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Physiological response of wild guanacos to capture for live shearing

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Abstract

Context. The use of wild guanacos (*Lama guanicoe*) through live capture and shearing may contribute to their conservation by providing an economic alternative to rural inhabitants. However, none of the biological impacts of this activity, including the physiological ones, have been addressed.

Aims. The aim of this work was to characterise the acute response of guanacos to stress after capture and shearing in terms of serum cortisol levels, heart rate, and body temperature.

Methods. The study was performed during 2006 and 2007 in La Payunia Reserve in western Argentina. In order to determine serum cortisol concentration, 128 blood samples were obtained and the unextracted sera were analysed by radioimmunoanalyses (RIA). Sex, age category, heart rate, body temperature and total restraint time (TRT) were also registered for each animal captured.

Key results. Serum cortisol levels were higher in guanacos captured and sheared during 2007 than in 2006, and male cortisol levels were consistently lower than female levels. No significant differences were observed in cortisol levels of the different age categories. A positive correlation was observed between TRT and serum cortisol concentration. The analyses of cortisol levels in relation to TRT showed differences between males and females. With handling periods longer than 80 minutes, females showed a delayed stabilisation in cortisol response when compared with males. Heart rate and body temperature showed no differences between year, sex or age categories.

Conclusions. The present results show that the stress response to capture and shearing in wild guanacos increased significantly with handling time. We recommend avoiding capture of large numbers of animals and keeping roundup duration short to reduce TRT.

Implications. This work provides new information that can improve guanaco welfare during handling and shearing and may have implications for the conservation of the species.

Additional keywords: animal welfare, body temperature, cortisol, heart rate, *Lama guanicoe*, South American camelids, sustainable use.

Introduction

The guanaco (*Lama guanicoe*) is the only native large herbivore with a wide distribution in South America (Franklin *et al*. 1997). This species shows a complex social structure that includes family groups, groups of males, and single males in their organisation (Franklin and Fritz 1991). Females exhibit induced ovulation and a gestation period between 10 and 11 months, after which a single offspring is born (Ruscitti 1994). In western Argentina, the study

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area, lambing is concentrated during the beginning of summer (December and January) (Ruscitti 1994).

Although still abundant in some areas, guanaco populations have been fragmented and total population size has declined from around 30 million to half a million individuals during the past century (Raedeke 1979; Franklin and Fritz 1991). Competition with sheep, hunting, and habitat degradation due to overgrazing have resulted in reduced guanaco densities, local extirpation, and restriction to marginal, low-quality habitats (Baldi *et al*. 2001, 2004). At present, ~95% of wild guanacos live in Argentina, primarily in the Patagonia region (Baldi *et al*. 2010). Today, sheep ranching is the main economic activity in Argentinean Patagonia and guanaco poaching is a common practice. Nevertheless, during the past decade, several sheep ranchers have begun to capture and shear wild guanacos to obtain high quality wool for export. Argentinean management policies for guanacos establish that only the wool taken from live animals is allowed to be sold (Baldi *et al*. 2006, 2010). This activity, if developed on the basis of knowledge of guanaco biology, could contribute to conservation of this species and rural development of the region. Although live capture and shearing could be a tool for guanaco conservation (Baldi *et al*. 2010), the biological impacts of capture and shearing of wild guanacos have not been addressed.

The handling of wild guanacos involves physical restraint for shearing, which can result in 'capture myopathy' syndrome (Williams and Thorne 1996), one of the main adverse consequences of stress in wild animals (Montané *et al*. 2003). Capture myopathy decreases the flow of oxygen and nutrients in the blood to the tissues, which increases the production of lactic acid, and causes extensive necrosis of skeletal muscle tissue. Also, the myoglobin released from dead muscle cells can cause fatal renal failure (Rogers *et al*. 2004). Shearing could also predispose guanacos to hypothermia because it affects their thermoregulatory mechanisms (de Lamo *et al*. 1998).

The measurement of stress related parameters is important for determining the least stressful capture and handling methods to reduce mortality after management and improve the wellbeing of wild species (Morton *et al*. 1995). Our objective was to characterise the acute response of wild guanacos to capture and shearing stress in terms of serum cortisol, heart rate, and body temperature.

Materials and methods

Study area

The Payunia Provincial Reserve covers approximately 4500 km^2 of which 2000 are state-owned lands and is located in the south of the province of Mendoza in Argentina (between 36°00′S and 36°36′S and 68°34′W and 69°23′W; Fig. 1). The climate is typically continental desert with average temperatures of 6°C in winter and 20°C in summer, and 255 mm of average annual precipitation. The semiarid biome dominates this region and is included in La Payunia Phytogeographic Province within the

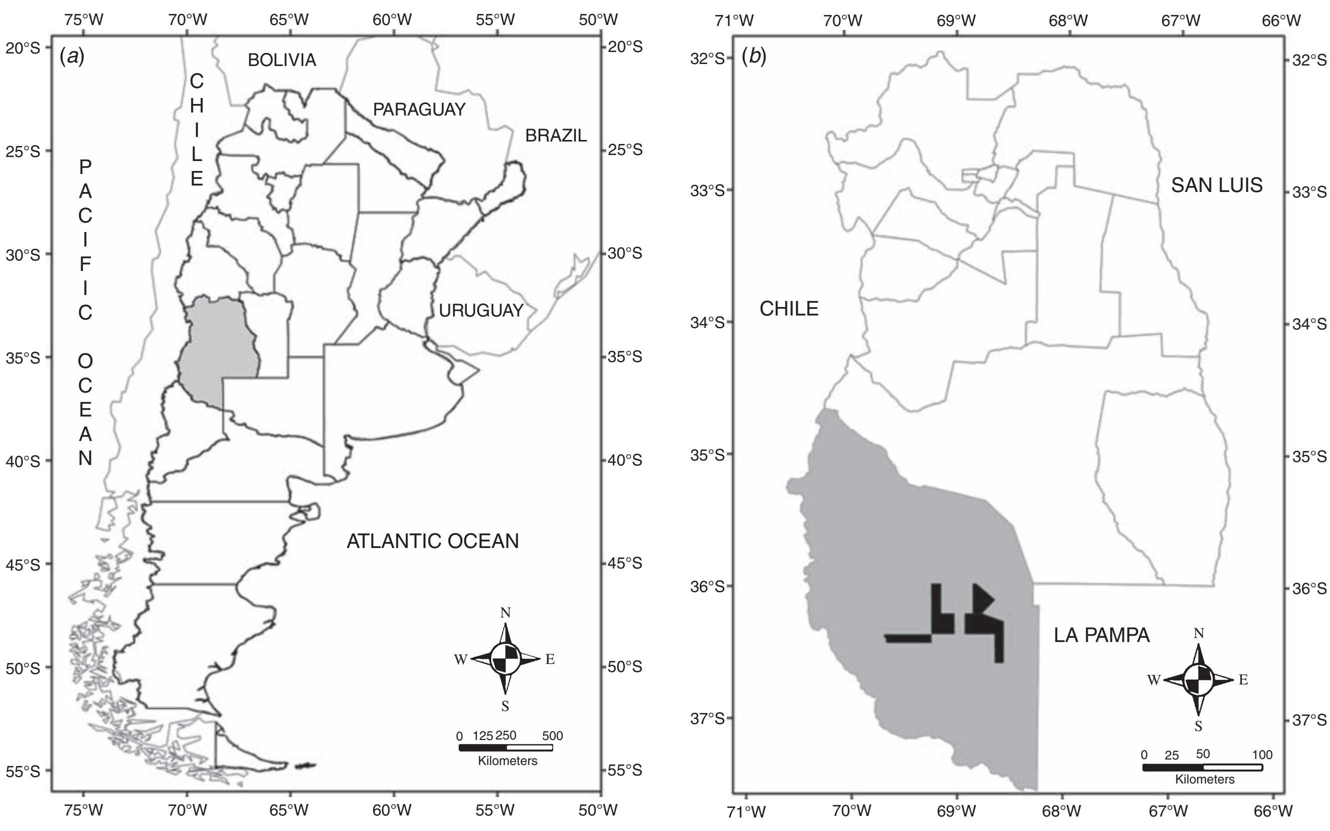

Fig. 1. (*a*) Location of the study site within Mendoza Province, Argentina. (*b*) Localisation of the state-owned lands in Payunia Provincial Reserve (in black) and Malargüe Department (in grey) within Mendoza Province.

Andean–Patagonian domain (Martínez Carretero 2004). La Payunia Reserve has suffered severe land deterioration due to overgrazing. In this region, human populations are scarce and goat breeding is the basis of the subsistence economy. Nevertheless, this area supports the most numerous migratory guanaco population in the central region of Argentina, with more than 10 000 individuals (Candia *et al*. 1993). In 2005, some inhabitants of this reserve began managing wild guanacos under strict standards of animal welfare (Marull and Carmanchahi 2008).

Capture and handling of wild guanacos

Wild guanacos were captured by horseback riders driving them towards a corral trap (Montes *et al*. 2006). This corral trap had two V-shaped arms, one pre-capture corral, a capture corral, three successive corrals, a holding pen and a shearing corral (Fig. 2). The arms were ~3500 m long and their distal opening was ~1500 m. To avoid escapes, the whole capture structure had a 2.5-m-high fence and the last pen was also enclosed with shade nets. The three successive corrals were lined with carpet to prevent the guanacos seeing outside the corral. The animals were removed from the holding pen, immobilised by binding the legs, then placed on a gurney and taken to the shearing machines. Shearing was carried out using an electric machine fed by a generator. The shorn individuals were sampled as described below, identified with numbered and coloured (according to sex) collars, and then released.

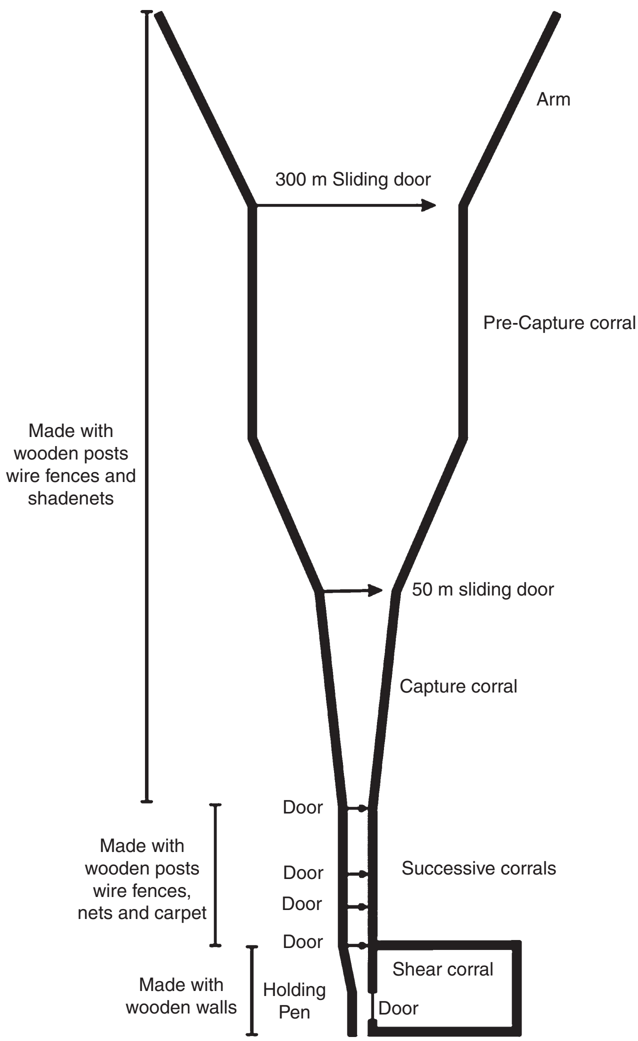

Fig. 2. Schematic design of the capture structure employed for the management of wild guanacos in La Payunia reserve.

The animals were handled according to the 'Good Practices of Wild Guanacos Handling Protocol' (Marull and Carmanchahi 2008). This protocol provides basic guidelines for the handling of guanacos in the interests of animal wellbeing, and considering that guanacos are wild animals not habituated to human contact.

Study animals

In total, 255 free-ranging guanacos were captured during September and October 2005–07, approximately 3 months before the period of parturition (Ruscitti 1994). One hundred and twenty-eight blood samples were taken. Sex and age categories of each individual are shown in Table 1. In 2005, only heart frequency and body temperature data were taken.

In order to determine the effect of handling time on cortisol levels, the total restraint time (TRT) of the animals was measured and was considered as the duration of roundup plus handling (capture, shearing, sample extraction, and liberation).

Blood sample collection and hormone analyses

Approximately 10 mL of blood was extracted from the femoral vein of each guanaco using a 10 mL syringe. Once obtained, the blood was allowed to clot at room temperature and centrifuged at 3300*g* for 20 min using a 12 V portable centrifuge, and serum samples were immediately frozen for analysis in the laboratory. The glucocorticoid concentration was measured in unextracted sera with a commercial ^{125}I-Cortisol radioimmunoassay kit (Cortisol RIA DSL-2000, Diagnostic Systems Laboratories, Inc., Webster, TX). To validate the method for guanaco serum, total steroids were extracted twice from 1 mL of selected serum samples with 5 mL diethyl ether. The organic phases were pooled, evaporated and resuspended in 1 mL of PBS (phosphate-buffered saline, pH 7.4). Initially, serial dilutions of extracted samples were run in parallel with the unextracted samples from the same animal in the same assay. As no significant differences were observed between extracted and unextracted samples, no extraction was performed afterwards. According to the manufacturer's report, the assay has a sensitivity of 0.01 ng mL^{-1} and the cortisol antiserum presents a cross-reactivity of 33.3% with prednisolone, 9.3% with corticosterone, 3.8% with 11-deoxycortisol, 2.2% with cortisone, 1.4% with prednisone and less than 1% with 17α-hydroxyprogesterone,

Table 1. Number of guanaco samples by year, sex and age categories collected in this study

Year	Males	Females	Yearlings	Juveniles	Adults	Total
2005	14	0	2	0	12	14
2006	36	15	26	3	17	51
2007	47	15	18	8	41	62

11-deoxycorticosterone, dexamethasone, testosterone, progesterone, epiandrosterone, dehydroepiandrosterone and oestradiol. The mean inter- and intra-assay coefficients of variation were 6.8% and 3.5% respectively.

In situ recording of physiological parameters

Heart rate and body temperature were recorded immediately after each captured guanaco was sheared, using a stethoscope and a lubricated rectal thermometer, respectively.

Statistical analyses

Factorial analysis of variance was used to compare cortisol levels among years, ages, sexes and management actions. The temperature and heart rate responses were analysed using correlation analysis. Linear and maximum likelihood models were built in R-program (version 2.9.0, www.R-project.org) to compare the effects of all variables, choosing the best model using Akaike's information criterion. The data were analysed using R-program and Statistica 7.0 (www.statsoft.com).

Results

Serum cortisol concentrations were higher in guanacos captured and sheared during 2007 than in 2006 ($F_{1,111} = 151.96$, $P < 0.0001$, Table 2). Likewise, there were significant differences between sexes ($F_{1,122} = 13.55$, $P < 0.001$), when each year was considered separately (2006: $F_{1,47} = 4.21$, $P = 0.05$; 2007: $F_{1,58} = 6.93$, $P = 0.01$ Table 2) the levels in males were consistently lower than in females. However, no significant differences were observed in the cortisol levels of the different age categories (2006: $F_{3,43} = 0.59$, $P = 0.63$; 2007: $F_{3,57} = 0.38$, $P = 0.77$).

As the total restraint time of the animals was significantly higher in 2007 (84.85 ± 2.13 min) than in 2006 (48.64 ± 0.33 min), an additional analysis was carried out to determine the factor responsible for the differences in cortisol levels between the two years.

A positive correlation was observed between restraint time and serum cortisol levels (multiple $r^2 = 0.57$, adjusted $r^2 = 0.56$; $F_{4,123} = 40.69$; $P < 2.2 \times 10^{-16}$; Table 3). Nevertheless, when all data were analysed together, circulating cortisol values tended to plateau after 80 min restraint time, despite the persistence of the stressor agent (restraint) (Fig. 3).

The analyses of cortisol levels for each sex in relation to restraint time showed a difference in response between males and females. For the first 80 min of handling, the values were similar for males and females, staying on an average of 11.6 ± 5.7 ng mL^{-1}, $n = 40$. After 80 min, females showed delayed stabilisation in the cortisol response when compared with males (AIC-males = 299.7, d.f. = 1, dAIC = 221.6, weight < 0.001; AIC-females = 78.1, d.f. = 1, dAIC = 0.0, weight = 1).

The different age categories did not differ in the effect that restraint time had on cortisol concentration (GLM, $F_{3,107} = 0.51$, $P = 0.67$).

Table 2. Guanaco cortisol levels by sex and age categories

	Cortisol ng mL^{-1} ± s.e. (n) 2006	2007
Overall	13.4 ± 1.0 (51)	36.6 ± 1.4 (63)
Males (all ages)	12.6 ± 1.2 (37)	34.1 ± 1.6 (48)
Females (all ages)	16.9 ± 1.8 (15)	44.7 ± 2.4 (15)
Yearlings	13.8 ± 1.5 (26)	37.7 ± 1.8 (19)
Young	16.7 ± 3.4 (8)	32.9 ± 4.3 (3)
Adult males	12.7 ± 1.2 (18)	36.4 ± 2.0 (41)
Adult females	12.4 ± 0.1 (3)	46.0 ± 3.0 (10)

Management effect on heart rate and body temperature

No significant differences were found in heart rate between years ($F_1 = 1.57$; $P = 0.23$), sexes ($F_{1,44} = 0.97$; $P = 0.53$), ages ($F_{3,88} = 0.86$; $P = 0.15$; Table 4), or the combination of sex and age categories ($F_{5,88} = 0.90$; $P = 0.32$).

Similarly, there were no differences in body temperature between years ($F_1 = 0.39$; $P = 0.53$), sexes ($F_{1,126} = 0.88$; $P = 0.58$), ages ($F_{3,126} = 0.94$; $P = 0.78$; Table 5), or sexes for

Table 3. Comparison between different models used to determine the effect of guanaco handling time, sex and age on serum cortisol levels

Model/functions	AIC	d.f.	dAIC	Weight
	Rational			
Michaelis–Menten	408.8	3	29.7	<0.001
Michaelis–Menten with scaling	379.1[A]	4	0.0	0.5
	Exponential-Based			
Monomolecular	398.6	3	19.5	<0.001
	Polynomials			
Linear	394.3	3	15.1	<0.001
No linear	379.1[A]	4	0.0	0.5

[A]Best goodness of fit.

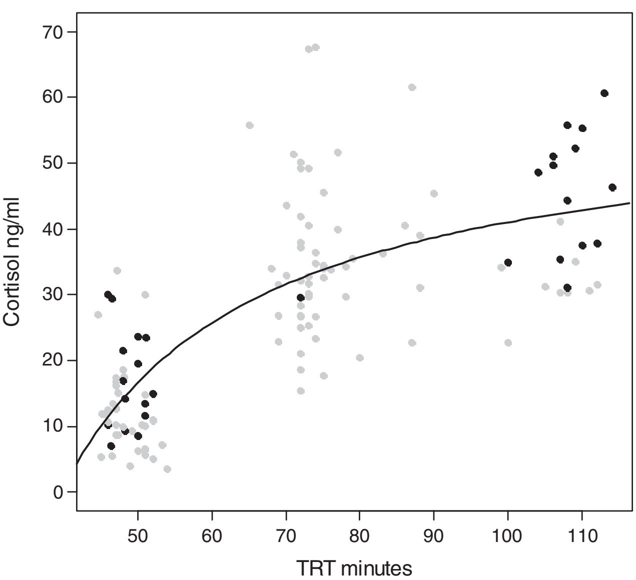

Fig. 3. Correlation between total restraint time (TRT) in guanacos and cortisol concentration in their blood. Black circles: females; grey circles: males.

Table 4. Heart rate of guanacos during handling

Categories	Heart rate Beats per min^{-1} ± s.e. (n) 2005	2006	2007
Males	80.36 ± 7.24 (14)	75.50 ± 6.22 (36)	82.41 ± 2.92 (47)
Females		77.31 ± 6.85 (15)	84.28 ± 6.30 (15)
Yearlings	67.00 ± 3.72 (2)	75.62 ± 7.58 (26)	82.78 ± 5.15 (18)
Young		77.30 ± 12.55 (8)	80.58 ± 8.34 (3)
Adults	82.58 ± 7.82 (12)	75.86 ± 6.83 (17)	82.41 ± 3.03 (41)
Mean	80.36 ± 7.24 (14)	75.63 ± 3.71 (51)	82.41 ± 2.41 (62)

Table 5. Guanaco body temperature during handling

Categories	Rectal temperature °C ± s.e. (n) 2005	2006	2007
Males	38.99 ± 0.22 (14)	39.66 ± 0.56 (36)	39.41 ± 0.09 (47)
Females		39.96 ± 0.44 (15)	39.15 ± 0.14 (15)
Yearlings	38.35 ± 0.51 (2)	39.56 ± 0.75 (26)	39.46 ± 0.17 (18)
Young		40.43 ± 1.56 (8)	39.72 ± 0.35 (3)
Adults	39.10 ± 0.23 (12)	39.89 ± 2.09 (17)	39.41 ± 0.10 (41)
Mean	38.99 ± 0.22 (13)	39.64 ± 0.32 (51)	39.42 ± 0.08 (62)

age categories ($F_{2,126} = 0.97$; $P = 0.89$). Also, there were no significant correlations between cortisol levels and heart rate ($r^2 = 0.02$; $F_{1,126} = 2.87$; $P = 0.09$), or body temperature ($r^2 = 0.003$, $F_{1,126} = 0.35$; $P = 0.55$). Finally, no significant correlations were found between total retention time and heart rate ($P = 0.39$), or body temperature ($P = 0.07$).

Discussion

The adrenocortical system is stimulated by unfamiliar situations in which the animal may experience helplessness or uncertainty (Frankenhaeuser 1986). Capture and shearing of wild guanacos may be considered stressful stimuli that may have important effects on the physiology of individuals. In addition, thermal stress may be induced by the loss of wool. Stress-induced physiological changes can lead to death in some animals (Reeder and Kramer 2005), differing among species and individuals, and depending on the stressor and individual immunologic and hormonal states (Cook *et al*. 2000). Therefore, appropriate practices should be used to limit excessive stressors, to maximise animal wellbeing and survival in management activities.

Previous reports of guanacos are scarce and in general refer to animals raised in captivity (Table 6). This study provides the first reported data of serum cortisol levels in managed wild guanacos. Our results show that serum cortisol values for wild guanacos increase significantly in correlation with handling time: longer restraint times correspond with higher serum cortisol levels.

The capture and physical restraint of an animal triggers a stress response that involves the sympathetic–adrenal–medulla axis, releasing catecholamines, and the hypothalamic–pituitary–adrenocortical axis, releasing corticosteroids (Norris 1995; Nelson 2000). Chronic and acute stressors have different significance to the wellbeing of the animal (Moberg 2000). The response to an acute stressor can be beneficial, e.g. glucocorticoid released can mobilise glucose and provide energy to escape a

Table 6. Cortisol values of guanacos under different management conditions

References: NS, not specified; CM, castrated males; M, Males

Condition	Sex	Age	Mean cortisol (s.e.) (ng mL^{-1})	n	Reference
Captive farming	NS	calf	8.3 (0.8)	8	Ríos *et al*. 2003
Captive farming	CM	adults	7.9 (1.0)	8	Zapata *et al*. 2004
Captive farming	M	calf	7.5 (2.5)	4	Zapata *et al*. 2002
Captive farming	NS	young	13.6 (5.0)	–	Le Roy 1999
Wild – captive	NS	newborn	30 (3.8)	53	Gustafson *et al*. 1998
Captive – farming	NS	young	4.7 (1.6)	12	Bas and González 2000
Captive – farming – shorn	NS	young	11.3 (9.4)	12	Bas and González 2000

predator, or have negative effects, causing capture myopathy, hypertension, or immune deficiencies (Williams and Thorne 1996). Chronic stress, on the other hand, can be detrimental to animals by decreasing reproductive and immune function over time (Harper and Austad 2000; Sapolsky *et al*. 2000). Both acute and chronic stressors can cause increases in plasma glucocorticoid levels, as well as changes in clinical parameters (Washburn and Millspaugh 2002; Montané *et al*. 2003). In this study, the increase in cortisol levels with manual restraint time is consistent with data reported for other mammals, such as black-backed jackal (Van Heerden and Bertchinger 1982), bighorn sheep (Kock *et al*. 1987), wild impala (Hattingh *et al*. 1990), mouflon (Marco *et al*. 1997), roe deer (Montané *et al*. 2003), and vicuñas (Bonacic *et al*. 2006).

The cortisol response can be triggered by behavioural patterns (Cavigelli 1999), social dominance (Creel 2001), or seasonality (Huber *et al*. 2003), and is essential for the adaptive short-term response to stressors (Sapolsky 1993). The complex interactions between these factors make baseline glucocorticoid values difficult to assess in free-ranging mammals (Reeder and Kramer 2005). Nevertheless, serum cortisol concentration can be used as an indicator of physiological stress and can be useful for evaluating differences among age or sex categories and type of handling.

Basal levels of serum cortisol concentration used in this study were based on guanacos bred in captivity. It is not known whether these levels represent basal levels of wild animals, so the comparison of values obtained in this study with cortisol levels previously reported for species in captivity (guanacos, vicuñas, alpacas, or lama) is only relative.

The difference in cortisol levels between guanaco males and females is in agreement with trends reported for other mammals, where females usually present higher glucocorticoid basal levels than males (Reeder and Kramer 2005). Moreover, females generally present a stronger response to stress when compared with males (Brett *et al*. 1983; Handa *et al*. 1994). Our results may confirm this, in that female cortisol values increased to higher levels than males and had longer retention times. This difference between the sexes is probably due to the central action of gonadal steroids (Handa *et al*. 1994). Higher cortisol levels in females may be related to pregnancy, a period of

Table 7. Heart rate and body temperature of guanacos under different management conditions
Data are from literature and this study

	Species/management condition				
	Castrated adult guanaco (in captivity)	Guanaco calf (in captivity)	Vicuña juveniles (in captivity)	Guanaco (wild)	South American domestic camelids (normal range)
Heart rate (average beats min^{-1} ± s.e. (*n*))	67.8 ± 3.0 (7)	83.0 ± 15.0 (16)	65.3 ± 4.6 (10)	79.6 ± 2.1 (123)	60–90
Body temperature (average °C ± s.e. (*n*))	–	–	38.1 ± 0.1 (10)	39.5 ± 0.1 (125)	37.5–38.9
Reference	Zapata *et al*. 2004	Bas and González 2000	Bonacic and Macdonald 2003	This study	Fowler 1998

high energy expenditure in the life history of females (Boonstra *et al*. 2001), in which cortisol levels have been demonstrated to increase during late stages (Reeder *et al*. 2004). Indeed, many of the adult females we studied may have been in the later stages of pregnancy.

Increases in heart rate and body temperature are considered to be indicators of a stress response to capture in wild ungulates (Montané *et al*. 2003). As reported, an important release of catecholamines, which are responsible for the heart rate increase at capture, is produced as a response of the autonomic nervous system to a stressor agent (Broom and Johnson 1993; Hopster and Blockhuis 1994). Captured animals may experience an increase in body temperature due to physical activity before capture, and also because of stress-induced hyperthermia (Broom and Johnson 1993). Nevertheless, this physiological parameter is time-dependent and body temperature increases during the first 10 min and returns to basal levels after 60 to 90 min (Moe and Bakken 1997).

The heart rate values of captured wild guanacos were, as expected, higher than those from castrated adult guanacos in captivity (Zapata *et al*. 2004), but lower than three-month-old guanacos kept in captivity (Bas and González 2000). The values we obtained were also in the range of those from vicuñas in captivity (Bonacic and Macdonald 2003), and domestic South American camelids (Fowler 1998; Table 7). Nevertheless, guanacos captured in 2007 had slightly higher heart rates than in 2006, perhaps due to higher levels of cortisol and longer handling times in the former, but more research is needed to determine other factors affecting the heart rate.

Finally, body temperatures of captured wild guanacos were higher than those described for domestic South American camelids and vicuñas in captivity (Fowler 1998; Bonacic and Macdonald 2003; Table 6). However, elevated body temperatures of wild guanacos were not correlated with increased cortisol levels, suggesting that body temperature could also be under the influence of atmospheric temperature. For instance, several guanacos in Payunia were penned during handling in small corrals that were lined with carpet to reduce visibility. The atmospheric temperature in these corrals was probably higher than the environmental temperature because there was no heat dissipation by wind.

Conservation and sustainable use of wild guanacos

Several development projects that promoted the utilisation of wild South American camelids were initiated in the 1980s within the framework of Integrated Conservation and Development Projects (ICDPs). These projects were based on economic incentives to promote the use of wildlife (Lichtenstein and Vilá 2003), and aimed to combine biodiversity conservation with improvements in the livelihood of rural people (Lichtenstein and Vilá 2003).

Shearing guanacos and vicuñas for wool can generate a valuable product, fleece, without harvesting wild populations (Vilá and Lichtenstein 2006; Sahley *et al*. 2007). Therefore, the live capture and shearing of wild guanacos could play an important role in the conservation of this species and its habitat, helping to reduce vegetation and soil degradation due to overgrazing by sheep. Given the wide distribution of the guanaco, their use has the potential to generate a positive economic incentive at the regional level in arid and semiarid ecosystems of Argentina. In this study, baseline data for a management system that does not require killing of wild animals are provided. In the light of the results obtained, it is recommended to avoid the simultaneous capture of a large number of animals, and to keep the duration of roundups short to reduce restraint time and improve animal welfare.

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