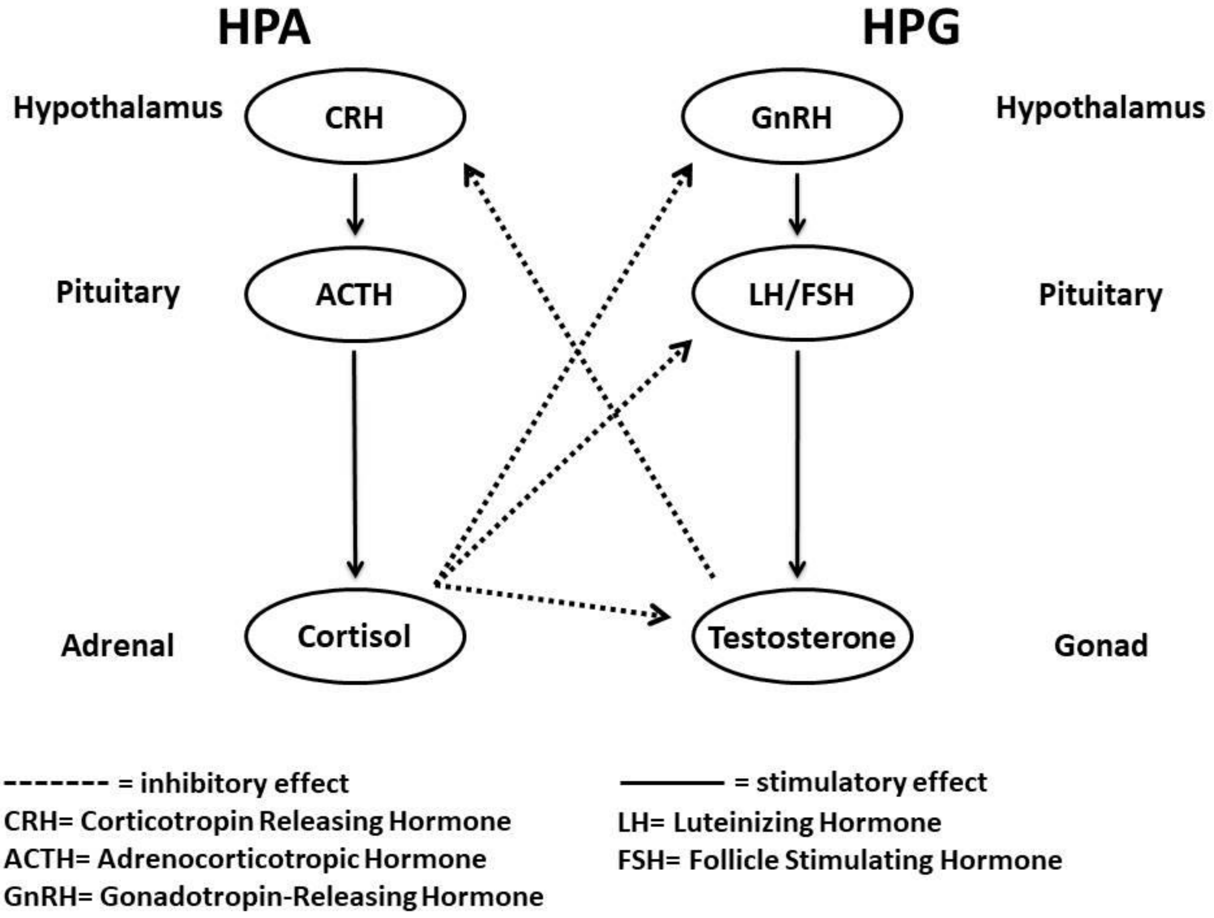

**Figure 2: The Hypothalamus-pituitary-adrenal (HPA) and Hypothalamus-pituitary-gonadal (HPG) axes: structures involved, hormonal and functional relations (adapted from Johnson et al. 1992)**

### 1.3.3 Hormones and social dominance

Social dominance and rank are often associated with physical traits (e.g., body size, antlers size) (Bartoš & Perner 1998; Kruuk et al. 2002; DeYoung et al. 2006) and related to high frequencies of agonistic interactions (Bartoš 1990; Bartoš & Losos 1997). A dominant position in the hierarchy is generally thought to be related to higher concentrations of androgens in the blood that will trigger a more aggressive behaviour (Bartoš 1990; Wingfield et al. 1990; Bartoš & Losos 1997) and lower pituitary/adrenocortical activity than the one of the submissive animals living with them (Rose et al. 1971). Conversely, subordinate individuals are expected to have low levels of androgens and high concentrations of corticosteroids (Brain 1980; Leshner 1980; Bartoš & Losos 1997), with the latter suppressing the first (Brain 1980). Dominant individuals are known to cause physiological stress to subordinates, while preventing themselves from stress (Bartoš et al., *in preparation*). However, no real consensus exists whether dominant or subordinate animals are those more "stressed" (Abbott et al. 2003; Sapolsky 2005; Hirschenhauser & Oliveira 2006; Bartoš et al. 2010). A convincing statement may be found in Sapolsky (1993), according to whom the degree of physiological and physical discomfort depends on the social setting in which it occurs. Indeed, during a major hierarchical reorganization the dominant individuals are the centre of social tension and are therefore expected to experience the greatest discomfort. Conversely, once the hierarchy has been stabilized, subordinate animals are those associated with the highest rates of so-called "stress".

To conclude the overview of the hormonal situation in deer it is necessary to briefly mention the effects that testosterone and cortisol have on each other.

It is known that cortisol can affect both the hypothalamic-pituitary complex and directly the gonads to inhibit the secretion of testosterone (Johnson et al. 1992; Sapolsky et al. 2000); however, cortisol and testosterone do not act independently when influencing the psychology of dominance and competition (Casto & Edwards 2016). Thus, the reciprocal relationship between the HPA and the HPG axes works so that the activation of one affects the function of the other and vice versa (e.g., Toufexis et al. 2014). Increasing testosterone requires increasing cortisol to fulfil the energy recourses. However, the concentration of the latter may reach the level of balance with the environmental demands and exceed the natural regulatory capacity of the organism, thus characterizing the stress situation (Koolhaas et al. 2011); moreover, an increased cortisol would become suppressive to the testosterone. Also the opposite is undesirable, as the suppressive effect of increasing testosterone over the cortisol may lead to a stage of reduced recovery of the neuroendocrine reaction (Koolhaas et al. 2011), that is why both the hormones should work in synergy to always guarantee an adequate supply of resources to the organism (Toufexis et al. 2014) in order to prevent the occurrence of otherwise unpleasant incidences.

### 1.4 Biological meaning and neuroendocrine regulation of the antlers

After describing the means of expression of dominance and the effects of the latter on the concentrations of both testosterone and cortisol, the next step is to describe the biological meaning of antlers and their neuroendocrine regulation -a topic that has been object of an extensive and long-lasting debate during the last century (e.g., Suttie et al. 1984; Barrell et al. 1985; Bubenik 1990; Suttie et al. 1991).

At it has already been described in the paragraph 1.1, in the red deer individuals of the two sexes live separated for most of the year, and eventually merge in the imminence of the rut. The mating system of the species is based on the males displaying their physical strength and characteristics indicating a good state of health, how they cope with the given environment, a better physical condition and the fact of being endowed with "better" genes [i.e., the roars (Clutton-Brock et al. 1979; Charlton et al. 2007), fighting abilities (Clutton-Brock et al. 1979), the antler size (Charlton et al. 2007; Bartoš & Bubenik 2011) and, to some –debatable- extent, the frequency and intensity of marking (Carranza et al. 1990)], and the females choosing the best one available – as the sex that chooses the mate is the one that will invest the most in parental care (Trivers 1972). The most valued stags will gather and defend a harem of females –ranging from a few to several individuals- with whom they will eventually mate.

According to the basic criteria of sexual selection, females are expected to mate selectively with high quality males (Darwin 1871), and to choose their mates according to signals that reliably indicate male quality (Smith 1991).

Authors have repeatedly suggested that antler size may be a good predictor of the bearer's quality to attract a mate (Bartoš et al. 2007; Bartoš & Bubenik 2011), and that it would play a key role in sexual selection as an indicator of individual quality (Kruuk et al. 2002; Bartoš et al. 2007). Thus, antler size may have the potential to serve as a signal to females of a male's quality (Darwin 1871; Bubenik 1982b; Ditchkoff et al. 2001), as shown in studies where more branched antler structure were typical for high-ranking males during the period of antler growth (Bartoš et al. 1987). Moreover, considerable antler size would also provide an evidence of its good genes, as the development of extravagant secondary sexual characters can be an honest advertisement of heritable male quality (Zahavi 1975, 1977; Ditchkoff et al. 2001; Bartoš et al. 2007). Last, Malo et al. (2005) found that a global measure of relative antlers size and complexity is associated with relative testes size and sperm velocity in red deer. Thus, by selecting mates with a large and branched antler structure, females selected those who were most successful in social competition during the period of antler growth and those being fertile in the rut and/or having the competitive ability to ejaculate.

There are a number of reviews focused directly or indirectly on the neuroendocrine regulation of the antlers (e.g., Bubenik 1982b; 1990; Price & Allen 2004; Price et al. 2005a; 2005b). Collectively, these studies have taken into consideration a wide range of hormones, such as insulin-like Growth Factor-1 (IGF-1, Suttie et al. 1985), prolactin (Barrell et al. 1985), luteinizing hormone (LH, Suttie et al. 1991), and testosterone (Bubenik 1982b; 1990; Bartoš et al. 2012).

Two of these hormones (i.e., LH and prolactin) did not represent ideal candidates; in fact, LH is regulated by the GnRH and it works on the production of testosterone in the HPG (being therefore somehow related to the testosterone itself). Instead, prolactin may have a facilitating effect on antler growth, but its role as stimulatory hormone is not supported.

IGF-1 could have been a good candidate (e.g., Suttie et al. 1985), as this hormone is responsible of tissue and bone growth. However, it promotes the growth of the individual, rather than the growth of the antlers, and the consistency of the results available in literature is probably due to the fact that the individuals that they studied for their project were young (Bartoš et al. 2009).

Finally, testosterone remained as last candidate.

As it has been reviewed in Bartoš et al. (2012), even though the development of the antlers is a multi-factorial process that has kept busy generations of scientists, an association between antler growth and an increase of testosterone concentrations has invariantly been found across several species of cervids [e.g., the white-tailed deer (Mirarchi et al. 1977; Brown et al. 1983a), the roe deer (*Capreolus capreolus*) (Sempéré & Boissin 1981, 1982; Sempere et al. 1992), the red deer (Bartoš et al. 2009), the axis deer (*Axis axis*) (Loudon & Curlewis 1988), the fallow deer (Rolf & Fischer 1990; 1996), the rusa deer (*Cervus* (*Rusa*) *timorensis*) (van Mourik & Stelmasiak 1990), and the pudu (*Pudu puda*) (Reyes et al. 1997)], as also have the other effects of increasing [i.e., cessation of antler growth by mineralization (Bubenik 1982b), shedding of the velvet (Waldo & Wislocki 1951), and attachment of the mineralized antler to the pedicle (Bubenik 1982b)] and decreasing [i.e., antler casting (Bubenik 1982b; Goss 1983)] levels of testosterone.

Experiments on castrated animals have furthermore confirmed these assumptions. For example, in their experiments on castrated white-tailed deer, Wislocki et al. managed to reverse the effects of castration on antler growth by artificial administration of testosterone (Aub & Wislocki 1946; Wislocki et al. 1947).

The key seems to be in the concentration of this hormone. Low levels of testosterone initiate the growth of the pedicles and, subsequently, of the antlers in roe deer, whereas high concentrations prevented the growth of the pedicles in the same species (Tachezy 1956, as it appears in Bartoš et al. 2012). Small concentrations of androgens stimulated the development of the antlers also in male Sika deer (*Cervus nippon*) (Shilang & Shanzhi 1983). For these reasons, it has been suggested -and several studies on roe deer (Sempéré & Boissin 1982; Sempéré & Lacroix 1982; Sempere et al. 1992), white-tailed deer (Brown et al. 1983a, b), North-American elk (*Cervus elaphus canadensis*, now *Cervus canadensis*) (Haigh et al. 1984), red deer (Suttie et al. 1984; Barrell et al. 1985; Suttie et al. 1991), and fallow deer (Rolf & Fischer 1990; Bartoš et al. 2000) have successively confirmed- that the new antler growth may be initiated by a testosterone pulse (i.e., a short-lasting hormonal peak) (Fig.3) .

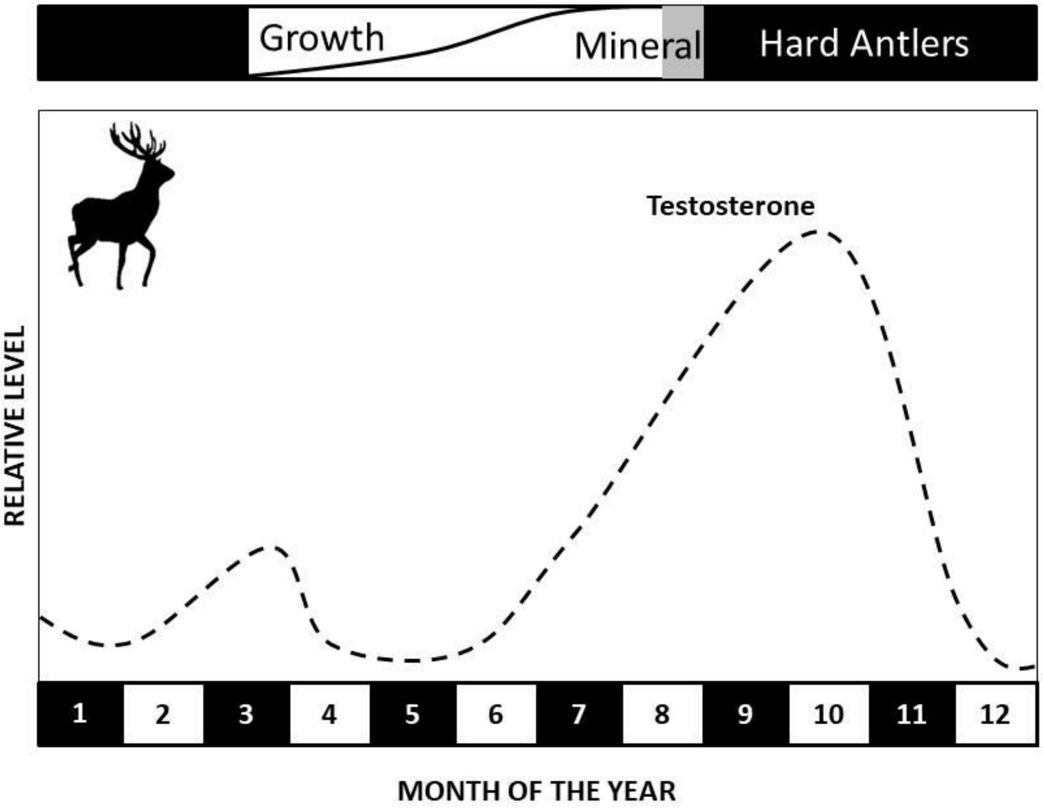

**Figure 3: Stylized time course of hormonal levels during the antler cycle of a red deer (adapted from Bartoš et al. 2012)**

Testosterone promotes the development of secondary sexual characteristics across many species of the animal kingdom (Ketterson & Nolan 1999; Hau 2007) and its concentrations can be modified throughout a feedback mechanism, as explained in the paragraph 1.3, offering an accurate, reliable and precious piece of information on the general state of health and vigour of the male during the previous season to the females that have to make the crucial mate choice.

The social position and the related agonistic activity of males during the velvet period influence antlers weight, length and number of points, suggesting that the antler size is a consequence of the previous social position and not vice versa (Bartoš et al. 1987; Bartoš 1990).

Thus, there is evidence of a relation between dominance-related behaviour, antler cycle timing and antler growth in several species of deer (Bartoš & Bubenik 2011; Bartoš et al. 2012). As the testosterone “pulses” happen during the period of lowest hormonal levels, the more dominant the male is, the stronger and more frequent testosterone pulses during the “low-level season”

they are supposed to have. Males of higher rank cast their antlers first and tend to shed the velvet earlier than the subordinates [as in Bartoš (1985); (1990), but see Forand et al. (1985) and Bowyer (1986) for conflicting results]. The earlier shedding of the velvet in high-ranked males may be a consequence of the stimulatory effects of social interactions on the level of testosterone among dominant individuals; conversely, the elevated levels of glucocorticoids typically found in subordinated animals would depress the levels of testosterone and postpone the time of antlers cleaning. Many authors have suggested that time of antlers cleaning is fully dependent on age. However, as the dominant males are not necessarily always the oldest (Bartoš 1986), the time of antlers casting and cleaning seem to be more tightly connected to the social status of the animals, rather than to their age (Bartoš 1980).
As of the effects of the males' rank on the antler growth, the connection between the former and the latter seems logical. Males' behaviour may influence the physiology of the antlers from the very first stages of the antler growth, at the beginning of the velvet time. The more dominantly an animal behaves, the higher will be the levels of testosterone in its blood and, consequently, the more the growth of the portion of antler developing in that very period will be enhanced (Bartoš 1990). As further proof of the tight connection between rank and testosterone and of the delicate balance between the former and the latter, a study on fallow deer has showed that males gaining a higher rank through fighting other males exhibited enhanced growth of that part of the antler that was just growing. This situation would change, if the male lost his position (Bartoš & Losos 1997).

For all of the above-mentioned reasons, we decided to focus on the role of testosterone as promoter of the antler growth and trigger of aggressive behaviour, using the antlers as record of the hormonal levels during the period of antler growth.

### 1.5 Bartoš et al. (2010): a new point of view

In the study from 2010, Bartoš *et al*. discovered that small changes in the social environment could profoundly affect the relationship between the rank and hormonal levels in the red deer (Bartoš et al. 2010).

They noticed that, being the centre of social friction with individuals of the same rank, adult males kept showing high levels of cortisol and that this had a suppressive effect on the concentration of testosterone, resulting in a negative relationship between rank and testosterone levels in adults.

By adding young males to a group of adult red deer males (i.e., giving them the chance to attack much smaller individuals rather than equally sized rivals) they expected to achieve a change in agonistic behaviour of the adults. Thus, they predicted that the new social environment would have had a calming effect on the adult stags (yet not affecting the hierarchy), decreasing their mean cortisol concentrations and eventually enhancing a positive relationship between testosterone and rank. As expected, during the experiment the hierarchy was maintained stable, even though the adult stags addressed the greatest part of their interactions –whose number did not vary significantly- against the smaller and, obviously, submissive individuals. This increased the concentrations of testosterone in the adult, dominant animals and decreased their concentrations of cortisol, permitting the occurrence of a “typical” physiological response (i.e., subordinate individuals showing higher cortisol levels than the dominant ones). On the other hand, as expected, the younger sparring partners showed the highest levels of cortisol due to the permanence in a condition of continuous discomfort.

This suggested that, among adult deer, to maintain the rank position was an uncertain venture causing a stress reaction comparable to the one seen in subordinate young males, which were exposed to permanent attacks from dominant adults. Social stress in adult males was the cost of dominance, not a consequence of subordination (Sands & Creel 2004; Bartoš et al. 2010). High-ranked individuals had the greatest physiological signs of stress when living in a competitive social situation with equal-sized adult conspecifics. The presence of smaller and obviously submissive individuals created a situation in which dominant male red deer attacked others with a low risk of failure (Bartoš 1986) .

This study has not only confirmed the suppressive effect of cortisol on testosterone (and not vice versa) but has also produced a new cause of reflection.

Thus, Bartoš et al. (2010) have highlighted that dominance itself does not provide enough information. As each individual differs from the other, it is imaginable to believe that the way each individual perceives the fact of being involved in an agonistic encounter will differ. In other words, the inconsistency of the results of many deer studies may be caused by the difference in how the dominant individuals themselves perceive the dominant position, how aggressive they are, how keen they are on attacking others and whom they attack. A simple assessment of the rank position within a group and of its relation to antler development may therefore not be sufficient (Bartoš & Bubenik 2011).

### 1.6 New insights to an "old" topic

When investigating the relationships between hormones (i.e., testosterone and cortisol) and social relations, authors focused predominantly on aggressive behaviour, as primarily related to dominance relationships and having an effect on growth and formation of antlers (Bartoš et al. 2000; 2009; 2012). However, dominance relationships are not the only type of social relationships existing in animal communities, as social bonding – established and maintained by socio-positive behaviours- has also been documented and should, therefore, be considered for its important role (Sachser et al. 1998).

Analysis of the grouping of red deer males throughout the antler growth period showed that individuals tended to associate with others of similar rank and age (Appleby 1983a; Bartoš & Perner 1985). Such an association needs not be permanent, as groups may disintegrate and some individuals may afterwards be observed alone.

Assuming that rank and dominance are not the only keys to understand the physiological dynamics in the red deer (see paragraph 1.5), in this project we focused on how individual males perceive their social position within a given group, adding two new "points of view" to a long lasting and already widely discussed topic.

We considered both the "Social buffering" (Cohen & Wills 1985) and the "Challenge hypothesis" (Wingfield et al. 1990), two aspects not taken in account in previous deer studies when analysing the relationship between social behaviour and physiological response.

"Social buffering" ["the Buffering hypothesis" (Cohen & Wills 1985; Hennessy et al. 2009; Hostinar et al. 2014; Gunnar & Hostinar 2015; Kiyokawa & Hennessy 2018)] includes the ability of a social partner to reduce potential stress responses. This may have positive effects on physiological responses, particularly those on behavioural reactions during stress (Hennessy et al. 2009). The presence of a close social partner attenuates the reactivity of the HPA axis and buffers against the potentially adverse effects of physiological stress (Young et al. 2014). In horses, elephants, hyenas, dolphins, and several primate species, some individuals form friendships that may last for years and many friendships are formed between unrelated individuals (Seyfarth et al. 2012; Young et al. 2014). In red deer, “friendly” behaviour between the males -such as mutual grooming, etc.- is hardly ever seen. Therefore, we decided to regard as a potentially “friendly” behaviour when two males spent prolonged time close together not attacking each other.

Second, to evaluate the effect of male-male competition during the period of antler growth, we applied a new approach extending the original Wingfield et al.’s “Challenge hypothesis” (Wingfield et al. 1990). This hypothesis deals with naturally-occurring and experimentally-induced elevated testosterone levels during the breeding season that are positively related to aggressive and dominant behaviours, especially when the social status of a dominant male is threatened. Our extension focuses on the elevation of testosterone concentrations due to male-male competition (Wingfield et al. 2019; Ball & Balthazart 2020; Moore et al. 2020) during the non-breeding season, especially whenever an important situation arises for the attacking animal. Such a situation may occur, for example, when the rank position of a male within the group is challenged.

### 1.7 Animal personality

In animal behavioural science, between-individual variation was traditionally considered as a “noisy” variation around an adaptive population mean (Wilson 1998; Dall et al. 2004). However, during the last decades research has increasingly developed statistical models for quantifying such variation (Dingemanse et al. 2010; Dingemanse & Wolf 2013; Araya-Ajoy & Dingemanse 2014; Martin et al. 2019), leading to the formulation of the concept of “animal personality”. Described as “*between-individual behavioural differences consistent across time and contexts*” (Sih et al. 2004; Pervin et al. 2005; Réale et al. 2007; Réale et al. 2010), personality has been linked to consistent inter-individual variation in ecologically relevant behaviours (Réale & Montiglio 2020), for instance foraging (Wilson & McLaughlin 2007), dispersal (Dingemanse & de Goede 2004), acquisition of dominance ranks (Fox et al. 2009), group joining preferences (Harcourt et al. 2009) or cognition (Boogert et al. 2018).

Researchers have identified several important axes of animal personality along which individuals can be placed [e.g., shyness – boldness, exploration – avoidance, aggressiveness, activity, sociability and  proactive – reactive stress coping styles (Koolhaas et al. 1999; Réale et al. 2007; Bergmuller & Taborsky 2010; Finger et al. 2018)] in a wide range of organisms, from mammals to birds, reptiles, fish and invertebrates (Gosling 2001; Sih et al. 2004; Wolf et al. 2007; Biro & Stamps 2008; Bell et al. 2009; Dingemanse & Wolf 2010; Freeman & Gosling 2010; Gartner & Weiss 2013; Weiss 2018).

Some personality characteristics such as boldness (Dahlbom et al. 2011), aggressiveness (Drent et al. 1996; Rodriguez-Santiago et al. 2020) and exploration (Verbeek et al. 1999) have been traditionally associated with a higher position in the hierarchy in many species, and it is known that changes in the dominance status can have repercussions on the behaviour or survival of the individuals whose social status has been affected (Rudin et al. 2016).

Despite the fact that the number of species in personality studies has steadily increased in the last decades, the literature investigating the consistency of behavioural patterns in cervids appears, to this day, still surprisingly scarce and counts only few studies (e.g., Bergvall et al. 2011; Jennings et al. 2013; Found & Clair 2016; Monestier et al. 2016). These studies focused on the trade-off between anti-predatory behaviour and foraging, pointing out that boldness but not dominance was related to time spent foraging in fallow deer (Bergvall et al. 2011), or on the inter-relationship between aggressiveness, willingness to fight, mating success and fitness in male rutting fallow deer (Jennings et al. 2013). Further, some studies looked at the influence of North-American elk personality on individual response to aversive conditioning and how this knowledge could help in reducing habituation in wild ungulates (Found & Clair 2016), or on the individual variation in acute stress response, focusing on the link between coping mechanisms and individual condition in captive roe deer (Monestier et al. 2016).

Surprisingly, the personality of red deer males has not been explored yet, therefore the extent to which some personality characteristics (e.g., boldness or aggressiveness) are related to some aspects of red deer male social behaviour (e.g., their position in the dominance hierarchy) is still unknown.

Following from Study II, we investigated the personality of adult red deer males and its links with naturally occurring behaviour to see if male red deer individuals consistently differed in their personality traits and whether the expected differences in male deer personalities could be linked to their differences in ranks -namely that the boldest and/or more aggressive males would also be higher in rank and vice versa- or not.

## 2. Material and methods

Observation of farmed red deer took place at a deer facility belonging to the Institute of Animal Science (V.Ú.Ž.V.) at Podlesek, Praha, Czech Republic (50°03'02.2"N 14°35'37.1"E). The experimental deer facility is an accredited research station according to the European and Czech laws for ethical use of animals in research. The experimental proposal n° MZe 1297 was approved by the Animal Care and Use Committee.

At this facility, tame captive red deer belonging to the same bachelor group since birth were available. The facility is composed of six enclosures of nearly identical size, covering an area of approximately 4 ha. Each enclosure (about 0.7 ha large) contained a shelter (a wooden, roofed barn with one side permanently open, covering an area of approximately 24 $m^2$), a water reservoir, and a mud pool for wallowing. The enclosures are interconnected by two (the first and the last enclosures) or three gates to the other enclosures, allowing the deer to move around and facilitating the operations of handling. This arrangement is designed to recreate the natural conditions, where animals of different sexes live segregated for most of the year and eventually merge for mating (Mitchell 1977; Clutton-Brock et al. 1982). The animals fed predominantly on the pasture and were supplemented with hay (*ad libitum*), and occasionally also with potatoes, apples, barley, soy and oats, together with a mineral/vitamin premix which amounted to an average of 0.7 kg/day/animal. They were identified with coloured, numbered collars.

For the specific information about the number of animals involved and the modality and type of data collected in each study, please refer to the material and methods section of the single studies.

# 3. Study I

## An effect of social buffering on cortisol-testosterone interactions and antler growth in red deer

### Introduction

Mammalian societies are complex systems, influenced and modified by numerous factors, both external and internal. Among the latter, inter-individual relationships surely play a key role in shaping social systems (Crook et al. 1976). Two different types of social relationship exist in mammalian social systems: dominance relationships (reviewed in the sections 1.2 and 1.3.3) and social bonding, which are established and maintained by socio-positive behaviours (Sachser et al. 1998). However, studies on several deer species conducted over the last few decades have mainly investigated the dominance relationships, linking them to the antler growth (e.g., Bartoš et al. 2000; 2009; 2012). In the red deer, temporary associations are extremely common, especially among animals of similar rank and age (Appleby 1983a; Bartoš & Perner 1985), but it is also common to observe animals conducting a solitary existence. Different associative preferences are surely affected by different responses to social stimuli, and their effects are reflected on the different stages of antler growth [e.g., timing of antler cleaning (Bartoš 1980), antler formation (Bartoš & Losos 1997)]. In the paragraph 1.3 we have already discussed the relations existing between social animals and physiological stress, explaining the difficulty of assessing whether are the dominant or the subordinate animals to exhibit the highest levels of stress.

When studying the relationships between dominance related behaviour and resulting testosterone and cortisol concentrations, authors have given great importance mainly to the aggressive behaviour, focusing very little on the perception that the individuals have of their position within a given group. In the wake of the study from Bartoš et al. (2010, described in detail in the section 1.5), where they found out that changes in the social environment of adult red deer males resulted in a change of the relationship between rank and concentrations of both testosterone and cortisol, we decided to focus on the –so far overlooked- individual perception of the single animals within the group, adding two aspects not taken into account in previous deer studies [e.g., “the Buffering hypothesis” (Cohen & Wills 1985; Hennessy et al. 2009; Hostinar et al. 2014; Gunnar & Hostinar 2015; Kiyokawa & Hennessy 2018) and the “Challenge hypothesis”  (Wingfield & Farner 1978; Wingfield et al. 1990), as described above in the section 1.6].

As testosterone has been proven to be a major hormone regulating antler growth (Bartoš et al. 2000; 2009), we believe that, in order to develop the largest antlers, a deer male would be faced to a trade-off situation in order to either achieve the highest protection against stress or to reach as high testosterone concentrations as possible. According to the “Buffering hypothesis" (Cohen & Wills 1985), it would be advantageous for such a male to be sociable, spending prolonged time close together with other individuals not attacking each other, thus minimalizing social stress. This should result in decreased cortisol concentrations, which in consequence would not suppress testosterone concentrations (Bartoš et al. 2012). The risks of such an approach would be that the testosterone concentrations would not need to be too high in order to develop larger antlers. An alternative to this would be to avoid a socially stable grouping, preventing the male to establish stable relationship with others during the period of antler growth. Whenever such a male would meet other males then, an encounter would be challenging, because its social status would be threatened (Wingfield et al. 1990; Hirschenhauser & Oliveira 2006; Ball & Balthazart 2020). As a result, testosterone concentrations should be increased and the antler growth should be greater, unless the -likely elevated cortisol concentrations would not be suppressive to the testosterone concentrations.

The problem is further complicated by the interaction between cortisol and testosterone. Although cortisol can act on the hypothalamus, the pituitary gland, and directly on the gonads to inhibit the secretion of testosterone (Johnson et al. 1992; Sapolsky et al. 2000), until recently it was common to think about cortisol and testosterone as acting independently one from the other to influence the psychology of dominance and competition (Casto & Edwards 2016). However, it exists a reciprocal relationship between the hypothalamic-pituitary-adrenal and the hypothalamic-pituitary-gonadal axes, wherein the activation of one affects the function of the other and vice versa (e.g., Toufexis et al. 2014). Basically, increasing testosterone requires increasing cortisol to fulfil the energy recourses. However, the concentrations of the latter may reach the level of balance with the environmental demands and exceed the natural regulatory capacity of the organism, thus characterizing the stress situation (Koolhaas et al. 2011, as reviewed in the section 1.3) and increased cortisol would become suppressive to testosterone.

We hypothesized that during the period of antler growth (i) some males will keep company with others for a long time, while others will not. If it was so, it would suggest two different tactics. In the first case, (ii) when aggregating with other males, a male will likely be in a socially stable situation resulting in lowering cortisol concentrations. (iii) Alternatively, males will be avoiding socially stable groupings. This would increase their aggressiveness and consequently testosterone concentrations to an extent not to be suppressed by also increasing cortisol levels: as a result, (iv) higher dominance status will reduce cortisol concentrations (dominant animals will have lower cortisol and increased testosterone).

Antler size will be taken as an arbiter of the evaluation which tactic will be more effective in producing larger antlers.

### Material and methods

Observation of farmed red deer took place at the deer facility belonging to the Institute of Animal Science (V.Ú.Ž.V.) at Podlesek, as described in the section 2.

Seventeen tame captive red deer males belonging to the same bachelor group since birth were kept for the whole observation period (from 1st May to 27th August 2014) in six, interconnected enclosures, allowing the deer to move around and aggregate with or separate from others. Together with the identifying, coloured, numbered collars, the animals were also equipped with GPS collars (Lotek Wireless Inc. GPS_3300).

### Data collection

The observation was performed in order to systematically record agonistic interactions between animals when competing for supplemental food. When the supplemental food was presented, it usually attracted all males either living in a group or individually so that for this occasion the males met even though, otherwise, they would have avoided any encounter with each other. The food was carried to the observation place in a wheelbarrow and presented in several piles to encourage the competition over a scarce resource. The piles were tilted from the wheelbarrow about 2 meters apart, in a number of piles equal to the number of the stags/2, in order to encourage the competition without exacerbating it. This method has already been proven valid in previous studies (e.g., Bartoš et al. 2010). Each observation session ranged from 20 to 60 min (depending how long the deer stayed at the site of supplementary feeding) and took place from 1 to 5 times per week (with an average equal to 3), in the morning (between 9.00 a.m. and 11.30 a.m.). All deer were tame and started the competition straightaway after the food was presented, running from one pile to another and trying to eat as much as possible. When the eating deer was attacked, it either escaped to another pile or defended itself. The observation started immediately after supplying the supplemental food and lasted until the deer ate it and all the animals left the place. All the observations were recorded on a voice recorder and then transcribed into a table using Microsoft Word Excel. We recorded any occurrence of an approach of one male to another, any attack, threat gesture, or fight (Bartoš 1982) which caused an apparent displacement of the approached individual. As in previous studies (Bartoš & Bubenik 2011), we determined the dominance status for each pair of males on the base of  the agonistic interactions. We regarded as dominant the male who won more agonistic encounters than he lost in the pair.

Inter-individual distances between stags were measured by GPS collars. Positions were programmed to be recorded once per hour. This enabled us to obtained 47.946.315 usable records of inter-individual distances during the observation period with an average of 176.273 records per dyad over the observation period, producing a reliable picture of the mean inter-individual spaces over the whole period.

Between May and August, we weighted the males once a month and collected blood sample in a physical restraining facility ("*crush*", see Fig. S1). Handling the deer in a crush has been used for routine manipulations. Therefore, all deer involved in the experiment were used to this procedure since they were born and had been handled the same way many times before the start of the experiment. No chemical restrain was used in addition to the physical restraint. We measured the antlers after casting in a way that has already been described earlier (e.g., Bartoš

& Bahbouh 2006), and used the total antler length (i.e., the final sum of the length of all tines, points and beams) as a dependent variable.

## Hormone analyses

Testosterone concentration was measured by RIA Kit from Beckam Coulter, code IM1087. The radioimmunoassay of testosterone is a competitive assay. Prior to the assay, samples (serum or plasma) were extracted with ethyl ether; the solvent was evaporated and the dry residues were re-dissolved in the recovery buffer of the kit. The re-dissolved extracts and calibrators were then incubated with $^{125}$I-labeled T, as tracer, in antibody-coated tube. Concentration range was up to 23 ng/mL, detection limit of the assay was 0.1 ng/mL, intra-assay-precision was 8.6% and inter-assay was 11.9%. Recovery of extraction step was 90%.

Cortisol concentration was determined by RIA Kit from Beckman Coulter, code IM1841. The radioimmunoassay of cortisol is a competitive assay. Samples and calibrators were incubated in monoclonal antibody-coated tubes with $^{125}$I-labeled cortisol tracer. Concentration range was up to 2000 nM, detection limit of the assay was 5 nM, intra-assay-precision was 9.4% and inter-assay was 12.6%.

## Statistics

All data were analysed with the aid of the SAS System (SAS, version 9.4).

For each male we collected inter-individual mean distance (metres) from each of the herd mates (i.e., 16 inter-individual mean distance per male) and other characteristics (listed in Table 1), including those between him and all other males (such as the number of attacks, wins, losses, etc.). For the analyses we used mean values of all countable variables over the whole period. Having 17 males with 16 relationships each, we obtained 272 dyadic records in total. These 272 dyads entered all statistical models always for the initiator of the interaction. Therefore, except for the inter-individual distance, the values of the countable variables entering the statistical model for the dyad X-Y was different from the one for the dyad Y-X, because the initiator in the dyad X-Y was the individual X and, vice versa, it was the individual Y in the dyad Y-X. That is why we needed to consider twice as many pair relationships than the number of inter-individual pairs available.

A cluster analysis (PROC CLUSTER, with TYPE=NOMINAL and METHOD=HIERARCHICAL) was used to divide the inter-individual distances between males into two groups, “Associates” (i.e., males keeping together) and “Distant” (i.e., those living apart) (i). For each male we then calculated “Proportion of Associates” (%) – a number of dyadic interactions with “Associates” divided by total number of pair interactions available. Because we expected differences between the groups in their agonistic interactions, we calculated mean ± SE for these groups (PROC MEANS).

In order to check for possible multicollinearity, we first calculated correlations (PROC CORR) between the individual countable variables involved (Table 1). A significant correlation was found between the Body weight at the beginning of the observation and at the end of the observation ($r = 0.91$, $P < 0.0001$), between the Body weight and Weight gain ($r = 0.83$, $P < 0.0001$), between Age and Body weight (at the beginning of the observation $r = 0.84$, $P < 0.0001$; at the end of the observation $r = 0.71$, $P < 0.0001$), and between Number of winning agonistic interactions and Number of lost agonistic interactions ($r = 0.34$, $P < 0.0001$). Across the models, where appropriate, count variables were log-transformed (natural logarithm transformation) to improve normality of residuals and to reduce skewness.

Since the issues analysed in this study represented more complex causality, we used advantages of the information-theoretic approach (IT-AIC) for estimating the effects of the factors on dependent variables (Burnham & Anderson 2002; Richards et al. 2011). There are warnings in the literature about the risks of using the Akaike Information Criterion (AIC, Akaike 1974) in case of nested and mixed models (e.g., Vaida & Blanchard 2005; Ward 2008). Therefore, we used the two most important and frequent model selection criteria (Ward 2008), i.e., AIC, and Bayesian methods (BIC, Schwarz 1978). Multiple information criteria are useful because each one was developed to optimize something different than the others. AIC is an example of efficient information criteria, while BIC is an example of consistent information criteria (Christensen 2018). We found justification for such a procedure in a study of Posada and Buckley (2004) where they argued and proved that AIC and BIC are able to simultaneously compare multiple nested or non-nested models and assess model selection uncertainty.

Associations were subsequently sought between cortisol concentrations (ii), testosterone concentrations (iii), or total antler length as dependent variables and the remaining fixed factors (Table 1) using a multivariate General Linear Mixed Model (PROC MIXED). Because we did not apply any random effect, the models were run as a fixed effect model (GLM). For each dependent variable we constructed a set of multiple *a priori* hypotheses always containing a hypothesized key factor of Association. Where appropriate, we included interaction terms. Specifically, for log-transformed Cortisol concentrations we set up 53 alternative hypotheses (Table TS1), for log-transformed Testosterone concentrations the hypotheses were 59 (Table TS2), and eventually, for Total antler length, the hypotheses were 203 (Table TS3). We generated all GLMs in Tables TS1, TS2, and TS3, respectively, and converted values of Fit statistics. The differences ($\Delta_i$) between the Fit statistic values (i.e., the smallest values indicating the best-fitting model) were sorted according to AIC values. Akaike weight *wi* can be interpreted as the probability that $M_i$ is the best model (in the AIC sense, that it minimizes the Kullback–Leibler discrepancy), given the data and the set of candidate models (e.g., Burnham & Anderson 2002). For the five models with the lowest AIC values, we therefore calculated Δ AIC, Akaike weights $w_i$, and we divided their Akaike weights $w_{min}/w_j$ (AIC Odds) to estimate the strength of evidence in favour of one model over the other (Burnham & Anderson 2002). Using the same formulas just replacing AIC by BIC values [as recommended by various authors (e.g., Buckland et al. 1997; Burnham & Anderson 2002; Wagenmakers & Farrell 2004)], we obtained analogically Δ BIC, BIC weights $w_i$, and BIC Odds. The advantage of this procedure is that, in comparison with AIC, BIC severely penalizes models with more parameters. Thus, the BIC weights $w_i$ are appreciably different from the AIC weights $w_i$ (Buckland et al. 1997).

Associations between the dependent variable and countable fixed effects are presented by fitting a random coefficient model using GLM as described by Tao et al. (2002). We calculated predicted values of the dependent variable and plotted them against the fixed effects with predicted regression lines. Least squares means (LSMEANs) were calculated for the categorical fixed effect Associations by computing the mean of each treatment and averaging the treatment means. These means of means were then used to compare the factors. In this way, the means were adjusted for the number of observations in each treatment.

| **Variable** | **Mean** | **SE** | **Lower 95% CL for Mean** | **Upper 95% CL for Mean** |
|---|---|---|---|---|
| Age | 4.82 | 0.48 | 3.81 | 5.84 |
| Testosterone concentration (ng/ml) | 0.28 | 0.03 | 0.21 | 0.36 |
| Cortisol concentration (ng/ml) | 82.68 | 5.92 | 70.12 | 95.23 |
| Number of attacked conspecifics [Number_attacked] | 8.65 | 1.23 | 6.05 | 11.25 |
| Sum of winning encounters [Wins] | 56.71 | 12.43 | 30.36 | 83.05 |
| Number of lost encounters [Losses] | 56.71 | 9.04 | 37.55 | 75.86 |
| Sum of all agonistic interactions (attacked others and being attacked) [Sum_interact] | 113.41 | 12.65 | 86.59 | 140.24 |
| Proportion of Associates of all dyadic relationship (%) [Proc_Ass] | 63.97 | 1.90 | 59.95 | 67.99 |
| Body weight at the beginning of the observation period (kg) [Weight1] | 109.12 | 7.00 | 94.27 | 123.97 |
| Body weight at the end of the observation period (kg) [Weight2] | 142.12 | 10.69 | 119.47 | 164.77 |
| Bodyweight gain over the period of observation (kg) | 33.00 | 5.27 | 21.83 | 44.17 |
| Relative bodyweight gain over the period of observation (%) | 20.44 | 3.42 | 13.21 | 27.68 |

| Variable | Levels |
|---|---|
| Association | Associates, Indifferent, Non-Associates |
| Dominance | Dominant, Subordinated |

**Table 1: List of potential fixed factors (mean, standard error, lower and upper 95% Confidence limit). Terms in square brackets are abbreviations used in defining a statistical model (n=17).**

## Results

(i) Although the males had known each other for an extended period before we started our observation, when they got into a six-time larger area, they either consistently associated with each other or consistently separated from each other over the five months. They kept this attitude despite the fact they were frequently attracted to supplementary food, and hence they broke the average inter-individual distances. The Cluster analysis divided the dyadic average distances between males into two convincingly well separated groups: “Associates” (number of pairs, mean ± SE; n=147, 25.53 ± 1.68 m) and “Distant” (n=125, 175.62 ± 4.13 m). Part of the “Distant” dyadic relationships revealed no attacks to others. Thus, the “Distant” dyads were further divided into “Indifferent” (n=51), i.e., those that were socially inactive (never attacking anybody) although they could be and were attacked by others, and “Non-Associates” (n=74), i.e., those attacking others. Thus the class variable “Association” used in further statistical analysis contained 3 levels of dyads (Associates, Indifferent, and Non-Associates). Mean frequencies (± SE) of the Sum of agonistic interactions according to Association is shown in Fig. 4.

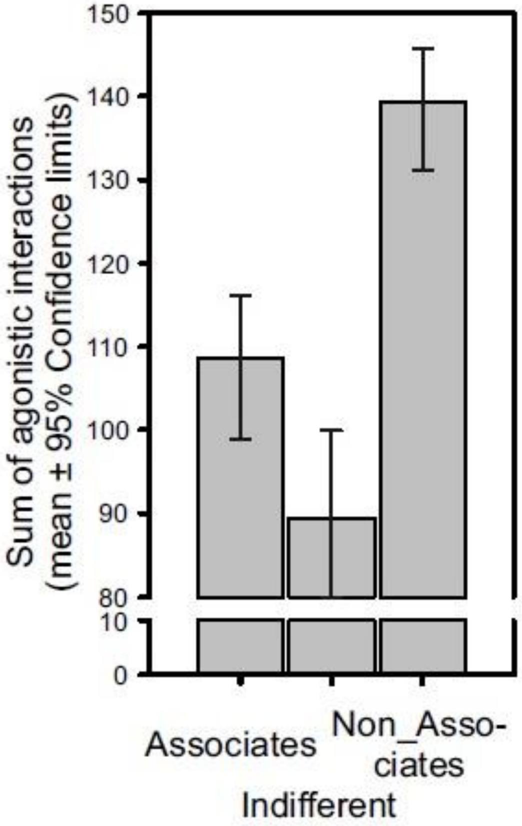

**Figure 4: Sum of agonistic interactions (means, lower and upper 90% confidence intervals) for Associates, Indifferent, and Non-Associates.**

On average, the Non-Associates showed the highest Sum of agonistic interactions in comparison to the groups Associates and Indifferent. Indifferent dyads showed the lowest Sum of agonistic interactions.

Table 2 shows five best-fitting models sorted according to the fitting statistics (starting with the smallest value) for the dependent variables log-transformed Cortisol concentrations, log-transformed Testosterone concentrations, and Total antler length. In all cases Δ AIC, AIC weights *$w_i$* and AIC Odds revealed comparable- if not even identical -results with Δ BIC, BIC weights *$w_i$* and BIC Odds. This strengthened the credibility of the results.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| GLM | AIC | Δ AIC | Akaike weights *wi* | AIC Odds | BIC | Δ BIC | BIC weights *wi* | BIC Odds |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Dependent variable log-transformed Cortisol concentrations** | | | | | | | | |
| Association Proc_Ass(Association) Testosteronet(Association) Weight2t(Association) | 115.67 | 0.00 | 0.91 | 1.00 | 119.24 | 0.00 | 0.91 | 1.00 |
| Association Proc_Ass(Association) Testosteronet(Association) Weight2t(Association) Dominance | 121.50 | 5.82 | 0.05 | 18.38 | 125.05 | 5.82 | 0.05 | 18.31 |
| Association Proc_Ass(Association) Testosteronet Sum_Interact | 123.11 | 7.44 | 0.02 | 41.23 | 126.69 | 7.45 | 0.02 | 41.55 |
| Association Proc_Ass(Association) Testosteronet Wins Losses | 124.66 | 8.99 | 0.01 | 89.44 | 128.23 | 9.00 | 0.01 | 89.96 |
| Association Proc_Ass(Association) Testosteronet Weight2t | 126.42 | 10.74 | 0.00 | 215.30 | 129.99 | 10.76 | 0.00 | 216.95 |
| **Dependent variable log-transformed Testosterone concentrations** | | | | | | | | |
| Association Cortisolt Weight2t Sum_Interactt | -381.23 | 0.00 | 0.56 | 1.00 | -377.65 | 0.00 | 0.56 | 1.00 |
| Association Cortisolt Weight2t | -380.70 | 0.53 | 0.43 | 1.30 | -377.11 | 0.53 | 0.43 | 1.30 |
| Association Cortisolt Weight2t Number_attacked | -373.97 | 7.26 | 0.01 | 37.76 | -370.38 | 7.26 | 0.01 | 37.76 |
| Association Cortisolt Weight2t Dominance | -369.79 | 11.44 | 0.00 | 304.31 | -366.21 | 11.43 | 0.00 | 303.74 |
| Association Cortisolt Weight2t Sum_Interactt Dominance | -369.70 | 11.53 | 0.00 | 319.26 | -366.12 | 11.52 | 0.00 | 318.06 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Dependent variable Total antler length** | | | | | | | | |
| Association Proc_Ass(Association) Wins Age*Weight2 Testosteronet | 2959.51 | 0.00 | 0.57 | 1.00 | 2963.08 | 0.00 | 0.57 | 1.00 |
| Association Proc_Ass(Association) Age*Weight2 Sum_Interact Testosteronet | 2960.10 | 0.59 | 0.42 | 1.34 | 2963.67 | 0.59 | 0.42 | 1.34 |
| Association Proc_Ass(Association) Age Sum_Interact*Wins Testosteronet | 2970.07 | 10.56 | 0.00 | 196.22 | 2973.64 | 10.56 | 0.00 | 196.22 |
| Association Proc_Ass(Association) Age Wins*Sum_Interact Testosteronet | 2970.07 | 10.56 | 0.00 | 196.22 | 2973.64 | 10.56 | 0.00 | 196.22 |
| Association Dominance Proc_Ass(Association) Wins Age Number_attacked Testosteronet | 2970.21 | 10.69 | 0.00 | 209.92 | 2973.77 | 10.68 | 0.00 | 208.72 |
| | | | | | | | | |

**Table 2: Five best-fitting models sorted according to fitting statistics (the smaller the better), AIC, Δ AIC, and BIC, Δ BIC for the dependent variables log-transformed Cortisol concentrations, log-transformed Testosterone concentrations, and Total antler length.**

(ii) The combination of factors of the GLMs with the lowest AIC and BIC values for the log-transformed Cortisol concentrations had substantial support, with a probability of 91% to be the correct model (Table 2). The model with the second lowest AIC and BIC values had odds over 18 times against it being the correct model when compared to the best model in the candidate set. This GLM contained fixed factors Association, Proportion of Associates nested within Association, log-transformed Testosterone concentrations nested within Association, and log-transformed Weight2 nested within Association.

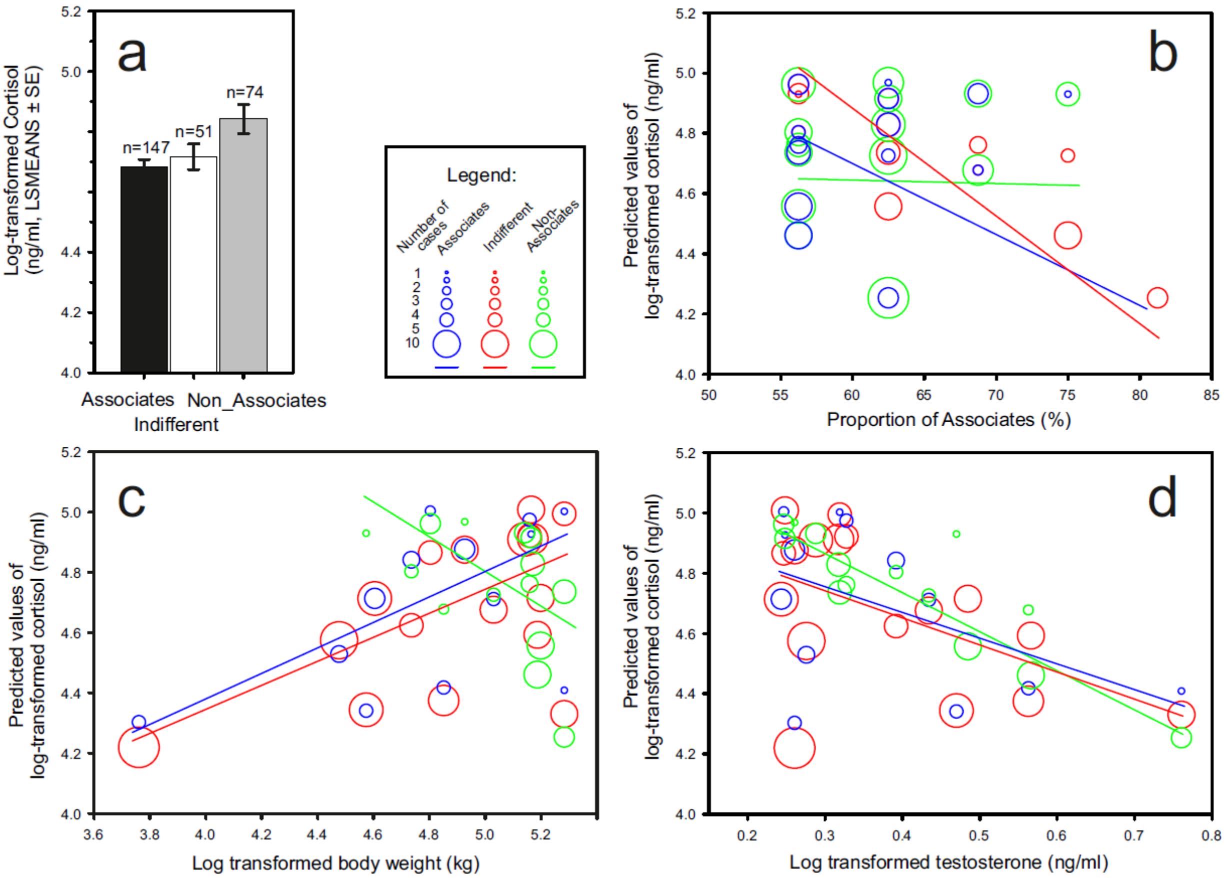

**Figure 5: Log-transformed concentrations of Cortisol (ng/ml, LSMEANs ± SE, n = number of dyads) and a) predicted values of log-transformed Cortisol concentrations (ng/ml) plotted against (b) proportion of Associates (%), (c) log-transformed Body weight (kg), and (and (d) log-transformed Testosterone concentrations (ng). All divided according to Association**

Associates had the lowest Cortisol concentrations, while the Non-Associates had the highest Cortisol concentrations (Fig. 5a). Cortisol concentrations in Associates and Indifferent decreased with increasing Proportion of Associates, while there was no such a relationship in the Non-Associates (Fig. 5b). On the contrary, Cortisol concentrations increased with increasing Body weight in the Associates and Indifferent dyads, while it was the opposite in the Non-

Associates (Fig. 5c). All the three categories of Association showed decreased Cortisol concentrations with increasing Testosterone concentrations (Fig. 5d).

(iii) For the dependent variable log-transformed Testosterone concentrations, the Δ AIC and Δ BIC values nominated two best-fitting GLMs covering similar combination of fixed effects (Table 2). The probability for being the correct model was analogous for these two combinations (56% vs 43%). The best-fitting GLM was only 1.3 times (odds) more likely to be the correct model. On the other hand, the probability of being the correct model was very low (0.01 %) for the third best-fitting GLMs, having odds about 38 times against it. Therefore, the third best-fitting GLM did not need to be considered. The effects of the two best-fitting GLMs were Association, log-transformed Cortisol concentrations, log-transformed Body weight, together with log-transformed Sum of agonistic interactions in one best-fitting GLM. In the second best-fitting GLM, instead of log-transformed Sum of agonistic interactions, there was the Number of Wins. There was apparently no variation in Testosterone concentrations within the Association (LSMEANs ± SE, Associates 0.38 ± 0.01, Indifferent 0.38 ± 0.02, and Non-Associates 0.38 ± 0.01). In no connection with the Association, Testosterone concentrations were generally decreasing with increasing Cortisol concentrations (Fig. 6a).

(iv)In contrast with our expectation, the effect of Dominance was not contained in any of the top fitting GLMS neither for Cortisol concentrations, nor for Testosterone concentrations or Total antler length (Table 2).

Total antler length had the highest number of potential fixed factors. Similarly, as with the Testosterone concentrations, there were two best candidates (Table 2). It is apparent that the selected best model is not convincingly the best. The odds ratio for the GLM with the highest probability to be the best model (57%) versus the second GLM (42%) in order is only 1.3. The odds ratio for the best model versus the third model in order is about 38. The two best-fitting GLMs contained Association, Proportion of Associates nested within Association, Age interacting with Body weight (Weight), and log-transformed Testosterone.

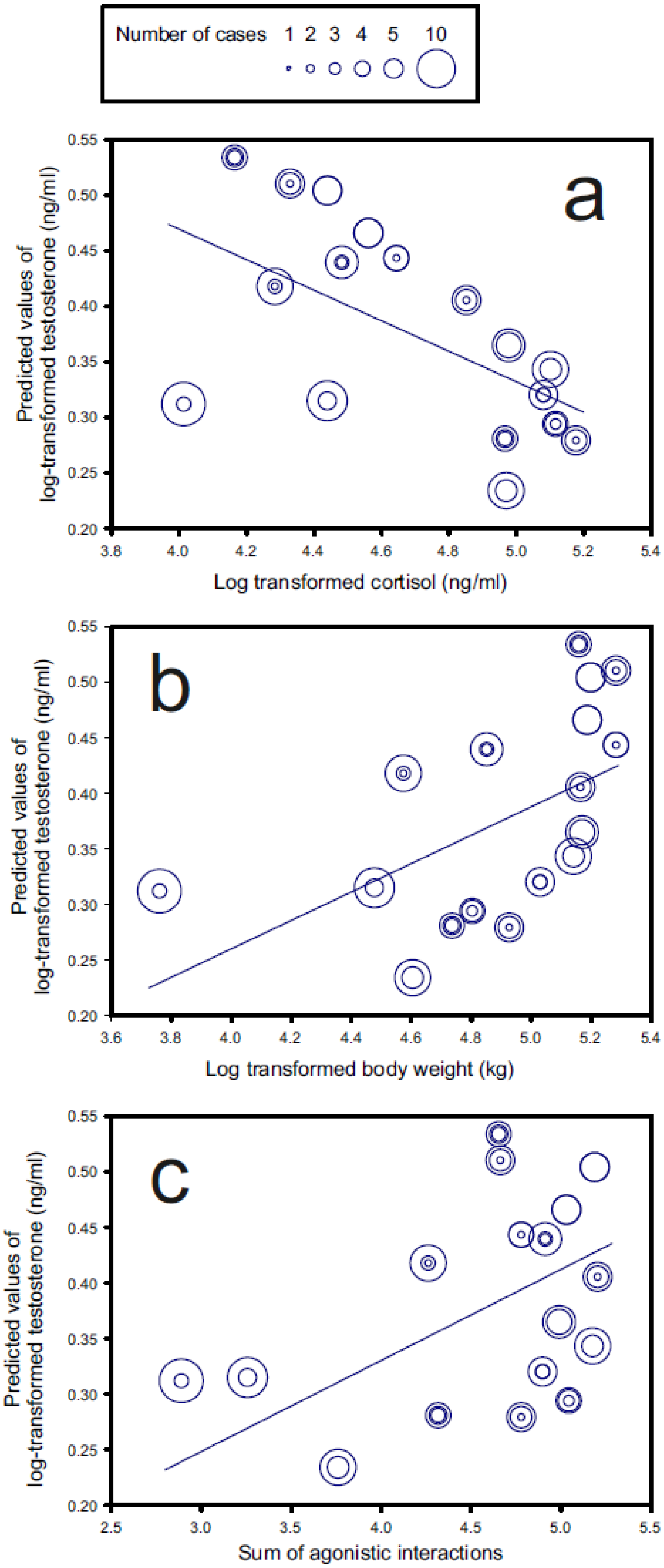

**Figure 6: Predicted values of log-transformed Testosterone concentrations (ng/ml) plotted against (a) log-transformed concentrations of Cortisol (ng/ml), (b) log-transformed Body weight (kg), and (c) the Sum of agonistic interactions**

The models differed by interchangeable highly correlated variables reflecting the aggressiveness of the males (i.e., Number of Wins and Sum of agonistic interactions). Although the effect of Association was part of the best-fitting GLMs, there was very weak indication of a greater Total antler length of Associates, being on average only 4 cm longer than the Indifferent's and Non-Associates' (Fig. 7a). Therefore, the trends of the rest of the fixed effects within the GLM were similar for all three categories of Association. Total antler length decreased with increasing Proportion of Associates (Fig. 7c). However, it increased with increasing log-transformed Testosterone concentrations (Fig. 7b), increasing Age in interaction with increasing Body weight (Fig. 7e) and, finally, with increasing Number of Wins (Fig. 7d) or Sum of agonistic interactions (Fig. 7f).

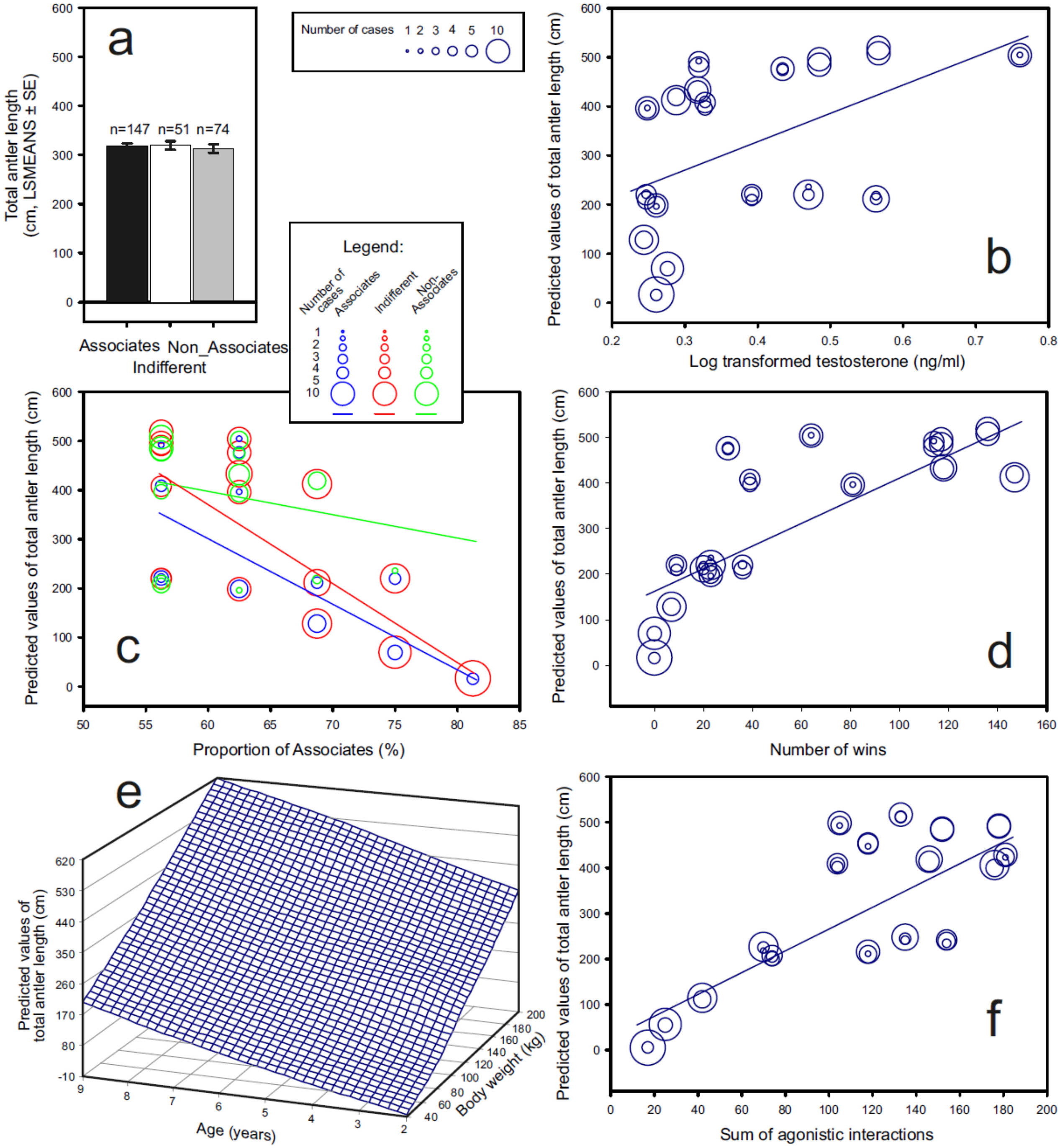

**Figure 7: Total antler length divided according to Association (cm, LSMEANs ± SE, n = number of dyads, a) and predicted values of Total antler length (cm) plotted against (b) log-transformed Testosterone concentrations (ng), (c) Proportion of Associates (%) according to Association, (d) Number of wins or (f) Sum of agonistic interactions, and (e) the interactions between Age (years) and Body weight (kg), and (d). All divided according to Association.**

## Discussion

As expected, (i) part of the males preferred the company of others for a prolonged time, while others did not. The frequency of close dyadic inter-individual distances (Associates) were slightly more common (58% of dyads) than far inter-individual distances (Non-Associates) showing that for the males it was more typical to aggregate than disintegrate during the period of antler growth. Still, more than 42% of dyads belonged to the males roaming around alone, being consistently Distant to all other males. Part of them, the Non-Associates (25% of all dyads), were increasingly aggressive, while the others, the Indifferent (17% of all dyads) remained alone but not attacking others. Since the greatest Sum of agonistic interactions was recorded in the Non-Associates, it supported the idea that at the beginning of the antler growth period the males may choose between alternative social environments with consequences on the aggressiveness and hence testosterone concentrations. We expected two alternative tactics. However, in reality, the data showed three alternatives: a first one -to keep together trying to be surrounded mostly by “friendly” individuals- a second one -to separate from others in order to avoid any agonistic interaction by not-attacking anybody- and a third one -to separate from a stable social situation while irregularly attacking others with an increased frequency.

Also the second prediction (ii) was fulfilled. During stressful situations (such as an attack from others), the presence of close social partners buffered against the adverse effects of increased physiological stress levels (Hennessy et al. 2009). Indeed, the Associates showed the lowest cortisol concentrations (Fig. 5a), and the cortisol concentrations of Associates and Indifferent decreased further with increasing proportion of Associates (Fig. 5b). Thus, to aggregate with others in a socially stable company was apparently a tactic benefitting from the suggested “Social buffering” effect (Cohen & Wills 1985). Association with “friendly” individuals appeared to be a stronger factor than the previously shown factor of Dominance (iv) (Bartoš & Losos 1997; Bartoš & Bubenik 2011).

This is an evidence of a stress-ameliorating effect of social bonding among red deer males under the conditions where the male might make decision whether to join “friends” or others and it represents the most important result of the present study. It supports the general effect of the "Social buffering hypothesis" (Cohen & Wills 1985), beyond the most frequently described mother-offspring, kin or pair bond (Kikusui et al. 2006; Hennessy et al. 2009; Young et al. 2014; Gunnar et al. 2015; Gunnar 2017; McNeal et al. 2017; Yirmiya et al. 2020). It also exceeds the hitherto defined area for buffering effect of males' affiliations in humans (Kirschbaum et al. 1995) and phylogenetically closely-related primates (Young et al. 2014). The results of this study may also contribute to the proposal suggested by Hennessy et al. (2009), according to which there are also many cases in which the ''social buffering" of the HPA axis exists, but has not been observed.

The reason why some males tended to keep in proximity of others or not could have been based perhaps on personality (Jennings et al. 2013; Found & Clair 2016; Esattore et al. 2020), inter-individual relationships and previous experience (Monestier et al. 2016), laterality and/or actual physical condition (Found & Clair 2016).

The fact that increasing Body weight supported an increase in cortisol concentration may have reflected an increasing energy demand of larger individuals, as has also been previously shown in the red deer (Bartoš et al. 2010). On the other hand, the suppressive effect of the increasing testosterone concentrations on the cortisol concentrations suggested that the males had reached the stage of stress characterized by a reduced recovery (uncontrollable) of the neuroendocrine reaction (Koolhaas et al. 2011). Otherwise increasing testosterone concentrations should have elicited also increasing cortisol concentrations to mobilize energy (Toufexis et al. 2014).

(iii) Males avoiding socially stable groupings and attacking others (Non-Associates) did increase their aggressiveness (Fig. 4). All the factors affecting the testosterone concentrations displayed an identical trend across the Association. With increasing agonistic activity (represented by the Sum of agonistic interactions, Fig. 6c) and with increasing size of the male (Fig 6b), the testosterone concentrations also increased. It has been previously shown that high-ranked red deer males are usually involved in the highest Number of agonistic encounters (e.g., Bartoš 1986; Bartoš et al. 2010). It comes from the definition of dominance in the pair-wise relationship, according to which the dominant individual in the dyad must display more attacks than the subordinated one. Thus, in principle, Dominance did affect the results. However, the variation in the Sum of agonistic interactions offered a more accurate representation than the either-or label of dominance.

Testosterone concentrations that did not differ between Associates, Indifferent, and Non-Associates can be interpreted, at first glance, as a non-existing support for the presumed effect of the “Challenge hypothesis”. However, two reasons should be considered. First, males had known each other for extended period. This was the cost for keeping full control over all the experimental animals. Although those who spent most of their time separated outside the main group were the most aggressive ones, competing with otherwise familiar males could not be as challenging as meeting a stranger individual. Second, in a follow-up to the present study, we have found out that even though the males showed behavioural plasticity to some extent, they maintained their diversified aggressive interaction-prone attitude over a three-year period, despite the experimental modifications of the social environment (Esattore et al. 2020). This would fit the opinion of Bell (2020) who has suggested that when individuals have a behavioural type that is somewhat stable over time or across situations, this could result in “behavioural spillovers” and limited plasticity. Then, one may expect hormones to change relatively slowly over time compared to behaviour (Bell 2020). Reviews analysing reports on the “Challenge hypothesis” have indicated the variation of the androgen response among the species (Hirschenhauser & Oliveira 2006; Moore et al. 2020), social stability of the group (Mendonça-Furtado et al. 2014) and the situations tested. It seems that, in general, an increase in androgen levels is a response to mating opportunities rather than a response to a challenge from another male (Moore et al. 2020). On the other hand, there was a visible trend of increasing testosterone concentrations to be dependent on the increased Number of agonistic interactions. Because the Non-Associated males displayed a higher Sum of agonistic interactions, after all, this could be taken as an indication of potential support to the “Challenge hypothesis”. Nevertheless, it needs further investigation.

Increasing cortisol concentrations revealed expected suppressive effect on testosterone concentrations, in agreement with a study realized at the same facility more than ten years earlier (Bartoš et al. 2010). The suppressive effect of the cortisol concentrations on the concentrations of testosterone is further evidence that the competition among males under the given conditions induces stress (Sapolsky et al. 2000; Koolhaas et al. 2011). However this suppression did not appear as strong as the stimulatory effect of aggression did. Moreover, the males preferring the company of non-fighting conspecifics could thus be protected "too much" against the challenging attacks. Lack of challenging encounters, therefore, could fail at producing sufficiently high testosterone concentrations that would stimulate the antler growth much more than what was recorded (Bartoš & Losos 1997; Bartoš et al. 2009).

(iv) Although there was no visible effect of Dominance on the concentrations of testosterone, the stimulatory effect of the increasing Sum of agonistic interactions on the this hormone (Fig. 6c) and, consequently, on the antler growth (Fig. 7d or Fig. 7f) suggested the involvement of an effect of Dominance throughout increased aggression of the males, as previously concluded for the cortisol concentrations. Such a role of the invisible but still present Dominance is in agreement with our previous studies (Bartoš & Bubenik 2011; Bartoš et al. 2012).

The study has presented the relationship between social interactions and antler growth as a dynamic process, involving at least three alternative tactics. Under our spatially restricted conditions, the tactic based on the ''Social buffering" effect (Cohen & Wills 1985) was suggesting very slight trend towards the development of larger antlers, with increased future potential chances for the reproductive success (Bartoš & Perner 1998; Kruuk et al. 2002; Morina et al. 2018). This was probably the reason why the cortisol concentrations in this study exceeded the natural regulatory capacity of an organism rather than primarily fulfilled the energy recourses (Koolhaas et al. 2011) and acted as suppressive social stress (e.g., Blanchard et al. 2001). This was anticipated in our previous studies dealing with rank – related behaviour and antler growth (reviewed in Bartoš et al. 2012). However, it is likely that under quite a different environment, either spatial or social, the tactic utilizing the suggested extended "challenging situations" (Wingfield et al. 1990) could be more effective than the social buffering. Thus, red deer males produced smaller antlers when they lived exclusively with females (one male and a group of females), but larger antlers in the presence of male rivals

(Carranza et al. 2020). Nevertheless, the suggested trade-off situation combining the “Social buffering hypothesis” (Cohen & Wills 1985; Hennessy et al. 2009; Young et al. 2014) and a possible “Challenge hypothesis” effect (Wingfield et al. 1990; Hirschenhauser & Oliveira 2006; Wingfield 2017; Moore et al. 2020) has to be elucidated in the future research, either in a less spatially-constrained situation or in free living populations. Still, our study animals, the red deer, should be taken as a model species with the possibility of applying this methodology to other social animals.

# 4. Study II

## *To beat or not to beat*: Behavioral plasticity during the antler growth period affects cortisol but not testosterone concentrations in red deer males

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### Introduction

As we have already described above (sections 1.1 and 1.2), the red deer is a polygynous ungulate with a high level of sociality and a complex social structure. In this species, males invest much energy in attempt to gain a high rank that will ensure them a privileged access to resources, such as food and mates (Bartoš 1982; Clutton-Brock et al. 1982) .

In our previous study (see Study I), we found that some individuals tended to keep in proximity with others, while others consistently avoided the rest of the herd. It was also observed that some of the animals would repeatedly attack other members of the group, while others were never seen attacking any other deer over the course of the entire period of observation. This variation in social behaviour significantly affected the concentration of cortisol and testosterone.

In general, not all animals perceive the interactions with others in the same way (Sapolsky 1994, 2002; Adkins-Regan 2005). It has been reported that the shy-bold continuum is a fundamental axis of behavioural variation (Wilson et al. 1994), ranging from "shy" (a more risk-averse individual) to "bold" (a more risk-prone individual) (Carere & Maestripieri 2013). Hence, the present study aims to investigate whether the inter-individual behavioural differences previously found (see Study I) might have reflected different and stable individual behavioural characteristics. A more "shy" animal is expected to repeatedly show a lower propensity to attack others across-seasons, meanwhile a high-frequency of attacks would be appropriate for a more "bold" individual (Carere & Maestripieri 2013). Studies performed on a wide variety of species have suggested that some individuals are consistently more aggressive, more exploratory or bolder than other individuals and that these behavioural differences are often heritable (Boake 1994; Stirling et al. 2002; Kölliker 2005; Van Oers et al. 2005). The evidence shows that "aggressiveness" is one of the most repeatable behaviours across taxa (Bell et al. 2009).

Given these past observations, it was initially hypothesized that, if attacking others was based on individual attitude, the red deer stags could be identified in terms of the number of attacks against others, with the categories of "Non-Fighters" (NF, i.e., "shy" animals rarely initiating aggressive encounters) and "Fighters" (F, i.e., "bold" individuals frequently initiating agonistic interactions). This behaviour would then be seen repeatedly in the following seasons and within diverse social situations. Thus, the NF males, who are naturally less keen on physical competition, are expected to avoid attacks; on the contrary, the F males are expected to attack others, due to their aggressive and physical-contact prone nature.

With regards to hormones, it is known that testosterone influences sexually selected behaviours (Pelletier et al. 2003; Hau 2007), e.g., stimulating aggression and territoriality (reviewed in Eisenegger et al. 2011) and promoting dominance displays (Bartoš et al. 2000; Mills et al. 2009). Increasing testosterone requires increasing cortisol to fulfil the energy recourses (Koolhaas et al. 2011) and a short-term production of glucocorticoids can be considered an adaptive adjustment to the social context (Wingfield et al. 1998), as long as it does not exceed the natural regulatory capacity of the organism (see section 1.3 for a more detailed description).

For these reasons, we expected the NF to have lower concentrations of cortisol than the F, and vice versa. Conversely, the F will have higher concentrations of testosterone than the NF, and vice versa.

## Material and methods

For this study, 18 tame red deer males were kept in a group always within three pens (for more details about the facility, see section 2). Although the experiment commenced with 18 deer, four males were removed from the data sets in 2016 and 2017 due to unpredictable events, reducing the final sample count to 14 individuals and resulting in an uneven number of individuals in the groups. In the analysis we omitted all data obtained from the removed males.

The study was developed in two steps: a preparatory one for 2015, in order to obtain information regarding basic characteristics of the males involved in the study, and an experimental one for 2016 and 2017 (data from both seasons in one dataset). First, during the period between June and August (i.e., during the most significant time of the antler growth period) in 2015 we observed the males when the animals were kept together within one group over the whole season to gather information enabling the recognition of the "NF" and "F" males. Second, to analyse the presumed consistency of the individual proneness to attack others, two different experimental settings were arranged and developed in a two-year timespan (2016, 2017). Both in 2016 and 2017 males were kept together over the winter until the last week of June, and were then split into two groups where they were observed. In September the animals were released back into one group until the next June.

Below, the method of grouping the animals is described in detail:

(i) In 2015 the males remained in the same group during the whole season. The instances of attacks were observed from June to August. To distinguish which males were more aggressive than others, a matrix of attacks was prepared between all members of the group over the period of observation. Given the results of the matrix, each male was subjectively labelled as either "NF" or "F" with respect to the number of attacks recorded during the sampling seasons. We created the group of "F" out all those animals that had shown a high number of initiated interactions and the "NF" with low number of initiated interactions – one "NF" male had never initiated any interaction. It resulted in two groups with a clear gap between them (Fig. 1 left). The labelling of males as NF or F will be further called "Fighting characteristic".

(ii) During the 2016 season, the males were split into two groups according to the Fighting characteristic and kept in separate enclosures. The first group consisted of NF (n=6) and the second group of F (n=8) males.

(iii) In the 2017 season, the herd was split into two groups, "Group 1" (n=8) and "Group 2" (n=6), each planned to be composed of an equal proportion of males which were classified as NF or F in 2015. However, due to the previously-mentioned removal of some males, Group 1 consisted of 3 NF males and 5 F males and Group 2 consisted of 3 NF males and 3 F males.

Experimental observations were focused on the recordings of agonistic encounters between the animals when competing for supplemental food. For each season, the observations of agonistic interactions were initiated at the beginning of April and terminated at the end of August. However, to ensure that the seasons were comparable, the data from the period of June to August were used each year. The observation session ranged from 20 to 60 minutes and took place for an average of 3 times per week, between 9.00 a.m. and 11.30 a.m., adjusting for one hour of observation for each observation session. Before the observation started, we supplied the deer with the previously-described mixture (see section 2). The food was carried to the observation place in a wheelbarrow and presented in several piles to encourage the competition over a scarce resource without excessively exacerbating it. The piles were tilted from the wheelbarrow about 2 meters apart, in a number of piles equal to the number of the stags/2. This method has already been proven valid by other studies using the same way to observe agonistic interactions in ungulates (Vymyslická et al. 2015), deer in particular (Dušek et al. 2007; Bartoš et al. 2010), regardless of the characteristics of the enclosure. The main point is that the animals were attracted to the observation point (a delimited area) and the observation continued until the food had been depleted and the animals left.

All deer were tame and started the competition straightaway without any delay. Deer immediately ran from one pile to another, trying to eat as much as possible. When the eating deer was attacked, it either escaped to another pile or repulsed the attack. The observation started at 9.00 immediately after supplying the supplemental food and lasted until the deer ate it and all animals left the place. When we divided the herd into two groups, the observation started at 9.00 in one of the two groups and continued with the other group after it was finished in the first one for the same time of observation. The order of the group changed regularly to avoid any bias in the observation. The time of observation was consistently very similar in both groups.

The weather affected the length of the observation rather than the composition of the groups (e.g., shorter time in hot days, etc.). Observations were recorded using a voice recorder and then transcribed into a table using Microsoft Excel. Any attack, threat or fight, which caused an apparent displacement of the approached individual, was recorded.

Each month (from June to August), the males were weighted and blood samples were collected from the jugular vein in a physical restraining facility (“*crush*”), as described in the Study I.

### Hormone analyses

Hormone concentrations were measured as described in the analogous section of the Study I.

### Statistics

All data were analysed using the SAS System (SAS, version 9.4). Values for the countable variables are presented in Table 3. Count variables “Body weight” and “Testosterone” were log-transformed (natural logarithm transformation) to improve normality of residuals and to reduce skewness.

To check for possible multicollinearity, we first calculated correlation (PROC CORR) between the individual variables listed in Table 3. A significant correlation was found only between the Age and Weight of the deer ($r=0.79$, $P<0.0001$). This correlation was so strong that applying the collinearity test was not meaningful.

The individuals labelled NF or F in 2015 were marked the same way in 2016 and 2017 for the analysis. We used a multivariate Generalized Linear Model (PROC MIXED). The model was applied in a different way for the preparatory and the experimental parts of the study. In 2015 the procedure was used as a fixed-effect model comparing all countable variables between NF and F males, to see whether the Fighting characteristic was the only variable for the males to differ. Therefore, for each of the traits (Table 3), the model always contained Fighting characteristic (NF and F) as a fixed effect. Then the model was revised with the addition of the other countable variables (Table 3) which might have potentially influenced the result. When appropriate, we also tested their interactions. Any factors which did not reach the level of statistical significance ($P > 0.05$) were dropped from the model.

For the analysis of the experimental aspect of the study, the GLM was designed for the repeated measures (i.e., in SAS, with REPEATED = Season, controlling the covariance structure across the two seasons, and the SUBJECT = identity of the male defining that the repeated observations belong to the same subject). The best-fitting covariance structure for the repeated measures (estimated according to Littell et al. 2000) appeared compound symmetric (TYPE=cs). For GLMs, the model always contained an interaction between Fighting characteristic and Season (2016 and 2017) as a fixed factor. Other factors were then added to the GLM (Table 1). Non-significant factors were dropped from the model and will not be mentioned any further.

Comparability and repeatability of measurement is a topic that many researchers have discussed (Nakagawa & Schielzeth 2010; Johnson & Waller 2018). Since a bias is expected in the sum of attacks between the seasons due to differing social environments, we chose Lin's Concordance correlation coefficient (calculated and described by Johnson & Waller 2018) and Kendall's tau-b correlation coefficient (using PROC CORR) to estimate a measure of association of the agonistic activities of the same subjects between the 2016 and 2017.

To check the first hypothesis that attacking others was based on the Fighting characteristic of each male, the sum of attacks per individual per season recorded between June and August ("Sum of attacks") was set as a dependent variable. To check the second hypothesis, Testosterone and Cortisol concentrations were used as the dependent variable and the GLMs were constructed as above.

Least squares means (LSMEANs) were calculated for the categorical fixed effects by computing the mean of each treatment and averaging the treatment means. These means of means were then used to compare the factors.

| | 2015 | | 2016 | | 2017 | |
|---|---|---|---|---|---|---|
| Variable | Mean | SEM | Mean | SEM | Mean | SEM |
| Sum of attacks | 116.43 | 17.5 | 406.43 | 44.5 | 385.57 | 36.88 |
| Body weight (kg) | 124.99 | 8.30 | 142.32 | 7.01 | 163.82 | 4.63 |
| Age (years) | 4.57 | 0.64 | 5.57 | 0.64 | 6.57 | 0.64 |
| Cortisol (ng/mL) in plasma | 113.78 | 11.15 | 106.29 | 7.53 | 140.18 | 16.19 |

|  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|
| Testosterone ng/mL) in plasma | 37.50 | 9.12 | 31.25 | 7.98 | 51.68 | 11.87 |
|  |  |  |  |  |  |  |

**Table 3: Count variables (mean ± SE) for observational seasons (2015, 2016, 2017)**

## Results

A total of 1630, 5690 and 5398 attacks were recorded in 2015, 2016 and 2017, respectively. For the analysis of the recorded attacks, data were adjusted for one hour of observation for each observation session.

In the preparatory period (2015), NF and F males differed only in the Fighting characteristic. The Sum of attacks was dependent on the Fighting characteristic ($F_{(1,\ 11)}$=6.41, P=0.02, Fig. 8 left) and log-transformed Body weight ($F_{(1,\ 11)}$=6.41, P=4.88 Fig.9). With increasing Body weight, the Sum of attacks also increased (Fig. 9).

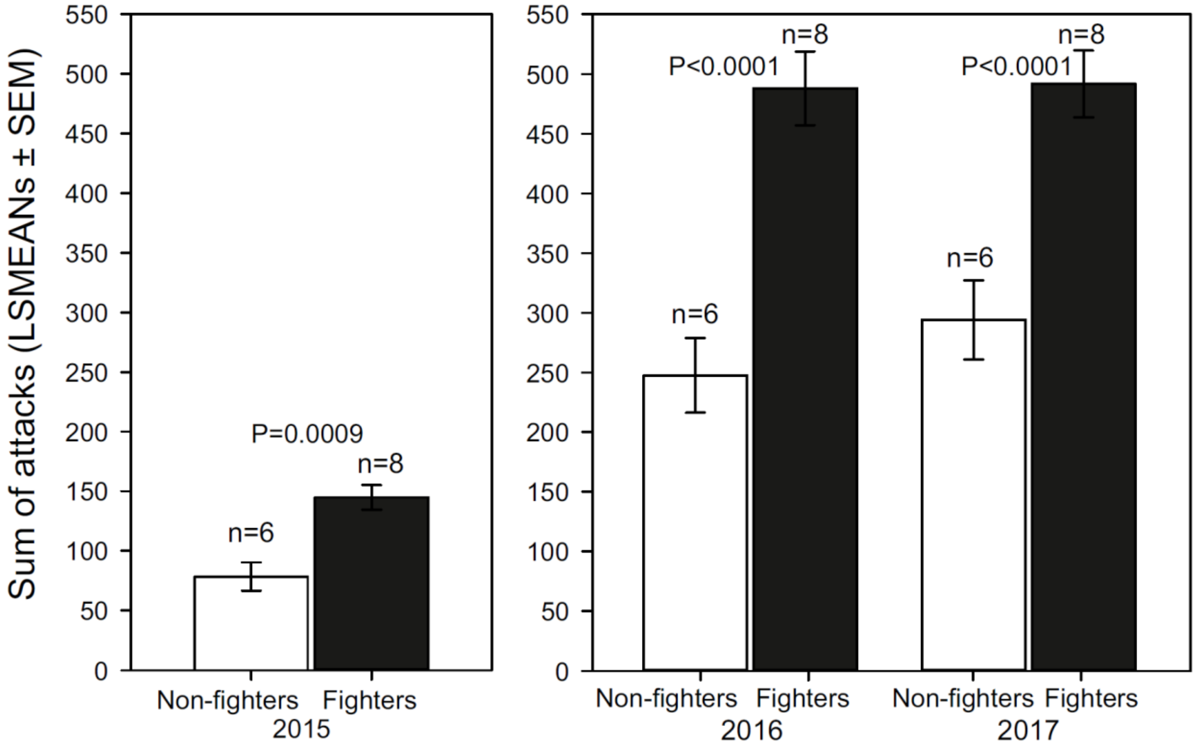

**Figure 8: Sum of agonistic interactions of Fighters and Non-fighters (LSMEANs ± SE) in 2015 (left), 2016, and 2017 (right).**

None of the other measures differed between NF and F males in 2015 (mean ± SE, Age - NF 4.83 ± 1.05, F 4.38 ± 0.84 years, $F_{(1,\ 11)}$=6.41, NS; Body weight - NF 124.41 ± 12.24, F 125.42 ± 11.97 kg, $F_{(1,\ 11)}$=6.41, NS; Cortisol - NF 114.53 ± 14.25, F 113.21 ± 17.18 ng/mL, $F_{(1,\ 12)}$=0.06, NS, Testosterone – NF 34.92 ± 9.91, F 39.43 ± 14.71 ng/mL, $F_{(1,\ 12)}$=0.02, NS).

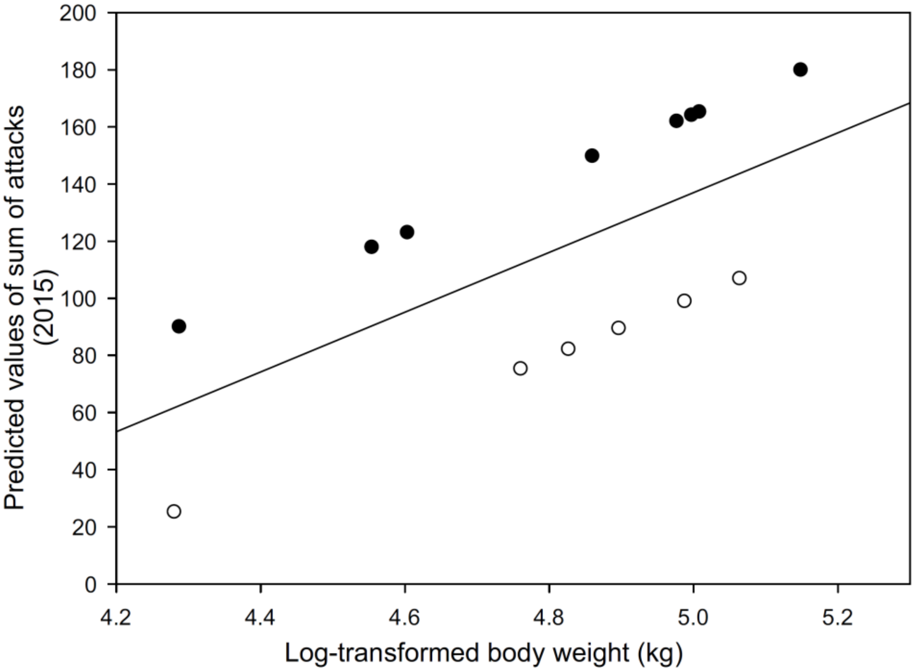

**Figure 9: Predicted values of the Sum of attacks plotted against log-transformed Body weight (kg) in 2015 (Fighters - black dots, Non-fighters – white dots).**

During the experimental phase of the study, the Sum of attacks was dependent on the interaction between Fighting characteristic and Season ($F_{(3,\ 19.8)}$= 13.55, P<0.0001, Fig. 8 right) and log-transformed Body weight ($F_{(1,\ 20.3)}$=7.89, P=0.01, Fig. 9). In both seasons, the F males attacked the others almost twice as much as the NF males on average, and there was no difference of the Sum of attacks between the seasons neither for the NF nor for the F males (Fig. 8, right). With increasing Body weight, the Sum of attacks decreased (Fig. 10). Lin's Concordance Correlation was relatively high ($\rho_c$=0.694, 95% CI: 0.190 – 0.909), as was Kendall's tau-b (tau=0.498, p<0.01).

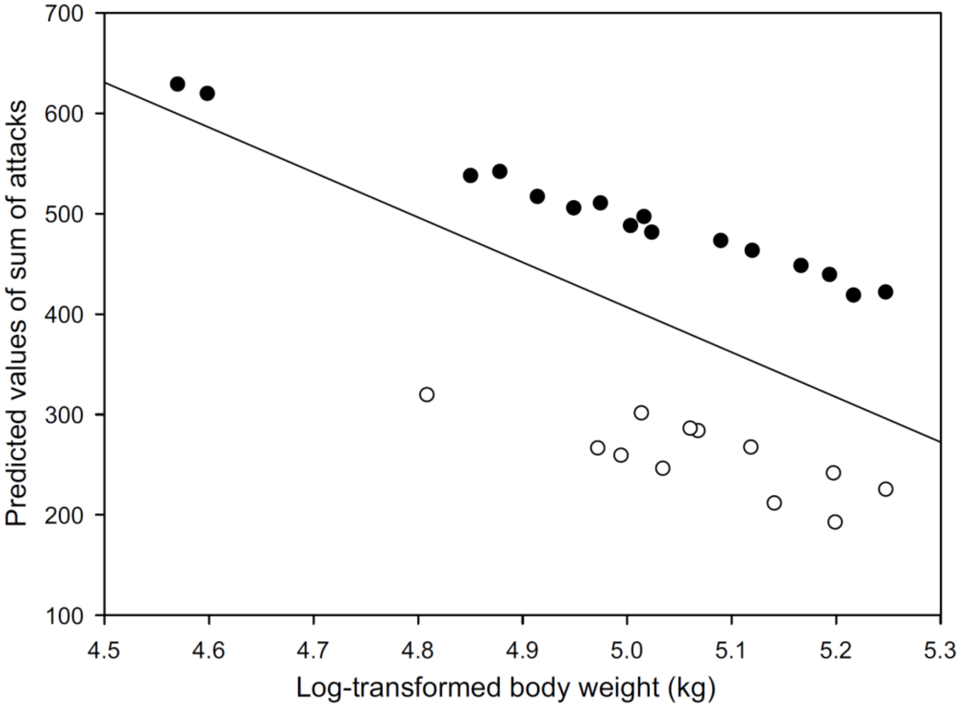

**Figure 10: Predicted values of the Sum of attacks plotted against log-transformed Body weight (kg) in 2016 and 2017 (Fighters - black dots, Non-fighters – white dots).**

Experimental changes in the social situation affected Cortisol concentrations by the interaction Fighting characteristic*Season ($F_{(3,\ 18.2)}$=3.48, P=0.04, Fig. 11 right) and between the Number of defeats and log-transformed Testosterone ($F_{(1,\ 23.7)}$=6.52, P=0.02, Fig. 12). The NF males tended to have (P=0.07 in 2016) or had (P=0.04 in 2017) higher Cortisol concentrations than the F males. The NF, as well as the F males, increased the Cortisol concentrations in 2017 when compared with 2016 (Fig. 11 right). It reached significance only in the F males, however, who doubled the Cortisol concentrations. The cortisol concentrations increased with increasing Number of defeats, as they did with increasing Testosterone concentrations. This was true independently of the season.

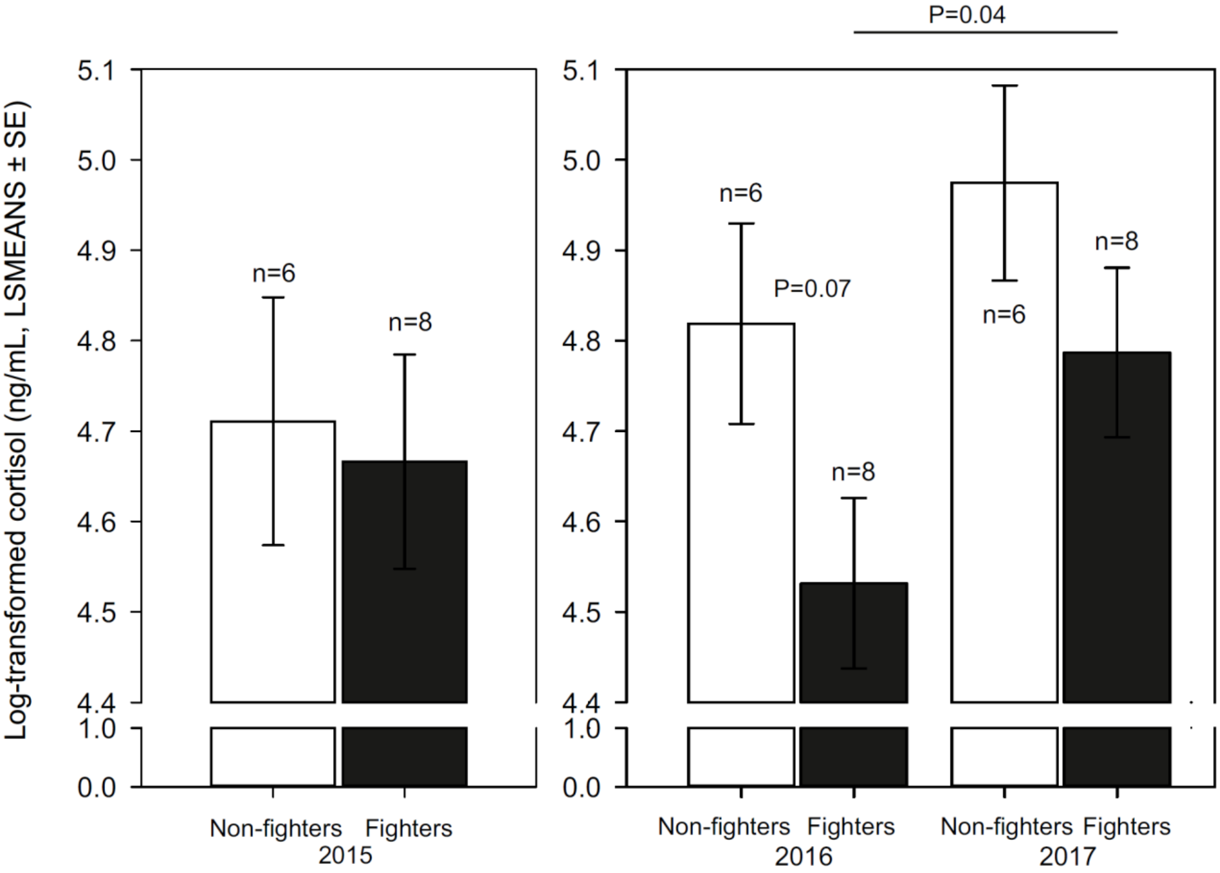

**Figure 11: Log-transformed Cortisol concentrations (ng/mL, LSMEANs ± SE) between Fighters and Non-Fighters in 2015 (left) and in 2016 and 2017 (right).**

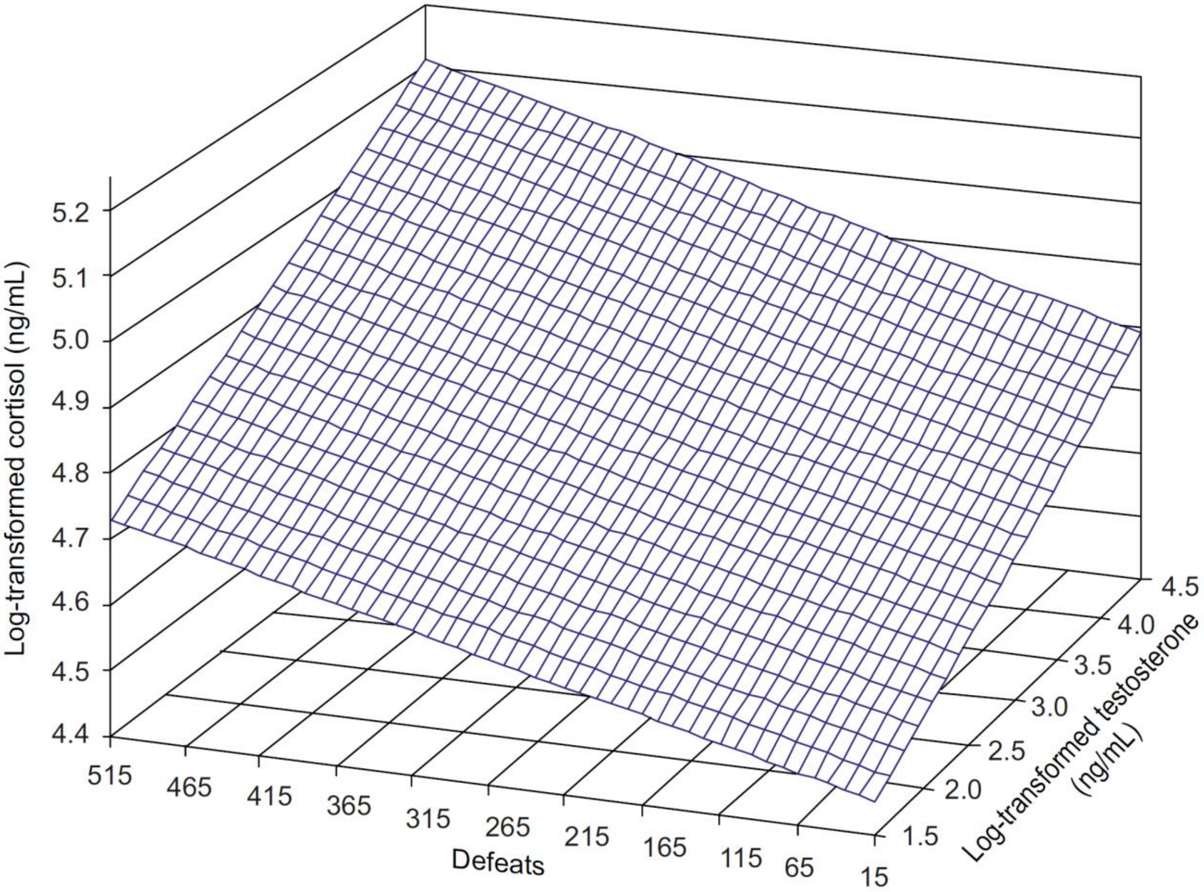

**Figure 12: Log-transformed Cortisol concentrations (ng/mL) plotted against the Number of defeats and log-transformed Testosterone concentrations (ng/mL) in 2016 and 2017 (right).**

The social environment in 2016 and 2017 affected testosterone concentrations according to the interaction Fighting characteristic*Season ($F_{(3,\ 18.2)}$=2.97, P=0.059, Fig. 13 right) in a very similar way as it did with the Cortisol concentrations. The NF males tended to have higher Testosterone concentrations than the F males. However, the pairwise comparison did not reveal any significant difference between the NF and F the males. Moreover, both the NF and the F males tended to increase the Testosterone concentrations in 2017 (compared to 2016), but only the F males had statistically higher T in 2017 than in 2016. The GLM was initially built with the interaction Fighting characteristic*Season. Once we added the Cortisol concentration as the second fixed factor, the effect of the interaction diminished in favour of highly significant dependency on Cortisol concentrations. Consequently the GLM containing the Cortisol concentration as the only fixed factor showed a high dependence of Testosterone concentrations on Cortisol concentrations ($F_{(1,\ 18.2)}$=12.08, P=0.003, Fig. 14).

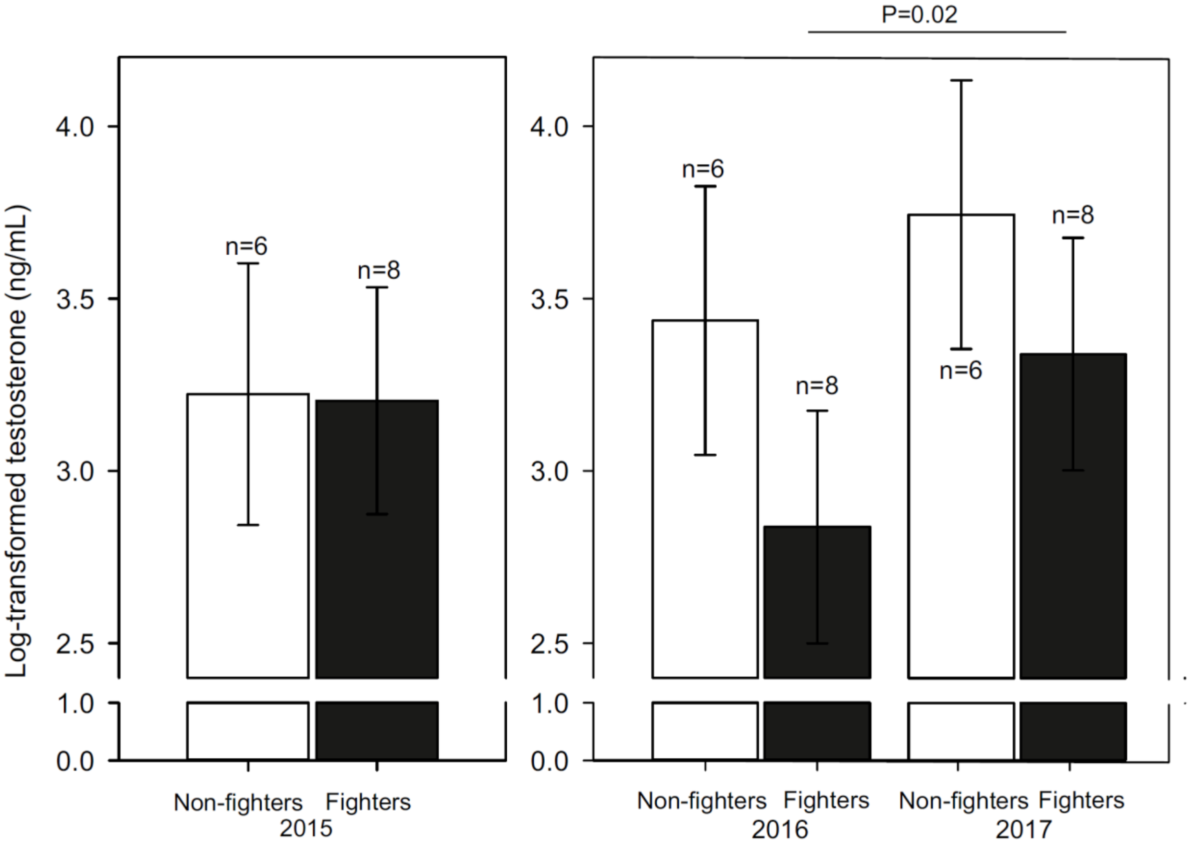

**Figure 13: Comparison of the log-transformed Testosterone concentrations (ng/mL, LSMEANs ± SE) between Fighters and Non-Fighters in 2015 (left) and in 2016 and 2017 (right).**

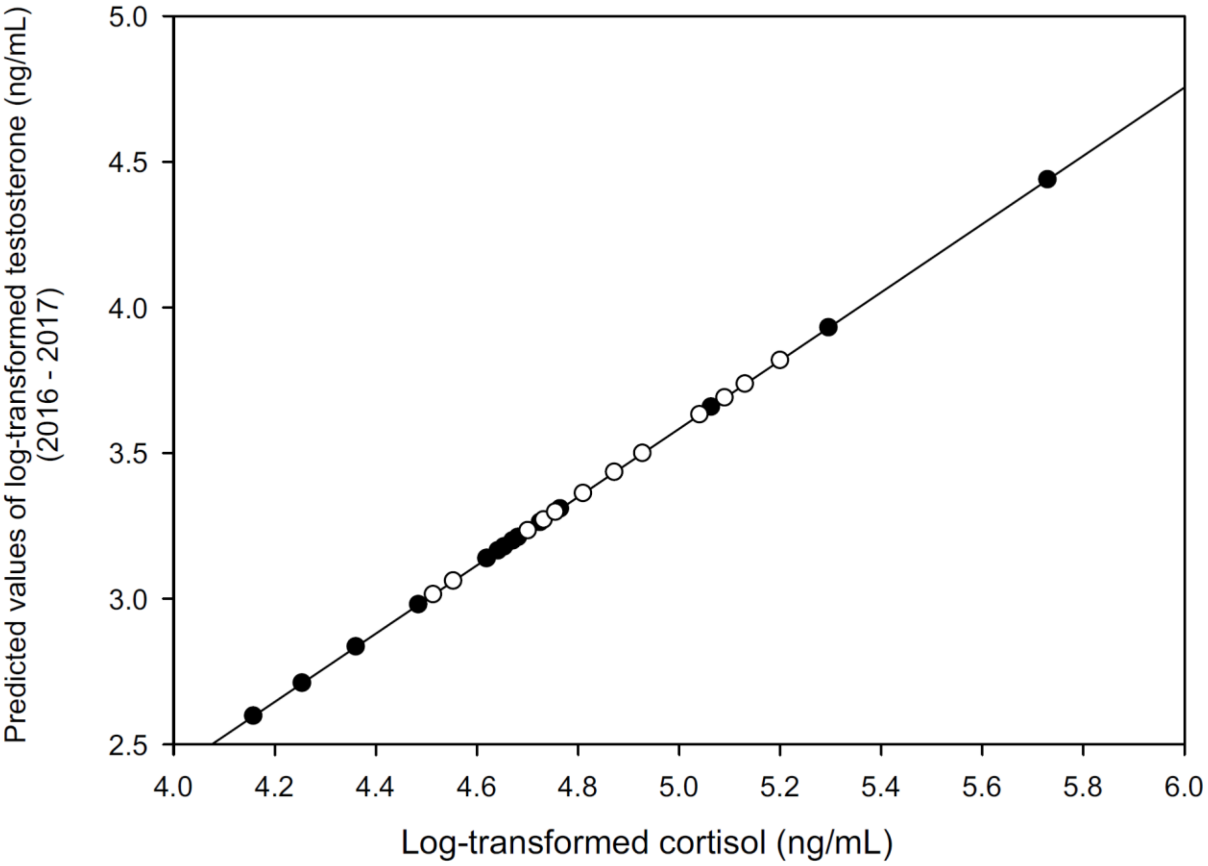

**Figure 14: Predicted values of log-transformed Testosterone concentrations (ng/mL) plotted against log-transformed Cortisol concentrations (ng/mL) in 2016 and 2017 (Fighters - black dots, Non-fighters – white dots).**

## Discussion

Given the expectations that the male members of the red deer group would engage with others consistently either in a “peaceful” (NF) way or in an “aggressive” (F) way, we investigated the differences in frequency of attacks to verify whether this engagement was due only to the individual fighting characteristics or not. This method was helpful to validate the accuracy of the successive division of the herd into two subgroups. In agreement with the expectations, it was found that the only measure which differed between the NF and the F in the preparatory period (2015) was the Fighting characteristic, and that none of the other measures happened to be different between the two groups. In this way, it was confirmed that the selected criterion of division was appropriate and that the differences between the two groups could be only ascribed to the different individual fighting attitude itself.

It was hypothesized that, after the division into two subgroups, the members of the NF group would have kept showing a lower number of attacks than the members of the F group, as a recognizable individual feature. Consistently with the initial expectations, our results showed that not only the NF males attacked others in fewer cases than the F males throughout both the two experimental seasons, but also that the F had a frequency of attacks double than that of the NF.

Curiously, the Sum of attacks and the Body weight of the animals were directly proportional during the preparatory period (2015), with the Sum of attacks increasing with the increase of the Body weight. However, it was inversely proportional in the experimental period (2016, 2017), with the Number of attacks increasing with the decrease of Body weight. It is likely that, being nested in a bigger group where the animals were accustomed to each other and the hierarchy was more or less defined (2015), the smallest animals did not take part in most of the interactions, leaving the agonistic encounters to the largest individuals. Changing the social context and turning it into a socially unstable environment (2016, 2017), also the smaller animals took the chance to improve their position in the hierarchy.

When splitting the socially stable group of males, it is widespread to register a sharp increase of attacks in the two smaller subgroups, because the hierarchy must be re-defined (Sapolsky 1983; Gust & Gordon 1991; Cavigelli & Pereira 2000; Kaburu & Newton-Fisher 2015). However, the individual behaviour can be adapted to different environmental conditions (Briffa et al. 2015) to allow the animal to appropriately respond to changing environmental stimuli (Coppens et al. 2010) (see "Behavioural plasticity" in Komers 1997). Moreover, it might be the case that less aggressive individuals show higher behavioural plasticity than the most aggressive ones (Natarajan et al. 2009). Thus, individuals with higher levels of a certain behavioural feature such as “aggressiveness” would not need to adjust their behavioural response to the different social context as they would be able, in any case, to achieve a sufficiently high level of fitness. Conversely, individuals with a lower expression of a specific behavioural trait must compensate for this gap by being able to adapt their behaviour with respect to the different environmental conditions (see "Compensatory hypothesis" in Betini & Norris 2012). In this particular study, an increase in attacking others did happen after each split of the whole herd into smaller groups both in 2016 and 2017. On the other hand, the increase of the frequency of the attacks was significantly more pronounced in the F than in the NF males (Fig. 8). Being in smaller and socially unstable groups, along with having the most aggressive individuals removed, the NF males may have taken advantage of the situation and started attacking each other more often than they used to in the complete large group. However, their individual characteristic of being less aggressive had not been completely lost, as they continued to show a significantly lower frequency of attacks than the F males who had been put in the same, socially unstable situation. Both methods of measuring the correlation coefficients which were used to analyse the observations throughout the study (Lin's Concordance correlation and Kendall's tau-b correlation) gave similar results, indicating relatively good concordance of the sum of attacks between 2016 and 2017. The fact that the NF males kept manifesting a lower frequency of attacks than the F males, as a sign of their less-interaction-prone nature, could represent the first element to start investigating the personality in the red deer.

Personality has been reported in several species (as reviewed in the paragraph 1.7); however, there has not yet been an extensive investigation of the personality in cervids (see Bergvall et al. 2011; Jennings et al. 2013; Monestier et al. 2016; Found & St. Clair 2018).

As already presented by Fox et al. (2009), extensive knowledge of animal personality could play a major role in understanding variation in ecologically relevant behaviours [e.g., dispersal (Dingemanse & de Goede 2004), acquisition of dominance ranks (Fox & Millam 2010), group joining preferences (Harcourt et al. 2009), foraging (Wilson & McLaughlin 2007)] and could therefore add another piece to the puzzle that is the biology of the deer species. Moreover, the application of this knowledge to a context of captivity may represent an important instrument in guaranteeing high welfare standards in animal production (Wechsler 1995; Müller & Schrader 2005; Van Reenen et al. 2005; Cockrem 2007; Koolhaas et al. 2011; Wolf & Weissing 2012), farms (Forkman et al. 1995; Veissier et al. 2012; Graunke et al. 2013) and zoos (Tetley & O'Hara 2012; Racevska & Hill 2017; Williams et al. 2019).

In terms of hormones, the experimental change in social situation affected the concentrations of cortisol, as there was an effect of both the interaction Fighting characteristic*Season and between the Number of defeats and log-transformed Testosterone. In contrast to the initial expectations, to split the males into the NF and the F groups did not reveal lower cortisol concentrations in the NF males. In fact, the very opposite occurred, as the NF males had (2016) or tended to have (2017) higher cortisol concentrations than the F males. In both groups, the concentration of cortisol was higher in 2017 than in 2016, reaching the significance only in the F males, which doubled the concentration of cortisol. Cortisol concentrations increased with increasing Number of defeats and increasing testosterone concentrations.

With respect to the testosterone concentrations in 2016 and 2017, it was observed that the social environment caused an effect according to the interaction Fighting characteristic*Season in a very similar way to the one recorded for the cortisol concentrations. The NF males tended to have higher testosterone concentrations than the F males; however, there was no significant difference between the two groups. As it can be imagined, higher levels of testosterone will trigger the aggressive behaviour (Pelletier et al. 2003) and promote a persisting agonistic behaviour, resulting in an increased metabolic demand to face the enhanced agonistic activity. Defeat itself represents a negatively perceived occurrence for the animals, especially since the possibility of improving their position in the hierarchy and consequently the possibility to have a privileged access to the resources are at stake. It was first hypothesized that the cortisol concentrations in the NF males had increased due to the new, possibly more hostile and distressing social environment, in which they had been placed. This would have also been supported by the relation that has been discovered between concentration of cortisol and the interaction between concentration of testosterone and Number of defeats. As it is logically explained, higher concentrations of testosterone trigger the aggressive behaviour, increasing the motivation of the animal to be involved in agonistic encounters. The enhanced physical activity thus requires a major metabolic effort and this, together with the discomfort deriving from losing a fight, would result in higher concentrations of cortisol. However, given the fact that no suppressive effects of cortisol concentrations on testosterone concentrations were observed, it may be suggested that the demands for increased cortisol concentrations did not reach the level of a stressful situation (Koolhaas et al. 2011) and, therefore, it should just be ascribed to the increased metabolic demands which derive from the enhanced social competition among the males (Koolhaas et al. 2011; Casto & Edwards 2016).

## Conclusion

Inter-individual differences in fighting attitudes have been shown to be consistent across different social environments. Thus, whereas the F males kept showing a higher inclination to fight after the split of the herd into two groups, also the NF males increased the frequency of attacks, even if this was lower than for the F males. However, despite the sharp increase of attacks that occurred in both groups due to an experimentally-induced social instability, the NF males kept manifesting a lower frequency of attacks than the F males, as a sign of their less-interaction-prone nature. Once half of the competing individuals were removed from their surroundings, it is clear that both the NF and the F took advantage of the situation and started competing more in order to reach a higher position in the social hierarchy. Therefore, both in 2016 and in 2017, splitting into two groups for three months induced the same motivation to compete and to improve their rank. Because this happened in both seasons, the actual social composition of the groups did not have any significant effect. This possibility was also supported by the evidence of an inversely proportional relationship between frequency of attacks and Body weight, a sign that not only the bigger animals were involved in the agonistic interactions but also those that had not tried to improve their rank in the preparatory period (when the frequency of attacks was directly proportional to the weight of the animals). As a consequence of the social instability, the NF males increased their cortisol concentrations in order to face the accentuated metabolic needs. The concentration of cortisol was also directly affected by the Number of defeats and by the log-transformed Testosterone concentration. This was likely due to the experimentally more challenging and hostile environment the NF were grouped into and the consequent higher energy demand necessary to face new threats and advantages of the new social situation. However, the testosterone concentrations did not differ between the groups. From the aspect of the physiology of behaviour, one might expect a correlation between aggression and testosterone (Lincoln 1972; Book et al. 2001; Giammanco et al. 2005; Martin et al. 2013). In a new social environment, it might be more difficult for the NF males to cope with a higher level of inter-individual agonistic interactions than for the F males. If so, the NF males' testosterone should have tended to be more elevated than in F males. In feedback, this would have resulted in the cortisol elevation as discussed above.

# 5. Study III

## *"How not to judge a deer by its cover": a personality assessment study on adult captive red deer males*

**Submitted to Behavioural Processes**

### Introduction

The phenomenon of animal personality has been one of the most intriguing challenges in behavioural research (Wolf et al. 2007; Wilson et al. 2019) and it is usually described as "*between-individual behavioural differences consistent across time and contexts*" (Sih et al. 2004; Pervin et al. 2005; Réale et al. 2007; 2010). During the last decades, animal personality research has flourished, also due to its connection with inter-individual variation in ecologically relevant behaviours (Réale & Montiglio 2020) and researchers have identified several important axes of animal personality along which individuals can be placed (e.g., shyness – boldness, exploration – avoidance, aggressiveness – cautiousness, fast and superficial explorers – slow and thorough explorers, proactive – reactive stress coping), and differences in activity and sociability (Koolhaas et al. 1999; Réale et al. 2007; Bergmuller & Taborsky 2010; Finger et al. 2018) in a wide range of organisms.

Despite the fact that the number of species in personality studies has steadily increased in the last decades, the literature investigating the consistency of behavioural patterns in cervids appears, to this day, still surprisingly scarce and counts only few studies (e.g., Bergvall et al. 2011; Jennings et al. 2013; Found & Clair 2016; Found & St. Clair 2018) , as already described in the paragraph 1.7.

Following the results found in the Study II, in this study we investigated the personality of adult red deer males and its links with naturally occurring behaviour, to see whether trait ratings assessed with questionnaires fully capture red deer behavioural variation as assessed with behavioural observations. In particular, we aimed to (i) describe personality of adult red deer males by using trait ratings and (ii) link deer personality with their dominance rank in bachelor groups.

We decided to focus only on adult males of the herd (n=11) that were minimum five years old, and up to ten years old (median age: 7). Thus, according to Bell et al.(2009), males' behaviour is more repeatable than the females' for two main reasons: first, testosterone would make the males to be more predictable than the females (Andrew & Rogers 1972; Wingfield & Hahn 1994) and second, due to sexual selection by females, because showing predictability in certain behavioural traits is a reliable predictor on males' behaviour in another context (Kokko 1998; Garamszegi et al. 2006; Bell et al. 2009).

We studied only adult animals since, as described in humans (Roberts & DelVecchio 2000), mallards (*Anas platyrhynchos*) (Butler et al. 2011) and red squirrels (*Sciurus vulgaris*) (Kelley et al. 2015), personality is shown to go through changes along with development (Stamps & Groothuis 2010; Class & Brommer 2016), and the consistency of behaviour increases with maturity (Øverli et al. 2007).

We predicted that the male red deer individuals would consistently differ in their personality traits.

Being inspired by the results of Study II, we wished to go deeper in details associated with the inter-individual differences and expected it would cover a complex personality.

Differently from the Study II, where we had focused just on aspects related to the agonistic activity across the different social contexts, in the Study III we extended the investigation of the inter-individual differences to more, unrelated contexts (namely a "stimuli-free" context, a context of competition over a scarce resource and a situation of possible discomfort due to human manipulation, as described in the material and methods of Study III).

Moreover, in the Study III we did not focus just on the agonistic behavioural repertoire of the species as in Study II, but we combined two different approaches: a "bottom-up" and a "top-down" approach. In the "bottom-up" approach we focused on the whole behavioural repertoire of the deer –not being anchored only to the agonistic activity as in the previous study- trying to depict a more complete picture of the behavioural patterns of the species across different situations. Conversely, in the "top-down" approach, three raters scored the animals to a list of fifteen items, trying to detect differences in how the items illustrated the characteristics of the single animals.

Last, we predicted that differences in male deer personalities would be linked to their differences in ranks, namely that the boldest and/or more aggressive males would have also been higher in rank and vice versa.

## Material and Methods

Twenty-five tame red deer males (11 adults aged 5-10 years, and 14 juveniles, aged up to 4 years) were kept in three inter-connected enclosures, covering an area of approximately 2 ha and housed separately from the females (for more details about the facility, see paragraph 2).

### *Behavioural Observations*

The observations were performed during three different situations: “normal” (i.e., no external stimuli), “feeding” (i.e., animals compete for a limited amount of food), and “handling” (i.e., animals undergo a situation of stress), between April and July 2018, between 9.00 a.m. and 11.00 a.m. During the “normal” and the “feeding” observations, the animals were observed in a group setting, whereas during the “handling” observations they were observed in an individual setting. All the observations were recorded on a voice recorder and successively transcribed into a table using Microsoft Excel. Questionnaire raters acted as observers during “normal”, “feeding” and “handling” situations, thus their overall impression of the deer was based on all three situations. However, for the calculation of the scores in the dominance hierarchy, we used only “normal” and “feeding” situations.

During the “normal” observations, the animals were free to roam through the paddocks without being intentionally enticed by external stimuli. The deer fed predominantly on the pasture or at the hay racks available in the paddocks. The focal observations of the animals during the “normal” time were performed between two and five times per week, with an average of three times per week. Each focal observation, carried out via focal continuous recording (Martin et al. 1993), lasted for 15 minutes/animal, with a maximum of three focal animals per session. Each animal was observed once per month, for a combined total time of approximately 16 hours per individual.

During the “feeding” observations, we provided the animals with additional food (i.e., a mixture of soy, oats and barley). The food was always presented in 8 piles (Number of the socially active animals/2) in order to let the competition escalate without exacerbating too much. The focal observations of the animals ranged between 30 and 45 minutes and was carried out via focal continuous recording (Martin et al. 1993). Due to the frenetic pace of this kind of observation, we recorded a maximum of one animal per session. Every animal was recorded once a month. Moreover, in order to obtain the hierarchy of the group, the most experienced rater performed also some group observations during feeding time (as already described for both Study I and Study II), recording all the interactions among all the deer, from the presentation of the food until its depletion. Due to the fast pace and the simultaneity of the interactions in this context, and the clear connotation of the interactions (i.e., meaning that an attack performed with legs would be labelled as “kick” by either a trained and a less-trained eye) we decided to rely just on our most experienced rater for the data collection.

Both “feeding” and “normal” observations focused on all social interactions between the focal animal and the rest of the herd (i.e., threats, agonistic encounters, socio-positive interactions, play, other, as summarized in Table 4), however, for the purpose of constructing a hierarchy, we especially focused on the agonistic interactions (i.e., any attack, threat or fight which caused an apparent displacement of the approached individual) during the feeding time. Moreover, each of the observers paid attention to the activity patterns of the focal animal together with the associations and feuds between the focal and other individuals. During both “normal” and “feeding” observations, we took care to avoid any interference between the observers. They performed their observations from different locations within the paddock and each observer had an observation schedule (randomly generated) so that they would never overlap in their observation.

The “handling” observations took place once per month, during the operations when the individuals‘general condition was checked. During this procedure the deer were confined in a restraining apparatus (i.e., “*crush*”). All the deer involved in the experiment had been regularly exposed and accustomed to this procedure. During the procedure, one of the observers scored the behavioural items presented in a pre-prepared table *ad hoc* (see Appendix Table TS4), in order to get a general impression of the inter-individual behavioural differences during this procedure. The behaviours scored during the procedure were mutually exclusive and covered the five stages of the handling process (i.e., creation of a subgroup from the herd, isolation of the animal from the subgroup, weighing, handling in the restraining apparatus, release). We treated the behaviors as binary variables, scoring them as 0 (i.e., behaviour not displayed during the handling) or 1 (i.e., behaviour displayed during the handling), based on the observation of the deer's reaction to the handling.

| | | |
|---|---|---|
| **Category** | **Behaviour** | **Description** |
| **Contact agonistic encounter** | Chase | a subject runs after another |
| | Kick | a subject performs an attack using at least one of the forelegs |
| | Flail | two subjects stand on the back legs and kick each other with the front legs |
| | Nibbling | a subject browses the other subject's fur. It causes displacement |
| | Pushing | a subject pushes another with the muzzle causing displacement |
| | Biting | a subject bites another subject |
| | | |
| **Non-contact agonistic encounter (Threat)** | | |
| | Head raise | a subject threatens another raising the head towards the back |
| | Tongue out | a subject approaches another while pulling out the tongue / grinding the teeth |
| | Ears back | a subject threatens another one pushing the ears backward |

|  |  |  |
|---|---|---|
|  | Imposition | a subject dominates another putting the head over its back (T position) |
|  | Direct look | a subject moves  the head toward another, causing a displacement |
|  | Standing threat | a subject stands on the back legs to threaten another, without any attempt to kick |
|  | Lips squishing | a subject squishes its lips |
|  | Displacement | the approach of a subject displaces another subject without any specific threat or attack |
|  |  |  |
| **Socio-positive interactions** | Grooming | as ”Nibbling”, without displacement |
|  | Rubbing | a subject al scratches its head over another. No displacement |
|  | Touching | a subject touches another with the muzzle. Does not cause displacement. |
|  |  |  |
| **Play** | Playing | two subjects chase each other with no offensive purpose |
|  |  |  |
| **Other** | Mounting | a subject mounts another (need not to be aggressive nor sexual) |
|  | Avoidance | a subject avoids to get close to another without engaging any kind of interaction |
|  |  |  |

**Table 4: Categories, behaviours and their descriptions, scored during “feeding” and “normal” observations (based on Clutton-Brock et al. 1982, Bartoš 1982).**

### *Personality assessment*

Two different methods are usually used to study personality, sometimes in conjunction, namely behavioural coding (the “bottom-up” approach”), and trait rating (the “top-down” approach) (Freeman & Gosling 2010; Šlipogor et al. 2020). Behavioural coding aims to capture the behavioural repertoire of a focal animal in either natural or experimental conditions. In contrast, trait ratings require a team of experienced raters to score a focal animal on a set of multiple behavioural descriptors (Freeman & Gosling 2010; Koski 2011).

To conduct the trait rating assessment, we compiled a personality questionnaire (Table 5), based both on the “bottom-up traits” (Stevenson-Hinde et al. 1980; Uher 2008, 2011a, b), relevant for the species’ behavioural repertoire, and “top-down traits”, previously used in other questionnaires on deer (e.g., Bergvall et al. 2011), elephants (e.g., Seltmann et al. 2018) and primates (Eckardt et al. 2015; Koski et al. 2017; Weiss 2017). Two of the three raters got acquainted with the animals for two months before the observation started; the third rater had known the animals for longer, as they had previously been the object of different studies. Successively, the raters conducted behavioural observations of the animals for four months (April-July 2018). Therefore, all raters were well familiar with the target animals and based their ratings both on the behavioural data collected and on their overall impressions of the animals. The questionnaire was written in English and included fifteen items describing behaviours representative for red deer with descriptions (see Table 5). The raters judged the propensity of each individual to display each specific behaviour on a 5-point scale, compiled as follows: 1- “Almost never”, 2- “Very rarely”, 3- “Occasionally”, 4- “Quite a lot”, 5- “Most of the time”. The raters were instructed not to discuss their ratings with each other in order to avoid mutual influence and interference. Each animal was rated by all three raters (December 2018-January 2019).

### *Dominance Hierarchy*

For the hierarchy construction, we calculated the Clutton-Brock Index (CBI) of dominance (Clutton-Brock et al. 1979) on the basis of the number of interactions won and lost by each of the individuals in “normal” and “feeding” situations, according to the formula

$CBI=(B+\Sigma b+1)/(L+\Sigma l+1)$, where B represents the number of individuals that the focal male defeated in one or more interactions, Σb represents the total number of individuals (excluding the focal animal) that those represented in B defeated, L represents the number of individuals by which the male was defeated and Σl represents the total number of individuals (excluding the male) by which those represented in L were defeated.

### *Statistical Analyses*

All statistical analyses were conducted with the R program for statistical computing, version 3.5.1 (R Core Team 2020). For initial statistical tests we used original rating scores as given by our three observers and the CBI index of dominance, as calculated from the formula above.

For personality assessment, we first ran an inter-rater reliability check for each variable (see Table 5), using Fleiss' Kappa (package irr, function kappam.fleiss (Gamer et al. 2012), which is suitable for ordinal categorical data (Seltmann et al. 2018). The Kappa values for each behavioural item can range from -1 (perfect disagreement) to +1 (perfect agreement). If the behavioural item's reliability showed statistical significance, the mean value among the three raters was used in subsequent analyses.

We created a correlation matrix (see Table TS5 in the supplementary material) to see if the data was well-correlated. To test for sampling adequacy, we ran the Kaiser-Meyer-Olkin test (KMO-test) and the Bartlett's Test of Sphericity (all functions from package psych. The analyses showed appropriate sampling adequacy (Keiser-Meyer-Olkin measure; KMO=0.74; Bartlett's test of Sphericity, $p < 0.001$). We combined three approaches to assess the number of factors to retain in the factor solution (Morton & Altschul 2019): i) eigenvalues > 1 (function eigen); ii) scree plot analysis and iii) Horn's Parallel Analysis with 10 000 iterations [package paran (Dinno 2012)]. Then, we ran a Principal Component Analysis (PCA) with a Varimax rotation, to investigate how these items were associated with each other as components. We further corroborated our results with Exploratory Factor Analysis (package psych; see Appendix, Table TS6) and a regularized Exploratory Factor Analysis (REFA) using the package fungible (Waller 2020) for small samples (Jung & Lee 2011) (see Appendix, Table TS7).

Further, we used Spearman's rank order correlations of the overall CBI index of each individual with the individual component scores of the obtained personality component (i.e., "Confidence/Aggressiveness", see Results). Component scores were obtained from the PCA output (package psych). We then calculated the links between the CBI index of each individual and the mean scores across raters of adjective items that we assumed would be linked to the hierarchy (i.e., "confident", "aggressive" and "submissive") using Spearman's rank order correlations.

## Results

### *Personality Assessment*

We found that five out of fifteen behavioural items showed a high overall agreement of the trait ratings between raters, namely "active", "aggressive", "confident", "submissive" and "stubborn" (Table 6).

| **Item** | **Description** | **Fleiss' Kappa** | **p-value** |
|---|---|---|---|
| **Active** | The deer moves around a lot, often at a fast pace, spends little time being still | 0.359 | <0.001 |
| **(Socially) Anxious** | The deer seems to be restless about everything and does not trust other individuals easily. It carefully controls the social surroundings | -0.075 | 0.455 |
| **Aggressive** | The deer causes harm or potential harm to other individuals, both deer and human | 0.301 | <0.001 |
| **Bullying** | A (larger) deer overreacts towards another animal of unbalanced size initiating a confrontational behaviour without a specific reason | 0.170 | 0.161 |
| **Calm** | The deer doesn't get easily excited and reacts to change in a relaxed, unhurried way | -0.046 | 0.63 |
| **Confident** | The deer behaves in an assured manner. It makes quick decisions and does not hesitate. It initiates the interactions and displaces other deer | 0.284 | <0.01 |
| **Excitable** | The deer over reacts to any change, easily excited from outside disturbances and is not calming down easily | -0.0839 | 0.388 |

| | | | |
|---|---|---|---|
| **Friendly** | The deer is not overly hostile towards others and initiates close contact to others within their group (scratching, rubbing , etc.) or lies/stands close to others ( <2m) whilst resting. | -0.0806 | 0.416 |
| **Inquisitive** | The deer readily explores new situations, objects, animals or people and tries to learn new things. | -0.0687 | 0.521 |
| **Opportunistic** | The deer seizes a chance as soon as it arises. | 0.00121 | 0.99 |
| **Playful** | The deer initiates play and joins in when play is solicited. | -0.128 | 0.345 |
| **Slow/Non active** | The deer moves and rests in a relaxed manner, moves slowly and deliberately, not easily hurried. | 0.134 | 0.146 |
| **Solitary** | The deer prefers to spend time alone and does not seek out contact with other deer. | 0.063 | 0.514 |
| **Submissive** | The deer gives in readily to others of a similar size and acts as though lower in rank to other deer( e.g., they will retreat or turn away in interactions). | 0.432 | <0.001 |
| **Stubborn** | The deer does not give up easily on some activity | 0.369 | <0.001 |
| | | | |

**Table 5: Questionnaire items with their descriptions, together with Fleiss' Kappa and p-values across raters.**

The smallest value of Fleiss' kappa was found in "playful" (Kappa = -0.128, p = 0.345), and the highest in "submissive" (Kappa = 0.432, p < 0.001). The repeatable items ranged from "confident" (Kappa = 0.284, p < 0.01) to "submissive" (Kappa = 0.432, p < 0.001). A scree plot test, with eigenvalues > 1, and a Horn's Parallel Analysis test with 10 000 iterations suggested 1 component (or 1 factor) for the remaining five items. We entered these five items into further PCA analyses, to investigate whether and how they were associated with each other as components. The PCA-solution was Varimax rotated, and loadings > ±0.4 were considered salient. Most items had high communalities, apart from "activity" (0.159), whose low communality renders the item unsuitable for contributing to PCA structure. PCA solution gave one principal component which explained 71.49 % of variance (Table 6).

| | Component | | | |
|---|---|---|---|---|
| | 1 | H2 | U2 | |
| Active | 0.4 | 0.16 | 0.841 | |
| Aggressive | **0.89** | 0.79 | 0.211 | |
| Confident | **0.93** | 0.86 | 0.141 | |
| Submissive | **-0.95** | 0.90 | 0.097 | |
| Stubborn | **0.93** | 0.86 | 0.136 | |

**Table 6: Mean adjective loadings in a Principal Component Analysis solution. Varimax rotation with a Kaiser normalization. Communalities (H2) indicate each variable's proportion of variance explained by the components. The remaining variance is unique variance (here U2), is the variance explained by specific factor and measurement error variance. Eigenvalues are as obtained by the PCA.**

This component had high positive loadings of “aggressive” (0.888), “confident” (0.927) and “stubborn” (0.930), and high negative loadings of “submissive” (-0.950), and thus we labelled it as “Confidence/Aggressiveness”. A separate exploratory factor analysis suggested the same personality model (see Table TS6 in the Appendix for more details). In addition, the regularized exploratory factor analysis corroborated that model (see Table TS7 in the Appendix for more details).

*Hierarchy Construction*

We recorded a total of 502 inter-individual agonistic interactions. The results of the CBI are shown in the Table 7.

| **Deer** | **CBI** |
|---|---|
| Vendelin | 4.5 |
| Bonsai | 1.64 |

| | |
|---|---|
| Scarface | 1.44 |
| Terrore | 0.96 |
| Duca | 0.90 |
| Africa | 0.86 |
| S.Coda | 0.81 |
| Tomas | 0.73 |
| Professor Galbani | 0.72 |
| Aggressore | 0.69 |
| Sangiovese | 0.58 |
| | |

**Table 7: Individuals of the group and their CBI index.**

### *Linking Personality with Dominance Hierarchy*

Individual personality component scores were correlated with the CBI score ($r_s = 0.45$, $p = 0.17$, $n=11$), yet not reaching the level of significance. The CBI score was, however, significantly linked to several adjective ratings that were connected with aggressiveness and thus predicted to be correlated with the rank of the individuals. Namely, we found a significant positive correlation between CBI and “confident” ($r_s = 0.66$, $p = 0.03$), and a significant negative correlation between CBI and “submissive” ($r_s = -0.68$, $p = 0.02$). The correlation between CBI and “aggressive” did not reach statistical significance ($r_s = 0.56$, $p = 0.14$).

## Discussion

In this study we explored inter-individual behavioural differences in red deer that could be ascribed to different personality traits, linking the latter with dominance hierarchy scores.

We created a novel questionnaire (Table 5) to investigate the personality of our captive adult male red deer population. We consulted several previous questionnaires on cervids and other mammal species, paying attention to and adding items relevant for this species’ behavioural repertoire. Using a combination of both “bottom-up” and “top-down” approaches, we carefully selected 15 behavioural items to be included in the questionnaire. In our study, only five of these 15 items showed an acceptable level of agreement between the raters, which overall corresponds to findings of behavioural consistency in other studies (Seltmann et al. 2018). However, the found agreement was much lower than in some other studies, where these items showed higher between-rater agreement (e.g., Lloyd et al. 2007; Bergvall et al. 2011; Seltmann et al. 2018). This was somewhat surprising. Since descriptions of the items were kept simple and all three raters had several months of observational experience with all focal deer, such an exposure time should have been enough to reliably rate deer behaviour. One explanation for low agreement might be our relatively small sample size: with the possibility to rate more individuals we might have been able to capture more reliably variation in deer behaviour (Sim & Wright 2005). Another explanation might be the difference in experience between the raters (Munch et al. 2019). Even though two raters got acquainted and collected observational data for several months, the third rater still had more experience with the focal individuals. Agreement has shown to be higher in behavioural ratings among novice raters than among novice and more experienced raters (Munch et al. 2019). However, when re-analysing our data with the more experienced rater excluded, Kappa values did not improve overall (see Appendix, Table TS8). Therefore, we do not believe that differences in experience have led to low agreement in ten out of fifteen behavioural items of our questionnaire. Perhaps the descriptions of these behavioural items did not reflect well enough the actual behavioural repertoire of the deer and thus should be revised and improved in the future (Koski 2011). The items with high agreement were related to general movement and agonistic interactions that are usually easily identifiable by observers (Meagher 2009). Nevertheless, four of the five behavioural items that showed acceptable agreement loaded on a single component which we labelled “Confidence/Aggressiveness”. This component contained behaviours that were of agonistic nature (positive loadings of “aggressive”, “confident” and “stubborn” and negative loadings of “submissive”). Deer scoring high on “Confidence/Aggressiveness” have “*caused harm to other deer and humans, made quick decisions and often initiated interactions and displaced other deer*” (as from the definition in Table 5). Further, they “*did not readily give in or gave up on certain activities and did not turn away in interactions*” (*ibidem*). It is safe to assume that the component we found describing variation in agonistic behaviour in red deer personality does not comprise their full behavioural variation, particularly as we did not include behaviours related to foraging or mate choice.

In general, individuality is a strong predictor of activity patterns in deer (Stache et al. 2013), their differences in neophobia (Monestier et al. 2017) and motivation to engage in fighting (Jennings et al. 2013). Moreover, individuality has ecological and evolutionary consequences, since it has been linked to diverse life-history traits in e.g., wild roe deer (Debeffe et al. 2014; Monestier et al. 2015; Bonnot et al. 2020). The component “Confidence/Aggressiveness” found in red deer was similar to “Dominance” in fallow deer, where the behavioural items “aggressive” and “submissive” loaded highly on this component (Bergvall et al. 2011). The studies on other ungulate species found similar personality components that describe agonistic or dominance behaviours, perhaps because the social rank is linked to life-histories (Favre et al. 2008) and is an important determinant of reproductive success in deer (Clutton-Brock et al. 1979). In our study we only investigated male individuals, which are known to invest a lot of energy in attainment of a high rank. Aggressive and confident behaviour might help male red deer to attain and keep high ranks, which in turn helps them to have access to resources and mates (Appleby 1980). It would be very interesting to assess also female red deer to investigate the presence of a “Confidence/Aggressiveness”, or perhaps a related “Dominance” personality component (i.e., as found in Bergvall et al. 2011).

The dominance hierarchy of our animals was assessed via repeated measures of focal behaviour (Briffa et al. 2015) and was roughly constant for the whole season. This usually happens among animals that know each other (Bartoš 1982), and where social dynamics do not suffer from unexpected events (e.g., a dominant deer suffers severe injuries, arrival of an unknown animal). Even though some animals were consistently more dominant than other animals, the hierarchy of our bachelor group was not linear, as this eventuality is, in fact, rare in this species [e.g., Clutton-Brock et al. (1982), Appleby (1983b), Bartoš and Bubenik (2011), but see Lincoln (1972) and (Mitchell 1977) for linear hierarchies]. Interestingly, not all dominant individuals perceived agonistic interactions in the same way (Esattore et al. 2020), and these inter-individual differences in interaction-proneness might be related to personality.

It has been suggested that whenever stable dominance hierarchies are an important part of the social structure of a species, findings on personality might be inflated. Thus, dominance hierarchies should be considered when investigating personality. When dominance hierarchies change, we could expect that personality traits change as well (e.g., Dingemanse & de Goede 2004; Rudin et al. 2016). We did not find an overall link between "Confidence/Aggressiveness" personality scores and deer's dominance hierarchy. However, we found positive links between the rank of the individuals and the agonistic item "confidence" loading highly in the factor solution, and possibly promoting a more interaction-prone behaviour. Namely, the individuals of higher rank were also assessed by raters as more confident. Conversely, we found a negative link between rank of the animal and the trait "submissive", suggesting that individuals that ranked higher in the bachelor group had lower ratings of submissiveness. Dominance status is sometimes associated with specific personality traits, even though the nature of this relationship appears to be complex (Drent et al. 2003; Rudin et al. 2016). For example, the personality component "Confidence" was positively correlated with the dominance rank in Hanuman langurs (*Semnopithecus entellus*) (Konečná et al. 2008) and the trait "Dominance" was positively linked to dominance strength in mountain gorillas (*Gorilla beringei beringei*) (Eckardt et al. 2015), but dominance rank was not explaining variation in personality traits of female bonobos (*Pan paniscus*) (Seyfarth et al. 2012). Furthermore, although "Aggressiveness" in graylag geese (*Anser anser*) was positively related to dominance rank (Kralj-Fišer et al. 2010), dominance was not related to boldness, exploration or activity in barnacle geese (*Branta leucopsis*) (Kurvers et al. 2009). Generally, individuals whose traits could be ascribed as proactive (i.e., those that score highly on "Aggressive" or "Confident") are more likely to initiate and win a physical contest (Sih et al. 2004; Garnham et al. 2019), as the initiative is often associated with a positive outcome of the aggression (Favati et al. 2014). Thus, more confident individuals will probably take greater risks in fights and, conversely, less confident individuals will be less interaction-prone (Briffa et al. 2015). If this risk-prone attitude is shown repeatedly and consistently (as shown in our preceding study, i.e., Esattore et al. 2020), the animal will be the winner of most of the fights, resulting in animals' overt dominance over others. However, these findings are rare and still inconclusive (e.g., Fox et al. 2009). The link between personality traits and contest behaviour is not always consistent, it is often context-dependent (Dingemanse & de Goede 2004; Briffa et al. 2015) and incorporating further physiological data in the study of personality and dominance can possibly help to further understand its complex nature (Briefer et al. 2015; Briffa et al. 2015; Finkemeier et al. 2019). Our finding that red deer position in the dominance hierarchy was linked to the variation in two behavioural items (i.e., “confident” and “submissive”) related to agonistic interactions is interesting for several reasons. First, a correlation between trait ratings and scores from an index estimated by behavioural observations gives reason to believe that personality assessed via questionnaires does reflect real behavioural variation. Even though there is still some scepticism in the field about the use of trait ratings in animal personality research, a manifold of studies have shown a clear link between trait ratings and behavioural coding (Konečná et al. 2008; Weiss et al. 2011; Carter et al. 2012; Eckardt et al. 2015). Second, the personality trait “Confidence/Aggressiveness” seems to be linked to the dominance rank in male red deer in a similar manner to previous studies in deer and other species.

## Conclusions

Even though we did pay attention to carefully construct our personality questionnaire, the explanations of several behavioural items were possibly not clear enough to result in acceptable agreement between raters. Therefore, as a general note, more care should go into the construction of questionnaires for personality assessment. In addition, it might be advisable that raters have lots of experience with the focal animals, more than the novice raters had in our study. Considering the found links between personality items and dominance hierarchy in red deer, and given the fact that both are long-term measures, more data is needed to verify the findings of our study. Studying the proximate mechanisms underlying variation in behaviour and dominance hierarchies (like stress, e.g., glucocorticoids, sex hormones, e.g., testosterone or autonomic nervous system, e.g., heart rate and heart rate variability), should provide more detailed knowledge about the interrelationship between personality and dominance.

Last, studies on different species either kept in zoos (Tetley & O'Hara 2012; Racevska & Hill 2017; Williams et al. 2019), different types of facilities or farms (Forkman et al. 1995; Veissier et al. 2012; Graunke et al. 2013) have shown the importance of knowing the individual characteristics of animals to set the best strategy in guaranteeing their welfare(Freeman & Gosling 2010; Robinson et al. 2016; Robinson et al. 2017). As personality has not been, at large, taken into account in management decisions, studies of individual behavioural phenotypes could improve the welfare of captive animals, and will definitely represent an important step forward in the development of this field.

## 6. General discussion

The main aims of this project were to verify the existence of different typologies of inter-individual relationships within the bachelor group. This was meant to be done both by verifying the existence of different associative patterns within the group – with part of the group of deer more keen on keeping together with other and a group less prone to keeping a lower inter-individual distance- and by investigating the individual keenness on attacking others – expecting, also with regards to this matter, two similarly antithetical strategies. We meant to link these differences to the concentrations of testosterone and cortisol recorded during the period of antler growth- imagining a *buffering* effect of positive social company against the negative effects of the competition-induced stress and a *boost* in the concentration of testosterone deriving from increased agonistic activity- taking the total antler length as product of the two different strategies. Last, imagining the inter-individual differences to be not only present but also deeply rooted within the nature of the animals, we intended to begin the investigation of the personality in the red deer.

In the first part of our study, the cluster analysis showed the existence of three convincingly well-separated groups representing three different strategies ("Associates" and "Distant", with the latter further divided into "Indifferent" and "Non-Associates") of animals, with the interactions being more common in the former than in the latter. As expected, some males preferred company of others, whereas others did not. As expectable, the "Indifferent" were alone and not attacking others.

The sum of agonistic interactions differed among the three different strategies, being higher in "Distant", and supporting the idea that, at the beginning of the antler growth period, the males choose between different and mutually exclusive social strategies.

During a stressful situation (i.e., an attack from a conspecific), the presence of close social partners buffered against the negative effects of increased physiological stress, as expected according to the concept of "Social buffering hypothesis" and confirmed by the lower levels of cortisol recorded in the "Associates". This evidence of a stress-ameliorating effect of social bonding among red deer males represents the most important result of this study, as it exceeds the so-far-defined area for buffering effect of males' affiliations, to date well-described only in in humans and primates.

Conversely, the males avoiding socially stable groupings had increased aggressiveness and increased levels of testosterone. However, there was no significant difference in concentrations of testosterone between types of Association, evidence that may –at fist- support a not-existent effect of the "Challenge hypothesis". However, it must be considered that the males knew each other for extended period therefore, even when competing with other males, an encounter with a known animal was not as challenging as meeting an unknown conspecific.

Moreover, reviews of the "Challenge hypothesis" have shown that the variation of the androgen responses is higher during mating opportunities, rather than during a hierarchical redefinition. Still, as testosterone increased with the number of interactions and the Non-Associates had higher sum of agonistic interactions, this could be taken as support of the "Challenge hypothesis" that will require further investigations.

Testosterone increased with agonistic interactions and size of the male, independently from the type of Association, while the concentrations of cortisol increased with the body weight, probably as a sign of increasing energy demand for larger individuals. The suppressive effect of increasing testosterone over cortisol suggested an uncontrollable state of stress due to lack of recovery in the males; conversely, the expected suppressive effect of high cortisol over testosterone was a clear index of a condition of stress deriving from prolonged competition among males. This suppressive effect, even if recorded, was not as strong as the stimulatory effect of aggression.

We found no significance of dominance as effect, neither for the concentration of cortisol and testosterone, nor for the total antler length. Moreover, "Association" itself seem to have a marginal effect on the total antler length of the "Associates", as it was only 4 cm longer than in the "Indifferent" and "Non-Associates".

Successively, we tested the consistency of the individual proneness to fight, expecting that the males would engage either in a “peaceful” (NF) way or in an “aggressive” (F) way and that these differences would have been kept across variations of the social composition of the group.

In agreement with our expectations we found out that the NF and the F differed only in the fighting characteristic and that, across different social contexts, the differences between the two groups could be only ascribed to the different individual fighting attitude itself.

After the separation in two subgroups, the NF kept showing a lower number of attacks than the F (the latter had a frequency of attacks double than the former).

The division of a socially stable group of animals usually leads to a sharp increase of attacks in the subgroups due to the need of re-define the hierarchy. The individuals, especially those with lower levels of a certain behavioural feature, can adapt their behaviour to the social context; in contrast, those that score high in certain behavioural trait would not need to adjust their behavioural response to the different social context as they would be able, in any case, to achieve a sufficiently high level of fitness. In our study we registered the physiological increase in attacking after each splitting -even started from the “light-weighted” animals”- but this was way more pronounced in F than in NF. It is likely that, as it usually happens, being in smaller and socially unstable groups along with having the most aggressive individuals removed, the NF males may have taken advantage of the situation and started attacking each other more often than they used to in the complete large group. However, their individual characteristic of being less aggressive had not been completely lost, as they had continued to show a significantly lower frequency of attacks than the F males who had been put in the same, socially-unstable situation.

In terms of hormones, the experimental changes in the social context affected the concentrations of cortisol in both the groups.

In contrast to our initial expectations, the levels of cortisol increased in both F and NF. It is interesting to notice that, in the NF -where we expected it to be lower- it neither had a suppressive effect on the testosterone nor reached the detrimental stage of stress, which is why we explained it as due to the increased metabolic needs deriving from enhanced agonistic activity. The concentrations of testosterone, being quite high in both the groups, did not differ much between NF and F, promoting a more persistent agonistic activity in both the groups.

Finally, the investigation of the personality of red deer via a 15-behavioural-items-composed questionnaire and successive linking with the dominance hierarchy resulted to be a more complicated matter that originally expected.

In our study, only five of these 15 items showed an acceptable level of agreement between the raters and, even so, the found agreement was much lower than in other studies.

Originally, we sought the reason of this in the difference experience between novice raters (2 out of 3) and expert rater. Thus, it is usually so that agreement among novice raters is higher than among novice and expert raters. However, even when removing the most experienced rater from the analysis, the agreement did not improve much. Therefore, we do not believe that the differences in experience have played a key role and led to a low agreement. Perhaps, the small size of the sample (not enough to catch all of the different shades of the behaviour) or the fact that the description of the items did not reflect well enough the actual behavioural repertoire –and should, therefore, be improved- played a major role and led to “weaker” results.

Nevertheless, four of the five behavioural items that showed acceptable agreement loaded on a single component which we labelled “Confidence/Aggressiveness”; this could describe variations in agonistic behaviour and was similar to what had already been found in other deer species.

We did not find an overall link between “Confidence/Aggressiveness” personality scores and deer’s dominance hierarchy but were able to find a positive link between the rank of the individuals and the agonistic item “confidence” and a negative link between rank of the animal and the trait “submissive”. This may mean that the personality traits assessed via questionnaire reflect real behavioural variation.

## 7. General conclusions

Our studies have shown that deer males show high versatility when it comes to choosing how to relate to conspecifics. Deer would choose whether to associate or not with conspecifics, eventually deciding also the type of relation to entertain with them. Adopting a more aggression-prone behaviour, deer would ensure themselves to have a high rank and a privileged access to the resources, boosting their concentration of testosterone and successfully defying the negative effects of cortisol. Conversely, by showing a less aggression-prone behaviour, the deer would benefit from the buffering effect of a positive, stable social company, extremely effective against the raise of the concentration of cortisol that may occur in an extremely competitive environment as the bachelor group is.

Inter-individual differences in fighting attitudes have been shown to be consistent across different social environments and, even in a modified and experimentally-induced unstable social context, it was evident the individual characteristic of the single individuals. The changes in the social environment affected the concentrations of cortisol more than they did with the testosterone; however, this should be attributed to an increased metabolic need rather than a new situation of stress.

As for the personality, it is likely that the inter-individual characteristics may be attributed to different personality traits. However, even though we paid attention to carefully construct the personality questionnaire, the understanding of it was probably not clear enough to result in an acceptable agreement between raters. Firmly believing that this field can offer several interesting applications, both to further understand the behaviour of wild animals and to set the most appropriate management strategies of the farmed ones, we advise that raters have lots of experience with the focal animals and to score a bigger group of animals on items easily understandable and representative of the behaviour of the species.

## 8. References:

Abbott DH, Keverne EB, Bercovitch FB, Shively CA, Mendoza SP, Saltzman W, Snowdon CT, Ziegler TE, Banjevic M, Garland Jr T. 2003. Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. Hormones and behavior **43**:67-82.

Adkins-Regan E 2005. Hormones and Animal Social Behavior. Princeton University Press.

Ahmad R, Sharma N, Mishra C, Singh NJ, Rawat GS, Bhatnagar YV. 2017. Security, size, or sociality: what makes markhor (Capra falconeri) sexually segregate? Journal of Mammalogy **99**:55-63.

Akaike H. 1974. A new look at the statistical model identification. IEEE transactions on automatic control **19**:716-723.

Andrew R, Rogers L. 1972. Testosterone, Search Behaviour and Persistence Nature **237**:343-346.

Appleby MC. 1980. Social rank and food access in red deer stags. Behaviour **74**:294-309.

Appleby MC. 1983a. Competition in a red deer stags social group - rank, age and relatedness of opponents. Animal Behaviour **31**:913-918.

Appleby MC. 1983b. The probability of linearity in hierarchies. Animal Behaviour **31**:600-608.

Araya-Ajoy YG, Dingemanse NJ. 2014. Characterizing behavioural 'characters': an evolutionary framework. Proceedings of the Royal Society B: Biological Sciences **281**:20132645.

Aub J, Wislocki G. 1946. The influence of testosterone on the growth of deer antlers. Pages 501-501. CANCER RESEARCH. AMER ASSOC CANCER RESEARCH PO BOX 11806, BIRMINGHAM, AL 35202.

Ball GF, Balthazart J. 2020. The neuroendocrine integration of environmental information, the regulation and action of testosterone and the challenge hypothesis. Hormones and behavior **123**:104574.

Barrell G, Muir P, Sykes A. 1985. Seasonal profiles of plasma testosterone, prolactin, and growth hormone in red deer stags. Biology of Deer Production, Roy Soc NZ Bull:185-190.

Barroso F, Alados CL, Boza J. 2000. Social hierarchy in the domestic goat: effect on food habits and production. Applied Animal Behaviour Science **69**:35-53.

Bartoš L. 1980. The date of antler casting, age and social hierarchy relationships in the red deer stag. Behavioural processes **5**:293-301.

Bartoš L. 1982. Reproductive and social aspects of the behaviour of "white" red deer. Saeugetierkd Mitt **30**:89-117.

Bartoš L. 1983. Some observations on the relationships between preorbital gland opening and social interactions in red deer. Aggressive Behavior **9**:59-67.

Bartoš L. 1985. Social activity and the antler cycle in red deer stags. Royal Society of New Zealand Bulletin **22**:269-272.

Bartoš L. 1986. Dominance and aggression in various sized groups of red deer stags. Aggressive Behavior **12**:175-182.

Bartoš L. 1990. Social status and antler development in red deer. Pages 442-459. Horns, Pronghorns, and Antlers. Springer.

Bartoš L, Bahbouh R. 2006. Antler size and fluctuating asymmetry in red deer (Cervus elaphus) stags and probability of becoming a harem holder in rut. Biological Journal of the Linnean Society **87**:59-68.

Bartoš L, Bahbouh R, Vach M. 2007. Repeatability of size and fluctuating asymmetry of antler characteristics in red deer (Cervus elaphus) during ontogeny. Biological Journal of the Linnean Society **91**:215-226.

Bartoš L, Bubenik G. 2011. Relationships between rank-related behaviour, antler cycle timing and antler growth in deer: behavioural aspects. Animal Production Science **51**:303-310.

Bartoš L, Bubenik GA, Kuzmova E. 2012. Endocrine relationships between rank-related behavior and antler growth in deer. Frontiers in Bioscience **4**:1111-1126.

Bartoš L, Losos S. 1997. Response of antler growth to changing rank of fallow deer buck during the velvet period. Canadian journal of zoology **75**:1934-1939.

Bartoš L, Perner V. 1985. Integrity of a red deer stag social group during velvet period, association of individuals, and timing of antler cleaning. Behaviour **95**:314-323.

Bartoš L, Perner V. 1998. Distribution of mating across season and reproductive success according to dominance in male red deer. Folia Zoologica (Czech Republic).

Bartoš L, Perner V, Losos S. 1988. Red deer stags rank position, body weight and antler growth. Acta Theriologica **33**:209-217.

Bartoš L, Perner V, Procházka B. 1987. On relationship between social rank during the velvet period and antler parameters in a growing red deer stag. Acta Theriologica **32**:403-412.

Bartoš L, Schams D, Bubenik GA. 2009. Testosterone, but not IGF-1, LH, prolactin or cortisol, may serve as antler-stimulating hormone in red deer stags (Cervus elaphus). Bone **44**:691-698.

Bartoš L, Schams D, Bubenik GA, Kotrba R, Tománek M. 2010. Relationship between rank and plasma testosterone and cortisol in red deer males (Cervus elaphus). Physiology & Behavior **101**:628-634.

Bartoš L, Schams D, Kierdorf U, Fischer K, Bubenik GA, Siler J, Losos S, Tomanek M, Lastovkova J. 2000. Cyproterone acetate reduced antler growth in surgically castrated fallow deer. Journal of Endocrinology **164**:87-95.

Bell AM. 2020. Individual variation and the challenge hypothesis. Hormones and behavior **123**:104549.

Bell AM, Hankison SJ, Laskowski KL. 2009. The repeatability of behaviour: a meta-analysis. Animal behaviour **77**:771-783.

Bergmuller R, Taborsky M. 2010. Animal personality due to social niche specialisation. Trends Ecol Evol **25**:504-511.

Bergvall UA, Schäpers A, Kjellander P, Weiss A. 2011. Personality and foraging decisions in fallow deer, Dama dama. Animal Behaviour **81**:101-112.

Bernstein IS. 1981. Dominance: The baby and the bathwater. Behavioral and brain sciences **4**:419-429.

Betini GS, Norris DR. 2012. The relationship between personality and plasticity in tree swallow aggression and the consequences for reproductive success. Animal Behaviour **83**:137-143.

Biro PA, Stamps JA. 2008. Are animal personality traits linked to life-history productivity? Trends Ecol Evol **23**:361-368.

Blanchard RJ, McKittrick CR, Blanchard DC. 2001. Animal models of social stress: effects on behavior and brain neurochemical systems. Physiology & behavior **73**:261-271.

Boake CR 1994. Quantitative genetic studies of behavioral evolution. University of Chicago Press.

Bonnot NC, Couriot O, Berger A, Cagnacci F, Ciuti S, De Groeve JE, Gehr B, Heurich M, Kjellander P, Kröschel M. 2020. Fear of the dark? Contrasting impacts of humans versus lynx on diel activity of roe deer across Europe. Journal of Animal Ecology **89**:132-145.

Boogert NJ, Madden JR, Morand-Ferron J, Thornton A. 2018. Measuring and understanding individual differences in cognition. The Royal Society.

Book AS, Starzyk KB, Quinsey VL. 2001. The relationship between testosterone and aggression: a meta-analysis. Aggression and Violent Behavior **6**:579-599.

Bourgoin G, Marchand P, Hewison AJM, Ruckstuhl KE, Garel M. 2018. Social behaviour as a predominant driver of sexual, age-dependent and reproductive segregation in Mediterranean mouflon. Animal Behaviour **136**:87-100.

Bowyer RT. 1984. Sexual Segregation in Southern Mule Deer. Journal of Mammalogy **65**:410-417.

Bowyer RT. 1986. Antler characteristics as related to social status of male southern mule deer. The Southwestern Naturalist:289-298.

Brain PF. 1980. Adaptive aspects of hormonal correlates of attack and defence in laboratory mice: a study in ethobiology. Pages 391-413. Progress in brain research. Elsevier.

Briefer EF, Oxley JA, McElligott AG. 2015. Autonomic nervous system reactivity in a free-ranging mammal: effects of dominance rank and personality. Animal behaviour **110**:121-132.

Briffa M, Sneddon LU, Wilson AJ. 2015. Animal personality as a cause and consequence of contest behaviour. Biology letters **11**:20141007.

Brown RD, Chao CC, Faulkner LW. 1983a. The endocrine control of the initiation and growth of antlers in white-tailed deer. European Journal of Endocrinology **103**:138-144.

Brown RD, Chao CC, Faulkner LW. 1983b. Hormone levels and antler development in white-tailed and sika fawns. Comparative Biochemistry and Physiology Part A: Physiology **75**:385-390.

Bubenik AB. 1982a. Proposal for standardized nomenclature for bony appendices in Pecora. Pages 187-194 in Brown RD, editor. Antler Development in Cervidae. Caesar Kleberg Wildl. Res. Inst., Kingsville, Texas.

Bubenik GA. 1982b. Endocrine regulation of the antler cycle. Antler development in Cervidae:73-107.

Bubenik GA. 1990. Neuroendocrine regulation of the antler cycle. Pages 265-297 in Bubenik GA, and Bubenik AB, editors. Horns, pronghorns and antlers. Evolution, morphology, physiology and social significance. Springer-Verlag, New York.

Buckland ST, Burnham KP, Augustin NH. 1997. Model selection: an integral part of inference. Biometrics:603-618.

Burnham KP, Anderson DR. 2002. A practical information-theoretic approach. Model selection and multimodel inference, 2nd ed. Springer, New York **2**.

Butler MW, Toomey MB, McGraw KJ, Rowe M. 2011. Ontogenetic immune challenges shape adult personality in mallard ducks. Proceedings of the Royal Society B: Biological Sciences **279**:326-333.

Carere C, Maestripieri D 2013. Animal personalities: behavior, physiology, and evolution. University of Chicago Press.

Carranza J, Alvarez F, Redondo T. 1990. Territoriality as a mating strategy in red deer. Animal Behaviour **40**:79-88.

Carranza J, Pérez-Barbería J, Mateos C, Alarcos S, Torres-Porras J, Pérez-González J, Sánchez-Prieto CB, Valencia J, Castillo L, de la Peña E. 2020. Social environment modulates investment in sex trait versus lifespan: red deer produce bigger antlers when facing more rivalry. Scientific Reports **10**:1-16.

Carter AJ, Marshall H, Heinsohn R, Cowlishaw G. 2012. Evaluating animal personalities: Do observer assessments and experimental tests measure the same thing? Behavioral Ecology and Sociobiology **66**:153-160.

Casto KV, Edwards DA. 2016. Testosterone, cortisol, and human competition. Hormones and Behavior **82**:21-37.

Cavigelli SA, Pereira ME. 2000. Mating Season Aggression and Fecal Testosterone Levels in Male Ring-Tailed Lemurs (Lemur catta). Hormones and Behavior **37**:246-255.

Charlton BD, Reby D, McComb K. 2007. Female red deer prefer the roars of larger males. Biology Letters **3**:382-385.

Christensen W. 2018. Agreeing to disagree: Using SAS® to make reasoned decisions when information criteria select different models. SAS Conference Proceedings: Western Users of SAS Software 2018.

Chrousos GP. 2009. Stress and disorders of the stress system. Nature reviews endocrinology **5**:374.

Ciuti S, Apollonio M. 2008. Ecological sexual segregation in fallow deer (Dama dama): a multispatial and multitemporal approach. Behavioral Ecology and Sociobiology **62**:1747-1759.

Class B, Brommer JE. 2016. Senescence of personality in a wild bird. Behavioral ecology and sociobiology **70**:733-744.

Clutton-Brock T, Albon S, Guinness F. 1986. Great expectations: dominance, breeding success and offspring sex ratios in red deer. Animal Behaviour **34**:460-471.

Clutton-Brock TH, Albon SD, Gibson RM, Guinness FE. 1979. The logical stag: adaptive aspects of fighting in red deer (Cervus elaphus L.). Animal behaviour **27**:211-225.

Clutton-Brock TH, Clutton-Brock Guinness FE, Albon S 1982. Red Deer. Behavior and Ecology of Two Sexes.

Cockrem JF. 2007. Stress, corticosterone responses and avian personalities. Journal of Ornithology **148**:169-178.

Cohen S, Wills TA. 1985. Stress, social support, and the buffering hypothesis. Psychological bulletin **98**:310.

Conradt L, Clutton-Brock T, Thomson D. 1999. Habitat segregation in ungulates: are males forced into suboptimal foraging habitats through indirect competition by females? Oecologia **119**:367-377.

Coppens CM, de Boer SF, Koolhaas JM. 2010. Coping styles and behavioural flexibility: towards underlying mechanisms. Philosophical Transactions of the Royal Society B: Biological Sciences **365**:4021-4028.

Crook JH, Ellis J, Goss-Custard J. 1976. Mammalian social systems: structure and function. Animal Behaviour **24**:261-274.

Dahlbom SJ, Lagman D, Lundstedt-Enkel K, Sundström LF, Winberg S. 2011. Boldness predicts social status in zebrafish (Danio rerio). PLoS One **6**:e23565.

Dall SR, Houston AI, McNamara JM. 2004. The behavioural ecology of personality: consistent individual differences from an adaptive perspective. Ecology letters **7**:734-739.

Darwin C. 1871. 1871The descent of man, and selection in relation to sex. London: Murray **415**.

Debeffe L, Morellet N, Bonnot N, Gaillard J-M, Cargnelutti B, Verheyden-Tixier H, Vanpé C, Coulon A, Clobert J, Bon R. 2014. The link between behavioural type and natal dispersal propensity reveals a dispersal syndrome in a large herbivore. Proceedings of the Royal Society B: Biological Sciences **281**:20140873.

DeYoung RW, Demarais S, Honeycutt RL, Gee KL, Gonzales RA. 2006. Social dominance and male breeding success in captive white-tailed deer. Wildlife Society Bulletin **34**:131-136.

Dingemanse NJ, de Goede P. 2004. The relation between dominance and exploratory behavior is context-dependent in wild great tits. Behavioral Ecology **15**:1023-1030.

Dingemanse NJ, Kazem AJ, Réale D, Wright J. 2010. Behavioural reaction norms: animal personality meets individual plasticity. Trends in ecology & evolution **25**:81-89.

Dingemanse NJ, Wolf M. 2010. Recent models for adaptive personality differences: a review. Philos Trans R Soc Lond B Biol Sci **365**:3947-3958.

Dingemanse NJ, Wolf M. 2013. Between-individual differences in behavioural plasticity within populations: causes and consequences. Animal Behaviour **85**:1031-1039.

Dinno A. 2012. Paran: Horn's Test of Principal Components/Factors. R package version 1.5.1.

Ditchkoff SS, Lochmiller RL, Masters RE, Hoofer SR, Bussche RAVD. 2001. Major-histocompatibility-complex-associated variation in secondary sexual traits of white-tailed deer (Odocoileus virginianus): evidence for good-genes advertisement. Evolution **55**:616-625.

Drent PJ, Oers Kv, Noordwijk AJv. 2003. Realized heritability of personalities in the great tit (Parus major). Proceedings of the Royal Society of London. Series B: Biological Sciences **270**:45-51.

Drent PJ, Verbeek ME, Boon A. 1996. Exploration, aggressive behaviour and dominance in pair-wise confrontations of juvenile male great tits. Behaviour **133**:945-963.

Dušek A, Bartoš L, Švecová L. 2007. The effect of a mother's rank on her offspring's pre-weaning rank in farmed red deer. Applied Animal Behaviour Science **103**:146-155.

Eckardt W, Steklis HD, Steklis NG, Fletcher AW, Stoinski TS, Weiss A. 2015. Personality dimensions and their behavioral correlates in wild Virunga mountain gorillas (Gorilla beringei beringei). Journal of Comparative Psychology **129**:26.

Eisenegger C, Haushofer J, Fehr E. 2011. The role of testosterone in social interaction. Trends in cognitive sciences **15**:263-271.

Esattore B, Villagrán M, Pluháček J, Komárková M, Dušek A, Kotrba R, Bartošová J, Bartoš L. 2020. To beat or not to beat: Behavioral plasticity during the antler growth period affects cortisol but not testosterone concentrations in red deer (Cervus elaphus) males. General and Comparative Endocrinology:113552.

Favati A, Leimar O, Løvlie H. 2014. Personality predicts social dominance in male domestic fowl. PLoS One **9**:e103535.

Favre M, Martin JG, Festa-Bianchet M. 2008. Determinants and life-history consequences of social dominance in bighorn ewes. Animal Behaviour **76**:1373-1380.

Finger J, Guttridge T, Wilson A, Gruber S, Krause J. 2018. Are some sharks more social than others? Short-and long-term consistencies in the social behavior of juvenile lemon sharks. Behavioral ecology and sociobiology **72**:17.

Finkemeier M-A, Oesterwind S, Nürnberg G, Puppe B, Langbein J. 2019. Assessment of personality types in Nigerian dwarf goats (Capra hircus) and cross-context correlations to behavioural and physiological responses. Applied Animal Behaviour Science **217**:28-35.

Forand KJ, Marchinton RL, Miller KV. 1985. Influence of Dominance Rank on the Antler Cycle of White-Tailed Deer. Journal of Mammalogy **66**:58-62.

Forkman B, Furuhaug I, Jensen P. 1995. Personality, coping patterns, and aggression in piglets. Applied Animal Behaviour Science **45**:31-42.

Found R, Clair CS. 2016. Behavioural syndromes predict loss of migration in wild elk. Animal Behaviour **115**:35-46.

Found R, St. Clair CC. 2018. Personality influences wildlife responses to aversive conditioning. The Journal of Wildlife Management **82**:747-755.

Fox RA, Ladage LD, Roth II TC, Pravosudov VV. 2009. Behavioural profile predicts dominance status in mountain chickadees, Poecile gambeli. Animal Behaviour **77**:1441-1448.

Fox RA, Millam JR. 2010. The use of ratings and direct behavioural observation to measure temperament traits in cockatiels (Nymphicus hollandicus). Ethology **116**:59-75.

Franklin WL, Mossman AS, Dole M 1975. Social Organization and Home Range of Roosevelt Elk.

Freeman HD, Gosling SD. 2010. Personality in nonhuman primates: a review and evaluation of past research. American journal of primatology **72**:653-671.

Gamer M, Lemon J, Fellows I, Singh P. 2012. irr: Various coefficients of interrater reliability and agreement. R package v. 0.84.

Garamszegi LZ, Hegyi G, Heylen D, Ninni P, De Lope F, Eens M, Møller A. 2006. The design of complex sexual traits in male barn swallows: associations between signal attributes. Journal of Evolutionary Biology **19**:2052-2066.

Garnham LC, Porthén SA, Child S, Forslind S, Løvlie H. 2019. The role of personality, cognition, and affective state in same-sex contests in the red junglefowl. Behavioral Ecology and Sociobiology **73**:149.

Gartner MC, Weiss A. 2013. Personality in felids: A review. Applied Animal Behaviour Science **144**:1-13.

Gaspar-Lopez E, Landete-Castillejos T, Estevez JA, Ceacero F, Gallego L, Garcia AJ. 2010. Biometrics, testosterone, cortisol and antler growth cycle in Iberian red deer stags (Cervus elaphus hispanicus). Reprod Domest Anim **45**:243-249.

Gaudin S, Chaillou E, Cornilleau F, Moussu C, Boivin X, Nowak R. 2015. Daughters are more strongly attached to their mother than sons: a possible mechanism for early social segregation. Animal Behaviour **102**:33-43.

Giammanco M, Tabacchi G, Giammanco S, Di Majo D, La Guardia M. 2005. Testosterone and aggressiveness. Medical science monitor **11**:RA136-RA145.

Gosling SD. 2001. From mice to men: what can we learn about personality from animal research? Psychological bulletin **127**:45.

Goss R. 1983. Deer Antlers: Regeneration. Function, and Evolution. New York: Academic.

Graunke KL, Nürnberg G, Repsilber D, Puppe B, Langbein J. 2013. Describing temperament in an ungulate: a multidimensional approach. PloS one **8**:e74579.

Gunnar MR. 2017. Social buffering of stress in development: A career perspective. Perspectives on Psychological Science **12**:355-373.

Gunnar MR, Hostinar CE. 2015. The social buffering of the hypothalamic–pituitary–adrenocortical axis in humans: Developmental and experiential determinants. Social neuroscience **10**:479-488.

Gunnar MR, Hostinar CE, Sanchez MM, Tottenham N, Sullivan RM. 2015. Parental buffering of fear and stress neurobiology: Reviewing parallels across rodent, monkey, and human models. Social neuroscience **10**:474-478.

Gust D, Gordon T. 1991. Female rank instability in newly formed groups of familiar sooty mangabeys (Cercocebus torquatus atys). Primates **32**:465-471.

Haigh J, Cates W, Glover G, Rawlings N. 1984. Relationships between seasonal changes in serum testosterone concentrations, scrotal circumference and sperm morphology of male wapiti (Cervus elaphus). Reproduction **70**:413-418.

Harcourt JL, Sweetman G, Johnstone RA, Manica A. 2009. Personality counts: the effect of boldness on shoal choice in three-spined sticklebacks. Animal Behaviour **77**:1501-1505.

Hau M. 2007. Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. BioEssays **29**:133-144.

Hennessy MB, Kaiser S, Sachser N. 2009. Social buffering of the stress response: diversity, mechanisms, and functions. Frontiers in neuroendocrinology **30**:470-482.

Hirschenhauser K, Oliveira RF. 2006. Social modulation of androgens in male vertebrates: meta-analyses of the challenge hypothesis. Animal Behaviour **71**:265-277.

Hostinar CE, Sullivan RM, Gunnar MR. 2014. Psychobiological mechanisms underlying the social buffering of the hypothalamic–pituitary–adrenocortical axis: A review of animal models and human studies across development. Psychological bulletin **140**:256.

Illius A, Gordon I. 1987. The allometry of food intake in grazing ruminants. The Journal of Animal Ecology:989-999.

Jennings DJ, Hayden TJ, Gammell MP. 2013. Personality and predictability in fallow deer fighting behaviour: the relationship with mating success. Animal Behaviour **86**:1041-1047.

Johnson EO, Kamilaris TC, Chrousos GP, Gold PW. 1992. Mechanisms of stress: A dynamic overview of hormonal and behavioral homeostasis. Neuroscience & Biobehavioral Reviews **16**:115-130.

Johnson HE, Bleich VC, Krausman PR, Koprowski JL. 2007. Effects of antler breakage on mating behavior in male tule elk (Cervus elaphus nannodes). European Journal of Wildlife Research **53**:9-15.

Johnson M, Waller J. 2018. Simple Methods for Repeatability and Comparability: Bland-Altman Plots, Bias, and Measurement Error.

Jung S, Lee S. 2011. Exploratory factor analysis for small samples. Behavior Research Methods **43**:701-709.

Kaburu SS, Newton-Fisher NE. 2015. Egalitarian despots: hierarchy steepness, reciprocity and the grooming-trade model in wild chimpanzees, Pan troglodytes. Animal behaviour **99**:61-71.

Kelley A, Humphries M, McAdam A, Boutin S. 2015. Changes in wild red squirrel personality across ontogeny: activity and aggression regress towards the mean. Behaviour **152**:1291-1306.

Ketterson ED, Nolan J, Val. 1999. Adaptation, exaptation, and constraint: a hormonal perspective. the american naturalist **154**:S4-S25.

Kidjo N, Serrano E, Bideau E, Gonzalez G. 2016. Is dominance the only factor determining access to food in an agonistic context? An experiment with captive male mouflon. acta ethologica **19**:69-79.

Kikusui T, Winslow JT, Mori Y. 2006. Social buffering: relief from stress and anxiety. Philosophical Transactions of the Royal Society B: Biological Sciences **361**:2215-2228.

Kirschbaum C, Prussner JC, Stone AA, Federenko I, Gaab J, Lintz D, Schommer N, Hellhammer DH. 1995. Persistent high cortisol responses to repeated psychological stress in a subpopulation of healthy men. Psychosomatic medicine **57**:468-474.

Kiyokawa Y, Hennessy MB. 2018. Comparative studies of social buffering: A consideration of approaches, terminology, and pitfalls. Neuroscience & Biobehavioral Reviews **86**:131-141.

Knight RR. 1970. The Sun River Elk Herd. Wildlife Monographs:3-66.

Kokko H. 1998. Should advertising parental care be honest? Proceedings of the Royal Society of London. Series B: Biological Sciences **265**:1871-1878.

Kölliker M. 2005. Ontogeny in the family. Behavior Genetics **35**:7.

Komers PE. 1997. Behavioural plasticity in variable environments. Canadian Journal of Zoology **75**:161-169.

Konečná M, Lhota S, Weiss A, Urbánek T, Adamová T, Pluháček J. 2008. Personality in free-ranging Hanuman langur (Semnopithecus entellus) males: Subjective ratings and recorded behavior. Journal of Comparative Psychology **122**:379-389.

Koolhaas J, Korte S, De Boer S, Van Der Vegt B, Van Reenen C, Hopster H, De Jong I, Ruis M, Blokhuis H. 1999. Coping styles in animals: current status in behavior and stress-physiology. Neuroscience & Biobehavioral Reviews **23**:925-935.

Koolhaas JM, et al. 2011. Stress revisited: a critical evaluation of the stress concept. Neurosci Biobehav Rev **35**:1291-1301.

Koski S. 2011. How to Measure Animal Personality and Why Does It Matter? Integrating the Psychological and Biological Approaches to Animal Personality. Pages 115-136.

Koski S, Buchanan-Smith H, Ash H, Burkart J, Bugnyar T, Weiss A. 2017. Common Marmoset (Callithrix jacchus) Personality. Journal of Comparative Psychology **131**.

Kralj-Fišer S, Weiß BM, Kotrschal K. 2010. Behavioural and physiological correlates of personality in greylag geese (Anser anser). Journal of Ethology **28**:363-370.

Kruuk LE, Slate J, Pemberton JM, Brotherstone S, Guinness F, Clutton-Brock T. 2002. Antler size in red deer: heritability and selection but no evolution. Evolution **56**:1683-1695.

Kurvers RH, Eijkelenkamp B, van Oers K, van Lith B, van Wieren SE, Ydenberg RC, Prins HH. 2009. Personality differences explain leadership in barnacle geese. Animal Behaviour **78**:447-453.

Leshner A. 1980. The interaction of experience and neuroendocrine factors in determining behavioral adaptations to aggression. Pages 427-438. Progress in brain research. Elsevier.

Li C, Littlejohn R, Corson I, Suttie J. 2003. Effects of testosterone on pedicle formation and its transformation to antler in castrated male, freemartin and normal female red deer (Cervus elaphus). General and Comparative Endocrinology **131**:21-31.

Lincoln G. 1972. The role of antlers in the behaviour of red deer. Journal of experimental zoology **182**:233-249.

Littell RC, Pendergast J, Natarajan R. 2000. Modelling covariance structure in the analysis of repeated measures data. Statistics in Medicine **19**:1793-1819.

Lloyd AS, Martin JE, Bornett-Gauci HLI, Wilkinson RG. 2007. Evaluation of a novel method of horse personality assessment: Rater-agreement and links to behaviour. Applied Animal Behaviour Science **105**:205-222.

Loe LE, Irvine RJ, Bonenfant C, Stien A, Langvatn R, Albon SD, Mysterud A, Stenseth NC. 2006. Testing five hypotheses of sexual segregation in an arctic ungulate. Journal of Animal Ecology **75**:485-496.

Loudon A, Curlewis J. 1988. Cycles of antler and testicular growth in an aseasonal tropical deer (Axis axis). Reproduction **83**:729-738.

Lovari S, Fattorini N, Boesi R, Bocci A. 2015. Male ruff colour as a rank signal in a monomorphic-horned mammal: behavioural correlates. The Science of Nature **102**:39.

Lowe VPW 1966. Observations on the dispersal of red deer on Rhum.

Main MB. 2008. Reconciling competing ecological explanations for sexual segregation in ungulates. Ecology **89**:693-704.

Mainguy J, Côté SD, Cardinal E, Houle M. 2008. Mating tactics and mate choice in relation to age and social rank in male mountain goats. Journal of Mammalogy **89**:626-635.

Malo AF, Roldan ER, Garde J, Soler AJ, Gomendio M. 2005. Antlers honestly advertise sperm production and quality. Proceedings of the Royal Society B: Biological Sciences **272**:149-157.

Martin AM, Presseault-Gauvin H, Festa-Bianchet M, Pelletier F. 2013. Male mating competitiveness and age-dependent relationship between testosterone and social rank in bighorn sheep. Behavioral Ecology and Sociobiology **67**:919-928.

Martin JS, Massen JJ, Šlipogor V, Bugnyar T, Jaeggi AV, Koski SE. 2019. The EGA+ GNM framework: An integrative approach to modelling behavioural syndromes. Methods in Ecology and Evolution **10**:245-257.

Martin P, Bateson PPG, Bateson P 1993. Measuring behaviour: an introductory guide. Cambridge University Press.

Mazzarone V, Mattiello S 2010. Il cervo in Italia: biologia e gestione tra Alpi e Appennini.

McElligott AG, Gammell MP, Harty HC, Paini DR, Murphy DT, Walsh JT, Hayden TJ. 2001. Sexual size dimorphism in fallow deer (Dama dama): do larger, heavier males gain greater mating success? Behavioral Ecology and Sociobiology **49**:266-272.

McNeal N, Appleton KM, Johnson AK, Scotti M-AL, Wardwell J, Murphy R, Bishop C, Knecht A, Grippo AJ. 2017. The protective effects of social bonding on behavioral and pituitary-adrenal axis reactivity to chronic mild stress in prairie voles. Stress **20**:175-182.

Meagher RK. 2009. Observer ratings: Validity and value as a tool for animal welfare research. Applied Animal Behaviour Science **119**:1-14.

Mendonça-Furtado O, Edaes M, Palme R, Rodrigues A, Siqueira J, Izar P. 2014. Does hierarchy stability influence testosterone and cortisol levels of bearded capuchin monkeys (Sapajus libidinosus) adult males? A comparison between two wild groups. Behavioural processes **109**:79-88.

Michel Eric S, Demarais S, Strickland Bronson K, Belant Jerrold L, Millspaugh Joshua J. 2016. Quantifying dominance of adult female white-tailed deer in the presence of abundant food. Behaviour **153**:49-67.

Mills SC, Grapputo A, Jokinen I, Koskela E, Mappes T, Oksanen TA, Poikonen T. 2009. Testosterone-mediated effects on fitness-related phenotypic traits and fitness. The American Naturalist **173**:475-487.

Mirarchi RE, Scanlon PF, Kirkpatrick RL, Schreck CB. 1977. Androgen levels and antler development in captive and wild white-tailed deer. The Journal of Wildlife Management:178-183.

Mitchell B, Staines, B.W., Welch, D. 1977. Ecology of Red Deer: A research review relevant to their management in Scotland. Institute of Terrestrial Ecology, Cambridge.

Monestier C, Gilot-Fromont E, Morellet N, Debeffe L, Cebe N, Merlet J, Picot D, Rames JL, Hewison AJM, Verheyden H. 2016. Individual variation in an acute stress response reflects divergent coping strategies in a large herbivore. Behavioural Processes **132**:22-28.

Monestier C, Morellet N, Gaillard J-M, Cargnelutti B, Vanpé C, Hewison A. 2015. Is a proactive mum a good mum? A mother's coping style influences early fawn survival in roe deer. Behavioral Ecology **26**.

Monestier C, et al. 2017. Neophobia is linked to behavioural and haematological indicators of stress in captive roe deer. Animal Behaviour **126**:135-143.

Monfort SL, Brown JL, Wildt DE. 1993. Episodic and seasonal rhythms of cortisol secretion in male Eld's deer (Cervus eldi thamin). Journal of Endocrinology **138**:41-49.

Moore IT, Hernandez J, Goymann W. 2020. Who rises to the challenge? Testing the challenge hypothesis in fish, amphibians, reptiles, and mammals. Hormones and behavior **123**:104537.

Morina DL, Demarais S, Strickland BK, Larson JE. 2018. While males fight, females choose: male phenotypic quality informs female mate choice in mammals. Animal Behaviour **138**:69-74.

Morton FB, Altschul D. 2019. Data reduction analyses of animal behaviour: avoiding Kaiser's criterion and adopting more robust automated methods. Animal Behaviour **149**:89-95.

Müller R, Schrader L. 2005. Behavioural consistency during social separation and personality in dairy cows. Behaviour **142**:1295-1312.

Munch KL, Wapstra E, Thomas S, Fisher M, Sinn DL. 2019. What are we measuring? Novices agree amongst themselves (but not always with experts) in their assessment of dog behaviour. Ethology **125**:203-211.

Nakagawa S, Schielzeth H. 2010. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biological Reviews **85**:935-956.

Natarajan D, de Vries H, Saaltink D-J, de Boer SF, Koolhaas JM. 2009. Delineation of violence from functional aggression in mice: an ethological approach. Behavior genetics **39**:73-90.

Øverli Ø, Sørensen C, Pulman KG, Pottinger TG, Korzan W, Summers CH, Nilsson GE. 2007. Evolutionary background for stress-coping styles: relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. Neuroscience & Biobehavioral Reviews **31**:396-412.

Pelletier F, Bauman J, Festa-Bianchet M. 2003. Fecal testosterone in bighorn sheep (Ovis canadensis): behavioural and endocrine correlates. Canadian Journal of Zoology **81**:1678-1684.

Pelletier F, Festa-Bianchet M. 2006. Sexual selection and social rank in bighorn rams. Animal Behaviour **71**:649-655.

Pervin LA, Cervone D, John O. 2005. Personality, Theory and Research. Jonh Wiley & Sons. Inc.

Posada D, Buckley TR. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Systematic biology **53**:793-808.

Price J, Allen S. 2004. Exploring the mechanisms regulating regeneration of deer antlers. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences **359**:809-822.

Price J, Faucheux C, Allen S. 2005a. Deer antlers as a model of Mammalian regeneration. Current topics in developmental biology **67**:1-48.

Price JS, Allen S, Faucheux C, Althnaian T, Mount JG. 2005b. Deer antlers: a zoological curiosity or the key to understanding organ regeneration in mammals? Journal of anatomy **207**:603-618.

Racevska E, Hill CM. 2017. Personality and social dynamics of zoo-housed western lowland gorillas (Gorilla gorilla gorilla). Journal of Zoo and Aquarium Research **5**:116-122.

Réale D, Dingemanse NJ, Kazem AJN, Wright J. 2010. Evolutionary and ecological approaches to the study of personality. Philosophical Transactions of the Royal Society B-Biological Sciences **365**:3937-3946.

Réale D, Montiglio P-O. 2020. Evolution of Adaptive Individual Differences in Non-human Animals. Pages 279-299. Adaptive Shyness. Springer.

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. Biological reviews **82**:291-318.

Reyes E, Bubenik GA, Lobos A, Schams D, Bartoš L. 1997. Seasonal levels of cortisol, IGF-l and triiodothyronin in adult male pudu. Folia Zool **46**:109-116.

Richards SA, Whittingham MJ, Stephens PA. 2011. Model selection and model averaging in behavioural ecology: the utility of the IT-AIC framework. Behavioral Ecology and Sociobiology **65**:77-89.

Roberts BW, DelVecchio WF. 2000. The rank-order consistency of personality traits from childhood to old age: A quantitative review of longitudinal studies. Psychological Bulletin **126**:3-25.

Robinson LM, Altschul DM, Wallace EK, Úbeda Y, Llorente M, Machanda Z, Slocombe KE, Leach MC, Waran NK, Weiss A. 2017. Chimpanzees with positive welfare are happier, extraverted, and emotionally stable. Applied animal behaviour science **191**:90-97.

Robinson LM, Waran NK, Leach MC, Morton FB, Paukner A, Lonsdorf E, Handel I, Wilson VA, Brosnan SF, Weiss A. 2016. Happiness is positive welfare in brown capuchins (Sapajus apella). Applied Animal Behaviour Science **181**:145-151.

Rodriguez-Santiago M, Nührenberg P, Derry J, Deussen O, Garrison LK, Garza SF, Francisco F, Hofmann HA, Jordan A. 2020. Behavioral traits that define social dominance are the same that reduce social influence in a consensus task. bioRxiv:845628.

Røed KH, Holand Ø, Smith ME, Gjøstein H, Kumpula J, Nieminen M. 2002. Reproductive success in reindeer males in a herd with varying sex ratio. Molecular Ecology **11**:1239-1243.

Rolf H, Fischer K. 1990. Serum testosterone (T) and 5-α-dihydrotestosterone (DHT) in male fallow deer (Dama dama L.): seasonality and age dependence. Comparative Biochemistry and Physiology Part A: Physiology **95**:445-452.

Rolf HJ, Fischer K. 1996. Serum testosterone, 5-α-dihydrotestosterone and different sex characteristics in male fallow deer (Cervus dama): a long-term experiment with accelerated photoperiods. Comparative Biochemistry and Physiology Part A: Physiology **115**:207-221.

Rose RM, Holaday JW, Bernstein IS. 1971. Plasma testosterone, dominance rank and aggressive behaviour in male rhesus monkeys. Nature **231**:366-368.

Ruckstuhl KE. 1998. Foraging behaviour and sexual segregation in bighorn sheep. Animal behaviour **56**:99-106.

Ruckstuhl KE. 2007. Sexual segregation in vertebrates: proximate and ultimate causes. Integr Comp Biol **47**:245-257.

Ruckstuhl KE, Neuhaus P. 2002. Sexual segregation in ungulates: a comparative test of three hypotheses. Biological Reviews **77**:77-96.

Rudin FS, Tomkins JL, Simmons LW. 2016. Changes in dominance status erode personality and behavioral syndromes. Behavioral Ecology:arw151.

Sachser N, Dürschlag M, Hirzel D. 1998. Social relationships and the management of stress. Psychoneuroendocrinology **23**:891-904.

Sands J, Creel S. 2004. Social dominance, aggression and faecal glucocorticoid levels in a wild population of wolves, Canis lupus. Animal behaviour **67**:387-396.

Santiago-Moreno J, Gómez-Brunet A, Toledano-Díaz A, Pulido-Pastor A, López-Sebastián A. 2007. Social dominance and breeding activity in Spanish ibex (Capra pyrenaica) maintained in captivity. Reproduction, Fertility and Development **19**:436-442.

Sapolsky R. 1993. The physiology of dominance in stable versus unstable social hierarchies. Primate social conflict:171-204.

Sapolsky RM. 1983. Endocrine aspects of social instability in the olive baboon (Papio anubis). American Journal of Primatology **5**:365-379.

Sapolsky RM. 1994. Individual differences and the stress response. Pages 261-269. Seminars in Neuroscience. Elsevier.

Sapolsky RM. 2002. 11 Endocrinology of the Stress-Response. Behavioral endocrinology:409.

Sapolsky RM. 2005. The influence of social hierarchy on primate health. Science **308**:648-652.

Sapolsky RM, Romero LM, Munck AU. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine reviews **21**:55-89.

Schwarz G. 1978. Estimating the dimension of a model. The annals of statistics **6**:461-464.

Seltmann MW, Helle S, Adams MJ, Mar KU, Lahdenpera M. 2018. Evaluating the personality structure of semi-captive Asian elephants living in their natural habitat. R Soc Open Sci **5**:172026.

Selye H. 1950. Stress–the physiology and pathology of exposure to systemic stress. Montreal: Acta Inc Med Publ.

Sempéré A, Boissin J. 1981. Relationship between antler development and plasma androgen concentrations in adult roe deer (Capreolus capreolus). Reproduction **62**:49-53.

Sempéré A, Boissin J. 1982. Neuroendocrine and endocrine control of the antler cycle in roe deer. Antler development in Cervidae. Kingsville, TX: Caesar Kleberg Wildlife Research Institute:109-122.

Sempéré A, Lacroix A. 1982. Temporal and seasonal relationships between LH, testosterone and antlers in fawn and adult male roe deer. European Journal of Endocrinology **99**:295-301.

Sempere A, Mauget R, Bubenik G. 1992. Influence of photoperiod on the seasonal pattern of secretion of luteinizing hormone and testosterone and on the antler cycle in roe deer (Capreolus capreolus). Reproduction **95**:693-700.

Seyfarth RM, Silk JB, Cheney DL. 2012. Variation in personality and fitness in wild female baboons. Proceedings of the National Academy of Sciences **109**:16980-16985.

Shilang Z, Shanzhi W. 1983. Studies of velvet antler production of sika deer in Fennessy P. F., and R DK, editors. Biology of Deer ProductiN. Roy Soc N Z.

Sih A, Bell A, Johnson JC. 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends in ecology & evolution **19**:372-378.

Sim J, Wright CC. 2005. The kappa statistic in reliability studies: use, interpretation, and sample size requirements. Physical therapy **85**:257-268.

Šlipogor V, Burkart JM, Martin JS, Bugnyar T, Koski SE. 2020. Personality method validation in common marmosets (Callithrix jacchus): Getting the best of both worlds. Journal of Comparative Psychology **134**:52.

Smith JM. 1991. Theories of sexual selection. Trends in Ecology & Evolution **6**:146-151.

Stache A, Heller E, Hothorn T, Heurich M. 2013. Activity patterns of European Roe Deer (Capreolus capreolus) are strongly influenced by individual behavior. Folia Zoologica -Praha- **62**:67-75.

Stamps JA, Groothuis TG. 2010. Developmental perspectives on personality: implications for ecological and evolutionary studies of individual differences. Philosophical Transactions of the Royal Society B: Biological Sciences **365**:4029-4041.

Stevenson-Hinde J, Stillwell-Barnes R, Zunz M. 1980. Individual differences in young rhesus monkeys: consistency and change. Primates **21**:498-509.

Stirling D, Réale D, Roff D. 2002. Selection, structure and the heritability of behaviour. Journal of Evolutionary Biology **15**:277-289.

Suttie J, Fennessy P, Crosbie S, Corson I, Laas F, Elgar H, Lapwood K. 1991. Temporal changes in LH and testosterone and their relationship with the first antler in red deer (Cervus elaphus) stags from 3 to 15 months of age. journal of Endocrinology **131**:467-474.

Suttie J, Lincoln G, Kay R. 1984. Endocrine control of antler growth in red deer stags. Reproduction **71**:7-15.

Suttie JM, Gluckman PD, Butler JH, Fennessy PF, Corson ID, Laas FJ. 1985. Insulin-like growth factor 1 (IGF-1) antler-stimulating hormone? Endocrinology **116**:846-848.

Tachezy R. 1956. Über den Einfluß der Sexualhormone auf das Geweihwachstum der Cerviden. Säugetierkundl. Mitt **4**.

Tao J, Littel R, Patetta M, Truxillo C, Wolfinger R. 2002. Mixed models analyses using the SAS system: Course notes. SAS Institute. Inc., Cary, NC:493.

Tetley CL, O'Hara SJ. 2012. Ratings of animal personality as a tool for improving the breeding, management and welfare of zoo mammals. Animal Welfare-The UFAW Journal **21**:463.

Toufexis D, Rivarola MA, Lara H, Viau V. 2014. Stress and the reproductive axis. Journal of neuroendocrinology **26**:573-586.

Trivers RL. 1972. Parental investment and sexual selection. Pages 136-180 in Campbell BG, editor. Sexual selection and the descent of man, 1871-1971. University of California Los Angeles, Aldine Publishing Company, Chicago.

Uher J. 2008. Comparative personality research: methodological approaches. European Journal of Personality **22**:427-455.

Uher J. 2011a. Individual behavioral phenotypes: An integrative meta-theoretical framework. Why “behavioral syndromes” are not analogs of “personality”. Developmental Psychobiology **53**:521-548.

Uher J. 2011b. Personality in nonhuman primates: What can we learn from human personality psychology? Pages 41-76. Personality and temperament in nonhuman primates. Springer.

Vaida F, Blanchard S. 2005. Conditional Akaike information for mixed-effects models. Biometrika **92**:351-370.

van Mourik S, Stelmasiak T. 1990. Endocrine mechanisms and antler cycles in rusa deer, Cervus (Rusa) timorensis. Pages 416-425. Horns, pronghorns, and antlers. Springer.

Van Oers K, De Jong G, Van Noordwijk AJ, Kempenaers B, Drent PJ. 2005. Contribution of genetics to the study of animal personalities: a review of case studies. Behaviour:1185-1206.

Van Reenen CG, O'Connell NE, Van der Werf JT, Korte SM, Hopster H, Jones RB, Blokhuis HJ. 2005. Responses of calves to acute stress: individual consistency and relations between behavioral and physiological measures. Physiol Behav **85**:557-570.

Veiberg V, Loe LE, Mysterud A, Langvatn R, Stenseth NC. 2004. Social rank, feeding and winter weight loss in red deer: any evidence of interference competition? Oecologia **138**:135-142.

Veissier I, Aubert A, Boissy A. 2012. Animal welfare: A result of animal background and perception of its environment. Animal Frontiers **2**:7-15.

Verbeek ME, Drent P, De Goede P, Wiepkema P. 1999. Individual behavioural characteristics and dominance in aviary groups of great tits. Behaviour **136**:23-48.

Vymyslická PJ, Brandlová K, Hozdecká K, Žáčková M, Hejcmanová P. 2015. Feeding rank in the Derby eland: lessons for management. African Zoology **50**:313-320.

Wagenmakers E-J, Farrell S. 2004. AIC model selection using Akaike weights. Psychonomic bulletin & review **11**:192-196.

Waldo CM, Wislocki GB. 1951. Observations on the shedding of the antlers of Virginia deer (Odocoileus virginianus borealis). American Journal of Anatomy **88**:351-395.

Waller NG. 2020. Fungible: Psychometric Functions from the Waller Lab. R package version 1.95.4.8.

Wang MY, Alves J, da Silva AA, Yang WK, Ruckstuhl KE. 2018. The effect of male age on patterns of sexual segregation in Siberian ibex. Scientific Reports **8**:9.

Ward EJ. 2008. A review and comparison of four commonly used Bayesian and maximum likelihood model selection tools. Ecological Modelling **211**:1-10.

Wechsler B. 1995. Coping and coping strategies: a behavioural view. Applied Animal Behaviour Science **43**:123-134.

Weiss A. 2017. Exploring factor space (and other adventures) with the Hominoid Personality Questionnaire. Pages 19-38. Personality in nonhuman animals. Springer.

Weiss A. 2018. Personality traits: a view from the animal kingdom. Journal of personality **86**:12-22.

Weiss A, Adams MJ, Widdig A, Gerald MS. 2011. Rhesus macaques (Macaca mulatta) as living fossils of hominoid personality and subjective well-being. J Comp Psychol **125**:72-83.

Williams E, Carter A, Hall C, Bremner-Harrison S. 2019. Exploring the relationship between personality and social interactions in zoo-housed elephants: Incorporation of keeper expertise. Applied Animal Behaviour Science **221**:104876.

Willisch CS, Neuhaus P. 2010. Social dominance and conflict reduction in rutting male Alpine ibex, Capra ibex. Behavioral Ecology **21**:372-380.

Wilson ADM, McLaughlin RL. 2007. Behavioural syndromes in brook charr, Salvelinus fontinalis: prey-search in the field corresponds with space use in novel laboratory situations. Animal Behaviour **74**:689-698.

Wilson AJ, Morrissey MB, Adams MJ, Walling CA, Guinness FE, Pemberton JM, Clutton-Brock TH, Kruuk LE. 2011. Indirect genetics effects and evolutionary constraint: an analysis of social dominance in red deer, Cervus elaphus. J Evol Biol **24**:772-783.

Wilson DS. 1998. Adaptive individual differences within single populations. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences **353**:199-205.

Wilson DS, Clark AB, Coleman K, Dearstyne T. 1994. Shyness and boldness in humans and other animals. Trends in Ecology & Evolution **9**:442-446.

Wilson V, Guenther A, Øverli Ø, Seltmann MW, Altschul D. 2019. Future Directions for Personality Research: Contributing New Insights to the Understanding of Animal Behavior. Animals **9**:240.

Wingfield JC. 2017. The challenge hypothesis: Where it began and relevance to humans. Horm Behav **92**:9-12.

Wingfield JC, Farner DS. 1978. The endocrinology of a natural breeding population of the white-crowned sparrow (Zonotrichia leucophrys pugetensis). Physiological Zoology **51**:188-205.

Wingfield JC, Goymann W, Jalabert C, Soma KK. 2019. Concepts derived from the challenge hypothesis. Hormones and behavior **115**:104550.

Wingfield JC, Hahn TP. 1994. Testosterone and territorial behaviour in sedentary and migratory sparrows. Animal Behaviour **47**:77-89.

Wingfield JC, Hegner RE, Dufty Jr AM, Ball GF. 1990. The" challenge hypothesis": theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. The American Naturalist **136**:829-846.

Wingfield JC, Maney DL, Breuner CW, Jacobs JD, Lynn S, Ramenofsky M, Richardson RD. 1998. Ecological bases of hormone—behavior interactions: the “emergency life history stage”. American Zoologist **38**:191-206.

Wislocki GB, Aub JC, Waldo CM. 1947. The effects of gonadectomy and the administration of testosterone propionate on the growth of antlers in male and female deer. Endocrinology **40**:202-224.

Wolf M, Van Doorn GS, Leimar O, Weissing FJ. 2007. Life-history trade-offs favour the evolution of animal personalities. Nature **447**:581.

Wolf M, Weissing FJ. 2012. Animal personalities: consequences for ecology and evolution. Trends Ecol Evol **27**:452-461.

Yirmiya K, Motsan S, Zagoory-Sharon O, Feldman R. 2020. Human attachment triggers different social buffering mechanisms under high and low early life stress rearing. International Journal of Psychophysiology.

Young C, Majolo B, Heistermann M, Schülke O, Ostner J. 2014. Responses to social and environmental stress are attenuated by strong male bonds in wild macaques. Proceedings of the National Academy of Sciences **111**:18195-18200.

Zahavi A. 1975. Mate selection—a selection for a handicap. Journal of theoretical Biology **53**:205-214.

Zahavi A. 1977. The cost of honesty (further remarks on the handicap principle). Journal of theoretical Biology **67**:603-605.

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## 10. List of scientific contributions

### 10.1 Impacted scientific papers

Saggiomo, L., **Esattore, B.**, & Picone, F., What are we talking about? Sika deer (*Cervus nippon*): A bibliometric network analysis, 2020, *Ecological Informatics*, *60*, 101146.

**Esattore B.**, Villagrán M., Pluháček J., Komárková M., Dušek A., Kotrba R., Bartošová J., Bartoš L., 2020 "To beat or not to beat: Behavioral plasticity during the antler growth period affects cortisol but not testosterone concentrations in red deer (*Cervus elaphus*) males." *General and Comparative Endocrinology* 297,113552.

Mori E., Mazza G., Saggiomo L., Sommese A., **Esattore B.,** 2017, Strangers Coming from the Sahara: An Update of the Worldwide Distribution, Potential Impacts and Conservation Opportunities of Alien Aoudad, 2017, Annales Zoologici Fennici, Vol. 54, No. 5–6, pp. 373-386

Saggiomo L., **Esattore B.**, Bartoš L., Evaluating the management success of an alien species through its hunting bags: the case of the sika deer (*Cervus nippon*) in the Czech Republic, submitted (Journal: Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis)

Saggiomo L., Bar V., **Esattore B.**, The fox who cried wolf: A keywords and literature trend analysis on the phenomenon of mesopredator release, under review (Journal: Ecological Complexity)

**Esattore B.**, Šlipogor V., Saggiomo L., Seltmann M.W., How not to judge a deer by its cover”: a personality assessment study on adult captive red deer males (*Cervus elaphus*), under review (Journal: Behavioural processes)

Bartoš L., **Esattore B.**, Peterka T., Kotrba R., Pluháček J., Ceacero F., Komárková M., Dušek A., Bartošová J., An effect of social buffering on cortisol-testosterone interactions and antler growth in red deer (*Cervus elaphus*), ready for submission

Cherin M., Breda M., **Esattore B.**, Holpin S., Hart V., Turek J., Iurino D.A., Pleistocene Fight Club: Early Pleistocene deer from Pantalla (Italy), in preparation

Ferretti F., **Esattore B.**, Bazzoni F., Rossi A.C., From the pan into the fire: roe deer, fallow deer and wolves in a Mediterranean area, in preparation

**Esattore B.**, Sensi M., Francia V., Saggiomo L., Tell me what you eat and I'll tell you… where you live: an updated review of the worldwide distribution and foraging ecology of the fallow deer (*Dama dama*), in preparation

### 10.2 Other papers

Sensi M., Mazza G., Mori E., **Esattore B.**, 2020, Valutazione ambientale del fiume Merse associata a campionamenti del granchio di fiume *Potamon fluviatile*, Atti della Società Toscana di Scienze Naturali, Atti Serie B

Urosevic M.I., **Esattore B.**, Saggiomo L., Ristic Z.A., Stojanac N., Animal welfare standards in Red deer (*Cervus elaphus*) farming, 2018, Arhiv veterinarske medicine, Vol. 11, No. 2, 11 – 20

Saggiomo L., Picone F., **Esattore B.**, Sommese A., 2017, An overview of understudied interaction types amongst large carnivores, Food Webs, 12, 35-39.

### 10.3 Publications in book of abstracts and conferences (divided by year):

#### 2020

Saggiomo L., **Esattore B.**, Bartoš L., Evaluating the management success of an alien species through its hunting bags: the case of the sika deer (*Cervus nippon*) in the Czech Republic, Online conference "Kostelecké inspirování 2020"

Dušek A., Jiříková K., **Esattore B.**, Kotrba R., Kott T., Bartoš L., Social dominance of female European red deer (*Cervus elaphus*): influence of lactation, age and sex hormones, Zoological Days, Olomouc, Czech Republic

**2019**

Dušek A., Jiříková K., Bartoš L., Kotrba R., Kott T., Kšáda V., Janovský P., **Esattore B.**, Pluháček J., Bartošová J., What influences the social dominance of female red deer (*Cervus elaphus*)?, 46th Czech and Slovak Ethological Society Conference, Bratislava, Slovakia

Dušek A., Jiříková K., Bartoš L., Kotrba R., Kott T., Kšáda V., Janovský P., **Esattore B.**, Pluháček J., Bartošová J., What influences the social dominance of female red deer (*Cervus elaphus*)?, 2019, Zoological Days, Brno, Czech Republic

Urosevic M.I., **Esattore B.**, Saggiomo L., Stojanac N., Beukovic D., Animal welfare standards in Fallow deer (*Dama dama*): regulatory compliance in European Union and Serbia, International Symposium on Animal Science (ISAS), Herceg Novi, Montenegro

**2018**

**Esattore B.**, Seltmann M., Šlipogor V., Saggiomo L., Rapti M.,How not to judge a …deer by its cover: a preliminary study on the personality of the Red deer (*Cervus elaphus*) males, 5th European Student Conference on Behaviour and Cognition, Wien, Austria, Oral presentation

**Esattore B.**, Villagrán M., Pluháček J., Komárková M., Dušek A., Kotrba R., Bartošová J., Bartoš L., *Meglio la qualità che la quantità:* possono la struttura sociale ed il comportamento degli individui influenzare la concentrazione di cortisolo in un gruppo di cervi (*Cervus elaphus*)?, 5th Behavioural Ecology Meeting, Chioggia (VE), Italy, Oral presentation

**2017**

**Esattore B.**, Villagrán M., Pluháček J., Komárková M., Dušek A., Kotrba R., Bartošová J., Bartoš L.,To beat or not to beat: "opportunistic" behaviour during antler growth period modifies cortisol concentration in red deer (*Cervus elaphus*) males, 4th European Student Conference on Behaviour and Cognition, České Budějovice, Czech Republic, Oral presentation

**Esattore B.**, Villagrán M., Pluháček J., Komárková M., Dušek A., Kotrba R., Bartošová J., Bartoš L.,To beat or not to beat: "opportunistic" behaviour during antler growth period modifies cortisol concentration in red deer (*Cervus elaphus*) males, Behaviour 2017 (35th International Ethological Conference |ASAB Summer Meeting 2017), Estoril, Portugal, Oral presentation