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Plains zebra (*Equus quagga*) adrenocortical activity increases during times of large aggregations in the Serengeti ecosystem

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ABSTRACT

Adverse environmental stimuli (stressors) activate the hypothalamic-pituitary-adrenal axis and contribute to allostatic load. This study investigates the contribution of environmental stressors and life history stage to allostatic load in a migratory population of plains zebras (*Equus quagga*) in the Serengeti ecosystem, in Tanzania, which experiences large local variations in aggregation. We expected higher fGCM response to the environmental stressors of feeding competition, predation pressure and unpredictable social relationships in larger than in smaller aggregations, and in animals at energetically costly life history stages. As the study was conducted during the 2016 El Niño, we did not expect food quality of forage or a lack of water to strongly affect fGCM responses in the dry season. We measured fecal glucocorticoid metabolite (fGCM) concentrations using an enzyme immunoassay (EIA) targeting 11β-hydroxyetiocholanolone and validated its reliability in captive plains zebras. Our results revealed significantly higher fGCM concentrations 1) in large aggregations than in smaller groupings, and 2) in band stallions than in bachelor males. Concentrations of fGCM were not significantly higher in females at the energetically costly life stage of late pregnancy/lactation. The higher allostatic load of stallions associated with females, than bachelor males is likely caused by social stressors. In conclusion, migratory zebras have elevated allostatic loads in large aggregations that probably result from their combined responses to increased feeding competition, predation pressure and various social stressors. Further research is required to disentangle the contribution of these stressors to allostatic load in migratory populations.

1. Introduction

In vertebrates, the hypothalamic-pituitary-adrenal axis is the neuroendocrine pathway by which glucocorticoids are released from the adrenal cortex in response to adverse environmental stimuli termed stressors (Creel et al., 2013; Hofer and East, 2012; Nelson et al., 2002). Glucocorticoids influence the expression of genes and gene pathways with diverse functions including those that mobilize energy required for behavioral and physiological responses to stressors (Phuc Le et al., 2005; Tung et al., 2012). The term allostatic load encompasses the cumulative energetic cost of maintaining homeostasis, fulfilling the physiological costs of daily life that vary with life history stage (Stearns, 1992), and costs of responding to environmental stressors (McEwen and Wingfield, 2003; Romero et al., 2009). Glucocorticoid (GC) concentrations are considered a useful indicator of allostatic load (Goymann and Wingfield, 2004; McEwen and Wingfield, 2003) and a proxy measure of the contribution of environmental stressors to allostatic load (e.g. Benhaiem et al., 2013; Clinchy et al., 2013; Nelson et al., 2002; Rubenstein and Shen, 2009). Environmental stressors known to elevate GC concentrations include conditions when competition for food is high or food abundance is low or unpredictable (Chinnadurai et al., 2009; Goymann et al., 2001; Jeanniard du Dot et al., 2009; Kitaysky et al., 2010), when predation pressure is high (Bao et al., 2017; Creel et al., 2009; Sheriff et al., 2015), when social environments are challenging (Creel et al., 2013; Hennessy et al., 2009; Hofer and East, 2012), unstable or unpredictable (Bassett and Buchanan-Smith, 2007; Benhaiem et al., 2012, 2013; Goymann et al., 2001; Tung et al., 2012) and when males compete for receptive females (Mooring et al., 2006; Pavitt et al., 2015).

In vertebrates the energetic costs of different life history stages vary

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and transitions between life history stages are often marked by glucocorticoid mediated metabolic changes (Crespi et al., 2013; McEwen and Wingfield, 2003). In mammals, lactation is the most energetically costly component of reproduction for females (Martin, 1984). In large herbivores that produce relatively large precocial offspring, late gestation can also be an energetically costly life stage (Fisher et al., 2002).

In wildlife, measurement of circulating GC usually requires interventions (capture, restraint and blood sampling) which can elevate GC, thereby affecting GC measurements (Kersey and Dehnhard, 2014). Also, pulsatile, episodic or diurnal GC secretion may affect measures of serum glucocorticoid levels (Aurich et al., 2015; Kersey and Dehnhard, 2014; Schmidt et al., 2010). Quantification of fecal glucocorticoid metabolites (fGCM) dispenses with the need for interventions and provides a cumulative fGCM concentration produced during the time of gut passage (Ganswindt et al., 2012; Kersey and Dehnhard, 2014; Schwarzenberger, 2007). Although GC responses to stressors are commonly measured in terms of fGCM concentrations (Goymann, 2012; Graham and Brown, 1996), species specific differences in steroid metabolism requires that an enzyme immune assay (EIA) must be validated for the study species to which it is applied (Touma and Palme, 2005). This typically involves an experimental adrenocorticotropic hormone (ACTH) challenge conducted on individuals of the target species in captivity (Bashaw et al., 2016; Benhaiem et al., 2012).

Most plains zebra (*Equus quagga*) (hereafter termed zebra) in the Serengeti ecosystem, in East Africa (~200,000 animals; IUCN, 2017) undertake a biennial migration in response to seasonal patterns of precipitation (Maddock, 1979; McNaughton, 1985). At the start of the wet season (in approximately November), zebra move from their dry season range in the north and west of the ecosystem through a transitional area to the short grass plains in the south-east of the ecosystem (Maddock, 1979), where they graze on vegetation containing essential minerals for pregnancy and lactation. As these minerals only occur in a few 'hotspots' elsewhere (McNaughton, 1990) they must be acquired in specific areas even though energy requirements can be fulfilled in many areas (MacNaughton and Banyikwa, 1995). When the wet season ends in May or early June, zebras leave the arid south-east and return to the north and west (Norton-Griffiths et al., 1975) which receives a higher annual rainfall than the south-east, and contains sources of surface water in the dry season (Kingdon, 1979). These migratory movements result in substantial local variation in the abundance and dispersion of zebra (Maddock, 1979; McNaughton, 1985). The dominant grass species and vegetation community on the short grass plains differs from those in the long grassland and woodland communities (MacNaughton and Banyikwa, 1995).

Plains zebras form stable groups termed family bands that contain one stallion and one to several unrelated mares and their subadult offspring. Stable social relationships and strong cohesion between females provides the core structure to zebra family bands and the socially dominant female in a band typically determines the direction of band movement (Klingel, 1969a,b). Agonistic interactions can occur between the female members of one family band and those in another band (Klingel, 1969b). Band stallions rarely actively defend mature female band members whereas both stallions and bachelors do compete to recruit adolescent mares in estrus (Klingel, 1969b). Females use body posture and olfactory cues to advertise when they are in estrus (Klingel, 1969b). Males leave their natal band when between one and four years old (hereafter termed bachelor males) and join bachelor groups that have stable membership and stable within group social relationships (Klingel, 1969b; Rubenstein, 1986). The stability of bachelor groups is unique among equids (Linklater, 2000; Ransom and Kaczensky, 2016). It is thought that bachelor males cannot successfully compete for access to receptive females before they are about 4 years old (Klingel, 2010, 2013). Females usually leave their natal family band during their first estrus when approximately 13 months old and few conceive before they are over two years old (Klingel, 1969a). In the Serengeti ecosystem there is a marked birth peak in the wet season which typically occurs on the short grass plains, although foals are born throughout the year. Pregnancy lasts 360–396 days, and lactation 7–11 months (Wackernagel, 1965), with postpartum estrus occurring approximately one week after birth (Klingel, 1969a).

In several mammalian species, GC concentrations are elevated when population densities are high or are experimentally increased (Boonstra and Boag, 1992; Caslini et al., 2016; Forristal et al., 2012; He et al., 2014; Rogovin et al., 2003). Agonistic interactions are typically elevated when competition for resources, such as food or mates is increased at higher population densities. Furthermore, for animals living in stable groups, the coalescence of groups into large aggregations will lead to a decline in the predictability of the social environment. Disentangling the effects of these environmental stressors on GC concentrations is problematic. In the Serengeti ecosystem, the migratory movements of zebra cause large fluctuations in the number of zebra in different locations (Maddock, 1979; McNaughton, 1985) but little is known how aggregation affects GC concentrations in migratory zebras.

This study assesses the impact of life history stage and environmental stressors on the allostatic load of migratory zebra. Energetically costly life history stages should increase allostatic load hence we expect higher fGCM levels in females during late pregnancy and lactation than in mature females in other life stages. As stallions aim to prevent estrus females in their band from mating with rival males (either bachelor males or other band stallions) and they also compete for estrus females in other family bands, we predict higher fGCM concentrations in family band stallions than in bachelors. We also consider the impact of three environmental stressors (feeding competition, predation pressure and an unpredictable social environment) on fGCM concentrations. We expect the highest fGCM response to these three stressors to occur in the largest category of zebra aggregations. More specifically we expect: 1) feeding competition to increase with aggregation size in adult animals; 2) predation pressure to increase when numerous predators, particularly 'commuting' spotted hyenas (*Crocuta crocuta*) are attracted to large aggregations of prey (Hofer and East, 1993, 1995). More specifically we expected predation pressure to be most pronounced in large aggregations in all age and sex categories, and for predation pressure to be similar in medium and small aggregations; 3) the negative effect of unpredictable social relationships to increase as the size of zebra aggregations increase. Strong social bonds between mature band females, mothers and their offspring, and males within bachelor groups should to some extent buffer zebra against this social stressor, at least in small and medium aggregations.

2. Materials and methods

2.1. Study site and sample collection

This study was conducted in the Serengeti NP, Tanzania, during the months of January–March, May–July and October 2016. In 2016, an El Niño climatic event resulted in above average rainfall in the Serengeti NP and an extension of rain into the dry season.

Zebras were sampled in two distinct habitat categories: the short grass plains in the south, and long grass, open woodland savannah in the center of the park (henceforth termed woodland boundary). Fecal samples were collected opportunistically, together with information on age, sex and reproductive state for sampled individuals, habitat type (short grass plains or woodland boundary) and the aggregation of zebras in the area. Zebra abundance within approximately 500 m in all directions of the research vehicle was estimated and categorized within three easily determined aggregation categories: small (≤ 50 zebras), medium (> 50 and ≤ 200 zebras), and large (> 200 zebras). In total 270 fecal samples were collected (in large $N = 124$, medium $N = 67$, small $N = 79$ aggregations of zebras). Following Klingel (1969b) we scored the age of animals in three categories: adult, subadult and foal using withers height and pelage (see Fig. 1). Reproductive state in adult females was visually assessed in three categories (Fig. 1): (i) lactating -