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Hormonal and behavioural stress responses to capture and radio-collar fitting in free-ranging pampas deer (Ozotoceros bezoarticus)

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Abstract

*This study evaluated the short-term hormonal and behavioural responses to capture and radio-collar fitting in free-ranging pampas deer (*Ozotoceros bezoarticus*). Twenty adult deer (eleven females and nine males) were captured in the South Pantanal wetland (Brazil) and equipped with VHF radio-collars (marked deer). Untreated adult deer of the same sex were randomly chosen as the control group (nine females and nine males). On the day following capture, an observer followed all deer for faecal collection and behaviour evaluation. Faecal samples were immediately refrigerated and frozen at –20°C within a maximum of 12 h. Faecal glucocorticoid metabolites (FGM) were measured using an 11-oxoaetiocholanolone enzyme immunoassay. A qualitative behaviour assessment and the consequences of capture were evaluated using pre-defined terminologies and scores. Flight distance was recorded using a range finder. FGM increased from 19–22 h after capture onwards and peak concentrations were five times (median) higher as the respective baseline values. FGM values of marked deer were significantly higher at 22–25 and 25-28 h compared with controls. Marked male but not female deer had significantly higher FGM values at 22–25 and 25–28 h compared with their baseline values. Marked deer were significantly more fearful, less sociable and defensive than controls. The absences of significant increases of FGM in the captured female deer may indicate that females are less prone to capture stress. The significantly more fearful, and less sociable and defensive patterns observed in marked deer may be relevant during capture of lactating females or in areas with high predator pressure.*

Keywords: *animal welfare, enzyme immunoassay, glucocorticoids, pampas deer, qualitative behaviour assessment, Southern Pantanal wetland*

Introduction

The capture and marking of free-ranging wildlife, including deer, can induce significant stress reactions (DeNicola & Swihart 1997). The challenge is to perform such procedures with minimal risk of injury or death to the animals. Evaluations of how the stress responses affect post-capture physiology and behaviour are also important (DelGiudice *et al* 2005; Morellet *et al* 2009).

Cervids demonstrate extreme physiological alteration during handling (Gasparinni *et al* 1997). Common problems related to physical or chemical restraint include trauma, hyper- or hypothermia (Boesch *et al* 2011), acid-base disturbances, hypoventilation, hypoxaemia (Caulkett 1997; Murray *et al* 2000; Read *et al* 2001; Read 2003) and capture myopathy (Conner *et al* 1987; Beringer *et al* 1996; Paterson 2007). Capture of cervids is typically conducted for blood sampling, morphological measurements and/or fitting of ear-tags and radio-collars (see DelGiudice *et al* 2005). The advantages of unconsciousness and immobility have stimulated studies regarding useful drug combinations to improve chemical restraint since 1953 (see Seal & Bush 1987). Most studies regarding capture focus on the efficacy of the capture technique or the effect of chemical restraint protocols. There is little or no information available concerning the consequences of post-capture stress on deer behaviour and hormonal responses, particularly in the Neotropical species.

Stress hormone assessment can be a useful tool in free-ranging deer to evaluate post-capture stress as it quantifies

changes in animal physiology. Non-invasive methods of measuring faecal glucocorticoid metabolites (FGM) have advantages over invasive methods (eg blood or saliva sample), because samples can be easily obtained, without additional stress and typically reflect changes in endocrine profile from a short-term, day-to-day or even long-term stress stimulus (Touma & Palme 2005; Sheriff *et al* 2011). Short-term stress is associated with an activation of the sympathetic nervous system, and the hypothalamic-pituitary-adrenal (HPA) axis, resulting in the secretion of catecholamines and glucocorticoids (Möstl & Palme 2002). When the stressor persists, the chronic stimulation of glucocorticoid secretion can lead to immune system suppression and disruption of reproduction (Dickens *et al* 2010). In general, the short-term stress responses have been described as beneficial for the animal, because they help an animal to cope with an acute stressor, such as the approach of a predator (Möstl & Palme 2002). Capture and restraint of an animal facilitates study of the maximal glucocorticoid response to an acute stressor as well as the maximal fight-or-flight response (Dickens *et al* 2010), which may result in distress (Moberg 1987), when the animal incurs a biological cost so large that it needs to divert resources away from normal functions to cope with this stress factor (threat). Capture myopathy, caused by overexertion of the muscles resulting in metabolic acidosis and secondary muscular necrosis, is an example of this situation, and has been stated as a major cause of mortality during capture of different species of mammal (Paterson 2007) including deer (Conner *et al* 1987; Beringer *et al* 1996).

Since individuals may differ in their capacity to cope with environmental challenges or may not even be able to cope in situations with unpredictable stressors (Koolhaas *et al* 1999), behavioural data may also improve information about how free-ranging deer cope with the capture and/or radio-collar-fitting stress and help to identify patterns of stress responses (eg sex differences).

In wild deer, just one study evaluated deer spatial behaviour during the 50 days after capture and radio-collar fitting. In this study, roe deer (*Capreolus capreolus*) males seemed to be more sensitive than females, showing a greater displacement of their home range ten days after release compared to their normal range. The authors suggested that this apparent sex difference in post-capture ranging behaviour may therefore be linked to a difference in social environment, with females exhibiting social ties with their fawns, and potentially with offspring from previous years, forming maternal clans and males exhibiting seasonal territorial behaviour (Morellet *et al* 2009). In a study that provides information about hierarchical rank, nature and frequency of social interactions, grazing activity and presence at the trough of captive red deer (*Cervus elaphus*) hinds a few days after radio-collar fitting, the authors observed a reduction (by, respectively, 40 and 32%) of grazing activity and presence at the trough compared to the time before the collar was fitted (Blanc & Brelurut 1997). The other studies usually focus on medium- or long-term post-capture stress (eg DelGiudice *et al* 2005; Konjévic *et al* 2011).

Pampas deer (*Ozotoceros bezoarticus*) are distributed from central Argentina to mid-western and north-eastern Brazil, eastern Bolivia, Paraguay and Uruguay (Cabrera 1943, 1960). This species prefers open habitats such as savannah, pampas, seasonal wetlands, and open grasslands (Merino *et al* 1997). In Brazil, two subspecies can be found: *O. bezoarticus bezoarticus* and *O. bezoarticus leucogaster* (Rodrigues *et al* 2007). The largest populations occur in the Emas National Park (Rodrigues 2003) and the Pantanal wetlands, respectively (Mourão *et al* 2000; Tomás *et al* 2001, 2011). The pampas deer is considered vulnerable according the Brazilian list of endangered species (Duarte *et al* 2012) and near threatened according the IUCN list (González & Merino 2008). This classification status can be attributed to habitat loss related to agricultural expansion, poaching, and diseases transmitted by cattle (Merino *et al* 1997). However, around 60,000 individuals (Mourão *et al* 2000) in high densities (Tomás *et al* 2001) can still be found in the Brazilian Pantanal.

It is often assumed that capture and radio-collar fitting have an insignificant long-term effect on animals (Moll *et al* 2008); however, the effects of capture on individual behaviour or short-term hormonal responses are rarely assessed in free-ranging animals, due to difficulties in following unmarked control animals (Côté *et al* 1998). To date, no study has evaluated the short-term effects of capture and radio-collar fitting correlating the hormonal and behavioural responses of wild cervids with those of free-ranging deer that are not captured. The evaluation of those short-term responses to stressors could help researchers interested in animal welfare to develop better strategies for capture and handling deer. It could also help understand how the monitored animal could change their normal and typical behaviour as a consequence of capture to minimise possible mistakes in interpretation of ecological data collected. Thus, the aim of this study was to evaluate short-term hormonal and behavioural stress responses to capture and radio-collar fitting in pampas deer (*O. bezoarticus leucogaster*) during the day following capture.

Materials and methods

This study was part of an anaesthesia experiment that was approved by the Animal Ethics and Welfare Committee (CEBEA) of the Faculty of Agrarian and Veterinary Sciences (FCAV-UNESP), Jaboticabal, Brazil (protocol number: 015077/09) and was granted a license (number: 21491-1) from the Brazilian Institute for Biodiversity Conservation (ICMBio).

Study area

The Pantanal is a seasonal floodplain located close to the geographic centre of South America at approximately 100 m elevation. It is drained to the west by the tributaries of the Paraguay River, which then flow southwards along the western border of the Pantanal (Mourão *et al* 2000). The study area covers the Nhumirim (a research station of Embrapa Pantanal) and Alegria ranch; both located in a region known as Nhecolândia (18° 59' 15"S; 56° 37' 03"W) in the South Pantanal wetland, Mato Grosso do Sul

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state, Brazil. The Nhecolândia has a rainy period during the summer (November to March) and the dry period lasts mostly from April to October. Floods occur from December to May in the study area, covering most of the open grasslands and seasonal freshwater ponds. During the dry periods, all that persist are some perennial rivers, remnant polls, creeks and lakes, and wildlife concentrate at these water bodies. The experiment was conducted during June and July of 2010. The mean (± SD) temperature, humidity and precipitation registered in the study area during this period were 21.0 (± 4.0)°C, 81.2 (± 9.2)% and 0.1 (± 0.9) mm, respectively.

Capture and handling

Twenty adult pampas deer were randomly captured and chemically restrained using two different anaesthesia protocols. Tiletamine-zolazepam (Zoletil 50, Virbac, Jururbatuba, Sao Paulo, Brazil)/xylazine (Sedimin, Könin, Buenos Aires, DC, Argentina; TZ/X; 2.5/1.0 mg kg^{-1}, intramuscular [IM]) was administered to six females and five males and ketamine (Vetaset, Ford Dodge Saúde Animal Ltda, Campinas, Sao Paulo, Brazil)/midazolam (Dormonid, Roche, Rio de Janeiro, Brazil)/xylazine (K/M/X; 6.0/0.45/0.3 mg kg^{-1}; IM) combinations administered to five females and four males. In the TZ/X group, three female and three male deer received intranasal oxygen supplementation at a flow rate of 3 L min^{-1}. In the K/M/X group, two female and two male deer received oxygen at the same flow rate. The drug doses were calculated for an estimated weight of 30 kg. After anaesthesia, xylazine was antagonised with 0.2 mg kg^{-1} of yohimbine hydrochloride (0.1 mg kg^{-1} IM and 0.1 mg kg^{-1} intravenous [IV]; Sigma Aldrich, São Paulo, Brazil). Yohimbine dose was calculated based on the measured bodyweight. Recovery time was recorded as the time between yohimbine administration and the deer being in a stable and upright position.

The capture consisted of approaching the deer, using a vehicle or on foot, performing circling manoeuvers/movements around the deer with the aim of progressively decreasing the distance between the darter and deer. When a distance of 15 to 25 m was achieved, the anaesthetic drugs were administered by dart, via a remote drug delivery system (Dist-Inject, Model 50, Basel, Switzerland). All deer were captured in the afternoon of the experimental days and equipped with VHF radio-collars (TXE-335C, Telenax, Playa Del Carmen, México). Deer were also ear-tagged to identify the individuals in case of problems with the radio-collars. Deer matching the treated individuals (sex) were chosen as the control group. On the day following capture and marking, the same observer followed the radio-collared deer ('marked group') and the control deer during daylight hours for faecal collection and behavioural recording.

Sample collection

Faecal samples were collected from the rectum of marked deer during anaesthesia (baseline; 0 h). On the day following capture, after the marked deer had been tracked via telemetry, the observer approached the deer by foot for collection of fresh faeces. Fresh faeces were also collected from the control group deer. The samples were stored immediately in plastic bags, identified and refrigerated in a thermic bag (2 L capacity, Bond Street, Rio Negro, Brazil) with three rigid ice packs (250 ml each) until freezing at –20°C (within a maximum of 12 h after collection). For statistical analysis, results of faecal samples were grouped according to the time of collection after capture (0, 16–19, 19–22, 22–25 and 25–28 h).

Storage experiment

To evaluate the stability of FGM in thermic bags and at ambient temperature, a storage experiment was conducted, similar to that described by Rehnus *et al* (2009). Four samples were used. These samples were collected at different days through the experimental period, from two randomly chosen unmarked males and females. Each sample was homogenised and divided into eleven subsamples and stored inside cryogenic tubes. A subsample was immediately frozen at –196°C, using liquid nitrogen (0 h), while the ten other subsamples were stored in thermic bags at ambient temperature for 1, 2, 4, 8 and 16 h, respectively, and then frozen at –196°C for 24 h and afterwards stored at –20°C until analysis.

FGM analysis

Faecal samples were lyophilised (Model LGA05, Web LMW Medizintechnik, Leipizig, Germany) for 24 h. Dried faeces were then pulverised and 0.5 g of the resulting powder weighed and extracted with 5 ml of methanol (80%; Palme *et al* 2013). After hand vortexing (30 s), the mixture was shaken for 12 h at room temperature, and vortexed again (30 s) before centrifugation (1,075 g; 15 min). An aliquot of supernatant of the extracts (0.5 ml) was evaporated to a dry state and shipped to the University of Veterinary Medicine, Vienna, Austria. Extracts were reconstituted in 0.4 ml of methanol (100%), followed by vortexing (1 min) and the addition of 0.1 ml of distilled water, prior to analysis. Levels of FGM were determined in the extracts (diluted 1:10 and 1:100 with assay buffer) by enzyme immunoassay (EIA).

Initially, two 11-oxoaetiocholanolone EIAs, developed for ruminants (Palme & Möstl 1997; Möstl *et al* 2002), were tested in faecal samples collected during anaesthesia (0 h) and after capture, from six marked deer (three males and three females), in order to biologically validate the method (Touma & Palme 2005). The EIA determining FGM with an 11,17-dioxoandrostane structure was chosen because higher amounts of immunoreactive FGM in the faeces were detected and a more pronounced increase after capture was

found with this EIA. All samples were analysed by this EIA only. The general procedures and cross-reactions of the chosen 11-oxoaetiocholanolone EIA are described in detail by Palme and Möstl (1997). The intra-assay coefficients of variation of the EIA were < 10% and the inter-assay coefficients (n = 17) were 9.6 and 13.8% for the high and low concentration pool samples, respectively. The detection limit of the assay was 4.8 ng g^{-1} faeces.

Behavioural observations

The behavioural observations began as soon as the collared and control deer were located on the day following marking and tagging. When deer were found alone they were described as solitary. In case deer were together with conspecifics, the group was described by the number of deer per different sex (females/males) and age classes (adult/juvenile). Distinctions were made by the same experienced observer based on the visual evaluation of the body size and antler development.

A qualitative behaviour assessment (QBA), similar to one used to assess animal welfare (Napolitano *et al* 2007, 2012; Stockman *et al* 2011), was performed in the mid-morning period after allowing the animals to adapt to the presence of the observer for at least 15 min. The QBA consisted of a rating form with pre-defined terminology (calm, indifferent, suspicious, alert, aggressive, fearful, sociable and defensive) and visual analogue scales of 12.5 cm (ranged from 'minimum' to 'maximum' with an uncategorised continuous line between these points) added to each term. The same trained observer recorded the pre-determined terms by ticking the scale at the appropriate point. This score was recorded as the measure of the distance in centimeters between the left 'minimum' point and the point where the observer's tick crossed the line. Two other quantitative terms were also evaluated, using the same scale mentioned for the QBA qualitative test, to evaluate foraging behaviour and the deer reactivity to the observer. The latter evaluates the avoidance of the observer, with deer not always fleeing during observation, but keeping walking as far as possible from the observer (more reactive). After the QBA test, flight distance was recorded using a laser rangefinder. The deer was calmly approached, from an initial distance of 100 m. When the deer fled, the flight distance was recorded.

The short-term consequences of capture for radio-collared deer were also evaluated by the observer at the end of the day following capture using the scores: 1) Good (deer without signs of lameness, ataxia or rejection by other deer); 2) Satisfactory (deer with slight lameness or ataxia); and 3) Bad (deer with severe ataxia and/or lameness and/or suffering rejection behaviour by other deer). These scores were considered the 'capture consequences scores'.

Statistical analysis

As FGM data were not normally distributed, logarithmic transformation (observation +1) was performed to normalise original data. Data were analysed by repeated measures ANOVA using a split-plot design, with the groups (marked and control), sexes (female and male), protocols of anaesthesia (TZ/X and K/M/X) or intranasal oxygen supplementation (with or without) as the principal source of variation in the whole plot and the time-frames (0, 16–19, 19–22, 22–25 and 25–28 h) and the interaction between groups or sexes and time-frames as the second source of variation in the subplot.

This analysis was followed by a *post hoc* Tukey test. In order to evaluate a possible link between the intensity of the HPA axis activation and the deer behaviour post-capture, we correlated the highest percentage (above baseline) of FGM increases in marked deer and the FGM mean values for control deer to behaviour data using the Spearman correlation test. The statistical analyses of FGM were performed using SAS 9.0 (SAS Institute Inc, Cary, NC, USA) and values of $P < 0.05$ were considered significant.

The QBA data from marked and control deer was analysed using Principal Component Analysis (PCA; Brscic *et al* 2009) to indicate which terms of QBA were most closely related. The PCA is usually used in QBA data to identify how different terms work together to better understand the studied group and also prepare the data for further analysis using other techniques. The values obtained on the visual analogue scale for each term of QBA were multiplied by their respective Euclidean distance obtained from PCA (using the first and second components) and followed by a Student's *t*-tests analysis. This analysis aimed to verify if the most closely related terms of QBA differed significantly between the groups. The reactivity to observer, foraging behaviour and flight distance were also analysed using Student's *t*-test. Possible differences between marked deer sexes for the capture consequences scores were analysed by the non-parametric Kruskal-Wallis test. The PCA analysis was performed using Statistica 7.0 (StatSoft Inc, Tulsa, Ok, USA). Values of $P < 0.05$ were considered significant.

Results

On the day following capture, one female anaesthetised with K/M/X without intranasal oxygen supplementation did not defaecate and was removed from analysis. Two additional marked females could not be followed during the whole light period on the day following capture due to technical difficulties with the telemetry receiver, but it was possible to collect one faecal sample during the first visualisation of these deer. Thus, it was possible to collect faeces from ten females and nine males (marked deer). The control group consisted of nine females and nine males. The mean (± SD) recovery time was 46 (± 35) min: (54 [± 47] min for deer anaesthetised with K/M/X and 41 [± 26] min for deer anaesthetised with TZ/X).

FGM increased significantly ($P < 0.01$) from 19–22 h after capture (0 h) onwards in marked deer and peak concentrations were 2 to 17 times (median: 5) higher than the baseline. FGM values of marked deer were significantly higher ($P < 0.01$) at 22–25 and 25–28 h when compared with the values of control deer at the respective time-frames (Figure 1). Marked male deer also had significantly higher FGM values at those intervals compared with their baseline values ($P < 0.01$). However, in marked female deer the

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Figure 1

Boxplots of FGM concentrations of free-ranging pampas deer (*Ozotoceros bezoarticus*), radio-collared after capture (marked group) or undisturbed (control group). Values are medians and quartiles. Sample size/time-frames after capture from marked (grey boxes) and control (open boxes) groups were 19/7/13/13/9 and 4/14/13/6, respectively. * Indicates significant differences ($P < 0.01$; Tukey test) between marked and control groups.

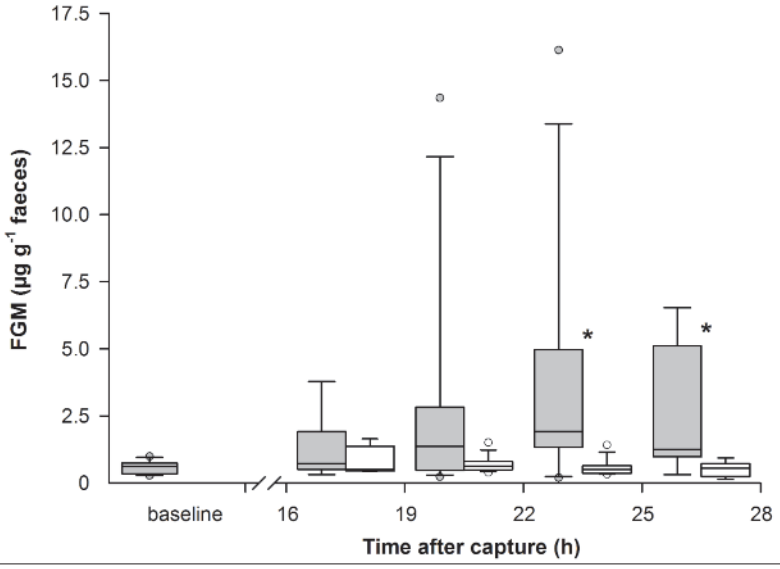
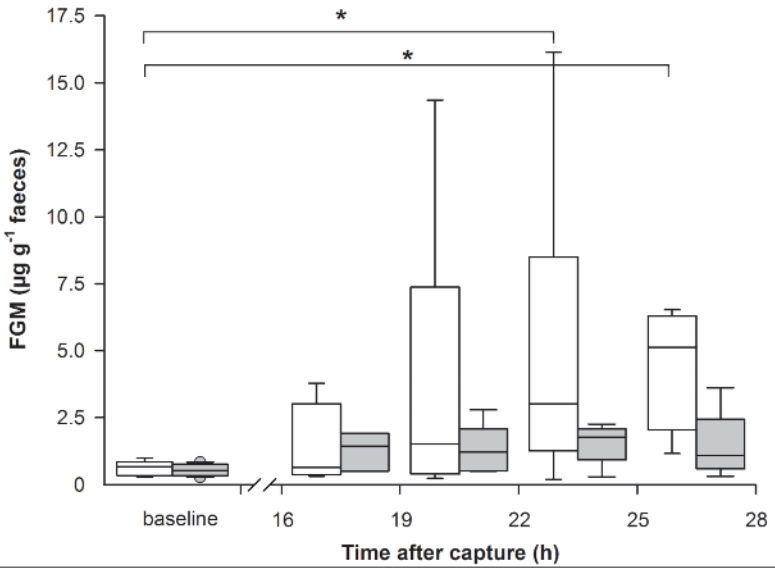


Figure 2

Boxplots of FGM concentrations after capture in both sexes of free-ranging pampas deer (*Ozotoceros bezoarticus*). Values are medians and quartiles. Sample size/time-frames after capture from male (open boxes) and female (grey boxes) deer were 9/4/8/8/4 and 10/3/5/5/5, respectively. * Indicates significantly higher FGM values ($P < 0.01$; Tukey test) in marked males at 22–25 and 25–28 h compared with their baseline values.



FGM values were not significantly different from the baseline (Figure 2). No significant differences were found in FGM values between deer anaesthetised with TZ/X or K/M/X, or between deer that were supplemented with oxygen and those that were not. The FGM concentrations did not vary significantly due to storage of faeces in the thermic bags (7.1 [± 5.6]°C) or at ambient temperature (21.3 [± 7.6]°C) for up to 16 h.

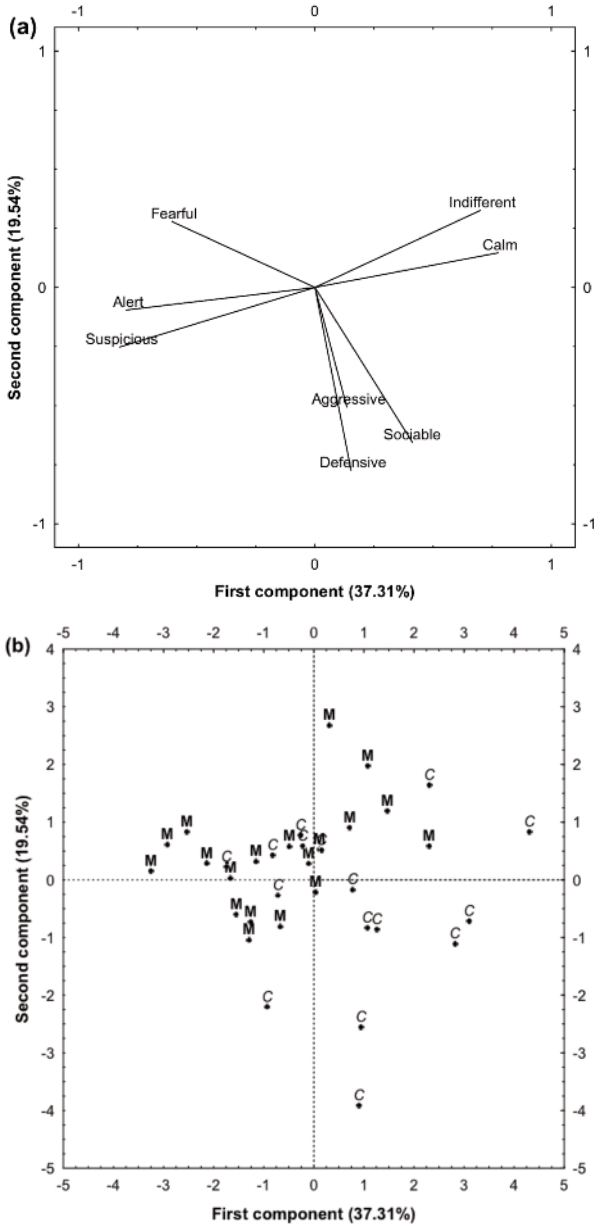
No significant differences for flight distances were found between marked (36.1 [± 23.6] m) and control deer (28.7 [± 14.8] m). However, flight distance of females (n = 9; 47.0 [± 24.1] m) was significant higher ($F = 1.73$; $P = 0.04$) than males (n = 9; 21.8 [± 21.4] m) in the marked group. Significant differences between marked and control deer or between sexes for reactivity were not observed. In marked deer the highest percentage of FGM increases demonstrated a negative correlation ($P = 0.01$; $r = –0.65$) with the reactivity to the observer.

The PCA analysis revealed two principal components (explaining 37.3 and 19.5% of total variation). The first component demonstrates a high negative correlation with the categories suspicious, alert and fearful and a high positive correlation with the categories calm, indifferent and sociable (Table 1, Figure 3). When the values obtained from the visual analogue scale of QBA were multiplied by the Euclidean distances of the terms of QBA obtained from PCA and then analysed by the Student's *t*-test, marked deer were significantly more fearful ($P = 0.02$), less sociable ($P = 0.02$) and defensive ($P = 0.04$) than control deer.

Table 1 Terms showing the highest positive and negative correlation with the factors 1 and 2 of PCA.

Terms	1st component	2nd component	3rd component	4th component	5th component
Calm	0.78	0.15	0.32	–0.25	–0.30
Indifferent	0.70	0.33	–0.41	0.06	–0.22
Suspicious	–0.83	–0.25	0.00	–0.06	–0.13
Alert	–0.80	–0.10	0.15	–0.46	–0.12
Aggressive	0.13	–0.51	0.50	0.66	–0.08
Fearful	–0.60	0.28	–0.49	0.47	–0.16
Sociable	0.41	–0.66	–0.38	–0.11	0.42
Defensive	0.15	–0.77	–0.31	–0.11	–0.46

Figure 3



Results of PCA analysis showing the variation in the first and second principle component with (a) the plot of terms and (b) the plot of cases whereby M = marked and C = control deer.

In the marked group, one male died on the post-capture day due to snakebite and another was euthanised three days after capture due to clinical signs of capture myopathy (which was confirmed by post mortem tests). Three males, including the snakebite victim, and one female limped and had severe ataxia after capture. These animals received score 3 in the capture consequences scores. All these deer were anaesthetised with K/M/X and no significant differences in the capture consequences scores were found between sexes.

The two marked males that died did not exhibit foraging behaviour after capture and consequently were removed from the foraging behaviour analysis. Foraging behaviour was not significantly different between marked and control deer. Female marked deer demonstrated significantly (Student's *t*-test; $P = 0.04$) less foraging behaviour than male deer on the day following capture.

Discussion

The current study aimed to evaluate short-term hormonal and behavioural responses to capture and radio-collar fitting in free-ranging pampas deer. Captured pampas deer had significantly higher FGM levels 22–25 and 25–28 h after capture compared with control deer. In males but not females, FGM concentrations were significantly higher than baseline levels during these intervals. The behavioural analysis demonstrated that females had a greater flight distance and less foraging behaviour than males (marked group) and that captured deer were significantly more fearful, less sociable and defensive than control deer. FGM increases in marked deer only correlated with reactivity to the observer.

For reliable monitoring of adrenocortical activity using FGM analyses it is important to validate the techniques used (Touma & Palme 2005; Sheriff *et al* 2011). An 11-oxoaetiocholanolone EIA (Palme & Möstl 1997) was chosen for FGM analysis. It detected biologically meaningful changes in FGM levels, resulting in peak concentrations five times (2 to 17 times) higher than the respective baseline in deer that experienced a relevant stressor (marked deer). A comparable increase (2.9 to 4.3 fold) was observed in fallow deer (*Dama dama L*) (Konjević *et al* 2011) 18 and 22 h after an adrenocorticotropic hormone

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(ACTH) challenge test using the same EIA as in the current study and this 11-oxoaetiocholanolone EIA has proven useful for measuring adrenocortical activity in several other ruminant species (Palme 2012). Stressors such as transport (Dehnhard *et al* 2001), human disturbance (Huber *et al* 2003b), husbandry systems (Christofoletti *et al* 2010), introduction to captivity conditions (Pereira *et al* 2006) and physical restraint in a chute system (Moll *et al* 2008) have also been described as strong enough stimuli for biological validation of FGM measurement in different deer species.

The absence of significant differences in FGM levels between female and male marked deer at baseline or between sexes in control deer suggest that sex differences in FGM excretion do not exist in pampas deer. Similar results were reported for red deer (Huber *et al* 2003a,b), brown brocket deer (*Mazama gouazoubira*) and marsh deer (*Blastocerus dichotomus*; Christofoletti *et al* 2010). However, in the current study, FGM concentration in females after capture and radio-collar fitting did not vary significantly from baseline in contrast to male marked deer. This result may reflect that: (i) in females peak concentration samples might have been missed; or (ii) female pampas deer were less prone to the mentioned stressors.

These females demonstrated significantly less foraging behaviour on the day following capture compared to males. Lack of food ingestion in females may have decreased defaecation during the light period on the day following capture (only 18 samples could be collected compared to 28 in male marked deer) and, consequently, could have increased delay times of excreted FGM. It was not possible to collect samples from all defaecations on the day following capture, as some deer did not permit an appropriate approach of the observer. This may have resulted in some faeces being missed, especially in female marked deer, which had longer flight distances than males. Even with the lower number of samples collected from females, an increase in FGM levels was observed; however, it was lower than in males (2 to 9 vs 3 to 17 times higher than respective baselines). The small sample size per time interval may account for the lack of discriminating power between sexes, as high individual variation in the FGM concentrations was observed.

The majority of studies evaluating FGM levels following stressor stimuli in cervids were conducted in males only (Dehnhard *et al* 2001; Pereira *et al* 2006) or do not mention sex differences in FGM increases after stress (Moll *et al* 2008). In brown brocket deer and marsh deer, sex-related differences in FGM levels following a change in husbandry system were not observed (Christofoletti *et al* 2010); however, only three males and three females per species were used and high individual variations may have masked sex differences in FGM increases. In a study in red deer hinds (Huber *et al* 2003b), chemically immobilised deer had, on average, a 774% lesser increase in FGM levels following ACTH than deer that were not immobilised, indicating that anaesthesia might interfere with FGM excretion.

Differences in coping styles have been reported to correlate with FGM levels in different species (Stöwe *et al* 2010), and two stress response patterns, proactive with lower activation of HPA axis and reactive with higher activation of HPA axis, can be distinguished (Koolhaas *et al* 1999). We observed that FGM increases in marked deer were negatively correlated with reactivity to the observer on the day following capture. This may be related to a reactive coping style described by Koolhaas *et al* (1999), where animals that exhibit a freezing behaviour in stressful situations typically demonstrate a pronounced HPA axis response after capture. We would also have expected that FGM increases in marked deer would correlate with flight distances registered on the day following capture, as described by Pereira *et al* (2006), but this was not observed. A possible explanation for these differences may be the small number of samples collected on the post-capture (n = 46) day from marked deer compared to the mentioned study (n = 186), which may increase the effect of individual variation on FGM analysis. It has already been suggested that female deer could be less sensitive to the capture and handling process than male deer, exhibiting lower displacement of their home range after ten days of capture and GPS radio-collar fitting than males (Morellet *et al* 2009). However, the spatial behaviour data in Morellet *et al* (2009) were not associated with the hormonal or visual behaviour data. The significantly longer flight distance observed in females when compared to males in the marked group and the observation that only marked males demonstrated significant increases in FGM concentrations (compared to baseline) in the present study also reinforces the theory that female pampas deer respond in a different manner to stressors, exhibiting proactive behaviour with longer flight distances and lower FGM increases. This proactive behaviour, which is also in agreement with a negative correlation observed between FGM increases and reactivity to the observer in marked deer, may also result in a reduced foraging behaviour of female marked deer, as they spent more time avoiding the presence of the observer than grazing. The significant lower foraging behaviour in female deer after capture and radio-collar fitting was previously reported in captive deer (Blanc & Brelurut 1997). However, the mentioned study did not include males for comparison.

The QBA test proved useful in evaluating differences between marked and control deer and it can infer behaviour by utilising objective visual analogue scales. The significantly more fearful behaviour, and less sociable and defensive patterns observed in marked deer may be relevant in captures that involve lactating females or females accompanied by juvenile fawns. Côté *et al* (1998) described a decline in mountain goat (*Oreamnos americanus*) kid-survival due to increased abandonment by mothers following anaesthesia. Diminished defensive behaviour after capture in marked deer may also be a concern in areas with high predator pressure: a study that evaluated the post-capture mortality in white-tailed deer (*Odocoileus virginianus*; DelGiudice *et al* 2005) described that 37.5% of deer killed by wolves within a 14-day period post-capture were

killed on the day following capture (DelGiudice *et al* 2005), indicating a high predation risk due to capture and handling.

The negative consequences of capture included limping and severe ataxia in 20% of the marked deer, which also might predispose animals to predation or late mortality due to capture myopathy as observed in white-tailed deer (Beringer *et al* 1996; DelGiudice *et al* 2005). The K/M/X combination used did not result in longer recovery times compared with TZ/X; however it did result in longer induction times (18.4 [± 12.4] min) when compared with TZ/X combination (11.7 [± 6.8] min) in pampas deer (unpublished data) and due to the possible extra-muscular activity during anaesthesia induction can be related with the high incidence of post-anaesthetic ataxia observed (Paterson 2007), excluding the snakebite deer. Half of the observed mortality rate (10%) was attributed to capture myopathy based on the necropsy and histological findings and is comparable to a rate of 4.9% observed in a study that evaluated deer mortality after anaesthesia without trap methods of capture (Duarte 2008). The snake incident that killed the other marked deer, occurred at maximum 12 h after anaesthesia recovery and, thus, was probably caused by that deer being less alert during this period. However, an accident bearing no relationship with the capture event cannot be excluded.

Animal welfare implications

Behaviour, foraging activity, survival and FGM were affected on the day following capture in pampas deer; thus, it is recommended to monitor the post-capture period carefully. The significantly more fearful, less sociable and defensive behaviour and the severe limping observed in marked deer after capture may be relevant during capture of lactating females or in areas with high predator pressure.

Conclusion

In conclusion, the FGM analysis revealed a possible difference in stress responses between female and male pampas deer. Females appeared to be less prone to capture and radio-collar-fitting stress than males. Further investigations, with greater numbers of animals, are necessary to achieve appropriate statistical power, and confirm these findings while further examining possible mechanisms for the observed sex differences.

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