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ORIGINAL ARTICLE

Stress response assessment during translocation of captive-bred Greater Rheas into the wild

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Abstract Translocation is an extensively used conservation tool that involves exposing animals to stressful situations that may influence the post-release survival. In this study, 20 Greater Rhea (*Rhea americana*) adults hatched and reared in captivity were translocated to a wildlife refuge. After transport and before release, animals were kept in pens at the liberation site to make a "soft-release" strategy. Fecal glucocorticoid metabolites (FGM) were monitored during pre-transportation, post-transportation and pre-release, and post-release phases as an indicator of the stress of translocation and acclimation to the new environment. During pre-transportation phase, FGM levels found were consistent with the baseline concentrations described for this species for males and females, respectively. On day 1 after transportation, FGM levels were increased in both sexes, returning to baseline values during the maintenance in the pens. Although the handling and transportation triggered an acute stress response, the procedures used and the soft release in pens allowed Rheas to reestablish quickly baseline FGM levels. After release into the novel wildlife refuge, FGM levels were increased again and remained similarly increased during the following 2 months of the study. Findings suggest a strong chronic stress response, probably triggered by a combination of many factors (i.e. novelty, attacks from predators, social interactions, human related disturbances such as poaching, vehicular noise, hunting dogs) that may reduce the bird's ability to solve new challenging situations, especially the illegal hunting pressure that seems to be a significant threat in this species.

Keywords Translocation · Stressors · Fecal glucocorticoid metabolites · Non-invasive monitoring · Near threatened species · Ratite

Zusammenfassung

Bewertung der Stress-Reaktion auf die Umsiedlung von in Gefangenschaft aufgezogenen Nandus (*Rhea americana*) ins Freiland.

Im Naturschutz werden Umsiedlungen häufig vorgenommen und stellen für die betroffenen Tiere stets einen besonderen Stress dar, der möglicherweise einen Einfluss auf ihr Überleben nach der Umsiedlung hat. In unserer Untersuchung wurden 20 adulte, in Gefangenschaft geschlüpfte und aufgezogene Nandus in ein Naturschutzgebiet umgesiedelt. Nach dem Transport, aber vor ihrer Freilassung, wurden die Tiere im Sinne einer „sanften Freilassung"zunächst noch in Gehegen innerhalb des Naturschutzgebiets gehalten. In den Phasen „vor Transport", „nach Transport", „vor Freilassung"und „nach Freilassung"wurden fäkale Glukococorticoid-Metaboliten (FGM) als Indikatoren für den Stress durch den Transport und durch das Einleben in die neue Umgebung gemessen Die FGM-Konzentrationen in der Phase vor dem Transport

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entsprachen den für beide Geschlechter bereits bekannten Basiswerten. Am Tag 1 nach dem Transport waren die Werte für beide Geschlechter erhöht, gingen aber während des Aufenthalts in den Gehegen wieder auf die Basiswerte hinunter. Obwohl das Einfangen und der Transport bei den Tieren eine akute Stress-Antwort auslösten, bewirkten die eingesetzten Methoden und die „sanfte Freilassung"in die Gehege, dass die Tiere ihre Basis-FGM-Konzentrationen rasch wieder einstellten. Nach der endgültigen Freilassung in das für sie neue Naturschutzgebiet stiegen die FGM-Werte wieder an und blieben danach noch zwei Monate lang auf dem erhöhten Niveau. Diese Ergebnisse weisen auf eine intensive Stress-Antwort hin, die von der Kombination vieler Faktoren (neue Umgebung, Angriffe durch Räuber, soziale Interaktionen, Störungen durch Menschen wie z.B. Wilderei, Fahrzeuglärm, streunende Hunde) getriggert wird und eventuell die Fähigkeit der Vögel vermindert, neuen Herausforderungen erfolgreich zu begegnen, vor allem der illegalen Jagd, die gerade für diese Art eine ganz besondere Bedrohung darstellt.

Introduction

Agricultural intensification over the last few decades in Argentina has resulted in reduction and fragmentation of landscapes inhabited by Greater Rheas (*Rhea americana*). The replacement of large areas of natural grasslands and planted pastures by annual crops, in particular soybean, has drastically decreased free ranging populations of this ratite (Bazzano et al. 2014; Giordano et al. 2008, 2010; Navarro and Martella 2008, 2011). This situation has led to the classification of the species as ''Near Threatened'' by the International Union for Conservation of Nature and Natural Resources (IUCN 2014). A population viability analysis previously developed for this species in an agro-ecosystem of central Argentina shows that given that the suitable habitat for Rheas might be drastically reduced in this region, the viability of Rhea populations in agro-ecosystems would be facing higher risks of local extinction, unless translocations are performed (Bazzano et al. 2014).

Despite the increasing use of translocation as a strategy to manage and conserve wildlife species, its success is typically low (Fischer and Lindenmayer 2000; Griffith et al. 1989). The failure is frequently attributed to the fact that translocated animals survive poorly during the first weeks after release (Armstrong and Seddon 2008; Cabezas et al. 2011; Wolf et al. 1996), mainly due to predation, starvation, disease, and dispersal (Dickens et al. 2010). Stress has also been proposed as a relevant physiological response affecting translocation that has not been fully considered in previous studies (Dickens et al. 2010; Teixeira et al. 2007). Stress responses include activation of the HPA axis and releasing glucocorticoids that are necessary to support adaptive behaviors (i.e. fight or flight response) (Harvey et al. 1984; Kuenzel and Jurkevich 2010). Therefore, stress responses are undoubtedly expected following all steps of the translocation (capture, transport, acclimatization, and release into an unfamiliar environment). If the birds are not quickly adapted to the new environmental/social situations, the physiological re-establishment of homeostasis via feedback mechanisms can be delayed (Blas 2015), and post-release survival may be affected. Different mechanisms may contribute to augment the risk of mortality, including suppression of the immune system (susceptibility to infectious disease), attenuation of subsequent adrenocortical fight- or flight-responses (susceptibility to predation), increase in energy expenditure and decrease in food intake, and/or feed conversion rate (susceptibility to starvation) (reviewed in Dickens et al. 2010; Teixeira et al. 2007). Thus, minimizing stress during translocation or reducing the adaptation time period could indirectly reduce many direct mortality risks simultaneously.

Some captive-release experiences with Greater Rheas have been carried out in Argentina with variable success (see Navarro and Martella 2008), but to date, no assessment of stress hormone responses to translocation has been conducted. Recent studies on Greater Rhea stress physiology demonstrate that this species shows an exacerbated adrenocortical stress response to an ACTH challenge in comparison to other bird species (Lèche et al. 2009, 2011). Moreover, short-term road transportation induced a more than 40-fold increase of the corticosterone basal levels (Lèche 2012), and altered behavioral responses after transport (Della Costa et al. 2013).

The highly stressful nature of Rheas, and the inescapable stress component of translocations, led us to undertake this study to evaluate fecal glucocorticoid metabolite (FGM) levels both in male and female Greater Rheas during different phases of the translocation procedure (pre-transportation, post-transportation and pre-release, and post-release). The results obtained will allow us to determine which phases are most stressful and/or critical, providing information that may help to take decisions aiming to improve future success during translocations.

The measurement of FGM levels excreted in the feces is considered an appropriate and effective non-invasive method for assessing stress in translocation programs (Aguilar-Cucurachi et al. 2010; Franceschini et al. 2008; Gelling et al. 2012). This methodology enables sample collection without disturbing or handling the animals, repeated sample collection during comparable daytime periods, and the assessment of long-term FGM patterns (Palme et al. 2005; Sheriff et al. 2011; Palme 2012). Indeed, fecal glucocorticoid metabolites have already been successfully used for monitoring adrenocortical activity in captive and wild Greater Rheas (Lèche et al. 2011, 2014).

Materials and methods

Study animals

Twenty adult Greater Rheas (11M:9F) hatched in captivity at the experimental farm at Córdoba Zoo, Argentina (31°25′31.79″S, 64°10′29.92″W) were randomly selected for this study. Eight months before the start of translocation, the animals were separated in two groups (6M:5F and 5M:4F) and housed in two outdoor pens. Pens were fenced with 2-m-high woven-wire mesh and had soil with bare ground. Food and water were provided ad libitum. Each animal was identified with Velcro leg-bands.

Release area

Within the natural range of this species, we selected as an appropriate release site Las Dos Hermanas Ranch, which is a privately owned wildlife refuge located in Arias, about 400 km southeast of Córdoba Zoo, Córdoba Province (Argentina) (33°40′S, 63°19′W). It has an area of 4189 ha with different types of habitats devoted to cattle raising and conservation purposes. Native vegetation in this area included grasslands, shrubs, salt flats, and saline marshes (Cantero et al. 1994). The main herbivores inhabiting the area were cattle, horses, sheep, and hares. Greater Rheas had not been seen since 2006. It is conceivable that they were subjected to hunting (ranch manager, personal communication). At the time of this study (2011–2012), hunting was controlled by the ranch's staff, which largely determined the choice of this site to carry out the translocation.

Translocation procedure

The transportation was realized during summer (breeding season). On the transportation day (07:30 pm) each of 14 individuals (7M:7F) were equipped with a CB-5 expansible collar with a radio-transmitter (Telonics, Mesa, AZ, USA). Then, the Greater Rheas were loaded and transported according to the procedure recommended by Navarro and Martella (2011). For loading, Rheas were individually caught by grasping the base of the wings and gently guided into the vehicle. They were transported maintaining their original group composition in two conditioned vehicles. Rheas were transported overnight, following Crowther et al. (2003), who proposed that this is potentially beneficial for reduction of stress and maintenance of welfare in Ostriches (*Struthio camelus*). After a 5 h trip (from 10:00 pm to 03:00 am), they arrived at the release site before dawn. Upon arrival, each Rhea group was immediately unloaded into a pen built with a plastic shade cloth at the release site. Rheas got off the vehicle on their own through a horizontal unloading ramp. Animals were housed there for 3 days and were provided with water and food ad libitum, following a soft-release strategy (Bellis et al. 2004). We decided to leave the birds in these pens for 3 days based on our preliminary works, which showed that 72 h after transportation Greater Rheas recover FGM basal levels (Lèche 2012) and behavioral activities are stabilized (Della Costa et al. 2013). On the afternoon of the third day, the lateral walls of the temporary pens were manually removed, allowing animals to leave the site and move away freely by themselves. After release, animals were tracked by direct observation or using a Telonics TR4 (168–172 MHz) portable receiver with a two-element Yagi Telonics antenna, and fecal samples were collected along three sampling periods (see "Fecal sample collection").

Fecal sample collection

Fresh fecal samples were collected at three phases of the translocation procedure. (1) Pre-transportation: at Córdoba Zoo before being transported. Samples were collected twice a day (morning: 10:00–12:00; afternoon: 14:00–18:00) during three sampling days preceding transportation (5, 3, and 1 days before transport). (2) Post-transportation and pre-release: after transport at release site during three consecutive days while Rheas were held in their pens. The first 2 days all fecal samples were collected between 8:00 and 20:00 and the third day between 8:00 and 14:00. (3) Post-release: at the release site during three sampling periods over time: 4–6, 25–34, and 58–60 days after release. Fecal samples were collected following observation of defecation when Greater Rheas were located, identified, and tracked.

Fecal samples were placed individually into a labeled plastic bag, and time of collection, location of collection and identification of the animal was recorded. Samples were frozen within 2 h of deposition, first on dry ice (avoiding direct contact) and then in a freezer at −22 °C until steroid analysis (Lèche et al. 2011).

It is important to recall that unlike most avian species, ratite urine is stored and excreted separately from feces (Stewart 1994) and, therefore, it is not mixed in dropping material.

Steroid analysis

Fecal glucocorticoid metabolites levels were determined using the commercial ^{125}I corticosterone RIA kit (MP Biomedicals, Costa Mesa, CA, USA) successfully validated for this species (Lèche et al. 2011). FGM concentrations are expressed as nanograms per gram of wet fecal matter (ng/g). Inter-assay variation for five assays was 9.4 % and average intra-assay variation was 3.9 %.

Data analysis

Statistical analyses were performed using the Infostat statistical software package (Di Rienzo et al. 2012).

FGM data during the three phases of translocation were evaluated performing mixed-model statistical analysis, considering the effects of days (see below), time of sampling (morning–afternoon), sex (male and female), and the effects of interactions between these factors. By including animal identity as a random variable, we are considering that the hormone concentration variable was measured in the very same individual each day and/or time of sampling, thus incorporating the "repeated sampling" effect into the analysis (Balzarini et al. 2008). For normality of residuals, FGM data during the pre-transportation and post-release phases were Ln-transformed, and data during post-transportation and pre-release phase were transformed to sequential ranks (Shirley 1987). Fisher's LSD was used for pair wise comparison. The significance level was set at 0.05.

The pre-transportation phase model considered Ln FGM concentration as the dependent variable, and days (5, 3, and 1 pre-transportation), sex, and time of sampling as fixed factors. We included Identity variance function to days and the animal as random factor (Mod 1).

The post-transportation and pre-release phase model considered rank FGM concentration as the dependent variable, and sex, days (1, 2, and 3 post-transportation), and time of sampling as fixed factors. We included identity variance function to days and animal as random factor (Mod 2). Because it was not possible to get a sample every day from each animal, the interactions sex × day × time of sampling and day × time of sampling were not possible to be included in the analyses. In particular, we evaluated the effect of sex and time of sampling on FGM levels during day 1, considering the dependent variable rank FGM concentration, sex, and time of sampling as fixed factors, and animal as random factor (Mod 3).

The post-release phase model considered Ln FGM concentration as the dependent variable, and sex and sampling periods (4–6, 25–34, and 58–60 days after release) as fixed factors, and animal as random factor (Mod 4).

We used a paired Student's *t* test to compare FGM levels obtained during each day of the post-transportation and pre-release phase, with the FGM average levels found during the pre-transportation phase. Post-release phase FGM levels were compared to the averaged pre-transportation phase levels, and post-release phase FGM levels were also compared to the levels found during day 3 of post-transportation and pre-release phase. Differences were considered significant at $P < 0.05$.

Results

Fecal glucocorticoid metabolite concentrations of Greater Rheas at the three phases of the translocation procedure are shown in Fig. 1.

The FGM average value obtained during pre-transportation phase was higher in males (13.31 ± 0.40 ng/g) than in females (6.99 ± 0.27 ng/g) ($F_{1,18} = 107.51$, $P < 0.01$, Mod 1). These FGM levels did not differ among days ($F_{2,90} = 0.61$, $P = 0.54$, Mod 1) nor time of sampling ($F_{1,90} = 3.34$, $P = 0.07$, Mod 1).

On day 1 after transportation, both male and female Rheas showed significantly increased FGM levels compared to the average pre-transportation values found in Cordoba Zoo ($T = 2.78$, $P < 0.05$ and $T = 4.27$, $P < 0.01$, for males and females, respectively). On the following days at the release pens (days 2 and 3 of post-transportation and pre-release phase), male FGM levels were found to be similar to pre-transportation values ($T = -1.22$, $P = 0.24$ and $T = -1.83$, $P = 0.10$, respectively). However, female FGM levels still remained increased during day 2 ($T = 2.52$, $P < 0.03$) and dropped to pre-transportation values on the 3rd day after transport ($T = 0.59$, $P = 0.51$).

A further analysis of the post- transportation and pre-release phase response showed that FGM levels were influenced by the passing of days interacting with the sex of the birds ($F_{2,75} = 3.58$, $P < 0.04$, Mod 2). While no differences were found between male and female FGM values on day 1, on the following days (days 2 and 3), males showed again higher FGM values than their females counterparts ($P < 0.05$ in both cases). In general, FGM levels were found to be similar between morning and afternoon ($F_{1,75} = 0.69$, $P = 0.41$, Mod 2). However, both male and female day 1 FGM levels significantly diminished ($F_{1,31} = 19.5$, $P < 0.01$, Mod 3) in the afternoon (16.08 ± 2.75 ng/g and 14.85 ± 2.85 ng/g, respectively) compared to morning (28.91 ± 3.57 ng/g and 32.08 ± 6.36 ng/g).

During the post-release phase, FGM values in both sexes were found again to be significantly increased compared to the averaged pre-transportation levels ($T = 6.03$, $P < 0.01$ for males; $T = 5.31$, $P < 0.01$ for females) and also compared to the levels found during day 3 of the post-

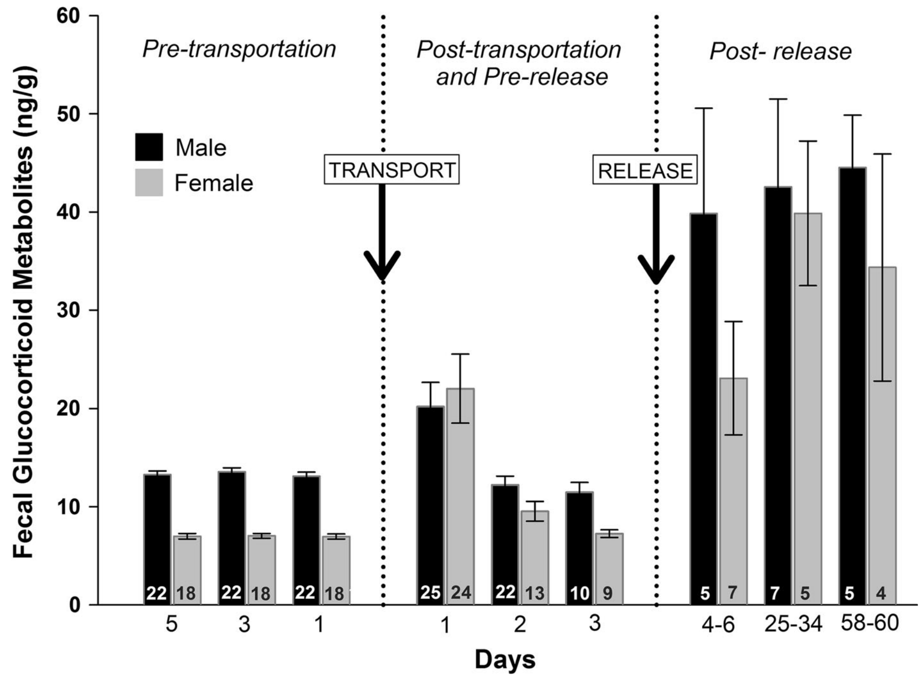

Fig. 1 Fecal glucocorticoid metabolites levels (mean + SE) in the three phases of translocation procedure for male and female captive Greater Rheas. Pre-transportation: sampling at days 5, 3, and 1 before transport ($n = 11$ for males; $n = 9$ for females). Post-transportation and pre-release: sampling at days 1–3 after transport ($n = 11$ for males; $n = 9$ for females). Post-release: sampling at days 4–6 ($n = 5$ for males; $n = 5$ for females), 25–36 ($n = 5$ for males; $n = 4$ for females), and 58–60 ($n = 4$ for males; $n = 4$ for females) after release. The number of samples collected for each group is given within the bar just above the axis

transportation and pre-release phase ($T = 7.46$, $P < 0.01$ for males; $T = 8.69$, $P < 0.01$ for females).

The FGM levels during post-release phase did not differ between sexes, nor did they change over the course of the sampling periods ($F_{1,12} = 1.80$, $P = 0.20$; $F_{2,15} = 0.96$, $P = 0.4$, respectively, Mod 4).

At the end of the study period (60 days following release), 13 Rheas survived (7M:6F). Mortality was attributed to natural predators (Puma $n = 1$ M), domestic predators (dogs $n = 2$ M:1F), and hunters ($n = 2$F). Another Rhea (1 M) was euthanized after it was found with a leg fracture.

Discussion

This study demonstrates that this translocation procedure leads to changes in the stress physiology of captive Greater Rheas. Fecal glucocorticoid metabolite levels increased after transportation, then quickly returned to baseline levels when Rheas were held in pens (soft-release strategy), and they increased again after release into a novel habitat; the values remained higher than pre-transportation levels over the whole sampling period.

Fecal glucocorticoid metabolite levels found at pre-transportation phase at Zoo Córdoba were similar to baseline FGM values reported by Lèche et al. (2015) for captive Greater Rheas during the reproductive period. Also, the present values were significantly higher in males than in females, probably due to the fights between them, as had been obtained by Lèche et al. (2015), this being characteristic of the mating system of this species.

Elevated FGM levels observed during the first day of post-transportation and pre-release phase, when animals were held in pens at the release area, suggest that transport/manipulation for about 5 h, as it would be expected, triggered an acute stress response. It is well known that transport normally exposes animals to a wide range of stressors (reviewed in Fazio and Ferlazzo 2003). The act of transportation leads to increased glucocorticoid secretion across a range of bird species including ratites (Lèche et al. 2013; Menon et al. 2014). In Greater Rheas, a particularly high stress response was observed, suggesting that manipulation and transport are potent stressors for this species. Rheas transported for 30 min show a 40-fold increase in the corticosterone levels (Lèche et al. 2013) and a 10-fold FGM peak between 4 and 6 h after transportation. FGM levels recovered baseline levels about 30 h post transportation (Lèche 2012). Because in the present study we were unable to collect fecal samples until 10 h after the onset of transport, it is possible that we have missed the glucocorticoid peak response, and the observed FGM levels were instead a point toward the recovery to pre-transportation levels. Because immediate release after transportation involves also a new stressful situation (the placement in a novel environment), the observed FGM levels after transportation could include both the effects of transportation and novel environment. However, considering that FGM are always giving a delayed information about blood circulating glucocorticoids (Palme 2005;

Touma and Palme 2005), and that in the afternoon of the day of placement in the pre-release pen FGM values were found near pre-release basal levels, it is highly likely that the new environment has in this case a minor effect on the birdś stress response (as it has very similar conditions to their original pens at the Zoo).

Interestingly, this study showed that the animals (mixed-sex groups) transported at night for 5 h in an adapted truck recovered baseline glucocorticoids levels in a shorter time lapse (16 h) than Rheas transported individually in the daytime for 30 min in wooden crates (1.5 m high × 1.5 m long × 0.90 m wide) (Lèche 2012). Thus, one can conclude that transport conditions used in the current study were less stressful than in the previous reported study. These results could be explained considering: (1) the usual response of Rheas to darkness is to sit, which provides greater stability for the birds while the transportation unit is in motion (Lèche, personal observation). In this sense, Crowther et al. (2003) have shown that during ostrich night transportation many of the birds choose to sit, and the sitting response resulted in a lowering of heart rate, suggesting a lower level of stress reaction; (2) temperatures are lower at night, which may also contribute to the lowering of heart rate, as observed in ostriches by Crowther et al. (2003). This is consistent with a recent publication showing that emus transported for 6 h in warm weather were more stressed than birds transported in cooler weather (Menon et al. 2014); (3) animals transported in groups are expected to experience fewer stress reactions than when transported in isolation (Kay and Hall 2009; Tamashiro et al. 2005). Moreover, familiarity with conspecifics within the release group or with those inhabiting the novel environment, such as family groups (Shier 2006) or social groups (Pinter-Wollman et al. 2009), can decrease stress related reactions (Guzman and Marin 2008; Marin et al. 2001). These results are interesting if we consider that a general strategy is to decrease the number and magnitude of exposure to stressors associated with all the phases of translocation involved (in this case transportation). This strategy helps to reduce the impact of chronic stress on the physiology and behavior of the animals and, therefore, to diminish the vulnerability of the translocated population to other potentially succumbing external factors (e.g. predators or pathogens) (Dickens et al. 2010; Teixeira et al. 2007).

As mentioned earlier, levels of FGM diminished to nearly baseline concentrations in the afternoon of the first day post-transportation (after 16 h) in Rheas of both sexes. Thus, apparently, the "soft- release" strategy used provided time to recover from stress induced by handling and transportation, and allowed Rheas to reestablish normal (pre-transportation) FGM levels. Additionally, this phase decreased novelty of the release site, since animals may have time to adjust to their new surroundings without additional stress of immediate needs to search for resources or facing predation. In several species, the increasing probability of establishment of a healthy population at the release site has been attributed to the "soft-release" procedure (Devineau et al. 2011; Parker et al. 2008; Tuberville et al. 2005). In contrast to studies demonstrating that captivity in the "acclimatization" enclosures is a stressful phase of translocation, we found a return to pre-transport baseline levels in the afternoon of the first day and during the third day of post-transportation and pre-release phase for males and females, respectively. Possibly, captive-bred birds are at least to some degree habituated to handling, enclosures and human presence, and thus they would be less sensitive to translocation compared to the wild-caught birds used in other published studies (Aguilar-Cucurachi et al. 2010; Franceschini et al. 2008). In this sense, FGM concentrations in captive-bred Whooping Cranes (*Grus americana*) also returned to basal levels within 1 week after transportation to another site (Hartup et al. 2005). Consistent with this, Jenni et al. (2015) also demonstrated in translocated captive Grey Partridges that after only 33 h of acclimatization, corticosterone levels were almost back to pre-transport levels.

During the post-release phase, FGM levels were increased and remained high up to 2 months following release, suggesting that at least within the time frame evaluated, the challenges presented in the new open environment generated a chronic stress in Rheas. After release, Rheas were exposed to many factors (novelty, human disturbance, attacks from predators, new social interactions), which may have triggered this chronic stress response. Novelty is a potent psychological stressor that stimulates both the glucocorticoid response as well as the fight-or-flight response in laboratory rodents (Weinberg and Wong 1986), farm animals (Lewis et al. 2008), and avian species (Beuving and Blokhuis 1997). In fact, studies often use introduction to a small novel environment as an experimental acute stressor (e.g. Kembro et al. 2008; Marin et al. 1997). Indeed, when the new environment implies a significantly increased and enriched ground extension to be explored, as the one used to translocate Rheas in this study (4189 ha), new threatening challenges are often presented, and therefore, the impact on bird behavior and physiology are expected to have long-term consequences. Human activity is normally accompanied by various kinds of environmental disturbances, for example, noise and vibration, due to the activity of people, machinery, and vehicles. Various studies showed that such stimuli can lead to stress responses in animals. Disturbance from traffic noise increased glucocorticoid stress hormone levels (e.g. corticosterone) in American Kestrel (*Falco sparverius*), and even induced reproductive failure (Strasser and Heath 2013). In American Flamingos (*Phoenicopterus ruber*) disturbances by tour boats reduced feeding time and, probably, reproduction (Galicia and Baldassare 1997). Anthropogenic activities and vehicular noise associated with hunting may also influence wildlife behavior (Ciuti et al. 2012) and stress hormones (Creel et al. 2002). Recently, Bryan et al. (2014) observed higher stress and reproductive hormone levels in wolves from heavily hunted populations. In our study, intensive poaching pressure was detected despite ranch control. Moreover, the presence of dogs was observed, being associated with hunters. Predators, especially when dealing with captive-bred animals, can be a source of stress (Boonstra et al. 1998). Although a natural predator of Rheas is the Puma (*Puma concolor*), nowadays, humans and dogs are to be considered the main predators (Martella and Navarro 2006; Navarro and Martella 2008, 2011; Vera Cortez et al. 2015). Social factors (e.g. attacks from dominant individuals, group restructuring) are also known to influence physiological states of vertebrates (McEwen and Wingfield 2003).

Generally, the duration of elevated stress response following release has been linked to the sensitivity of a species, the intensity and duration of stressors, and the number of stressors encountered (Dickens et al. 2010). Studies have demonstrated that physiological acclimatization (i.e. decline from elevated stress hormone levels) can require an extended period of time (Franceschini et al. 2008; Jachowski et al. 2012, 2013). Unfortunately, as our sampling was restricted to 60 days of release, we could not assess whether the Rheas were or not acclimatized to the novel environment, in the strict sense of exhibiting a decrease in their stress hormone levels.

Predation was the main cause of mortality during the 2 months after release. Teixeira et al. (2007) indicated that an impaired cognitive ability due to chronic stress can affect fight-or-flight performance making the animal less likely to escape a predator's attack. Thus, it could be possible that the high levels of FGM observed in this study cause the Rheas to be more susceptible to predation. However, a more intensive and prolonged sampling would be required to evaluate whether there is a relationship between high FGM levels and Rheas' mortality or survival.

Conclusions

Translocation leads to changes in the stress physiology of captive Greater Rheas. Although manipulation and transport triggers an acute stress response, the handling and transportation procedures utilized in this study minimized the stress. The "soft release" strategy seems to be important, since it provides time to recover from stress induced by handling and transportation and allowed Rheas to reestablish baseline FGM levels. However, after release, FGM increases and remained elevated up to at least 2 months following release. This result is consistent with an induced chronic stress state probably triggered by a combination of many factors (i.e. novelty, attacks from predators, social interactions, human related disturbances such as poaching, vehicular noise, hunting dogs) that may reduce the birds' ability to solve new challenging situations, especially the illegal hunting pressure that seems to be a significant threat to this species.

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