Snake assemblage in a disturbed grassland environment in Rio Grande do Sul State, southern Brazil: population fluctuations of *Liophis poecilogyrus* and *Pseudablabes agassizii*

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A mark-recapture study of a snake assemblage using pitfall traps with drift fences was carried out in a disturbed grassland environment (e.g. cattle breeding and cultivations), located in the Pampa Biome, in the central region of the Rio Grande do Sul State, southern Brazil. From February 2001 to January 2004 we caught 272 snakes belonging to 20 species from the following families: Elapidae (5%), Viperidae (10%), and Colubridae (85%). The assemblage had a unimodal seasonal pattern of activity, and the highest number of captures occurred between September and May. There was a positive and significant correlation between the number of captures and monthly minimum and maximum average temperatures. Recruitment was observed from January to April. During the study, the area was affected by human activities, which altered the community structure: Pseudablabes agassizii was negatively affected by habitat devastation while *Liophis poecilogyrus* took advantage of this. Our results reinforced the impression that *Pseudablabes agassizii* is a habitat specialist species. We extend the understanding of the susceptibility of this species to environmental destruction in open natural environments of South America, and propose its use as a potential bio-indicator of the Pampa biome. We also discuss the importance of conservation strategies for snakes in grasslands of southern Brazil.

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Fig. 1. Geographical position of Santa Maria municipality, Rio Grande do Sul State (adapted from: www.ufsm. br/).

Introduction

The composition of species within communities and ecological relationships between them are determined by ecological processes (Futuyma 1986), where geographical and evolutionary factors can determine the species composition, diversity, niche occupation, and other features related to the organization of a community (Ricklefs & Schluter 1993). Differences in the way in which the species use resources may originate within each unique assemblage, show a response to coexistence, or reflect a historical divergence (see Vitt et al. 1999). Unfortunately, human modifications of innumerable habitats are occurring very fast, which may extinguish natural communities (Vitt & Vangilder 1983). On a global scale, the main threat to biodiversity is the growing occupation of natural landscapes for human activities (Ehrlich 1988, Colli 2003). Unfortunately, there is a lack of data for the understanding of several traits in snake communities, as compared with those in other vertebrate groups (Vitt 1987).

Medium and long term studies involving snake communities in temperate regions are necessary, because populations may vary considerably from one year to another (Scott & Campbell 1982). In addition, the relative densities and/or relative abundances of the species fluctuate, and some of the species could disappear and be replaced (Scott & Campbell 1982).

Long-term studies are essential to gain data that could be used as a tool to evaluate the impact caused by human actions, mainly the effects of habitat reduction, which could contribute to population decline and local extinction (e.g. Ellstrand & Elam 1993, Tornhill 1993, Loeschcke *et al.* 1994, Primack & Rodrigues, 2001).

In this study, we have explored basic questions related to assemblage ecology: (1) which species make up the assemblage? (2) Which is the recruitment season? (3) How is the seasonal activity pattern? (4) Is there a correlation between temporal distribution and abiotic environmental features, such as temperature, precipitation and/or solar radiation exposition? (5) Did the species which compose the assemblage show populational fluctuations during our study?

Material and methods

Study site

This study was conducted in a grassland of the Pampa Biome, located in the Peripherical Depression, in the central region of the State of Rio Grande do Sul, southern Brazil (Fig. 1). According to the classification of Köppen, the area is a Cfa type climate (subtropical warm and humid or temperate warm and rainy). Precipitation is regular during the year, varying from 1500 to 1750 mm (Moreno 1961, Pereira *et al.* 1989, Budke *et al.* 2004). The study site (29°44'S, 53°45'W) is covered by Poaceae and used for cattle breeding. During the study, the area was burnt and cultivated (annual plants, e.g., corn and soybean).

Sampling

The sampling was conducted from February 2001 to January 2004, and each month was considered a sample unit (total number of sample units = 36). We used pitfall traps with drift fences: $30 \ 100$ -liter barrels were placed in an L-shaped line, $20 \ m$ away from each other. The pitfall line traps (plastic mesh) measured 1 m in

height and totalized 580 m in length. The barrels were left open continually from February 2001 to January 2004 and were reviewed daily in the warm season and every two days during the cold season. We sampled a total of 1095 consecutive days (26 280 barrel-hours). Artificial shelters were used and occasional encounters with snakes inside the study area were also considered. The collected specimens were individually marked by tattoo branding of ventral scales (adapted from Woodbury 1956, Ferner 1979) or subcutaneous microchip implants. For each individual, we registered the following data: snout vent length (SVL, mm), tail length (TL, mm), mass (g), sex, and presence of eggs or embryos (ventral palpation). After this procedure, the snakes were released 15 m from the capture site. Pregnant females were retained until oviposition and eggs were incubated using humid vermiculite (Shine 1983), at room temperature (mean of 25 °C). Newborns were marked, measured, sexed and released 15 m away from the sites where the females had been captured.

Minimum and maximum average temperatures and precipitation and solar radiation means (accumulated hours of sunlight) were obtained from the Estação Meteorológica of the Departamento de Fitotecnia, Universidade Federal de Santa Maria.

We were able to determine whether the snake was adult or juvenile for the following species: *Liophis poecilogyrus, Lystrophis dorbignyi, Oxyrhopus rhombifer, Sibynomorphus ventrimaculatus* and *Waglerophis merremii*, using previous studies that define the minimum SVL of mature individuals (A. P. Maciel unpubl. data, J. L. Oliveira unpubl. data, G. F. Maschio unpubl. data, R. B. Oliveira unpubl. data, R. S. Jordão unpubl. data).

Data analysis

Using the sample-based rarefaction method we constructed a species accumulation curve for the study area (Mao Tau) (Colwell 1994–2006, Colwell *et al.* 2004) with 95% confidence intervals (Gotelli & Colwell 2001). The function of richness (Mao Tau) was calculated as the accumulation function of species throughout the

total number of months studied (n = 36 sample units). The species accumulation curve (Mao Tau) was made without replacement using 100 sample randomizations. Species richness estimators were calculated to evaluate the sampling effort, using nonparametric incidence-based estimators (Bootstrap, Chao 2, ICE, Jacknife 1 and 2) and abundance-based data (ACE and Chao 1) (*see* Colwell 1994–2006, Colwell & Coddington 1994). All analysis of the species accumulation curves were performed with EstimateS 7.5 software (Colwell 1994–2006).

The correlation between the monthly capture incidence and abiotic features of the study site (temperature, precipitation and solar radiation) was tested by calculating Spearman's rank correlation coefficient (r_{c}) (Zar 1999) because the datasets according to the Shapiro-Wilk normality test (Zar 1999) were not normally distributed. To compare the abundance of two snake species (Liophis poecilogyrus and Pseudablabes agassizii) likely to be affected by environmental impacts in the study area we used the χ^2 -test (Zar 1999) on the observed and expected frequencies before and after the impacts. The expected frequencies of snakes, considering the proportional sample numbers before and after impacts, were: Liophis poecilogyrus (16.5 before, 36 after) and Pseudablabes agassizii (25 before, 56.8 after). The χ^2 -test was also used to compare the sexual proportion of each species. Spearman correlation and χ^2 -test were performed using the BioEstat 4.0 software (Ayres et al. 2005); differences were considered significant at p < 0.05. Rank species abundance plots (Magurran 2004) were made to compare the community structure before and after environmental impacts (burning and cultivation of annual plants).

Results

Composition, richness and abundance

During the 36 months, we captured 272 snakes from three families, 15 genera and 20 species. Of the 255 snakes that were captured and released (excluding 17 deaths), the recapture rate was 18.7%. We observed a smaller number of Elapidae (5%) and Viperidae (10%) snakes, as com-





Fig. 2. Species accumulation curve of sample-based rarefaction (Mao Tau) for the snake assemblage, sampled monthly between February 2001 and January 2004, in a disturbed grassland, Rio Grande do Sul State, Brazil. Dots indicate mean curve and bars indicate 95% confidence intervals created by 100 randomizations.

pared with that of Colubridae (85%).

The species accumulation curve (Mao Tau) did not reach stability (Fig. 2) and the species richness estimators produced estimates greater than the actual recorded species richness (Table 1). The minor estimate was recorded with a Bootstrap estimator (22.15 ± 0.56) and the major estimate was recorded with Jacknife 2 estimator (27.75 ± 1.63 species) (Table 1). There was no significant difference in the sexual proportion of species which composed the snake assemblage, except for *Oxyrhopus rhombifer* ($\chi^2 = 6.0$, p = 0.02), whose males were more abundant than females (Table 2).

Table 1	. Richness	estimator	s of snal	ke assen	nblage
sampling	js between	February	2001 and	I January	2004
in a dis	turbed gras	ssland, Rid	Grande	do Sul	State,
Brazil.					

Estimators	Mean ± SD		
ACE	24.46 ± 1.28		
ICE	24.48 ± 0.01		
Chao 1	26.25 ± 7.55		
Chao 2	26.25 ± 7.55		
Jack 1	24.86 ± 2.48		
Jack 2	27.75 ± 1.63		
Bootstrap	22.15 ± 0.56		

Seasonality and abiotic features

Capture incidence was higher from spring to early autumn (September to May) for the whole

Table 2. χ^2 -test of sexual differences in each species composing the snake assemblage sampled between February 2001 and January 2004 in a disturbed grassland, Rio Grande do Sul State, Brazil. ns = not significant.

Species	χ^2	p
Atractus reticulatus	0	ns
Boiruna maculata	1	ns
Bothrops alternatus	1	ns
Bothrops neuwiedi	1	ns
Echinanthera cf. occipitalis	1	ns
Liophis flavifrenatus	0.143	ns
Liophis jaegeri	1.28	ns
Liophis miliaris	0.2	ns
Liophis poecilogyrus	2.57	ns
Lystrophis dorbignyi	0.42	ns
Mastigodryas bifossatus	2.57	ns
Micrurus altirostris	1	ns
Oxyrhopus rhombifer	6	0.02
Philodryas aestiva	2	ns
Philodryas patagoniensis	0.33	ns
Pseudablabes agassizii	2.13	ns
Sibynomorphus ventrimaculatus	0.05	ns
Tantilla melanocephala	5	ns
Thamnodynastes hypoconia	4	ns
Waglerophis merremii	2.25	ns



Fig. 3. Accumulated seasonal incidence of a snake assemblage in a disturbed grassland: (A) general assemblage; (B) newborns; (C) adult males; and (D) adult females (February 2001 to January 2004), Rio Grande do Sul State, Brazil.

assemblage (Fig. 3A). Newborns were recorded mainly at the end of summer (February and March), probably representing the main factor responsible for the peak in abundance (*see* Fig. 3B). We recorded a higher number of adult females than males at the end of the highest activity period of the assemblage (March/April) (Fig. 3C and D).

There was a positive correlation between the capture incidence and monthly average minimum and maximum temperatures ($r_s = 0.60$, p < 0.01 and $r_s = 0.47$, p < 0.01, respectively), but there was no correlation with solar radiation ($r_s = 0.02$, p = 0.89) or with precipitation ($r_s = 0.24$, p = 0.16) (see Fig. 4).

Environmental modifications

There were three major anthropogenic perturbations in the area — either burning of the area or the implementation of annual crops — that took place in September 2001, August 2002 and August 2003. After January 2002, we observed alterations in the abundance of two species of snakes: a progressive increase of Liophis poecilogyrus and a constant decline of Pseudablabes agassizii (Fig. 5). The latter was the most abundant species from February to December 2001 (n = 26, 28% of the total number of captures) but was not found again after April 2003. The observed frequency of P. agassizii was significantly different than expected by chance when we compared frequencies before and after the environmental impacts ($\chi^2 = 43.7$, p < 0.001, df = 1). In contrast, L. poecilogyrus was the most captured species after the environmental impacts (n = 64, 35%) and there was a significant difference in its observed frequency before and after the impacts ($\chi^2 = 21.9, p < 0.01, df = 1$). The rank species abundance plots corroborated the event of changes in the community structure after the environmental impacts (burning and cultivation) (Fig. 6A and B) in the study area. The community showed a greater diversity (greater evenness in the species abundance) before (Fig. 6A) than after the environmental impacts, when it was dominated by Liophis poecilogyrus (Fig. 6B).



Fig. 4. Correlation between capture incidence of snakes and monthly minimum and maximum average temperatures (A, B, respectively), accumulated hours of sunlight (C) and accumulated monthly precipitation (D) between February 2001 and January 2004, in a disturbed grassland, Rio Grande do Sul State, Brazil.



Fig. 5. Monthly incidence of *Liophis poecilogyrus* and *Pseudablabes agassizii* in a disturbed grassland, Rio Grande do Sul State, Brazil.

Fig. 6. Relative abundance of snake species for an assemblage studied in a disturbed grassland, Rio Grande do Sul State, Brazil, (A) before and (B) after environmental impacts. Species: Atractus reticulatus (Ar), Boiruna maculata (Bm), Bothrops alternatus (Ba), Bothrops neuwiedi (Bn), Echinanthera cf. occipitalis (Eo), Liophis flavifrenatus (Lf), Liophis jaegeri (Lj), Liophis miliaris (Lm), Liophis poecilogyrus (Lp), Lystrophis dorbignyi (Ld), Mastigodryas bifossatus (Mb), Micrurus altirostris (Ma), Oxyrhopus rhombifer (Or), Philodryas aestiva (Pae), Philodryas patagoniensis (Pp), Pseudablabes agassizii (Pa), Sibynomorphus ventrimaculatus (Sv), Tantilla melanocephala (Tm), Thamnodynastes hypoconia (Th) and Waglerophis merremii (Wm).



Discussion

Composition, richness and abundance

Species richness found in our study corresponded to 27.5% of the species recorded from the State of Rio Grande do Sul (n = 73; Di-Bernardo *et al.* 2003). The low recapture index recorded in this study indicates an assemblage composed of large populations (Begon *et al.* 1995), but the effect of high snake mobility cannot be rejected as an alternative hypothesis to explain this result. Both the species accumulation curve and the species richness estimators indicated a larger number of species in the study area. The species accumulation curve was not asymptotic, which means that there could be other species at the site that were not registered (Fig. 2). In most studies concerning biodiversity inventories, mainly in tropical ecosystems, the cumulative curves do not reach stability (Santos 2003), and thus, the absence of an asymptotic species accumulation curve indicates that with further collection effort, there may have been an increase in the number of species recorded in the area. The results of the species richness estimators corroborated the tendency observed in the species accumulation curve and the estimate most likely varied according to the sensibility of the estimators to species that were not very abundant or were infrequent in the samples. Thus, we considered that at least four species recorded in similar neighboring areas (*Liophis almadensis*, *L. anomalus*, *Philodryas olfersii* and *Thamnodynastes strigatus*) may occur in the study area (Cechin *et al.* 2002 and http://www.biotaneotropica.org.br/v5n1/pt/ abstract?inventory+BN02705012005).

In the Neotropical region there are three lineages of Colubridae: Colubrinae, Xenodontinae from Central America (Dipsadinae, sensu Zaher 1999) and Xenodontinae from South America (Greene 1997). In the south of Brazil, these two first lineages are poorly represented (S. Z. Cechin unpubl. data). For instance, only four Xenodontinae species from Central America (*Atractus reticulatus, Sibynomorphus ventrimaculatus*, and the incertae sedis of this subfamily: *Echinanthera* cf. occipitalis, and Thamnodynastes hypoconia, sensu Zaher 1999) and two species of Colubrinae (Mastigodryas bifossatus and Tantilla melanocephala) were registered at this study site (Table 3). The majority of the assemblage are species of the South-American Xenodontinae lineage, as recognized by Cadle (1984). This could be explained from a historical perspective as this group has a large representation in South America, where approximately 60 of the 93 American genera occur, while Colubrinae snakes are represented by nearly 12 of the 32 genera existent in all of the Americas (Duellmann 1979). The great incidence of Xenodontinae in the assemblage studied by R. B. Oliveira (unpubl. data) in sand dunes on the northern coast of the State of Rio Grande do Sul, follows a general tendency observed in snake communities of South America, that the more to the south a community is located, the higher the proportion of representatives of this group. This explanation

Table 3. Snake richness and abundance recorded in a disturbed grassland in Rio Grande do Sul State, Brazil. *n* = number of individuals, FR = relative frequency of captures (%). * Allocated in subfamily as *incertae sedis* (sensu Zaher 1999).

	п	FR
Colubridae		
Colubrinae		
Mastigodryas bifossatus (Raddi, 1820)	19	6.98
Tantilla melanocephala (Linnaeus, 1758)	5	1.85
Dipsadinae		
Atractus reticulatus (Boulenger, 1885)	2	0.73
* <i>Echinanthera</i> cf. <i>occipitalis</i> (Jan, 1863)	1	0.37
Sibynomorphus ventrimaculatus (Boulenger, 1885)	19	6.98
*Thamnodynastes hypoconia (Cope, 1860)	4	1.47
Xenodontinae		
Boiruna maculata (Boulenger, 1896)	1	0.37
Liophis flavifrenatus (Cope, 1862)	30	11.03
Liophis jaegeri (Günther, 1858)	7	2.58
Liophis miliaris (Linnaeus, 1858)	5	1.85
Liophis poecilogyrus (Wied, 1825)	79	29.04
Lystrophis dorbignyi (Duméril, Bibron and Duméril, 1854)	21	7.72
Oxyrhopus rhombifer Duméril, Bibron and Duméril, 1854	24	8.82
Philodryas aestiva (Duméril, Bibron and Duméril, 1854)	2	0.73
Philodryas patagoniensis (Girard, 1857)	3	1.1
Pseudablabes agassizii (Jan, 1863)	32	11.76
Waglerophis merremii (Wagler, 1824)	15	5.51
Elapidae		
Elapinae		
Micrurus altirostris (Cope, 1860)	1	0.37
Viperidae		
Crotalinae		
Bothrops alternatus Duméril, Bibron and Duméril, 1854	1	0.37
Bothrops neuwiedi Wagler, 1824	1	0.37
Total	272	100

could also be applicable to the assemblage of our study, since the two sites (ours and Oliveira's) are relatively close.

The most abundant snake was Liophis poecilogyrus, a species that is probably tolerant of disturbed areas, followed by Pseudablabes agassizii, a species sensitive to environmental degradations as indicated by our results (see below) and those recorded in the Brazilian cerrado (Marques et al. 2006). The low incidence of snakes from Viperidae and Elapidae families differentiates this assemblage from the majority of assemblages studied in Brazil (e.g. in Amazonas State [M. Martins unpubl. data], in São Paulo State [O. A. V. Marques unpubl. data, and R. L. Sawaya unpubl. data], in Rio Grande do Sul State [S. Z. Cechin, unpubl. data]). We believe that this difference between the studies is caused by the different sampling methods applied, because in our study we did not use visual search associated with pitfall traps, as did the other authors. Visual search could be favorable for encountering sit-and-wait species (Viperidae).

In relation to sampling techniques, Greenberg et al. (1994) suggest that all capture mechanisms (e.g., pitfall traps with drift fences) are biased, because they are selective. Small species with surface activities are easier to catch with pitfall traps or funnel traps, than large snakes (Campbell & Christman 1982, Enge & Marion 1986). For the pitfall trap with drift fence system, the length, number, height and arrangement of fences can affect the sampling result (Campbell & Christman 1982, Vogt & Hine, 1982, Jones 1986, Bury & Corn 1987, Corn & Bury 1990). In addition to biased sampling techniques, the home range, daily and seasonal movement patterns, and micro-habitat fidelity may also influence the efficiency of the capture (Gibbons & Semlistch 1982, Bury & Corn 1987, Corn & Bury 1990). A single pitfall system does not capture all species at proportions representative of their real abundance, which makes it difficult to estimate the population or the relative abundance and diversity between habitats (Corn 1994).

The sexual proportion differed only in *Oxyrhopus rhombifer*, the males of which were more abundant than females. Perhaps the main factor which contributed to differences in the sexual proportion is the faster maturation of

males. The greatest incidence rate from one of the genders may be determined by differences related to feeding, activity pattern, and reproduction, as well as biased sampling (Parker & Plummer 1987). Males tend to move more than females and, consequently, become more exposed during the reproductive season (Whitng *et al.* 1996, Greene 1997) to pitfall traps. However, considering that there were no differences in the sexual proportion in others species, we believe that the discrepancy recorded for *O. rhombifer* may be more a chance effect than biased sampling.

Seasonality and abiotic features

The unimodal pattern observed in this study corroborates the results found in subtropical regions (e.g. R. B. Oliveira unpubl. data), where temperature is considered a limiting factor for most snake species.

Temperature and rain are the climatic variables with the greatest impact on snake activity patterns (Gibbons & Semlitsch 1987). The absence of a correlation between capture incidence and precipitation is due to the regular distribution of rain throughout the year, without a dry or wet season in the area.

The great incidence of females during September and October may be due to the search for oviposition sites (Reinert 1984, Shine 1988, Graves & Duvall 1993) (Fig. 3D). Larger numbers of males were captured from August to March (Fig. 3C) due to the search for reproductive partners. Therefore, we believe that reproduction is generally seasonal in our region, corroborating other studies (e.g. M. Di-Bernardo unpubl. data, R. B. Oliveira unpubl. data).

The intense activity observed in March and April is related to recruitment (Fig. 3B). Gibbons and Semlitsch (1987) suppose that the difference between juvenile and adult activity patterns is due to differences in selective pressures (e.g., diet, predators), as is demonstrated for a variety of taxa (*see* Mushinsky 1987); although the entry of new individuals into the population, hatching, and reproductive cycles would explain the influence of recruitment on the seasonal activity pattern.

Environmental modifications

Changes in abundance after human disturbance at our study site show that *P. agassizii* is negatively affected by habitat degradation and that *L. poecilogyrus* takes advantage of it. *P. agassizii* is a poorly collected snake species (S. Z. Cechin pers. obs.) which is not threatened with extinction in Brazil. However, the species is considered threatened in the Brazilian State of Minas Gerais and of maximum priority for conservation in Uruguay (Morales Fagundes & Carreira Vidal 2000). In Argentina, this species was also considered not threatened (Scrocchi *et al.* 2000), although with only a few reports (Giraudo 2001).

In the recently published study about the ecology of *P. agassizii*, this snake was considered a habitat-specialist for a "cerrado" habitat (shrubby grassland or 'campo sujo') in the southeast of Brazil and highly susceptible to environmental destruction, probably because of its underground microhabitat foraging specificity and egg-laying activities (Marques *et al.* 2006). In this area of southeastern Brazil no specimen of *P. agassizii* was recorded in the impacted areas (such as *Eucalyptus* groves) surrounding the "cerrado" of the Conservation Unit (Marques *et al.* 2006).

Actual data show that over 180 000 km² of southern Brazil were covered by grasslands (Leite & Klein 1990). This physiognomy is considered threatened due to excessive cattle breeding, burning, invasion of exotic species and conversion of native vegetation to cultivated areas. Often, only a few portions of the native vegetation inserted into a landscape that is predominantly agricultural remain (Risser 1997, Porto 2002, Bencke 2003), especially in LEDC countries. In addition, although native grasslands are considered important stores of genetic diversity, they are usually neglected due to the low rate of endemism (Risser 1997), which results in only a few areas of this native habitat being effectively protected in Conservation Units (Mantovani & Silva 2002). This sad situation persists in Rio Grande do Sul State, mainly due to the great extension of monocultures such as soybeans, and more recently, due to projects involving the transformation of extensive areas of grasslands from the Pampa biome (including those indicated according to MMA [2002] as prioritaries for fauna and flora conservation) into *Eucalyptus* forests for cellulose extraction. Thus, species that occur predominantly in opened natural environments, such as *P. agassizii* in the Pampa biome, are at risk because of the progressive destruction of this vegetation type.

Conclusions

Snake fauna of the Pampa biome has not been sufficiently studied, when compared with other ecosystems this environment houses a smaller number of species (e.g., Atlantic Forest). However, analyzing the area where this study was conducted, the species richness can be considered high as compared with the total number of species recorded in the State of Rio Grande do Sul. Snake activity was associated with the season of higher temperatures, and it followed the typical unimodal pattern recorded in subtropical regions.

Our results showed that the structure of the snake community was affected by environmental impacts and reinforced the impression that *Pseudablabes agassizii* is a habitat-specialist species. We extended the understanding of the susceptibility of *P. agassizii* to environmental destruction in open natural environments of South America and propose its use as a potential bioindicator for the Pampa biome. We believe that creation of Conservation Units in the Pampa, an open field biome profoundly modified by human activity, as well as inclusion of *Pseudablabes agassizii* in the list of threatened fauna in Brazil should result from this study.

The species that occur mainly in open natural environments, such as *P. agassizii* in the Pampa biome, are at risk due to the progressive destruction of this vegetation type. Long-term studies of Pampa communities are necessary to produce results that may be used as a base for the conservation of the species that occur in this biome.

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