Diet preference and density of the Greater Rhea (*Rhea americana*) in grasslands of the Flooding Pampa, Argentina

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ABSTRACT: The Greater Rhea (*Rhea americana*) has been greatly affected by habitat alteration and hunting. Density of rheas was estimated by monthly surveys along transects from June 1996 to November 1997 in 760 ha of coastal grasslands in the Flooding Pampa, Buenos Aires Province, Argentina. Fresh feces and vegetation samples were collected in spring 1996, and in winter and spring 1997 to study diet and resource selection. Feces were macroscopically processed, and the vegetal fraction was prepared for micro histological analysis. Vegetation was sampled in quadrants, separated by species and dried to obtain the percentage of dry weight to estimate availability. Mean density was 0.22 ± 0.04 rheas/ha. Dicots were always preferred in relation to their availability in the grassland, and monocots were not preferred in neither of the two spring seasons studied. Monocots were consumed more frequently than dicots in winter. Legumes were preferred in both spring seasons, with Black Medic (*Medicago lupulina*) the most consumed species. Perennial Ryegrass (*Lolium perenne*) was the most represented monocot, but it was always consumed in proportions similar to its availability. Grasses growing in warm seasons were never preferred. The diet of Greater Rheas in these grasslands was generalist and almost completely herbivorous, showing preference for dicots, mainly legumes. Rheas were more selective in spring, when the availability of the vegetation was higher than in winter.

KEY-WORDS: food habits, herbivory, microanalysis, Ratites.

INTRODUCTION

The Greater Rhea (Rhea americana) is a South American ratite endemic to Argentina, Bolivia, Brazil, Paraguay, and Uruguay. In Argentina, this bird is characteristic of the tall grass steppe of the Pampas (Folch 1992). The Pampas occupy the eastern plains of Argentina, between 32°S and 39°S covering an area of over 500,000 km², with annual rainfall ranges between 750 and 1100 mm (Bucher & Nores 1988). This area is being heavily modified by land-use practices (Bilenca & Miñarro 2004, Viglizzo et al. 2011). Populations of rheas have been greatly affected by grassland destruction, fragmentation of their habitat, and hunting for their skin and meat (BirdLife International 2014). Overgrazing, soil compaction, and fire caused the elimination of tall grasses and their replacement by shorter grass species, giving way before long to agriculture. The economic development of the region produced an increase in the human population and the construction of roads, resulting in a high hunting pressure. Although still locally common, particularly in protected areas and some large ranches, the number of rheas has been greatly reduced, to the point of local extinction in many areas (Bucher & Nores 1988). The Greater Rhea has been categorized as Near Threatened (IUCN 2015), and Threatened in Argentina (López-Lanús *et al.* 2008).

The Greater Rhea lives in open plains generally in mixed groups of males, females and juveniles of five to 50 individuals (Folch 1992). They spend a high proportion of their time foraging in open grasslands (Reboreda & Fernández 1997, Azevedo *et al.* 2010), sometimes together with other wild and domestic herbivores as Pampas Deer *Ozotoceros bezoarticus* (Parera 2002), cattle and sheep, which is advantageous for all to detect threats at a distance (Folch 1992). Rheas also forage in agroecosystems (Comparatore & Yagueddú 2007, Giordano *et al.* 2008). In these agroecosystems, rheas use grasslands and crops as sometimes they cross internal fences (pers. obs.). They are omnivorous, although

most of their diet is vegetal including leaves (some thorny species), seeds, fruits, and roots (Folch 1992). They also consume insects and small vertebrates such as birds, snakes, fish and rodents (Raikow 1968, Folch 1992, Martella et al. 1996, Silva et al. 2001, Azevedo et al. 2006, Comparatore & Yagueddú 2007, Masat et al. 2011). They are coprophagous, as they were seen feeding on fecal material deposited a few minutes earlier by other rheas (Raikow 1968). Rheas also eat crop weeds (Comparatore & Yagueddú 2007, Bernard 2012) and other plants rejected by cattle, and often eat burr-like seeds, which get tangled in sheep's wool (Folch 1992). As a consequence, the diet overlap with domestic herbivores is low (Martella et al. 1996, Vacarezza 2001, Vacarezza et al. 2001, Pereira et al. 2003). It is worth noting that rheas do not have a crop, but have a very large gizzard and a large cecum which help digestion (Angel 1996). Also, they ingest small stones, which aid the gizzard in grinding (Folch 1992).

Intensive and extensive breeding of rheas has been increasing as an alternative animal production (Maceira *et al.* 2003, Feld *et al.* 2011). The maintenance of rheas through effective management or the breeding of individuals for animal production requires knowledge of their diet requirements. The diet of Greater Rheas in coastal natural grasslands of the Flooding Pampa is unknown. Previous studies at Pila (Yagueddú & Rossi 1985) and Ayacucho (Vacarezza 2001, Vacarezza *et al.* 2001), on diet overlap among rheas and domestic herbivores, were carried out in the inner Flooding Pampa, concluding that the former prefers forbs (legumes and non-legumes), and the latter grasses. So, competition for forage among these species would be low.

The objectives of this study were: a) analyze the dietary habits of the Greater Rhea in coastal grasslands of the Flooding Pampa; and b) estimate rheas' density in the same area. According to the forage abundance hypothesis (MacArthur & Pianka 1966, Schoener 1971), rheas in this area are expected to be more selective in spring, when the availability of the vegetation is higher than in winter. This study complements two others in the region, where the dietary habits in crop fields (Comparatore & Yagueddú 2007) and the habitat use in grasslands (Herrera *et al.* 2004) were described.

METHODS

The study was conducted in the Flooding Pampa (Batista *et al.* 2005), Buenos Aires Province, Argentina, Estancia Medaland (37°22'–37°27'S; 57°12'–57°7'W), considered Valuable Grassland Area (Bilenca & Miñarro 2004). This farm is dominated by natural and modified grasslands with small crop areas, which are rapidly expanding due

to changes in land-use. Data were collected over 760 ha of grasslands during the spring 1996, and the winter and spring 1997.

Animal density was estimated from June 1996 to November 1997 with monthly complete surveys (n = 18), walking along ten parallel transects covering the whole area (760 ha) using 10 x 50 binoculars, being careful to count animals only once. The number of animals counted each month was divided by the entire area to obtain 18 density values, which were then averaged to calculate mean density of rheas during the studied period.

Twenty fresh feces were collected in spring 1996, 10 in winter 1997 and 10 in spring 1997, with at least 300 m apart from one another in each season. Then, they were processed in the laboratory to separate pebbles, shells, animal and vegetal material. Plant material was prepared for microanalysis (Sparks & Malecheck 1968) to determine its botanical composition. This vegetal material was dried for 24 h in a forced air oven at 60°C and was then ground over a 1 mm (16 mesh) sieve screen to reduce all plant fragments to a uniform size (Sparks & Malecheck 1968). A representative amount of each fecal sample was soaked in 50% bleach for 30 to 60 s to clear the material, and was then washed to remove the bleach. Each sample was analyzed individually, five slides were prepared from each sample, and 20 microscopic fields were observed from each slide. So, data from 100 microscopic fields were registered for each sample. Species fragment density per field was recorded (Yagueddú et al. 1998). Then, the percentage of each item in the diet (species, Class of species, group of species) was calculated as the proportion of the total number of fragments of each item in the 100 observed fields in relation to all counted fragments of the sample. Species were grouped according to their seasonality and digestibility (Mattson 1980). Also, to recognize the species from the fecal fragments, epidermis patterns of all plant species present in the grassland were performed (Dizeo de Strittmatter 1973).

Animal material in the diet involved whole squeezed caterpillars (around 5 cm long) tangled with vegetation. These were counted and then hydrated to facilitate its identification on the basis of their colors and jaws. Their bodies were reduced to their exoskeletons and their cephalic capsules were in good condition, allowing the determination of the species with a key (Pastrana & Hernández 1978/79).

To analyze resource availability, vegetation was clipped at ground level from 50 randomly placed quadrants (2 m x 2 m) in each season. Plants were manually separated by species and dried at 60°C for 2 days to obtain the percentage of dry weight of each vegetal species and the total dry weight/ha (kg DM/ha).

Bonferroni interval (BI) for the observed proportion of use (Neu *et al.* 1974, Byers *et al.* 1984) was calculated

 $(\alpha = 0.05)$ to analyze the selectivity of species, Class of species (monocots and dicots) and group of species (legumes, other dicots, cool season grasses - C3, warm season grasses - C4, and other monocots). If vegetal availability (expected percentage of use) did not fall within the respective confidence interval for the observed percentage in feces, the difference between diet and availability was regarded as significant. So, if the percentage of the species, Class or group available in the grassland felt below the lower limit of its associated confidence limit, it was considered preferred. If this percentage fell above the upper limit of its associated confidence limit, it was not preferred. Reciprocally, if vegetal availability fell within the respective confidence interval for the observed percentage in feces, the difference between diet and availability was not significant.

RESULTS

The number of individuals in the study area varied between 120 (May 1997) and 215 (November 1996) with a mean density of 0.22 ± 0.04 rheas/ha (n = 18).

Pebbles and seashells were found in all feces, but their composition was mainly vegetal. From the 40 collected feces, only one contained animal material: 67 True Armyworms *Pseudaletia* (=*Mythimna*) *adultera*, (Noctuidae) in spring 1996.

In spring 1996, 34 vegetal species were found in feces. Black Medic (*Medicago lupulina*) was the most consumed species, then Perennial Ryegrass (*Lolium perenne*), the Mouse Ear (*Dichondra microcalyx*) and the Salt Water Couch (*Paspalum vaginatum*) (Table 1). In winter 1997, 27 species were found, and the most consumed species were Black Medic and Perennial Ryegrass, then Saltgrass (*Distichlis* spp.) and Chickweed (*Stellaria media*) (Table 1). In spring 1997, 30 species were found: Black Medic was the most consumed

species, then thistles (*Carduus acanthoides*, *Cirsium vulgare*, *Cynara cardunculus*, *Onopordon acanthium*), Salt Water Couch and Perennial Ryegrass (Table 1). Black Medic was the most common species in the three studied seasons, and was consumed more than available in both springs (BI 1.4 - 65.4 in 1996; BI 5.0 - 73.7 in 1997). Perennial Ryegrass was the most represented monocot in the diet and was always consumed in the same proportion as available (Tables 1 and 2).

In spring diets, dicots were more consumed than monocots, and during the whole study dicots were always preferred respect to their availability (56.88% \pm 16.98; BI 32.6 – 81.1 in spring 1996; 38.67% \pm 11.04; BI 4.2 – 73.2 in winter 1997; 65.75% \pm 12.63; BI 40.7 – 90.8 in spring 1997). Monocots were not preferred in spring (43.29% \pm 16.99; BI 19.6 – 66.7 in 1996; 34.25% \pm 12.63; BI 9.2 – 59.3 in 1997), but they were consumed in the same proportion as available in the grassland in winter. Also, monocots were more consumed than dicots in winter (Figure 1).

Legumes were preferred in spring $(32.20\% \pm 14.29;$ BI 4.6 – 59.8 in 1996; 41.93% ± 24.14; BI 5.2 – 78.6 in 1997), but in winter, although this group was common in the diet of rheas, the difference between consumed $(25.68\% \pm 8.51)$ and available $(4.96\% \pm 6.62)$ was not significant. Warm season grasses were not preferred in any season (23.13% ± 13.92; BI -0.02 – 46.3 in spring 1996; 21.93% ± 8.52; BI -0.12 - 55.6 in winter 1997; 19.08% ± 8.81; BI -0.11 – 49.5 in spring 1997, Figure 2A-C). Saltgrass and Smooth Cordgrass (Spartina spp.) were not preferred in spring 1996 (BI -8.7 - 14.4 and BI -1.7 -1.8, respectively), and Buffalo Grass (Stenothaphrum secundatum) and Smooth Cordgrass were not preferred in winter 1997 (BI -13.8 - 23.8 and BI -2.1 - 2.2, respectively) (Tables 1 and 2). The remaining plant groups (other dicots, cool season grasses and other monocots) were consumed in the same proportion as available in the grassland.

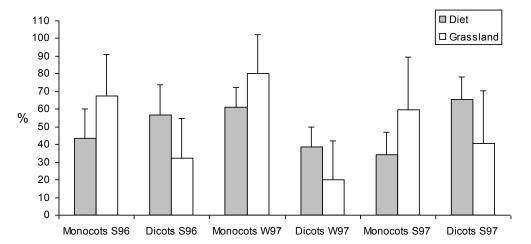


FIGURE 1. Percentage of vegetal Class and standard deviation in the diet of Greater Rhea (*Rhea americana*) and grassland in spring 1996 (S96), winter 1997 (W97), and spring 1997 (S97).

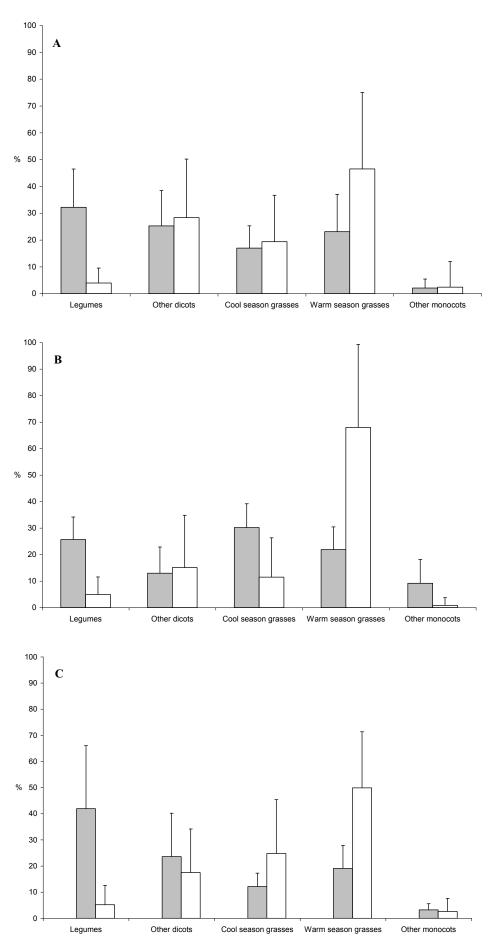


FIGURE 2. Percentage of vegetal groups and standard deviation in the diet of Greater Rhea (*Rhea americana*) and grassland in (A) spring 1996, (B) winter 1997, and (C) spring 1997. Grey = Diet, White = Grassland.

Vegetal availability was 1626.67 ± 1031.65 kg DM/ha in spring 1996, 1208.50 ± 564.81 kg DM/ha in winter 1997, and 3341.30 ± 1531.86 kg DM/ha in spring 1997. Grassland species, either native or adventive, were adapted to low wet soils, typical of the Flooding Pampa (Cabrera & Zardini 1993). Vegetal availability in spring 1997 was twice the one in spring 1996, and 50% of the diet of rheas consisted of Black Medic and thistles. Alternatively, in spring 1996, 50% of the diet was composed of Black Medic, Perennial Ryegrass and

Mouse Ear. In winter 1997, with the lowest availability, 50% of the diet of rheas was composed of Black Medic and Perennial Ryegrass (Table 1).

Among the species that appeared in the grassland, but not in the diet, the Saltwort (*Sarcocornia ambigua*) stands out. In spring 1996, vegetation samples had an important proportion (9.00% \pm 21.33), but it was not found in feces. Other species that did not appear in the diet and had low representation in the grassland (<1%) were *Galium aparine*, *Conium maculatum* and *Bupleurum teniusimum*.

TABLE 1: Botanical composition in percentage of the number of fragments of each species over the total number of fragments (Mean ± SD) in the diet of the Greater Rhea (*Rhea americana*) in Argentinean grasslands. N: native, A: adventive, C: cultivable, W: weed (Cabrera & Zardini 1993). *Statistically significant differences between consumption and availability.

Species in diet		Spring 1996 n = 20	Winter 1997 n = 10	Spring 1997 n = 10
Legumes				
Medicago lupulina (Black Medic)	А	30.42 ± 13.81*	22.60 ± 10.29	39.39 ± 25.86°
Trifolium repens (White Clover)	AC	1.02 ± 2.06	0.16 ± 0.39	2.38 ± 2.60
Adesmia incana	Ν	0.75 ± 1.12	2.89 ± 8.42	0
<i>Medicago arabica</i> (Spotted Medic)	А	0	0	0.18 ± 0.49
Other dicots				
<i>Malvella leprosa</i> (Alkali Mallow)	Ν	0.66 ± 1.03	0.07 ± 0.12	1.31 ± 1.13
Phyla canescens (Hairy Fogfruit)	Ν	3.63 ± 2.79	0	2.99 ± 3.95
Stellaria media (Chickweed)	А	3.59 ± 3.27	9.81 ± 9.63	4.76 ± 4.16
Ambrosia tenuifolia (Lacy Ragweed)	Ν	3.72 ± 3.58	0.83 ± 1.49	0.65 ± 1.02
Dichondra microcalyx (Mouse Ear)	Ν	9.05 ± 9.17	0.95 ± 1.76	1.51 ± 3.31
Mentha pulegium (Pennyroyal)	А	1.19 ± 2.33	0	0.67 ± 1.48
<i>Plantago</i> spp. (Plantain)	Ν	3.01 ± 4.81	1.33 ± 2.30	1.01 ± 2.49
Leontodon taraxacoides (Lesser Hawkbit)	А	0.24 ± 0.45	0	0
Thistles	AW	0	0	10.00 ± 9.49
<i>Centaurea</i> spp. (Star Thistles)	AW	0	0	0.25 ± 0.69
Rapistrum rugosum (Annual Bastardcabbage)	AW	0	0	0.47 ± 1.00
Eryngium spp. (Sea Holly)	Ν	0.09 ± 0.22	0	0
Cool season grasses				
Lolium perenne (Perennial Ryegrass)	AC	9.06 ± 7.60	22.58 ± 11.53	8.32 ± 4.44
Poa spp. (Meadow Grass)	Ν	1.20 ± 1.36	0.37 ± 0.74	1.44 ± 1.70
Bromus unioloides (Rescue Grass)	NC	1.75 ± 4.11	5.58 ± 6.03	0.41 ± 1.05
Hordeum bonariense (Barley)	Ν	1.94 ± 2.95	0.09 ± 0.27	0.28 ± 0.56
Festuca arundinacea (Tall Fescue)	AC	0.57 ± 1.16	0.15 ± 0.25	0
<i>Stipa neesiana</i> (Needle Grass)	Ν	1.55 ± 2.45	1.37 ± 1.39	0.49 ± 0.96
Avena sativa (Common Oat)	AC	0.18 ± 0.37	0	1.21 ± 1.71
Chaetotropis elongata	N	0.74 ± 1.38	0.09 ± 0.29	0
Warm season grasses				
Stenothaphrum secundatum (Buffalo Grass)	Ν	3.89 ± 5.96	4.95 ± 5.75*	5.18 ± 5.39
<i>Leersia hexandra</i> (Rice Grass)	Ν	2.54 ± 2.30	0.12 ± 0.29	1.38 ± 1.86
Thinopyrum ponticum (Tall Wheat Grass)	AC	2.67 ± 2.73	1.22 ± 2.00	0
Paspalum vaginatum (Saltwater Couch)	Ν	8.76 ± 7.36	3.10 ± 4.39	9.15 ± 3.04
Distichlis spp. (Salt Grass)	Ν	$2.86 \pm 1.83^*$	10.06 ± 7.11	1.27 ± 2.00
<i>Setaria geniculata</i> (Bristle Grass)	Ν	0.62 ± 1.40	0.25 ± 0.67	0
Sporobolus indicus (Smut Grass)	Ν	1.57 ± 2.82	2.11 ± 2.27	0.22 ± 0.42
<i>Cynodon dactylon</i> (Bermuda Grass)	AW	0.15 ± 0.28	0.06 ± 0.20	0
Bothriochloa laguroides (Silver Bluestem)	Ν	0.02 ± 0.08	0	1.72 ± 2.35
Spartina spp. (Smooth Cordgrass)	N	$0.05 \pm 0.19^*$	$0.05 \pm 0.16^*$	0
Other monocots				
Carex bonariensis	Ν	1.39 ± 3.55	4.06 ± 4.44	0.84 ± 1.66
Eleocharis flavescens (Yellow Spikerush)	Ν	0	0	0.96 ± 2.71
Cyperus rotundus (Nut Grass)	А	0	0	0.31 ± 0.88
Scirpus sp. (Sedges)	Ν	0.40 ± 0.55	0	0.91 ± 1.06
Sisyrinchium platense (Blue-eyed Grass)	Ν	0.03 ± 0.09	0.03 ± 0.09	0
Juncus imbricatus (Folded Rush)	Ν	0.27 ± 0.80	5.10 ± 6.78	0.21 ± 0.59

Species in grassland	Spring 1996 n = 50	Winter 1997 n = 50	Spring 1997 n = 50
a <i>Medicago lupulina</i> (Black Medic)	1.29 ± 2.85	0.78 ± 2.66	4.40 ± 7.55
b <i>Lolium perenne</i> (Perennial Ryegrass)	7.67 ± 8.43	5.57 ± 9.85	11.80 ± 14.27
c Dichondra microcalyx (Mouse Ear)	0.76 ± 2.59	0	1.20 ± 2.90
d Bromus unioloides (Rescue Grass)	0.52 ± 2.40	0.57 ± 2.71	0.40 ± 1.26
e Paspalum vaginatum (Saltwater Couch)	3.67 ± 9.49	0.43 ± 2.09	8.50 ± 14.93
f Stenothaphrum secundatum (Buffalo Grass)	13.19 ± 18.17	24.22 ± 27.79	10.00 ± 15.61
g <i>Distichlis</i> spp. (Salt Grass)	14.52 ± 27.25	14.65 ± 28.80	6.00 ± 11.94
h Spartina spp. (Smooth Cordgrass)	14.62 ± 29.42	26.09 ± 42.60	9.10 ± 18.81

TABLE 2: Grassland botanical composition in percentage of dry weight (Mean \pm SD) of the most common species in the diet of Greater Rheas (a, b, c, d, e) and less represented species in relation to their availability (f, g, h).

DISCUSSION

Diet of Greater Rheas in coastal natural grasslands of Argentina was generalist and predominantly herbivorous. These animals consumed a large number of vegetal species, but preferred dicots, mainly the legume M. lupulina. On the other hand, monocots were not preferred in neither of the two springs and were consumed in the proportion as similar to their availability in the grassland in winter. Martella et al. (1996) also found that the Greater Rhea preferred wild short-lived forbs and Alfalfa (Medicago sativa), but showed no preference for grasses, while Paoletti & Puig (2007) and Puig et al. (2013), found that the Lesser Rhea (Pterocnemia pennata) showed preference for forbs and shrubs with dominant grasses not selected. Even though rheas feed selectively, the Greater Rhea in this study showed a higher selectivity with increasing resources (forage abundance hypothesis), while the diet selectivity of the Lesser Rhea decreased with increasing resources (selective quality hypothesis) as it is predicted for arid environments (Puig et al. 2013).

Although Greater Rhea did not prefer monocots in any of the two spring seasons, the high proportion of cool season grasses in the diet in winter, principally L. perenne, could be due to their lower fiber content and to the low availability of dicots at that time of the year. It is in this season when diet of rhea and cattle can overlap (Pereira & Quintana 2009). Legumes preference in both springs could be related to their high nitrogen (N) content (Mattson 1980). In general, short-lived, fastgrowing species require and therefore contain more N than long-lived, slower-growing species (Mattson 1980). The non-preference for warm season grasses (C4) in all studied seasons could be due to their lower N content and higher fiber content in comparison to cool season grasses (C3) (Mattson 1980). Similar results were found by Madanes et al. (2010) for Greater Rhea in the lower Paraná River Basin.

Despite the low availability of the most consumed

species, rheas seek them and use their bill to select these plants when they are small. Besides, they ate native and adventive species, therefore, what they eat would depend on their preferences based on species quality, availability, and phenological stage of the plants. In the study site, rheas preferred sites near streams in all seasons (Herrera et al. 2004), probably due to the presence of riparian communities dominated by dicots as well as available water for drinking. Also, domestic animals like cattle, sheep and horses, used these areas near water sources. These large groups of wild and domestic herbivores gain the benefit of protection from predators and hunters (Farias & Canepuccia 2001, Carro & Fernández 2009, Barri et al. 2012). Moreover, in grasslands of the Flooding Pampa, these domestic herbivores prefer grasses, so their diet overlap with rheas would be low (Vacarezza 2001, Vacarezza et al. 2001).

Mean density of rheas was greater in neighboring crops (0.43 rheas/ha in the wheat area in spring 1996, and 0.40 rheas/ha in the oat area in spring 1997, Comparatore & Yagueddú 2007) than in the natural grasslands sampled in the present study (0.22 rheas/ha) in the same property and in the same counting period. This could be related to the fact that rheas ate weeds and animal plagues of these crops (Comparatore & Yagueddú 2007). The large number of True Armyworms found in one feces in the grassland in spring 1996, shows that rheas can cross fences to the neighboring wheat crop attacked by these caterpillars that move all together. True Armyworms were present in many feces collected in the neighboring wheat crop in the same season (Comparatore & Yagueddú 2007). The absence of caterpillars in feces collected in spring 1997 could be a consequence of a different neighbor crop (i.e. oat instead of wheat, pers. obs.). Oat is not a preferred crop for True Armyworms (SENASA 2016). Incorporating a high-protein item to the diet when it appears shows an opportunistic behavior. A food selection study in captivity supports the above. When minced meat was offered to rheas, the animals spent

between 12 and 29% of the time of the experiment eating it (Comparatore & Herrera 1998). This opportunistic behavior was also found for the Greater Rhea in a wetland region of Argentina (Pereira & Quintana 2009). Birds tend to be opportunistic foragers and often make ready use of superabundant food resources (Szaro *et al.* 1990). Furthermore, Southwood (1977) sustains that in disturbed environments, such as the one where this study was conducted, individuals, populations, and species should feed more flexibly.

Rheas can endure in agroecosystems that include grasslands, pastures and crops (Comparatore & Yagueddú 2007, Bernard 2012). Conservation problems for rheas may occur with the rapid increase of the area used for grain production and consequently low habitat diversity (Giordano *et al.* 2008, 2010, Codesido *et al.* 2012). This emphasizes the importance of coordinating actions to conserve grassland bird areas as exposed by Di Giacomo & Krapovickas (2005).

In conclusion, in the studied grasslands, rheas were more selective in spring, when the availability of the vegetation was high, and they preferred dicots (mainly legumes) and rejected monocots. In winter, when the availability of vegetation was low, they consumed legumes and monocots in the same proportion as available in grassland. Also, rheas showed an opportunistic behavior consuming caterpillars when they appeared.

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