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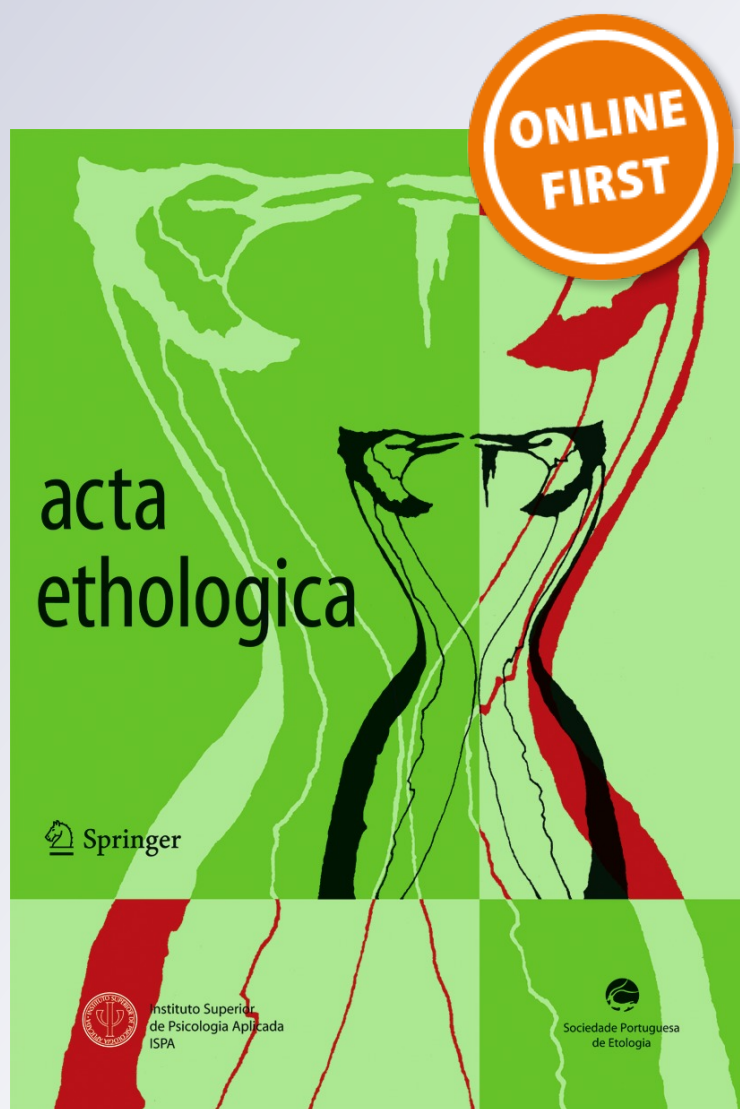
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Efficiency of antipredator training in captive-bred greater rheas reintroduced into the wild

**Marilina Vera Cortez · Diego J. Valdez ·
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Abstract High post-release mortality of captive greater rheas reintroduced into the wild might be mitigated with antipredator training that helps released individuals recognize their predators. We analyzed whether captive-bred greater rheas conserve antipredator behavior, and evaluated the efficiency of antipredator training by recording survival after reintroduction into the wild. Training involved 12 individuals and consisted of a stimulus representing the natural predator (taxidermized puma) paired to an aversive stimulus (simulated capture). The control stimulus consisted of an innocuous object (chair) that was not paired to the aversive stimulus. Thirty and 60 days after the last training session, we presented the trained and control individuals (nine untrained individuals) to the predator stimulus, which was not paired to the aversive one. All of the greater rheas showed vigilant and running behaviors in the presence of the predator model. Trained individuals did not discriminate between a predator and a non-predator stimulus but they recognized the predator up to 2 months later. Survival was nil 8 months after release. However, only one individual was killed by a puma, whereas the remaining individuals died due to dog attack and poaching. Training did not increase survival of reintroduced greater rheas because of the failure to consider other potential predators, such as dogs or humans. Therefore, captive breeding might have affected greater rheas' behavior by preventing them from recognizing man as a predator.

Introduction

Wild populations of greater rhea (*Rhea americana*) are declining as a consequence of natural habitat loss and poaching (Martella and Navarro 2006; Bazzano et al. 2007; Giordano et al. 2008; Bazzano 2010). In central Argentina, they have been reduced and isolated, and currently occur in highly fragmented agroecosystems (Giordano et al. 2010); as a consequence, the species is categorized as near threatened (IUCN 2013). Predictive models indicate that, should the current land-use conversion rates continue in this region, greater rhea populations might become fragmented and reduced to critical limits (Bazzano et al. 2014). However, this situation could be mitigated by reintroducing captive individuals (Navarro and Martella 2008; Bazzano et al. 2010; Navarro and Martella 2011). Indeed, there are records of successful translocations (Bellis et al. 2004; Navarro and Martella 2004; Martella and Navarro 2006) and that the genetic similarity between wild and captive populations is still preserved in central Argentina (Alonso Roldán et al. 2011).

Reintroduction of individuals is a widely used tool in wildlife management, despite the high post-release mortality rates generally recorded, mainly due to predation (Teixeira et al. 2007). In greater rhea, translocation success has mainly depended on the banning of poaching and the absence of the puma (*Puma concolor*), the only wild predator of the species at present (Navarro and Martella 2004). As observed in other species, failure of reintroductions might be related to the captive condition of the individuals being released, since captivity would not provide animals with the experiences necessary to effectively recognize predators and therefore ensure survival in their natural habitat (Kleiman 1989). Thus,

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captivity may induce changes and deficiencies in certain behaviors, such as foraging and hunting, social and reproductive interactions, locomotor capacities and antipredator behavior (Rabin 2003). These behavioral changes might be a disadvantage for animals reintroduced into the wild (Mc Phee and Silverman 2004); hence, experts suggest the need for training animals in recognizing predators prior to reintroduction (Kleiman 1989; Beck et al. 1994).

The use of antipredator training, involving the simultaneous presence of a predator and an aversive event, has shown an increase in the capacity of reintroduced individuals to recognize a predator (Griffin et al. 2000). This procedure has provided positive results in mammals (Miller et al. 1994; Griffin and Evans 2003), fish (Vilhunen 2006; Mesquita and Young 2007), birds (Maloney and McLean 1995; Azevedo and Young 2006a, b) and amphibians (Teixeira and Young 2013). However, few studies have demonstrated the relationship between training and post-release survival. For example, trained individuals of several species, such as red-legged partridges (*Alectoris rufa*), little owl (*Athene noctua*), houbara bustards (*Chlamydotis undulata macqueenii*), and prairie dog (*Cynomys ludovicianus*), had higher survival rates than untrained ones (Van Heezik et al. 1999; Shier and Owings 2006; Alonso et al. 2011; Gaudioso et al. 2011). Studies focused on antipredator training in captive greater rheas showed that the individuals were able to recognize a predator model (taxidermized puma) 3 months post-release (Azevedo and Young 2006b). It was later demonstrated that greater rheas could modify their behavior in the presence of predator and non-predator models, although they were not able to discriminate between them (Azevedo et al. 2012b). Therefore, Azevedo et al. (2012b) proposed that captive greater rheas should receive antipredator training before reintroduction.

In this context, the aim of this study was to analyze whether captive greater rheas receiving antipredator training maintained antipredator behaviors as well as to evaluate the efficacy of training by measuring survival after release into the wild.

Materials and methods

Study animals

For the study, we used 21 adult greater rheas between 2 and 3 years old bred under captive conditions at the experimental farm of Córdoba Zoo, Argentina (31°25′31.79″S, 64°10′29.92″W). These animals were identified with Velcro legbands and separated into two groups: the Antipredator Training group, comprising six males and six females; and the Control group, comprising five males and four females. Both groups were kept in separate corrals, with water and food (Vasquetto®) being provided ad libitum throughout the experiment.

Antipredator training

The antipredator training and memory test followed Griffin et al. (2001) and Azevedo and Young (2006a, b). Training was conducted between May and July 2011 and consisted of exposing greater rhea individuals to a stimulus (representing a predator) along with an aversive experience. The predator stimulus was mimicked by a taxidermized puma (*Puma concolor*) that was presented on a moving platform, appearing and disappearing from an opaque cubic structure located on one side of the corral. The aversive stimulus was a person, dressed in black to camouflage the silhouette, who appeared and disappeared from the lateral cubic structure, carrying a net and simulating animal chase. The control (innocuous) stimulus consisted of a chair that was used to detect if animals responded to the predator model and not simply to the movement of the platform. The training corral had all the walls covered with a dark and opaque plastic shade cloth, so that animals could not see what was happening outside the pen.

Each training session was filmed using a SAMSUNG camera MODEL SMX-F43BM/XB6 equipped with a 52× optical zoom. Each video recording was 18–19 min long. Once the animal was inside the corral, the behavior prior to predator presentation was recorded for 2 min. The predator model was then presented for 8–10 s, followed by the aversive stimulus. Finally, the predator stimulus was repeated. In total, conditioning lasted about 80–100 s, followed by 15 min video recording (following the method of Azevedo and Young 2006a, b). Training with models (puma and chair) was performed in a similar way, but only the appearance of the puma was paired to the presentation of the aversive stimulus.

Each greater rhea received five sessions with the puma model and five sessions with the chair model. Taking into account that training sessions might be considered a stressful situation as well as the findings of Della Costa et al. (2012), who reported that individuals undergoing a stressful experience show stress-related behaviors for up to 3 days, we decided to conduct training sessions at 3 to 5 days intervals.

Memory test

The memory test was performed twice, 30 and 60 days after the last training session, between August and September 2011. The same 18–19 min test was applied to both trained and untrained (Control) individuals, and consisted of exposing the animals to the presence of the predator model without the aversive stimulus, with the aim of comparing behavioral responses between groups (according to Azevedo and Young 2006a, b). Trained individuals were tested in the training corral, whereas control animals were tested in a corral that was adjacent to their maintenance corral, and which was prepared similarly to the training corral (i.e., the walls were also covered with black shade cloth). This procedure was

followed due to it was not possible to transport control animals to the corral used for training sessions and memory tests of trained individuals, because the distance between corrals would have affected control animals' behavior. A few days after the memory tests were performed, a female of the Antipredator Training group suffered everted cloaca and died, but this event was not associated with the memory tests. Hence, this group was composed of 11 individuals (6 males and 5 females).

Behavior analysis

Greater rheas' behavior was analyzed in each of the training sessions and memory tests. For this purpose, an ethogram was generated by observing individuals' behaviors and following Azevedo and Young (2006a, b), Della Costa et al. (2012) and Sales et al. (2000) (Table 1). The behaviors were quantified using focal sampling with continuous recording in each session. Total time allocated to each behavior was expressed as seconds for both models (puma and chair).

Release method and radio tracking

Eight individuals from the Antipredator Training group and six from the Control group were equipped with CB-5 expansion collars (Telonics, Mesa, Arizona, USA). All 20 greater rheas were transported, maintaining the original group composition, in conditioned vehicles as proposed by Navarro and Martella (2011). The selected area was *Estancia Las Dos Hermanas Wildlife Refuge* (33°40′S, 63°19′W), in Arias, province of Córdoba (Argentina), 400 km away from the zoo. Once at the selected site, the animals were housed in temporary corrals built with plastic shade cloth and provided with water and food, following a soft-release strategy (Bellis et al. 2004). After 2 days, the lateral walls of the temporary corrals were manually removed, allowing animals to leave the site and move away by themselves. After release, each individual was tracked by direct observation during the first week, and for 4–19 consecutive days per month for 8 months, using a Telonics TR4 (168–172 MHz) portable receiver with a two-element Yagi Telonics antenna. Each individual was tracked

two to four times a day, at intervals greater than 2 h, to minimize dependency on successive radiolocations (Bellis et al. 2004).

Survival analysis

Survival of greater rheas was analyzed (expressed in days) from release up to 8 months later, and causes of deaths were recorded.

Statistical data analysis

Statistical analyses were performed using Infostat software (Di Rienzo et al. 2012). Behavioral data obtained from individuals that were presented the predator (puma) and non-predator (chair) models during training sessions were evaluated using a mixed model statistical analysis. Besides examining the effect of the two presented models (chair and puma) on behavioral differences, as expressed in the objectives, we also considered the effects of session days (1 and 5), sex (male and female) and the possible interactions that may have occurred between models and sessions and between models and sex. On the other hand, the interaction between sessions and sex was not analyzed because it did not seem to have biological significance. The correlation among observations over time was taken into account. So, a variable was generated to indicate the order in which sessions was performed. For each behavior, the analysis included individuals as a random effect. When necessary, response variables were transformed for normal distribution of residuals.

The data obtained from the memory tests were analyzed with non-parametric tests because they did not meet the requirements for parametric statistics. First, the possible differences between the memory test sessions (1 and 2) for each of the groups were compared using a Friedman non-parametric analysis. Then, the possible differences between the mean individual responses of Antipredator Training and Control groups were analyzed with a Kruskal-Wallis test. An *a posteriori* comparison was performed with a Fisher's LSD test (*alpha*=0.05).

Table 1 Description of greater rheas' behaviors

Antipredator	Running[a]	Runs parallel to wire-fences or across the pen
	Vigilant[a]	Still, standing, observes with head up
	Alert[a]	Walks from one side to the other, parallel to lateral wire-fences
	Hiding[a]	Hides behind a tree
	Jumping[a]	Jumps and hits the body against the wire-fence
	Defecation[a]	Excretes feces and urine
Relax	Foraging	Pecks the grass, the ground or shrubs while walking or being still
	Walking	Walks across the entire pen
	Resting	Sits with the neck straight or on its body

[a] Indicates those related to antipredator behaviors

A chi-square test was used to compare survival between Antipredator Training and Control groups. All the results are expressed for each group as the mean±standard error. A significance level of 0.05 was employed in all statistical analyses. All the analyses were performed using raw or transformed data; however, the figures show the means and standard deviations of time devoted to each behavior, expressed in percentage of time. Only those results showing significant differences are shown in the figures.

Results

Antipredator training

Behavioral responses of Antipredator Training group

Behavior responses were observed throughout the training period, both after exposure to predator (taxidermized puma) and control (chair) models. Of all the behaviors evaluated, only running, vigilant, defecation and walking showed significant differences between models presented (Fig. 1).

Description of the observed antipredator behaviors

"Running" Only in the first session greater rhea individuals allocated more time to this behavior when exposed to the puma than to the chair ($F=3.77$, d.f.=4, $P=0.007$). In the remaining sessions there were no differences in running behavior due to the two models. No interaction between models and the sex of individuals was observed ($F=0.21$, d.f.=1, $P=0.64$) (Fig. 1).

"Vigilant" Individuals devoted more time to this behavior when exposed to the chair than to the puma. This response was influenced by the sex of animals ($F_{model-sex}=5.9$, d.f.=1, $P=0.017$). Males exhibited this behavior for a significantly longer period with the chair than with the puma. No significant differences were observed throughout the sessions ($F=0.58$, d.f.=4, $P=0.67$) (Fig. 1).

"Alert" This behavior was not influenced by the presence of the two models ($F=0.68$, d.f.=1, $P=0.41$) or the sex of individuals ($F=0.74$, d.f.=1, $P=0.41$). No differences were observed in alert behavior among sessions ($F=0.72$, d.f=4, $P=0.58$); there were no interactions between models and sessions ($F_{model-session}=0.94$, d.f.=4, $P=0.44$) or between models and sexes ($F_{model-sex}=1.99$, d.f.=1, $P=0.16$).

"Hiding" Mean times devoted to this behavior did not differ between models ($F=0.26$, d.f.=1, $P=0.61$), sexes ($F=0.00059$, d.f.=1, $P=0.98$) or sessions ($F=0.59$, d.f.=4, $P=0.67$). The tests showed that there was no interaction between the factors studied for this behavior ($F_{model-session}=2.45$, d.f.=4, $P=0.05$; $F_{model-sex}=2.84$, d.f.=1, $P=0.10$).

"Jumping" Mean time allocated to "jumping" did not differ between models ($F=0.09$, d.f.=1, $P=0.77$), sessions ($F=0.99$, d.f.=4, $P=0.42$) or sexes ($F=0.68$, d.f.=1, $P=0.43$). There was also no effect of the interaction of the factors analyzed ($F_{model-session}=0.27$, d.f.=4, $P=0.89$; $F_{model-sex}=1.02$, d.f.=1, $P=0.31$).

"Defecation" Although low frequencies of defecation behavior were recorded, sex of individuals influenced the response to the models ($F_{model-sex}=7.26$, d.f.=1, $P=0.008$). Thus, females responded similarly to both models presented, whereas males exhibited this behavior more frequently when presented with the puma than with the chair (Fig. 1).

Description of the observed behaviors associated with relaxation

"Foraging" Individuals exhibited this behavior as a response to the presence of the models, with no difference in time allocated to both models ($F_{model}=2.28$, d.f.=1, $P=0.13$), between sessions ($F_{sessions}=0.73$, d.f.=4, $P=0.57$) or sexes ($F_{sex}=4.28$, d.f.=1, $P=0.07$). No interactions were observed between models and sessions ($F=1.50$, d.f.=4, $P=0.20$) or between models and sex ($F=0.23$, d.f.=1, $P=0.63$).

"Walking" Greater rheas allocated more time to this behavior in the presence of the puma than of the chair model ($F=5.74$, d.f.=1, $P=0.018$). No significant differences were observed among sessions nor was there an effect of sex on response ($F_{session}=2.45$, d.f.=4, $P=0.05$; $F_{sex}=0.02$, d.f.=1, $P=0.89$) (Fig. 1).

"Resting" Although with low frequency, there were variations in the time devoted to this behavior among sessions, but without significant differences between models ($F_{model}=1.80$, d.f.=1, $P=0.18$), among sessions ($F=1.04$, d.f.=4, $P=0.39$), sex ($F=1.00$, d.f.=1, $P=0.34$) or their interactions ($F_{model-session}=0.72$, d.f.=4, $P=0.58$; $F_{model-sex}=1.98$, d.f.=1, $P=0.16$).

Memory tests

Antipredator Training group responses

In both memory tests, individuals belonging to the Antipredator Training group exhibited the same behaviors observed in the training sessions, except for "resting", which was not detected. In the second memory test, greater rheas on average allocated more time to the alert behavior than the first one (Friedman test $X^2=8.80$, $P=0.013$). The opposite was
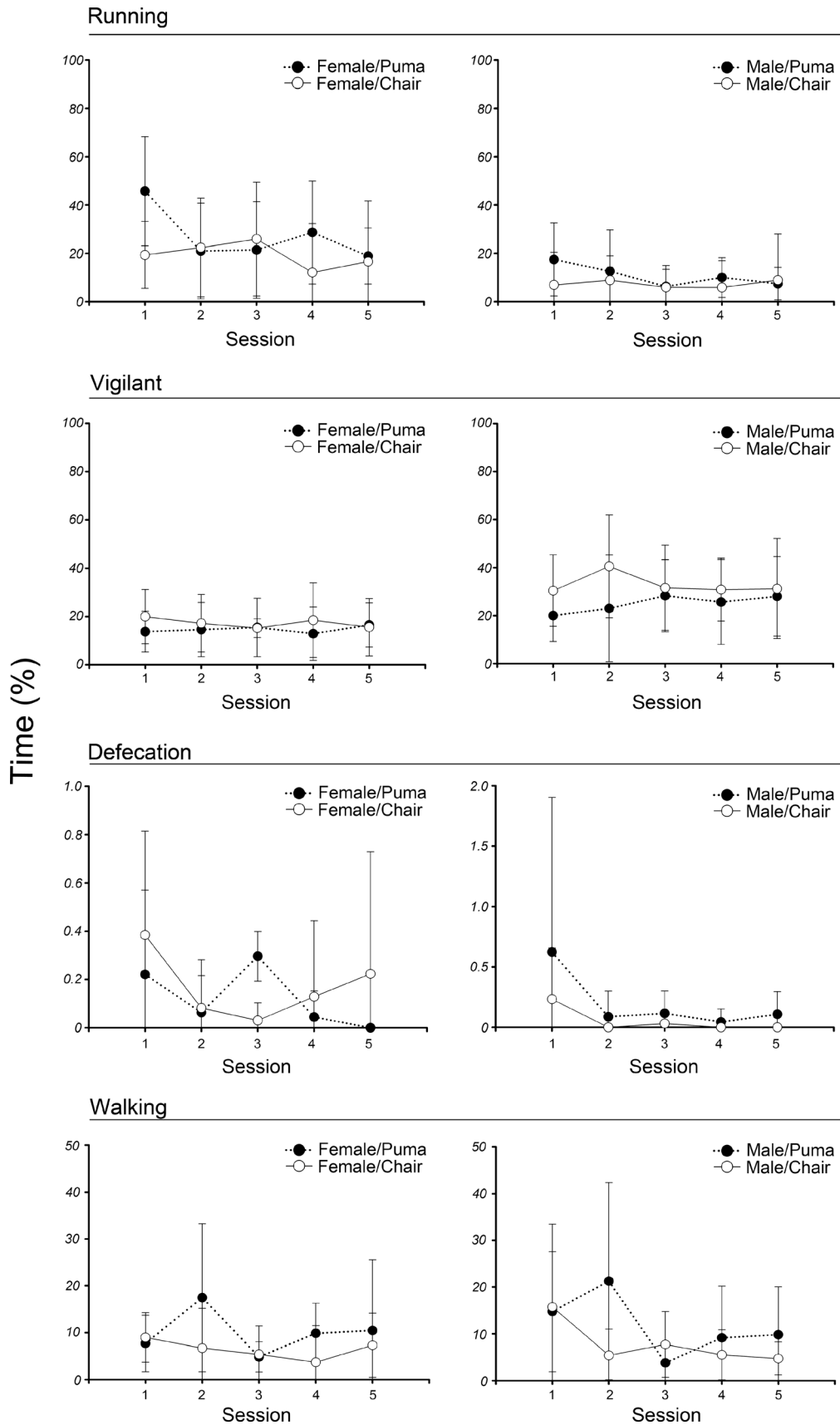
Fig. 1 Behaviors that changed significantly during antipredator training sessions in the group of trained greater rheas. Means and standard deviations of percentage of time devoted to each behavior are shown for puma and chair models, differentiated by sex

detected in "foraging" and "walking" behaviors ("foraging" $X^2=5.50$, $P=0.039$; "walking" $X^2=17.47$, $P=0.002$) (Fig. 2)

Control group responses

The Control group individuals showed the same behaviors as the individuals in Antipredator Training group, except for "foraging" and "resting", which were not recorded in either test. The remaining behaviors were observed in the two memory tests, but without significant differences (Friedman test: "alert" $X^2=0.10$, $P=0.76$; "running" $X^2=3.57$, $P=0.10$; "hiding" $X^2=0.31$, $P=0.59$; "jumping" $X^2=1.00$, $P=0.35$; "vigilant" $X^2=1.00$, $P=0.35$; "defecation" $X^2=0.00$, $P=>0.99$; "walking" $X^2=1.00$, $P=0.35$).

The comparison of the memory tests between Antipredator Training and Control groups showed that Control individuals on average allocated more time to "alert" and "jumping" behaviors than Antipredator Training ones (Kruskal-Wallis test: "alert" $H=5.17$, d.f.=1, $P=0.023$; "jumping" $H=9.78$, d.f.=1, $P=0.001$). The remaining behaviors did not show significant differences between groups ("running" $H=0.85$, d.f.=1, $P=0.36$; "hiding" $H=1.21$, d.f.=1, $P=0.23$; "vigilant" $H=0.25$, d.f.=1, $P=0.62$; "defecation" $H=0.99$, d.f.=1, $P=0.21$; "foraging" $H=1.64$, d.f.=1, $P=0.06$; "walking" $H=0.85$, d.f.=1, $P=0.35$) (Fig. 3).

Survival to translocation and post-release

No greater rheas were hurt or died during manipulation or transport. A female from the Control group was killed by poachers between 8 and 19 days post-release (dpr) and a male of the same group had to be euthanized because of a fractured leg due to a fall. Between 30 and 40 dpr, one Control male and one male and one female from the Antipredator Training died for dog attacks. The dead body of one Antipredator Training male was found with signs of puma attack. Fourteen individuals remained alive between 40 and
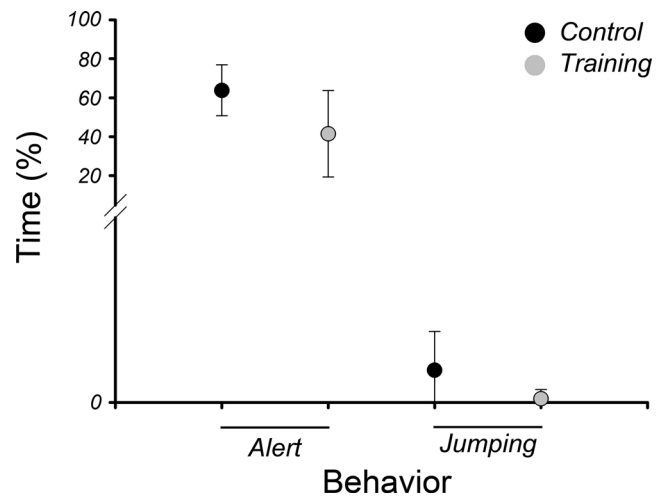

Fig. 3 Behaviors that changed significantly in the memory tests of the Control and Antipredator Training groups of greater rheas when exposed to the puma model. Means and standard deviations of percentage of time allocated to each behavior are shown

90 dpr: three males and three females from the Control group and four males and four females from the Antipredator Training group. This difference was non-significant ($X^2=1.17$, d.f.=1, $P=0.28$). At 120 dpr, only seven individuals were alive, three males and one female from the Control group and two females and one male from the Antipredator Training group.

Dog attacks and poaching were the causes of those deaths occurring between 40 and 120 dpr. During the 120 to 320 dpr period, it was not possible to locate the signals of some of the radio-tracked animals, so the tracked area was expanded to cover 1,718 ha. However, those animals were not found nor were the radio signals detected, except for one case in which we found a collar on the ground of a plot. At 320 dpr, survival was nil. Death causes were similar in both training and control groups, and were consistent with the causes recorded previously. Considering all released animals, and according to the bone remains found, 50 % of deaths ($n=10$) were caused by
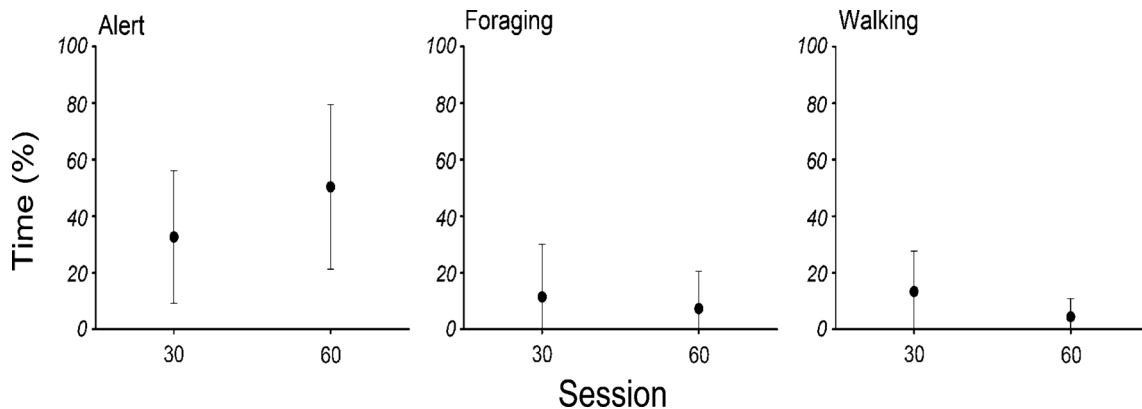

Fig. 2 Behaviors that changed significantly during memory test sessions of greater rheas of the Antipredator Training group. Means and standard deviations of percentage of time are shown for each session devoted to each behavior when the puma model was presented

dog attack, 40 % ($n=8$) by poaching and only one by puma attack. There were no significant differences between control and training groups ($X^2=2.22$, d.f.$=3$, $P=0.53$).

Discussion

Greater rheas exhibited the expected behaviors in the presence of the predator model. However, individuals also showed antipredator behaviors when the chair was presented, with greater amounts of time devoted to "vigilant" behavior. This result shows that greater rheas did not discriminate between a predator and a strange but innocuous object, confirming the recent proposal of Azevedo et al. (2012b). This aspect needs to be taken into account when undertaking the release of captive-bred animals into the wild, because it is important that they conserve the capacity to recognize a possible threat in the objects they encounter. Greater rheas should be able to distinguish between a predator and a non-predator model, so that once released, they do not respond to the presence of species that do not pose a definite risk. Consequently, individuals would avoid the loss of energy and time allocated to vigilance (Ferrari et al. 2009), and might invest time in other activities such as feeding or reproduction.

Another important difference between the presented models was that the greater rheas allocated more time to the "vigilant" behavior (at the expense of "resting") when the chair model was present. This result might be related to some morphological characteristics of the chair with respect to the puma. While the puma is brown and could be confused with the environment, the chair was white, contrasting with the corral background and floor colors, which made the chair presence more evident. For this reason, individuals may have allocated more time to vigilance. The "vigilant" behavior also varied with sex, with males investing more time to this behavior than females, which is in agreement with the behavior of the species in the wild (Reboreda and Fernandez 1997).

On the other hand, individuals allocated more time to "running" when presented with the puma than with the chair. This result might also be explained by some specific characteristics that the animals would recognize in the predator model, such as the position of the quadruped body and the location of the eyes in the front (Griffin and Evans 2003). The animals can possibly identify these characteristics and therefore, in the incident, they would respond more appropriately by escaping (Azevedo and Young 2006a).

Furthermore, we observed that males defecated more frequently in the presence of the puma model than of the chair, whereas females did not show differences between models. It has been reported that stressful or fear-inducing events can increase defecation behavior (Sanger et al. 2000; Mignon-Grasteau et al. 2003; Haas et al. 2010). However, during transport stress, the greater rhea did not modify the number of defecations (Della Costa et al. 2012). In the present study, the predator model may have been a more acute stressor than transport, inducing an immediate metabolic stress response for facilitating an antipredator response (running or hiding) and, therefore, increase defecation.

In the memory tests of the trained animals, "vigilant" and "running" behaviors were observed in the presence of the predator model, with "resting" being absent. This result suggests that greater rheas maintain the expected responses to a predator model after the end of training, which is consistent with the results of Azevedo and Young (2006a, b). Moreover, our results confirm that, under captive conditions, the greater rhea does not need a stimulus other than the predator, as stated by Griffin et al. (2000). This may be attributed to the relatively short time that this species has been in captivity compared with generational time and life expectancy of adults (Alonso Roldán et al. 2011), suggesting that these individuals still maintain the antipredator behaviors despite the captive conditions.

The comparison of the studied groups shows that greater rheas recognize any strange object, exhibiting antipredator behaviors even though the model is not pared to an aversive stimulus. This suggests that greater rheas can maintain the adequate behaviors when released into the wild, with no need for specific training as was done in this study.

The survival analysis showed that, although the first deaths occurred in the Control group, animals died independently of the group to which they belonged, i.e., regardless of whether they had been trained or not. This fact shows, contrary to what was expected, that antipredator training did not increase the chances of survival of captive-bred greater rheas that were released into the wild. Although the sample was relatively small and differences may appear with a larger sample, increasing the sample size would be economically expensive and would pose a risk for this threatened species. Finally, survival was nil 8 months after translocation. However, it should be noted that only one individual was predated by a puma, whereas most of the greater rheas died due to dog attacks or poaching, suggesting that training could have contributed, in part, to the individuals' capacity to recognize the puma as a natural predator.

According to the present results, future training tests should incorporate humans and dogs as predator models, since greater rheas do not associate man with a possible predator because they are in contact with humans during breeding and manipulation in captivity (Azevedo et al. 2012a). As for deaths produced by dogs, it may be necessary to perform tests and training to determine if greater rheas recognize dogs as predators. Although dogs might be predators nowadays (Navarro and Martella 2002), they were not present during the evolutionary history of greater rheas (Del Hoyo et al. 1992; Dani 1993).

Conclusions

Our work shows some relevant points to be considered in the design of future translocations for greater rhea conservation: (1) the training method used showed that greater rheas conserve antipredator behaviors, although they fail to distinguish between a predator and non-predator model; (2) training did not contribute to an increase in survival of reintroduced greater rheas; (3) inefficiency of training was based on the failure to consider other potential predators, such as dogs and humans; (4) contact with humans during captivity may affect the individuals' behavior, preventing them from recognizing man as a possible predator; (5) as effective poaching control is very difficult to accomplish, it would be important to conduct training that considers man as the principal predator, since humans play a key role in the success (or failure) of reintroductions.

Integrity of research and reporting The manuscript submitted for publication complied with the current laws of Argentina.

Conflict of interest The authors declare that they have no conflict of interests.

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