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Physiological and behavioral indices of short-term stress in wild vicuñas (*Vicugna vicugna*) in Jujuy Province, Argentina

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ABSTRACT

The management of wild vicuñas can trigger a stress response that may compromise welfare. In Santa Catalina, Jujuy Province, Argentina, indices of short-term stress associated with capture, handling, and shearing were studied in 105 wild vicuñas (*Vicugna vicugna*). The study included 2 groups (n = 59 and n = 46) of wild vicuñas captured in 2 consecutive days. Independent variables analyzed included sex, restraint time, and groups. Cortisol, creatine kinase, glucose, white blood cells, temperature, heart rate, and respiratory frequency were higher than published values. Respiratory rate increased during handling and correlated with holding time and group size, while heart rate decreased. Packed cell volume was higher in females. Cortisol concentrations differed between restraint groups and sex and inversely correlated with agonistic behavior. The most common behavior was increased vigilance. Sternal recumbency increased over holding time. During handling procedures, frequency of sudden movements like kicking and attempts to stand increased as restraint time increased. Females vocalized more than males. In conclusion, the methods used triggered measurable changes suggestive of short-term stress that appeared to be physiologically tolerated by the vicuñas.

KEYWORDS

Argentinean Puna; capture; stress; wild vicuñas

Current projects for the conservation and sustainable use of wild South American camelids (SACs), vicuñas (*Vicugna vicugna*), and guanacos (*Lama guanicoe*) include their capture to obtain live shorn fiber. Therefore, investigations of the stress response and its management in camelids are of vital importance.

Stress can be defined as the biological response elicited when an individual perceives a threat to his/her homeostasis (Moberg, 2000). It is known that the capture and handling of nonhabituated, nonhuman animals is stressful and potentially risky in wildlife management and can play a critical role in the capacity of individuals to survive and reach homeostasis (Bonacic & Macdonald, 2003).

A stressful event stimulates the sympathetic nervous system and activates the hypothalamic-pituitary-adrenal axis, resulting in the release of catecholamines and glucocorticoids, respectively (Moberg, 1987). This release triggers a response that involves a cascade of events indicated by changes in hematological, serum biochemical, clinical, and behavioral variables (Casas-Díaz, Marco, Lopez-Olvera, Mentaberre, & Lavin, 2010; Guyton & Hall, 2000; Williams & Thorne, 1996) that can

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then be used to indicate the level of response to a stressor (Meyer, Fick, Matthee, Mitchell, & Fuller, 2008).

The elevation of glucocorticoids in response to stress is beneficial and its function is to reach homeostasis, but it can be detrimental if it is prolonged by a persistent stressor (Romero & Butler, 2007).

The physiological response to stressors in higher vertebrates also includes the release of catecholamines and dopamine, which results in immediate variations in clinical and biochemical parameters related to the flight-or-fight response such as body temperature and heart and respiratory rates, reflecting a short-term stress response (Von Borell, 2000). A less acute response (minutes to hours) can be observed in blood glucose, plasma cortisol activity, and creatine kinase (CK), while some parameters may change within hours to days, such as total protein (TP) and blood urea nitrogen (Bonacic, Feber, & Macdonald, 2006; López-Olvera, Marco, Montané, & Lavín, 2006; Mentaberre et al., 2010; Zapata et al., 2004). Some behavioral responses to acute stress during capture include an increase in the frequency, duration, and intensity of alarm, defense, avoidance, or flight behaviors (Montané, Marco, Lopez-Olvera, Manteca, & Lavín, 2002). Long-term stress can be reflected in a decrease in body weight, impaired growth, and compromised immune response (Fraser & Broom, 1997).

Previous research on wild guanacos has demonstrated that cortisol concentrations varied with sex (lower in males than females) and correlated with total restraint time as well as with agonistic behavior and vocalizations (Carmanchahi et al., 2011; Taraborelli et al., 2011). Similarly, captive guanacos undergoing transport had increased cortisol concentrations and heart rates (Zapata et al., 2004).

Previous results from captured wild vicuñas have shown that longer restraint times were associated with significantly greater packed cell volume (PCV), CK, and glucose concentrations, thereby increasing the risk for capture myopathy; those animals subjected to longer periods of chase and restraint had significantly higher concentrations of cortisol (Arzamendia, Bonacic, & Vilá, 2010; Bonacic et al., 2006; Bonacic & Macdonald, 2003; Gimpel & Bonacic, 2006).

Studies that assess stress during capture are crucial for improving management techniques (Arzamendia et al., 2010; Arzamendia & Vilá, 2012). It is ideal for any project including management of the capture and shearing of wild vicuñas to follow appropriate animal welfare protocols (Bonacic, Arzamendia, & Marcoppido, 2012) to minimize or avoid negative consequences, as vicuña mortalities have often been reported to exceed 10% of the animals (Gimpel & Bonacic, 2006; B. Vilá, personal observation).

The objective of this study was to determine the short-term stress response of a previously unmanaged population of wild vicuñas to capture and handling in Santa Catalina, Jujuy Province, northwest of Argentina. Physiological, biochemical, hematological, and behavioral parameters were measured as indicators of a short-term stress response to chasing, sampling, and shearing.

Materials and methods

Animals and captures

In November 2012, two capture events on consecutive days (Groups 1 and 2) were planned of free-living, unmanaged vicuñas for shearing purposes in Santa Catalina (65º 08´W; 22º 08´S), Jujuy Province, in the Argentinean-Bolivian border of the Andean Puna. A total area of 10 km^2 with a mean density of 6 vicuñas/km^2 to 19 vicuñas/km^2 was chosen as the capture area.

Each day, captures started early in the morning (07:00), with different herding distances and holding times, which were defined as the time period between herding initiation and when the animals were enclosed within the corral. Eighty people herded the animals by holding ropes with colored plastic strips (acting as a visual barrier) and walking toward a funnel system consisting of

fenced slides (500 m long and 2 m high) built with a strong and semitransparent plastic net (10 cm × 10 cm mesh size), which prevented the escape of any vicuñas from the funnel.

Two extra nets buried across the entrance closed the trap securely. These net systems ended in a subdivided corral with a prehandling corral and a shearing area. Gates connected these corrals, and plastic covers blocked the view between them to facilitate the sampling and shearing of the vicuñas (Arzamendia et al., 2010; Baldo, Arzamendia, & Vilá, 2013).

In the technical report, suggested recommendations for restraint and handling include the animal welfare criteria for management of the vicuña (*Vicugna vicugna*) from the SAC Specialist Group (in Spanish; Bonacic et al., 2012). Using these recommendations, three people physically restrained the vicuñas and blindfolded them before they left the prehandling corral and until their release. Each animal was removed from the corral and placed over a carpet for blood sampling, physiological data collection, and shearing. Therefore, group size decreased over time.

We recorded the sex of the animals and their age classes (based on the eruption and wear of incisor teeth), which were estimated as adults (3 years and older), juveniles (1–2 years old), and crias (younger than 1 year old; Yacobaccio, 2006). The animals were marked with a numbered necklace made of flexible plastic. All the vicuñas were physically restrained, and no medications (anesthetics or tranquilizers) were used to capture or manage them. Daily environmental temperatures were requested from the nearest weather station: Climate and Water Institute, National Institute of Livestock and Agronomic Technology. Marked animals (n = 105) were monitored in the study area, following the vicuñas on foot or in vehicles, for up to 15 days after capture for evaluation of postrelease mortality.

Physiological sampling of clinical, hematological, and biochemical variables

Body temperature (°C) was measured using a digital thermometer via the rectum; heart rate (beats per minute) and respiratory rate (respirations per minute) were measured using a stethoscope and by observing the thorax, respectively. These parameters were measured twice, at prehandling and before release (posthandling sampling and shearing). Trained veterinarians collected samples and performed clinical observations.

Blood samples were collected immediately, 6 min (median time) after taking each individual from the prehandling corral, from the jugular vein of those vicuñas deemed to be healthy (health indicators taken into account included absence of ocular, nasal, and anal secretions; presence of skin lesions; presence of external parasites; and normal ear mobility) and in an optimal nutritional status (by palpation of ribs, spine, and pelvis) who were older than 1 year and female with no signs of pregnancy (by observations of palpable fetal movements and abdominal distension). Ten millimeters of blood were taken from each vicuña. Five milliliters were placed in commercial tubes with an anticoagulant ethylenediaminetetraacetic acid k3 (EDTA), and the remaining 5 mL were placed in tubes for serum collection. Blood samples were kept in a refrigerated box until they were processed in the laboratory.

Glucose concentration was measured from fresh blood with a portable glucometer (Ascensia Entrust, Bayer, Buenos Aires, Argentina). Total protein concentration was measured by a portable refractometer (General Tools REF 312 ATC). Total white blood cells (WBC) were counted in a Neubauer (Boeco, Germany) counting chamber using standard procedures. Packed cell volume was measured using a microhematocrit tube and was then centrifuged (Haematospin 1400, Hawksley, Lancing, England) at 14,000 G for 6 min. Blood samples were centrifuged at 1,500 G, the serum was removed, and then it was frozen at −20°C. Cortisol concentration was analyzed with a commercial enzyme-linked immuno assay (ELISA) kit (Cortisol KAPDB 270, Dia Source Immunoassay SA, Belgium), widely used in ungulates (Pighin et al., 2013, 2015). Lactate concentrations were calculated with a commercial enzymatic kit (L-Lactate, manual procedure, RANDOX Ltd., Crumlin, Co. Antrim, United Kingdom). Creatine kinase was determined using an optimized Ultraviolet (UV) method Creatin-Kinase N-Acetylcystine (CK-NAC) UV Wiener Laboratories, Rosario, Argentina).

An analysis of covariance (ANCOVA) was conducted to study physiological variables, with captured group and sex as explanatory variables and using corral holding time and group size as covariables. Each physiological parameter was checked for normality and homoscedasticity (Gurevitch & Scheiner, 1993; Underwood, 1997). Transformed values (ln) were used for CK values, and respiratory rate, temperature, cortisol, and TP variables were transformed to ranks (Shirley, 1987). The data were analyzed using the Infostat program (Di Rienzo et al., 2008).

Behavioral variables

In both groups, scan sampling (n = 29) with instant records (Lehner, 1996) was used to observe animals in the prehandling corral every 5 min until they were handled. The number of vicuñas in the corral and their ages (classified as adults and crias from the observation of physical size) were recorded. Behaviors were recorded with a camera through a small hole in the walls of the corrals to avoid disturbing the corralled vicuñas, and they were calculated as the proportion of animals performing each behavior pattern over the total number of scans (Martin & Bateson, 1986).

Only juveniles and adult vicuñas (n = 66) were analyzed using focal sampling (Martin & Bateson, 1986) during handling and shearing, following the vicuña ethogram reported by Vilá, Bonacic, Arzamendia, Wawrzyk, and Lamas (2004). Scans and focal sampling used the same behavioral categories (standing, increased vigilance, walking, running, lying in sternal recumbency, allogrooming, and mounting). Events recorded were agonistic behaviors (abrupt movements such as jumping and kicking and aggressive encounters or fighting), vocalizations, urination, and defecation. Likely relationships between physiological variables and behavioral variables were analyzed using nonparametric correlations (Grandin, 1997).

An ANCOVA was used to study the mean proportion of animals in each behavior category in each area of the corral as a dependent variable, using sex, corral, and event as explanatory variables and corral holding time and group size as covariables. An ANCOVA was used to analyze each behavior category during handling as the dependent variable, using sex and event as explanatory variables and corral holding time as a covariable. Behavioral variables were checked for normality and homoscedasticity (Gurevitch & Scheiner, 1993; Underwood, 1997) and were transformed to ranks (Shirley, 1987). A Tukey test for multiple comparisons was used after the ANCOVA; $p < .05$ was considered significant. The data were analyzed using the Infostat program (Di Rienzo et al., 2008).

Results

Physiological sampling of clinical, hematological, and biochemical variables

A total of 105 free-ranging, unmanaged vicuñas were captured on 2 consecutive days. Fifty-nine vicuñas (29 females, including 13 crias, 4 juveniles, and 12 adults; and 30 males, including 11 crias, 8 juveniles, and 11 adults) were captured on the 1st day (Group 1), with a herding distance of 4.11 km throughout the highlands and plain landscape. On the 2nd day (Group 2), 46 vicuñas (15 females, including 2 crias, 3 juveniles, and 10 adults; and 31 males, including 14 crias, 8 juveniles, and 9 adults) were captured with a herding distance of 2 km on the plain landscape. The Day 1 (Group 1) environmental mean temperature was 14°C (range = 7.7°C–20.3°C), while Day 2 (Group 2) mean temperature was 14.8°C (range = 6.6°C–22.9°C).

Herding time was 120 min the 1st day (Group 1) and 60 min the 2nd day (Group 2), with similar speeds of chasing (2 km/hr) for both events. The mean holding time (inside corral facilities) was higher during the 1st day (mean = 256.25 min, SD = 82.24 min, range = 127 min–390 min) compared with the 2nd day (mean = 204.81 min, SD = 95.077, range = 53 min–350 min). Mean handling time (for sampling and shearing) per animal was 13.31 min for Group 1 and 9.92 min for Group 2 (minimum = 1 min and maximum = 29 min; H = 1.81, p = .17).

Table 1. Values of clinical, hematological, and biochemical parameters of vicuñas from Santa Catalina, Jujuy Province, compared with published data.

Variable	*n*	Santa Catalina (1)	Cieneguillas (2)	Captive vicuñas (3)	Domestic camelids (4)
Initial Temp. (°C)	51	39.29 ± 0.07 (38.1–41)	39.7	38.1 (37.5–38.9)	37.5–38.9
Final Temp. (°C)	43	39.38 ± 0.08 (38.4–419)	39.55		
Initial Heart Rate (bpm)	51	84.33 ± 2.74 (42.4–136)	66.15	65.3	60–90
Final Heart Rate (bpm)	43	78.14 ± 2.56 (56–140)	57.5		
Initial Respiratory Rate (rpm)	51	39.22 ± 1.2 (24–68)	32.7	20.2	10–30
Final Respiratory Rate (rpm)	43	47.35 ± 2.75 (24–116)	46.35		
Packed Cell Volume (%)	21	38.48 ± 51 (19–51)	Nd	39.5 (27–45)	25–44.5
Total White Blood Cells (cells/µl)	32	1,0765.26 ± 584.46 (4,640–17,750)	Nd	7370	8,000–22,000
Total Protein (g/dL)	32	6.06 ± 0.08 (5.2–7.6)	5.43	Nd	4.7–7.3
Glucose (mg/dL)	32	149.32 ± 5.01 (78–189)	132	100.3 (95–150)	86–163
Lactate (mmol/L)	30	13.58 ± 1.16 (5.6–29.89)	Nd	Nd	Nd
Creatine kinase (ln)	29	5.99 ± 0.16 (4.05–8.05)	4.785	4.93 (0–137)	0–70
Cortisol (nmol/L)	27	366.33 ± 31.50 (162.78–775.21)	143.4	29.9 (18–24)	Nd

Reference values: (1) this study; (2) Arzamendia et al., 2010; (3) Bonacic et al., 2003; (4) Fowler, 2004. rpm = respirations per minute; bpm = beats per minute; ln = transformed values; Nd = no data.

No prerelease mortality (during chasing, capture, handling, or shearing) was reported. Postrelease mortality was not observed during the 15-day postrelease monitoring period. Mean values of clinical, hematological, and biochemical parameters of the wild vicuñas captured and sampled in this study are depicted in Table 1.

In general, comparing prehandling and posthandling measures revealed that the respiratory rate showed a tendency to increase during the handling procedure, while heart rate decreased during the same period. In contrast, body temperature remained constant (Table 1).

The initial respiratory rate and final respiratory rate did not show significant differences between captured groups or between sexes, but it positively covaried with holding time and group size (Table 2, <link rid="fig1">Figure 1). Females showed significantly lower final rectal temperatures

Table 2. Results of analyses of covariance.

Source		Group	Sex	Group × Sex	Corral holding time	Group size		
Variable	*N*	F, P	F, P	F, P	F, P	coefficient	F, P	coefficient
Initial Heart Rate	51	1.93, 0.17	0.86, 0.36	3.19, 0.08	2.44, 0.12	−0.13	1.25, 0.27	−0.77
Final Heart Rate	51	0.09, 0.76	2.42, 0.13	0.84, 0.36	0.91, 0.74	−0.03	0.0004, 0.99	0.004
Initial Respiratory Rate	43	1.06, 0.31	0.24, 0.62	3.06, 0.09	1.77, 0.18	0.12	1.67, 0.20	0.92
Final Respiratory Rate	43	3.94, 0.06	0.23, 0.63	0.55, 0.46	15.74, 0.02*	0.19	6.43, 0.01*	1.59
Initial Temperature		1.09, 0.30	0.3, 0.59	3.07, 0.09	0.00005, 0.94	0.01	0.37, 0.55	0.42
Final Temperature		0.06, 0.80	3.34, 0.08	6.31, 0.02*	0.19, 0.67	0.03	0.11, 0.74	0.19
White Blood Cell Count		0.59, 0.451	0.05, 0.83	0.35, 0.56	0.65, 0.43	24.4	1.09, 0.31	−245
Packed Cell Volume		0.73, 0.407	9.1, 0.009*	0.47, 0.50	0.56, 0.46	0.05	0.19, 0.67	0.22
Glucose		1.91, 0.18	1.44, 0.24	4.1, 0.05*	0.9, 0.35	−0.2	2.14, 0.16	−2.6
Lactate		0.11, 0.74	0.79, 0.38	0.02, 0.96	0.97, 0.33	−0.1	0.7, 0.41	−0.4
Cortisol		3.19, 0.09	5.07, 0.03*	12.9, 0.002*	1.86, 0.19	−0.08	1.96, 0.17	−0.66
Creatine kinase (ln)		1.19, 0.28	0.53, 0.47	1.78, 0.19	0.53, 0.47	0.01	0.11, 0.74	0.02
Total Protein		0.82, 0.37	1.37, 0.25	0.15, 0.71	1.1, 0.30	0.09	1.71, 0.20	0.83

Note. Effect of capture group (day), sex, and corral holding time—with corral and group size as covariables—on the clinical (rank values), hematological, and biochemical parameters.
F = value of statistic F; P = probability value; ln = transformed values. *Significant differences.

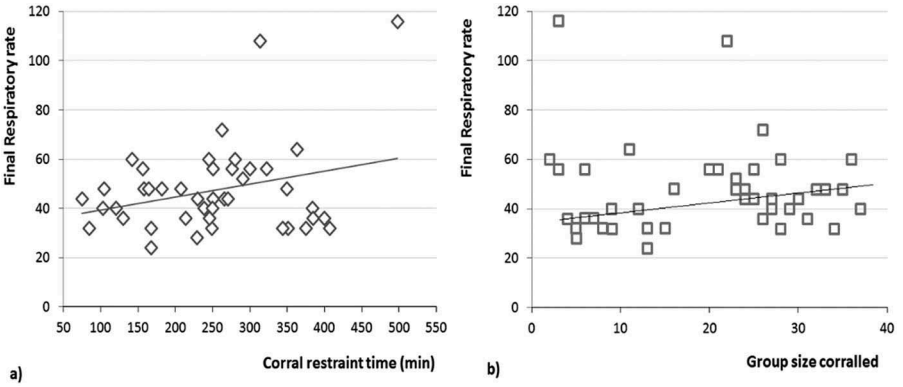

Figure 1. Final respiratory rate (respirations per minute) in relation to (a) corral holding time and (b) group size. Note the general trend of respiratory rate to increase with both parameters

than that of males (39.06°C and 39.48 °C, respectively) during the 1st day, while this measure was higher in Group 2 for both sexes (39.6°C; Table 2).

Regarding blood parameters, PCV differed between sexes (Table 2), with females having higher values than males (42.2 ± 0.88% vs. 33.22 ± 2.4%, respectively; Tukey test, $p < .05$).

The vicuñas' cortisol serum samples fit the curve proposed by the kit. Captured groups correlated with sex for serum cortisol concentrations (Table 2). Cortisol values for males from Group 2 were the lowest level observed (217.96 ± 2.95 nmol/mL) compared with vicuñas from Group 1 (360 nmol/mL) and females in Group 2 (430.5 ± 80.67 nmol/mL) $F(3, 29) = 6.27$, $p = 0003$, Tukey test $p < .05$ (<link rid="fig2">Figure 2).

Blood glucose values (Table 1) also showed correlations between captured groups and sex (Table 2). Blood glucose was higher in males ($M = 177.9 \pm 17.5$ mg/dL) than females ($M = 169.9 \pm 16.8$ mg/dL) in Group 1; however, for Group 2, this relationship was reversed, with females

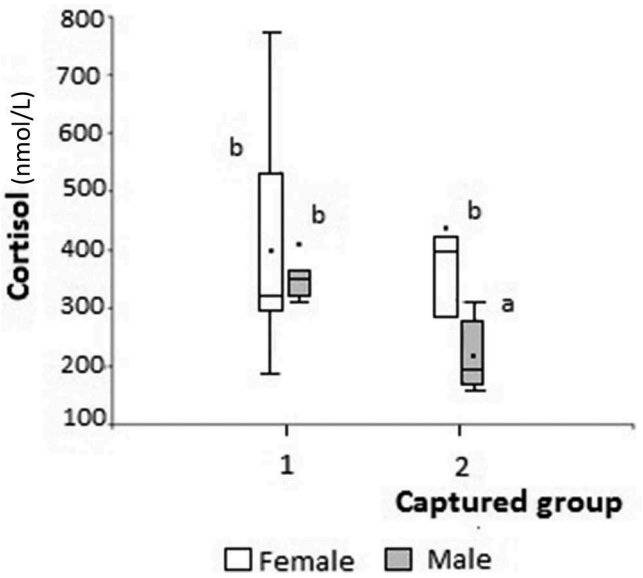

Figure 2. Boxplot of serum cortisol (nmol/L) by captured group and sex. Different letters indicate significant differences (Tukey's test, $p < .05$). Error bars indicate the deviation of the cortisol value.

showing higher values than males (M = 145.10 ± 18.54 mg/dL; males M = 114.42 ± 17.5 mg/dL; Tukey test $p < .05$). Neither captured group nor sex or their interaction significantly affected CK concentrations, TP, or lactate variables (Table 2).

Behavioral variables

The most common behavior observed in both corrals was increased vigilance (72 ± 3 SE, percentage of animals standing with head up and ears erect and staring) without variation between corrals, group size, or restraint time. Group size covaried positively with increased-vigilance event frequency (n = 29, F = 5.05, df = 1, *coefficient* [*coef.*] = 0.034, p = .15) and vocalizations (n = 29, F = 4.36, df = 1, *coef.* = 0.36, p = .047). The frequency of movements such as jumping (1.5 vs. 0 occurrences/time; n = 29, F = 8.01, df = 1; p = .009) and walking (9% vs. 7%; n = 29, F = 13.66, df = 1, p = .0011) was higher in the prehandling corral and negatively correlated with restraint time (n = 29, walk F = 21.96, df = 1, *coef.* = −0.83, p = .0001; jump F = 4.99, df = 1, *coef.* = −2.34, p = .035).

With the decreased group size and increased total captive time, sternal recumbency frequency increased (1% in the prehandling corral and 11% in the prerelease corral; n = 29, group size, F = 21.38, df = 1, *coef.* = −0.59, p = .0001; time, F = 35.07, df = 1, *coef.* = −5.72, $p < .0001$).

In the prerelease corral, grooming behavior (2% vs. 0%) was more frequently observed (n = 29, F = 24.96, df = 1, $p < .0001$), together with other behaviors (such as feeding, attempt to eat, or submission; 1% vs. 0%; (n = 29, F = 5.49, df = 1, p = .027), than it was in the prehandling corral.

The frequency of sudden movements such as kicks (n = 66, F = 4.92, df = 1, *coef.* = 0.64, p = .03) and attempts to stand (n = 66, F = 6.97, df = 1, *coef.* = 0.64, p = .01) during handling (sampling and shearing) depended on restraint time, with more agonistic behaviors being observed with increasing restraint time (minimum = 1 min, maximum = 29 min, M_{time} = 12 min; <link rid="fig3">Figure 3).

In contrast, and also during handling, vicuñas in Group 1 spent more time quiet (Group 1, 99 ± 0 SE %; Group 2, 94 ± 4 SE %; n = 66, F = 6.54, df = 1, p = .013), and this behavior decreased as restraint time increased (n = 66, F = 6.12, df = 1, *coef.* = −0.70, p = .016). Females vocalized more often than males (5% vs. 1%; n = 66, F = 7.24, df = 1, p = .009).

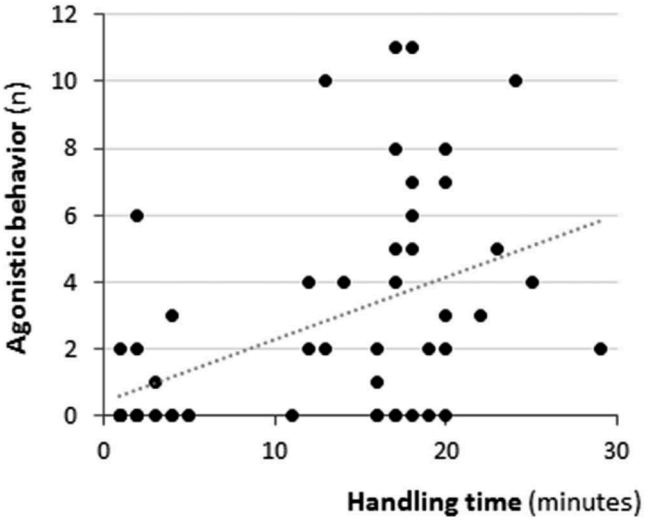

Figure 3. Agonistic behavior (abrupt movements such as jumping and kicking and aggressive encounters or fighting) in relation to handling time.

Relationships between physiological and behavioral variables

Vicuñas with increased cortisol levels (*coef.* = − 0.1, $p = .06$, $R^2 = .54$, $N = 7$) spent more time quiet (*coef.* = 0.00023, $p = .05$, $R^2 = .56$) and had decreased rates of agonistic behavior (kicking, jumping, aggressive encounters or fighting). These correlations were not significant for Group 1 ($p > .10$).

Discussion

Wild vicuñas are stressed by the capture and shearing process (Gimpel & Bonacic, 2006). In this study, most of the parameters measured fell within the normal range described for camelids (Fowler, 2004). However, other parameters were significantly higher compared with previous vicuñas' specific physiological parameters in wild and captive conditions (Arzamendia et al., 2010; Bonacic et al., 2006; Bonacic & Macdonald, 2003).

Heart rate is one of the most widely used indicators of acute stress (Broom & Johnson, 1993). For Santa Catalina vicuñas, heart rate was higher than previously reported in captured vicuñas in other regions of Argentina (Arzamendia et al., 2010) and Chile (Bonacic et al., 2006). In wild animals, heart rate increases during the capture event due to the release of short half-life catecholamines induced by physical activity and handling (Mentaberre et al., 2010; Porges, 1985). In this study, heart rate decreased and stabilized by the end of the shearing process in both males and females.

While the short serum half-life of catecholamines could partially explain this decrease, it could also reflect some degree of physiological habituation as a response to careful, low-impact handling techniques, such as blindfolding, which maintains a quiet handling environment, and partial shearing with scissors as opposed to electrical shearing machines. This pattern was also observed in previously captured vicuñas in Argentina (Arzamendia et al., 2010) and in guanacos after transportation (Zapata et al., 2004). This stabilization has also been reported in tranquilized wild roe deer (Mentaberre et al., 2010; Montané et al., 2003).

The respiratory rate was higher than that shown by previously reported data (Arzamendia et al., 2010; Bonacic, Macdonald, & Villouta, 2003). The increase observed during the handling process, especially in Group 2, could be associated with higher environmental temperatures necessitating an increase to dissipate heat or due to respiratory compensation for a probable metabolic acidosis (Barasona, Ramón López-Olvera, Beltrán-Beck, Gortázar, & Vicente, 2013; Gregory, 2004). The increased respiratory rate could be associated with stress-induced hyperthermia (Montané et al., 2003). Mean body temperature in this study was higher than previously described for SACs (Fowler, 2004) and lower than that of vicuñas previously captured in other regions in Argentina (Arzamendia et al., 2010), but it was similar to that of vicuñas captured in Chile (Bonacic et al., 2006). Meyer et al. (2008) reported that impalas (*Aepyceros melampus*) in captivity who habituated best to handling procedures had smaller changes in body temperature, lower plasma cortisol concentrations, and less irritable behavior compared with nonhabituated animals.

The increase in PCV and total WBC observed in most of the lactating females may be associated with splenic contraction due to stimulation of α-adrenergic receptors (Ganong, 2002) and partly to a reduction in plasma volume (Cross, Mackintosh, & Griffin, 1988). Females also had an increase in their leukocyte count compared with males. This result is similar to the findings of Zomborszky et al. (1996), who detected high WBC counts in red and fallow deer females. However, the use of sedative solution as well as fear and excitement may have affected the total and differential WBC count in fallow deer (Zomborszky et al., 1996).

Total protein values were similar to those reported in a previous study in wild vicuñas in Cieneguillas, Argentina (Arzamendia et al., 2010).

Overall glucose concentrations in this study were within normal references described for SACs, but they were higher than those described for vicuñas captured in Argentina (Arzamendia et al., 2010) and Chile (Bonacic et al., 2003, 2006). In this study, serum glucose levels in females were lower than those in males in Group 1 but higher than those of males in Group 2. The increase in blood

glucose values for females could be associated with the action of adrenocorticotrophic hormones, glucocorticoids, and adrenaline that mobilized amino acids from body proteins, thereby promoting gluconeogenesis and glycogenolysis with the concomitant conversion of α-ketoacids to glucose as observed during pregnancy (Beitz, 2004; Poljičak-Milas et al., 2009).

The higher blood glucose values detected in captured males in Group 1 is in agreement with observations of other authors who reported a linear relation between elevated cortisol concentrations and elevated plasma glucose in several species (Zapata et al., 2004).

Cortisol values have been widely used as stress indicators (Ingran et al., 1999; Möstl & Palme, 2002; Palme, Rettenbacher, Touma, El-Bahr, & Möstl, 2005). Santa Catalina vicuñas had higher cortisol values compared with wild Chilean vicuñas (Bonacic et al., 2003, 2006) and other Argentinian populations (Arzamendia et al., 2010). Cortisol concentration in Group 1 and in females of Group 2 was higher than that of males in Group 2; a high interindividual variation was evident and has been widely reported (Goymann et al., 2001; Ingram et al., 1999; Moberg, 1985; Montané et al., 2003). Blood collection time varied between groups regarding corral holding time, which was higher during the 1st day (range = 127 min–390 min) than during the 2nd day (range = 53 min–350 min), which could explain individual variations in cortisol levels. In other studies, cortisol levels peaked at 90 min and 120 min postcapture and were higher for females (Arzamendia et al., 2010).

Capture and handling often will increase muscle enzyme activity due to increased cell permeability, cell damage, or hemolysis (Duncan & Prasse, 1986). Increases in enzymes (Alanine Aminotrasferase (ALT), Aspartato Aminotrasferase (AST), CK, and Lactate Dehydrogenase (LDH)) have been reported in stressed ungulates in the wild. In this study, lactate concentrations were similar to those observed in other wild ungulates (Barasona et al., 2013; Montané et al., 2003; Poljičak-Milas et al., 2009), with higher values in females that might be associated with increased metabolic demand during late pregnancy, which leads the animal into an anaerobic metabolic state.

In this study, longer restraint time in the corral was associated with lower lactate concentrations in the vicuñas. This decrease in serum lactate concentrations could indicate that lactate concentrations were returning to baseline by the time the vicuñas were sampled after being restrained and immobilized in the corral.

The CK concentrations observed were higher than values reported by Arzamendia et al. (2010) and also were positively correlated with higher cortisol values. During the 1st day, distance covered and chasing time were greater, the landscape was more difficult to navigate, and mean restraint time was higher compared with the 2nd day. Also, the number of corralled vicuñas during the 1st day was larger than on the 2nd day. All these factors could influence the capacity of the vicuñas to cope with stress and could explain the higher values in hormones and enzyme concentrations observed on the 1st day.

During handling, our results showed that stress response behaviors (agonistic behaviors such as kicking and struggling behaviors such as defense and aggression) and vocalizations (taken as a sign of discomfort or stress response behavior) increased with restraint time. Other studies have shown fighting, alarm calls, and attempts to escape as acute stress indicators in response to handling (Grandin, 1997; Henry, 1992; Morgan & Tromborg, 2007; Taraborelli et al., 2011). Distress in vicuñas and in guanacos (Taraborelli et al., 2011) would be characterized by more frequent agonistic behavior in the corrals and by higher vocalization rates during manual immobilization for shearing.

Time in the corral and handling were factors that modulated muscular fatigue and exhaustion, as reflected in lying behavior in the prehandling corral and quiet behavior during handling. The latter was correlated with an increase in cortisol, as has been reported in many species (Henry, 1992). While some authors have recommended the use of tranquilizers when dealing with wildlife (Barasona et al., 2013; Casas-Díaz et al., 2010; Edebes & Raath, 1999; Montané et al., 2003), we chose not to sedate the vicuñas because the drug-induced state of drowsiness meant

they would have been unable to escape from natural predators, who have reported in the area. This project used the recommended techniques to limit physiologic and behavioral stress including blindfolding, a quiet handling environment, and minimized handling time, thereby decreasing stress and allowing these vicuñas to reach a calm state and to be able to better cope with the situation.

Conclusion

Capture-related factors such as herding distance, overall capture time, and restraint time had a measurable effect on stress indicators in wild vicuñas. The use of an adaptive management framework allowed for modifications and improvements in these factors on the 2nd day of capture, resulting in lower values for most of the analyzed parameters.

We conclude that the capture, sampling, and shearing methods used here resulted in a stress response; however, the vicuñas were apparently able to cope with the stress, as evidenced by the absence of acute posthandling complications, morbidity, or mortality.

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