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ORIGINAL ARTICLE

Stress in wild Greater Rhea populations *Rhea americana*: effects of agricultural activities on seasonal excreted glucocorticoid metabolite levels

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Abstract In recent years, wild populations of Greater Rhea (*Rhea americana*) have declined drastically, due mainly to the conversion of grassland into cropland as a result of intensive, specialized agricultural practices. In this study we test the potential stressful effects of agricultural activities on this ratite by assessing their adrenocortical response. Specifically, we compared fecal glucocorticoid metabolite (FGM) levels of rheas living in two areas under very different land use: grasslands mainly used for cattle grazing and agro-ecosystems intensively used for crop production. Groups of rheas were observed during 45–50 min from a distance of approximately 1 km to avoid any potential disturbance. Therefore, no matching of each fecal sample with a particular sex or individual within the group was possible. Radioimmunoassay of fecal samples from 269 individuals indicated no significant differences in mean concentrations of FGM from the two habitats sampled. In the agro-ecosystem we found no overall effect of agricultural practices on the birds' FGM levels. However, during the dry season FGM concentrations were significantly higher, which may represent a stress response to the low availability of food in the post-harvesting season. In contrast, no increase in FGM levels was registered during the dry season in the grassland, where food was available throughout the year. In this environment the highest increases in FGM levels coincided with the reproductive period, likely due to the frequent agonist encounters between males at this time of the year. Our findings, therefore, suggest that the agricultural practices have to be viewed as chronic environmental stressors for Greater Rhea populations living under such conditions. The present results support earlier research showing detrimental impacts of agricultural activities on this species, which inhabits the most productive regions of South America.

Keywords Field endocrinology · Greater Rhea · Stress · Fecal glucocorticoid metabolites · Human disturbance · Agricultural practices

Zusammenfassung

Stress in freilebenden Nandupopulationen Rhea americana: Effekte landwirtschaftlicher Aktivitäten auf saisonale Glukokortikoidmetabolitspiegel im Kot

In den letzten Jahren haben freilebende Populationen des Nandus (*Rhea americana*) drastisch abgenommen, hauptsächlich wegen der Umwandlung von Grünland in Ackerland infolge intensiver, spezialisierter landwirtschaftlicher Methoden. In dieser Studie testen wir mögliche Stresseffekte

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landwirtschaftlicher Aktivitäten auf diesen Laufvogel, indem wir seine adrenokortikale Antwort abschätzen. Wir haben die Spiegel von Glukokortikoidmetaboliten (FGM) im Kot von Nandus verglichen, die in zwei Gebieten mit sehr unterschiedlicher Landnutzung leben: Grünland, auf dem hauptsächlich Rinder grasen, und Agrarökosysteme, die intensiv für den Pflanzenbau genutzt werden. Die Nandugruppen wurden 45-50 min lang aus einer Entfernung von etwa 1 km beobachtet, um jegliche potenzielle Störung zu vermeiden. Daher war es nicht möglich, Kotproben einem bestimmten Geschlecht oder Individuum innerhalb der Gruppe zuzuordnen. Radioimmunassays der Kotproben von 269 Individuen zeigten keine signifikanten Unterschiede in der mittleren FGM-Konzentration zwischen den beiden untersuchten Habitaten. Im Agrarökosystem fanden wir keinen Gesamteffekt landwirtschaftlicher Methoden auf die FGM-Spiegel der Vögel. Während der Trockenzeit waren die FGM-Konzentrationen jedoch signifikant höher, was eine Stressantwort auf die geringe Nahrungsverfügbarkeit in der Zeit nach der Ernte widerspiegeln könnte. Im Gegensatz dazu wurde im Grünland, wo Nahrung das ganze Jahr über verfügbar war, kein Anstieg der FGM-Spiegel festgestellt. Hier fiel der stärkste Anstieg der FGM-Spiegel mit der Fortpflanzungsperiode zusammen, wahrscheinlich weil es zu dieser Zeit häufig zu aggressiven Begegnungen zwischen Männchen kommt. Unsere Befunde deuten daher darauf hin, dass landwirtschaftliche Methoden als chronische Umweltstressfaktoren für Nandupopulationen, die unter solchen Bedingungen leben, betrachtet werden müssen. Die vorliegenden Ergebnisse stützen frühere Forschungsergebnisse, die schädliche Auswirkungen landwirtschaftlicher Aktivitäten auf diese Vogelart zeigten, welche die produktivsten Regionen Südamerikas bewohnt.

Introduction

Human-induced habitat changes are one of the main factors influencing environmental quality. A growing body of evidence indicates that the well-being of wild animal populations is being threatened by deteriorating environmental quality as a consequence of habitat fragmentation (Rangel-Negrín et al. 2009; Janin et al. 2011), urban environments (French et al. 2008; Zhang et al. 2011), noise (Blickley et al. 2012; Bruintjes and Radford 2013), ecotourism (Malo et al. 2011; Thiel et al. 2011; Maréchal et al. 2011), proximity to roads (Garcia Pereira et al. 2006; Crino et al. 2011; Dietz et al. 2013), pollution (Dauwe et al. 2005; Markman et al. 2011; Alaya-Ltifi et al. 2012), and other anthropogenic effects (Hopkins and Durant 2011; Jaimez et al. 2012; Carrete et al. 2013).

Animals respond to unpredictable and disruptive changes in their environment by modifying their physiology and behaviour to cope with such stimuli and promote survival (Walker et al. 2005). In vertebrates, the activation of the hypothalamic–pituitary–adrenal axis results in an increase in circulating glucocorticosteroids (corticosterone in birds; Romero 2004) to mobilize energy stores and to augment locomotor activity, foraging behaviour, escape or irruptive behaviour, time spent foraging and food intake rate (reviews in Wingfield et al. 1998; Sapolsky et al. 2000). These responses allow animals to overcome the negative effects of stressors and re-establish homeostasis in the best possible physical conditions (Wingfield and Kitaysky 2002). In the short term a stress response is adaptive, but it is physiologically costly and can have negative effects on essential catabolic body processes when sustained over extended periods (Sapolsky 2002). For instance, long-term high levels of glucocorticoids can have negative effects on the health of an organism by inhibiting growth, suppressing the immune system, or inhibiting reproductive functions (Sapolsky 1992; Wingfield and Romero 2001; Nazar and Marin 2011); ultimately, long-term stress affects individual fitness and may alter population dynamics (Tarlow and Blumstein 2007; Busch and Hayward 2009; Creel et al. 2013; Crespi et al. 2013).

The Greater Rhea (*Rhea americana*) is a flightless bird endemic to South America and an icon of the pampas grasslands. Free-ranging populations of this ratite have declined drastically in recent years, and the species has been classified as “Near Threatened” by the International Union for Conservation of Nature and Natural Resources (IUCN 2012). The geographical distribution of the Greater Rhea includes one of the most productive agricultural regions in Argentina, and the species is, therefore, affected by the progressive reduction and fragmentation of suitable habitats (Bazzano 2010; Giordano et al. 2008, 2010; Martella and Navarro 2006; Navarro and Martella 2008; 2011). The combined impact of local and global economic factors in recent decades has made agriculture currently more profitable than cattle production (Brown et al. 2005; Elverdín et al. 2011), which resulted in the replacement of large areas of natural grasslands and planted pastures by annual crops, in particular soybean (*Glycine max*). Giordano et al. (2008) found that in a predominantly cattle-grazing area, Greater Rhea populations were abundant and showed a wide and uniform spatial distribution, whereas in an agricultural area, individuals occurred in small and isolated clusters. The intensive use of land for agricultural purposes and the associated anthropogenic activities may be a possible cause of the decline in free-ranging Greater Rhea populations. In order to predict the long-term sustainability of wild populations of this species, it is important to improve our understanding of the physiological effects of threats caused by intensive agricultural production.

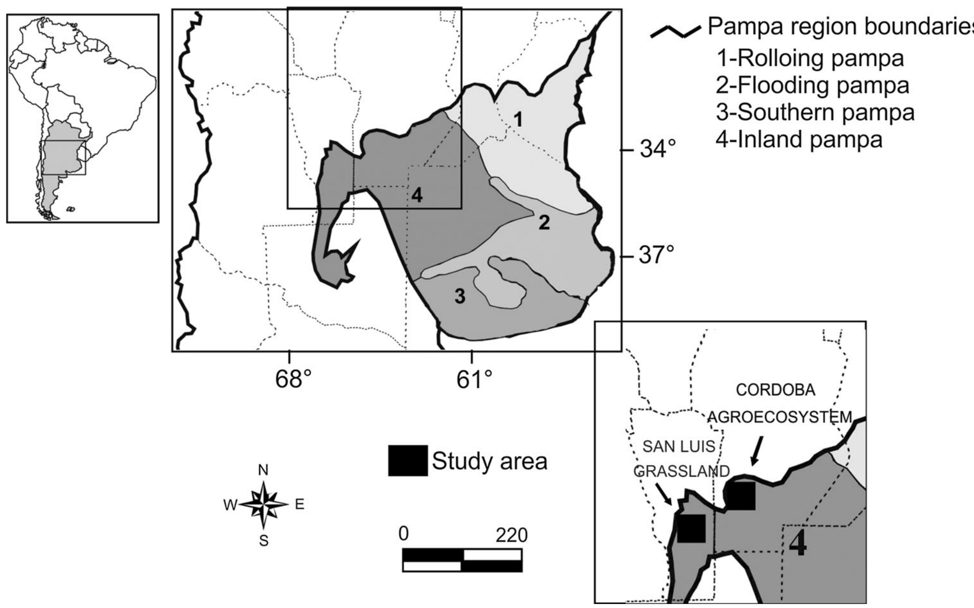

Fig. 1 Location of the two study areas in the Inland pampa within the Argentine pampas region. Figure taken from Giordano et al. (2010)

We wanted to test whether repeated human activities involved in agricultural practices may be viewed as chronic environmental stressors for the Greater Rhea; foraging activities are frequently disturbed/interrupted, and, particularly after harvesting, food availability is low. Cattle-raising pastures, on the other hand, provide an environment compatible with the development of viable populations of Greater Rhea, largely because of the year-round availability of alfalfa, their preferred food (Martella et al. 1996). Therefore, we predicted that stress hormone levels such as glucocorticoids are higher among Greater Rheas living in agro-ecosystems than in conspecifics living in less disturbed grasslands. In order to test this prediction, we measured the fecal glucocorticoid metabolite (FGM) levels of Greater Rhea in two areas under different land use: (a) grassland area mainly devoted to cattle grazing, and (b) agro-ecosystem intensively used for crop production (Fig. 1). Measuring stress responses non-invasively by using FGM levels from excreta offers a number of advantages, including sample collection with minimal disturbance to the animals and repeated sample collection during comparable daytime periods, and it enables the assessment of long-term FGM patterns (Palme et al. 2005). FGMs have been extensively used as adrenocortical activity indicators for several bird species including the Greater Rhea (Lobato et al. 2010; Lèche et al. 2011; Thiel et al. 2011; Busso et al. 2013).

Materials and methods

Study area

Fieldwork was conducted in two areas of the Argentine Pampas region (Fig. 1). The study location sites were selected on the basis of their conservation status: (a) a semi-natural grassland area located in the south-central portion of San Luis province (ca. 4,943 km^2, 34.43°S–65.37°W), which still resembles the natural habitat of rheas; and (b) an agro-ecosystem, close to the grassland area, located in the southwest of Córdoba province (ca. 4,006 km^2, 33.69°S–64.92°W). The San Luis study area covers almost all the grassland of the westernmost pampas region, the last relic of semi-arid grassland, where habitat conversion has been less extensive (Demaría et al. 2008).

The study areas differ in their average annual rainfall, which decreases westwards from 900 mm in Córdoba to 500 mm in San Luis (Ghersa et al. 2002). Rainfall is concentrated between October and April in both areas. The selected grassland is characterized by sandy soils and rolling hills with fixed and moving dunes (Anderson et al. 1970). Maximum summer temperatures can peak at 43 °C, and winter temperatures can be as low as -15 °C. Vegetation is mostly composed of native grasses (*Sorghastrum pellitum*, *Elyomurus muticus*, *Bothriochloa springfieldii*, *Chloris retusa*, *Schizachyrium plumigerum*, *Eragrostis lugens*, *Sporobolus subinclusus*, *Aristida spegazzini*, *Poa ligularis*, and *Poa lanuginosa*), with small patches of *Geoffrea decorticans*, *Prosopis caldenia*, and *Prosopis alpataco* trees (Anderson et al. 1970). Exotic planted pastures such as *Medicago sativa* (alfalfa), *Eragrostis curvula* (weeping lovegrass), and *Digitaria eriantha* (pangola grass) were introduced to increase carrying capacity for livestock on ranches (Demaría et al. 2003). Land in this study area is mostly used for cattle grazing and is only sporadically devoted to crop production because of the low annual rainfall, which in turn has contributed to maintaining its natural physiognomy (Guerschman et al. 2003).

Unlike the neighbouring grassland study area, the agro-ecosystem has been severely transformed by agriculture and cattle-raising over the past 150 years (Díaz-Zorita et al. 2002; Viglizzo et al. 2005). The climate is temperate, with mean temperatures of 33 °C in summer and 1.6 °C in winter. The area is characterized by flat to gently rolling dunes. The vegetation was originally composed of grasslands and forests, but the landscape is currently dominated by corn (*Zea mays*) and soybean (*G. max*) crop cultures (Díaz-Zorita et al. 2002; Guerschman and Paruelo 2005). The intensive crop production gives rise to a considerable degree of anthropogenic disturbance caused by the heavy traffic of agricultural machinery, tractors, trucks, and people during the planting and harvesting seasons. Free-ranging animal populations, therefore, have to contend with a constantly changing environment.

Sampling procedures

Greater Rheas excrete two types of feces: cecal and rectal. The former are shapeless and odorous, of a viscous, creamy consistency, and are eliminated only two or three times a day. Rectal feces, excreted more frequently, are solid and fibrous, uniform in shape, and odorless. Since previous findings suggested that rectal samples are more appropriate for monitoring adrenocortical activity in this species, only this type of feces was collected in the present study (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, they are not mixed in the dropping material.

Fresh fecal samples of adult Greater Rheas were collected in the study areas from April 2009 through April 2011. The sampling periods were selected according to extreme rates of anthropogenic disturbance intensity in the agro-ecosystem: zero or very low (June 2009, 2010, September 2009) and very high [harvesting (April 2009, 2010, 2011) and planting (October 2009 and 2010, November 2010, December 2010)]. Each sampling period lasted 1 or 2 weeks, commencing with the search for rhea groups. Once a group was sighted, the individuals were observed without disturbances and until several of them were observed defecating. After an observation time between 45 and 50 min, the samples were collected. Considering that the group of rheas usually ambulated during foraging, and individuals defecate every 2 h or more, this time frame was selected to minimize potential multiple sampling of a same individual within the group. Data on the number of individuals per group, landscape features (natural grasslands, exotic, or crops), temperature, weather, and anthropogenic activities (planting, harvesting, vehicular traffic, and human presence) were recorded while waiting for the rheas to defecate. Since the animals were observed from a distance of approximately 1 km in order to avoid disturbing them, it was not possible to match each fecal sample with a particular sex or individual within each group. In order to minimize the probability of re-sampling members of the same group, we moved forward at least 1.5 km until another different group of birds was observed and then proceeded to collect their samples. As Greater Rhea density is lower in the agro-ecosystem than in the grassland (4.08 ± 0.36 versus 0.34 ± 0.04 individuals/km^2, respectively; Bazzano 2010), sampled groups in the former were relatively small (2–12 individuals) compared with those in the latter (10–60 individuals).

Each fecal sample was placed individually into a labeled plastic bag which was kept on dry ice (avoiding direct contact) until it was stored in a freezer at −22 °C, where all samples remained until the steroid analysis was performed.

Precipitation data were obtained from a weather station of the National Meteorological Service of Argentina.

FGM analysis

The concentration of FGM in Greater Rhea samples was measured with a commercial ^{125}I corticosterone radioimmunoassay kit (MP Biomedicals, Costa Mesa, CA, USA), which has been validated for this species (Lèche et al. 2011). We followed the procedures provided by the manufacturer except that all reagent volumes were halved. FGM concentrations are expressed as nanograms per gram of wet fecal matter (ng/g). Cross-reactivity of corticosterone antisera was reported by the manufacturer to be 100 % with corticosterone and <1 % for other steroids. Fecal sample intra-assay and inter-assay variations were 3.9 and 8.3 %, respectively.

Statistical analysis

Data on FGM were analyzed using a generalized linear mixed model. The dependent variable was the FGM concentration, and the independent variables were the type of habitat (agro-ecosystem or grassland) and the combined variables year and month, habitat and year, and rhea sampling group. The model included fixed effects (habitat, year, month and the interaction between variables) and random effects (habitat and year, and sampling group). LSD Fisher tests were performed for post hoc analysis. The FGM concentrations are presented as mean ± standard error, and $P < 0.05$ was taken as a significant difference.

Results

A total of 269 fecal samples of Greater Rheas were collected: 161 from the grassland (41 rhea groups) and 108

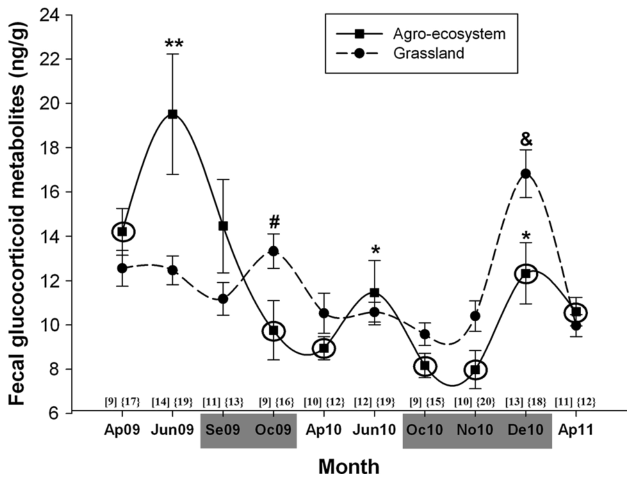

Fig. 2 FGM concentrations (mean ± SE) of Greater Rhea in grassland and agro-ecosystems. Sampling periods in *gray* correspond to the breeding season of the species. *Black circles* show the periods related to planting and harvesting. $^{**}P < 0.05$ compared with all other sampling periods within the agro-ecosystem; $^{*}P < 0.05$ compared with samples collected in Apr 2010, Oct 2010, and Nov 2010 within the agro-ecosystem, and $P < 0.05$ compared with all other sampling periods in the grassland. $^{\#}P < 0.05$ compared with samples collected in Sep 2009, Apr 2010, Jun 2010, Oct 2010, Nov 2010, and Apr 2011 in the grassland. Sample sizes per mean for the agro-ecosystem [] and grassland { } are shown at the bottom of the figure

from the agro-ecosystem (31 rhea groups). The 2-year seasonal profiles of FGM concentrations of Greater Rheas in the two habitats are shown in Fig. 2. No significant overall differences in FGM levels were detected between the agro-ecosystem and grassland areas ($F_{(1,4)} = 0.0004$; $P = 0.98$). However, a significant interaction was found between habitat and year × sampling period ($F_{(10,53)} = 2.16$; $P = 0.03$). Therefore, it is clear that even though the two habitats did not differ in their overall total means, particular differences arose when the sampling year, the month, and the environment are considered in the analysis, as shown below.

In the agro-ecosystem, high values were registered throughout most of 2009, with a marked increase in June (April: 14.20 ± 1.05; June: 19.51 ± 2.72; September: 14.46 ± 2.37) and a decrease later in October (10.28 ± 1.95). In 2010 there was also an increase in June, and although this is lower than in June 2009, it was significantly higher than April and October 2010. FGM levels remained low in October and November 2010, and an additional increase was observed in December 2010. FGM concentrations did not differ among the months associated with different agricultural practices ($F_{(1,290)} = 0.38$; $P = 0.54$). Thus, the observed effects were not directly due to disturbances, e.g. by machines, but rather occurred with a delay.

The observed FGM profile was much more constant in the grassland than in the agro-ecosystem throughout the 2-year study (CV 30.33 and 18.45 for agro-ecosystem and grassland, respectively). Only two FGM peaks were observed, and both were in months corresponding with the mating season of the species: October 2009 (13.32 ± 1.91) and December 2010 (16.82 ± 0.81).

Discussion

In this study we have shown that agricultural activities have an effect on the seasonal variation of FGM excretion in feces of free-ranging Greater Rheas. Unexpectedly, no direct effect of agricultural activities (planting, harvesting, vehicular traffic, human presence) on the seasonal FGM levels of Greater Rheas was found. It appears that the delayed peaks were due to the low availability of food in the phase after harvesting. Thus, intensive agricultural practices are an important chronic environmental stressor for rheas living in agro-ecosystems. It is important to recall that in this study, feces collected from groups could not be assigned to individuals, or by sex of the birds; therefore, it is possible in some cases that the results may be affected/confounded by this methodological constraint.

In the post-harvest period (April–May) in the agro-ecosystem, and until new plants begin to emerge (end of September–beginning of October), the land was practically devoid of vegetation. This low availability of food after harvesting, together with the lowest temperatures of the year in the autumn and winter that occur particularly when the bird's energy requirements are higher, could explain the higher levels of FGM observed in Greater Rhea during these months (April, June, and September). Corticosterone is well known to accelerate metabolism, and is suggested to initiate food-searching behaviour during periods of food restriction (Astheimer et al. 1992). Elevated corticosterone levels also serve to mobilize energy stores via protein catabolism (Harvey et al. 1984), and starvation was shown to increase corticosterone to stress levels to induce severe protein catabolism and a behavioural change (e.g. Cherel et al. 1988). In our samples, corticosterone release was integrated over several hours. Therefore, it was not possible to know whether the elevated FGM concentrations are due to the classical stress response (for example, to starvation) or a chronic elevation of baseline levels.

Higher levels of fecal glucocorticoids associated with the reduction of food availability during the dry season have also been observed in others species (Garcia Pereira et al. 2006, 2010; Rangel-Negrín et al. 2009). In order to maintain adequate homeostasis levels during periods of food shortage, particularly when temperatures are low, the animals have higher energy demands. This scarcity may obligate individuals to increase their foraging effort (e.g. by increasing day ranges) in order to maintain net food intake levels. Although the present study does not include systematic behavioural measurements, rheas in the agro-ecosystem were clearly observed constantly moving forward during foraging, whereas those in the grasslands fed at one place for longer periods. These observations are in agreement with previous studies undertaken by Bellis et al. (2004), who found that the home range size and movements of Greater Rhea depend on the distribution, abundance, and quality of the available forage. Thus, for example, the home range of Greater Rhea in an area with ample availability of alfalfa (as already mentioned, the preferred food of this species, Martella et al. 1996), was on average three times smaller (2.4 km^2; Bellis et al. 2004) than that of Greater Rhea in areas lacking this pasture (6.69 km^2; Bazzano 2010). Furthermore, given that the productivity of this type of habitat depends directly on the amount of precipitation, the higher levels of FGM observed in June 2009 compared to those in June 2010 could be attributed to the lower levels of precipitation registered in 2008 (528 mm) versus 2009 (810 mm). Years with higher rainfall increase the availability of food in the dry season of the following year, which would help to reduce the stress level of species in habitats with intense agricultural production.

In support of this hypothesis, we found that in the grasslands, where ample forage—in particular alfalfa—is available throughout the year, no increase in the level of glucocorticoids was registered during the dry season. In this environment, the highest increases in FGM levels coincided with the reproductive period, possibly owing to the overall higher density of Greater Rhea in the grasslands than in the agro-ecosystem Bazzano 2010), and thus the greater likelihood of agonistic encounters between males, which are common for this time of the year during harem formation (Martella et al. 1994; Codenotti and Alvarez 2001). In fact a study undertaken in a captive animal facility also showed that male Greater Rheas have higher levels of FGM during the reproductive season, whereas in females, the level is constant throughout the year (Lèche 2012). However, it should also be considered that not only animal encounters but also the increased metabolic rate during the reproductive phase might be reasons for increased corticosterone, as proposed by Romero (2002). It could also be considered that peaks of FGM occurring prior to the breeding season may be the result—particularly for females—of increased energetic demands associated with preparation of reproductive physiology (e.g. developing oocytes).

In the agro-ecosystem an increase in glucocorticoids was also observed in December 2010, which could reflect the expected higher levels in males during this season (Lèche 2012). However, taking into account that the sampling in December 2010 coincided with the planting season, the possibility that the observed peak could be attributed to the stress caused by the seasonal anthropogenic activity, or by a combination of both factors, cannot be ignored. The absence of an increase in other months of the reproductive period could be due to the sex ratio composition of groups sampled in this study, and that, on average, a much higher number of female feces were collected during these months, which may have biased the observed mean FGM levels in the sample "pool" (Lèche 2012). In fact, Greater Rheas present a seasonal variation of the sex ratio with flocks (4–50 animals) of both sexes and a sex ratio near unity in the non-breeding season and harems (1 or 2 males with 2–12 females), solitary males, and mixed flocks (females, males and yearlings) during the breeding season (Bruning 1974).

In summary, our findings suggest that the courtship behaviour of male Greater Rheas and the shortage of food are the main factors leading to stress responses in this species. In future studies it would be advisable to determine the sex and individual genotype of each feces sampled to avoid potential bias of the observed seasonal patterns. The present study supports earlier research (Martella et al. 1996; Navarro and Martella 2008, 2011; Giordano et al. 2010) showing negative impacts of agricultural activities on this species whose area of distribution coincides with the most productive regions of South America. Findings clearly suggest that every effort to minimize periods of food shortage should be taken. For example, keeping natural vegetation corridors along agro-cultural habitats, combining agriculture with cattle breeding, and planting alfalfa, at least along the edges of fields in the agro-cultural habitats, would be advisable strategies to help maintain this endangered species.

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References

Alaya-Ltifi L, Chokri MA, Selmi S (2012) Breeding performance of passerines in a polluted oasis habitat in southern Tunisia. Ecotoxicol Environ Saf 79:170–175

Anderson DL, Del Águila JA, Bernardon AE (1970) Las formaciones vegetales de la provincia de San Luis. Rev. Inv. Agropecuaria INTA, serie 2. Biología y Producción Vegetal 7:83–153

Astheimer LB, Buttemer WA, Wingfield JC (1992) Interactions of corticosterone with feeding, activity and metabolism in passerine birds. Ornis Scand 23:355–365

Bazzano G (2010) Fragmentación del hábitat: consecuencias comportamentales y demográficas en el Ñandú (*Rhea Americana*). PhD thesis in Biological Sciences at Universidad Nacional de Córdoba, Argentina

Bellis LM, Martella MB, Navarro JL, Vignolo PE (2004) Home range of greater and lesser rhea in Argentina: relevance to conservation. Biodivers Conserv 13:2589–2598

Blickley JL, Blackwood D, Patricelli GL (2012) Experimental evidence for the effects of chronic anthropogenic noise on abundance of Greater Sage-Grouse at leks. Conserv Biol 26(3):461–471

Brown A, Martínez Ortiz U, Acerbi M, Corchera J (2005) Situación ambiental argentina, 3rd edn. Fundación Vida Silvestre Argentina, Buenos Aires

Bruintjes R, Radford AN (2013) Context-dependent impacts of anthropogenic noise on individual and social behaviour in a cooperatively breeding fish. Anim Behav 85(6):1343–1349

Bruning DF (1974) Social structure and reproductive behavior of the Greater Rhea. Living Bird 13:251–294

Busch DS, Hayward LS (2009) Stress in a conservation context: a discussion of glucocorticoid actions and how levels change with conservation-relevant variables. Biol Conserv 142:2844–2853

Busso JM, Dominchin MF, Marin RH, Palme R (2013) Cloacal gland, endocrine testicular, and adrenocortical photoresponsiveness in male Japanese quail exposed to short days. Domest Anim Endocrinol 44(3):151–156

Carrete M, Bortolotti GR, Sánchez-Zapata JA, Delgado A, Cortés-Avizanda A, Grande JM, Donázar JA (2013) Stressful conditions experienced by endangered Egyptian vultures on African wintering areas. Anim Conserv 16:353–358

Cherel Y, Robin JP, Walch O, Karmann H, Netchitailo P, Le Maho Y (1988) Fasting in king penguin. I. Hormonal and metabolic changes during breeding. Am J Physiol Regul Integr Comp Physiol 254:R170–R177

Codenotti TL, Alvarez F (2001) Mating behavior of the male Greater Rhea. Wilson Bull 113(1):85–89

Creel S, Dantzer B, Goymann W, Rubenstein DR (2013) The ecology of stress: effects of the social environment. Funct Ecol 27:66–80

Crespi EJ, Williams TD, Jessop TS, Delehanty B (2013) Life history and the ecology of stress: how do glucocorticoid hormones influence life-history variation in animals? Funct Ecol 27:93–106

Crino OL, Van Oorschot BK, Johnson EE, Malisch JL, Breuner CW (2011) Proximity to a high traffic road: glucocorticoid and life history consequences for nestling white-crowned sparrows. Gen Comp Endocrinol 173:323–332

Dauwe T, Janssens E, Pinxten R, Eens M (2005) The reproductive success and quality of blue tits (*Parus caeruleus*) in a heavy metal pollution gradient. Environ Pollut 136:243–251

Demaría MR, Mcshea WJ, Koy K, Maceira NO (2003) Pampas deer conservation with respect to habitat loss and protected area considerations in San Luis, Argentina. Biol Conserv 115: 121–130

Demaría MR, Suárez IA, Steinake DF (2008) Reemplazo y fragmentación de pastizales pampeanos semiáridos en San Luis, Argentina. Ecología Austral 18:55–70

Díaz-Zorita M, Duarte GA, Grove JH (2002) A review of no-till systems and soil management for sustainable crop production in the subhumid and semiarid Pampas of Argentina. Soil Tillage Res 65:1–18

Dietz MS, Murdock CC, Romero LM, Ozgul A, Foufopoulos J (2013) Distance to a road is associated with reproductive success and physiological stress response in a migratory landbird. Wilson J Ornithol 125(1):50–61

Elverdín JH, Maggio A, Muchnik J (2011) Facteurs d'ancrage territorial des activités agricoles. Soja et élevage en Argentine. Économie Rurale 322:64–78

French SS, Fokidis HB, Moore MC (2008) Variation in stress and innate immunity in the tree lizard (*Urosaurus ornatus*) across an urban-rural gradient. J Comp Physiol B Biochem Syst Environ Physiol 178:997–1005

Garcia Pereira RJ, Barbanti-Duarte JM, Negraõ JA (2006) Effects of environmental conditions, human activity, reproduction, antler cycle and grouping on fecal glucocorticoids of free-ranging Pampas deer stags (*Ozotoceros bezoarticus bezoarticus*). Horm Behav 49:114–122

Garcia Pereira RJ, Granzinolli MAM, Barbanti-Duarte JM (2010) Annual profile of fecal androgen and glucocorticoid levels in free-living male American kestrels from southern mid-latitude areas. Gen Comp Endocrinol 166(1):94–103

Ghersa CM, Ferraro DO, Omacini M, Martínez-Ghersa MA, Perelman S, Satorre EH, Soriano A (2002) Farm and landscape level variables as indicators of sustainable land-use in the Argentine Inland-Pampa. Agric Ecosyst Environ 93:279–293

Giordano PF, Bellis L, Navarro JL, Martella MB (2008) Abundance and spatial distribution of Greater Rhea (*Rhea americana*) in two sites on the pampas grasslands with different land use. Bird Conserv Int 18:63–70

Giordano PF, Navarro JL, Martella MB (2010) Building large-scale spatially explicit models to predict the distribution of suitable habitat patches for the Greater Rhea (*Rhea americana*), a near-threatened species. Biol Conserv 143(2):357–365

Guerschman JP, Paruelo JM (2005) Agricultural impacts on ecosystem functioning in temperate areas of North and South America. Glob Planet Change 47:170–180

Guerschman JP, Paruelo JM, Burke IC (2003) Land use impacts on the normalized difference vegetation index in temperate Argentina. Ecol Appl 13:616–628

Harvey S, Phillips JG, Rees A, Hall TR (1984) Stress and adrenal function. J Exp Zool A 232:633–645

Hopkins WA, DuRant SE (2011) Innate immunity and stress physiology of eastern hellbenders (*Cryptobranchus alleganiensis*) from two stream reaches with differing habitat quality. Gen Comp Endocrinol 174:107–115

IUCN (2012) IUCN red list of threatened species. Version 2012.2. http://www.iucnredlist.org. Downloaded on 13 June 2013

Jaimez NA, Bribiescas RG, Aronsen GP, Anestis SA, Watts DP (2012) Urinary cortisol levels of gray-cheeked mangabeys are higher in disturbed compared to undisturbed forest areas in Kibale National Park, Uganda. Anim Conserv 15(3):242–247

Janin A, Léna JP, Joly P (2011) Beyond occurrence: body condition and stress hormone as integrative indicators of habitat availability and fragmentation in the common toad. Biol Conserv 144:1008–1016

Lèche A (2012) Evaluación de la respuesta adrenocortical de estrés en ñandúes (Rhea americana) criados en condiciones artificiales y en vida silvestre. PhD thesis in Biological Sciences. Universidad Nacional de Córdoba, Argentina

Lèche A, Busso JM, Navarro JL, Hansen C, Marin RH, Martella MB (2011) Non-invasive monitoring of adrenocortical activity in Greater Rhea (*Rhea americana*) by measuring fecal glucocorticoid metabolites. J Ornithol 152:839–847

Lobato E, Moreno J, Merino S, Morales J, Tomás G, Martínez J, Vásquez RA, Kuchar A, Möstl E, Osorno JL (2010) Arrival date and territorial behavior are associated with corticosterone metabolite levels in a migratory bird. J Ornithol 151(3):587–597

Malo JE, Acebes P, Traba J (2011) Measuring ungulate tolerance to human with flight distance: a reliable visitor management tool? Biodivers Conserv 20(14):3477–3488

Maréchal L, Semple S, Majolo B, Qarro M, Heistermann M, MacLarnon A (2011) Impacts of tourism on anxiety and physiological stress levels in wild male Barbary macaques. Biol Conserv 144:2188–2193

Markman S, Müller CT, Pascoe D, Dawson A, Buchanan KL (2011) Pollutants affect development in nestling starlings *Sturnus vulgaris*. J Appl Ecol 48(2):391–397

Martella MB, Navarro JL (2006) Proyecto Ñandú. Manejo de *Rhea americana* y *R. pennata* en la Argentina. In: Bolkovic ML, Ramadori DE (eds) Manejo de fauna en Argentina: proyectos de uso sustentable. Dirección de Fauna Silvestre, Secretaría de Ambiente y Desarrollo Sustentable, Buenos Aires, Argentina, pp 39–50

Martella MB, Navarro JL, Sahade R, Tatián M, Burgos A (1994) Breeding system of the Greater Rhea (*Rhea americana)*. J Ornithol 135:123

Martella MB, Navarro JL, Gonnet JM, Monge SA (1996) Diet of greater Rheas in an agroecosystem of central Argentina. J Wildl Manag 60:586–592

Navarro JL, Martella MB (2008) The relevance of captive breeding to conservation of native ratites in Argentina: an overview. Aust J Exp Agric 48:1302–1307

Navarro JL, Martella MB (2011) Ratite conservation: linking captive-release and welfare. In: Glatz P, Lunam C, Malecki I (eds) The welfare of farmed ratites. Springer, New York, pp 237–258

Nazar FN, Marin RH (2011) Chronic stress and environmental enrichment as opposite factors affecting the immune response in Japanese quail (*Coturnix coturnix japonica*). Stress 14(2): 166–173

Palme R, Rettenbacher S, Touma C, El-Bahr SM, Möstl E (2005) Stress hormones in mammals and birds comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. Ann N Y Acad Sci 1040:162–171

Rangel-Negrín A, Alfaro JL, Valdez RA, Romano MC, Serio-Silva JC (2009) Stress in Yucatan spider monkeys: effects of environmental conditions on fecal cortisol levels in wild and captive populations. Anim Conserv 12:496–502

Romero LM (2002) Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. Gen Comp Endocrinol 128:1–24

Romero ML (2004) Physiological stress in ecology: lessons from biomedical research. Trends Ecol Evol 19:250–255

Sapolsky RM (1992) Neuroendocrinology of the stress response. In: Becker JB, Breedlove SM, Crews D, McCarthy MM (eds) Behavioral endocrinology. Massachusetts Institute of Technology Press, Nueva York, pp 287–324

Sapolsky RM (2002) Endocrinology of the stress-response. In: McCarthy MM (ed) Behavioral endocrinology. MIT Press, Cambridge, pp 409–450

Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 21:55–89

Stewart J (1994) Ratites. In: Ritchie BW, Harrison GJ, Harrison LR (eds) Avian medicine: principles and applications. Wingers, Lake Worth, pp 1284–1326

Tarlow EM, Blumstein DT (2007) Evaluating methods to quantify anthropogenic stressors on wild animals. Appl Anim Behav Sci 102:429–451

Thiel D, Jenni-Eiermann S, Palme R, Jenni L (2011) Winter tourism increases stress hormone levels in the Capercaillie *Tetrao urogallus*. Ibis 153:122–133

Viglizzo EF, Frank FC, Carreño L (2005) Situación ambiental en las ecoregiones pampa y campos y malezales. In: Acerbi M, Corcuera J, Brown A, Martínez Ortiz U (eds) Situación ambiental Argentina. Fundación Vida Silvestre Argentina, Buenos Aires, pp 261–269

Walker BG, Boersma PD, Wingfield JC (2005) Field endocrinology and conservation biology. Integr Comp Biol 45:12–18

Wingfield JC, Kitaysky AS (2002) Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? Integr Comp Biol 42:600–609

Wingfield JC, Romero LM (2001) Adrenocortical responses to stress and their modulation in free-living vertebrates. In: McEwen B (ed) Comprehensive physiology, supplement 23: handbook of physiology, the endocrine system, coping with the environment: neural and endocrine mechanisms. Oxford University Press, New York, pp 211–234

Wingfield JC, Breuner C, Jacobs J, Lynn S, Maney D, Ramenofsky M, Richardson R (1998) Ecological bases of hormone-behavior interactions: the 'emergency life history stage'. Am Zool 38:191–206

Zhang S, Lei F, Liu S, Li D, Chen C, Wang P (2011) Variation in baseline corticosterone levels of Tree Sparrow (*Passer montanus*) populations along an urban gradient in Beijing, China. J Ornithol 152:801–806