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Monitoring stress in captive and free-ranging African wild dogs (*Lycaon pictus*) using faecal glucocorticoid metabolites

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ABSTRACT

An understanding of stress physiology is important for species management because high levels of stress can hamper reproduction and affect an individual's ability to cope with threats to their survival, such as disease and human–wildlife conflict. A commonly used indicator of stress, faecal concentrations of cortisol metabolites (FCM), can be used to assess the impact of social, biological and environmental factors. Measurements of FCM are particularly valuable for endangered species that are logistically challenging to study and where non-invasive techniques are preferred. As the second most endangered canid in Africa, the African wild dog (*Lycaon pictus*) has been the focus of considerable conservation research, yet there is still little understanding of factors associated with stress, in either captive or free-ranging populations. The present study therefore aimed to determine whether stress levels differ between captive and free-ranging populations, and to detect social, biological and environmental factors that are stressful in these populations. Faecal samples were collected from 20 captive and 62 free-ranging animals. Within free-ranging populations, the sexes differed significantly, but there was no effect of social status, age or breeding period for either sex. Captive females had higher FCM concentrations than free-ranging females. In captive populations, FCM concentrations differed among zoos and with reproductive status in females, but were not related to age class or group-housing structure. In conclusion, FCM is a useful indicator of stress and should be considered an integrative aspect of management, for both *in situ* and *ex situ* African wild dog populations.

1. Introduction

An individual's response to stressful factors has important ramifications for their ability to survive and reproduce. An acute stress response leading to a release of glucocorticoids (GC) does not normally affect the health status of an individual and may even be beneficial, provided the individual can rapidly regain homeostasis (Asa, 2010; Keay et al., 2006; Moberg, 1985). On the other hand, the well-known adverse effects of stress on reproductive health (Matteri et al., 2000; Moberg, 1985; Rivier and Rivest, 1991; Wingfield and Sapolsky, 2003) are associated with long-term production of GC (Ladewig, 2000; Moberg, 2000; Tilbrook et al., 2000). The outcomes include disruption of ovulation and expression of sexual behaviour, inhibition of embryo implantation and reductions in sex hormone production (Moberg, 1985; Rivier and Rivest, 1991; Wingfield and Sapolsky, 2003). These negative consequences for reproduction can be critical for individuals and for populations, so it is important to detect and mitigate highly stressful social, environmental or biological factors, particularly in the management of endangered species (Millspaugh and Washburn, 2004).

The management of endangered species varies considerably between captive and free-ranging populations because it is difficult to mimic the natural environmental and social conditions for *ex situ* populations. Thus, when examining GC patterns in these populations, it is essential to consider different potential causes of stress. For example, captive individuals face no threat from predators and are not exposed to the daily stress of finding food and shelter, but instead may experience negative social interactions due to confined space and lack of cover. In a study of captive clouded leopards (*Neofelis nebulosa*), where reproductive success in captivity has been poor, Wielebnowski et al. (2002) observed increased GC concentrations in individuals with small enclosure heights and limited keeper contact. Alternatively, anthropogenic factors, such as human activity from roads and villages, could be a source of stress in free-ranging but not captive populations

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(Bhattacharjee et al., 2015; Creel et al., 2002; Van Meter et al., 2009). It is difficult to determine whether stressors in captivity are more severe than those in a free-ranging environment, particularly as some individuals may cope with stress better than others (Ladewig, 2000; Millspaugh and Washburn, 2004; Sapolsky, 1994), and repeated exposure may diminish stress responses through habituation (Ladewig, 2000). However, in studies with cheetah (*Acinonyx jubatus*), Canada lynx (*Lynx canadensis*), spider monkeys (*Ateles geoffroyi*) and sparrows (*Zonotrichia spp.*), GC concentrations were higher in captive than in free-ranging individuals (Fanson et al., 2011; Marra et al., 1995; Rangel-Negrin et al., 2009; Terio et al., 2004). If the level of stress is indeed higher in captive animals than in free-ranging animals, then it might help explain poor reproductive success in captivity in some species (Carlstead and Shepherdson, 1994).

African wild dogs (*Lycaon pictus*) are highly social, cooperative breeders. Reproduction is typically restricted to the dominant pair who maintain their dominance through displays of aggression associated with submission by subordinates (Creel et al., 1997a; Frame et al., 1979). In previous studies, dominant individuals were found to have higher faecal GC concentrations than subordinates throughout the year (Creel et al., 1997a), an observation that was initially counter-intuitive and led to the suggestion that GCs are not mediators of reproductive suppression (Barja et al., 2008; Creel, 2005). High GC values in dominants might have been explained by aggression, but Creel (2005) considered this unlikely and concluded that aggression alone could not explain the persistence of high concentrations throughout the year because most aggression is confined to the mating period. Therefore, although aggression is likely to induce an acute stress response, adrenal activity is still poorly understood in African wild dogs. Most other studies of GC patterns in this species have been limited to the effects of immobilisation, handling and translocation (Comizzoli et al., 2009; Creel et al., 1997b; de Villiers et al., 1995, 1997), so further investigations into potential biological causes of chronic stress in both captive and free-ranging populations are still needed.

In the present study, we chose to measure GC non-invasively in collected faeces because this approach would minimise our potential effect on stress levels. We focused on the metabolites that have previously been measured in African wild dogs (de Villiers et al., 1995, 1997; Monfort et al., 1998; Santymire and Armstrong, 2009) and that are commonly used to asses stress (Touma and Palme, 2005). We hypothesised that faecal concentrations of GC metabolites in wild dogs would (a) be higher in captive than in free-ranging animals, as found in previous studies, (b) vary with conditions of captivity; and (c) be affected by age and reproductive status, in both captive and free-ranging animals.

2. Methods

2.1. Animals and sample collection

Faecal samples were collected from captive female wild dogs in five zoos in either 2008 or 2009 in Europe (Artis Zoo, Port Lympne Wild Animal Park, West Midland Safari and Leisure Park and Zoo Duisburg) during the reproductive season (June–November) and Australia (Perth Zoo) at the end of the reproductive season (June). No samples were collected from males because too few animals were available during the study period. The collection protocol used in European zoos has been described previously (Van der Weyde et al., 2015) and a similar protocol was followed in Australia. For free-ranging animals, faecal samples were collected opportunistically from up to eight packs in Hluhluwe-iMfolozi Park (South Africa), during the reproductive season (February–July) in 2010 and 2011. At least one individual per pack was VHF-radio-collared to allow regular tracking of the pack when they were in vehicle-accessible areas. Most packs ranged in areas within the park that included roads and human activity, and only a couple of packs ranged in areas with limited vehicle access. These packs have been followed intensively for several years, so all individuals could be identified and their ages to the nearest month were known (range 0.6–10 y). Packs were followed from a vehicle and samples from identified individuals were collected within 15 min of defecation and stored for 0.5–4 h on ice until frozen. For each sample, the time of day, delay to freezing, date, individual ID and pack ID were recorded. A total of 531 samples were used for analysis, comprising 297 from captive females ($n = 20$), 96 from free-ranging females ($n = 18$) and 138 from free-ranging males ($n = 44$).

2.2. Faecal extractions

The extraction method for samples from captive animals has been described previously (Van der Weyde et al., 2015). Logistical issues made transporting frozen samples from free-ranging populations difficult, so samples were first oven dried over low heat in the field and then transported to Australia before extraction. In brief: 0.25 g wet sample was weighed into a plastic tube, vortexed with 4 mL 100% methanol for 20 min, and then centrifuged for 20 min (4 °C, 3000*g*). The supernatant was decanted into clean glass tubes, dried under air, capped and stored at −20 °C until assay.

2.3. Cortisol radioimmunoassay

Cortisol was assayed using a GammaCoat Cortisol ^{125}I kit (DiaSorin, North Ryde, Australia). Major cross-reactions were: cortisol (100%), prednisolone (25.5%), 6-methylprednisolone (14.5%), cortisone (10.3%), 11-deoxycortisol (9.8%), 6β-hydrocortisone (4.2%) and prednisone (3.8%); all other cross-reactions were below 1%. We followed kit instructions except that we doubled the volume of standards and extracts and added three extra standards (1.25, 2.5 and 5 ng/mL) below the minimum provided by the kit using kit products. Extracted samples were reconstituted with 1 mL 100% methanol, sonicated for 20 s and vortexed briefly before being diluted 1:2–1:5 in phosphate buffer. Three samples were diluted at several volumes to demonstrate parallelism with the standard curve. Sensitivity was <1.25 ng/mL and the limit of detection ranged from 3.9–17.9 ng/g. The intra-assay coefficient of variation was 14 ± 3.9% ($n = 9$) and the inter-assay coefficient of variation was 13.2%, 11.5% and 26.2% for high, medium and low concentrations, respectively. Cortisol is not present in wild dog faeces (Monfort et al., 1998) so results here are considered to represent faecal cortisol metabolite (FCM) concentrations (Palme, 2005).

2.4. Data analysis

Statistical analyses were aided by SPSS version 20.0 (SPSS Inc., IBM, Chicago, IL, USA). Linear mixed models (LMM) using

Table 1
Factors tested in each of the analyses using linear mixed models.

Analysis	Population differences
1	*Population*
2	Factors affecting free-ranging wild dogs
	Sex
	Social status + Age (y) (Females and Males separately)
	*Social status + Breeding period + Social status * Breeding period (Females and Males separately)*
3	Factors affecting captive wild dogs
	Reproductive status + Age class + Zoo + Group housing