

Behavioural response of free-ranging guanacos (*Lama guanicoe*) to land-use change: habituation to motorised vehicles in a recently created reserve

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Abstract

Context. Guanacos, the only native ungulates inhabiting Patagonian arid lands, are perceived by local people as a threat to livestock production and, consequently, uncontrolled hunting and harassment are widespread across the region. In 2005, a traditional sheep ranch (RSP) was converted into a wildlife reserve, offering the opportunity to assess changes in guanaco tolerance to motorised vehicles after harassment ceased.

Aims. The aims of the present study were to address factors influencing guanaco flight response on RSP, to assess inter-annual variation in flight responses after management changed and to compare guanaco response to cars among the RSP population, neighbouring ranches with traditional management and a southern population (C2B) that has had effective protection since the early 1970s.

Methods. Field surveys using available roads at RSP were conducted during a 4-year period to assess inter-annual changes in guanaco flight probability. Current estimates of flight probability at RSP were then compared with point estimates obtained from neighbouring ranches and the C2B population.

Results. We found that flight probability at RSP decreased as groups were located farther from the transect line and groups with at least one juvenile were more likely to flee than were adult-only groups. Flight probability decreased progressively during the study and significant differences with initial conditions emerged during the fourth year of monitoring. The current flight response observed at RSP is consistent with an intermediate state between neighbouring ranches and C2B population.

Key conclusion. Our results support the hypothesis that guanacos can become rapidly habituated to vehicles if harassment ceases and subsequent traffic acts as a neutral stimulus for enough time.

Implications. Finally, we discuss how our results may be helpful for other recently created reserves and ecotourism oriented projects.

Additional keywords: flight response, hunting, motor vehicles.

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Introduction

During the past few decades, there has been an increasing interest in understanding and quantifying wildlife responses to anthropogenic stressors because of their potential implications for conservation and management efforts (Whittaker and Knight 1998; Stankowich 2008; Bejder *et al.* 2009). Simple behavioural reactions are among the most obvious responses an animal makes to a threatening stimulus and have been widely used to address questions about the effects and impacts of disturbance (Beale 2007). In addition to complementary ecological and physiological information, behavioural studies have helped identify some of the potential consequences of human-induced

stress, such as changes in resource-use patterns, avoidance of preferred areas and increased energy expenditure (Beale 2007).

Habituation, as well as sensitisation, are often cited as consequences of sustained human-wildlife interactions (Whittaker and Knight 1998), although there has been some debate on the proper use of these terms (Bejder *et al.* 2009). Following Bejder's review (2009), sensitisation is referred to as the 'increased behavioural responsiveness over time when animals learn that a repeated stimulus has significant consequences for the individual' (Richardson *et al.* 1995), whereas habituation is referred to as the 'the waning of a response as a result of the exposure to a repeated, neutral

stimuli' (Thorpe 1963). Both responses constitute learning processes that reflect an individual's cumulative experience with humans, including the number and outcome of exposures to anthropogenic stimuli over the animal's lifetime (Knight and Temple 1995). Sensitisation and habituation processes often correlate with temporal variation in tolerance level. Sensitisation implies a sequential decrease in tolerance over time, whereas habituation might be accompanied by an increase in the intensity of disturbance that an individual tolerates without responding in a defined way (Nisbet 2000; Bejder *et al.* 2009). Therefore, tolerance level is considered a behavioural state that can be measured at a single point in time, and the magnitude and direction of the temporal changes in tolerance indicate the occurrence of habituation or sensitisation processes. Spatial and seasonal variation in wildlife tolerance to specific human activities has been studied extensively, particularly among ungulate species in recreational settings (Taylor and Knight 2003; de Boer *et al.* 2004; Stankowich 2008). Although taking into consideration that time dimension is a prerequisite to properly address these processes, empirical studies on habituation often do not account for sequential variation in responses. This is presumably due to practical and financial limits, which usually restrict the sampling effort to short periods or to a single point in time (Bejder *et al.* 2009).

Among the conventional indicators of human-induced stress, flight response is frequently used to assess the relative level of disturbance or harassment which a population is exposed to (Taylor and Knight 2003; Stankowich 2008). Many factors influence the decision to flee, and the way animals optimise the trade-off between the costs and benefits of staying versus escaping can vary among species, populations and individuals (Ydenberg and Dill 1986; Stankowich 2008). For example, flight response by females with vulnerable offspring by their side is often greater than that of males or females without young, and this behaviour is consistent across many ungulate species (Stankowich 2008). However, some individuals may reduce their response to an increased disturbance because they are willing to accept the greater risk in finding food or mates (Lima and Bednekoff 1999). Group size, habitat structure and weather conditions (de Boer *et al.* 2004) might also play a role in flight decisions and individual responses might be ultimately determined by the interactive effects of the factors influencing risk perception (Frid 1997, 2003; Stankowich 2008).

Guanacos are the dominant wild ungulate inhabiting the arid and semiarid ecosystems of South America, even though their populations have been drastically reduced since European colonisation (Franklin 1982). At present, despite the marked heterogeneity in the conservation status of their populations, most of them have continued to decline since the introduction of domestic livestock (Baldi *et al.* 2006). With guanaco and sheep (*Ovis aries*) diets overlapping significantly and water being extremely scarce, the native herbivore is perceived by land owners and locals to be a threat to livestock production (Baldi *et al.* 2001, 2004, 2006). Consequently, uncontrolled hunting and poaching are widespread across Patagonian ranches (Baldi *et al.* 2010). Hunters usually shoot guanacos from dirt roadways and, thus, within heavily hunted populations, whenever a car is detected, flight responses are conspicuous. Flight frequency of guanaco is higher within

protected areas where poaching is common, than within reserves with effective protection (Donadio and Buskirk 2006). This lower tolerance level suggests that sensitisation to vehicle-related stimulus has occurred in heavily hunted populations. Guanacos have shown a great plasticity in various aspects of their ecology, such as diet selection (Puig 1995; Puig *et al.* 1996, 1997), social organisation and migratory movements (Franklin 1983; Puig and Videla 1995), habitat use (Puig and Videla 2000) and anti-predator behaviour (Marino 2010). Because flight events entail at least energy and time costs (Ydenberg and Dill 1986), behavioural adaptive changes leading to habituation to human presence might also be expected if harassment is discontinued.

In 2005, a local non-governmental organisation purchased a 7300-ha ranch (RSP) that was formerly dedicated to sheep production and then turned it into a private wildlife reserve, Refugio de Vida Silvestre San Pablo de Valdés (RSP). A permanent warden has been supervising visitor activities and preventing harassment towards wildlife since 2006 (Burgi *et al.* 2011). Wildlife-monitoring programs oriented to assess changes in key ecosystem components after the management change began in 2008 and have continued up to present, providing an opportunity to test the occurrence of guanaco habituation to human presence. The present study aimed to assess temporal variation in guanaco tolerance towards motorised vehicles at RSP since management practices changed and harassment to wildlife ceased. The specific objectives were (1) to address factors influencing guanaco flight response towards motorised vehicles at RSP, (2) to assess temporal variation in flight responses at RSP and (3) to compare guanaco response to cars among RSP population after 5 years of effective protection, with the following two contrasting harassment-level scenarios: neighbouring ranches with traditional sheep-management practices and a southern population (C2B) which has had effective protection since the early 1970s. The underlying hypothesis was that as car-related stimulus changed from negative to neutral, guanacos at RSP became progressively more tolerant to motor vehicles and this habituation was reflected in the waning of their flight response.

Materials and methods

Study species

Guanaco breeding system is classified as a resource-defence polygyny (Franklin 1983). The main social units in this system are family groups, bachelor groups and solo males; mixed groups can also be found in migratory populations (Franklin 1983). Family groups are composed of an adult male and one or more adult females with their offspring from the year ('chulengo', i.e. an individual younger than 1-year old). Family members usually form highly cohesive and behaviourally synchronised units. Bachelor groups are composed mostly of adult males and yearlings, and migratory-mixed groups are composed of both sexes and all age classes (Raedeke 1979; Franklin 1983). Each family male defends its territory from the intrusion of other guanacos that are not members of its group (Raedeke 1979; Franklin 1983). Guanaco population can be sedentary or migratory. In migratory populations, family groups leave their territories after the reproductive season (Raedeke 1979; Franklin

1983), whereas in sedentary ones, family groups remain within their territories all year round (Burgi 2005).

Study area

Península Valdés (PV) is a 4000-km² area located in Chubut, Argentine Patagonia, and it is divided into ~90 private ranches and six small coastal reserves. Effectively protected areas in PV are particularly oriented to the conservation of seabird and pinniped colonies, whereas most terrestrial wildlife occurs within private land. Extensive sheep ranching is the main productive activity in this area (Burgi *et al.* 2011); however, some landowners implemented eco-tourism as a complementary activity to wool production because Península Valdés was declared a World Natural Heritage site by the United Nations in 1999. RSP is located in the southern section of Península Valdés (42°36'S, 64°10'W). A detailed description of RSP environmental features and vegetation communities is given in Burgi *et al.* (2011). Management shift was completed by 2006 when the last sheep were removed from the ranch. Simultaneously, a permanent warden began to control and poaching as well as harassment practices (i.e. the use of horses and dogs to chase animals off-road) ceased. Since RSP guanaco monitoring program began in 2008, population density, distribution and behaviour have been surveyed on an annual basis. Guanaco densities at RSP have been increasing since management changed – from 3.9 ± 1.1 guanacos km⁻² in 2006 to 16.5 ± 2.8 guanacos km⁻² in 2011 – whereas the density at neighbouring ranches remained below one guanaco km⁻² during the same period (Burgi *et al.* 2011). Telemetry studies conducted near RSP (Burgi 2005) and the lack of seasonal changes in family-group density (A. Marino, unpubl. data) indicate that guanacos in this area are sedentary.

Cabo Dos Bahías (C2B) is a small wildlife reserve (1700 ha) located at south-eastern Chubut (44°55'S, 65°31'W), 300 km from Península Valdés. As well as at RSP, the vegetation in this area is characteristic of the Patagonian Province and is composed of shrublands and grasslands (Beeskov *et al.* 1987). C2B has had effective protection since it was created in 1973 (Cévoli 2005), with a permanent warden that supervises visitor activities. Tourists visit the reserve frequently and guanacos are so habituated to human presence that their vigilance effort is almost null (Marino 2010) and continue foraging or resting even when observers approach them as close as 20 m (A. Marino, pers. obs.). Post-reproductive estimation of population density at C2B in 2011 was 58.7 ± 5.5 guanacos km⁻².

Observations

Ground line-transect surveys were conducted at RSP during April 2008, January 2009, February 2010 and 2011, along available dirt roads and tracks. A total of 23.2 km, distributed among five transects, was surveyed twice on successive days in 2008 and 2011, and three times in 2009 and 2010. A similar survey was conducted once across 11.7 km of available roads at C2B during March 2011. In 2011, RSP surveys were extended 33.5 km outside the reserve, with transects being distributed among six neighbouring ranches. Surveys were conducted from an open pick-up vehicle (see Burgi *et al.* 2011 for detailed methods). For every guanaco group encountered,

group size, group composition (number of adults and chulengos) and behavioural response of the group were recorded. The distance between the vehicle and the location of the animals was measured using a laser rangefinder and was referred to as the minimum vehicle-group distance (MVG distance). The following criterion was used to decide where to stop the vehicle to measure MVG distance: whenever the animals did not show an evasive response to the vehicle and remained in the same location, the vehicle was stopped when the minimum distance between the group and the road (i.e. perpendicular distance to the road) was reached. If the group started an evasive response as the vehicle approached (walked or galloped away), the vehicle was stopped and the distance between vehicle and the location where the animals were standing right before moving was measured. It is important to note that MVG distance differs from flight initiation distance (i.e. the distance at which the animals flight in response to an observer that is directly approaching them) often used in behavioural studies because, in this case, the group is not being directly approached because the observers' trajectory is dictated by the available roads and tracks. Therefore, some groups might be far enough from the road to remain indifferent to the passing vehicles. Observed groups were classified according to group type into family groups (an adult male with one or more females, with or without chulengos), bachelor groups (composed mostly of adult males and yearlings, including solo males that were considered as bachelor groups of size one), or as undetermined, if the animals were too far to assess group type (Pedrana *et al.* 2009). In addition, groups were classified according to the presence or absence of chulengos. Bachelor groups and all-adult families were pooled into a single category of groups without chulengos, whereas families with at least one chulengo were classified as groups with chulengos. Both factors, namely, group type and presence of chulengos, were considered in the analysis so as to distinguish whether it was the social category or the presence of more vulnerable young that explained the different responses among groups. Observed behavioural response was classified into the following categories: alert – at least one member of the group stopped foraging and became alert but the group stayed in the same location; walking away – the animals slowly moved away from the vehicle; and fleeing – animals ran away in response to the approaching vehicle. A fourth category was defined as indifferent, with animals remaining foraging or resting in the same place, without evident signs of disturbance. In all occasions, when the members of the group moved, they did it cohesively and in the same direction. Effective strip width varied between 400 and 500 m (i.e. estimate of the strip effectively sampled when estimating abundance by *Distance sampling*) (Buckland *et al.* 1993) and the farthest groups were detected within 1 km from the transect line. Behavioural categories were easily assessed even for farthest groups. High winds preclude proper observations and because windy days were avoided to conduct surveys, sampling effort in RSP differed among the years. Rainy days are extremely infrequent during eastern Patagonian summer; therefore, between-survey variation in weather conditions is assumed to be negligible. Although there is some heterogeneity in vegetation communities in the study areas, all of them are open grasslands and short shrublands and we believe that differences in detection and escape opportunities were trivial. We assumed that including transect identity in our

models would be enough to account for any spatial heterogeneity in guanaco responses; therefore, we excluded habitat structure from the analysis. Transects were located in open plains and low hills, and the previous argument applies also to topography. However, transects differed in traffic intensity. A public road that goes through the northern section of the reserve is used many times a day. The warden's house access is used almost once a day and the southern tracks are visited weekly.

Statistical analysis

To address the factors affecting flight response and its changes over the study period, the proportion of guanaco groups that fled when detecting the vehicle was modelled using a generalised linear mixed model (Crawley 2007). The observations of groups that walked away and groups that stayed in the same location, either alert or indifferent, were pooled into a single category of non-fleeing groups. Therefore, all the observations were classified into fleeing or non-fleeing groups. This binary response was modelled assuming a Bernoulli distribution for the error term and a complementary log–log link function (Crawley 2007). Regarding the fixed part of the model, the independent factors considered were Year, Group type (three levels: families, bachelors and undetermined) and Presence of chulengos (two levels: groups with and groups without chulengos); the independent variables were Group size and MVG distance. MVG distance was included to account for the relatively increased flight probability of the groups that were closer to the transect line. To account for the lack of independence among observations collected on the same day (survey) and in the same transect, Survey and Transect nested within Survey were included as random factors. Parameters for fixed factors have been expressed as differences from the reference level, which was the first treatment considered. The slopes of the regression lines corresponding to the reference level are represented by the parameters for the variables. Then, estimated standard errors were used to test whether the difference between the reference level and the other levels was significantly greater than zero, considering an α level of 0.05 (Crawley 2007). Model simplification was carried out by dropping terms that showed non-significant differences between factor levels or slopes that did not differ significantly from zero.

To account for possible changes in guanaco distribution relative to road location that might affect behavioural responses over the study period and to complement the information obtained from the binomial model, MVG distance at RSP was log-transformed and considered as a response variable as well. This variable was modelled assuming a normal distribution for the error term and an identity link

function (Crawley 2007). A set of linear mixed models, including Year, Presence of chulengos and Behavioural reaction (four levels; indifferent, alert, walk and flight) as fixed effects, and Survey and Transect nested within Survey as random effects, was fitted to the MVG distance data (Crawley 2007).

Finally, 2011 data from RSP were compared with data from C2B and the neighbouring ranches, by fitting another linear model and assuming a Bernoulli distribution for the error term and a complementary log–log link function. Model fitting was performed using the lme4 package and the 2.9.2 version of R (The R Foundation for Statistical Computing, www.r-project.org, verified 26 June 2012) software.

Results

In total, 326 groups were observed during 11 surveys conducted between 2008 and 2011 at RSP. Sample sizes and average MGV distances are shown in Table 1. Overall, 24% ($n=78$) of the groups were indifferent to the presence of the vehicle, 32% ($n=106$) stayed in the same place where they were located but became alert when the vehicle was detected, 22% ($n=71$) walked away slowly and 22% ($n=71$) fled. To describe flight events on a general basis, it is worth noting that family groups escaped in a cohesive and stereotypical way; usually, mothers triggered the escape response and started running in the front of the group, with their offspring by their side, the rest of the females followed them and finally the adult male ran at the back, sometimes waiting for the entire group to move or placing itself between the females and the threatening subject until they were various metres apart. Family males used to accompany flight sequence with alarm calls and head movements, seemingly directed to group members. In contrast, bachelor groups showed flight events that were disorganised and less cohesive.

The minimal adequate model for flight probability at RSP included the effect of the MVG distance, the presence of chulengos in the group and the differences between years. The probability of a group showing a flight response significantly decreased as the animals were located farther from the transect line (Table 2, Fig. 1). The presence of chulengos had the strongest influence on flight response. Groups having at least one young individual were, on average, 23% more likely to flee in response to an approaching vehicle (Table 2). Guanaco groups fled progressively less between successive years and statistically significant differences with initial conditions emerged during the fourth year of the study (Table 2, Fig. 2). Partial aliasing (i.e. information from one explanatory variable is partially contained within another) (McCullagh and Nelder 1983) between group type and presence of chulengos precluded the inclusion of both factors in the same model. However, when

Table 1. Mean (s.d.) group size (GS), sample size (N) and average minimum distance between the group and the vehicle (m) at RSP wildlife reserve

Year	Groups without chulengos			Average distance (s.d.)	Groups with chulengos	
	Bachelors GS (N)	Families GS (N)	Undetermined GS (N)		GS (N)	Average distance (s.d.)
2008	1 (9)	2 (1)	4.6 (11)	277.0 (186.6)	8.5 (32)	317.4 (133.7)
2009	5.3 (25)	5.6 (14)	5.4 (26)	355.4 (247.8)	8.4 (43)	449.9 (250.8)
2010	8.8 (22)	5.4 (8)	3.7 (10)	390.1 (193.6)	9.8 (46)	475.6 (276.5)
2011	6.7 (21)	5.7 (4)	2.9 (11)	385.4 (207.6)	9.8 (43)	357.5 (209.8)

Table 2. Parameter estimations for flight-probability at RSP wildlife reserve, s.e., *t*-values and their probabilities
Non-significant fixed terms are included with informative purposes but final-model parameters were estimated without them

Final model (differences and slopes)	Estimate	SE	<i>t</i> pr.
A. Reference level (2008, groups without chulengos)	-0.45	0.47	0.34
B. Groups with chulengos	1.10	0.32	<0.001
C. 2009	-0.89	0.51	0.082
D. 2010	-1.02	0.53	0.057
E. 2011	-1.31	0.56	0.019
F. Distance	-0.002	0.001	0.005
Non-significant terms			
G. Group size	0.007	0.02	0.761
Interactions			
B × C	1.17	0.83	0.157
B × D	1.01	0.91	0.265
B × E	1.04	0.97	0.279
B × F	0.000	0.00	0.935
F × C	0.001	0.003	0.548
F × D	0.001	0.003	0.550
F × E	0.003	0.003	0.243
Random effects	Variance	s.d.	
Surveys	0.017	0.612	
Transects within surveys	0.375	0.129	

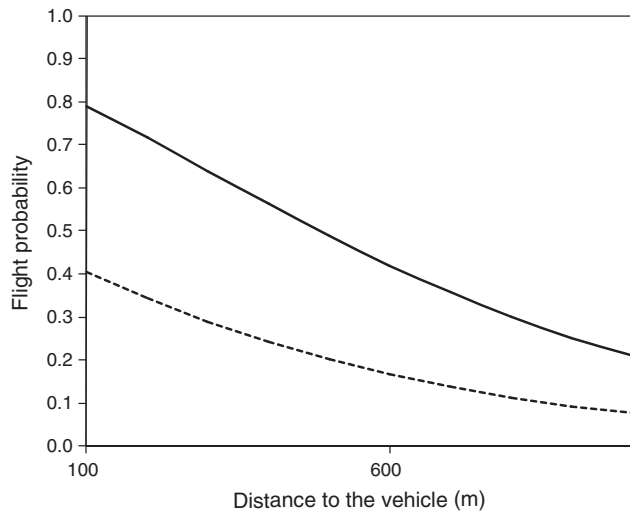


Fig. 1. Model predictions for flight probability as a function of the distance between the group and the vehicle, for groups without chulengos (dotted line) and groups with chulengos (full line) (RSP wildlife reserve).

group type was considered instead of the presence of chulengos, the difference between family and bachelor groups in flight probability was not statistically significant (difference = -0.5, s.e. = 0.37, $z_{(314)} = -1.35$, $P = 0.176$), which was true as well in the difference between families and undetermined groups (difference = -0.7, s.e. = 0.44, $z_{(314)} = -1.61$, $P = 0.106$). This confirmed that the increase in flight probability was explained by the presence of chulengos rather than by the social category. Regarding random terms, between-transect variation was relatively high. A subsequent analysis to explore the direction and magnitude of these differences, including transect identity ($n = 5$) as a fixed factor instead of a random one, indicated that

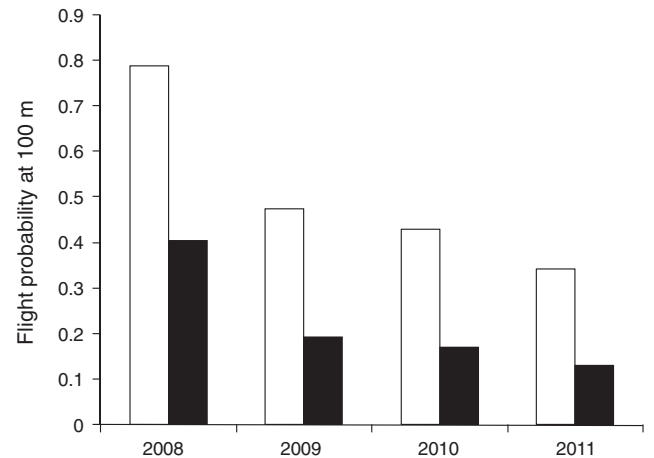


Fig. 2. Model predictions for flight probability of groups located 100 m apart from the vehicle at RSP wildlife reserve during the studied period; groups with chulengos (white bars) and groups without chulengos (black bars).

groups with chulengos located at 100 m from the road had a 33–38% higher probability of fleeing in the less used transects than in the ‘public road’ or the ‘warden’s house access’ that were used almost every day, and this difference was statistically significant (difference = 1.65, s.e. = 0.56, $z_{(316)} = 2.94$, $P = 0.003$).

The complementary analysis of MGV distance indicated that there were no differences among the years in our sample (difference 2008–2009 = 0.08, s.e. = 0.14, $t_{(40)} = 0.53$, $P = 0.596$; 2008–2010 = 0.25, s.e. = 0.15, $t_{(40)} = 1.65$, $P = 0.106$; 2008–2011 = 0.05, s.e. = 0.16, $t_{(40)} = 0.33$, $P = 0.739$). The average MGV distance recorded was similar between fleeing groups and groups that started walking in response to the approaching vehicle (difference = 0.097, s.e. = 0.13, $t_{(273)} = 0.76$,

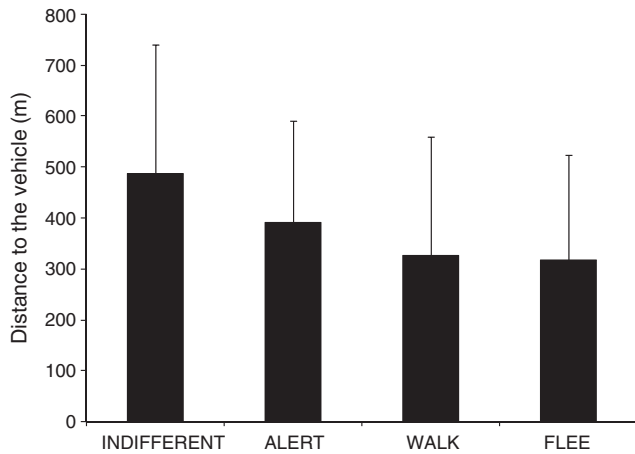


Fig. 3. Average minimum group-vehicle distance at RSP wildlife reserve. Scale bars represent standard deviation.

$P=0.444$; Fig. 3). In contrast, groups that stayed alert in the same location where they were when detected were on average 98.5 m farther from the vehicle than those that fled (difference in log scale = 0.40, s.e. = 0.12, $t_{(273)}=3.40$, $P<0.001$). Groups that remained indifferent to the presence of the vehicle were on average 183.4 m farther than were fleeing groups (difference in log scale = 0.65, s.e. = 0.13, $t_{(273)}=5.05$, $P<0.001$). Last, after fitting behavioural reaction, groups with chulengos were significantly farther from the vehicle than were all adult groups (difference = 0.2, s.e. = 0.086, $t_{(273)}=2.39$, $P=0.017$). However, retransformed values on the natural scale indicated that this difference was only 45 m.

Regarding C2B, 54 groups were observed during the survey conducted in 2011, and 29 of them had at least one chulengo. None of the observed groups fled in our presence. Moreover, 43 (90%) continued resting or foraging in the same place where they were located before we approached, whereas the other 10% walked away slowly. Minimal MGVD distance recorded was 27 m. Average MGVD distance was similar between C2B and RSP (difference = 0.35, s.e. = 0.16, $t_{(1)}=2.26$, $P=0.265$). There were no statistical differences in flight probability between C2B and RSP 2011 data (difference = 8.8, s.e. = 15.5, $t_{(131)}=0.57$, $P=0.570$).

After surveying more than 30 km along the ranches neighbouring RSP, only five guanaco groups were observed and none had chulengos among their members. Four (80%) of these groups fled in response to the vehicle. A plain comparison of 2011 data suggested that guanacos at RSP were less likely to flee than were the guanacos in the neighbouring ranches (difference = -2.92, s.e. = 1.16, $z_{(82)}=-2.53$, $P=0.011$); however, a larger sample size outside the reserve would be required to confirm this result.

Discussion

Factors affecting flight response

The probability of fleeing in response to an approaching vehicle decreased as groups were located farther from the transect line and this effect was consistent across the years. This result suggests that guanacos perceive a higher risk when they are closer to the

threatening subject. A similar result was found in a small guanaco population heavily visited by tourists at Ischigualasto Provincial Park, San Juan, Argentina (Malo *et al.* 2011). A study on moose (*Alces alces*) behaviour in response to snowmobiles showed that traffic affected individuals that were close to the trails, displacing them to less favourable habitat (Colescott and Gillingham 1998). It is important to note that the lack of between-year differences in MGVD distance recorded (considering all fleeing and non-fleeing groups) suggests that group distribution relative to road location did not vary appreciably during the study. Therefore, the observed differences in flight responses might be a consequence of changes in guanaco risk perception rather than an effect of changing location relative to the roads. Among factors affecting flight probability, group composition showed the greatest influence. Groups with at least one chulengo among their members had a significantly greater probability of fleeing than did groups without them, even when the former seemed to be located farther from the road than were all-adult groups. In a previous study on South American camelids, flight frequency of groups with juveniles was higher than that of adult-only groups in areas with poaching, although this effect was statistically weak (Donadio and Buskirk 2006). In the previously cited study aimed at assessing the effect of tourists on guanaco behaviour, groups with chulengos also had the highest likelihood of flight in response to a vehicle when compared with adult-only groups or solo individuals (Malo *et al.* 2011). Increased flight response of mothers is common in many ungulates species (Stankowich 2008) and presumably related to the mothers' effort to compensate increased vulnerability of their offspring. In the case of guanaco family groups, whose flight response is highly cohesive, mothers' reaction might trigger the escape response of the entire group. This hypothesis is supported by the observations of mothers and chulengos running ahead of the fleeing groups. Mothers' sensitivity might decrease as the juveniles grow up and become less vulnerable. Although intra-annual differences were not assessed in the present study, seasonal changes in mothers' tolerance to human presence can be expected, as was found in reindeer (*Rangifer tarandus*) (Haskell *et al.* 2006).

We found no effect of group size on flight probability, in accordance with findings of other studies among camelids (Donadio and Buskirk 2006). However, Malo *et al.* (2011) did encounter a group-size effect on guanaco flight probability. The direction and intensity of the group-size effect on flight response shows a huge variation among and within species, and it is likely to be influenced by many variables (Stankowich 2008). Further research is needed to understand the factors underlying the different responses among guanaco populations.

Last, it is worthwhile to point out the high level of between-transect variability in guanaco flight response observed in RSP. Flight probability was considerably higher in weekly visited transects than in roads that were used on a daily basis. Even though particularly designed studies are needed to address the effect of traffic intensity, this preliminary result suggests that it may be a major factor affecting guanaco flight responses. A previous study showed that reindeer living in an area with medium level of human activity, with/without hunting, had weaker responses than did individuals living in an area rarely frequented by humans and with no hunting. This result supported the authors' prediction that reindeer exposed to high levels of

human activity were likely to habituate to human presence (Colman *et al.* 2001).

Temporal variation in flight response

Regarding temporal variation, flight probability decreased progressively and significant differences with initial conditions emerged during the fourth year of the study. The current flight response at RSP is consistent with an intermediate state between the contrasting settings of traditional managed ranches (80% of the groups fled) and C2B reserve, where guanacos did not flee at all. However, a larger sample size within private ranches will be needed to confirm this result. RSP outcomes indicated increased tolerance to traffic and were in accordance with a change in the guanaco's perception of the potential consequences of staying instead of fleeing when detecting an approaching vehicle, which supports the habituation hypothesis. In 2008, surveys were conducted in April, whereas in subsequent years, they were conducted in January–February. As previously suggested, mothers might show seasonal variation in their sensitivity to passing vehicles and this difference in survey dates may confuse our interpretation of temporal variation results. Because chulengos are born during November, mothers might be more sensitive during January–February when chulengos are younger; therefore, we would expect our habituation results to be the same or even more significant if surveys had been conducted on the same date every year. One of the few studies that have addressed habituation in an ungulate species according to Bejder *et al.* (2009) criteria is Haskell *et al.* (2006). Temporal variation in reindeer tolerance to oilfield infrastructure was observed in their summer range, following a northward spring migration from wintering areas in northern Alaska (Haskell *et al.* 2006). The authors found that this short-term habituation, determined by a measured decrease in overall avoidance of roads, reoccurred annually. We failed to find another ungulate study accounting for inter-annual variation with which to compare our mid-term habituation results. Although less studied among ungulates, habituation to humans has been well documented in primates. Since flight response is a major obstacle for observing the behaviour of wild apes, primatologists usually dedicate a preliminary period of their studies to habituate individuals to human presence (Bertolani and Boesch 2008; Jack *et al.* 2008). In a systematic study on chimpanzees (*Pan troglodytes*), Bertolani and Boesch (2008) found that habituation varied with sex and reproductive status and suggested that the critical factors affecting the rate of habituation were the frequency of human contact and the outcomes of these experiences.

If we consider that guanacos are long-lived animals (up to 14 years in the wild) (Amaya and von Thüngen 2001), our 4-year results indicate a rapid behavioural adjustment to recently changed conditions at RSP. In sedentary populations, such as the ones surveyed in the present study, guanaco family groups are territorial and remain roughly in the same location all year round (i.e. there is a high overlap between seasonal home ranges of the same group) (Burgi 2005). This fact could help explain the relatively fast habituation to traffic observed at RSP. Remaining in the same location might increase the relative exposure to repeated stimuli of passing vehicles, when compared with

dwelling animals where this exposure would appear occasionally. Second, guanacos fleeing in response to humans often enter other group territories and aggressive reactions of territorial males are triggered (A. Marino, pers. obs.), thus increasing the relative cost of fleeing. Both processes presumably help accelerate the wane of the flight response in absence of human harassment.

Final considerations

Our results suggest that, if harassment ceases and negative stimuli are replaced by neutral stimuli, guanacos can adjust their tolerance level rapidly. Guanacos within recently created reserves or ranches beginning eco-tourism exploitation might need some period of exposure to passing vehicles to learn that staying put has no negative consequences such as before, and those groups living far from more intensively used roads are expected to wane flight responses later than those living in more visited areas. There has been extensive debate on the pros and cons of habituation because its occurrence neither always implies beneficial outcomes for wildlife, nor that the animals are unaffected by the addressed disturbance (Bejder *et al.* 2009). However, flight events *per se* have further negative and observable consequences for guanacos in the short-term, besides the obvious extra energy expenditure, suggesting that this type of habituation within free-poaching areas might be beneficial. In guanaco populations habituated to human presence, such as at Torres del Paine National Park, Chile, mothers remain around their neonates and even might display aggressive behaviour towards the researcher if the chulengo is captured (Franklin and Johnson 1984). In contrast, in less tolerant populations, mothers usually escape with the rest of the group if a threat is detected. Most new born guanacos do not flee but remain hidden in vegetation, waiting for the return of the mother (V. Burgi, pers. comm.). But if females refuse to return in response to sustained disturbance, flight events may derive in permanent mother–neonate separation, increasing neonatal mortality. In addition, wire fences used to define paddocks and ranch limits are a threat to guanacos, particularly for young individuals because they often get entangled and die (González 2010; Rey 2010), or because mother–offspring reunion may fail if the young is left behind the fence when the group flees. The results obtained in the present study indicated that a guanaco population less habituated to human presence or which has recently experienced intense poaching might have a relatively greater proportion of groups fleeing in response to motor vehicles, and these groups will probably be those having chulengos among their members. Family and bachelor groups usually use different areas (Franklin 1983), a pattern that is evident in sedentary populations (A. Marino, unpubl. data) and can be easily assessed by local managers. This information might be useful if alternative traffic-circuits can be set within reserves during the birthing season to reduce flight frequency, especially near fences, until family groups become habituated to human presence. A similar measure was recommended after assessing the effect of tourists on guanaco responses (Malo *et al.* 2011). Although further research is required to assess the significance of these actions in terms of net conservation benefits, relatively low operation costs might warrant implementation when the aim is to recover extremely low-density populations. Overall, these results

highlight guanacos' ability to rapidly habituate to human presence if neutral stimuli operate for a few years, reinforcing the potential for tourist-oriented use of this species, as was already suggested by Malo *et al.* (2011). Finally, future studies on guanaco habituation rates should take into account the influence of traffic-intensity differences, in addition to group composition and group-vehicle distance, when assessing flight probability as a measure of tolerance to motorised vehicles.

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