Behavioral responses to short-term transport in male and female Greater Rheas (*Rhea americana*) reared in captivity

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ABSTRACT Animal transport is an indispensable practice in species that need to be moved for management or commercial purposes. However, transport may have negative effects on individuals' welfare. The aims of the present work were to determine if the behavioral responses of adult Greater Rheas (*Rhea americana*) bred in captivity are sensitive to short-term transport and if males and females differ in their posttransport behavioral activity and recovery. Eight males and 8 females were placed in individual pens and allowed 6 d to habituate (d 1 to 6) before transport procedure. On the transport day (d 7), half of the birds (4 males and 4 females) were randomly assigned to a transport group that was captured and handled to be placed into the crates, exposed to a 30-min transport stressor, and immediately returned to their pens. Four transports with 1 different male and female each time were performed. The other half remained undisturbed and were used as controls. Behavior of all individuals was video-recorded during habituation days, after transport on d 7, and on the 2 following days (d 8 and 9) to evaluate pre- and posttransport behavioral activity for 2 h per day. No significant behavioral changes were observed during the last 2 d of the habituation period (d 5 and 6), suggesting that Greater Rheas were adapted to the housing conditions before transport. After transportation, several behaviors were affected: transported males and females showed null resting, transported females also showed reduced preening and increased vigilance ($P < 0.05$), whereas transported males showed increased drinking ($P < 0.05$) compared with their respective control groups. The results suggest that behavioral responses of captive-bred Greater Rheas are sensitive to short-term transport (which includes handling) and that males and females differ in their posttransport behavioral activity, recovering their overall basal levels on the third day posttransportation.

Key words: ratite, stress response, transportation, welfare, sex difference

INTRODUCTION

Short-, medium-, and long-term transportation is a common practice in species that need to be moved for management or commercial purposes. Animals are transported for a variety of reasons, including breeding, biomedical purposes, slaughter, and translocation. As a side effect, transport exposes the animals to a wide range of stressors, including capture and handling, prolonged food and water restriction, novelty, confinement, and intermittent movement (reviewed in Fazio and Ferlazzo, 2003). Thus, transported individuals may undergo different stress levels that have deleterious effects on their health and welfare and on yield and quality of their by-products (Nielsen et al., 2011). In farm animals, transportation is also considered a great stress factor that has different adverse effects (Cockram, 2007). For example, transport stress disrupts normal lives of animals and results in aggressive behavior (Geverink et al., 1998), reduces live weight gain (Karaman, 2009), increases susceptibility to diseases (Hansson et al., 2005), and impairs immune function (Stanger et al., 2005). Transport stress induces injuries, animal mortality, and poorer quality animal products and therefore can cause huge economic losses to the animal industry (Warriss et al., 2005; Yue et al., 2010).

The response of the animal to the different stressors of transport and handling determines welfare during these events (Cafazzo et al., 2012; Hoffman and

Lühl, 2012). Hence, there is a growing interest among scientists and producers to evaluate and understand the effects of transportation with the aim of designing new management procedures and therefore preserving animal welfare (Miranda-de la Lama et al., 2012). For example, Vosmerova et al. (2010) showed that transport of broilers under conditions of low ambient temperatures in winter represents a more stressful event than transport during fall and summer, and Crowther et al. (2003) demonstrated that transportation at night is potentially beneficial for the reduction of stress and maintenance of welfare in ostriches (*Struthio camelus*).

The Greater Rhea (*Rhea americana*) is bred in farms and zoos, either in intensive or semiextensive systems. In the former system, animals are kept in pens (ranging in area from 1,000 m^2 or less to 2,000 m^2) and fed a diet composed of processed feed and chopped alfalfa, whereas in the semiextensive system rheas are housed in paddocks greater than 1 ha, where they graze on alfalfa and clover. Greater Rheas are captive bred for conservation purposes—as genetic reservoirs and a source of individuals for reintroduction into the wild—and for commercialization of meat, skin, feathers, fat, and eggs (Navarro and Martella, 2008, 2011). Transportation is a frequent practice in the production of Greater Rheas; however, behavioral responses triggered by transport have still not been studied in this species. A recent evaluation of the adrenocortical response to a physiological stressor (adrenocorticotropin challenge) in captive animals showed that they respond with a release of very high levels of corticosterone (Lèche et al., 2009, 2011). Specifically, in response to 30 min of transportation, plasma corticosterone levels increased more than 40-fold the basal levels, suggesting that transportation is a very potent stressor for rheas (Lèche et al., 2012). This study focuses on behaviors that would provide information about a wide variety of the rhea's response repertoire, covering, for example, indicators of comfort (preening and resting), antipredator (vigilance, ambulation), and maintenance (feeding and drinking) behavior among others. Considering that male and females also would present different behavioral strategies due to the sex roles that they play within the social structure of this species, there is a wide variety of expected changes that transport stressors should influence over time. Here we evaluate posttransport behavioral activity, addressing 2 main questions: are the behavioral responses of captive adult Greater Rheas sensitive to short-term (30 min) transport? If so, do males and females differ in their respective posttransport behavioral activity and recovery?

MATERIALS AND METHODS

Birds and Experimental Design

The study was conducted on the Experimental Farm of the zoo of Córdoba city, Argentina (31°S, 64°W), during the nonbreeding season of Greater Rhea (March to April) to minimize the occurrence of behavioral patterns associated with courtship and mating, which might distort the interpretation of results. Eight 12-mo-old adult males and 8 same-age females were evaluated. They were randomly selected from a stock of 30 individuals hatched and bred in captivity at the zoo.

This study was part of a project that included the noninvasive glucocorticoid hormonal assessment of pre- and poststress response of Greater Rheas, which involved sequential fecal sample collection from each individual throughout a day. Therefore, birds were individually housed during the 9 d of the experiment, to ensure identification of fecal samples. However, it is important to note that birds from the same treatment group (transport or control) were only physically but not visually or audibly isolated (see below). Birds were allowed 6 d to habituate to their new pens before the transport procedure was applied. The length of the habituation period (6 d) was chosen considering previous preliminary behavioral observations and the report of Lèche et al. (2011) showing basal plasma and fecal corticosterone levels at the end of that period. Half of the animals (4 males and 4 females) were randomly assigned to a transported group, whereas the other half remained undisturbed and were used as controls. Thus, 4 experimental groups of 4 individuals each were established: transported males, transported females, control males, and control females. Because these large-sized, strong birds need to be carefully handled, the study was carried out during 4 consecutive periods of 9 d each (4 different batches) and in consequence 4 transports in different days. Therefore, each batch consisted of one representative individual of each experimental group: one male and one female of the transport group and one male and one female of the control group. Birds were offered food (processed feed for chickens and chopped alfalfa) and water ad libitum in the home pens throughout the experimental period. Water was offered in individual drinkers. Processed feed (Vasquetto, Córdoba, Argentina) was provided in individual dishes placed on the floor and chopped alfalfa was provided directly on the floor. None of the individuals evaluated in this study had been transported before.

Enclosures

Four dirt-floored pens were used (one for each representative individual of each experimental group); in each pen (6 m in length × 4 m in width, fenced with 2-m-high woven-wire mesh), a digital recording camera (CCD-C384 Nova Spirit DSP, H3 Color Vari-Focal Camera, Tokyo, Japan) was mounted 7 m high to capture the entire site. The 4 cameras were connected to a portable computer, where the video files were stored. Shade-cloth curtain covered the woven-wire mesh lateral side of the enclosures that separated the adjacent birds of the other treatments to minimize visualization. The male and female of the same treatment (either transported or control) were only separated by the wo-

ven-wire mesh, and therefore they were only physically isolated.

Procedure

Males and females were placed in individual pens and allowed 6 d to habituate to their new enclosure. During this habituation period before transport (d 1 to 6), each individual behavior was video-recorded for 2 h every day (1000 to 1200 h) to determine whether birds were adapting to the housing conditions. The behavioral studies were performed during that period considering preliminary observations where it was noticed that at that time frame (1 h after feeders were replenished) birds are active and show a wide variety of behaviors. After noon, the environmental heat rises and birds became more passive. Taking into account that photoperiod, circadian rhythms, or both can also affect behavior and performances, and to make behavioral registrations along testing days comparable, reducing potential confounding environmental variables, every day birds were evaluated at the exact same time period.

On the transport day (0930 h, d 7), one male and one female (previously randomly assigned to the transport group) were caught by grasping the base of the wings and individually gently guided by walking approximately 20 m from the pen to the individual wooden crate (0.95 long × 0.55 wide × 1 m high) that contained an alfalfa bedding (usually used to transport this species). The crates were loaded individually by 2 workmen and placed in the back of a pickup truck. The entire loading procedure took less than 5 min for each pair of animals. Then, the vehicle traveled for 30 min on a paved road at an average speed of 50 km/h. The transport speed was set as a starting point to obtain information about the transport effects on this species and is considered a regular average speed for transportation of these birds among the very few farms that keep them in captivity. After this round-trip, the crates were immediately unloaded and the animals were returned to their original pens. The entire transport stress procedure included capture and brief handling of the animals to load them into the crates, as well as the actual 30-min transport exposure. Behaviors of transported and control individuals were immediately video-recorded during the following 2 h (1000 to 1200 h, d 7). The same video-recording and data collection scheme was used on the 2 following days (d 8 and 9). This procedure was performed with the aim of evaluating the posttransport behavioral activity, based on our preliminary behavioral observations showing that 2 d after transport would be enough for full activity recovery and also considering the reports by Lèche et al. (2009, 2011), which showed that Greater Rheas recover from an adrenocortical stimulation, decreasing to pretreatment levels in less than 24 h postchallenge.

Behavioral activity of transported individuals was considered recovered when no significant differences from that of control individuals were observed. As con-

sidered by Adams et al. (2011), we assumed that 2 h of observations per day were sufficient to characterize the behavioral changes over the study period. Individuals were captured and manipulated by the same experienced handler throughout the experiment, who cleaned the pens and replenished water and food every morning (0900 h). This procedure took less than 10 min. All the trips were made along the same route, using the same vehicle driven by the same driver.

Behavioral Analysis

The following behavioral variables were analyzed during both the habituation and posttransport periods: time spent resting, preening, vigilant, feeding, drinking, foraging, ambulating, the distance ambulated, and number of defecations (Table 1). Each behavioral variable and the corresponding X/Y position data were recorded at 1-s intervals using the software *Behavior Collect* (Tietjen, 1981). The time spent on each behavior was calculated by adding the number of time intervals during which the animal was performing that behavior during the 2-h observation per day. Distance traversed by each individual was calculated using the X/Y data. The number of times each individual defecated was also considered because increased defecation has been frequently regarded as an indicator of stressful situations in poultry (Jones et al., 1995; Mignon-Grasteau et al., 2003; Haas et al., 2010).

Statistical Analysis

Statistical analyses were performed using the Infostat statistical software package (InfoStat, 2010). Behavioral data during the habituation period were evaluated performing mixed-model statistical analysis. Pearson coefficient correlations were performed for all male-female (separated by the fence) paired samples to evaluate independence of data. Because no significant correlations were observed for all behavior variables but resting and vigilant, samples from males and females were considered as independent. Thus, the mixed-model statistical analysis examined the effects of habituation days (1 to 6), sex (male and female), and the interaction between these factors. The model also considered the transport batches and animal as random effects.

Posttransport behavioral data were also compared by mixed-model statistical analysis that evaluated the effects of treatment (transport and control), days of posttransport (7, 8, and 9), sex (male and female), and the interaction among these factors. The model for each behavioral variable also considered batch and animal as random effects.

Resting, preening, feeding, drinking, and ambulating time data were square-root transformed, and data on time spent vigilant were transformed to sequential ranks (Shirley, 1987), for normality of residuals.

The Fisher least significant difference test was used for post-hoc analysis. Differences were considered sig-

Table 1. Description of behaviors of Greater Rheas[1]

Behavior	Description
Resting	Sitting with head raised or down, in a crouched position
Preening	Using the beak to preen part of the individual's own body while standing or sitting
Vigilant	Standing with head raised, motionless, or performing minimum (<0.4 m)
Feeding	Eating processed feed directly from dish or that scattered immediately around the dish
Drinking	Drinking water directly from water drinker
Foraging	Pecking the ground and alfalfa while standing or sitting
Defecation	Number of times an individual excretes feces or urine
Ambulating	Moving at steps longer than 0.4 m
Distance ambulated	Distance traversed moving at steps long than 0.4 m

[1]The descriptions are based on Reboreda and Fernandez (1997) and Sales et al. (2000).

nificant at $P < 0.05$. All nontransformed values are expressed as mean $\pm$ SEM.

RESULTS

Pretransport Habituation Period

Habituation days had a significant main effect on the time Greater Rheas allocated to resting and preening (Figure 1A, $F_{5,70} = 7.65$, $P < 0.001$, and Figure 1B, $F_{5,70} = 11.62$, $P < 0.001$, respectively). During d 1, those times were significantly lower ($P < 0.05$) than on the following days. No significant changes in these behaviors were detected during the last 2 d of the habituation period (d 5 and 6). The time allocated to resting and preening was not significantly influenced by sex.

The time spent feeding was significantly affected by sex and over the course of the habituation days (Figure 1C, $F_{1,11} = 5.56$, $P < 0.04$ and $F_{5,70} = 6.59$, $P = 0.001$, respectively). On d 1, birds spent more time feeding ($P < 0.05$) than on the following days, and no significant changes were observed among d 2 to 6.

Distance ambulated was influenced by sex (Figure 1D, $F_{1,11} = 5.89$, $P < 0.03$). No significant changes were found in the distance ambulated over habituation days (Figure 1D, $F_{5,70} = 1.49$, $P < 0.20$).

The time spent ambulating was influenced by habituation days and sex (Figure 1E, $F_{5,70} = 2.63$, $P = 0.03$, and $F_{1,14} = 5.77$, $P = 0.03$, respectively). Females spent more time ambulating ($P < 0.04$) than males during d 1 to 4, and no significant differences between sexes or changes between d 5 and 6 were detected.

Vigilance, drinking, foraging, and number of defecations did not differ between sexes nor did these behaviors change over the course of the habituation days.

Short-Term Transport Effects

Transportation significantly altered the time Greater Rheas allocated to resting (Figure 2A, $F_{1,9} = 10.82$, $P < 0.001$), with a significant effect of days posttransportation ($F_{2,24} = 3.43$, $P < 0.05$). Immediately after transportation (d 7), resting was null in all transported females and males. On d 9, no significant differences between groups were observed.

Preening time was significantly influenced by transportation in combination with sex (Figure 2B, $F_{1,9} = 4.85$, $P = 0.05$). Days posttransportation also affected this behavior ($F_{2,24} = 17.59$, $P < 0.001$). The time that transported females spent preening on d 7, 8, and 9 was significantly lower ($P < 0.05$) than that of control females and transported males. This activity did not differ between transported and control males on any of the study days.

Vigilance behavior was significantly influenced by transportation in combination with sex (Figure 2C, $F_{1,9} = 5.22$, $P < 0.05$). Transported females spent significantly more time in vigilance than control females and transported males on d 7 ($P < 0.05$), with no differences on d 8 and 9. No significant differences between transported and control males were observed.

A significant sex effect was observed for the time spent feeding (Figure 2D, $F_{1,9} = 7.41$, $P < 0.03$, respectively) where males spent significantly more time feeding than females. No significant differences between transported and control birds were observed.

Drinking time was significantly influenced by transportation in combination with sex (Figure 2E, $F_{1,9} = 11.02$, $P < 0.01$). Transported males spent more time drinking than control males and transported females. The time allocated to drinking did not differ between transported and control females.

Transportation showed a significant effect on the distance ambulated (Figure 2F, $F_{1,9} = 5.74$, $P < 0.04$), and on the effects of sex and days posttransportation ($F_{2,24} = 3.37$, $P = 0.05$). The distance ambulated by transported birds was greater ($P < 0.05$) than that of control birds. Males and females differed in the distance ambulated on d 7 and 8, with no differences on d 9.

The time spent ambulating showed a similar pattern to the distance ambulated. Only a trend toward a combined effect of transport and sex (Figure 2G, $F_{1,9} = 2.91$, $P < 0.12$) was observed. Therefore, no differences between transported and control individuals on any of the study days were statistically evidenced.

Time allocated to foraging and number of defecations did not differ between transported and control individuals on any of the study days. No significant differences were observed in any of the behavioral variables evaluated between control females and control males on any of the study days.
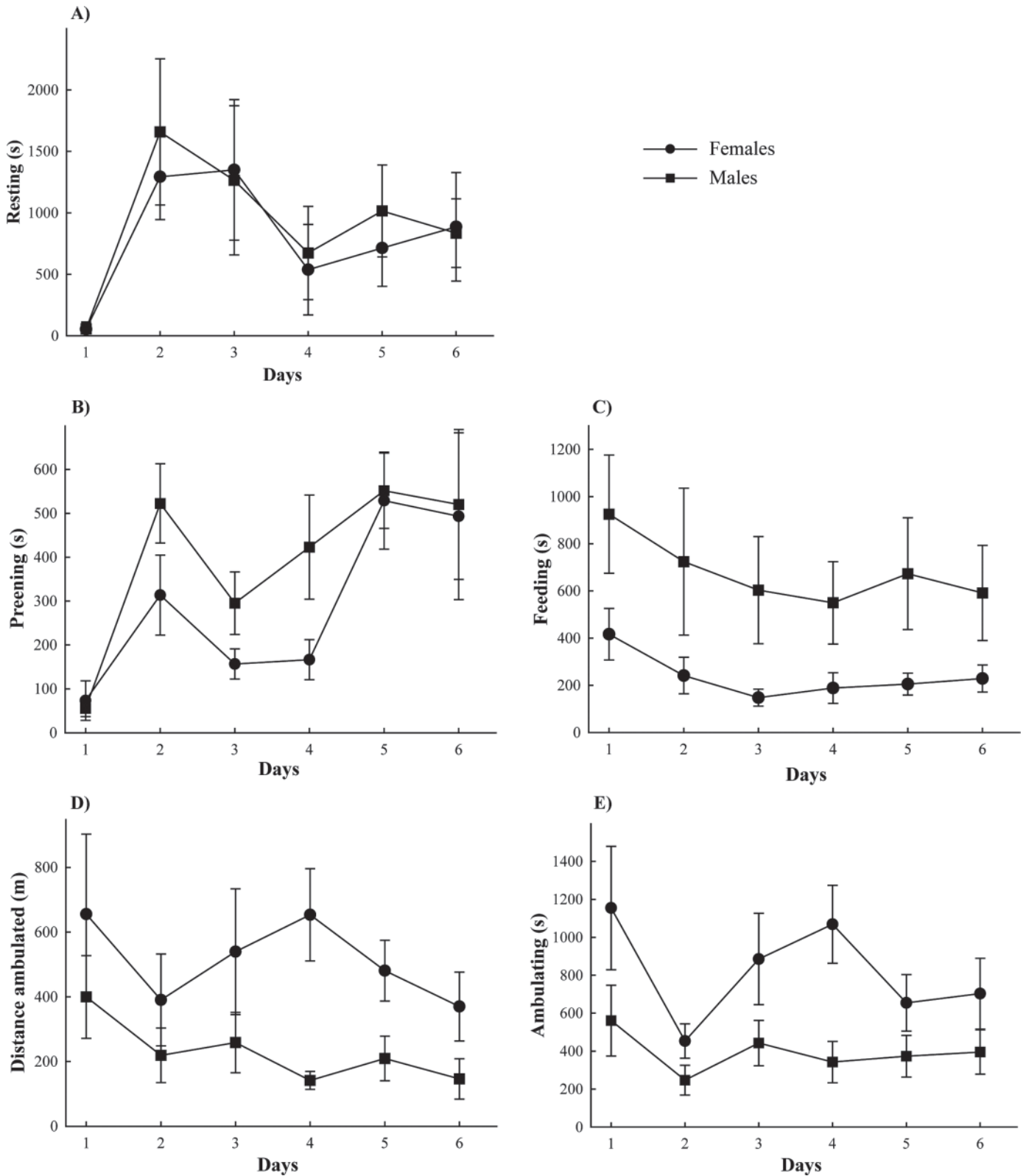
Figure 1. Means (±SEM) of behavioral activities during the habituation period (d 1 to 6), from a total of 2 h of observation per day.

DISCUSSION

The behavioral data obtained during the 6-d period after birds were placed in their pens (before transport exposure) showed, as expected, some differences between sexes and rapid changes on the first habituation days. Nevertheless, no changes were observed during the last 2 d of this habituation period (d 5 and 6), suggesting that the behaviors evaluated were already stabilized by the end of that period, and therefore, birds were adapted to the pretransport housing conditions. The present habituation findings may have relevance
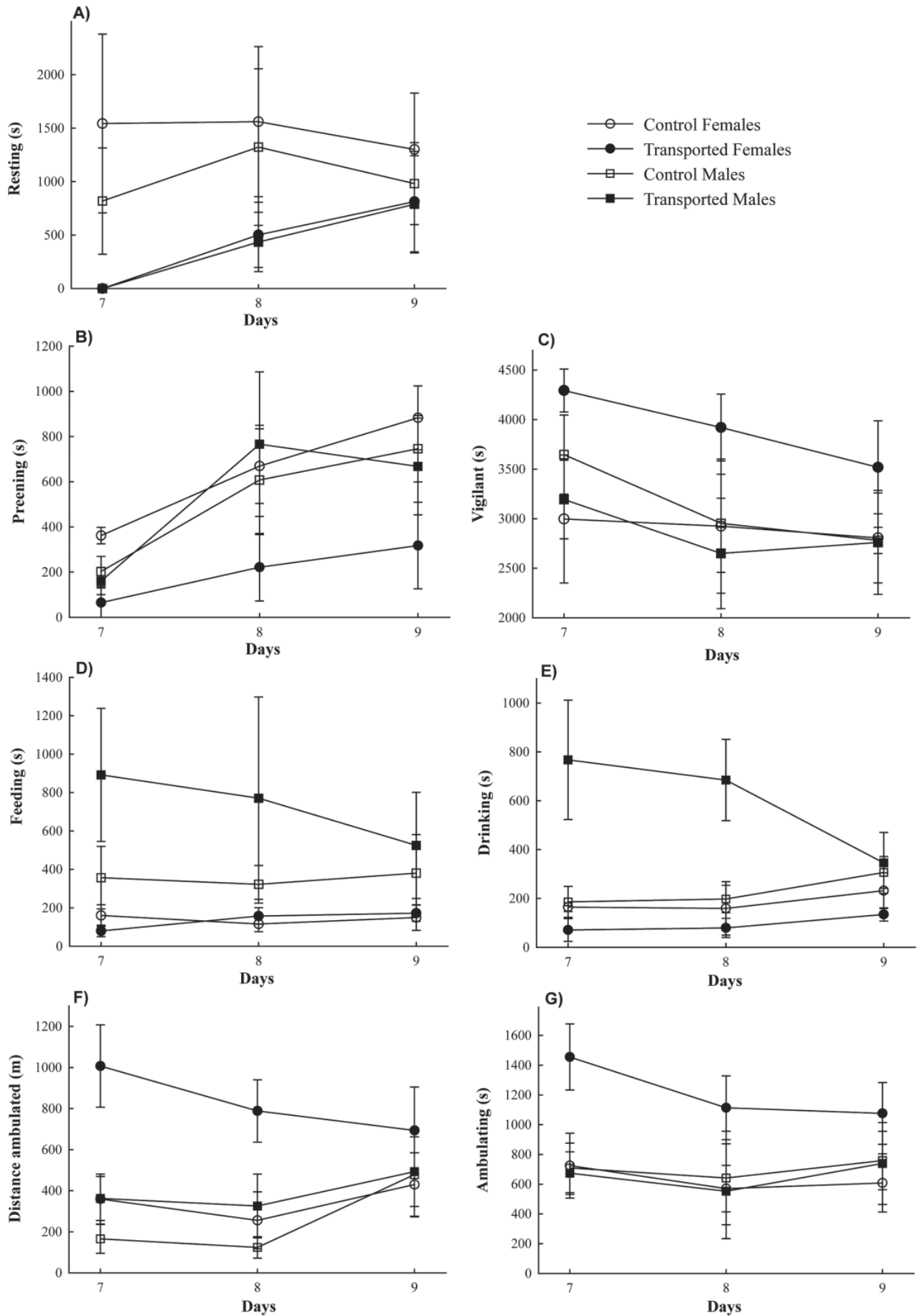
Figure 2. Means (±SEM) of behavioral activities during days posttransportation (d 7 to 9), from a total of 2 h of observation per day.

for zoos and other establishments where animals are usually kept in individual pens for the purpose of quarantine, medical treatments, and avoidance of direct social aggression or recovery from physical lesions (Hosey et al., 2008).

Short-term transport of captive Greater Rheas altered the time they spent resting, preening, vigilant, drinking, and ambulating, especially just after transport; such alterations differed between males and females in most cases. Wild males remain alone during incubation and chick care, and conversely, females live in group year-round (Bruning, 1974; Fernández and Reboreda, 1998); this behavior may explain why males recover their normal behavior more rapidly than females after an individual housing after the transport. The transported rheas recovered most of the behavioral activity displayed by their control counterparts 48 h after transportation (on d 9 of the experiment).

In females and males, resting behavior was null during the 2 h after transportation. This behavior is considered very important in birds because it contributes to the adaptation of the individual to environments subjected to stressful conditions (Blokhuis, 1984; Malleau et al., 2007). Thus, alteration of resting might have deleterious effects on welfare of individuals (Leeson et al., 1995; Appleby et al., 2004). In the present study, we do not know if animals compensated this lack of resting behavior later during the day of transport and the real effects of this stress on the performance of birds. However, in any case efforts should be made to avoid further posttransport stress situations that may delay bird recovery.

The observed reduction in preening time in response to transport was consistent with that reported for 2 genetic lines of laying hens after transportation (Cheng and Jefferson, 2008). Our results also showed a day of transportation effect. Because significant differences in preening behavior were still found on d 9, we assume that this activity was not fully recovered, at least at the frame time assessed. One possible explanation is that animals reduced this activity during the morning due to the presence of the caretaker at 0900 h and the negative experience of the last contact with humans on the day of transport. However, it should be noted that our animals were hatched and bred in captivity at the zoo, where they are handled by humans daily since hatch. No signs of stress were noticed because of the presence of humans within or in the proximity of the enclosure after transportation. Furthermore, while feeders were being replenished, birds approached the feeders while the caretaker was still present within the pen, and thus, no clear evidence of human-induced stress (avoidance, vigilance, and so on) or other behavior alterations was noticed. The second explanation could be a general reduction in preening along the day that in the present study cannot be confirmed. In any case, females' behavioral response in terms of preening and the recovery of this activity may be more sensitive to short-term transport than that of males. However, again, being isolated from other females after transport could have an important effect on this response.

Females increased time allocated to vigilance immediately after transport, whereas in males, this behavior was not affected by transport. This increased vigilance can be an adaptive response to a risky situation, as shown in domestic hens by Rutherford et al. (2003). In addition, given that under natural conditions Greater Rhea males devote more time to vigilance than females (Reboreda and Fernández, 1997; Sales et al., 2000), the observed increase in vigilance exhibited by females could be due to the absence of males or other females in the same pen during the study period. A similar trend was reported in the domestic fowl (Odén et al., 2005), for hens raised in groups without males.

The time that transported males allocated to drinking strongly increased compared with control males, and even to transported females. Considering that males and females were transported in similar-sized crates, males may have suffered higher dehydration than females during the stressful event because of their larger body mass. Heat stress and dehydration appear to be important issues in ostriches, particularly during transport (Mitchell, 1999; Crowther et al., 2003), and BW losses in transported broilers were also related to an increased demand for evaporative heat loss (Mitchell et al., 2003). On the other hand, a 30-min trip may have not been long enough to induce significant dehydration, and therefore, the increase observed in time devoted to drinking in males may also be a consequence of other causes, such as the increased time spent feeding observed in males during the habituation days and also after transportation in comparison with females. However, the increase in time devoted to drinking in males should be further explored to draw more accurate conclusions. Nevertheless, regardless of the mechanism involved in the increased drinking behavior, these results suggest that an ad libitum water supply should be always available immediately after transport or exposure to other stressors. However, it is true that total consumption of water along the day was not considered in the present study. Therefore, we do not know if a higher consumption of water during the morning was being compensated with a reduction in its consumption during the rest of the day and, consequently, what we are seeing is just a return to similar circadian rhythms in d 9 between control and transported animals.

Taking into account that one of the behavioral indicators of discomfort is attempting to escape (Grandin, 1997), the increase in the time ambulated observed in females compared with males might be associated with an attempt to escape induced by new stressful situations resulting from the transport procedure or being isolated by itself. However, increased female ambulatory activity may be also due to an attempt to reinstate social contact with the male conspecifics located in the nearby pen after stressor exposure. Accordingly, in studies involving broiler and Japanese quail, Marin et al. (2001), Jones et al. (2002), and Guzmán and

Marin (2008) showed that when birds are physically isolated, a stressful situation causes an increase in time spent near congeners or moving in their proximity. The fact that during the first 4 d females spent more time ambulating than males would confirm that for isolated females this behavior can be a good indicator of stress when coping with a new situation. Social support seems more prevalent in females (Scheiber et al., 2009), so further research is required in poultry to elucidate if social partners can provide advantages in coping with stressful situations (Rault, 2012).

Time allocated to foraging was not significantly altered by transport, possibly due to the high individual variability observed and the limited number of animals assessed in the present study; indeed, some transported birds had increased foraging time, whereas others reduced this activity compared with control individuals.

The number of defecations was not altered by transport. The fact that in rheas it is not possible to record fecal and urine excretion differentially may have precluded the detection of the effects of stress by transport on this behavioral and physiological response.

Overall, in response to stress by transport and at the expense of preening and resting, Greater Rhea females increased vigilance, whereas males increased time devoted to drinking, suggesting that males and females adopted different behavioral strategies to cope with stressful situations. Behavioral differences between adult male and female Greater Rheas were detected during the breeding season both in captivity (Sales et al., 2000) and in the wild (Reboreda and Fernández, 1997). In the present work, which was conducted during a nonbreeding season, differences were observed for feeding behavior and time of ambulation, in which a sex effect, but not a sex and transport interaction, were found. It is true that the lack of differences in the interaction could be due to the low number of animals used in the present study. In any case, it would be interesting to evaluate the effects of transport or other stress factors on birds during the breeding season because male and female behavioral coping strategies might be different from those observed in the present work.

In the present study, the transport stressor consisted of a series of components, including the handling procedure that, in turn, is composed of the catching procedure and the gently guided walking of birds into the crate. Overall, our results suggest that a 30-min transport and handling-associated event can alter behavioral dynamics of Greater Rheas, with more negative effects on females. Considering that transport is a frequent and necessary practice in the management of Greater Rheas (Navarro and Martella, 2008), further studies on the behavioral responses are needed, including physiological assessments after transport, to help generate management standards for optimization of the welfare of this ratite during captivity.

Social behavior appeared in Greater Rheas, as the major part of species that are predated, mainly as a strategy to increase safety. The necessity to look for other partners to enhance security increases before or after exposure to a stressful or a dangerous situation, as for example the presence of a predator. As mentioned before, some differences were found between male and female behavior. Because transport procedure also involves handling of animals, it should be considered that the presence of humans after that negative experience may have affected females and males in a different way, probably due to the higher necessity of females looking for other partners.

The results of the present work indicate that behavioral responses of captive-bred Greater Rheas are sensitive to short-term transport and that male and female differ in their posttransport behavioral activity, recovering the overall basal levels from the second to third day. Female preening was the only behavior that remained different from controls at the end of the study, suggesting that a short-transport procedure may affect females for longer periods than males. However, the present study is based on observations of behavior at one specific time frame of the day, and it is unknown if the change observed is linked to the circadian rhythms of certain behaviors or a real change in the overall activity during the day. The findings highlight the importance of considering sex-specific posttransport management procedures to help the birds cope with the stressor effects. A possible effect would be the need for females to be in a group, especially after a stressful experience. Therefore, further studies should evaluate the effectiveness of group versus individual transport as an approach to reduce the stress induced by transportation on Greater Rhea or the allocation of females in a group after transportation.

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