PHYSIOLOGICAL MEDIATION OF SOCIAL BEHAVIOR IN GROUP-LIVING CARNIVORES

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ABSTRACT

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Social behavior and relationships between conspecifics have led to the characteristic of obligate group living in some species. Benefits of these longer-term social groupings include assistance with predator defense, foraging, and infant care, which often outweigh the associated individual costs of group-living, such as increased competition for mates, food and other scarce resources. Social interactions between peers in such social groups include affiliative, aggressive, communicative, and/or cooperative behaviors. These behaviors are the result of both environmental factors and the individual's genetic, epigenetic, endocrine and neural mechanisms that affect fitness and that evolve through natural selection. My dissertation investigates the physiological mediation of these social behaviors and their relationship to the costs and benefits of group living in social carnivores. Chapter One reviews the endocrine mechanisms that mediate cooperative breeding in mammalian carnivores, focusing on reproductive suppression and alloparental care. My review indicates that breeding carnivores typically have higher levels of a suite of reproductive hormones than do non-breeders, while the effect of glucocorticoids on reproductive suppression appears to be sex specific. In my remaining chapters, I use the spotted hyena (*Crocuta crocuta*), a gregarious carnivore living in complex social groups, as my model organism in my study of the physiological mediation of complex social interactions. Chapter Two presents and validates a novel non-invasive method of collecting saliva from juvenile spotted hyenas that allows for detection of short-term fluctuations in glucocorticoid concentrations. I examine how social behaviors, such as aggression and play, affect glucocorticoid concentrations, and find that receiving aggression increases glucocorticoid levels while emitting aggression reduces them. Chapter Three investigates the social and physiological mechanisms that lead to hyenas' cooperative mobbing behavior, through exploration of when mobs occur, which hyenas participate, and what potential benefits might accrue. My findings indicate that individuals participate in this type of collective behavior primarily to obtain benefits for themselves, and that their decision to participate is a function of the characteristics and internal state of potential cooperators as well as their immediate social environment.

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INTRODUCTION

Social behavior – interaction among conspecifics – is fundamental to the survival of all mammals. At a minimum, social interactions with members of the opposite sex are necessary to find mates and produce offspring. However, many species go well beyond this minimum, exhibiting a wide range of social behaviors that function in foraging, defense, mate competition, mate choice, and parental care (Smith et al., 2017a). In some species, the advantages of social behavior have even resulted in obligate or facultative group living (Krause and Ruxton, 2002). In these species, peer-based same-sex associations, or selective affiliative relationships, are common and may ultimately provide the basis for stable, long-term animal groupings (Beery, 2019). Although parent-offspring and male-female bonding are now fairly well understood (Numan and Young, 2016), relatively little work has been done on aspects of prosocial behavior that are unrelated to reproduction. Group-living species therefore offer a valuable opportunity to study a different facet of affiliation: social interactions and relationships between peers within a social group.

Life in social groups carries costs and provides benefits to group-members. Benefits of group living are numerous, and include decreased predation risk, increased foraging efficiency, thermoregulatory benefits, information exchange, access to mates, and access to helpers for infant care. Costs of group living, however, can also be high, including competition with other group-members for food, mates, and other limited resources, potential disease transmission, and infanticide (Ebensperger et al., 2012). Nonetheless, sociality is widespread, occurring in all major lineages of mammals (Smith et al., 2017a).

An individual’s fiercest competitors and strongest allies are often members of its social group, as illustrated by the cooperation and conflict that characterize many intraspecific interactions. Social behaviors and interactions between peers may be affiliative, aggressive, communicative and/or cooperative, among others, and individuals show dramatic plasticity in their ability to switch rapidly between different social behaviors. Despite this plasticity, the occurrence of social behavior, like other phenotypes, ultimately depends on the underlying physiological mechanisms that regulate its expression (Seebacher and Krause, 2017). Social interactions thus result from a combination of environmental conditions and an individual’s genetic, epigenetic, endocrine and/or neural mechanisms, which affect fitness and evolve via natural selection (Hofmann et al., 2014). It is essential to describe these mechanisms in order to understand how social behavior is perceived and performed by individuals, both within and across species (Robinson et al., 2019). To understand social behavior, we must study both proximate (causation and development) and ultimate (adaptation and evolution) points of view (Tinbergen, 1963).

The study of social evolution thus requires an understanding of the physiological, social and environmental factors that enable social behaviors to emerge (Alexander, 1974). In my dissertation, I investigate social behaviors, their physiological mediation, and their relation to the costs and benefits of group living. In Chapter One, I review the endocrine mechanisms mediating cooperative breeding in terrestrial species of mammalian carnivores, with a focus on aspects of reproductive suppression and alloparental care (Montgomery et al., 2018). My results show that breeding carnivores typically have higher circulating levels of a suite of reproductive hormones than do helpers. The effect of glucocorticoids on reproductive suppression, however, appears to be sex-specific, as breeding males typically have higher glucocorticoid levels than non-breeding subordinates, but females exhibit no clear trends in this relationship. Overall, I echo other researchers in calling for more non-invasive studies of behavioral endocrinology within the order Carnivora, as many long-term studies currently lack endocrine data sufficient to address remaining questions regarding the physiological mediation of social behavior (Smith et al., 2017b).

In my remaining chapters, I focus on spotted hyenas (*Crocuta crocuta*) as a model organism for understanding the physiological bases of social behavior. Spotted hyenas are gregarious carnivores that live in large mixed-sex groups, called clans, of 6-130 individuals, with a mean of 29 hyenas per clan across Africa (Holekamp and Dloniak, 2010). Clans are fission-fusion societies in which all group-members know one another individually, rear their cubs together at a communal den, and defend a common territory (Kruuk, 1972), yet clan members spend much of their time alone or in small subgroups (Smith et al., 2008). Due to female philopatry and male dispersal, the large clans found in east Africa are comprised of multiple matrilines of adult females and their offspring, and several adult immigrant males (Frank, 1986a). Each clan is structured by a strict linear dominance hierarchy with natal animals ranking above immigrants (Kruuk, 1972); natal animals “inherit” their rank from their mother in a process called maternal rank inheritance (Frank, 1986b), whereas immigrant males queue for rank in a tenure-based hierarchy (East and Hofer, 2001). In most respects, clans of spotted hyenas bear little resemblance to other social carnivores but are instead remarkably similar in their structure, size, and complexity to the female-bonded societies of many cercopithecine primates (Holekamp et al., 2015). Because of their complex social lives and the prevalence of both cooperative and competitive social behaviors, spotted hyenas are an ideal species in which to examine the physiological mediation of social behavior.

For group-living animals like spotted hyenas, daily life occurs within the context of the social environment. Social interactions between peers occur frequently, and these interactions may act as social buffers to stress or as stressors in their own right (Beery et al., 2020). However, identifying direct consequences of particular social behaviors in natural populations is difficult, as most methods of non-invasive sampling reflect hormone concentrations averaged over hours or days instead of over only a few minutes. In Chapter Two, I present and validate a novel non-invasive method of collecting saliva from juvenile spotted hyenas that allows for the detection of short-term changes in concentrations of glucocorticoids, which are important mediators of metabolism and energy, and which are released in high concentrations from the adrenal gland to mobilize resources during stressful events. I then examine how glucocorticoids vary with respect to ecological variables and demographic characteristics of the hyenas sampled, and I describe a glucocorticoid daily rhythm in juvenile hyenas. Finally, I examine how social behaviors, such as aggression and social play, affect endogenous glucocorticoids, and find that while receiving aggression increases glucocorticoid concentrations, emitting aggression actually reduces glucocorticoids.

Like many complex social species, spotted hyenas frequently cooperate, joining forces to defend a common territory against conspecifics, to guard their kills, and to secure resources from sympatric carnivores (Boydston et al., 2001; Lehmann et al., 2017). In Chapter Three, I use hyenas' cooperative mobbing against lions to investigate social and physiological mechanisms facilitating collective action in these dangerous contests. I ask when mobs occur, who participates, and what the payoffs might be for those who participate (or costs for those who don't). Overall, my investigation of cooperative mobbing behavior in spotted hyenas supports the idea that collective behavior occurs largely to gain individual benefits. Mobbing is most likely to occur when the overall costs are lowest: mainly, when more hyenas are present and when male lions are absent. Furthermore, characteristics that suggest a stronger individual, such as being female (the larger sex), prime-aged (for both sexes), and higher-ranking (for both sexes), predict likelihood of mobbing, as do higher concentrations of both glucocorticoids and testosterone. When fighting with lions over a recent kill, individuals who mob are more likely to feed from the defended or acquired food. Finally, participation is also influenced by individual motivation and social facilitation; individuals are more likely to mob when they are hungry and when their maternal relatives and closely bonded associates are present in the group. Our findings demonstrate that cooperation in a complex society with differentiated, dynamic relationships is maintained through a complicated web of interacting factors that are dependent on the characteristics and internal state of potential cooperators, as well as the immediate social environment.

Data in this dissertation arise from the Mara Hyena Project, which was founded in 1988 by Kay Holekamp and Laura Smale, and which has supported multitudes of undergraduate, graduate, and post-doctoral researchers over the decades. My research is thus based on the work of dozens of graduate students, research assistants, and lab staff, from the United States, Kenya, and other nations. Furthermore, all of the work in this dissertation is the result of wonderful intellectual collaborations with other researchers in the lab and elsewhere. Therefore, I use the first-person plural, rather than the first-person singular, throughout the remainder of this dissertation.

CHAPTER ONE:

PHYSIOLOGICAL MECHANISMS MEDIATING PATTERNS OF REPRODUCTIVE SUPPRESSION AND ALLOPARENTAL CARE IN COOPERATIVELY BREEDING CARNIVORES

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ABSTRACT

Although cooperation represents a long-standing evolutionary puzzle, field studies on social carnivores have contributed greatly to our understanding of the selective forces favoring cooperative breeding. Despite these insights, our grasp of the proximate mechanisms facilitating cooperation in carnivores remains surprisingly limited. Here we provide an overview of our current knowledge of the endocrine mechanisms mediating cooperative breeding in terrestrial species belonging to the mammalian order Carnivora. We focus primarily on aspects of reproductive suppression and alloparental care. We find few studies on the topic, with some of the best studies focusing on the behavioral endocrinology of cooperative breeding in canids (dogs) and herpestids (mongooses). Overall, these studies suggest that breeding females typically have higher circulating levels of estrogen, luteinizing hormone, progesterone, and prolactin than do non-breeding adult females. We also find that among males, testosterone levels are often elevated in breeders compared to non-breeding adult males. The effect of glucocorticoids on reproductive suppression in carnivores appears to be sex-specific: breeding males typically have higher glucocorticoid levels than their non-breeding subordinates, but there is no clear pattern for breeding females. Finally, elevated levels of prolactin and oxytocin are consistently associated with alloparental care in cooperatively breeding carnivores, whereas testosterone and glucocorticoids are often lower in individuals who participate in alloparenting. Taken together, our synthesis elucidates striking gaps in our knowledge of carnivore physiology, especially the endocrine mechanisms promoting alloparental care, and we identify important areas for future research.

INTRODUCTION

The vast majority of species (~85-90%) belonging to the mammalian order Carnivora (here referred to as “carnivores”) are solitary such that conspecifics interact only to mate or raise young (Bekoff et al., 1984; Wilson and Mittermeier, 2009). Here we focus on “social carnivores,” those terrestrial members of the order Carnivora whose individuals interact frequently with one another, forming social groups called societies (Smith et al., 2017b). Specifically, these species regularly cooperate with group-mates to hunt large game, defend resources, guard against predators, attack others, and/or rear young (Clutton-Brock, 2006; Smith et al., 2012). Researchers have long recognized the social carnivores as an important taxonomic group for understanding the evolutionary origins and maintenance of cooperation (Alexander, 1974; Eberhard, 1975; Macdonald, 1983; Schaller and Lowther, 1969).

Cooperatively breeding members of the social carnivores are particularly fascinating because their social systems are characterized by alloparental care and often by some degree of reproductive suppression. Alloparental care, defined as any investment in the evolutionary fitness of non-descendent offspring (e.g., born to others), includes all behaviors in which individuals guard, groom, carry, play with, feed, or nurse the offspring of others (Creel and Creel, 1991). In contrast, reproductive suppression in cooperative breeders occurs when individuals beyond the age of sexual maturity fail to raise young of their own, regardless of the mechanism involved (Creel and Macdonald, 1995). The extent of cooperative care of young varies widely among carnivore species, ranging from joint territorial defense to the nursing and provisioning of unrelated offspring (Clutton-Brock, 2016). The degree of care often varies with the extent of reproductive suppression within social groups (Macdonald and Moehlman, 1982). Social carnivores that engage in some form of cooperative breeding include species belonging to the canid (dog), felid (cat), herpestid (mongoose), hyaenid (hyena), mustelid (weasel), and procyonid (coati) families (Isler and van Schaik, 2012; Lukas and Clutton-Brock, 2012).

Carnivores are typically categorized as communal breeders, facultative cooperative breeders, or obligate cooperative breeders based on the degree to which non-parents assist in the cooperative care of offspring born to others, regardless of how breeding is shared within the group (Clutton-Brock, 2016; Smith et al., 2017a). Among communal breeders, such as African lions (*Panthera leo*), spotted hyenas (*Crocuta crocuta*), and banded mongooses (*Mungos mungo*), most females breed during each reproductive cycle and participate in some alloparental care, although temporarily non-breeding females and males may also contribute to the care of young born to the group (Lewis and Pusey, 1997). In facultative cooperative breeders, such as black-backed jackals (*Canis mesomelas*) and Arctic foxes (*Vulpes lagopus*), both parents and non-breeding helpers alike care for the young, but the number of helpers is small, and parents may successfully raise their young with no helper assistance (Clutton-Brock, 2006). Obligate cooperative breeders, such as African wild dogs (*Lycaon pictus*) and meerkats (*Suricata suricatta*), require assistance from non-breeding helpers to successfully raise offspring; in these groups, non-breeding helpers often provide the majority of care to the young, and their number typically exceeds the number of breeders within these groups (Lukas and Clutton-Brock, 2012).

The benefits of cooperative breeding for offspring survival and fitness are widely established among many taxa, including various social carnivore species (Brown et al., 1982; Jennions and Macdonald, 1994; Moehlman and Hofer, 1997; Snowdon, 1996; Solomon, 1991). In contrast to our broad understanding of the evolutionary function of cooperative breeding (Smith, 2014), our knowledge of the physiological mechanisms mediating reproductive suppression and alloparental care in mammals remains surprisingly limited (Schradin et al., 2018). Social carnivores offer a rare opportunity to study the physiological mechanisms of cooperative breeding within a comparative framework. Carnivores face a unique set of ecological pressures, such as extreme fluctuations in prey availability and high protein diets, which may have led to the evolution of different physiological trade-offs than those experienced by cooperative breeders in other mammalian taxa (Rosenbaum and Gettler, 2018). Our goal here is therefore to provide an updated synthesis of the hormonal aspects of cooperative breeding, namely reproductive suppression and alloparental care, in social mammals within the order Carnivora. We aim to identify key gaps in our knowledge about the physiological basis for cooperation in social carnivores in an effort to propel this area of research forward within a comparative context.

REPRODUCTIVE SUPPRESSION

Sexually mature adults of either sex may fail to breed when reproductive behavior is inhibited, reproductive physiology is suppressed, or both occur. Behavioral inhibition includes avoidance of inbreeding in animals living in natal groups (Clutton-Brock et al., 2001; O’Riain et al., 2000), direct interference in mating attempts (Creel et al., 1992; Packard et al., 1985), infanticide (Cant, 2000; Ebensperger, 1998), or the inability to find a suitable mate (Hinton et al., 2013; O’Riain et al., 2000). Physiological suppression usually involves dysfunction of the hypothalamic-pituitary-gonadal (HPG) axis, leading to degradation in gonadal endocrine function, gametogenesis, and maintenance of pregnancy (Saltzman, 2010).

Most mammalian studies of the physiological mechanisms mediating reproductive inhibition have examined HPG axis activity to determine whether non-breeders are physiologically capable of reproducing. In breeders, gonadotropin-releasing hormone (GnRH) is released from neurosecretory cells in the hypothalamus, causing the pituitary to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH). In turn, LH and FSH stimulate the gonads to facilitate gametogenesis and the production of gonadal steroids such as testosterone in males and estrogen and progesterone in females. These gonadal hormones then feed back to the brain and pituitary, which further regulate secretion of GnRH, LH, and FSH (Nelson, 2011). Dysfunction at any point in this loop can potentially impair reproduction, creating a non-breeding adult (Saltzman, 2010).

Failure to breed in mammals may thus arise from many physiological causes. In males, for example, inadequate levels of GnRH, FSH, or LH may prevent spermatogenesis or lead to insufficient testosterone, which could reduce mating behavior (Newell-Fugate et al., 2012). In females, the suppression of reproductive function may occur if inadequate estrogen buildup in the female fails to signal receptivity to males, if the absence of a peak in GnRH or LH levels produces unsuccessful ovulation, or if insufficient levels of progesterone fail to establish or maintain pregnancy (Creel and Macdonald, 1995). Non-reproductive females may also display pseudopregnancy, an ovulatory but non-pregnant state that includes elevated progesterone levels and physical changes such as an extended abdomen and lactation (Asa and Valdespinot, 1998; Concannon, 2009).

Stress-induced reproductive suppression

In addition to those actions regulated by gonadal steroids, stress hormones (glucocorticoids, GCs) may be involved in regulating reproductive suppression and alloparental care in mammals. GCs, which are released from the adrenal cortex, can suppress HPG axis activity through inhibition of GnRH, and thus LH and FSH, in both males and females (Nelson, 2011). Pioneering studies showed that losing fights triggers a significant increase in circulating levels of GCs in captive rodents (Bronson and Eleftheriou, 1964; Louch and Higginbotham, 1967). These studies generated the 'stress of subordination' hypothesis, which posits that social stressors act to physiologically suppress subordinate reproduction in cooperative breeders (Creel, 2001; Wingfield and Sapolsky, 2003). However, while data from some free-living species support this hypothesis [e.g., olive baboons, *Papio anubis* (Sapolsky, 1982)], a review by Creel (Creel, 2001) reported that for five of the six studied species of free-living mammalian cooperative breeders, dominants had higher GCs than subordinates. These data initiated the ‘stress of dominance’ hypothesis, which suggests that dominant individuals endure the highest degree of social stressors, likely because aggression is stressful to both the perpetrator and the recipient (Creel et al., 2012). In the wild, dominant animals of many species engage in higher rates of aggressive behavior than subordinate group members, as dominants must participate in aggressive behavior to maintain their status while subordinates can potentially evade aggression via avoidance of dominants (Creel, 2001). These studies have clarified that dominance hierarchies, social stability, and other features that differentiate wild groups from captive ones likely influence this endocrine relationship (Sapolsky, 2005).

More recent work has suggested that the processes used to acquire and maintain social dominance, not the dominant or subordinate status itself, may determine the physiological mediation of reproductive suppression (Creel et al., 2012). For example, although subordinates may have lower overall GC levels than dominants, their GC levels may spike at much higher concentrations than dominants while they are contesting status, and it is these higher GC concentrations which may suppress reproduction in subordinates (Young et al., 2006). Clearly, further research will be required to fully elucidate the role of GCs in reproductive suppression in cooperatively breeding mammals, and studies of social carnivores may aid in these efforts.

ALLOPARENTAL CARE

The physiological mechanisms providing the proximate control of alloparenting present a second avenue for understanding mammalian cooperative breeding behavior. In breeding females, specific hormones such as estrogen, progesterone, prolactin, and oxytocin act upon the brain and peripheral structures (i.e. mammary glands) to promote maternal care (Asa, 1997; Kenkel et al., 2017). These endocrine stimuli are closely linked to pregnancy, parturition, and lactation (Nelson, 2011). However, these processes do not occur in the non-breeding helpers engaging in maternal-like behavior in cooperatively breeding societies, raising the question of whether and how alloparenting may be activated by hormonal events in these females (Schradin et al., 2018).

Allonursing, or the nursing of non-offspring infants, is physiologically costly to the female, but may provide allosuckled infants with increased growth, transferred immune compounds and improved survival rates (MacLeod and Lukas, 2014; Roulin, 2002). In mammalian mothers, two hormones primarily control lactation: prolactin, which stimulates milk secretion, and oxytocin, which stimulates milk ejection (Hill et al., 2016). Allolactators are frequently females who were recently pregnant but may have lost their own litters, suggesting that allonursing is also linked closely with the endocrinology of pregnancy (MacLeod et al., 2013). Pseudopregnancy, which is particularly common in canids, may likewise cause spontaneous lactation and thus allow for allonursing behavior (Creel et al., 1991; Gobello et al., 2001). However, it remains possible that allonursing may result from misdirected care or from milk theft, which may occur because group-living forces a female to rear her young in close proximity to others (Lewis and Pusey, 1997).

Both juvenile and adult males participate in alloparental behavior in a variety of mammalian species (Kleiman and Malcolm, 1981), yet relatively little is known about the role hormones play in the mediation of paternal and alloparental care in males (de Bruin et al., 2016; Nelson, 2011). Hormones similar to those influencing maternal behaviors might facilitate paternal behavior, namely testosterone, prolactin, and oxytocin (Wynne-Edwards and Reburn, 2000). However, the paucity of data on male alloparental hormones is further exacerbated by the confounding seasonal changes that most male mammals undergo during the breeding period, making it difficult to distinguish between hormonal changes driven by the environment versus by parental status (de Bruin et al., 2016).

Some experiments have been conducted to reveal the role of each hormone in terms of alloparental investment, particularly in rodents and primates (this issue); however, reports on larger mammals are extremely scarce and lack experimental support (de Bruin et al., 2016; Soares et al., 2010). Even for cooperative breeders, most endocrine studies remain correlative, where increases or decreases in hormone levels are related to breeding status or periods of offspring dependence. Improving our understanding of the hormonal basis of alloparental behavior in a wide range of mammalian species, including carnivores, may clarify both the proximate and ultimate basis for cooperative breeding.

LITERATURE REVIEW

We conducted a systematic literature review to investigate the endocrine basis of cooperative breeding in terrestrial members of the mammalian order Carnivora. Specifically, our goal was to synthesize knowledge of the hormones involved in reproductive suppression (including pseudopregnancy) and alloparental care in social carnivores. We first compiled a list of species exhibiting at least one form of alloparental care (e.g., cooperative defense, allonursing/alloprovisioning) from recent reviews of cooperative breeding and alloparental care (Isler and van Schaik, 2012; Lukas and Clutton-Brock, 2012). Included in our initial list were: 1) all carnivore species categorized as either cooperative or communal breeders by (Lukas and Clutton-Brock, 2012) and 2) all gregarious carnivore species exhibiting any form of alloparental care as in (Isler and van Schaik, 2012). This yielded 37 species, which belonged to the families Canidae (20 species), Eupleridae (1 species), Felidae (1 species), Herpestidae (6 species), Hyaenidae (3 species), Mustelidae (3 species), and Procyonidae (3 species; Table 1.6).

Using this list of 37 species, we performed a search in Google Scholar for the Latin name of each species and one of the seven classes of hormones identified in Saltzman (2010) as a potential mediator of cooperative breeding and alloparental behavior (Table 1.1). Thus, our literature searches contained one Latin name and each one of the following hormone terms individually: “luteinizing hormone,” “androgen,” “testosterone,” “estrogen,” “estradiol,” “progestogen,” “progesterone,” “glucocorticoid,” “cortisol,” “prolactin,” and “oxytocin.” We then repeated each search by also adding the terms “cooperative breeding” or “alloparental care” in the search field for each pair of candidate species and hormone. For example, for meerkats, we ran the following three separate queries for the hormone oxytocin: 1) “*Suricata suricatta*” “oxytocin”, 2) “*Suricata suricatta*” “oxytocin” “cooperative breeding” and 3) “*Suricata suricatta*” “oxytocin” “alloparental care”.

Of our original list of 37 species of carnivores with possible cooperative breeding and/or alloparental care (Table 1.6), we identified publications that contained data about these hormones for only 13 species. Of these, the bush dog (*Speothos venaticus*), European badger (*Meles meles*), maned wolf (*Chrysocyon brachyurus*), and spotted hyena (*Crocuta crocuta*) exhibit rare (if any) cases of allonursing and/or alloprovisioning behavior (Dugdale et al., 2010; Holekamp and Smale, 1990). All four of these species were therefore excluded from this review.

In total, endocrine data relevant to cooperative breeding and alloparental care were only available for nine species of social carnivore. Our literature review yielded six canid species [coyote (*Canis latrans*), gray wolf (*C. lupus*), Ethiopian wolf (*C. simensis*), African wild dog, Arctic fox, red fox (*V. vulpes*)] and three herpestid species [meerkat, dwarf mongoose (*Helogale parvula*), banded mongoose] in which non-breeding adults regularly engaged in cooperative breeding, allonursing, and/or alloprovisioning. For each of these nine species, we first provide a brief overview of the cooperative breeding system as background to our discussion on the hormones mediating these behaviors (Table 1.2). Then, we quantify the hormone levels for breeders relative to non-breeders and for helpers relative to non-helpers in an effort to understand the extent to which each candidate hormone acts, on average, to suppress reproduction or promote alloparenting behavior across social carnivores (for details, see Table 1.7 and Table 1.8).

FEMALE REPRODUCTIVE SUPPRESSION AND PSEUDOPREGNANCY

Reproductive suppression occurs in certain groups of social carnivores when a subset of sexually-mature females delay their age of first reproduction after puberty or fail to breed altogether (Creel and Creel, 1991). Our review synthesizes the current knowledge about hormones involved in reproductive suppression and pseudopregnancy, as discussed below for the canid and herpestid families (Figure 1.1, Table 1.3).

Canidae

As is true for most free-living carnivores, scientific knowledge regarding female reproductive physiology is limited in canids, primarily due to the difficulty of obtaining adequate sample sizes for analysis. As such, the majority of existing knowledge comes from captive studies, where a social group is arbitrarily created for cooperative breeders (Figure 1.1, Table 1.3). Most of the studied canids share many reproductive attributes, including monoestrus cycles, long proestrus and luteal phases, behavioral suppression, and spontaneous ovulation with pseudopregnancy (Asa and Valdespinot, 1998; Van der Weyde et al., 2015).

In the canid estrus cycle, estrogen concentrations typically increase during proestrus and decline during estrus regardless of pregnancy. Estrus begins with a surge in LH, after which progesterone rapidly rises. In pregnant female canids, progesterone remains elevated until parturition, and in pseudopregnant females progesterone remains elevated throughout an extended luteal phase approximating the length of gestation. High progesterone concentrations during the luteal phase are dependent on both LH and prolactin, both of which are also elevated (Concannon, 2009). Most wild canids appear to follow this general pattern of hormone secretion throughout the estrus cycle (Asa and Valdespinot, 1998; Van den Berghe et al., 2012).

Studies of captive gray wolves reveal no hormonal differences between pregnant and pseudopregnant females, including LH, progesterone, and estrogen (Packard et al., 1985; Seal et al., 1979). Thus, these studies find no evidence of physiological reproductive suppression in grey wolves, with most subordinate females exhibiting pregnancy or pseudopregnancy rather than failing to ovulate (Packard et al., 1985; Seal et al., 1979). Studies of captive coyotes and African wild dogs provide similar results, with pregnant and pseudopregnant individuals showing no differences in estrogen or progesterone (Carlson and Gese, 2008; Newell-Fugate et al., 2012; Van der Weyde et al., 2015).

Results from captive studies of Arctic foxes and red foxes are similar to those from wolves and coyotes. At the beginning of estrus, foxes exhibit no differences in LH or progesterone based on breeding or dominance status (Hartley et al., 1994; Mondain-Monval et al., 1985; Valberg and Farstad, 1992). In red foxes, progesterone concentrations remain similar between breeders and non-breeders throughout pregnancy (Hartley et al., 1994); in Arctic foxes, however, both estrogen and progesterone concentrations are higher in pregnant than non-pregnant females towards the end of gestation (Sanson et al., 2005).

Unfortunately, it is impossible to know whether what is observed in captivity is also true in the wild, which makes studies of wild cooperatively breeding carnivores even more valuable. To our knowledge, researchers have investigated sex steroid hormones and reproductive suppression in wild populations of only two canid species: African wild dogs and Ethiopian wolves (Table 1.3).

Free-living female African wild dogs appear to exhibit some physiological reproductive suppression. Dominant females have significantly higher estrogen and progesterone concentrations during estrus than subordinates (Creel et al., 1997);

however, subdominant females do cycle and ovulate despite their lower estrogen concentrations (Van den Berghe et al., 2012). It is likely that ovulation occurs in all female African wild dogs, but that behavioral suppression prevents copulation by subordinate females (Van den Berghe et al., 2012); for example, lower estrogen concentrations may make subordinates less attractive to males and thus less likely to mate (Creel and Macdonald, 1995). Ovulation without conception then results in a period of pseudopregnancy that likely increases rates of alloparenting behavior provided by subordinate females (Van den Berghe et al., 2012). While allolactation and subsequent allonursing are rare in African wild dogs, subdominant females provision pups throughout the denning period (Creel et al., 1997; Malcolm and Marten, 1982).

The reproductive physiology of Ethiopian wolves includes physiological suppression of subordinate females, and possible pseudopregnancy and allonursing of pups (van Kesteren et al., 2013). During the estrus cycle, significantly higher concentrations of estrogen are found in dominants than in subordinates, suggesting that subordinates are reproductively suppressed during the mating season (van Kesteren et al., 2013). Unlike in African wild dogs, estrus is observed during the mating season in all dominant females, but not in subordinate females, which further suggests that dominance status significantly affects a female’s probability of coming into estrus (van Kesteren et al., 2013). However, dominant and subordinate females have indistinguishable progesterone concentrations during the dominant’s pregnancy, suggesting that elevated progesterone and possibly pseudopregnancy promote allonursing by subordinates (Asa and Valdespinot, 1998; van Kesteren et al., 2013).

Herpestidae

Due to their smaller size, the herpestids have been well-studied in the wild, and they exhibit a range of patterns of reproductive suppression for adult females (Figure 1.1, Table 1.3). In the banded mongoose, all subordinate females typically ovulate and breed, suggesting no difference in sex steroid levels due to status (Cant, 2000). In the dwarf mongoose, baseline estrogen concentrations are higher in dominants than in subordinates, a difference magnified during estrus, when estrogen levels in dominants are more than triple that of their subordinates (Creel et al., 1992). Estrogen levels remain elevated in dominants throughout pregnancy until parturition. Among subordinates, these low baseline levels of estrogen may lead to low mating rates by reducing their attractiveness as mates; for those who do manage to mate, the low estrogen concentrations during estrus could cause a failure to establish pregnancy (Creel et al., 1992). Pseudopregnancy and spontaneous lactation can also occur in the dwarf mongoose, and are hormonally characterized by higher estrogen levels than in other non-pregnant, non-lactating individuals (Creel et al., 1991).

In meerkats, dominants appear to enforce reproductive suppression using aggression, and often temporary eviction from the group, to impose stressors upon subordinates. During their pregnancy, dominant females become more aggressive towards subordinate females, which increases GCs in subordinates during their dominant’s pregnancy (Dantzer et al., 2017*a*; Young et al., 2006). Pregnant dominant females also temporarily evict some subordinate individuals from the group; while evicted, these females experience extremely high GC levels that cause downregulation of the reproductive system, including reduced conception and increased abortion rates

(Young et al., 2006). Subordinate females also have generally lower baseline levels of LH and estrogen (Carlson et al., 2004; Clutton-Brock et al., 2001; Davies et al., 2016; O'Riain et al., 2000), which are not related to the temporary evictions nor attributed to any chronic stress of subordination (Young et al., 2008). Despite subordinates' lower baseline levels of reproductive hormones, recent work has demonstrated that all adult females regardless of rank possess adequate hormone levels for full-term pregnancies (Dimac-Stohl et al., 2018). Reproductive suppression in meerkats therefore appears to be primarily the result of behavioral interference by the dominant female via eviction of pregnant subordinates or infanticide of subordinate pups (Dimac-Stohl et al., 2018; Young et al., 2008). Allonursing is far more common in meerkats than in dwarf mongooses, occurring in roughly 50% of litters (MacLeod et al., 2013).

MALE REPRODUCTIVE SUPPRESSION

In some carnivore species, male reproductive hormones are suppressed in subordinates (non-breeders) when in the presence of their dominants (breeders; Figure 1.1, Table 1.4). For example, in both captive and wild African wild dogs, dominant males have significantly higher testosterone concentrations than subordinates during the mating season (Creel et al., 1997; Newell-Fugate et al., 2012), suggesting that dominant males may be able to suppress their subordinates' testosterone levels (Johnston et al., 2007). In one study, although the dominant male had testosterone concentrations at least 20 times that of subordinates, subordinates and dominants had similar testicular volumes and sperm production during the mating season (Johnston et al., 2007); this is unusual in that elevated testosterone usually supports greater testes size and sperm production (Nelson, 2011). In banded mongooses, dominant males have higher testosterone concentrations than subordinates during the mating season, but not during the rest of the year (Sanderson, 2012). In contrast, although dominant Ethiopian wolves have higher overall testosterone concentrations than subordinates, this difference is not significant during the mating season (van Kesteren et al., 2012). This indicates that subordinate male Ethiopian wolves are likely behaviorally suppressed, and observations suggest that subordinates are often prevented from mating by the dominant male (van Kesteren et al., 2012).

Other carnivore species, however, show no rank- or breeding-related differences in testosterone levels (Figure 1.1, Table 1.4); in species with high reproductive skew, reproduction is therefore behaviorally suppressed. Subordinate male gray wolves and dwarf mongooses have testosterone levels indistinguishable from those of dominants, but are prevented from mating by the dominant male (Creel et al., 1993, 1992; Packard et al., 1985). Likewise, dominant and subordinate male meerkats have similar levels of both LH and testosterone (Carlson et al., 2004; Davies et al., 2016; Moss et al., 2001; O'Riain et al., 2000). In these species, failure to breed by reproductively mature males appears to be the result of direct behavioral interference by dominant males rather than endocrine deficiencies.

STRESS-INDUCED REPRODUCTIVE SUPPRESSION

The effect of GCs on reproductive suppression in social carnivores appears to be sex specific. In male carnivores, most of the data on stress hormones (Figure 1.1, Table 1.4) support the 'stress of dominance' theory, which predicts that dominants have higher

GC concentrations than subordinates. In canids, this theory is supported by cooperatively breeding gray wolves and Ethiopian wolves (Sands and Creel, 2004; van Kesteren et al., 2012), although in African wild dogs the relationship between rank and GC levels is debated (Creel et al., 1997; Van der Weyde et al., 2016). Similarly, in the herpestid family, dominant male meerkats have the highest GC concentrations, followed by natal subordinates, then immigrant subordinates (Carlson et al., 2004), while no rank- or breeding-based differences are found in dwarf mongoose males (Creel, 2005).

In female carnivores, rank and breeding relationships with GCs are species-specific (Figure 1.1, Table 1.3), and there is no clear pattern within either the canid or herpestid families. In free-living canids, dominant gray wolf females display higher GC levels with no decrease in fertility (Sands and Creel, 2004), while no difference in GCs is found between dominant and subordinate Ethiopian wolves (van Kesteren et al., 2013). In female African wild dogs, the relationship between rank and GC levels is again under debate (Creel et al., 1997; Van der Weyde et al., 2016). For red foxes, GC levels are elevated in non-pregnant females, indicating a possible stress-related mechanism in their reproductive suppression (Hartley et al., 1994). In herpestids, subordinate female meerkats have higher GCs than dominants during their dominant’s pregnancy or while temporarily evicted from the group (Dantzer et al., 2017a; Young et al., 2006). Aside from these periods, however, dominant and subordinate female meerkats have similar GC levels (Barrette et al., 2012; Young et al., 2008). Among banded mongooses, rank-related maternal stress during gestation leads to reduced reproductive success in subordinate females (Sanderson et al., 2015), although dominant dwarf mongoose females exhibit higher GC levels than subordinates without any apparent fertility costs

(Creel, 2005).

ALLOPARENTAL CARE

Studies of the endocrine basis of alloparental care are limited to only five carnivore species, with the great majority of work focusing on meerkats (Figure 1.2, Table 1.5). Meerkats engage in many strictly alloparental behaviors, including babysitting, feeding, and teaching pups how to forage (Clutton-Brock et al., 2001; Doolan and Macdonald, 1999; Thornton and McAulife, 2006). Peptide hormones such as prolactin and oxytocin, which are associated with parental care across vertebrates (Kenkel et al., 2017), likewise promote alloparental care in meerkats. Male meerkats who opt to remain at the nest and babysit on a given day have higher plasma levels of prolactin earlier in the day (Carlson et al., 2006b). Increased prolactin is non-significantly associated with increased pup-feeding behavior (Carlson et al., 2006a), and peripheral administration of oxytocin also results in increased pup-feeding behavior and time spent associating with pups (Madden and Clutton-Brock, 2011). Gonadal steroids such as testosterone seem to have little effect on alloparental care in meerkats, including babysitting and pup-feeding (Carlson et al., 2006a, 2006b), although a negative correlation between testosterone levels and pup-feeding rates exists in males during their extraterritorial prospecting periods (Young et al., 2005). In banded mongooses, high testosterone concentrations predict lower babysitting effort in the following days (Sanderson, 2012).

The association of alloparental behavior with GCs is more variable, even within a single carnivore species (Figure 1.2, Table 1.5). In meerkats, individuals of both sexes who opt to remain at the nest and babysit have lower levels of GCs (Carlson et al., 2006b; Dantzer et al., 2017b). Although females with high GCs spend more time associating with pups (Santema et al., 2013), experimental manipulation of GCs shows that females with the lowest levels of GCs are more frequent in their pup-feeding behavior (Dantzer et al., 2017b). However, experimentally elevated levels of GCs increased the amount of provisioning by males (Dantzer et al., 2017b). Similarly, in naturalistic conditions, males with higher GC levels are more likely to participate in pup feeding (Carlson et al., 2006a). In banded mongooses, however, males with low GC concentrations prior to the care period are more likely to provision pups (Sanderson et al., 2014). There thus appears to be little consistency in the effects of GCs on alloparenting behavior, suggesting that other factors such as age, sex, and reproductive experience may be at play (Dantzer et al., 2017b).

In canids, prolactin likely moderates alloparental behavior (Figure 1.2, Table 1.5). However, there is little research that specifically disentangles alloparental care behavior from other temporal hormone and behavior changes in these seasonal breeders. Gray wolves of both sexes experience a rise in prolactin during the pup-rearing period, which could increase their alloparental input (Kreeger et al., 1991). While all females regardless of pregnancy status experience this increase in prolactin, lactating wolf mothers exhibit the highest prolactin levels (Kreeger et al., 1991). Likewise, in Arctic foxes, all females exhibit a seasonal increase in prolactin coinciding with lactation, although prolactin is highest in lactating mothers (Mondain-Monval et al., 1985; Valberg and Mondain-Monval, 1992). In coyotes and red foxes, however, prolactin rises significantly in pregnant and lactating females, but does not exhibit much change in non-pregnant individuals (Carlson and Gese, 2008; Hartley et al., 1994). In addition to prolactin's effects in canid females, pseudopregnancy and the corresponding changes in sex steroid hormones are thought to be a major component of the high levels of alloparental care (including allonursing) exhibited by subordinate females (Asa and Valdespinot, 1998), although we could find no experimental evidence to support this.

CONCLUSION

Our study elucidates a strong bias towards endocrine research investigating mechanisms underlying reproductive suppression, but not alloparental care, within cooperatively breeding members of the order Carnivora. In general, our results show that breeding carnivores tend to have higher circulating levels of a suite of reproductive hormones than do non-breeders (Figure 1.1, Table 1.3, Table 1.4). Breeding females typically have higher levels of estrogen, LH, progesterone, and/or prolactin than do other non-breeding adults, and breeding males often have elevated levels of testosterone compared to non-breeders. The effect of GCs on reproductive suppression in carnivores, however, appears to be sex-specific, as breeding males typically have higher GC levels than non-breeding subordinates, but females exhibit no clear trends in this relationship. These same reproductive and stress hormones control reproduction in other mammalian species, including primates and rodents (Beehner and Lu, 2013; Holmes et al., 2009), suggesting that a common group of hormones mediates the reproductive physiology of mammalian cooperative breeding. At low levels, these hormones likely act to suppress reproduction for non-breeding adults in cooperatively-breeding societies.

With respect to the endocrine basis of alloparental care in carnivores, we identify few published studies detailing the mechanisms shaping helping behaviors themselves, although these studies tend to follow the same general pattern observed in other mammals (Kenkel et al., 2017; Ziegler, 2000). Overall, these studies indicate that elevated levels of prolactin and oxytocin, but reduced levels of testosterone and glucocorticoids, are associated with increased alloparental care (Figure 1.2, Table 1.5). We could find no studies that investigated the effects of progesterone or estrogen on alloparental care in carnivores, although both hormones are thought to influence affiliative and cooperative behavior across mammals (Gangestad and Grebe, 2017; Soares et al., 2010).

The patterns revealed here allow us to extend recent work investigating the endocrine basis of paternal care in carnivores (de Bruin et al., 2016). Importantly, we echo de Bruin et al. (de Bruin et al., 2016) in emphasizing the skewed research focus in carnivore studies, which, despite a number of long-term studies (Smith et al., 2017b), often lack endocrine data sufficient to address the issues explored in this review. We are surprised to see how few studies actually used fecal samples for their endocrine analysis, especially given recent advancements in non-invasive hormone monitoring (Kersey and Dehnhard, 2014; Sheriff et al., 2011). Future work should utilize these non-invasive hormone sampling methods to fill these gaps in our knowledge and to identify the shared endocrine mechanisms promoting alloparental care in carnivores.

Whereas the evolutionary advantages of alloparental care have been extensively documented (Creel and Creel, 1991; Macdonald and Moehlman, 1982), our review reveals that the endocrine mechanisms mediating these behaviors have yet to be the subject of systematic study across cooperatively breeding species in the order Carnivora. Undoubtedly, this is a fruitful avenue for future studies, both for correlative research performed on free-living species and for experimental manipulations such as those conducted in meerkats (Dantzer et al., 2017b; Madden and Clutton-Brock, 2011). Although we recognize the challenges associated with performing these endocrine studies in ecological contexts (Smith et al., 2017c), there is also a need for studies investigating how hormonal mechanisms may mediate aspects of cooperative breeding beyond babysitting, allonursing, and alloprovisioning. Parallel investigations should thus aim to clarify the endocrine basis of group defense, predator detection, and communal denning in an effort to document the hormones promoting cooperation of all kinds in mammalian carnivores. Together, insights revealed here in combination with future avenues of inquiry are contributing to the emerging view that hormones play a central role in shaping the lives of social carnivores in particular and mammals in general.

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APPENDICES

APPENDIX A:

FIGURES AND TABLES

Table 1.1. Candidate hormones mediating reproductive suppression and alloparental care in mammals.

Hormone	**Actions in breeders**
Androgen (testosterone)	Sex steroid hormone; stimulates male secondary sexual characteristics and sperm production
Estrogen (estradiol)	Sex steroid hormone; regulates female reproductive cycles
Glucocorticoid (cortisol)	“Stress” steroid hormone; regulates energy balance, mediates “fight or flight” response and trade-offs in immune function and reproduction
Luteinizing hormone	Sex steroid hormone; triggers ovulation in females
Oxytocin	Peptide hormone; associated with lactation, pair-bonding and orgasm
Progestogen (progesterone)	Sex steroid hormone; supports pregnancy
Prolactin	Peptide hormone; supports pregnancy and stimulates milk production

Table 1.2. Biology of cooperatively breeding carnivores with endocrine data.[a]

Family	Species	Breeding system	Group size	Reproductive skew	Helping behavior
Canidae (Dogs)	**Coyote** (*Canis latrans*)	Monogamous pair bond (basic unit), but facultatively cooperative (may be joined by associates)	2-10 individuals	60-90% of females breed; 70% of yearlings produce litters	Pups are reared by breeding pair and associates (usually adult siblings)
	Gray wolf (*Canis lupus*)	Monogamous dominant pair bond (basic unit), but facultatively cooperative (often joined by offspring)	5-12 individuals (up to 36)	Dominant pair breeds, but other females suppressed unless food abundant	Den attendance and provisioning of food by helpers
	Ethiopian wolf (*Canis simensis*)	Obligate cooperative breeders living in multi-male philopatric packs comprised of daughters of alpha female	3-13 adults	Only 60% of females breed, but dominant female breeds every year	Helpers patrol, allonurse (50% of individuals exhibit pseudopregnancy), and provision pups
	African wild dog (*Lycaon pictus*)	Obligate cooperative breeders with one dominant pair (basic unit) in pack	4-9 individuals (up to 30) adults and yearlings	One dominant breeding pair usually suppresses reproduction of others; subordinates may breed but are rarely successful	All pack members provision mothers and pups by regurgitating meat; babysitting and group defense also occurs
	Arctic fox (*Vulpes lagopus*)	Monogamous pair (basic unit), but may be communal (one to several closely related breeding pairs may share a den)	2-12 individuals (up to 18)	One dominant breeding pair usually suppresses reproduction of yearling females	Non-breeding yearlings help by provisioning young with meat
	Red fox *(Vulpes vulpes)*	Communal (one male shares den with two breeding females), pair bond (monogamous pair), or facultatively cooperative (breeding pair and several non-breeding related female helpers)	2-7 individuals	Female reproductive suppression varies with population size and food density, enforced via infanticide, harassment and fetal reabsorption	Non-breeding female helpers may feed, groom, and babysit pups; adoption occurs and females allonurse

Table 1.2. (cont'd)

Family	Species	Breeding system	Group size	Reproductive skew	Helping behavior
Herpestidae (Mongooses)	**Dwarf mongoose** (*Helogale parvula*)	Obligate cooperative breeders comprised of dominant breeding pair, their offspring and other adult males and females	8-9 individuals (up to 32)	High reproductive skew reinforced socially (via infanticide) and hormonally; plural breeding; dominant female produces 73% of litters	Subordinates (mainly females) babysit (carry, guard against conspecifics and predators), allonurse (via pseudopregnancy), provision, and groom pups
	Banded mongoose (*Mungos mungo*)	Obligate cooperative breeders in cohesive groups comprised of closely related adult males and females along with their immature offspring	9-28 individuals (typically 15, up to 75)	Dominant males breed most often and guard females; limited skew because all adult females breed synchronously	Helpers (mainly young non-breeding males, but also breeding females) babysit (carry, guard) and provision pups
	Meerkat (*Suricata suricatta*)	Obligate cooperative breeders (2-3 family units, including adult males, adult females, and young of the dominant breeding pair)	4-9 individuals (up to 49)	Dominant female produces 75% and dominant male produces 80% of litters; infanticide and evictions enforce female reproductive suppression	Non-breeding helpers babysit (carry, guard), allonurse, and provision pups

[a] All information summarized from Wilson and Mittermeier (2009).

Figure 1.1. Endocrine basis of reproductive suppression in carnivores. Box plots representing the extent to which breeding individuals in carnivore species belonging to Canidae (dog family) or Herpestidae (mongoose family) possess circulating levels of hormones that are, on average, relatively higher (indicated as a positive value), equivalent to (indicated as a zero value), or relatively lower (indicated as a negative value) than non-breeding members of that same species. Data points represent individual species. Data are available for estrogen, luteinizing hormone (LH), progesterone, and prolactin for females only and testosterone for males only. Glucocorticoid (GCs) data are available for both sexes.

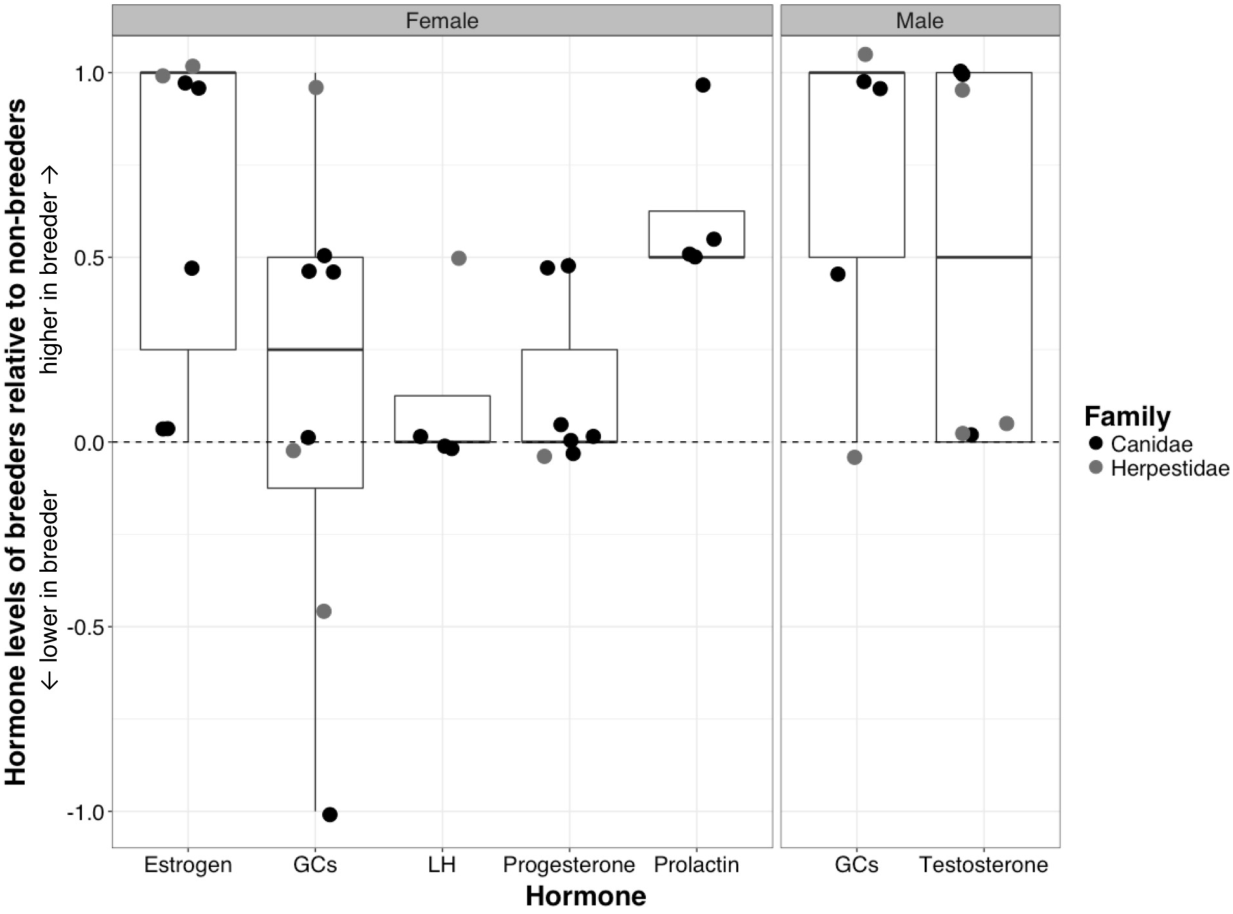

Table 1.3. Endocrine basis of reproductive suppression in adult female carnivores.

Species	Category	Luteinizing hormone	Estrogen	Progesterone	Prolactin	Glucocorticoids
Coyote *(Canis latrans)*	Breeder vs. non-breeder[a]		No differences (Carlson and Gese, 2008)	No differences (Carlson and Gese, 2008)		
	Pregnant vs. non-pregnant[b]		Little difference (Carlson and Gese, 2008)	No differences (Carlson and Gese, 2008)	No differences in first half of gestation, higher in pregnant females in second half of gestation (Carlson and Gese, 2008)	
Gray wolf *(Canis lupus)*	Breeder vs. non-breeder	No differences (Seal et al., 1979)	No differences (Seal et al., 1979)	No differences (Packard et al., 1985; Seal et al., 1979)		No differences (Packard et al., 1985); higher in dominants (Sands and Creel, 2004)
	Pregnant vs. non-pregnant	No differences (Seal et al., 1979)	No differences (Packard et al., 1985; Seal et al., 1979)	No differences (Packard et al., 1985; Seal et al., 1979)	Higher in pregnant/lactating females (although all females exhibit seasonal increase) (Kreeger et al., 1991)	No differences (Packard et al., 1985)
Ethiopian wolf *(Canis simensis)*	Breeder vs. non-breeder		Higher in dominant females (van Kesteren et al., 2013)			No differences (van Kesteren et al., 2013)
	Pregnant vs. non-pregnant			No differences (elevated in all females) (van Kesteren et al., 2013)		

Table 1.3. (cont'd)

Species	Category	Luteinizing hormone	Estrogen	Progesterone	Prolactin	Glucocorticoids
African wild dog *(Lycaon pictus)*	Breeder vs. non-breeder		Higher in dominant females during estrus (Creel et al., 1997)	Higher in dominant females during estrus (Creel et al., 1997)		Higher in dominant females (Creel et al., 1997); no differences (Van der Weyde et al., 2016)
	Pregnant vs. non-pregnant			No differences between pregnant & pseudo-pregnant (Newell-Fugate et al., 2012)		No differences between pregnant and pseudo-pregnant (Van der Weyde et al., 2016)
Arctic fox *(Vulpes lagopus)*	Pregnant vs. non-pregnant	No differences (Mondain-Monval et al., 1985)	Higher in pregnant females at end of gestation (Sanson et al., 2005)	Higher in pregnant females in second half of gestation (Sanson et al., 2005; Valberg and Farstad, 1992)	Higher in pregnant females in second half of gestation (Mondain-Monval et al., 1985; Valberg and Mondain-Monval, 1992)	Higher in pregnant females at end of gestation (Sanson et al., 2005)
Red fox *(Vulpes vulpes)*	Pregnant vs. non-pregnant	No differences (Hartley et al., 1994)		Little difference (Hartley et al., 1994)	Higher in pregnant females in second half of gestation (Hartley et al., 1994)	Lower in pregnant females (Hartley et al., 1994)
Dwarf mongoose *(Helogale parvula)*	Breeder vs. non-breeder		Higher in dominant females (Creel et al., 1992)			Higher in dominant females (Creel, 2005)
	Pregnant vs. non-pregnant		Higher in dominant females (Creel et al., 1992)			

Table 1.3. (cont'd)

Species	Category	Luteinizing hormone	Estrogen	Progesterone	Prolactin	Glucocorticoids
Banded mongoose *(Mungos mungo)*	Breeder vs. non-breeder					No differences prior to conception or in first trimester of gestation (Sanderson et al., 2015)
	Pregnant vs. non-pregnant					Lower in dominant females during second and third trimesters (Sanderson et al., 2015)
Meerkat *(Suricata suricatta)*	Breeder vs. non-breeder	Higher in dominant females (O'Riain et al., 2000); no differences (Carlson et al., 2004)	Higher in dominant females (Carlson et al., 2004; Clutton-Brock et al., 2001; Davies et al., 2016; Dimac-Stohl et al., 2018; Young et al., 2008)	No differences (Dimac-Stohl et al., 2018)		Higher in dominant females (Carlson et al., 2004); no differences (Barrette et al., 2012; Young et al., 2008)
	Pregnant vs. non-pregnant					No differences (Barrette et al., 2012); higher in subordinate females (Dantzer et al., 2017a)

[a] Breeder vs. non-breeder: hormone differences evaluated during the breeding season.
[b] Pregnant vs. non-pregnant: hormone differences evaluated during the pregnancy period between cycling individuals.

Table 1.4. Endocrine basis of reproductive suppression in adult male carnivores.

Species[a]	Category	Testosterone	Glucocorticoids
Gray wolf *(Canis lupus)*	Breeder vs. non-breeder[b]	No differences (Packard et al., 1985)	Higher in dominant males (Sands and Creel, 2004)
Ethiopian wolf *(Canis simensis)*	Breeder vs. non-breeder	Higher in dominant males (van Kesteren et al., 2012)	Higher in dominant males (van Kesteren et al., 2012)
African wild dog *(Lycaon pictus)*	Breeder vs. non-breeder	Higher in dominant males during breeding season (Creel et al., 1997; Johnston et al., 2007; Newell-Fugate et al., 2012)	Higher in dominant males (Creel et al., 1997); no differences (Van der Weyde et al., 2016)
Dwarf mongoose *(Helogale parvula)*	Breeder vs. non-breeder	No differences (Creel et al., 1993, 1992)	No differences (Creel, 2005)
Banded mongoose *(Mungos mungo)*	Breeder vs. non-breeder	Higher in dominant males during breeding season (Sanderson, 2012)	
Meerkat *(Suricata suricatta)*	Breeder vs. non-breeder	No differences (Carlson et al., 2004; Davies et al., 2016; Moss et al., 2001)	Highest in dominant males, then natal subordinates, then immigrant subordinates (Carlson et al., 2004)

[a] No data were available for coyotes *(Canis latrans)*, Arctic foxes *(Vulpes lagopus)*, or red foxes *(Vulpes vulpes)* for either of the candidate hormones.

[b] Breeder vs. non-breeder: hormone differences evaluated during the breeding season.

Figure 1.2. Endocrine basis of alloparenting in carnivores. Box plots representing the extent to which individuals engaging in alloparental care (helpers) possess circulating levels of hormones that are, on average, relatively higher (indicated as a positive value), equivalent to (indicated as a zero value), or relatively lower (indicated as a negative value) than individuals who do not engage in alloparental care (non-helpers). All data come from Herpestidae (mongoose family) and include both sexes. Data points represent either males or females of a single species. Data are available for glucocorticoids (GCs), oxytocin, prolactin, and testosterone.

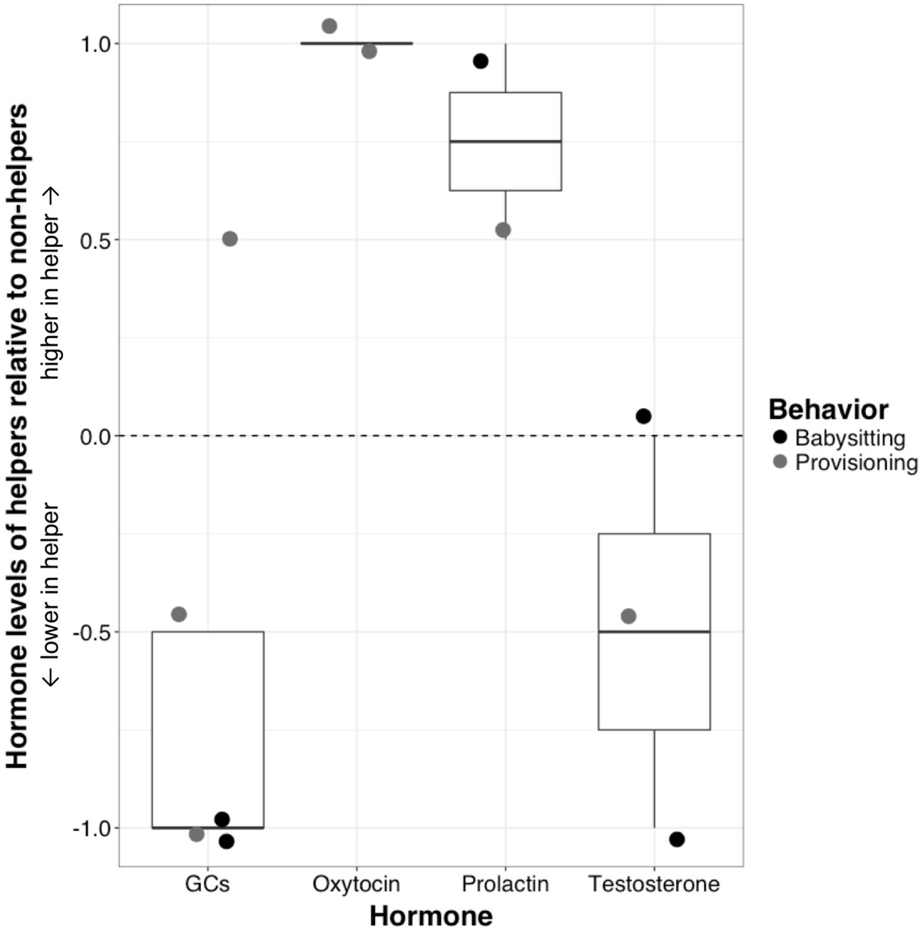

Table 1.5. Endocrine basis of alloparental behaviors in both sexes of cooperatively breeding carnivores.

Species[a]	Sex	Testosterone	Prolactin	Oxytocin	Glucocorticoids
Gray wolf *(Canis lupus)*	Both		High prolactin during lactation period (Kreeger et al., 1991)		
Ethiopian wolf *(Canis simensis)*	Male	Does not decrease during denning (van Kesteren et al., 2012)			
African wild dog *(Lycaon pictus)*	Male	Does not decrease during denning (Creel et al., 1997)			Does not vary within breeding period (Van der Weyde et al., 2016)
Arctic fox *(Vulpes lagopus)*	Female		Higher in lactating females, seasonal increase in all females coinciding with lactation (Mondain-Monval et al., 1985)		
Banded mongoose *(Mungos mungo)*	Male	Males with low testosterone were more likely to babysit pups in following days (Sanderson, 2012)			Males with low cortisol prior to the care period were more likely to provision pups (Sanderson et al., 2014)

Table 1.5. (cont'd)

Species	Sex	Testosterone	Prolactin	Oxytocin	Glucocorticoids
Meerkat *(Suricata suricatta)*	Male	No differences prior to choosing to babysit (Carlson et al., 2006b); no differences prior to pup-feeding (Carlson et al., 2006a); prospecting males had elevated levels of testosterone and reduced pup-feeding rates (Young et al., 2005)	Higher in males prior to choosing to babysit (Carlson et al., 2006b); no differences prior to pup-feeding in full model, but prolactin higher in males prior to pup-feeding without cortisol in model (Carlson et al., 2006a)		Lower in males prior to choosing to babysit (Carlson et al., 2006b); higher in males prior to pup-feeding (Carlson et al., 2006a); males with higher GCs exhibited increased pup-feeding (Dantzer et al., 2017b)
	Female				Females dosed with cortisol increased time spent close to pups (Santema et al., 2013); females with lower GCs exhibited increased pup-feeding (Dantzer et al., 2017b)
	Both			Individuals dosed with oxytocin were more generous in proportion of food fed to pups & spent more time close to pups (Madden and Clutton-Brock, 2011)	Individuals dosed with cortisol showed no differences in pup feeding (Santema et al., 2013); individuals dosed with mifepristone increased babysitting (Dantzer et al., 2017b)

[a] No data were available for coyotes *(Canus latrans)*, red foxes (*Vulpes vulpes)*, or dwarf mongooses (*Helogale parvula)* for any of the candidate hormones. No studies measured the effects of luteinizing hormone, estrogen or progesterone on alloparental care.

APPENDIX B:

SUPPLEMENTAL FIGURES AND TABLES

Table 1.6. Initial data set of species used in literature review.

Family	Candidate species	Endocrine data	Source
Canidae	Golden jackal *(Canis aureus)*	no	(Isler and van Schaik, 2012; Lukas and Clutton-Brock, 2012)
Canidae	Coyote (*Canis latrans*)	yes	(Isler and van Schaik, 2012; Lukas and Clutton-Brock, 2012)
Canidae	Gray wolf (*Canis lupus*)	yes	(Isler and van Schaik, 2012; Lukas and Clutton-Brock, 2012)
Canidae	Black-backed jackal (*Canis mesomelas*)	no	(Isler and van Schaik, 2012; Lukas and Clutton-Brock, 2012)
Canidae	Ethiopian wolf (*Canis simensis*)	yes	(Lukas and Clutton-Brock, 2012)
Canidae	Maned wolf (*Chrysocyon brachyurus*)	yes	(Isler and van Schaik, 2012)
Canidae	Dhole (*Cuon alpinus*)	no	(Isler and van Schaik, 2012)
Canidae	African wild dog (*Lycaon pictus*)	yes	(Isler and van Schaik, 2012; Lukas and Clutton-Brock, 2012)
Canidae	Raccoon dog (*Nyctereutes procyonoides*)	no	(Isler and van Schaik, 2012)
Canidae	Bat eared fox (*Otocyon megalotis*)	no	(Isler and van Schaik, 2012)
Canidae	Pampas fox (*Pseudalopex gymnocercus*)	no	(Isler and van Schaik, 2012)
Canidae	Bush dog (*Speothos venaticus*)	yes	(Isler and van Schaik, 2012)
Canidae	Gray fox (*Urocyon cinereoargenteus*)	no	(Isler and van Schaik, 2012)
Canidae	Island fox (*Urocyon littoralis*)	no	(Isler and van Schaik, 2012)
Canidae	Indian fox (*Vulpes bengalensis*)	no	(Isler and van Schaik, 2012)
Canidae	Cape fox (*Vulpes chama*)	no	(Isler and van Schaik, 2012)
Canidae	Arctic fox (*Vulpes lagopus*)	yes	(Isler and van Schaik, 2012; Lukas and Clutton-Brock, 2012)
Canidae	Swift fox (*Vulpes velox*)	no	(Isler and van Schaik, 2012)
Canidae	Red fox (*Vulpes vulpes*)	yes	(Isler and van Schaik, 2012)
Canidae	Fennec fox (*Vulpes zerda*)	no	(Isler and van Schaik, 2012)
Eupleridae	Ring-tailed mongoose (*Galidia elegans*)	no	(Isler and van Schaik, 2012)
Felidae	African lion (*Panthera leo*)	no	(Isler and van Schaik, 2012; Lukas and Clutton-Brock, 2012)

Table 1.6. (cont'd)

Family	Candidate species	Endocrine data	Source
Herpestidae	Yellow mongoose (*Cynictis penicillata*)	no	(Isler and van Schaik, 2012)
Herpestidae	Ethiopian dwarf mongoose (*Helogale hirtula*)	no	(Lukas and Clutton-Brock, 2012)
Herpestidae	Dwarf mongoose (*Helogale parvula*)	yes	(Isler and van Schaik, 2012; Lukas and Clutton-Brock, 2012)
Herpestidae	Egyptian mongoose (*Herpestes ichneumon*)	no	(Isler and van Schaik, 2012)
Herpestidae	Banded mongoose (*Mungos mungo*)	yes	(Isler and van Schaik, 2012; Lukas and Clutton-Brock, 2012)
Herpestidae	Meerkat (*Suricata suricatta*)	yes	(Isler and van Schaik, 2012; Lukas and Clutton-Brock, 2012)
Hyaenidae	Spotted hyena (*Crocuta crocuta*)	yes	(Isler and van Schaik, 2012; Lukas and Clutton-Brock, 2012)
Hyaenidae	Brown hyena (*Hyaena brunnea*)	no	(Isler and van Schaik, 2012; Lukas and Clutton-Brock, 2012)
Hyaenidae	Aardwolf (*Proteles cristatus*)	no	(Isler and van Schaik, 2012)
Mustelidae	Smooth-coated otter (*Lutrogale perspicillata*)	no	(Isler and van Schaik, 2012)
Mustelidae	European badger (*Meles meles*)	yes	(Isler and van Schaik, 2012; Lukas and Clutton-Brock, 2012)
Mustelidae	Giant otter (*Pteronura brasiliensis*)	no	(Isler and van Schaik, 2012)
Procyonidae	White-nosed coati (*Nasua narica*)	no	(Isler and van Schaik, 2012; Lukas and Clutton-Brock, 2012)
Procyonidae	Ring-tailed coati (*Nasua nasua*)	no	(Isler and van Schaik, 2012)
Procyonidae	Kinkajou (*Potos flavus*)	no	(Isler and van Schaik, 2012)

Table 1.7. Data set of values used to create Figure 1.1. Values were assigned based on studies listed in Table 1.3 and Table 1.4. Values were assigned for each species, hormone, and sex combination as follows: 1) all studies showed that breeders had higher levels than non-breeders; 0.5) some studies showed that breeders had higher levels than non-breeders; 0) most studies showed no hormone differences between breeders and non-breeders; -0.5) some studies showed that breeders had lower levels than non-breeders; -1) all studies showed that breeders had lower levels than non-breeders. Studies which showed that breeders have different hormone levels than non-breeders for only part of the breeding period were assigned 0.5 or -0.5.

Species	Family	Hormone	Sex	Value
Canis latrans	Canidae	Estrogen	Female	0
Canis lupus	Canidae	Estrogen	Female	0
Canis simensis	Canidae	Estrogen	Female	1
Lycaon pictus	Canidae	Estrogen	Female	1
Vulpes lagopus	Canidae	Estrogen	Female	0.5
Helogale parvula	Herpestidae	Estrogen	Female	1
Suricata suricatta	Herpestidae	Estrogen	Female	1
Canis lupus	Canidae	GCs	Female	0.5
Canis lupus	Canidae	GCs	Male	1
Canis simensis	Canidae	GCs	Female	0
Canis simensis	Canidae	GCs	Male	1
Lycaon pictus	Canidae	GCs	Female	0.5
Lycaon pictus	Canidae	GCs	Male	0.5
Vulpes lagopus	Canidae	GCs	Female	0.5
Vulpes vulpes	Canidae	GCs	Female	-1
Helogale parvula	Herpestidae	GCs	Female	1
Helogale parvula	Herpestidae	GCs	Male	0
Mungos mungo	Herpestidae	GCs	Female	-0.5
Suricata suricatta	Herpestidae	GCs	Female	0
Suricata suricatta	Herpestidae	GCs	Male	1
Canis lupus	Canidae	LH	Female	0
Vulpes lagopus	Canidae	LH	Female	0

Table 1.7. (cont'd)

Species	Family	Hormone	Sex	Value
Vulpes vulpes	Canidae	LH	Female	0
Suricata suricatta	Herpestidae	LH	Female	0.5
Canis latrans	Canidae	Progesterone	Female	0
Canis lupus	Canidae	Progesterone	Female	0
Canis simensis	Canidae	Progesterone	Female	0
Lycaon pictus	Canidae	Progesterone	Female	0.5
Vulpes lagopus	Canidae	Progesterone	Female	0.5
Vulpes vulpes	Canidae	Progesterone	Female	0
Suricata suricatta	Herpestidae	Progesterone	Female	0
Canis latrans	Canidae	Prolactin	Female	0.5
Canis lupus	Canidae	Prolactin	Female	1
Vulpes lagopus	Canidae	Prolactin	Female	0.5
Vulpes vulpes	Canidae	Prolactin	Female	0.5
Canis lupus	Canidae	Testosterone	Male	0
Canis simensis	Canidae	Testosterone	Male	1
Lycaon pictus	Canidae	Testosterone	Male	1
Helogale parvula	Herpestidae	Testosterone	Male	0
Mungos mungo	Herpestidae	Testosterone	Male	1
Suricata suricatta	Herpestidae	Testosterone	Male	0

Table 1.8. Data set of values used to create Figure 1.2. Values were assigned based on studies from banded mongooses *(Mungos mungo)* and meerkats *(Suricata suricatta)* listed in Table 1.5. Values were assigned for each species, hormone, sex, and alloparental behavior combination as follows: 1) all studies showed that helpers had higher levels than non-helpers; 0.5) some studies showed that helpers had higher levels than non-helpers; 0) most studies showed no hormone differences between helpers and non-helpers; -0.5) some studies showed that helpers had lower levels than non-helpers; -1) all studies showed that helpers had lower levels than non-helpers.

Species	Hormone	Sex	Behavior	Value
Mungos mungo	GCs	Male	Provisioning	-1
Suricata suricatta	GCs	Male	Babysitting	-1
Suricata suricatta	GCs	Female	Babysitting	-1
Suricata suricatta	GCs	Male	Provisioning	0.5
Suricata suricatta	GCs	Female	Provisioning	-0.5
Suricata suricatta	Oxytocin	Male	Provisioning	1
Suricata suricatta	Oxytocin	Female	Provisioning	1
Suricata suricatta	Prolactin	Male	Babysitting	1
Suricata suricatta	Prolactin	Male	Provisioning	0.5
Mungos mungo	Testosterone	Male	Babysitting	-1
Suricata suricatta	Testosterone	Male	Babysitting	0
Suricata suricatta	Testosterone	Male	Provisioning	-0.5

CHAPTER TWO:

MEASURING SALIVARY CORTISOL IN WILD CARNIVORES

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ABSTRACT

Salivary analyses provide a useful alternative to fecal and urinary analyses in non-invasive studies of behavioral endocrinology. Here we use saliva to assess cortisol levels in a wild population of spotted hyenas (*Crocuta crocuta*), a gregarious carnivore living in complex social groups. We describe a novel non-invasive method of collecting saliva from juvenile hyenas, validate a salivary cortisol assay for use in this species, and investigate several questions regarding the endocrinology of spotted hyenas. Using nearly 300 samples collected from over 70 juveniles, we obtained evidence of a daily rhythm in salivary cortisol concentrations with two declining phases (or at least two peaks), possibly associated with their crepuscular activity patterns. We also found that cortisol varied across juvenile hyenas according to litter size and intra-litter rank, but surprisingly did not vary with age, sex, or social rank relative to the rest of the group. Finally, we examined how social behaviors, such as aggression and play, affected salivary cortisol, and found that receiving aggression increased cortisol concentrations while emitting aggression reduced them.

INTRODUCTION

Our ability to non-invasively assess the physiological condition of wild animals has advanced our understanding of the relationship between physiology and other aspects of their biology. This has been particularly important in field studies of larger mammals, which, for both practical and ethical reasons, cannot be trapped and sampled easily in the field. In particular, techniques for assessing the metabolic products of steroid hormones in feces and urine have permitted investigation of important questions in ecology, evolution, ethology, and animal conservation and welfare (Behringer and Deschner, 2017; Kersey and Dehnhard, 2014; Palme, 2019).

Despite these technical advances, however, several methodological challenges remain when measuring metabolic products in feces and urine. First, excreted metabolites may originate from several different sources within the endocrine system [e.g., androgens may originate from either the gonads or the adrenal system], and their origins may be difficult to pinpoint once they have been excreted (Goymann, 2012; Preis et al., 2011). Second, excreted metabolites may be so alike in structure that determining the metabolites' native plasma hormone is not possible [e.g., metabolites from cortisol and testosterone can cross-react in different assays (Pribbenow et al., 2017; Touma and Palme, 2005)]. Third, assays might measure hormones from prey animals in the predator's diet that have passed through its gut without having any biological impact (Goymann, 2012; von der Ohe et al., 2004). Fourth, fecal and urinary metabolite measures represent integrated endocrine variation over periods of hours or days (Behringer and Deschner, 2017; Palme et al., 2005). Although these measures are well-suited for investigating baseline endocrine states, they provide limited information about real-time or short-term variations in endocrine physiology.

Salivary analyses provide a useful complement to fecal and urinary analyses in studies of behavioral endocrinology. First, salivary hormone sampling allows for a real-time assessment of endocrine status, including diurnal variation in hormone production (Cross and Rogers, 2004; Heintz et al., 2011) and short-term physiological responses to behavioral interactions (Horváth et al., 2008; Wobber et al., 2010). For example, following stress onset, cortisol levels in saliva and in plasma rise in parallel, with salivary cortisol lagging only minutes behind plasma cortisol (Beerda et al., 1998; Riek et al., 2019). Second, salivary concentrations of steroid hormones accurately reflect the unbound plasma concentrations of those hormones because the lipid-soluble steroids transfer from blood to saliva via rapid passive diffusion along a concentration gradient (Kirschbaum and Hellhammer, 1989; Wood, 2009); salivary cortisol is thus highly correlated with plasma cortisol concentrations across species (Sheriff et al., 2011). Third, saliva collection permits repeated sampling in much smaller windows of time than can be achieved with urine or feces. Fourth, a wide range of salivary analytes are now available, including analytes that cannot be measured as excreted metabolites. Salivary studies now include measures of steroid [e.g., glucocorticoids, androgens, progestogens, estrogens (Behringer and Deschner, 2017)] and peptide hormones [e.g., oxytocin, vasopressin (MacLean et al., 2018)], correlates of sympathetic axis function [e.g., alpha amylase (Higham et al., 2010)], and measures of immune function and health [e.g., secretory immunoglobulin A, C-reactive protein (Lensen et al., 2015)].

Use of saliva collection for hormone measurement has recently become widespread for both captive animals and wild primates, but is not broadly used for other free-living species, including carnivores. Here we collected saliva from members of a wild population of spotted hyenas (*Crocuta crocuta*) in the Maasai Mara National Reserve, Kenya. Spotted hyenas are large, gregarious carnivores that live in matriarchal social groups called ‘clans’ (Kruuk, 1972). Spotted hyenas are unusual in that they are a ‘sex-role-reversed’ species: adult females are larger and more aggressive than adult males and the females’ genitalia are heavily ‘masculinized’ (Glickman et al., 2006). Due to these peculiar traits, much work has been done on the endocrine physiology of spotted hyenas using either plasma (usually from captive individuals) or feces (usually from wild populations). Most of this work has been with adults, documenting, among other things, how late-pregnancy androgen exposure affects aggressive behavior in female spotted hyenas (Dloniak et al., 2006a; Holekamp et al., 2013) and how fecal glucocorticoid metabolites vary with social and ecological variables (Goymann et al., 2001; Van Meter et al., 2009). Other work has investigated how social behavior and social interactions affect endocrine physiology in hyenas, demonstrating that social interactions with and around females are correlated with higher androgen concentrations in adult males (Dloniak et al., 2006b; Goymann et al., 2003).

However, there remain a number of gaps in our knowledge of spotted hyena physiology – and that of carnivores in general – that are difficult to fill using fecal hormone metabolites. First, cortisol exhibits clear circadian variation in the saliva and plasma of many mammals and birds. Diurnal animals have elevated glucocorticoid concentrations at the end of the dark phase of a light-dark cycle to promote energy availability for locomotion and foraging, and the pattern is reversed in nocturnal animals (Kumar Jha et al., 2015). However, many carnivore species, including hyenas, are crepuscular, being most active around twilight (Holekamp and Dloniak, 2010); we still know little about circadian cortisol rhythms in species with these activity patterns.

Second, many carnivore species raise their young in dens (Noonan et al., 2015; Wolff and Peterson, 1998), which can impede fecal sampling because researchers are often unwilling to disturb individuals at such sites. Unfortunately, this is true for infant spotted hyenas, which rarely defecate away from the den. This leads to a gap in our understanding of endocrine changes occurring throughout ontogeny, as we are seldom able to collect fecal samples from den-dwelling juveniles.

Third, saliva sampling in primates has recently begun to elucidate the short-term physiological consequences of social behavior (Hohmann et al., 2009; Leeds et al., 2018). Like primates, social carnivores display complex social behavior; spotted hyenas in particular have evolved behaviors that produce a social system very similar to that of cercopithecine primates (Holekamp et al., 2015). However, we do not know if the proximate mechanisms mediating these complex social behaviors are evolutionarily similar or disparate across taxa. Comparing primates and hyenas with respect to the physiological mediation of, and reactions to, specific behaviors should shed considerable light on the relationship between the fitness consequences of social behavior and the proximate mechanisms mediating its occurrence.

Here, we present methods for collecting and analyzing saliva from wild carnivores. We performed field testing of free-living juvenile spotted hyenas, exploring different materials for hyenas to chew on to capture saliva and various flavorings to act as attractants. First, we investigated whether our cortisol assay of hyena saliva samples met analytical validation criteria and performed adrenocorticotropic hormone (ACTH) stimulation tests on three wild adult spotted hyenas to permit clear physiological validation of our sampling methods. Second, we assessed how methodological covariates affected obtained measures of cortisol, and we documented the nature of diurnal variation in cortisol concentrations among wild juvenile hyenas. Third, we determined how cortisol values varied with respect to biologically meaningful demographic characteristics of the hyenas tested. Finally, we inquired how short-term social behavior affects cortisol concentrations. To our knowledge, these data represent the first systematic attempt to collect and measure salivary hormones from wild carnivores without capture.

METHODS

Between 2015 and 2018, we studied hormones and behavior in three clans of wild spotted hyenas in the Maasai Mara National Reserve in southwestern Kenya; these clans have been monitored continuously since 2008. We monitored clans daily during two observation periods, in the morning from 0530 h to 0930 h and in the evening from 1700 h to 2100 h. When we encountered a subgroup of one or more hyenas separated from other group-members by at least 200 m, we initiated an observation session and recorded the identities of all hyenas present, using their unique spot patterns and ear damage to recognize individuals. Sessions lasted from five minutes to a few hours, and ended when behavioral interactions ceased, and/or observers left that individual or group.

In all sessions, we performed scan-sampling (Altmann, 1974) upon arrival and every twenty minutes throughout each session to determine the identities and activities of hyenas present. We also recorded common agonistic and affiliative behaviors using all-occurrence sampling (Altmann, 1974). Aggressive interactions included threat behaviors with intention movement (stand over, displace), body movement (lunge, chase, snap), or aggressive contact (bite, bite shake) (Kruuk, 1972). Affiliative behaviors included greeting, social sniffing, and allo-grooming (Smith et al., 2015). Greetings occur when two hyenas stand parallel to one another and face in opposite directions to sniff the other’s anogenital region (Smith et al., 2011), and social sniffs occur when multiple hyenas lean against one another to sniff a spot on the ground or a grass stalk together (Kruuk, 1972). We recorded all occurrences of allo-grooming, except for those within mother-infant pairs. We also recorded social play behavior, which we defined as two or more individuals engaged together in chasing, wrestling, jumping or chewing on one another (Tanner et al., 2007). We recorded social play behavior using two-min scan sampling, where sampling was initiated when social play was observed, repeated at two-min intervals as long as any social play behavior was occurring, and terminated when at least two scan samples had passed with no social play behavior. If hyenas began to play later in the session, the two-min sampling protocol was re-started.

Saliva sample collection

Saliva samples were collected from juvenile hyenas at communal dens using a hand-held pole apparatus (Figure 2.1) modified after Lutz et al. (2000). To enable safe and effective saliva sampling from carnivores, we lengthened and strengthened the apparatus, modified the swab, and modified the bait. We used a solid piece of rope instead of commercially available saliva swabs, as even juvenile hyenas have sharp teeth and strong bite force (Binder and van Valkenburgh, 2000). We also used a high-fat bait instead of bait with high sugar content, as hyenas, like many carnivores, have lost some sweet-taste receptors (Jiang et al., 2012).

Our device consisted of a 1-meter PVC pipe (diameter 2.5 cm) with a bolt crossing the pipe near the bottom and secured with a nut. A 9-inch piece of solid braid polyester rope (3/8-inch diameter, Quality Nylon Rope) was knotted at the top and coated with ~1 tsp of vegetable fat (Kimbo, Bidco Africa; Figure 2.1A). Polyester rope was chosen for its limited effect on steroid hormone concentrations (Gröschl et al., 2008; Hansen et al., 2008), and vegetable fat was chosen as bait due to its wide availability in Kenya and low phytoestrogen content (Verleyen et al. 2002). The baited rope was inserted into the PVC pipe and the bolt secured under the knot to hold the rope in place (Figure 2.1B). A single known juvenile hyena was allowed to chew on the rope for 1-5 min (Figure 2.1C,D), after which the apparatus was withdrawn (Kobelt et al., 2003). To prevent contamination from other food sources, juveniles who had fed or nursed within the past 10 min were not permitted to chew the saliva collection device (Petrullo et al., 2016). After saliva collection, the white rope was inspected for traces of blood, because blood contamination may artificially elevate measured salivary cortisol levels (Kivlighan et al., 2004). If no traces of blood were found, the saturated portion of the rope was cut away, placed in a Salivette tube with a perforated inlay and stored at room temperature for the remainder of the observation period.

Upon return to camp, samples were centrifuged for 10 min, and the saliva collected at the bottom of the tube was again inspected for possible blood contamination: a pink coloration will appear at a blood concentration of 0.1–0.2% (Wood, 2009). If the saliva appeared clear or white, it was transferred to a cryotube with a disposable pipette and frozen in liquid nitrogen. In association with each saliva sample, we recorded the identity of the hyena, the date, the start and stop time of chewing, and the time at which each sample was frozen in liquid nitrogen. Saliva volume was not recorded because cortisol levels are unaffected by salivary flow rate across species (Kirschbaum and Hellhammer, 1989; Sheriff et al., 2011).

Physiological validation: ACTH stimulation tests

In June-July 2015, we performed ACTH stimulation tests on three wild adult hyenas (2 males, 1 female) (Goymann et al., 1999). Hyenas were anesthetized with 6.5 mg/kg tiletamine-zolazepam (Telazol, Zoetis) diluted in 3 mL distilled water, administered intramuscularly in a pressurized dart fired from a CO_2-powered rifle (Holekamp and Sisk, 2003). Immobilizations took place between 0700 and 0900 hours. Within 10-15 min of darting, we drew our first blood sample from the jugular vein of each individual into a heparinized vacutainer tube. Approximately 20 min after the first blood draw, we injected 0.25 mg of synthetic ACTH (Cortrosyn, Amphastar Pharmaceutical) diluted in 1 mL distilled water into the thigh muscle of a back leg. Serial blood samples were drawn at 15-min intervals from the first blood draw until 90 min after ACTH injection. Serial saliva samples were taken at the same 15-min intervals as the blood samples by carefully pipetting saliva out of the mouth and into a cryotube using a disposable pipette. To keep the hyena hydrated over the 2-hour test period, we injected a 20 mL bolus of saline solution under the loose skin of the hyena’s neck or legs every 15-20 min. Supplementary doses of Telazol were administered as necessary throughout this procedure to maintain deep anesthesia.

After placing the anesthetized hyena in a safe and shaded place to recover from anesthesia, we returned to camp. Saliva samples were inspected for possible blood contamination (Wood, 2009) and then frozen in liquid nitrogen. Blood samples were centrifuged for 10 min; plasma was then drawn off, aliquoted, and stored in liquid nitrogen until it was shipped on dry ice to the United States, where it was stored at -80°C until assay. Plasma samples were assayed in duplicate using a corticosterone radioimmunoassay kit (MP Biomedicals CortiCote RIA kit, #06B256440); further details of this plasma assay are published in Holekamp and Smale (1998).

Salivary cortisol assay

All saliva samples were treated identically for the remainder of the analysis. Within one year, samples were transported on dry ice to a -20°C freezer in the USA. Prior to analysis, samples were thawed, briefly vortexed, and centrifuged at 3000 rpm at 12°C for 15 min. Centrifuged samples often had a coating of vegetable fat on top; clear saliva was pipetted out from below the vegetable fat and stored in a fresh microcentrifuge tube for future analysis. Samples were then either re-frozen or assayed immediately.

To measure concentrations of salivary cortisol, samples were assayed in duplicate using a cortisol enzyme immunoassay kit already validated in humans and some animals (Salimetrics Cortisol Enzyme Immunoassay Kit, #1-3002). Cross-reactivity of the antibody with steroids was as follows: cortisol: 100%; dexamethasone: 19.2%; prednisolone: 0.568%; corticosterone: 0.214%; 11-deoxycortisol: 0.156%; cortisone: 0.130%; triamcinolone: 0.086%; 21-deoxycortisol: 0.041%; progesterone: 0.015%; testosterone: 0.006%. All other steroids tested: < 0.004%. Analytical sensitivity for cortisol was 0.007 ug/dL.

Analytical validation

A parallelism validation demonstrated that our assay could reliably measure cortisol in saliva samples of varying concentrations by comparing the curve of a serial dilution of sample pool against the curve created by standardized amounts of synthetic hormone (Brown et al., 2004). In our validation, we assayed a serial dilution (6 dilutions) of a hyena saliva pool; we then modeled the percent hormone binding as a function of the calculated cortisol concentrations of the pool dilutions and of the kit standards. We included an interaction term between type (pool versus standards) and cortisol concentration; if the interaction term is non-significant, it indicates that the slopes of the curves of pool dilutions and of the kit standards are parallel.

An accuracy validation demonstrated that concentrations of hormone measured with our assay corresponded to the true concentration of that hormone in samples by adding a known amount of hormone to the sample pool (Brown et al., 2004). In our validation, we spiked diluted aliquots of hyena saliva pool (80% binding) with a small amount of each kit standard, such that each assayed aliquot was 50% hyena saliva pool and 50% kit standard. We then calculated the recovery of the hormone added to the pool via the kit standard as the amount observed (cortisol measured by assay) divided by the amount expected (based on known concentration of kit standards).

A precision validation demonstrated that our assay results were consistent both within and between assays (Brown et al., 2004). To demonstrate that the assay results were consistent within an assay, we ran a dilution of the hyena saliva pool (80% binding) 6 times in the same assay (intra-assay CV). To demonstrate that the assay results were consistent between assays, we ran the same low (70% binding) and high concentration (20% binding) kit controls in all assays, as well as the same aliquot from the hyena saliva pool (45% binding).

Temporal effects

Most mammals exhibit a daily circadian rhythm in their cortisol concentrations (Kumar Jha et al., 2015). To determine whether juvenile hyenas' cortisol varies predictably across the day, we explored the distribution of logged cortisol concentrations relative to the time of day at which that sample was collected (Figure 2.6). Juvenile hyenas dwelling in the communal den are most active around dawn and dusk, when mothers visit the den to socialize and to nurse their cubs. Thus, we investigated the relationship between time and juvenile cortisol concentrations in two ways, 1) morning vs. evening and 2) relative to the time of sunrise/sunset.

To determine whether the observed daily patterns in juvenile cortisol was associated with maternal presence at the den, we used GPS data collected from adult females in 2012-2014 to document maternal presence at dens. GPS radio collars (Vectronic Aerospace, Germany) were deployed in 2012 on 10 adult females from two of the three clans in our study. Collars were programmed to record GPS locations hourly from 1600 h to 1000 h, and also once at 1300 h, for a total of 20 location fixes per 24-hour period. We determined maternal presence at dens by calculating the proportion of each female's total fixes that occurred within 100m of the clan's active communal den (Greenberg, 2017). Further details about the GPS collars and analysis are available in Greenberg (2017).

Statistical analysis

All analyses were conducted using R Version 3.6.3 and R Studio Version 1.2.5042. Prior to any analysis, we explored our data by investigating outliers, distribution and collinearity (Zuur et al., 2010). Forest plots were created using R package sjPlot (Lüdecke, 2020a), and all other plots were created using R package ggplot2 (Wickham et al., 2020).

For the physiological validation, we performed a Pearson’s product-moment correlation on logged values of cortisol concentrations in paired saliva and plasma samples taken from hyenas during ACTH stimulation tests. We also built a simple linear model, where we modeled the logged cortisol concentrations as a function of time relative to ACTH injection, the sample type (plasma versus saliva), and the interaction between time and sample type.

Modeling cortisol concentrations in juvenile spotted hyenas

Because this is the first time, to our knowledge, that saliva has been collected from wild spotted hyenas for hormone measurement, we investigated many methodological covariates with the potential to influence observed cortisol concentrations. We first ran a series of bivariate models, where we modeled cortisol concentrations as a function of individual methodological variables, including hyena chew time on the saliva device (minutes), time between collection and freezing of saliva sample (hours), time between collection and assay of saliva sample (months), and number of freeze-thaw cycles undergone by each sample.

Next, we built a global model of methodological, ecological, and demographic variables with the potential to influence measured cortisol concentrations (Table 2.1). Here we included any methodological variables that were significantly associated with cortisol concentrations in the above bivariate models. We included collection time of day (AM/PM) and collection time relative to sunrise/sunset (min) to determine if there were any temporal effects. We included an interaction between time of day and time relative to sunrise/set in case the cortisol concentrations changed at different rates in the morning versus evening. We included daily temperature (both minimum and maximum) and precipitation to account for potential thermal stress (de Bruijn and Romero, 2018). Daily temperatures were measured (°C) with an outdoor min/max thermometer, and daily rainfall was measured (mm) using a standard plastic rain gauge. We included an interaction between minimum and maximum temperature in case thermal stress was due to the change in temperature rather than the minimum/maximum. We included monthly prey density to account for potential maternal nutritional stress (Dloniak, 2004). We monitored prey availability during biweekly surveys by counting all wild herbivores within 100 m of 2-3 line transects (1.5-5.4 km long) in each clan territory (Holekamp et al., 1999). Prey density was calculated as prey counted per square kilometer based on the number of animals sighted during line transect surveys. For each month of our study, we calculated the mean prey density within the territories of each of our study clans and assigned that prey density to any saliva samples collected in that clan during that month.

Lastly, we included the demographic covariates of hyena age, sex, maternal social rank, and litter status. Cortisol concentrations differ between the sexes and vary throughout ontogeny in many species (Behringer and Deschner, 2017). Furthermore, social rank, measured here by maternal rank and litter status, is known to affect cortisol concentrations in some species (Creel et al., 2012). We calculated the age in months of each hyena from which we obtained a saliva sample; we estimated juvenile birthdates (to ± 7 days) from their size and pelage when they were first seen above ground (Holekamp et al., 1996). We only sampled juvenile hyenas, or hyenas who were less than 24 months of age (Glickman et al., 1992), due to safety concerns. All hyenas were sexed based on the morphology of the erect phallus (Frank et al., 1990). Because spotted hyenas exhibit maternal rank inheritance (Engh et al., 2000), we assigned juveniles the same social rank as that of their mother at the time of sampling. The social rank of each adult female hyena was determined based on the occurrence of submissive behavior during dyadic agonistic interactions (Strauss and Holekamp, 2019a); for each clan, standardized social ranks were calculated annually for adult females using the MatReorder method in R package DynaRank (Strauss, 2019). We assigned litter status for each juvenile to one of three categories based on whether we ever observed that juvenile with a littermate. If not, juveniles were considered 'singletons.' If we did observe a littermate at any point in time, we determined the 'dominant' and the 'subordinate' juvenile based on the outcome of aggressive interactions between the littermates during early life (Smale et al., 1995). We included interactions between age and sex, and between age and rank, to account for the possibility of different developmental trajectories based on sex or rank. We also included an interaction between rank and prey density to account for the effect of rank on access to resources for adult females (Holekamp et al., 1996).

Prior to creating our global model, we tested model predictors for multicollinearity using both correlation coefficients and variance inflation factors (VIFs), and we removed collinear predictors until none were collinear, with all correlation coefficients ≤ 0.7 and all VIFs ≤ 3 (Harrison et al., 2018). Numeric model predictors were z-score standardized immediately before modeling using the scale function in R to allow comparison of coefficients (Harrison et al., 2018). We performed model selection on the global model using the dredge function in R package MuMIn (Bartoń, 2020). The top model was visually inspected to confirm assumptions regarding multicollinearity, normality of residuals, normality of random effects, heteroscedasticity, and homogeneity of variance using R package performance (Lüdecke et al., 2020). We also inspected groups and observations for disproportionate influence on the model; removal of identified outliers (n = 2) did not change interpretation of results and were thus left in the dataset. Between-group comparisons of litter status were conducted using a Tukey post-hoc test for multiple comparisons of means in R package multcomp (Hothorn et al., 2020). Predicted cortisol values for plotting were obtained using the ggpredict function in R package ggeffects (Lüdecke, 2020b).

Investigating behavioral correlates

Studies have demonstrated a predictable relationship between stressful events and cortisol excretion in saliva across species. In humans, peak concentrations in saliva are reached 20-30 min after the onset of the stress condition (Kirschbaum and Hellhammer, 1989). Most animal studies focusing on the effects of behavior or events on cortisol concentrations use a saliva collection time of 15-30 min post-behavior/event [lambs (*Ovis aries*; Chapagain et al., 2014); cattle (*Bos taurus*; Wagner et al., 2013); rhesus macaques (*Macaca mulatta*; Petrullo et al., 2016); common marmosets (*Callithrix jacchus*; Cross et al., 2004); bonobos (*Pan paniscus*; Hohmann et al., 2009)]. In domestic dogs (*Canis lupus familiaris*), salivary cortisol levels peak at 20 min post-stressor (Buttner et al., 2015; Horváth et al., 2008); cortisol is elevated within 10 min after exposure to a stressor, remains elevated at 30 min, and returns to baseline within 60 min (Beerda et al., 1998). Based on these numbers, we used a 10-30 min window post-behavior to investigate how social behavior may affect cortisol concentrations in wild hyenas.

Using our field notes, we categorized saliva samples based on the specific behavioral interactions involving the sampled hyena in the 10-30 min before saliva sample collection. Samples were only included in the analyses if the individual exhibited a single type of behavior during the time window to avoid conflicting behavioral signals [e.g., the sample would be recorded as active if the only behaviors recorded were active and were not resting]. We then used the residuals from the top model explaining salivary cortisol concentrations to investigate the effect of social behavior on cortisol concentrations. We built a simple linear model, where residuals were modeled as a function of the behavior category. Between-group comparisons were conducted using Tukey HSD tests.

We built two models based on two separate assignments of saliva samples to groups. For our first model, or our ‘general’ model, samples were categorized as ‘rest’ (lie down or sit), ‘non-social active’ (wander, walk, lope, investigate, etc.), ‘aggression’ (either emitting or receiving aggression), or ‘affiliation’ (greet, social sniff, groom, social play). For our second model, we re-categorized saliva samples based on the occurrence of specific vigorous affiliative or aggressive behaviors; we wanted to compare behaviors of similar activity level, due to cortisol’s metabolic functions, but different valence. Thus, for our second model, or our ‘vigorous’ model, we compared samples in which the individual had emitted aggression (‘aggressor’), received aggression (‘recipient’), or engaged in social play (‘play’) in the 10-30 min before the sample was collected.

RESULTS

Validation of salivary cortisol measurement in spotted hyenas

Analytical validation. Our assay passed all three analytical validation tests. For parallelism, there was no significant interaction between cortisol concentrations and the type of sample (pool vs. standard) ($t = 2.175$, $p = 0.060$), indicating that the sample pool dilution curve and the standard curve were parallel. Mean cortisol recovery was 99.8 ± 9.2%, indicating the accuracy of our salivary cortisol measurements across concentrations. Our intra-assay CV was 7.4%, while our inter-assay CVs were 10.2% (low concentration control; n = 15 assays), 4.7% (high concentration control; n = 15 assays), and 13.2% (hyena saliva pool; n = 7 assays), indicating the precision of our salivary cortisol measurements.

Physiological validation. Our results indicated that cortisol measured in saliva closely reflects that measured in plasma in spotted hyenas (Figure 2.2). The correlation between cortisol measured in saliva versus plasma was 82.7% ($p < 0.001$). A linear mixed model indicated that the time relative to ACTH injection was significantly correlated with both cortisol concentrations ($\beta = 0.031$, $p < 0.001$) and sample type ($\beta = -2.71$, $p < 0.001$), but was not correlated with the interaction between time and sample type ($\beta = 0.001$, $p = 0.904$), indicating that cortisol concentrations in plasma and saliva increased at similar rates. Cortisol (ug/dL) measured in saliva was 37% of the cortisol (ug/dL) measured in plasma.

Temporal effects

Juvenile spotted hyenas exhibited two daily phases of decline in their salivary cortisol levels throughout the day, once around sunrise and once around sunset (Figure

2.3A). This indicates that there must be at least two peaks in salivary cortisol concentrations, although we cannot determine when the troughs and peaks occur, nor can we rule out the possibility that there are more than two peaks. These declining phases (and the peaks that must precede them) seem to correspond with maternal activity at the communal den (Figure 2.3B).

Juvenile cortisol concentrations

Methodological covariates. Neither variation in chew time nor time between collection and freezing had any systematic influence on measured concentrations of salivary cortisol. Chew time (mean = 3.5 min, range = 1-8) was not associated with cortisol concentrations in a bivariate model ($\beta = 0.027$, $p = 0.670$). The time a sample spent at room temperature after collection and prior to being frozen in liquid nitrogen (mean = 2.1 hours, range = 0.6-5.0) was also not associated with cortisol concentrations in a bivariate model ($\beta = 0.011$, $p = 0.871$). Time between collection and assay (mean = 9.1 months, range = 2.3-32.3) and number of freeze-thaw cycles (mean = 2 cycles, range = 1-4) were significantly associated with cortisol concentrations in bivariate models and were thus included in the global model of cortisol concentrations.

Juvenile cortisol model. Our top model (Figure 2.4A) predicting salivary cortisol concentrations in juvenile hyenas included time between collection and assay ($\beta = -0.40$, $p < 0.001$; Figure 2.4B), indicating that cortisol concentrations decreased as storage time increased. Samples collected in the evening had higher cortisol than samples collected in the morning ($\beta = 0.47$, $p < 0.001$; Figure 2.4B), and samples collected before sunrise or sunset contained more cortisol than samples collected later that same morning or evening, respectively ($\beta = -0.21$, $p < 0.001$; Figure 2.4B). Samples collected on hotter days had higher cortisol than samples collected on cooler days (β = 0.24, p < 0.001; Figure 2.4B). Samples from dominant littermates had lower cortisol than samples from either subordinate littermates or singleton juveniles, although subordinate and singleton juveniles did not differ (Tukey post-hoc test: [dominant - subordinate]: β = -0.53, p = 0.058; [dominant - singleton]: β = -0.68, p = 0.007; [subordinate - singleton]: β = -0.15, p = 0.810; Figure 2.4C). Number of freeze-thaw cycles, minimum temperature, precipitation, prey density, age, sex, and social rank were not included in the top model or any model within 6 AIC of the top model.

Behavioral correlates of cortisol concentrations

In our general behavior model, none of the various activity states we recorded (active, rest, affiliation, or aggression) differed significantly from one another (all p > 0.2; Figure 2.5A). In our vigorous behavior model, aggressors had lower cortisol concentrations than recipients of aggression or individuals engaging in social play (Tukey post-hoc test: [aggressor - recipient]: β = -0.56, p = 0.089; [aggressor - play]: β = -0.85, p = 0.007; [aggressor - active]: β = -0.45, p = 0.140; Figure 2.5B); cortisol concentrations did not differ significantly among other sample types (all p > 0.2; Figure 2.5B).

DISCUSSION

We successfully measured cortisol in the saliva of wild hyenas. We fully validated our rope and bait device as a method for measuring cortisol concentrations non-invasively in juvenile hyenas (Figure 2.1, Figure 2.2). In total, we collected nearly 300 saliva samples from more than 70 individuals over three years. We hope that our detailed description of our methods provides other wildlife researchers with a relatively simple way to measure salivary cortisol in other wild carnivores.

Although we only sampled saliva from our subjects during morning and evening observation periods, we nevertheless established that a daily cortisol rhythm must exist: we documented two clear phases of decline in salivary cortisol concentrations, one around dawn and one around dusk. We found that cortisol covaried with daily temperature and correlated with maternal den attendance, but not with other socio-ecological covariates. We also found that cortisol varied across juvenile hyenas from different types of litters, but surprisingly did not vary with age, sex, or social rank. Most importantly, we were able to observe the effects of short-term engagement in specific types of vigorous behavior on the salivary cortisol concentrations of young hyenas.

Methodological considerations for other researchers

The literature is divided on most of the methodological covariates we tested. Studies indicate that salivary cortisol is stable when stored at room temperature for up to 3 days (Gröschl et al., 2001), but disagree on stability at -20°C, with one study finding no change in measured cortisol after 1 year (Garde and Hansen, 2005) and another finding a 9% decrease in measured cortisol after only 1 month (Toone et al., 2013). The effect of freeze-thaw cycles on salivary cortisol also varies. Gröschl et al. (2001) found that 5 freeze-thaw cycles resulted in a 10% decrease in salivary cortisol concentrations, whereas Garde and Hansen (2005) reported that 4 freeze-thaw cycles did not affect cortisol concentrations. We found that hyena saliva can be stored up to 5 hours at room temperature prior to freezing with no effect on measured cortisol concentrations, but that concentrations decrease across months and years during storage at -20°C. Our results also indicate that the number of freeze-thaw cycles (up to 4) may affect cortisol concentrations depending on other covariates. The amount of time that a hyena spent chewing on the collection device had no effect on measured cortisol; this is consistent with results obtained from domestic dogs, in which up to four minutes of chewing had no effect on measured cortisol (Kobelt et al., 2003). Overall, the variation in results from hyenas and other species suggests that all future studies should assess these covariates for their specific sampling procedures.

Daily cortisol rhythm

We found evidence of a daily rhythm in the salivary cortisol concentrations of juvenile spotted hyenas (Figure 2.3), which are most active around dawn and dusk and at night, particularly in the warmer portions of the species' geographic range. Although we were unable to sample saliva outside our normal observation hours, we nevertheless observed two distinct periods of decline in salivary cortisol, one around sunrise and one around sunset (Figure 2.3A). Concentrations in samples that we collected at the beginning of these sampling periods were similar, as were concentrations at the end. This pattern points to the conclusion that there are two peaks and two troughs in what is likely a bimodal rhythm of salivary cortisol, although the possibility of more peaks and troughs cannot be ruled out. We suspect that this reflects the bimodal distribution of activity in spotted hyenas, as adult hyenas exhibit peaks in activity just before dawn and dusk (Kolowski et al., 2007). This bimodal distribution of activity is especially evident in juvenile hyenas living at the communal den, who comprised the majority of individuals sampled in our dataset. Mothers of juvenile hyenas typically visit the communal den in the morning and again in the evening to nurse their cubs (Figure 2.3B), producing peaks in both activity and nursing there twice per day (White, 2007). This observed daily cortisol rhythm could be generated either internally (i.e., by a circadian clock), by responses to daily changes in the environment (e.g., the arrival of mothers at the den), or by an interaction between the two. For example, activity or food could entrain an internally driven cortisol rhythm, a process that has been reported in many other species (Boulos and Terman, 1980).

To our knowledge, a bimodal cortisol rhythm has been documented in only two other mammals, the domestic pig (*Sus scrofa domesticus*; Hillmann et al., 2008; Ruis et al., 1997) and the Sudanian grass rat (*Arvicanthis ansorgei*; Verhagen et al., 2004). Activity patterns in wild boar (*Sus scrofa*) have historically been crepuscular, although these animals are currently more nocturnal than crepuscular due to recent anthropogenic disturbance (Gaynor et al., 2018; Robert et al., 1987). Domestic pigs are diurnal, although they exhibit bimodal peaks in both activity and feeding behavior (de Haer and Merks, 1992; Robert et al., 1987). Although domestic pigs are typically fed twice daily, their bimodal cortisol rhythm could reflect a true endogenous pattern and not just a direct response to humans; this was supported by a study that found no effect of feeding method (ad libitum or fed twice daily) on salivary cortisol concentrations (De Leeuw and Ekkel, 2004).

Sudanian grass rats are diurnal/crepuscular rodents from western Africa. Their locomotor activity and free corticosterone concentrations are tightly associated, with both peaking around dawn and dusk (Verhagen et al., 2004). Furthermore, their body temperature also displays this bimodal rhythm, reflecting the close links between activity, corticosterone, and body temperature (Cuesta et al., 2009). Given that cortisol secretion is tightly coupled with awakening in both diurnal and nocturnal species (Kumar Jha et al., 2015), it is possible that a 12 h rhythm evolved in crepuscular species, thus retaining cortisol's tight link with activity.

Cortisol covaries with temperature and litter status

Ecological covariates

Maximum temperature was positively correlated with salivary cortisol concentrations (Figure 2.4). Although this trend could potentially arise from concentration of saliva due to dehydration, we do not see the same trend in salivary testosterone analyzed in the same samples (unpublished data), suggesting that dehydration cannot explain our results. Instead, our results might reflect a seasonal trend of higher cortisol during the hottest parts of the year (Figure 2.7). Juveniles may experience thermal stress when transitioning from their underground burrows, where temperatures are relatively cool and quite stable (Anderson and Richardson, 2005; Whittington-Jones et al., 2011), to the higher temperatures above ground, as they do in the evenings when mothers arrive at the communal den to nurse their young. Alternatively, hyena mothers might experience heat stress during the hottest parts of the year, and juveniles may then receive elevated glucocorticoids via their mothers' milk and absorb those glucocorticoids into their own systematic circulation (Hollanders et al., 2017). We found no effects of minimum temperature on cortisol concentrations; juvenile hyenas may be able to easily regulate cold stress via huddling together (Gilbert et al., 2010), as is often observed on cooler mornings.

In contrast to ambient temperature, precipitation had no systematic effect on cortisol concentrations in young hyenas; whereas variation in ambient temperature is associated with higher cortisol concentrations in many vertebrates, the evidence is weaker for an association between cortisol concentrations and local precipitation (de Bruijn and Romero, 2018). Finally, we found no effect of local prey abundance on salivary cortisol in juvenile hyenas.

Demographic covariates

Litter status was correlated with salivary cortisol concentrations, such that singletons had the highest cortisol values, followed by subordinate juveniles from twin litters, with dominant juveniles from twin litters having the lowest cortisol concentrations (Figure 2.4C). Our findings correspond to those of Benhaiem et al. (2013), who found that subordinate littermates had higher fecal glucocorticoid metabolite (fGCM) concentrations than dominants; dominant littermates frequently aggress on subordinate littermates in order to monopolize maternal milk (Smale et al., 1995; Wahaj and Holekamp, 2006). Finding similar patterns of litter status on glucocorticoids in both feces and saliva, which measure hormones over different time scales, indicates that litter status likely has a profound effect on the endocrine physiology of juvenile hyenas.

In contrast with the published literature on fGCMs in juvenile spotted hyenas (Benhaiem et al., 2013; Greenberg, 2017), we found no effect of either age or sex on salivary cortisol concentrations. The earlier studies found that juvenile females had higher fGCMs than males, and that both sexes experienced a decrease in fGCMs as they approached reproductive maturity. The differences between our studies have at least two possible explanations. First, sex differences in gut composition could lead to sex differences in fGCMs but not in salivary cortisol (Goymann, 2012; Rojas et al., 2020). Second, juveniles sampled via saliva tended to be much younger than juveniles sampled via feces; whereas the mean age for saliva sampling was 7.3 months (range 2.2-23.4), the mean age of fecal sampling was 14.6 months (range 3.1-24.0). In fact, Greenberg (2017) found no age effect on fGCMs in hyenas aged 6-12 months, which comprise 64% of our saliva samples. Maternal rank did not explain glucocorticoid concentrations in either saliva or feces (Greenberg, 2017), nor does social rank explain fGCMs in adults (Dloniak, 2004).

Behavior affects short-term cortisol concentrations

Although a correlation between glucocorticoid concentrations and aggressive behavior has been established in a wide range of taxa, most studies of wild animals are unable to evaluate the immediate effects of emitting or receiving aggression (but see Wittig et al., 2015). Saliva sampling allows for precisely this short-term assessment of glucocorticoids because, in contrast to glucocorticoids measured in feces, each saliva sample reflects the preceding minutes rather than hours or days (Wood, 2009). Here, we found that receiving aggression was correlated with elevated salivary cortisol (Figure 2.5B). In laboratory animals, elevated levels of glucocorticoids are often discovered in individuals that have recently lost a fight or been targets of aggression, and concentrations may even correlate with the actual amount of aggression received (Hsu et al., 2006). We also found that emitting aggression was correlated with decreased salivary cortisol (Figure 2.5B). In other species, the effect of emitting aggression, or winning, is more variable, with some species exhibiting no change and others exhibiting increases in glucocorticoids similar to that of losers (Hsu et al., 2006).

Most work documenting the short-term effects of aggressive interactions is done in laboratory species, where group-level hierarchies are often absent. Outcomes of aggressive interactions in these species are thus uncertain, although many animals use proxies such as body size or markings to reduce uncertainty (Holekamp and Strauss, 2016). In wild spotted hyenas, however, a strict linear dominance hierarchy leads to highly predictable outcomes of aggressive interactions: the vast majority of individuals who emit aggression receive only submission in response, and many aggressive interactions involve no physical contact but instead are primarily an exchange of threat and appeasement signals. Thus, we suspect that emitting aggression is not inherently stressful in spotted hyena society, as individuals can direct aggression toward subordinates with very little risk. In fact, emitting aggression might be a form of stress relief in much the same way as redirected aggression, or scapegoating (Kazem and Aureli, 2005). Given that these animals are highly attuned to dominance relationships within the group (Engh et al., 2005), confirmation of dominance over subordinates through ritualized aggression might actually buffer individuals against socially-induced stress.

Conclusion

Our work confirms that salivary analyses offer a useful alternative to fecal and urinary analyses in naturalistic studies of the behavioral endocrinology of wild carnivores. Our results also demonstrate that salivary hormone measurements are particularly useful for assessing short-term effects of specific behavioral interactions, while also accounting for other ecological or demographic variables likely to affect hormone concentrations. Finally, our work raises many new questions and opens pathways for further research exploring phenomena such as daily rhythms in hormone concentrations and variation due to litter size and composition.

ACKNOWLEDGEMENTS

We first and foremost thank all past and present members of the Mara Hyena Project for their work collecting and curating the data presented here. We especially thank Spencer Freeman, Michael Kowalski, Molly McEntee, and Olivia Spagnuolo for their hard work collecting saliva samples. We thank Teera Losch at the University of Michigan Core Assay Facility (UM CAF) for her help assaying saliva samples for cortisol. We thank the Office of the President of Kenya, the National Commission for Science, Technology, and Innovation, the Kenya Wildlife Service, the Narok County Government, and the Mara Conservancy for permission to conduct this research. This work was supported by National Science Foundation (NSF) grants to KEH and by research grants from the American Society of Mammalogists (ASM) and the Society for Integrative and Comparative Biology (SICB) to TMM.

APPENDICES

APPENDIX A:

FIGURES AND TABLES

Figure 2.1. Methods for collecting saliva from juvenile spotted hyenas. Photos by Erin Person and Jadelys Tonos. **A.** Pieces of polyester rope knotted at the top and coated with vegetable fat. **B.** Baited rope inserted into the PVC pipe, with a bolt secured under the knot. **C.** Research assistant safely collecting saliva from a hyena. **D.** Juvenile hyena depositing saliva on the collection rope.

Figure 2.2. Physiological validation of saliva as a measure of plasma cortisol in three wild spotted hyenas. Dots represent sampling points, and lines are drawn between each sampling point for visualization. The vertical gray line represents the time of ACTH injection. Raw cortisol concentrations are standardized within sample type for visualization. Cortisol concentrations from samples collected prior to the ACTH injection were averaged and plotted at time 0 for visualization.

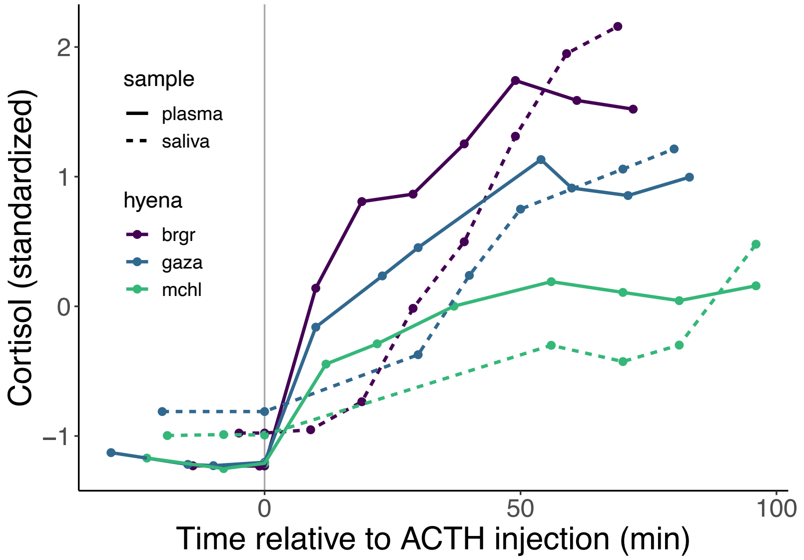


Figure 2.3. Temporal effects on salivary cortisol concentrations. **A.** Cortisol concentrations in saliva samples collected from juvenile spotted hyenas. Samples were collected from three clans between 2015-2018, primarily at the communal den. Each bin represents the average cortisol concentrations from all juvenile samples collected within that hour. **B.** Proportion of time spent at the communal den by GPS-collared adult female hyenas (n = 10) who were nursing juveniles in two of the same three clans between 2012-2014. GPS collar data from Greenberg (2017).

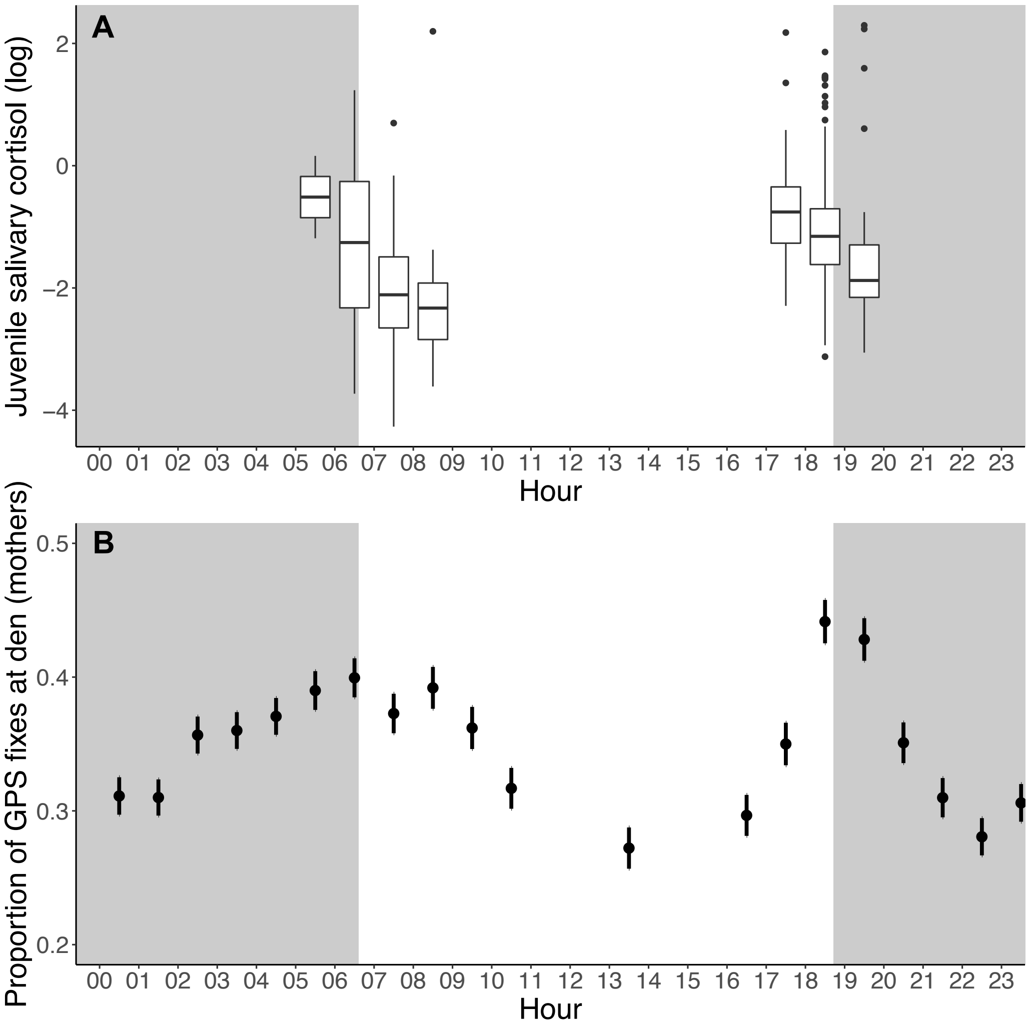


Figure 2.4. Top model of predicted salivary cortisol concentrations (n-samples = 261, n-hyenas = 71). **A.** Dots depict coefficient estimates, lines depict 95% confidence intervals, and asterisks depict significance at the following p-values: * = 0.05; ** = 0.01; *** = 0.001. **B-C.** Lines (or dots) depict estimated marginal means and shaded areas (or vertical lines) depict 95% confidence intervals. Asterisks depict significance in a Tukey post-hoc test at the following p-values: * = 0.05; ** = 0.01; *** = 0.001.

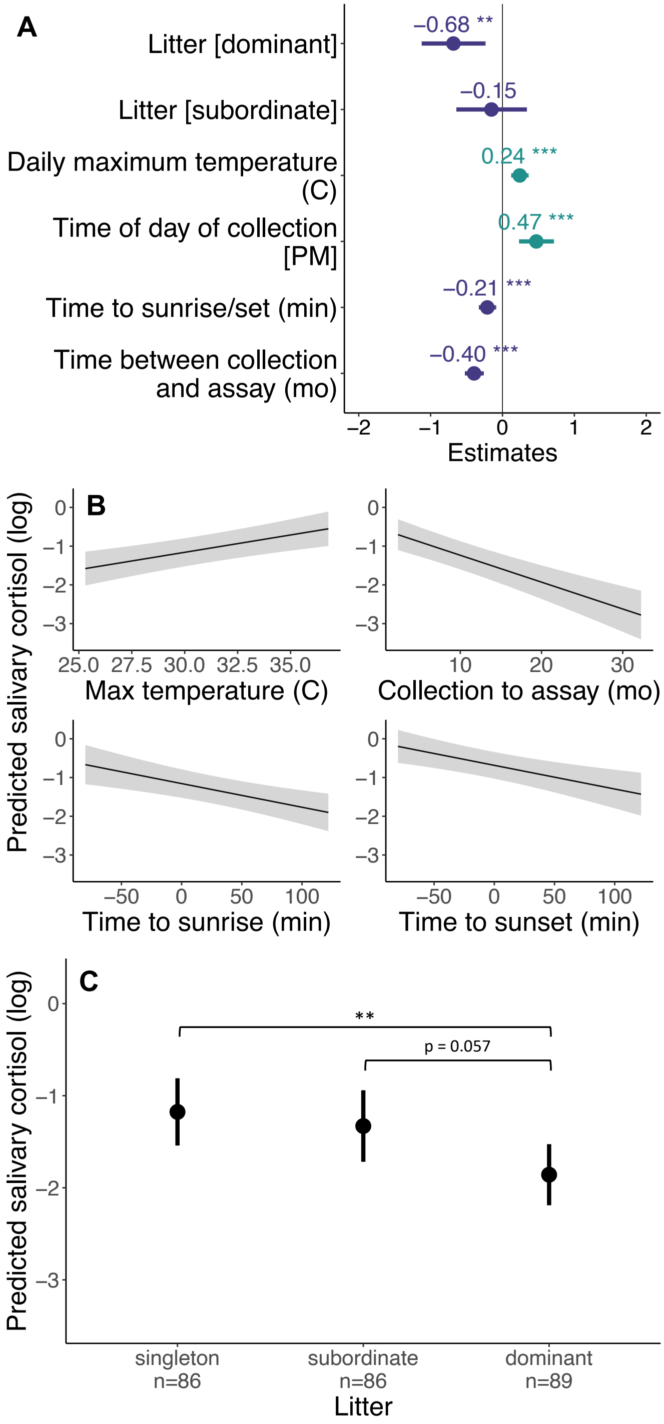
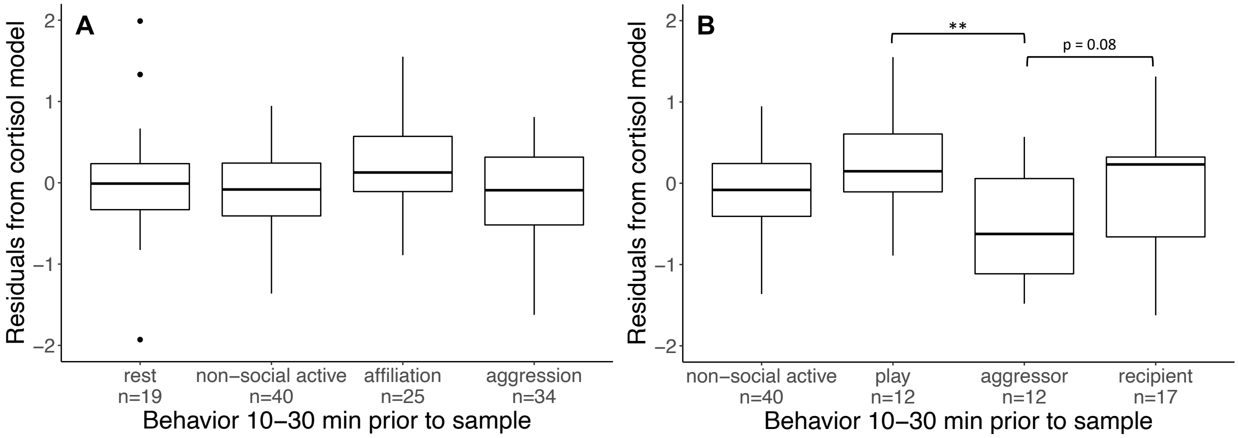


Figure 2.5. Effect of behavior on juvenile salivary cortisol concentrations. Samples categorized based on individual behavior in the 10-30 min prior to sampling. Residuals taken from model in Figure 2.4. **A.** Samples categorized based on general behavior. **B.** Samples categorized based on occurrence of specific vigorous behaviors. Asterisks depict significance in a Tukey HSD test at the following p-values: * = 0.05; ** = 0.01; *** = 0.001.



APPENDIX B:

SUPPLEMENTAL FIGURES AND TABLES

Figure 2.6. Scatterplot of logged juvenile salivary cortisol concentrations as a function of time of day in the morning (left) versus evening (right). Gray line represents the best fit linear model.

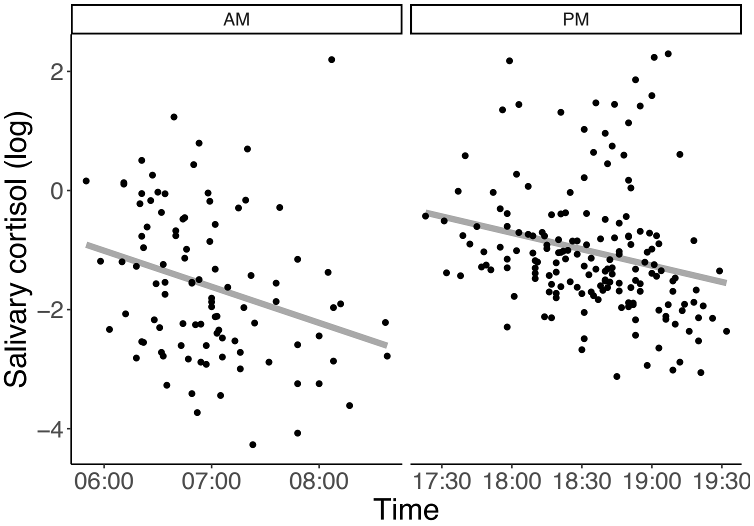
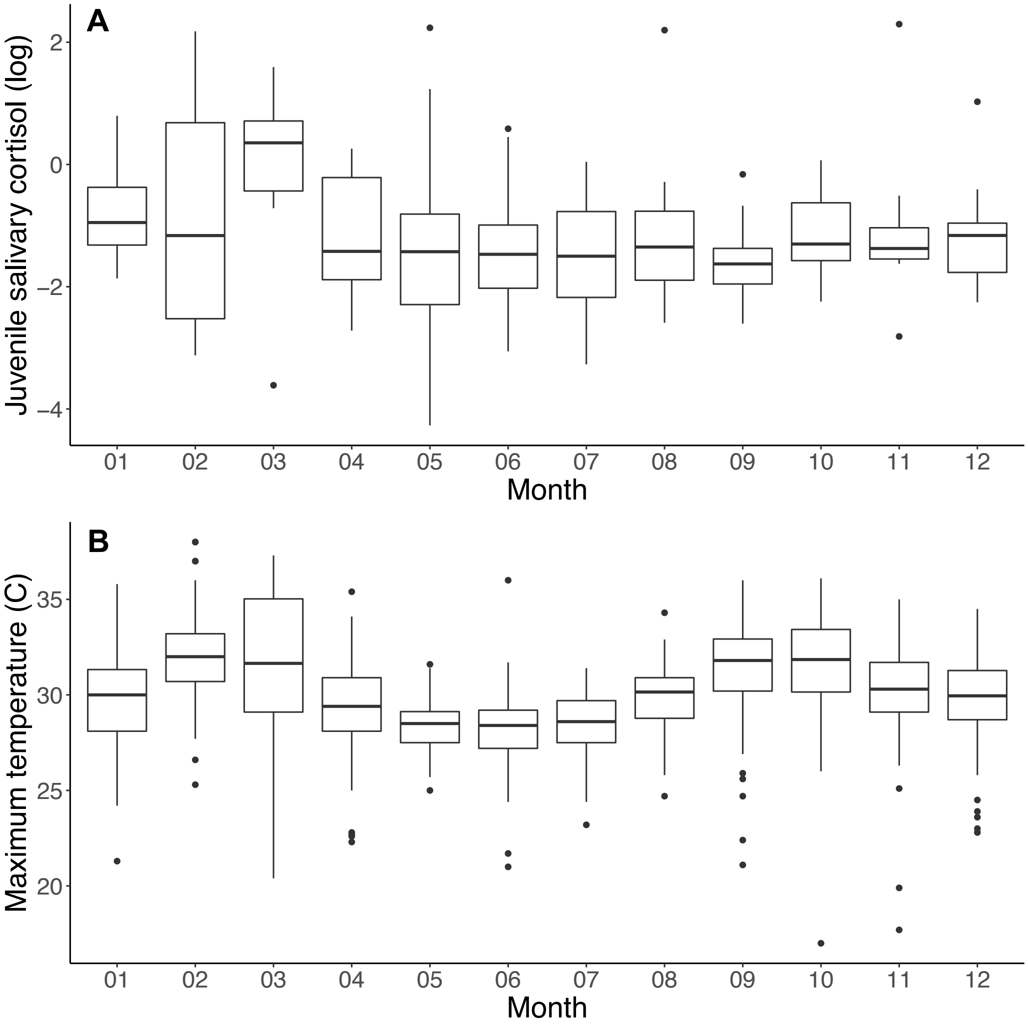


Table 2.1. Description of outcome variables and predictors used in model selection for model of logged salivary cortisol concentrations in wild juvenile hyenas. (Bolded terms remain in the top model.)

Outcome variable	Main effects	Definition	Interaction effects	Random effects
Salivary cortisol concentration (log)	**Collection to assay**	Time between collection and assay of sample (months)	Time of day x Time relative to sunrise/set Minimum temperature x Maximum temperature Prey density x Maternal rank Age x Sex Age x Maternal rank	Hyena ID
	Freeze-thaw	Number of freeze-thaw cycles		
	Time of day (AM/PM)	Collection time of day (AM/PM)		
	Time relative to sunrise/set	Collection time relative to sunrise/sunset (min)		
	Minimum temperature	Daily minimum temperature (°C)		
	Maximum temperature	Daily maximum temperature (°C)		
	Precipitation	Daily rainfall (mm)		
	Prey density	Number of prey animals sighted per square kilometer of transects during sample month		
	Age	Age of hyena (months)		
	Sex	Sex of hyena		
	Maternal rank	Social rank of hyena's mother during calendar year of sample		
	Litter (dominant, subordinate, singleton)	Dominant (dominant juvenile of twin litter), subordinate (subordinate juvenile of twin litter), singleton (no littermate)		

Figure 2.7. Effect of temperature on salivary cortisol concentrations. **A.** Average monthly concentrations of salivary cortisol in wild juvenile hyenas. **B.** Average monthly maximum temperatures recorded during 2015-2018.



CHAPTER THREE:

SOCIAL AND DEMOGRAPHIC TRAITS PREDICT COOPERATION DURING INTER-SPECIFIC CONFLICT

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ABSTRACT

Collective action problems arise when cooperating individuals suffer costs of cooperation while the benefits of cooperation are received by both cooperators and defectors. In these cases, cooperation is perplexing because “cheaters” benefit the most by avoiding costs of cooperation. Nevertheless, costly cooperative behaviors are observed across taxa. Cooperative mobbing behavior is a prime example of a collective action problem, because the benefits derived from repelling a predator accrue to individuals that do not participate, while only the participating individuals face the risk of predation. Here we study cooperative mobbing behavior of lions by spotted hyenas (*Crocuta crocuta*), and ask 1) when mobbing occurs, 2) who participates in mobbing, and 3) who benefits from mobbing. First, we find that mobbing was most likely to occur when the overall costs were lowest: mainly, when more hyenas were present and when male lions were absent. Second, we find that mobbing participants tended to be the strongest individuals, as measured by sex, rank, and age. Mobbing participation was also promoted by real-time affiliative behavior (greetings), as well as by the presence of closely bonded individuals and kin. Third, we find evidence of direct individual benefits of mobbing participation, as individuals that mobbed were more likely to feed at the carcass than individuals that did not mob. Our findings demonstrate that overcoming collective action problems in a complex society with differentiated, dynamic

relationships is achieved through a complicated web of interacting factors that are dependent on the characteristics and internal state of potential cooperators, as well as the immediate social environment. Overall, this suggests that these behaviors may be a venue in which social selection favors individuals that attend to the physiological and social characteristics of their groupmates in order to safely navigate these dangerous interactions together.

INTRODUCTION

Humans and many other animals carry out activities collectively with group-mates because the net benefits derived from cooperating exceed those that can be achieved individually (Olson, 1965). These benefits of collective action are thought to underlie a wide range of social behaviors across species (Alexander, 1974). However, when collective behaviors entail costs, it is a challenge to understand how they can persist, because each individual could potentially avoid costs by “cheating” – that is, an individual who does not participate in the collective costs (e.g., the behavior) but still partakes in the collective benefits (Nunn and Lewis, 2001). Understanding the factors that facilitate or impede collective action is a central problem in evolutionary biology as well as all of the behavioral sciences.

Much work in the field of collective action focuses on the coordination of movement in spatially cohesive groups, best epitomized by behaviors such as schooling and flocking (Sumpter, 2010). Most of these studies focus on gregarious species that form large, ephemeral groups, and thus these studies have assumed that group members are identical and have indistinguishable relationships with other group-members (Vicsek and Zafeiris, 2012). However, this assumption of homogeneity does not hold for species living in complex social groups, where individual differences in behavior and differentiated social relationships are critical to social functioning (Bergman and Beehner, 2015). In fact, recent evidence suggests that this assumption may not hold even in these larger, more transient schools and flocks (Jolles et al., 2017; Ling et al., 2019); rather, individual and relational differences appear to affect collective action across species and societies (Jolles et al., 2020).

Group members in many animal societies engage in collective action during intra- or inter-specific conflicts to drive away predators or to defend access to territory, offspring, or resources such as food, water, and shelter (Dugatkin, 1997). However, even in species where participation in such conflicts appears to be collective, individual participation is often quite variable (Kitchen and Beehner, 2007). Therefore, cooperation is usually not a true collective action involving all members of a social group but is rather a “joint action” by a subset of individuals (Willems et al., 2015). The group-level cooperation observed during joint action is thus an emergent property based on individual-level decisions regarding participation. These decisions are likely governed by an individual’s internal state, current ecological conditions, and social influences (Jolles et al., 2020; van den Bos et al., 2013). Understanding these decisions should reveal the benefits and costs for each individual in the social group, as well as the feedback effects of others’ decisions to cooperate or defect (Nunn and Lewis, 2001). Which individuals participate in joint action, and what can that tell us about why an individual would choose to do so?

For the individual decision-maker, participation in intra- or inter-specific conflicts is potentially costly, involving opportunity and energetic costs, risk of injury or death, and the possibility of exploitation by non-participating group members, or cheaters (Caro, 2005; Nunn and Deaner, 2004). Because individuals that do not participate in these conflicts avoid paying costs while receiving benefits obtained by the group (and thus gain the greatest net benefits), cooperation suffers from a collective action problem (Olson, 1965). Highly related group members can gain indirect, kin-selected fitness benefits from cooperating (Hamilton, 1964; Nunn and Lewis, 2001). For non-kin, however, the participants in group action are thought to be those that accrue the largest direct benefits from the conflict (Olson, 1965). For instance, high-ranking individuals with priority of access to resources acquired or defended by joint action may be more likely to participate than other group members (Gavrilets and Fortunato, 2014). Social incentives can also provide indirect benefits to participants by enhancing their reputation with potential coalition partners or mates (Dugatkin and Godin, 1992; Zahavi, 1995). Better understanding of the direct and indirect benefits that drive participation in conflict will help elucidate how cooperation evolves and is maintained in differentiated societies despite the selective benefits of cheating.

Cooperative mobbing behavior, which frequently occurs during intra- or inter-specific conflicts, is a prime example of the collective action problem, and yet it is commonly observed in many groups of animals, including fish, birds, and mammals (Caro, 2005; Dugatkin, 1997). Mobbing behavior occurs when two or more individuals in a high state of arousal synchronously approach or attack a threatening stimulus in the environment (Curio, 1978). This cooperative behavior is costly to participants, because 1) it involves considerable expenditure of energy and risk of injury, and 2) it often results in benefits to both cooperating and defecting group members via increased access to contested resources or decreased risk by driving away a predator (Crofoot, 2013; Dugatkin and Godin, 1992). These are exactly the conditions under which cheating is expected to destabilize cooperation (Dugatkin, 1997; Nunn and Lewis, 2001), suggesting that cooperative mobbing behavior can shed new light on the factors explaining individual variation in participation during collective action.

Spotted hyenas (*Crocuta crocuta*) employ cooperative mobbing behavior when engaged in intra-specific conflict with neighboring hyena groups or in inter-specific conflict with lions (*Panthera leo*) (Boydston et al., 2001; Lehmann et al., 2017). Hyenas often recruit group-mates to locations where they encounter lions by emitting long-distance vocalizations (Gersick et al., 2015). Once multiple hyenas are present, individuals may cooperate to mob the lions, approaching them as a cohesive group and vocalizing loudly together, behaviors that can enable the hyenas to overwhelm the lions and drive them away (Kruuk, 1972). Mobbing appears to enhance fitness in spotted hyenas by increasing their probability of feeding when competing with lions for control of a food resource (Lehmann et al., 2017). However, there are considerable fitness costs associated with this form of cooperation, as lions are significantly larger and stronger than hyenas. An attack from a lion can result in serious injury or death for a mobbing hyena, and lions are the leading cause of hyena mortality in many hyena populations (Périquet et al., 2015; Watts and Holekamp, 2009).

Spotted hyenas live in large mixed-sex groups, called clans, of 6-130 individuals, with a mean of 29 hyenas per clan across Africa (Holekamp and Dloniak, 2010). Clans are fission-fusion societies in which all members know one another individually, rear their cubs together at a communal den, and defend a common territory (Kruuk, 1972), yet clan members spend much of their time alone or in small subgroups (Smith et al., 2008). Due to female philopatry and male dispersal, the large clans found in east Africa are comprised of multiple matrilines of adult females and their offspring, and several adult immigrant males (Frank, 1986a). Each clan is structured by a strict linear dominance hierarchy with natal animals ranking above immigrants (Kruuk, 1972); natal animals “inherit” their rank from their mother in a process called maternal rank inheritance (Frank, 1986b), whereas immigrant males queue for rank in a tenure-based hierarchy (East and Hofer, 2001).

Social rank is critically important in hyena society because it determines priority of access to resources and the ability of individual group members to usurp food from clanmates (Frank, 1986b). Spotted hyenas experience intense competition associated with feeding on ungulate carcasses, which are extremely rich but highly ephemeral food resources in the Mara ecosystem (Frank, 1986b; Jones et al., 2016). An adult spotted hyena can consume one-third of its body weight in one meal, and a group of hungry hyenas can reduce a large antelope to a few scattered bones in less than 30 minutes (Kruuk, 1972). As female reproductive output in this species is strongly dependent on the quality and quantity of food consumed, high-ranking females reproduce at much higher rates than do their low-ranking counterparts, which translates into large fitness effects of rank (Holekamp et al., 2012). Although mean relatedness among clan-mates is very low (Van Horn et al., 2004), clan-mates frequently cooperate in 1) coalitionary aggression, which maintains the dominance hierarchy (Strauss and Holekamp, 2019b), 2) the acquisition and defense of food (Holekamp et al., 1997b), 3) territory advertisement and defense during border patrols and inter-clan wars (Boydston et al., 2001), and 4) interactions with lions (Lehmann et al., 2017).

Lions and spotted hyenas are the largest and most abundant large carnivores in many east African ecosystems, and each of these two species represents the other's main competitor for resources, with a dietary overlap of 69% (Périquet et al., 2015). Both species kleptoparasitize one another, with hyenas losing 12% of their food to lions and lions losing 17% of their food to hyenas (Périquet et al., 2015). This makes mobbing in spotted hyenas particularly interesting, because lions are both the primary cause of mortality for hyenas and their primary competitors for resources. Mobbing by spotted hyenas can thus be understood theoretically as both mobbing-as-predator-harassment (Crofoot, 2013; Dugatkin and Godin, 1992) and mobbing-as-resource-defense (Kitchen and Beehner, 2007; Willems et al., 2015). Here, we asked three questions aimed at identifying the mechanisms driving cooperation in spotted hyenas' risky mobbing behavior. First, when does cooperative mobbing occur? Second, who participates in cooperative mobbing behavior? Third, who benefits from cooperative mobbing?

We addressed these questions using a wild population of spotted hyenas in the Maasai Mara National Reserve, Kenya, which we have been following since 1988. We combined long-term demographic and ecological data from four clans with detailed behavioral data on participation in cooperative mobbing events during observation sessions (hereafter, "sessions") in which lions and hyenas interacted. In our population, lions and hyenas co-occurred in an average of 4 sessions per clan per month, and the two species interacted, or directed behavior at one another, in 44% of those sessions

(Green et al., 2019; Lehmann et al., 2017). During these interspecific interactions, hyenas frequently engaged in cooperative mobbing behavior, which occurs when a group of two or more hyenas jointly approach within 10 m of at least one lion (Figure 3.1). This comprehensive, long-term dataset provided a rare opportunity to address the question of why individuals in complex societies choose to participate in collective action, and what benefits they receive.

First, we inquired which contextual, environmental, and social characteristics predict when cooperative mobbing occurs across all sessions in which lions and hyenas interact. Lions and hyenas are likely to interact at carcasses or the communal den, both of which are valuable resources for hyenas, although motivation to guard a food resource may depend on current resource availability such as local prey density (Lehmann et al., 2017). Hyenas are also more likely to wrest control of a carcass from lions when risks to individual hyenas are lower [i.e., when the ratio of lions to hyenas is lower and when male lions are absent (Cooper, 1991; Höner et al., 2002)]. Recent studies of collective action have further indicated the importance of affiliative behavior and social allies to the emergence of collective behavior (Farine et al., 2016; Ling et al., 2019). We therefore expected that the occurrence of mobbing behavior should vary based on the costs and benefits of the current situation and the social environment of the individuals present.

Second, we examined which social, demographic, and physiological characteristics predict which hyenas participate in cooperative mobbing and which individuals are present but choose to defect. Individual sex and social rank are known to affect participation in intergroup encounters in primates (Kitchen and Beehner, 2007);

social rank in particular is considered important in determining which individuals participate in collective action (Gavrilets and Fortunato, 2014). Both body condition and age have the potential to affect a hyena's ability to escape lions (due to either physical ability or experience) and may therefore affect participation in mobbing behavior (Abolins-Abols and Ketterson, 2017). Steroid hormones like glucocorticoids and testosterone have been shown to influence cooperation and risk-taking behavior in several species, including humans (Kurath and Mata, 2018; Trumble et al., 2015). Like many primates (Silk et al., 2003), spotted hyenas form long-lasting, stable social bonds that positively affect fitness (Ilany et al., 2015; Smith et al., 2007), and several studies indicate that primates are more likely to participate in collective action when their social allies or kin are present (Kitchen and Beehner, 2007). Hyenas also engage in ritualized greeting behavior, which functions to promote cooperation, aid in reconciliation, and reinforce social bonds (Smith et al., 2011); we thus expected that this real-time affiliative behavior might affect an individual's short-term motivation to cooperate. Finally, individuals may be more likely to participate in collective action when potential mates are present in the "audience", particularly if mobbing acts as a demonstration of fitness aimed at conspecifics (Dugatkin and Godin, 1992). Thus, we predicted that individual decisions to mob would be influenced by a variety of social, demographic, and physiological characteristics.

Third, we investigated which individuals benefit from participating in cooperative mobbing in an effort to evaluate the respective benefits of cheating versus cooperating. It is difficult to formulate mutually exclusive predictions because multiple proposed benefits of mobbing may accrue simultaneously (Crofoot, 2013). Nevertheless, one way that individuals might benefit from collective mobbing is through communal benefits, which are shared among all the individuals in the social group. While often tenuous and hard to measure, in hyenas two such communal benefits might be defense against intraguild predators (lions) and information exchange between lions and hyenas (Dugatkin and Godin, 1992; Graw and Manser, 2007). However, in light of the individual differences we observed in mobbing participation, we expected that individual benefits, as well as communal benefits, might be important in the expression of collective action.

One individual direct benefit that might arise from this collective behavior is access to resources gained or defended by mobbing (Willems et al., 2015). If so, we predicted that mobs should be more likely to occur at sessions where higher quality and/or larger food items were present. We also predicted that hyenas that are hungrier, or that are more motivated to gain food, may be more likely to participate in cooperative mobbing; participation in collective action based on nutritional state has been demonstrated in other species (Krause et al., 1992). Most importantly, we predicted that hyenas that participate in mobbing would be more likely to obtain food.

Another direct benefit that may arise from cooperative action is protection of close relatives or vulnerable offspring from predators, allowing participants to gain inclusive fitness benefits (Hamilton, 1964). Spotted hyenas invest heavily and over long periods of time in their offspring, even supporting weaned offspring during feeding competition with conspecifics (Kruuk, 1972; Watts et al., 2009). Thus, we predicted that females with at least one juvenile offspring present would be more likely to participate in cooperative mobbing against lions than females with no young present.

To address these three questions, we built a series of logistic mixed effect models (Table 3.1, Table 3.2, Table 3.3) and performed model selection on biologically relevant global models to determine which predictors were important determinants of 1) when mobbing occurs, 2) who participates in mobbing, and 3) who benefits from mobbing.

RESULTS

Mobbing occurrence

Between 1988-2016, lions and multiple hyenas across 4 clans interacted in 325 sessions satisfying criteria for inclusion in our analyses (see Methods). Spotted hyenas mobbed in 41.8% of these sessions, with a median of 2 mobs per session (mean 3.1, range 1-40) and a median of 4 hyenas per mob (mean 5.1, range 2-16).

In our mobbing occurrence model (Model A: n = 321 complete cases; Figure 3.2A; Table 3.4), mobbing was more likely to occur when more (compared to fewer) hyenas were present (β-hyenas = 0.62, p = 0.001; Figure 3.2B) and when male lions were absent (compared to present) (β-male lions = -0.85, p = 0.007; Figure 3.2B). Local prey density had a positive effect on the probability of mobbing (β-prey = 0.29, p = 0.026; Figure 3.2B). The number of individuals that engaged in greeting behavior during the session also increased the probability of mobbing (β-greeters = 0.83, p < 0.001; Figure 3.2B). A marginal interaction between presence of male lions and number of hyenas present indicated that the effect of many hyenas overwhelms the negative effect of male lion presence (β-male lions x hyenas = 0.60, p = 0.094; Figure 3.2C). A marginal interaction between number of greeters and number of hyenas present indicated that greetings facilitate mobbing behavior when only a few hyenas are present but do not affect mobbing behavior when many hyenas are present (β-greeters x hyenas = -0.32, $p = 0.066$; Figure 3.2D). Session length, session context, number of lions present, and mean association index of hyenas present were not included in the top model or any model within 6 AIC of the top model.

Because of the strong effect of adult male lion presence, we ran two subsequent models dividing sessions into those with and without adult male lions to investigate whether the number of lions had a continuous effect as a predictor of mobbing behavior. We did not find support for this. Our top model of the occurrence of mobbing at sessions with adult male lions (Model K: n = 113 complete cases; Table 3.4) did not include the term for number of lions or for number of adult male lions, nor did any models within 6AIC of the top model. Similarly, our top model of the occurrence of mobbing at sessions without adult male lions (Model L: n = 212 complete cases; Table 3.4) did not include the term for number of lions, nor did any models within 6AIC of the top model.

Mobbing participation

Our participation dataset consisted of 4740 individual mob-hyena combinations, with 492 unique hyenas present for 344 total mobs in 119 observation sessions involving lions and hyenas. In 33% (n = 1577) of mobbing opportunities, focal hyenas participated in the mob (“mobbers”), while in the remaining 67% (n = 3163) of mobbing opportunities, focal hyenas were present but did not participate (“defectors”).

In our hyena participation model (Model B: n = 4383 complete cases; Figure 3.7; Table 3.4), females were more likely to mob than males (β-male = -1.04, $p < 0.001$). Focal individuals of age 7.6 (range 0.2-21.2 years) were most likely to mob (β-age = 0.72, $p < 0.001$; $\beta\text{-age}^2 = -0.40$, $p < 0.001$). Based on the clear effects of both age and sex, we divided all subsequent analyses by age and sex class.

Adult female hyenas

In our female participation model (Model C: n = 2175 complete cases; Figure 3.3A; Table 3.4), focal females that were 6.0 years old (range 2.0-21.2 years) were most likely to mob (β-age = 0.03, p = 0.775; β-age^2 = -0.13, p = 0.016; Figure 3.3B). Higher-ranking females were marginally more likely to mob than lower-ranking individuals (β-rank = 0.19, p = 0.073; Figure 3.3B). Focal females with a higher concentration of fecal testosterone metabolites (fTMs) may be more likely to mob than females with lower fTMs, but this effect was non-significant (β-fTMs = 0.14, p = 0.146; Figure 3.3B). Females that had engaged in greeting behavior during the 5 min before the mob formed were more likely to mob than those that had not greeted (β-greeted = 1.12, p < 0.001; Figure 3.3B). Focal females that were close associates of the current mobbers were more likely to participate in that mob than females that were weakly associated (β-association index = 0.31, p = 0.026; Figure 3.3B), and focal females that were maternally related to a larger proportion of the current mobbers were more likely to mob than those that were related to a smaller proportion of mobbers (β-maternal relatedness = 0.24, p = 0.010; Figure 3.3B). An interaction between whether the focal hyena greeted and its social rank indicated that greeting facilitated mobbing behavior for low-ranking hyenas, but did not affect mobbing behavior for higher-ranking individuals (β-greeted x social rank = -0.78, p = 0.005, Figure 3.3C). An interaction between association index and social rank indicated that the focal individual's association index with the mobbing hyenas increased their likelihood of mobbing when they were high-ranking, but not when they were low-ranking (β-association index x social rank = 0.22, p

= 0.025, Figure 3.3D). Reproductive state was not included in the top model or any model within 6 AIC of the top model, and concentration of fecal glucocorticoid metabolites (fGCMs) was not included in the top model or any model within 4 AIC of the top model.

Adult male hyenas

In our male participation model (Model D: n = 783 complete cases; Figure 3.4A; Table 3.4), focal males that were 6.6 years old (range 2.0-16.9 years) were most likely to mob (β-age = 0.24, p = 0.354; β-age^2 = -0.50, p = 0.008; Figure 3.4B). Higher-ranking males were more likely to mob than their lower-ranking counterparts (β-rank = 1.15, $p < 0.001$; Figure 3.4B). Concentration of fGCMs was included in the top model but was not significant (β-fGCMs = 0.03, p = 0.833; Figure 3.4B). Focal males that were close associates of the current mobbers tended to be more likely to participate in that mob than males that were weakly associated (β-association index = 0.33, p = 0.083; Figure 3.4B). A marginal interaction between fGCMs and social rank indicated that fGCMs had a stronger positive effect on mobbing for high- than low-ranking males (β-fGCMs x social rank = 0.34, p = 0.053, Figure 3.4C). Dispersal status, fTMs, and whether the focal hyena greeted were not included in the top model or any model within 6 AIC of the top model.

Because most hyena clans contain two different types of adult males (those that have dispersed and those that have not yet dispersed), we ran two subsequent models dividing adult males into immigrant males and natal males. In our model of adult immigrant male participation in cooperative mobbing events (Model M: n = 551 complete cases; Figure 3.8A,B; Table 3.4), focal males that were 4.4 years old (range

2.1-16.9 years) were most likely to mob (β-age = -0.45, p = 0.046; β-age^2 = -0.20, p = 0.188). Higher-ranking males were more likely to mob than lower-ranking individuals (β-rank = 0.98, $p < 0.001$). Association index with mobbers was included in the top model but was not significant (β-association index = 0.17, p = 0.429). Focal males were marginally less likely to mob when more potential mates, or reproductively active females, were present (β-potential mates = -0.58, p = 0.056). Concentration of fTMs and whether the focal hyena greeted were not included in the top model or any model within 6 AIC of the top model. Concentration of fGCMs was included in models within 2 AIC of the top model but not in the top model.

In our model of adult natal male participation in cooperative mobbing events (Model N: n = 326 complete cases; Figure 3.8C,D; Table 3.4), social rank and association index with mobbers were included in the top model but were not significant (β-rank = 0.40, p = 0.222; β-association index = -0.52, p = 0.149). Focal males that were maternally related to a larger proportion of the current mobbers were more likely to mob than those that were related to a smaller proportion of mobbers (β-maternal relatedness = 0.69, p = 0.027). An interaction between social rank and maternal relatedness indicated that being closely related to the mobbers may facilitate mobbing behavior for low-ranking hyenas but not for higher-ranking individuals, although this effect was non-significant (β-maternal relatedness x social rank = -0.51, p = 0.107). Age and whether the focal hyena greeted were not included in the top model or any model within 6 AIC of the top model.

Juvenile hyenas

In our juvenile participation model (Model E: n = 1153 complete cases; Figure

3.9A), focal juveniles that were older were more likely to mob than younger juveniles (β = 1.74, $p < 0.001$). Sex was included in the top model but was not significant (β-male = -0.34, p = 0.390). An interaction between age and sex indicated that sex differences in mobbing may emerge early in life, but this effect was non-significant (β-age x sex = -0.66, p = 0.123, Figure 3.9B). Social rank, association index, maternal relatedness, and whether or not the hyena greeted were not included in the top model or any model within 6 AIC of the top model.

Benefits to mobbers

Resource defense

If hyenas mob to obtain or defend food resources, we predicted that mobs would be more likely to occur at sessions at which higher quality and/or larger food items were present. We did not find support for this prediction. Our top model of the occurrence of mobbing at sessions with food (Model F: n = 218 complete cases; Table 3.4) did not include the terms for carcass freshness or carcass size, nor did any models within 6AIC of the top model.

Second, we predicted that focal hyenas in poorer nutritional states would be more likely to participate in mobbing at sessions with food. Our results support this prediction. In our model of whether adult hyenas mobbed during sessions with food (Model G: n = 423 complete cases; Figure 3.5A; Table 3.4), “obese” individuals were less likely to mob than either “fat” or “normal” individuals (Tukey post-hoc test for belly size: [obese - normal]: β = -2.56, p = 0.054; [obese - fat]: β = -2.67, p = 0.044; Figure 3.5B), although there was no difference in mobbing participation between “normal” and “fat” individuals (Tukey post-hoc test for belly size: [fat - normal]: β = 0.11, p = 0.946;

Figure 3.5B). Focal individuals were also less likely to mob at larger carcasses (Tukey post-hoc test for carcass size: [extra-large - medium]: $\beta = -3.85$, $p = 0.019$; [extra-large - large]: $\beta = -2.60$, $p = 0.044$; [large - medium]: $\beta = -1.25$, $p = 0.389$; Figure 3.5C). The age (β-age = 0.49, $p = 0.003$; β-age^2 = -0.20, $p = 0.021$) and social rank (β-rank = 0.74, $p < 0.001$) of the focal hyena also significantly affected an individual's probability of mobbing, as shown in earlier models (Models C and D).

Lastly, we predicted that hyenas that mob would be more likely to obtain food, both immediately after the mob and in the session overall. We found moderate support for this prediction. In our model of adult hyenas feeding in the 5 min after a mob occurred (Model H: $n = 1040$ complete cases; Figure 3.6A; Table 3.4), high-ranking focal individuals were more likely to feed than low-ranking individuals (β-rank = 0.54, $p = 0.001$). Individuals of age 6.8 (range 2.0-21.2 years) were most likely to feed (β-age = 0.06, $p = 0.736$; β-age^2 = -0.26, $p = 0.007$). Importantly, focal individuals that mobbed were significantly more likely to feed than individuals that defected, even after controlling for social rank (β-mobber = 0.66, $p = 0.002$). A marginal interaction between whether the focal hyena mobbed and its social rank indicated that mobbing facilitates feeding behavior among low-ranking hyenas but does not affect feeding among high-ranking individuals (β-mobber x social rank = -0.40, $p = 0.065$; Figure 3.6B). MobID was dropped as a random effect in the top model because it explained no variance.

Our model of hyena feeding during the session overall (Model I: $n = 671$ complete cases; Table 3.4) did not include the term for whether or not a focal hyena mobbed during the session, nor did any models within 6 AIC of the top model.

Kin defense

If hyenas mob to defend kin, we predicted that adult females would be more likely to mob when their juvenile offspring are present. Our results support this prediction. In our model (Model J: n = 1140 complete cases; Figure 3.10A; Table 3.4), focal females were more likely to mob when their own offspring were present (β-own juvenile = 0.42, p = 0.044; Figure 3.10B). The focal hyena's age (β-age = -0.21, p = 0.097; β-age^2 = -0.06, p = 0.467), social rank (β = 0.17, p = 0.306), association index (β = 0.32, p = 0.102), maternal relatedness (β = 0.33, p = 0.014), and whether or not the hyena greeted (β = 0.60, p = 0.072) also remained in the top model, as did two interactions: greeted x social rank (β = -0.89, p = 0.027) and association index x social rank (β = 0.30, p = 0.045). These terms all showed similar trends to the top model predicting adult female participation in cooperative mobbing events (Model C), which were discussed above. Reproductive state and fTMs were not included in the top model nor any model within 6 AIC of the top model, and fGCMs was not included in the top model or any model within 5 AIC of the top model.

DISCUSSION

Our investigation of cooperative mobbing behavior in spotted hyenas supports the idea that collective behavior occurs largely to gain individual benefits. First, mobbing was most likely to occur when the overall costs were lowest: mainly, when more hyenas were present, when no male lions were present, and when individual hyenas were particularly motivated (as proxied using recent greeting behavior). Furthermore, characteristics that suggest a stronger individual, such as being female (the larger sex), prime-aged (for both sexes), and higher-ranking (for both sexes), predicted likelihood of mobbing. Finally, the individuals that were more likely to mob were also more likely to partake in the benefits. Individuals that mobbed were more likely to mob when they needed food and when their maternal relatives and closely bonded associates were present in the group. Our findings demonstrate that cooperation in a complex society with differentiated, dynamic relationships is maintained through a complicated web of interacting factors that are dependent on the characteristics and internal state of potential cooperators, as well as the immediate social environment.

Mobbing occurrence and participation

We found conflicting evidence that groups of hyenas assess the potential immediate benefits, as neither the proximity of a valuable resource (the clan's communal den or a prey carcass) nor carcass size affected the probability that a mob would form (Table 3.1, Model A). In fact, 24% of sessions with mobbing occurred in sessions away from either dens or prey carcasses. Further, mobs were more likely to occur when local prey densities were higher, suggesting that competition for resources was not a driving factor of mobbing occurrence (Figure 3.2). However, resource competition may affect mobbing participation, as the probability of feeding immediately after a mob was greater for cooperators than defectors (Figure 3.6).

Instead, hyenas may assess the situational risks of mobbing, as mobs were more likely to occur in sessions where more hyenas were present (diluting the risk) and male lions were absent (reducing the risk) (Figure 3.2). Interestingly, the presence of large numbers of hyenas appeared to overwhelm this negative effect of adult male lion presence; larger group sizes may reduce individual perception of risk and thus increase boldness and facilitate risk-taking behaviors (Webster and Ward, 2011; Zajonc, 1965). In primates, individual participation in intergroup contests can also be mediated by numerical assessment of relative group sizes (Van Belle and Scarry, 2015). Hyenas, however, appear not to assess their potential risk via numeric odds, as the ratio of lions to hyenas, typically considered important in interspecific conflict (Cooper, 1991; Höner et al., 2002), was not included any top models (Table 3.1, Model A; Table 3.2, Model F; Table 3.3, Models K,L). Because hyenas are cognitively capable of numerical assessment (Benson-Amram et al., 2011), it is possible that the effect of a single adult male lion changed the lion versus hyena numeric odds in such an extreme way that the ratio of lions to hyenas no longer mattered when assessing risk.

Real-time affiliative behavior via greeting ceremonies importantly facilitated both the occurrence of mobs and individual participation in mobbing behavior. Greetings were especially important to mobbing occurrence when fewer hyenas were present and therefore social facilitation based on subgroup size was weaker (Figure 3.2). Ritualized greetings could potentially help hyenas increase group cohesion, coordinate mobbing behavior, and increase cooperative success, as occurs in other gregarious carnivores (Estes and Goddard, 1967). In addition, adult females that greet prior to mob formation were more likely to participate in that mob than those that did not, and greeting behavior can wipe out the negative effect of rank for low-ranking females who, when they greet beforehand, were more likely to mob than low-ranking individuals that fail to greet (Figure 3.3). Greetings are known to re-affirm social bonds (Smith et al., 2011), increasing the likelihood of support from allies and potentially recruiting allies during risky situations (Mercier et al., 2017; Whitham and Maestripieri, 2003). Because higher-ranking individuals were more likely to mob overall, greetings may be especially important to low-ranking females with respect to re-affirming social bonds and recruiting mobbing partners. This large effect of greeting behavior in both mobbing occurrence and mobbing participation demonstrates how influential individual-level decisions are on the group-level cooperation observed during joint action.

We were unsurprised to find that social rank, which is a crucial aspect of hyena social life, structures their cooperative mobbing behavior. As predicted, higher-ranking individuals were most likely to participate in mobbing (Figure 3.3, Figure 3.4, Figure 3.8), probably because they can accrue the largest direct benefits from any food acquired via mobbing (Gavrilets and Fortunato, 2014). High-ranking individuals are also usually in the best physical condition due to their priority of access to resources (Flies et al., 2016; Lewin et al., 2015), which may reduce a hyena's overall risk of being caught by a lion. However, the effects of social rank on mobbing were often mediated by other important social variables, including greetings, social relationships, and kinship.

Hyena participation in cooperative mobbing was also promoted by long-term social relationships with both kin and non-kin (Figure 3.3, Figure 3.4). Interestingly, this was true for both adult males and adult females, despite adult males having weaker social bonds within the group (Smith et al., 2007; Turner et al., 2018). Having many trusted allies available likely increases individual participation in mobbing, as close associates and kin are likelier to offer support when engaging in risky behavior (Massen and Koski, 2014). Furthermore, social bond strength, which represents one potential gauge of what support to expect from a potential ally, increases cooperative success across a wide range of species (Ebenau et al., 2019).

We found small effects of hormone concentrations on mobbing behavior, where higher fTMs promoted mobbing behavior in females and higher fGCMs interacted with social rank to promote mobbing in higher-ranking males (Figure 3.3, Figure 3.4). Both effects were non-significant but were retained in the top model explaining participation in mobbing behavior. Testosterone and glucocorticoids can affect cooperative behavior, as demonstrated in a variety of other species (Kurath and Mata, 2018; Trumble et al., 2015). Although our results may indicate that these hormones have little effect on mobbing behavior in spotted hyenas, our small sample size makes this conclusion premature at present. For each female, we collected a mean of 6 (median = 4) fecal samples per lifetime, and for each male, a mean of 4 (median = 2) samples, which are very small sample sizes relative to those obtained from primates living in cohesive societies.

Individual benefits of mobbing

Our results indicated that mobbing increases access to food for spotted hyenas. Past research suggested that mobbing increases the probability of any one hyena at the session feeding, but did not address individual benefits of mobbing (Lehmann et al., 2017). While hyenas that mob were not more likely to obtain food in the session overall (Table 3.2, Model I), mobbers – especially low-ranking individuals – were more likely than non-mobbers to get food immediately after mob occurrence (Figure 3.6). Given how quickly a group of hyenas can reduce a carcass to bones (Kruuk, 1972), food obtained by mobbers during or immediately after mobbing could be enough to promote this risky behavior, especially for low-ranking individuals who would not ordinarily be able to access the carcass. Note however that this food reward is not sufficient to entice all low-ranking individuals to cooperate, as high-ranking individuals were still most likely to mob. Likewise, hyenas are less likely to mob at extra-large carcasses such as hippos and elephants (Figure 3.5); these carcasses last for days in the Mara (Jones et al., 2016), so the risk of mobbing to gain access may be unnecessary when simply waiting will yield rewards. Obese hyenas are also less likely to participate in mobbing than thinner individuals (Figure 3.5), perhaps because they are already satiated or because their obesity may impair their movement. In total, individual hyenas appear to alter their mobbing behavior based on their own likelihood of gaining food later in the session.

Mobbing may also have inclusive fitness benefits in spotted hyenas (Hamilton, 1964), where mobbing participants may choose to mob to support and protect close relatives or vulnerable offspring from predators. Adult females were more likely to mob with maternal kin (Figure 3.3) and when their own juvenile offspring are present (Figure 3.10), supporting this hypothesis. Den-dwelling offspring are safe from lions within the communal den (Cooper, 1993; East et al., 1989), which could also explain why hyenas are not more likely to mob at the communal den than at sessions where no immediate resources are present (Table 3.1, Model A).

In addition to any direct benefits, cooperative individuals may reap long-term indirect benefits, such as social incentives, or benefits granted them by their fellow group members. For example, mobbing individuals may strengthen their social bonds and cement alliances with potential coalition partners (Maklakov, 2002; Zahavi, 1995). In support of this theory, we found a clear effect of the social environment on mobbing, as both social behavior and social allies affected mobbing occurrence and participation. Alternatively, cooperative individuals might improve their reputation with potential mates, thus enhancing their mating success (Dugatkin and Godin, 1992; Zahavi, 1995). We did not find support for this hypothesis, as immigrant males, who sire the vast majority of cubs, were actually less likely to mob when breeding females were present than when they were absent (Figure 3.8). However, we believe this primarily reflects the low rank of immigrant males in hyena society: immigrant males were more likely to mob at sessions where they were relatively high-ranking (few females present) than at sessions where they were relatively low-ranking (many females present).

Ultimately, the best way to test these potential indirect social benefits is with a long-term dataset. If individuals that frequently engage in cooperative mobbing improve their reputation with potential mates, then immigrant males that are more cooperative overall should sire more cubs over their lifetime. If individuals that frequently cooperate strengthen their social bonds with allies, then individuals that are more cooperative overall should have stronger social bonds and greater social benefits than expected, such as increased feeding tolerance at carcasses (Smith et al., 2007). Overall, we expect that there may be other, less immediate social benefits of participation in collective action aside from those suggested here, and we hope to explore those indirect benefits in the near future.

Why act collectively?

Cooperative mobbing behavior is a prime example of a collective action problem, because the benefits derived from mobbing can accrue to individuals that do not participate in the mob (Nunn and Lewis, 2001). Here our overarching goal was to deepen our understanding of collective action in complex societies. The striking variation among individuals and relationships in these groups complicates decision-making based on current situational variables, individual attributes, and the dynamic social environment. Furthermore, we find that while there are some immediate direct benefits of collective action, many of the benefits appear to be less tangible ones involving the social environment. This is exemplified by the strong effects of greeting, social ties, and kinship on mobbing behavior. Overall, our results demonstrate how the coordination of collective action is contextualized within the broader social environment of a society characterized by many different types of social relationships. Cooperation in hyena society appears to depend on both individual attributes and the strength of social relationships. This suggests that these behaviors may be a venue in which social selection favors individuals that attend to the physiological and social characteristics of their groupmates in order to safely navigate these dangerous interactions together.

METHODS

From 1988-2016, we monitored four clans of wild spotted hyenas in the Maasai Mara National Reserve in southwestern Kenya. We monitored one clan from 1988-2016 and three clans from 2008-2016. We monitored clans daily during two observation periods, in the morning from 0530-1030 and in the evening from 1600-2100. When we encountered a subgroup of one or more hyenas, we initiated an observation session and recorded the identities of all hyenas present within a 200 m radius, using their unique spot patterns and ear damage to recognize individuals. We also recorded the number, sex, and age class of all lions found (Whitman and Packer, 2006). Sessions lasted from 5 min to several hours and ended when behavioral interactions ceased, and observers left that individual or group. Using all-occurrence sampling (Altmann, 1974), we recorded arrivals and departures of individual hyenas, agonistic interactions, unsolicited submissive signals, and affiliative interactions such as greetings. Greetings occur when two partners stand parallel to one another and face in opposite directions to sniff the other’s anogenital region (Kruuk, 1972). We also performed scan-sampling (Altmann, 1974) every 20 min throughout each session to determine hyenas present and the identities of all nursing individuals.

Throughout each session involving both lions and hyenas, we recorded all mobbing events using all-occurrence sampling. We operationally defined “mobbing” as a group of two or more hyenas, usually side-by-side and within 1m of one another, with tails bristled over their backs, approaching within 10 m of at least one lion (Figure 3.1; Lehmann et al., 2017). In association with each mobbing event, we recorded the identities of all participating hyenas and the number, sex, and age class of the lions being approached. Three trained research assistants (SG, KK, LM) extracted data on lion-hyena sessions from detailed written field notes. To ensure consistency, all data extraction work was overseen by TMM and KDSL.

Throughout each lion-hyena session in which a kill or carcass was present, we recorded hyena feeding behavior. Because lion-hyena sessions are often very chaotic (and thus the ability of the observer to record feeding behavior varies), we created a simple feeding dataset of one-zero sampling for each hyena present at each session. For each minute of each session, we recorded whether or not a focal hyena was observed feeding. To be conservative, any minutes in which a focal hyena was not directly observed feeding were coded as “not feeding.”

Because hyena societies are fission-fusion and most individuals spend the majority of their time alone or in small subgroups, we measured social relationship strength among individuals by calculating association indices, as done previously (Holekamp et al., 1997a). Association indices were calculated for each dyad in each session using R package asnipe (Farine, 2019) based on patterns of association over the previous 365 days. Thus, we calculated the association index within the dyad of hyenas A and B as $(A+B_{\text{together}}) / [(A_{\text{without B}}) + (B_{\text{without A}}) + (A+B_{\text{together}})]$ where $(A+B_{\text{together}})$ represents the number of observation sessions in which A and B were both present, $(A_{\text{without B}})$ represents the number of sessions in which A was observed but B was not present, and $(B_{\text{without A}})$ represents the number of sessions in which B was observed but A was not present.

Model predictors

Variables calculated for each observation session

Session length: The length of the observation session in minutes.

Session context: We assigned each session to one of three contexts: “food” sessions occurred within 200 m of a kill or carcass, “den” sessions occurred within 200 m of an active hyena den, and locations of all remaining sessions were categorized as “other” sessions (Boydston et al., 2003).

Prey density: For each session, we calculated the current prey density on a monthly basis. We monitored prey availability during biweekly surveys by counting all wild herbivores within 100 m of 2-3 line transects (1.5-5.4 km long) in each clan territory (Holekamp et al., 1999; Lehmann et al., 2017). Prey density was calculated as prey counts per square kilometer based on the number of animals sighted during line transect surveys. For each month of our study, we calculated the prey density within the territories of each of our study clans and used the monthly number of standard deviations above or below the yearly mean prey density to determine standardized prey availability for each clan during each month of study (Lehmann et al., 2017).

Number of hyenas present: The total number of hyenas present at the session.

Number of lions present: The total number of lions present at the session.

Male lions present: Whether or not adult male lions were present at the session.

Number of adult male lions present: The total number of adult male lions present at the session.

Number of hyenas that greet (greeters): For each session, we quantified the amount of affiliative behavior observed as the total number of individuals present that engaged in greeting behavior during that session.

Mean association index: For each session, we quantified the strength of social ties between individuals present in that session as the mean association index of all dyads present at that session.

Carcass freshness: At food sessions, we categorized the carcass as “fresh” (prey was recently killed) or “old” (prey was killed over 24 h earlier).

Carcass size: At food sessions, we recorded the species, sex, and age class (where possible) of the carcass and tracked its consumption throughout the session. Based on observer descriptions, carcass size was later categorized by prey species age and weight (Kingdon, 2015) as small (< 20 kg), medium (20-100 kg), large (100-500 kg), and extra-large (> 500 kg) (Table 3.5). Small and medium categories were later combined due to the limited sample size for small carcasses.

Variables calculated for each focal hyena

Age: We estimated cub birthdates (to ± 7 days) from their size and pelage when they were first seen above ground (Holekamp et al., 1996). We considered individuals of both sexes to be juveniles until 24 months of age, after which they were considered adults (Glickman et al., 1992). All immigrant males were classified as adults; whenever possible, their birthdates (to ± 6 months) were estimated via tooth measurements obtained during routine immobilizations or necropsies (Van Horn et al., 2003). Here we calculated the age in years of each focal hyena on the session date, and we fit a quadratic effect of age for all adults because very young and very old hyenas may be less likely to mob.

Sex: All hyenas were sexed based on the morphology of the erect phallus (Frank et al., 1990).

Social rank: The social rank of each hyena was determined based on the occurrence of submissive behavior during dyadic agonistic interactions (Strauss and Holekamp, 2019a). For each clan, separate social ranks were calculated annually for adult females and adult males using the MatReorder method in R package DynaRank (Strauss, 2019). For each year, the sex-specific hierarchies were then combined, with all adult females dominant to all adult immigrant males (Kruuk, 1972), and an annual standardized rank was calculated for each adult within each clan. Juveniles in our dataset were assigned the same rank as their mother for each year until they either became adults at age 2 (females) or dispersed between ages 2-5 (males). Males that did not disperse were added to the top of the adult immigrant male hierarchy when they reached 5 years of age (East and Hofer, 2001). Here we calculated the social rank of each focal hyena as its social rank in the calendar year of the session.

Reproductive state (females): Reproductive states of all adult females were continuously monitored to determine periods of pregnancy and lactation, as described earlier (Holekamp et al., 1996). A female was considered “nulliparous” until her first parturition, determined by direct observation of her first litter or by the observation of pink scar tissue on the posterior surface of her phallus, which tears during birth (Frank and Glickman, 1994). Conception dates were determined by subtracting a 110 day gestation period from a cub’s date of birth (Kruuk, 1972) and/or by observations of fresh tears in a female’s phallus, indicating recent parturition. A female was considered “pregnant” from conception until a cub’s date of birth and “lactating” from the day after birth until the latest weaning date of a cub from that litter. Weaning dates for each cub were calculated based on observations of nursing conflicts and observations of cubs subsequently seen with their mother when no nursing occurred (Holekamp et al., 1996). Cubs that did not successfully wean were considered “dead before weaning,” and their disappearance date was substituted for their weaning date in the calculation of maternal reproductive state. A focal female that was neither nulliparous, pregnant, nor lactating on the session date was assigned to reproductive state “other.”

Dispersal status (males): Dispersal status of all adult males was based on dispersal behavior: focal adult male dispersal status was considered “immigrant” if the individual had immigrated from his natal clan into the study clan and “natal” if the male was born in the study clan, regardless of age. Dispersal status is a good proxy for reproductive status in males because immigrant males sire 97% of all juveniles born in the clan (Engh et al., 2002), and immigrant males have higher testosterone and higher ejaculate quality than do age-matched adult natal males (Curren et al., 2013; Holekamp and Sisk, 2003).

Fecal glucocorticoid metabolites (fGCMs): For each adult focal hyena, we assigned a lifetime measure of fGCMs. Our measure represents the underlying distribution of each focal hyena's glucocorticoid levels as compared to the population average for their sex. See Fecal Hormone Concentrations for further information.

Fecal testosterone metabolites (fTMs): For each adult focal hyena, we assigned a lifetime measure of fTMs. Our measure represents the underlying distribution of each focal hyena's testosterone levels as compared to the population average for their sex. See Fecal Hormone Concentrations for further information.

Greeted: We measured individual affiliative behavior as whether or not the focal hyena had engaged in a greeting during the five minutes before the mob. Five minutes is frequently used as a window to measure the effects of a behavior in this species (Pangle and Holekamp, 2010).

Association index with mobbers: We measured the strength of social ties between a focal hyena and the mobbing individuals to evaluate the effect of relationship strength on the probability that a focal hyena would mob. For each focal hyena, we calculated the mean association index between the focal individual and all mobbing individuals; in other words, we averaged the association index of all dyads involving the focal individual and each mobbing individual.

Maternal relatedness with mobbers: Throughout our study, we established maternity for all resident hyenas based on nursing associations (Holekamp et al., 1996). To evaluate the effect of kinship on the probability that an individual would mob, we calculated the proportion of mobbers to whom the focal individual was related. Because many sires are immigrant male hyenas for whom we currently lack paternity data, relatedness was evaluated solely on the basis of maternal kin. For each focal hyena, we calculated the proportion of mobbers to whom the focal individual was closely maternally related as the number of mobbers to whom that individual was either a mother, offspring, or sibling, divided by the total number of mobbers.

Potential mates present: For immigrant males, we evaluated the audience effect of potential future mates. We first calculated the number of reproductively active females present as the number of adult females (age > 2 years) present. Because the number of adult females present correlated strongly with the total number of hyenas present (correlation coefficient > 0.7), we measured potential mates present for each focal immigrant male as the proportion of individuals present during the mob that were adult females.

Belly size: We recorded the belly size of all focal adult hyenas upon first sighting as one of four states: “gaunt” hyenas were very skinny with hipbones protruding; “normal” hyenas were fit but not fat; “fat” hyenas had a big full belly; and “obese” hyenas had a truly monstrously giant belly (Watts and Holekamp, 2008). Gaunt and normal categories were later combined due to the tiny sample size of gaunt hyenas.

Own juvenile offspring present: For each adult female focal hyena, we calculated whether or not her juvenile was present by asking whether or not she was the biological mother of any juveniles (age < 2 years) present during the mob.

Modeling occurrence of cooperative mobbing

Here, we restricted our dataset to observation sessions in our four study clans where lions and hyena interacted. We operationally defined interspecific interactions as occurring when lions and hyenas directed behavior at one another and/or when lions and hyenas approached within 10 m of one another (Lehmann et al., 2017). We further filtered to sessions with field notes of high-enough quality to be certain that mobbing events were recorded if they were observed. Finally, we filtered to sessions where at least 2 hyenas were present because, by definition, multiple hyenas are required for mobbing to occur. We fit a generalized linear model (specifically, logistic regression using the logit link function) using R package glmmTMB (Magnusson et al., 2020). Our response variable was binomial: whether or not a mob occurred during that session. Fixed effect covariates included key environmental and contextual characteristics with the potential to affect mobbing occurrence (Model A in Table 3.1). We included interactions of session length x number of hyenas present and session length x number of hyenas that greet (greeters) to control for the possible correlation between observation time and number of hyenas or greetings observed. We included interactions between number of hyenas present and number of lions present, and between number of hyenas present and male lions present based on past work indicating that the ratio of lions to hyenas present can affect mobbing behavior (Cooper, 1991; Höner et al., 2002). We included interactions between hyena and lion variables (number of hyenas present, number of lions present, male lions present) and social variables (number of greeters, mean association index) to test whether social behavior could help overcome the barriers to mobbing we documented earlier (Lehmann et al., 2017). No random effects were included in this model.

We ran one subsequent version of this model modifying our global model of mobbing occurrence (Model A in Table 3.1), in which we separated sessions by whether adult male lions were present (Model K in Table 3.3) or absent (Model L in Table 3.3) and inquired whether the number of lions had a continuous effect as a predictor of mobbing occurrence.

Modeling individual participation in cooperative mobbing

Here, we restricted our dataset to observation sessions in our four study clans where mobbing occurred and where the identities of more than 90% of mobbing participants were known. For each mob during these sessions, we determined which hyenas were present when the mob occurred based on the arrival and departure times of all hyenas in the session. Each focal hyena present during a mobbing event was coded as either a participant (mobber) or non-participant (defector) for that particular mobbing event. We then assigned relevant demographic, physiological, and social variables to each focal hyena; first, we assigned an age, social rank, reproductive state (females), and dispersal status (males) to each focal hyena present. We assigned a measure of fGCMs and fTMs to each adult hyena present from which we had collected at least one fecal sample (see Fecal Hormone Concentrations). Lastly, we assigned social context measures to each focal hyena present, including whether or not the focal hyena had greeted in the five minutes prior to a mob (“greeted”), the average association index of the focal hyena with mobbers (“association index”), and the proportion of mobbers to which the focal hyena was closely maternally related (i.e., mother, sibling, or offspring of the focal hyena; “maternal relatedness”).

To investigate hyena participation in cooperative mobbing events, we fit a series of logistic mixed-effect models using R package glmmTMB (Magnusson et al., 2020).

Our response variable was binomial: whether or not the focal hyena participated in that mob. Fixed effect covariates included key demographic, physiological, and social characteristics with the potential to affect mobbing participation (Models B-E in Table 3.1). All models included random intercept covariates of hyena identity and of mob nested within session. Clan was not included as a random intercept because it explained only 2.2% of the variance in participation (intraclass correlation coefficient = 0.022).

We built five logistic mixed-effect models to test the effects of five different variable sets on five different categories of hyenas. The first of these models (Model B in Table 3.1), looked at all hyenas in the dataset to determine whether hyena age or sex affected overall mobbing participation.

Female participation model. The second model (Model C in Table 3.1) was restricted to all adult females (age > 2 years) and included key demographic, physiological, and social characteristics with the potential to affect mobbing participation in adult females. We included interactions between social rank and other variables because social rank critically structures hyena social relationships (Smith et al., 2007). We also included an interaction between fGCMs and fTMs based on the dual-hormone hypothesis, which suggests that these two hormones together regulate aspects of social behavior (Mehta and Prasad, 2015; Pfattheicher et al., 2014).

Male participation models. The third model (Model D in Table 3.1) was restricted to all adult males (age > 2 years) and likewise included key demographic, physiological, and social characteristics with the potential to affect mobbing participation in adult males. We included interactions between social rank and other variables, as well as an interaction between fGCMs and fTMs. We were not able to include the term for maternal relatedness in this model because many of these individuals were immigrant males for which we do not currently have relatedness data. We were also not able to include an interaction between age and social rank due to its collinearity with social rank.

In addition, we ran two subsequent models modifying the adult male model (Model D in Table 3.1), in which we separated males by their dispersal status, either immigrant or natal. For the immigrant male model (Model M in Table 3.3), we included a term for potential mates. We also removed hyena identity as a random effect from the global model because it explained no variance. For the natal male model (Model N in Table 3.3), we included the term for maternal relatedness but did not include the term for potential mates because immigrant males sire 97% of all juveniles born in our study clans (Engh et al., 2002). We also did not include hormones in the natal male model due to sample size considerations.

Juvenile participation model. Our final model (Model E in Table 3.1) was restricted to all juveniles (age < 2 years) and included key demographic and social characteristics with the potential to affect mobbing participation in juvenile hyenas. We included three interactions, age by sex, age by social rank, and sex by social rank. Hormones were not included in the juvenile model because juveniles do not show adult hormone concentrations until they reach reproductive maturity at two years of age (Glickman et al., 1992).

To ensure that we were measuring the effect of affiliative social interactions and not just that of social interactions more generally, we re-ran top models that included a term for whether or not a hyena greeted to also include a term for whether or not an individual engaged in an aggressive interaction in the five minutes prior to the mob occurring. In none of these models was the aggression term included in the top model, whereas the affiliative term remained in top models, confirming that our greeting measure captures the effect of affiliation specifically and not of social interactions more generally.

Modeling individual benefits of cooperative mobbing

To investigate potential resource benefits of mobbing, we fit four logistic mixed-effect models (Models F-I in Table 3.2). For all analyses of resource benefits, we restricted our dataset to sessions with food present, and further restricted our participants to focal adult hyenas (age > 2 years), as juvenile resource acquisition and defense are strongly dependent on adult support (Engh et al., 2000; Watts et al., 2009). If hyenas mob to obtain or defend food resources, we predicted that mobs would be more likely to occur at sessions where higher quality and/or larger food items were present (Model F in Table 3.2). Here, we modified our global model of the probability of mobbing occurrence (Model A) by including a term for food quality (“carcass freshness”) and for food size (“carcass size”).

In our second model (Model G in Table 3.2), we predicted that hyenas that were hungrier, or those in a poorer nutritional state, would be more likely to participate in mobbing at sessions with food. Here, we fit a logistic mixed-effects model with a binomial response variable: whether or not the focal hyena mobbed during the session. We restricted our analysis to focal adult hyenas at sessions in which observers had recorded at least one non-normal belly size to create more even categorical distributions for belly size. This model included the following fixed effects: age, sex, social rank, belly size, carcass size, and carcass freshness. We also included interactions between social rank and belly size and between social rank and carcass size because of the large effect that social rank has on resource acquisition (Frank, 1986b).

Lastly, we predicted that hyenas that participate in mobbing would be more likely to obtain food, both immediately after the mob and during the remainder of the observation session. For these analyses, we restricted our dataset to mobs (Model H in Table 3.2) or sessions (Model I in Table 3.2) where at least one hyena fed, and we coded each hyena present as either a mobbing participant or defector (variable “mobber”). We built two logistic mixed-effects models to test these predictions, where the response variable was binomial: whether or not that hyena fed. Both models included the following fixed effects: focal hyena age, sex, and social rank, carcass freshness and size, and interactions between social rank and mobber and between mobber and carcass size. Model H investigated the probability of the hyena getting food within 5 minutes after the mob and included a fixed effect of whether or not the focal hyena participated in that mob. Here, for each mob, our response variable was whether or not the focal hyena fed in the five minutes following the mob. Model I investigated the probability of a hyena getting food later in the session and included a fixed effect of whether or not the focal hyena mobbed during the session. Here, for each session, our response variable was whether or not the focal hyena fed anytime between the first mobbing event and 30 minutes after the final mobbing event. We excluded later feeding data to reduce feeding observations due to hyena turnover at the carcass as some hyenas become satiated, and we used 30 minutes as our cut-off because a group of hyenas can reduce a large carcass to skin and bones in under 30 minutes (Kruuk, 1972). We also removed hyena identity as a random effect from the global model (Model I) because it explained no variance.

To investigate potential inclusive fitness benefits of mobbing, we fit a single logistic mixed-effect model (Model J in Table 3.2). If hyenas mob to help kin, we predicted that adult females would be more likely to mob when their own juvenile offspring (age < 2 years) were present. To enable strict comparison based on offspring presence, we restricted our analysis to mobs where at least one juvenile was present and to adult female focal hyenas with at least one offspring alive in the clan on the session date. We then modified our global female model (Model C) by including a term for whether or not her own juvenile offspring was present at the session.

Statistical analysis

All analyses were conducted using R Version 3.6.3 and R Studio Version 1.2.5042. We first performed data exploration by investigating outliers, distribution and collinearity (Zuur et al., 2010). We tested all global model predictors for multicollinearity using both correlation coefficients and variance inflation factors (VIFs), and we removed collinear predictors until none were collinear, with all correlation coefficients ≤ 0.7 and all VIFs ≤ 3 (Harrison et al., 2018). All numeric model predictors were z-score standardized immediately before modeling using the scale function in R to allow comparison of coefficients (Harrison et al., 2018). We performed model selection on the global model using the dredge function in R package MuMIn (Bartoń, 2020). The top models as determined by AIC criterion are depicted in the figures and tables here and in our supplementary information. All top models were visually inspected to confirm assumptions of multicollinearity, normality of residuals, normality of random effects, heteroscedasticity, and homogeneity of variance using R package performance (Lüdecke et al., 2020) and R package DHARMa (Hartig, 2020). We also used R package DHARMa to inspect all groups and observations for disproportionate influence on the models, but none warranted exclusion. Between-group comparisons were conducted using Tukey post-hoc tests for multiple comparisons of means in R package multcomp (Hothorn et al., 2020). Forest plots were created using R package sjPlot (Lüdecke, 2020a) and all other plots were created using the ggpredict function in R package ggeffects (Lüdecke, 2020b) to obtain predicted values and R package ggplot2 (Wickham et al., 2020) to create the plots from those values.

Fecal hormone concentrations

Fecal sample collection, extraction, and assay

Between 1993-2016, we collected fecal samples during observation sessions anytime we saw a known hyena defecating. Sample collection, extraction, and storage all followed protocols previously validated for use in spotted hyenas (Dloniak et al., 2004; Van Meter et al., 2008). Briefly, samples were collected, mixed, aliquoted, and frozen in liquid nitrogen within 12 hours of collection. Fecal samples were later lyophilized and ground into a fine powder, and hormone extraction was performed by heating and shaking the fecal powder in ethanol.

Fecal glucocorticoid metabolite assays

To measure concentrations of fecal glucocorticoid metabolites (fGCMs), samples were assayed in duplicate using a corticosterone radioimmunoassay kit (MP Biomedicals ImmuChem Double Antibody Corticosterone ^{125}I RIA kit). Assays were performed by five different individuals between 2000-2018. Further details of the fGCM assay, as well as analytical and biological validations, are published in Dloniak (2004), Greenberg (2017), and Van Meter et al. (2009).

Fecal testosterone metabolite assays

To measure concentrations of fecal testosterone metabolites (fTMs), samples were assayed in duplicate using a testosterone radioimmunoassay kit (MP Biomedicals ImmuChem Double Antibody Testosterone ^{125}I RIA kit). Extracts were diluted 1:5 using the steroid diluent provided in the kit. Assays were performed by three different individuals at the Core Assay Facility at the University of Michigan between 2016-2018. Cross-reactivity of the antibody with steroids was as follows: testosterone: 100%; 5α-dihydrotestosterone: 3.40%; 5α-androstane-3β, 17β-diol: 2.20%; 11-oxotestosterone: 2.00%; 6β-hydroxytestosterone: 0.95%; 5β-androstane-3β, 17β-diol: 0.71%; 5β-dihydrotestosterone: 0.63%; androstenedione: 0.56%; and epiandrosterone: 0.20%. All other steroids tested: < 0.01%. The minimum detection limit of the assay was 0.015 ng/mL.

We demonstrated parallelism, accuracy, and precision to validate our fTM assay (Brown et al., 2004). First, we determined parallelism by modeling the percent binding as a function of the concentrations of varying dilutions of a mixed-sex fecal extract pool and of the RIA standard curve. There was no significant interaction between the concentrations and the type of sample (pool vs. standard) ($t = 0.845$, $p = 0.415$), indicating that the slopes of these lines are parallel. Second, we determined the accuracy of the assay by spiking each standard with a diluted aliquot of the fecal extract pool. Mean recovery was 117.0 ± 11.7%, indicating accuracy of our fecal measurements. Lastly, we determined the precision of the assay using two methods: (1) we ran a dilution of the fecal extract pool (50% binding) 8 times in the same assay (intra-assay CV), and (2) we ran low (80% binding) and high concentration (25% binding) dilutions of the mixed-sex fecal extract pool in all assays (inter-assay CV). Our intra-assay CV was 11.7%, while our inter-assay CV was 19.4% (low concentration pool) and 10.3% (high concentration pool) (n = 15 assays), indicating the precision of our fecal measurements.

We performed a biological validation by demonstrating that fTM measurements matched the patterns previously documented for plasma testosterone in spotted hyenas. Adult immigrant males had higher fTMs than adult natal males ($t = 4.08$, $df = 184$, $p < 0.001$) (Dloniak et al., 2004; Holekamp and Smale, 1998). Pregnant females had higher fTMs than lactating females ($t = -7.63$, $df = 221$, $p = p < 0.001$) (Dloniak et al., 2004).

Calculation of fecal hormone best linear unbiased predictors (BLUPs)

Prior to our main analysis, we summarized and controlled for confounding variables that might potentially bias estimates of fGCMs and fTMs (which are based on multiple observations or values per hyena), such that each hyena ultimately had a single mean value for each hormone. Hormones were analyzed separately in each sex because both fGCMs and fTMs have sex-specific effects in the spotted hyena (Dloniak et al., 2004; Van Meter et al., 2009). For our female models, each hyena had a mean of 6.4 fecal samples (median 4, range 1-61). For our male models, each hyena had a mean of 3.9 fecal samples (median 2, range 1-34).

We fit four linear mixed-effect models, one for each hormone and sex, where the outcome of interest was the log of the hormone measurement (either fGCMs or fTMs) for a given fecal sample. Fixed-effect covariates included key methodological and demographic characteristics with the potential to affect fecal hormone measurements: time of fecal deposition (AM/PM), years between fecal deposition and hormone assay, individual that performed the hormone assay, hyena reproductive state at fecal deposition (females), hyena age in years at fecal deposition, and hyena social rank at fecal deposition (Dloniak et al., 2004; Van Meter et al., 2009). For males, we were not able to include hyena dispersal status at fecal deposition due to its collinearity with hyena age and social rank. Our models also included a random intercept for hyena identity to account for correlations among the repeated fecal samples. For each fecal hormone and sex, we created a model including all above predictors using R package lme4 (Bates et al., 2020); these models, including coefficients and sample sizes, are described in Table 3.6.

We extracted the individual-level random intercept estimates (or BLUPs) from each hormone model using the ranef function in R package lme4 (Bates et al., 2015). These BLUPs represent the underlying distribution of the deviation of each hyena's hormone levels in comparison to the population average for their sex for that particular hormone, while controlling for variation in fecal sampling (Gelman and Hill, 2007). This method has been previously used to consolidate repeated measurements of a given variable into a single value per individual without making assumptions about the underlying distribution of the data (Laubach, 2019).

ACKNOWLEDGEMENTS

We first and foremost thank all past and present members of the Mara Hyena Project for their work collecting and curating the data presented here. We especially thank Stephanie Dloniak, Page Van Meter, and Erin Person for their help assaying fecal samples for hormones, as well as Teera Losch at the University of Michigan Core Assay Facility (UM CAF). We thank Andrew Dennhardt at the Michigan State University Center for Statistical Training and Consulting (MSU CSTAT) for his considerable help with our statistics and model selection. We thank the Office of the President of Kenya, the National Commission for Science, Technology, and Innovation, the Kenya Wildlife Service, the Narok County Government, the Naboisho Conservancy, and the Mara Conservancy for permission to conduct this research. This work was supported by a National Institutes of Health (NIH) grant and National Science Foundation (NSF) grants to KEH and by a MSU Dr. Marvin Hensley Research Grant to TMM.

APPENDICES

APPENDIX A:

FIGURES AND TABLES

Figure 3.1. A group of hyenas mobbing a lioness. Unknown photographer.

Table 3.1. Description of outcome variables and predictors used in model selection for mobbing occurrence and participation. (Bolded terms remain in the top model.)

Outcome variable	Main effects	Definition*	Interaction effects	Random effects
A. Mobbing occurrence: Logistic model				
(T/F): Whether a mob occurred during the session	Session length	Length of observation session in minutes	Session length x Number of hyenas present Session length x Number of greeters Number of hyenas present x Number of lions present **Number of hyenas present x Male lions present** **Number of greeters x Number of hyenas present** Number of greeters x Number of lions present Number of greeters x Male lions present Association index x Number of hyenas present Association index x Number of lions present Association index x Male lions present	None
	Session context (food, den, other)	Food (a kill or carcass present), den (an active hyena den present), other (all other sessions)		
	Prey density	**Number of standard deviations from the annual mean prey density**		
	Number of hyenas present	**Total number of hyenas present at the session**		
	Number of lions present	Total number of lions present at the session		
	Male lions present (T/F)	**(T/F): whether adult male lions were present at the session**		
	Number of hyenas who greet (greeters)	**Number of hyenas who engage in greeting behavior during the session**		
	Mean association index	Mean association index of all dyads present based on association data from the previous 365 days		
B. Mobbing participation by all hyenas: Logistic mixed-effects model				
(T/F): Whether a hyena participated in the mob	**Age**	**Age of focal hyena in years**	None	Session ID-Mob ID Hyena ID
	Sex	**Sex of focal hyena**		

Table 3.1. (cont'd)

Outcome variable	Main effects	Definition*	Interaction effects	Random effects
C. Mobbing participation by adult female hyenas: Logistic mixed-effects model				
(T/F): Whether a hyena participated in the mob	**Age**	**Age of focal hyena in years**	Age x Social rank Social rank x Fecal glucocorticoids Social rank x Fecal testosterone **Social rank x Greeted** **Social rank x Association index** Social rank x Maternal relatedness Fecal glucocorticoids x Fecal testosterone	Session ID-Mob ID Hyena ID
	Social rank	**Social rank of focal hyena during calendar year of session**		
	Reproductive state (nulliparous, pregnant, lactating, other)	Nulliparous (never given birth), pregnant (pregnant with at least one cub), lactating (nursing at least one cub), other (cycling or between pregnancies)		
	Fecal glucocorticoids	Fecal glucocorticoids BLUP for focal hyena		
	Fecal testosterone	**Fecal testosterone BLUP for focal hyena**		
	Greeted (T/F)	**(T/F): whether focal hyena engaged in greeting behavior in five minutes prior to mob**		
	Association index with mobbers	**Mean association index between focal hyena and mobbers based on association data from the previous 365 days**		
	Maternal relatedness with mobbers	**Proportion of mobbers to whom focal hyena is closely maternally related**		
D. Mobbing participation by adult male hyenas: Logistic mixed-effects model				
(T/F): Whether a hyena participated in the mob	**Age**	**Age of focal hyena in years**	**Social rank x Fecal glucocorticoids** Social rank x Fecal testosterone Social rank x Greeted Social rank x Association index Fecal glucocorticoids x Fecal testosterone	Session ID-Mob ID Hyena ID
	Social rank	**Social rank of focal hyena during calendar year of session**		
	Dispersal status (natal, immigrant)	Natal (born in clan), immigrant (immigrated into clan)		
	Fecal glucocorticoids	**Fecal glucocorticoids BLUP for focal hyena**		
	Fecal testosterone	Fecal testosterone BLUP for focal hyena		
	Greeted (T/F)	(T/F): whether focal hyena engaged in greeting behavior in five minutes prior to mob		
	Association index with mobbers	**Mean association index between focal hyena and mobbers based on association data from the previous 365 days**		

Table 3.1. (cont'd)

Outcome variable	Main effects	Definition*	Interaction effects	Random effects
E. Mobbing participation by juvenile hyenas: Logistic mixed-effects model				
(T/F): Whether a hyena participated in the mob	**Age**	**Age of focal hyena in years**	**Age x Sex** Age x Social rank Sex x Social rank	Session ID-Mob ID Hyena ID
	Sex	**Sex of focal hyena**		
	Social rank	Social rank of focal hyena during calendar year of session		
	Greeted (T/F)	(T/F): whether focal hyena engaged in greeting behavior in five minutes prior to mob		
	Association index with mobbers	Mean association index between focal hyena and mobbers based on association data from the previous 365 days		
	Maternal relatedness with mobbers	Proportion of mobbers to whom focal hyena is closely maternally related		

*See Model Predictors section in Methods for a detailed description of these variables.

Table 3.2. Description of outcome variables and predictors used in model selection for potential individual benefits of mobbing. (Bolded terms remain in the top model.)

Outcome variable	Main effects	Definition*	Interaction effects	Random effects
F. Are mobs more likely to occur at sessions with higher quality and/or larger food? Logistic model				
(T/F): Whether a mob occurred during the session	**Session length**	**Length of observation session in minutes**	**Session length x Number of hyenas present** **Session length x Number of greeters** Number of hyenas present x Number of lions present **Number of hyenas present x Male lions present** Number of greeters x Number of hyenas present Number of greeters x Number of lions present Number of greeters x Male lions present Association index x Number of hyenas present Association index x Number of lions present Association index x Male lions present	None
	Prey density	Number of standard deviations from the annual mean prey density		
	Number of hyenas present	**Total number of hyenas present at the session**		
	Number of lions present	Total number of lions present at the session		
	Male lions present (T/F)	**(T/F): whether adult male lions were present at the session**		
	Number of hyenas who greet (greeters)	**Number of hyenas who engage in greeting behavior during the session**		
	Mean association index	Mean association index of all dyads present based on association data from the previous 365 days		
	Carcass freshness (fresh, old)	Fresh (the prey was recently killed), old (the prey was killed >24h ago)		
	Carcass size (medium, large, extra-large)	Medium (<20-100 kg), large (100-500 kg), extra-large (>500 kg)		
G. Are hyenas in poorer nutritional condition more likely to mob at sessions with food? Logistic mixed-effects model				
(T/F): Whether a hyena mobbed during the session	**Age**	**Age of focal hyena in years**	Social rank x Belly size Social rank x Carcass size	Session ID Hyena ID
	Sex	Sex of focal hyena		
	Social rank	**Social rank of focal hyena during calendar year of session**		
	Belly size (normal, fat, obese)	**Normal (fit but not fat), fat (big full belly), obese (monstrously giant belly)**		
	Carcass freshness (fresh, old)	Fresh (the prey was recently killed), old (the prey was killed >24h ago)		
	Carcass size (medium, large, extra-large)	**Medium (< 20-100 kg), large (100-500 kg), extra-large (> 500 kg)**		

Table 3.2. (cont'd)

Outcome variable	Main effects	Definition*	Interaction effects	Random effects
H. Are hyenas who mob more likely to feed immediately after the mob? Logistic mixed-effects model				
(T/F): Whether a hyena fed in the five minutes after the mob	**Age**	**Age of focal hyena in years**	**Social rank x Mobber** Mobber x Carcass size	Session ID** Hyena ID
	Sex	Sex of focal hyena		
	Social rank	**Social rank of focal hyena during calendar year of session**		
	Mobber (T/F)	**(T/F): whether focal hyena participated in the mob**		
	Carcass freshness (fresh, old)	Fresh (the prey was recently killed), old (the prey was killed >24h ago)		
	Carcass size (medium, large, extra-large)	Medium (< 20-100 kg), large (100-500 kg), extra-large (> 500 kg)		
I. Are hyenas who mob more likely to feed in the session? Logistic mixed-effects model				
(T/F): Whether a hyena fed during the session	Age	Age of focal hyena in years	Social rank x Mobber Mobber x Carcass size	Session ID***
	Sex	**Sex of focal hyena**		
	Social rank	**Social rank of focal hyena during calendar year of session**		
	Mobber (T/F)	(T/F): whether focal hyena mobbed during the session		
	Carcass freshness (fresh, old)	Fresh (the prey was recently killed), old (the prey was killed >24h ago)		
	Carcass size (medium, large, extra-large)	Medium (< 20-100 kg), large (100-500 kg), extra-large (> 500 kg)		

Table 3.2. (cont'd)

Outcome variable	Main effects	Definition*	Interaction effects	Random effects
J. Are female hyenas more likely to mob when their juvenile offspring are present? Logistic mixed-effects model				
(T/F): Whether a hyena participated in the mob	**Age**	**Age of focal hyena in years**	Age x Social rank Social rank x Fecal glucocorticoids Social rank x Fecal testosterone **Social rank x Greeted** **Social rank x Association index** Social rank x Maternal relatedness Social rank x Juvenile present Fecal glucocorticoids x Fecal testosterone	Session ID-Mob ID Hyena ID
	Social rank	**Social rank of focal hyena during calendar year of session**		
	Reproductive state (pregnant, lactating, other)	Pregnant (pregnant with at least one cub), lactating (nursing at least one cub), other (cycling or between pregnancies)		
	Fecal glucocorticoids	Fecal glucocorticoids BLUP for focal hyena		
	Fecal testosterone	Fecal testosterone BLUP for focal hyena		
	Greeted (T/F)	**(T/F): whether focal hyena engaged in greeting behavior in five minutes prior to mob**		
	Association index with mobbers	**Mean association index between focal hyena and mobbers based on association data from the previous 365 days**		
	Maternal relatedness with mobbers	**Proportion of mobbers to whom focal hyena is closely maternally related**		
	Own juvenile offspring present (T/F)	**(T/F): whether focal hyena's own juvenile offspring present during the mob**		

*See Model Predictors section in Methods for a detailed description of these variables.
**The random effect of Mob ID was removed from this global model because it explained no variance.
***The random effect of Hyena ID was removed from this global model because it explained no variance.

Figure 3.2. Top model of the predicted probability of mobbing occurrence in sessions where lions and hyenas interact (Model A: n-sessions = 321). **A.** Dots depict coefficient estimates, lines depict 95% confidence intervals, and asterisks depict significance at the following p-values: * = 0.05; ** = 0.01; *** = 0.001. **B-D.** Lines (or dots) depict estimated marginal means and shaded areas (or vertical lines) depict 95% confidence intervals. **D.** Number of greeters was analyzed as a continuous variable but is depicted categorically for illustrative purposes.

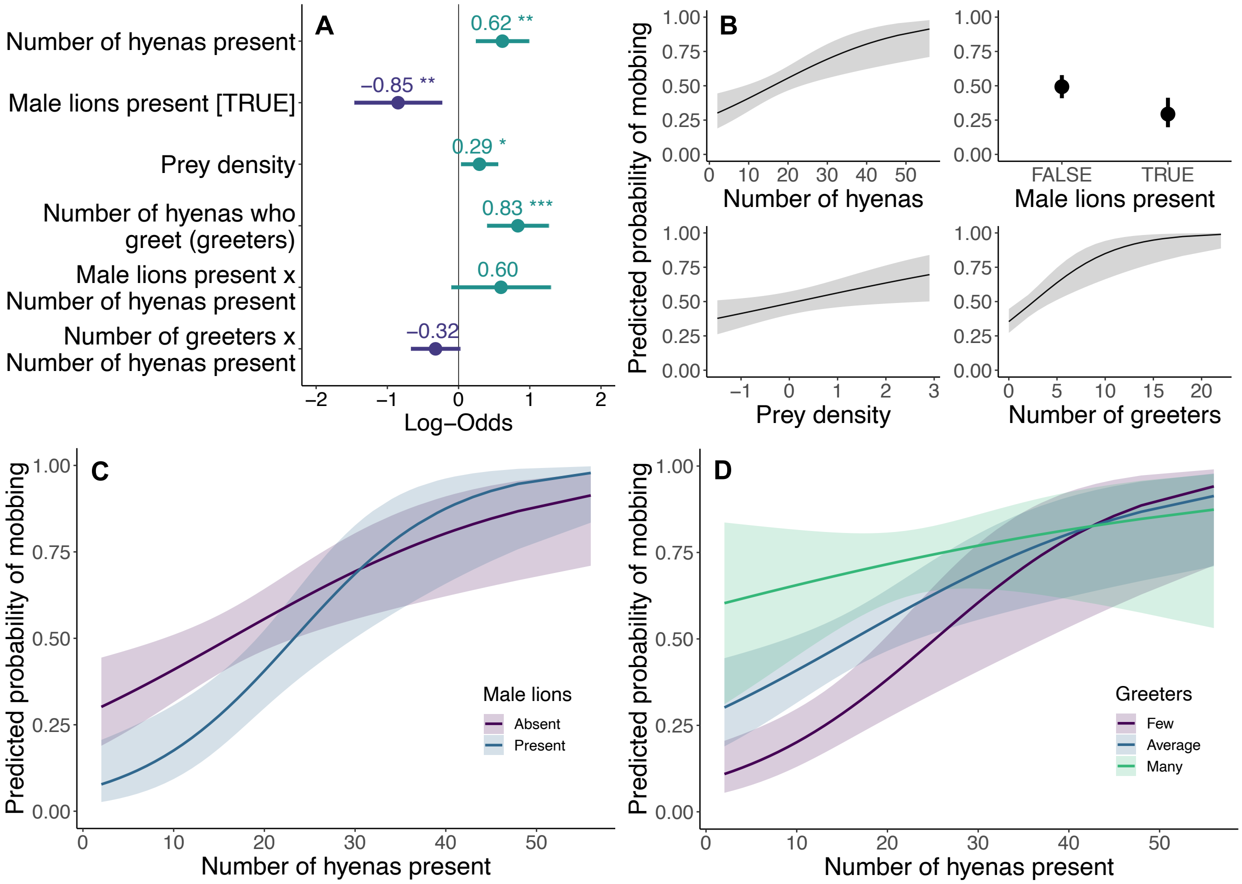


Figure 3.3. Top model of the predicted probability of mobbing participation by adult female focal hyenas (Model C: n-focal hyenas = 2175; n-sessions = 108; n-mobs = 322; n-unique hyenas = 141). **A.** Dots depict coefficient estimates, lines depict 95% confidence intervals, and asterisks depict significance at the following p-values: * = 0.05; ** = 0.01; *** = 0.001. **B-D.** Lines (or dots) depict estimated marginal means and shaded areas (or vertical lines) depict 95% confidence intervals. **D.** Association index with mobbers was analyzed as a continuous variable but is depicted categorically for illustrative purposes.

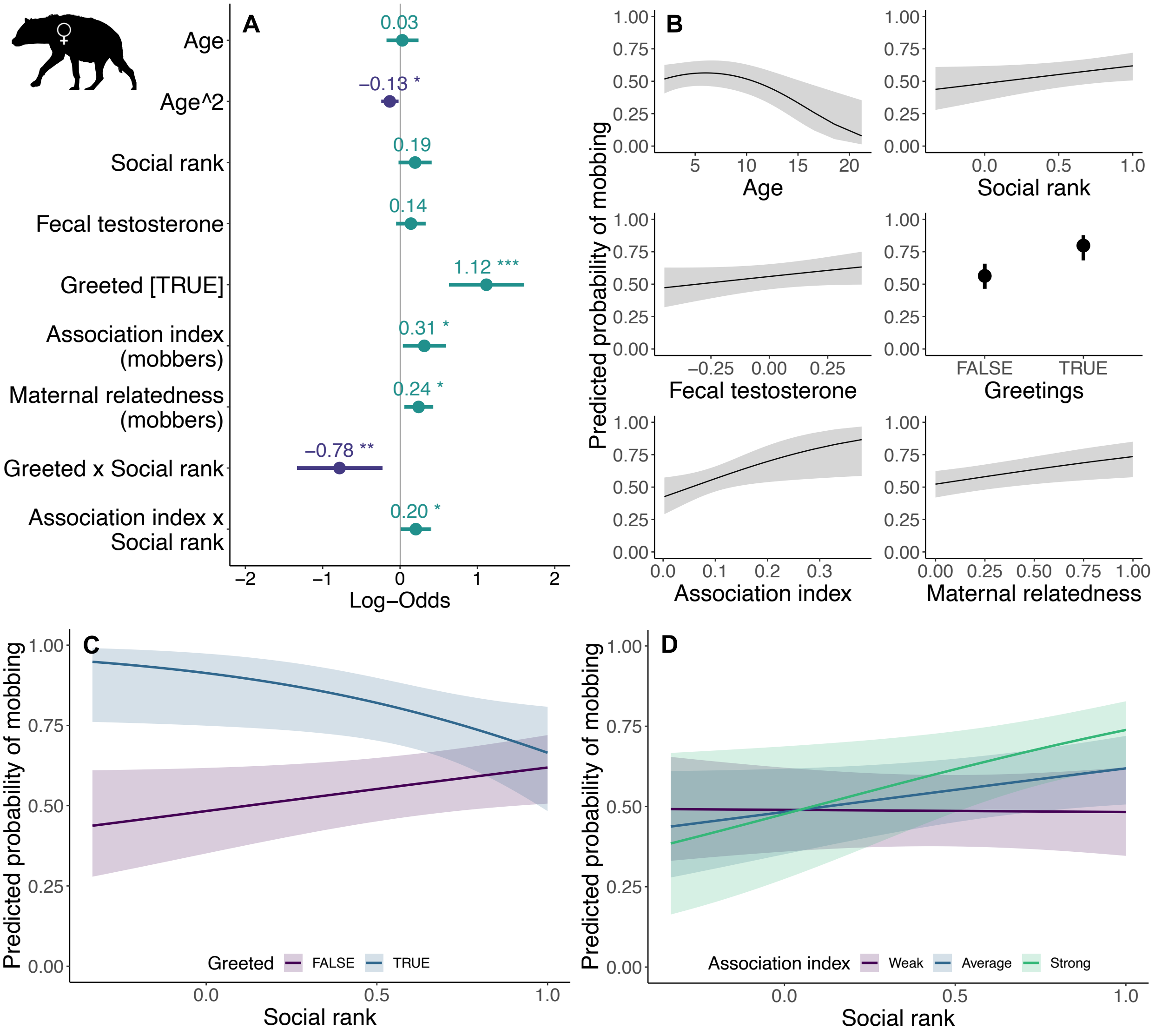


Figure 3.4. Top model of the predicted probability of mobbing participation by adult male focal hyenas (Model D: n-focal hyenas = 783; n-sessions = 90; n-mobs = 268; n-unique hyenas = 92). **A.** Dots depict coefficient estimates, lines depict 95% confidence intervals, and asterisks depict significance at the following p-values: * = 0.05; ** = 0.01; *** = 0.001. **B-C.** Lines depict estimated marginal means and shaded areas depict 95% confidence intervals. **C.** Fecal glucocorticoid BLUPs were analyzed as a continuous variable but are depicted categorically for illustrative purposes.

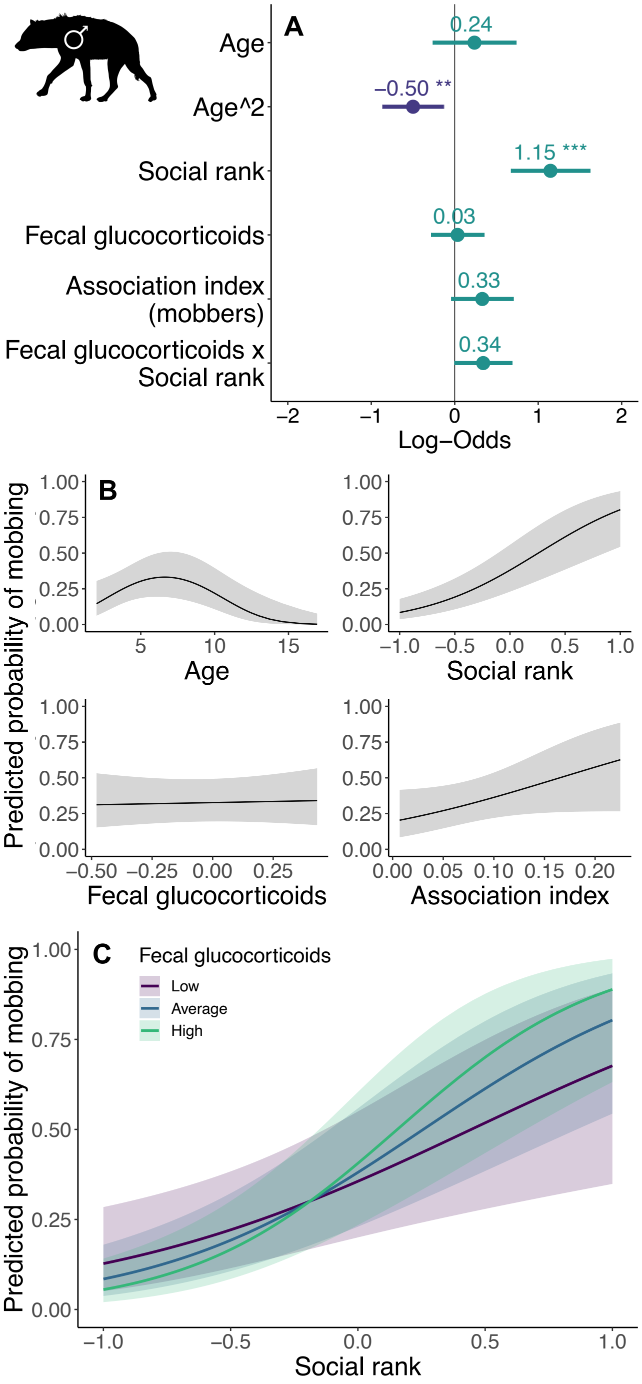


Figure 3.5. Top model for probability of mobbing participation for adult hyenas in sessions with food (Model G: n-focal hyenas = 423; n-sessions = 34; n-unique hyenas = 196). **A.** Dots depict coefficient estimates, lines depict 95% confidence intervals, and asterisks depict significance at the following p-values: * = 0.05; ** = 0.01; *** = 0.001. **B-C.** Dots depict estimated marginal means and vertical lines depict 95% confidence intervals. Asterisks depict significance in a Tukey post-hoc test at the following p-values: * = 0.05; ** = 0.01; *** = 0.001.

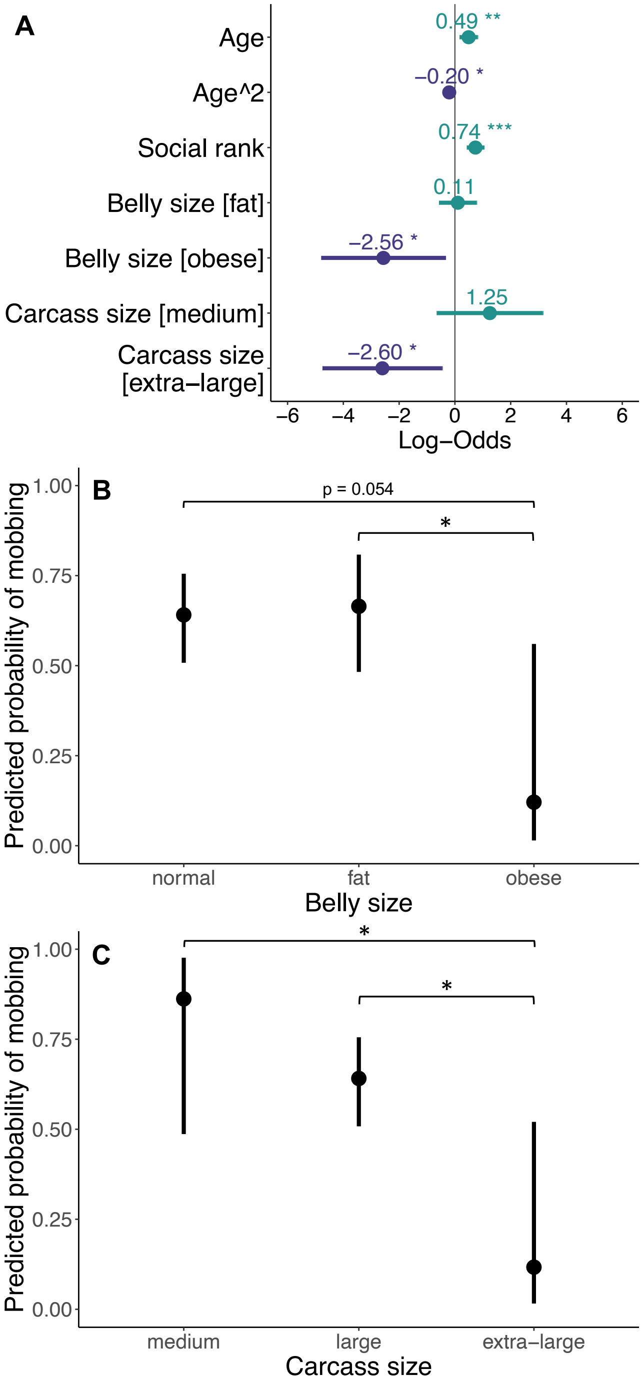
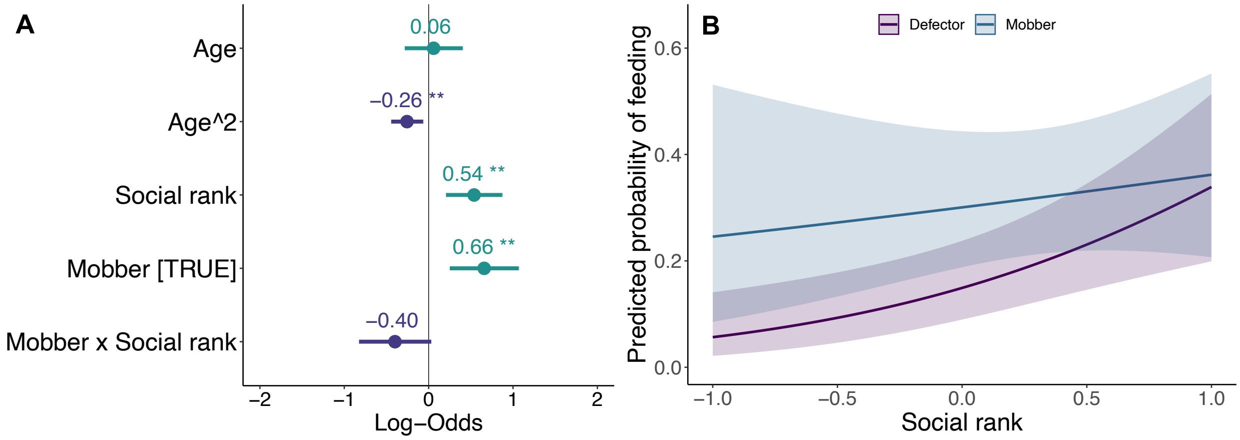


Figure 3.6. Top model of the predicted probability of the focal hyena feeding during the five minutes immediately after a mob (Model H: n-focal hyenas = 1040; n-sessions = 40; n-mobs = 90; n-unique hyenas = 182). **A.** Dots depict coefficient estimates, lines depict 95% confidence intervals, and asterisks depict significance at the following p-values: * = 0.05; ** = 0.01; *** = 0.001. **B.** Lines depict estimated marginal means and shaded areas depict 95% confidence intervals.



APPENDIX B:

SUPPLEMENTAL FIGURES AND TABLES

Table 3.3. Description of outcome variables and predictors used in model selection for supplementary models of mobbing occurrence and participation. (Bolded terms remain in the top model.)

Outcome variable	Main effects	Definition*	Interaction effects	Random effects
K. Mobbing occurrence at sessions with adult male lions: Logistic model				
(T/F): Whether a mob occurred during the session	Session length	Length of observation session in minutes	Session length x Number of hyenas present Session length x Number of greeters Number of hyenas present x Number of lions present Number of greeters x Number of hyenas present Number of greeters x Number of lions present Association index x Number of hyenas present Association index x Number of lions present	None
	Session context (food, den, other)	Food (a kill or carcass present), den (an active hyena den present), other (all other sessions)		
	Prey density	**Number of standard deviations from the annual mean prey density**		
	Number of hyenas present	**Total number of hyenas present at the session**		
	Number of lions present	Total number of lions present at the session		
	Number of adult male lions present	Total number of adult male lions present at the session		
	Number of hyenas who greet (greeters)	**Number of hyenas who engage in greeting behavior during the session**		
	Mean association index	Mean association index of all dyads present based on association data from the previous 365 days		

Table 3.3. (cont'd)

Outcome variable	Main effects	Definition*	Interaction effects	Random effects
L. Mobbing occurrence at sessions without adult male lions: Logistic model				
(T/F): Whether a mob occurred during the session	Session length	Length of observation session in minutes	Session length x Number of hyenas present Session length x Number of greeters Number of hyenas present x Number of lions present **Number of greeters x Number of hyenas present** Number of greeters x Number of lions present Association index x Number of hyenas present Association index x Number of lions present	None
	Session context (food, den, other)	Food (a kill or carcass present), den (an active hyena den present), other (all other sessions)		
	Prey density	Number of standard deviations from the annual mean prey density		
	Number of hyenas present	**Total number of hyenas present at the session**		
	Number of lions present	Total number of lions present at the session		
	Number of hyenas who greet (greeters)	**Number of hyenas who engage in greeting behavior during the session**		
	Mean association index	Mean association index of all dyads present based on association data from the previous 365 days		
M. Mobbing participation by adult immigrant male hyenas: Logistic mixed-effects model				
(T/F): Whether a hyena participated in the mob	**Age**	**Age of focal hyena in years**	Social rank x Fecal glucocorticoids Social rank x Fecal testosterone Social rank x Greeted Social rank x Association index Social rank x Potential mates Fecal glucocorticoids x Fecal testosterone	Session ID-Mob ID**
	Social rank	**Social rank of focal hyena during calendar year of session**		
	Fecal glucocorticoids	Fecal glucocorticoids BLUP for focal hyena		
	Fecal testosterone	Fecal testosterone BLUP for focal hyena		
	Greeted (T/F)	(T/F): whether focal hyena engaged in greeting behavior in five minutes prior to mob		
	Association index with mobbers	**Mean association index between focal hyena and mobbers based on association data from the previous 365 days**		
	Potential mates present	**Proportion of hyenas present who are adult female hyenas**		

Table 3.3. (cont'd)

Outcome variable	Main effects	Definition*	Interaction effects	Random effects
N. Mobbing participation by adult natal male hyenas: Logistic mixed-effects model				
(T/F): Whether a hyena participated in the mob	Age	Age of focal hyena in years	Social rank x Greeted	Session ID-Mob ID
	Social rank	**Social rank of focal hyena during calendar year of session**	Social rank x Association index	Hyena ID
	Greeted (T/F)	(T/F): whether focal hyena engaged in greeting behavior in five minutes prior to mob	**Social rank x Maternal relatedness**	
	Association index with mobbers	**Mean association index between focal hyena and mobbers based on association data from the previous 365 days**		
	Maternal relatedness with mobbers	**Proportion of mobbers to whom focal hyena is closely maternally related**		

*See Model Predictors section in Methods for a detailed description of these variables.
**The random effect of Hyena ID was removed from this global model because it explained no variance.

Table 3.4. All top logistic mixed-effects models presented in the manuscript.

Predictors	**Log-Odds**	**SE**	**p**
A. Mobbing occurrence (n-obs = 321)			
Number of hyenas present	0.62	0.19	0.001
Male lions present [TRUE]	-0.85	0.31	0.007
Prey density	0.29	0.13	0.026
Number of hyenas who greet (greeters)	0.83	0.22	<0.001
Male lions present x Number of hyenas present	0.60	0.36	0.094
Number of greeters x Number of hyenas present	-0.32	0.18	0.066
Marginal R^2 = 0.366			
B. Mobbing participation by all hyenas (n-obs = 4383, n-sessions = 117, n-mobs = 342, n-hyenas = 431)			
Age	0.72	0.09	<0.001
Age^2	-0.40	0.05	<0.001
Sex [male]	-1.04	0.16	<0.001
ICC = 0.52			
Marginal R^2 / Conditional R^2 = 0.090 / 0.566			
C. Mobbing participation by adult female hyenas (n-obs = 2175, n-sessions = 108, n-mobs = 322, n-hyenas = 141)			
Age	0.03	0.10	0.775
Age^2	-0.13	0.06	0.016
Social rank	0.19	0.11	0.073
Fecal testosterone	0.14	0.10	0.146
Greeted [TRUE]	1.12	0.25	<0.001
Association index (mobbers)	0.31	0.14	0.026
Maternal relatedness (mobbers)	0.24	0.09	0.010
Greeted x Social rank	-0.78	0.28	0.005
Association index x Social rank	0.20	0.10	0.038
ICC = 0.50			
Marginal R^2 / Conditional R^2 = 0.096 / 0.546			
D. Mobbing participation by adult male hyenas (n-obs = 783, n-sessions = 90, n-mobs = 268, n-hyenas = 92)			
Age	0.24	0.26	0.354
Age^2	-0.50	0.19	0.008
Social rank	1.15	0.24	<0.001
Fecal glucocorticoids	0.03	0.16	0.833
Association index (mobbers)	0.33	0.19	0.083
Fecal glucocorticoids x Social rank	0.34	0.18	0.053
ICC = 0.64			
Marginal R^2 / Conditional R^2 = 0.125 / 0.682			

Table 3.4. (cont'd)

Predictors	**Log-Odds**	**SE**	**p**
E. Mobbing participation by juvenile hyenas (n-obs = 1153, n-sessions = 88, n-mobs = 269, n-hyenas = 247)			
Age	1.74	0.33	<0.001
Sex [male]	-0.34	0.39	0.390
Age x Sex	-0.66	0.43	0.123
ICC = 0.66			
Marginal R^2 / Conditional R^2 = 0.179 / 0.720			
F. Mobbing occurrence at sessions with food (n-obs = 218)			
Number of hyenas present	0.48	0.22	0.031
Male lions present [TRUE]	-0.87	0.37	0.019
Number of hyenas who greet (greeters)	0.64	0.22	0.003
Session length	0.06	0.19	0.752
Number of hyenas present x Male lions present	0.75	0.43	0.081
Number of hyenas present x Session length	0.18	0.20	0.386
Number of greeters x Session length	-0.45	0.28	0.105
Marginal R^2 = 0.335			
G. Mobbing participation by hyenas of variable body condition at sessions with food (n-obs = 423, n-sessions = 34, n-hyenas = 196)			
Age	0.49	0.17	0.003
Age^2	-0.20	0.09	0.021
Social rank	0.74	0.16	<0.001
Belly size [fat]	0.11	0.34	0.760
Belly size [obese]	-2.56	1.14	0.024
Carcass size [medium]	1.25	0.97	0.197
Carcass size [extra-large]	-2.60	1.10	0.018
ICC = 0.30			
Marginal R^2 / Conditional R^2 = 0.183 / 0.428			
H. Feeding immediately after the mob (n-obs = 1040, n-sessions = 40, n-hyenas = 182)			
Age	0.06	0.17	0.736
Age^2	-0.26	0.10	0.007
Social rank	0.54	0.17	0.001
Mobber [TRUE]	0.66	0.21	0.002
Mobber x Social rank	-0.40	0.22	0.065
ICC = 0.47			
Marginal R^2 / Conditional R^2 = 0.092 / 0.521			

Table 3.4. (cont'd)

Predictors	**Log-Odds**	**SE**	**p**
<u>I. Feeding during the session</u> (n-obs = 594, n-sessions = 53)			
Sex	-0.33	0.29	0.251
Social rank	0.15	0.15	0.312
ICC = 0.37			
Marginal R^2 / Conditional R^2 = 0.015 / 0.384			
<u>J. Mobbing participation by adult female hyenas</u> (n-obs = 1140, n-sessions = 81, n-mobs = 274, n-hyenas = 100)			
Age	-0.21	0.13	0.097
Age^2	-0.06	0.09	0.467
Social rank	0.17	0.17	0.306
Greeted [TRUE]	0.60	0.33	0.072
Association index (mobbers)	0.32	0.19	0.102
Maternal relatedness (mobbers)	0.33	0.13	0.014
Own juvenile offspring present [TRUE]	0.42	0.21	0.044
Greeted x Social rank	-0.89	0.40	0.027
Association index x Social rank	0.30	0.15	0.045
ICC = 0.47			
Marginal R^2 / Conditional R^2 = 0.113 / 0.530			
<u>K. Mobbing occurrence at sessions with adult male lions</u> (n-obs = 113)			
Number of hyenas present	1.25	0.34	<0.001
Prey density	0.64	0.24	0.008
Number of hyenas who greet (greeters)	0.80	0.31	0.011
Marginal R^2 = 0.515			
<u>L. Mobbing occurrence at sessions without adult male lions</u> (n-obs = 212)			
Number of hyenas present	0.58	0.20	0.003
Number of hyenas who greet (greeters)	0.84	0.28	0.002
Number of greeters x Number of hyenas present	-0.42	0.21	0.049
Marginal R^2 = 0.251			
<u>M. Mobbing participation by adult immigrant male hyenas</u> (n-obs = 551, n-sessions = 80, n-mobs = 203)			
Age	-0.45	0.22	0.046
Age^2	-0.20	0.15	0.188
Social rank	0.98	0.23	<0.001
Association index (mobbers)	0.17	0.22	0.429
Potential mates (present)	-0.58	0.30	0.056
ICC = 0.61			
Marginal R^2 / Conditional R^2 = 0.139 / 0.661			

Table 3.4. (cont'd)

Predictors	Log-Odds	SE	p
<u>N. Mobbing participation by adult natal male hyenas</u> **(n-obs = 326, n-sessions = 69, n-mobs = 207, n-hyenas = 72)**			
Social rank	0.40	0.33	0.222
Association index (mobbers)	-0.52	0.36	0.149
Maternal relatedness (mobbers)	0.69	0.31	0.027
Maternal relatedness x Social rank	-0.51	0.32	0.107
ICC = 0.64			
Marginal R^2 / Conditional R^2 = 0.120 / 0.687			

Figure 3.7. Top model for probability of mobbing participation by all hyenas (Model B: n-focal hyenas = 4383; n-sessions = 117; n-mobs = 342; n-unique hyenas = 431). **A.** Dots depict coefficient estimates, lines depict 95% confidence intervals, and asterisks depict significance at the following p-values: * = 0.05; ** = 0.01; *** = 0.001. **B.** Lines depict estimated marginal means and shaded areas depict 95% confidence intervals.

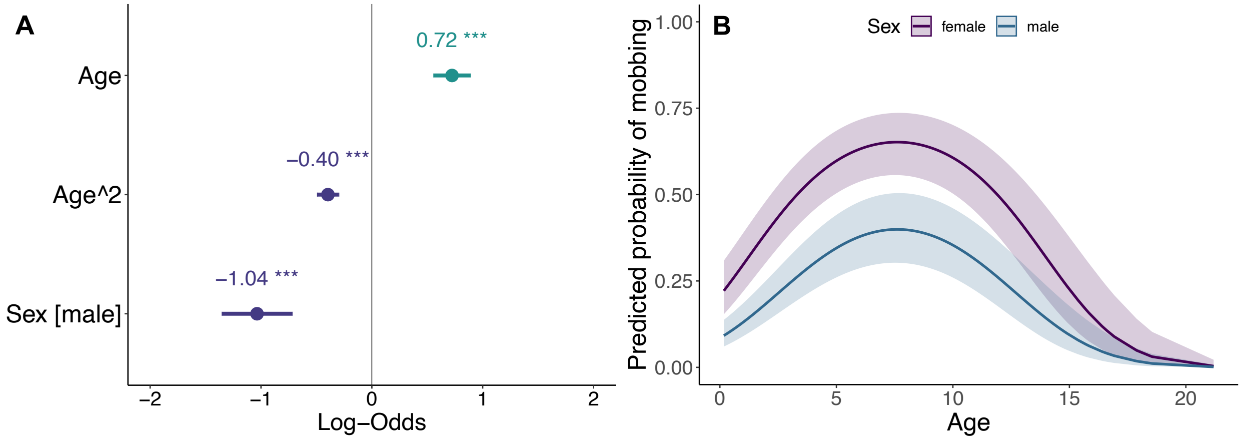


Figure 3.8. Top models for probability of mobbing participation by immigrant and natal adult male hyenas. **A-B.** Top model for probability of mobbing participation by adult immigrant male hyenas (Model M: n-focal hyenas = 551; n-sessions = 80; n-mobs = 203; n-unique hyenas = 50). **C-D.** Top model for probability of mobbing participation by adult natal male hyenas (Model N: n-focal hyenas = 326; n-sessions = 69; n-mobs = 207; n-unique hyenas = 72). **A,C.** Dots depict coefficient estimates, lines depict 95% confidence intervals, and asterisks depict significance at the following p-values: * = 0.05; ** = 0.01; *** = 0.001. **B,D.** Lines depict estimated marginal means and shaded areas depict 95% confidence intervals.

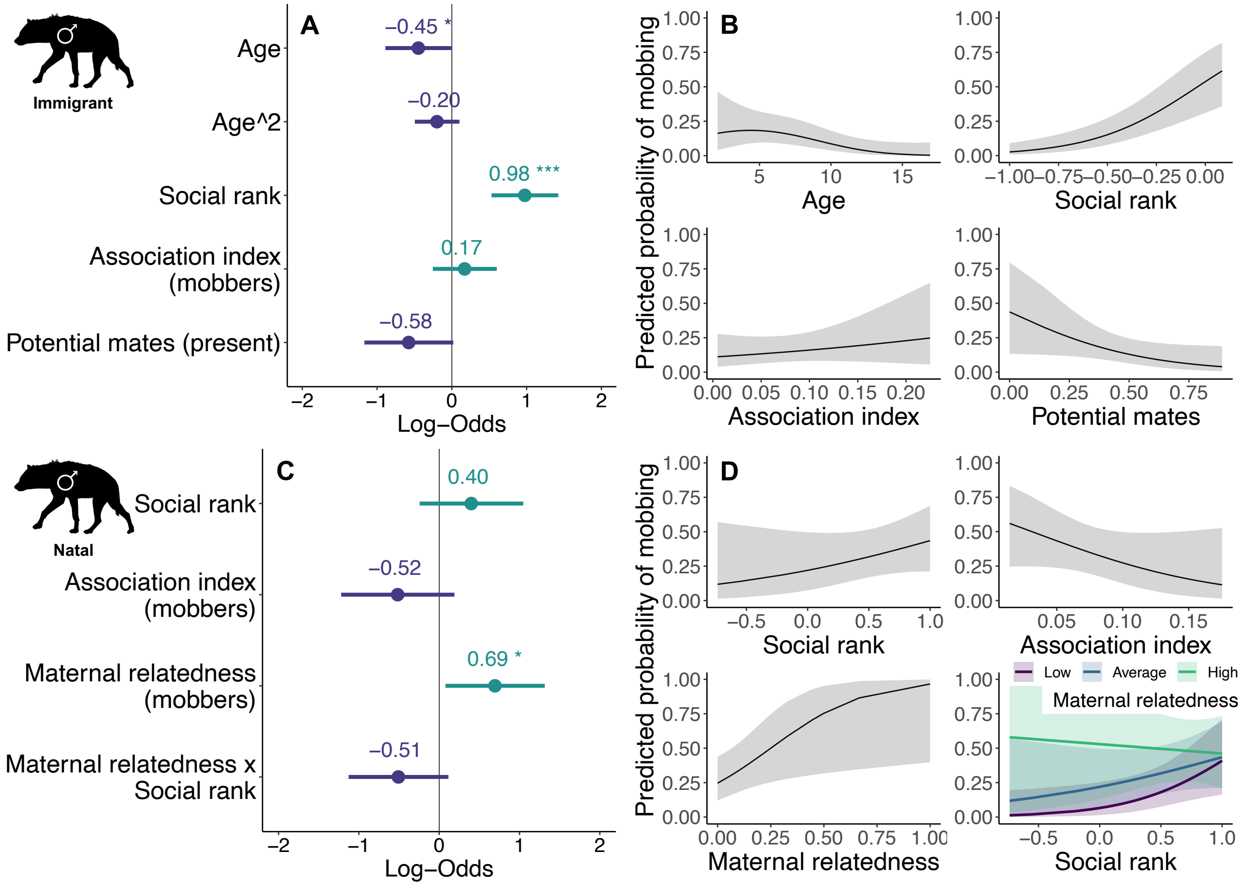


Figure 3.9. Top model for probability of mobbing participation by juvenile hyenas (Model E: n-focal hyenas = 1153; n-sessions = 88; n-mobs = 269; n-unique hyenas = 247). **A.** Dots depict coefficient estimates, lines depict 95% confidence intervals, and asterisks depict significance at the following p-values: * = 0.05; ** = 0.01; *** = 0.001. **B.** Lines depict estimated marginal means and shaded areas depict 95% confidence intervals.

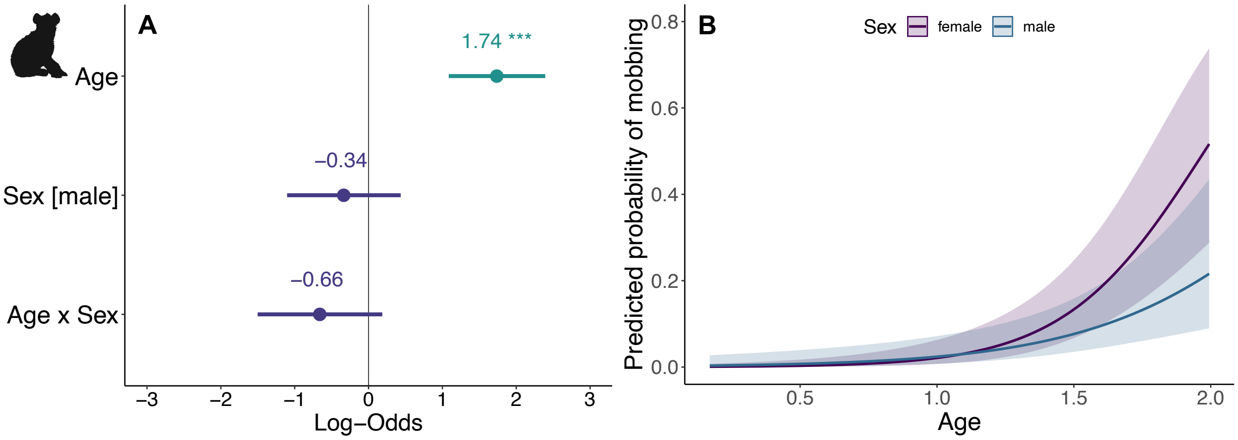


Figure 3.10. Top model of the predicted probability of mobbing participation by adult female focal hyenas (Model J: n-focal hyenas = 1140; n-sessions = 81; n-mobs = 274; n-unique hyenas = 100). We restricted our analysis to mobs where at least one juvenile was present and to adult female focal hyenas with at least one offspring alive in the clan on the session date. **A.** Dots depict coefficient estimates, lines depict 95% confidence intervals, and asterisks depict significance at the following p-values: * = 0.05; ** = 0.01; *** = 0.001. **B.** Dots depict estimated marginal means and vertical lines depict 95% confidence intervals.

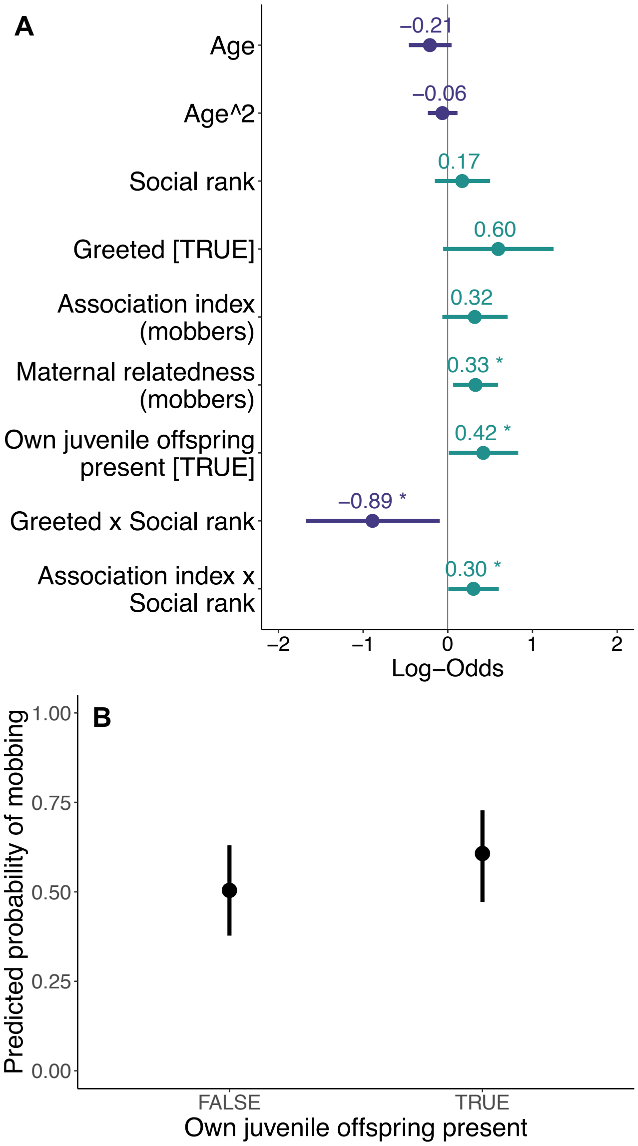


Table 3.5. Categorization of carcass size based on prey species and prey age.

Prey species	Carcass size	
	Age adult	**Age juvenile**
Buffalo (*Syncerus caffer*), Elephant (*Loxodonta africana*), Giraffe (*Giraffa camelopardalis*), Hippopotamus (*Hippopotamus amphibius*)	XL	XL
Domestic cow (*Bos taurus*), Hartebeest (*Alcelaphus buselaphus*), Topi (*Damaliscus lunatus*), Wildebeest (*Connochaetes gnou*), Zebra (*Equus quagga*)	L	M
Domestic goat (*Capra aegagrus*), Domestic sheep (*Ovis aries*), Grant's gazelle (*Nanger granti*), Impala (*Aepyceros melampus*), Thompson's gazelle (*Eudorcas thomsonii*), Warthog (*Phacochoerus africanus*)	M	S

Table 3.6. Generalized linear models used to estimate BLUPs for fecal glucocorticoid metabolites and fecal testosterone metabolites in female and male hyenas.

Predictors	**Estimates**	**SE**	**p**
Fecal glucocorticoids (log) for female hyenas (n-samples = 1544, n-hyena = 239)			
Hyena age at defecation	0.08	0.04	0.062
Hyena social rank at defecation	0.06	0.04	0.102
Hyena reproductive state at defecation [nulliparous]	-0.18	0.10	0.069
Hyena reproductive state at defecation [pregnant]	0.73	0.10	**<0.001**
Hyena reproductive state at defecation [lactating]	0.33	0.09	**<0.001**
Hyena reproductive state at defecation [other]	0.42	0.12	**<0.001**
Time of day of defecation [PM]	-0.44	0.05	**<0.001**
Hormone assay by [JRG-UM]	-0.10	0.09	0.283
Hormone assay by [JRG-UNO]	0.06	0.09	0.502
Hormone assay by [PVM]	0.52	0.11	**<0.001**
Hormone assay by [TMM]	0.01	0.10	0.956
Time between defecation and assay	0.05	0.03	0.121
ICC = 0.18			
Marginal R^2 / Conditional R^2 = 0.141 / 0.298			
Fecal testosterone (log) for female hyenas (n-samples = 1471, n-hyena = 235)			
Hyena age at defecation	0.14	0.03	**<0.001**
Hyena social rank at defecation	0.13	0.03	**<0.001**
Hyena reproductive state at defecation [nulliparous]	-0.07	0.08	0.416
Hyena reproductive state at defecation [pregnant]	0.90	0.09	**<0.001**
Hyena reproductive state at defecation [lactating]	0.33	0.07	**<0.001**
Hyena reproductive state at defecation [other]	0.57	0.10	**<0.001**
Time of day of defecation [PM]	-0.15	0.04	**<0.001**
Hormone assay by [ESP]	-0.04	0.06	0.549
Hormone assay by [TL]	-0.65	0.07	**<0.001**
Time between defecation and assay	0.04	0.04	0.241
ICC = 0.11			
Marginal R^2 / Conditional R^2 = 0.242 / 0.322			

Table 3.6. (cont'd)

Predictors	**Estimates**	**SE**	**p**
Fecal glucocorticoids (log) for male hyenas (n-samples = 907, n-hyena = 231)			
Hyena age at defecation	0.13	0.05	**0.010**
Hyena social rank at defecation	-0.10	0.05	**0.039**
Time of day of defecation [PM]	-0.52	0.07	**<0.001**
Hormone assay by [JRG-UM]	0.06	0.12	0.647
Hormone assay by [JRG-UNO]	0.27	0.11	**0.015**
Hormone assay by [PVM]	0.66	0.15	**<0.001**
Hormone assay by [TMM]	-0.22	0.12	0.068
Time between defecation and assay	0.23	0.05	**<0.001**
ICC = 0.13			
Marginal R^2 / Conditional R^2 = 0.146 / 0.257			
Fecal testosterone (log) for male hyenas (n-samples = 848, n-hyena = 219)			
Hyena age at defecation	0.18	0.04	**<0.001**
Hyena social rank at defecation	-0.01	0.04	0.834
Time of day of defecation [PM]	-0.08	0.06	0.173
Hormone assay by [ESP]	-0.21	0.09	**0.022**
Hormone assay by [TL]	-0.69	0.10	**<0.001**
Time between defecation and assay	0.15	0.05	**0.002**
ICC = 0.07			
Marginal R^2 / Conditional R^2 = 0.103 / 0.162			

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