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Seasonal, daily activity, and habitat use by three sympatric pit vipers (Serpentes, Viperidae) from southern Brazil

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ABSTRACT

Viperid snakes are widely distributed in the South America and the greater distribution range of the family is found at the Crotalinae subfamily. Despite the abundance of this snakes along their geographic distribution, some ecological aspects remain unknown, principally at subtropical areas. In the present study, we evaluated the activity (daily and seasonal) and the use of the habitat by *Bothrops diporus*, *B. jararaca* and *B. jararacussu*, in an Atlantic Forest area at southern Brazil. We observed higher incidence of viperid snakes during the months with higher temperatures, while no snakes were found during the months with lower temperatures. The data suggest the minimum temperature as environmental variable with the greatest influence on the seasonal activity of this species. Considering the daily activity, we observed a tendency of snakes to avoid the warmest hours. *Bothrops jararacussu* tend to avoid open areas, being registered only inside and at the edges of the forest. We compared our results with previous studies realized at tropical areas and we suggest the observed seasonal activity as an evolutive response, despite the influence of the different environmental variables, according to the occurrence region.

Key words: Parque Estadual do Turvo, Rio Grande do Sul, Atlantic Forest, *Bothrops*, activity pattern.

INTRODUCTION

Ecological studies using snakes as a model organism have become more frequent in recent decades (e.g., Huey et al. 1989, Shine and Bonnet 2000, Blouin-Demers and Weatherhead 2001, Miller et al. 2011), mainly due to their greater

plasticity in habitat use, behavior, and ecology, when compared to other vertebrate groups (e.g., Ford and Siegel 1989, Madsen and Shine 1993). In addition, information about their natural history are important for understanding the wide variety of habits that many species have adopted through their life story, generating important data for employing conservation measures (Greene 1997).

The family Viperidae is widely spread, with more diversity in the tropics of Central America, South America and Southeast Asia (see Campbell

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and Lamar 2004). Most radiation occurred within the subfamily Crotalinae, and in South America representatives can be found in all ecosystems (Martins et al. 2001). In Brazil, the members of this subfamily are generally abundant in inventories and studies of snake assemblages (Martins and Oliveira 1998, Santos et al. 2005, Zanella and Cechin 2006, Pontes et al. 2008, Sawaya et al. 2008, Hartmann et al. 2009a, b, Guizoni-Jr et al. 2009, Centeno et al. 2008), with exceptions (e.g., Winck et al. 2007, Martins et al. 2012). However, studies are still incipient in remnants of Atlantic Forest in southern Brazil, where *Bothrops diporus* (Cope 1862), *Bothrops jararaca* (Wied 1824), and *Bothrops jararacussu* Lacerda, 1884 occur in sympatry.

Closely related species that occur in sympatry usually differ in some degree in some of the major axes of the hyper-dimensional niche (*sensu* Hutchinson 1959): time, space, and food (see Pianka 1986). Regarding space use, some species are more adapted to inhabit different types of vegetation (e.g., natural or degraded, more closed or more opened), and these limitations are mainly related to several features of the species natural history (e.g., nest site, alimentary resource, thermal regulation) (see Gibbons and Semlistch 1987, Martins et al. 2001, Hartmann and Marques 2005). In terms of temporal activity, snake species may vary over the year (seasonally) and throughout the day. Snakes of sites with temperate climates tend to show more restrictions on seasonal activity than the tropical species (May et al. 1996) because in most cases seasonality is related to the breeding activities (see Mushinsky 1987). Meanwhile, daily activity tends to be more related to the activities of foraging and thermoregulation (see Gibbons and Semlistch 1987). Studies with *Bothrops* in tropical areas usually classified them as nocturnal snakes, with activity peak corresponding to the rainy season (Campbell and Lamar 2004). In southern Brazil, the data on viperids are still incipient, with most studies designed to recognize patterns related to

reproduction, seasonal activity (e.g., Hartmann et al. 2004), and diet and habitat use (e.g., Hartmann et al. 2005). In the present study, we sought to assess the habitat use by three sympatric species of the genus *Bothrops*. We also pursued to elucidate if the pattern of seasonal and daily activity of three species in subtropical Brazilian territory is similar to the known congeneric species occurring in tropical climate.

MATERIALS AND METHODS

STUDY AREA

The study was conducted in Turvo State Park (Parque Estadual do Turvo, PET), situated in the northwest region of the state of Rio Grande do Sul, Brazil (27°07'-27°16' S; 53°48'-54°04'W). The area has a variation in altitude between 100 and 400 m, belonging to the municipality of Derrubadas. With 17,491 ha, the conservation unit is inserted within the Atlantic Forest biome domain (Instituto Brasileiro de Geografia e Estatística, IBGE 2010). The vegetation type is semi-deciduous forest, belonging to the Misiones core dominion in seasonal tropical (TSFR; *sensu* Prado 2000), and represents the largest remaining area of such vegetation in the state (Secretaria do Meio Ambiente do Estado do Rio Grande do Sul, SEMA 2005).

The climate in PET is characterized as subtropical sub-humid with dry summer (Maluf 2000). During the study the average temperature was 20.5°C, with two seasons defined by temperature. The first, with higher temperatures, comprised the months from November to March, with an average of 25°C. The second, corresponded to the season with lower temperatures, and ranged from May to September, with average temperature of 15.7°C. Rainfall during the study period was 2231.8 mm, with monthly averages of 185.9 mm. The months of lowest rainfall were April and June, with 26.20 mm and 42.5 mm, respectively. Climatic data during this study were obtained from the National Institute of Meteorology (Instituto Nacional de

Meteorologia, INMET 2010) from the weather station situated in the city of Iraí, 56 km distant from the study area. Data comprised temperature (mean monthly minimum, monthly average, and maximum monthly average), precipitation (monthly rainfall) and insolation.

SAMPLING

We conducted one year of monthly sampling during 10 days (from April, 2009 to March, 2010) in areas pertaining to PET, and in surrounding areas. The categories of vegetation considered for this study included: i) forest interior, at least 1 km away from the edges of the conservation unit, which influence the natural hydroperiod of the ponds of the PET; ii) forest edge, which comprise transitional environments between the areas of cultivation and forest; and iii) degraded areas, which correspond to the matrix surrounding the conservation area with degraded natural vegetation and/or replaced by cultivation or pastures. For analysis of daily activity, we considered the time of day as a sample, while for seasonal analysis, we considered the months as samples (total of samples = 12). To avoid the bias of the different number of sampled hours of day and night, we calculated the rate of activity of these periods (day and night) as the number of minutes divided by recorded snakes within 24 hours (day = 660 minutes; night = 360 minutes). To capture the snakes, we used different methods in combination: pitfall traps with drift-fence, time-constrained search, road search, support of registered workers, and occasional encounters. The pitfall traps (Cechin and Martins 2000) were installed in eight stations, each consisting of a row of four plastic containers of 0.8 X 0.4 m (height x diameter), with a capacity of 136 liters, buried in the ground level, placed every 15 m, and united by a plastic fence with height of 0.75 m. In total we used 32 containers and about 480 m of drift-fences. Four stations were placed in forest edge, 20 m far from the opened vegetation area. Other four stations were placed in forest interior,

three kilometers far from forest edge. Each station was placed at least a kilometer apart from the nearest one. The traps were opened 24 hours previous to first review, and remained open uninterruptedly during five days per month. The traps were checked each morning, and the sampling effort was measured in hours of open traps. To accomplish time-constrained search (TCS) (Martins and Oliveira 1998), we walked through transects without pre-defined orientation with slow dislocation in each vegetation category considered. Sampling using TCS was accomplished for three hours during the day (between 08:00-12:00 or 14:00-19:00 hours) and three hours at night (between 19:00-24:00 hours), dispensing the same effort at all daily hours. The sampling effort and the rate of encounter of snakes were measured in person-hours of visual search. During road search (ROS) (Hartmann et al. 2011) we recorded the number of active snakes (e.g., dislocation, basking, etc.) in roads inside PET as well as the roads around the park. During sampling, the forward speed was up to 30 km/h. The sampling effort was quantified in kilometers driven. Records made by registered workers (RW) (Bernarde and Abe 2006) were performed by local residents trained to handle snakes and to collect individual data. Residents were equipped with protective items, trained for handle the animals, and properly registered at the licensor organ (SEMA and Instituto Brasileiro de Meio Ambiente e dos Recursos Naturais Renováveis IBAMA). We considered occasional encounters (OE) those records done during off-hours search (TCS and ROS) (Sawaya et al. 2008, Hartmann et al. 2009a).

We consider snakes to be active when dislocating, or exposed with the neck in the shape of "S" and with the head forming an angle of up to 20° to the ground performing stalking activity (Oliveira and Martins 2001). Snakes were considered inactive when they were sheltered, or neck flat against the body forming an angle of less than 20° relative to the ground (Oliveira and Martins 2001).

DATA ANALYSIS

To compare the abundance of viperids and environmental variables (minimum temperature, rainfall and insolation), we used multiple linear regression. To this end, climate data and abundance were transformed into logarithm (ln) to ensure homoscedasticity of data (Zar 1999). To calculate the rate of encounter in day and night periods, we used the number of captures divided by minutes of sampling over 24 hours. To statistical analysis, we utilized the software Statistica 8.0 (STATSOFT 1984-2006). To analyze the use of mesohabitats by snakes (degraded area, forest edge and forest interior) we utilized analysis of variance via data randomization (see Legendre and Legendre 1998) with 10,000

iterations. Each specimen was considered a sample ($N=97$; G.L.=96), and each species as a group ($N_{\text{groups}}=3$). As resemblance measure, we utilized Gower index. This analysis was conducted through MULTIV 2.78b (Pillar 2006).

RESULTS

We recorded 97 viperid snakes in the area: *Bothrops diporus* ($N=63$), *Bothrops jararaca* ($N=21$) e *Bothrops jararacussu* ($N=13$). Active individuals were recorded from October to March, with the highest occurrence during the months of January, February and March (Figure 1). During the months of lower ambient temperatures (June, July and August), there was no record of snakes (Figure 1).

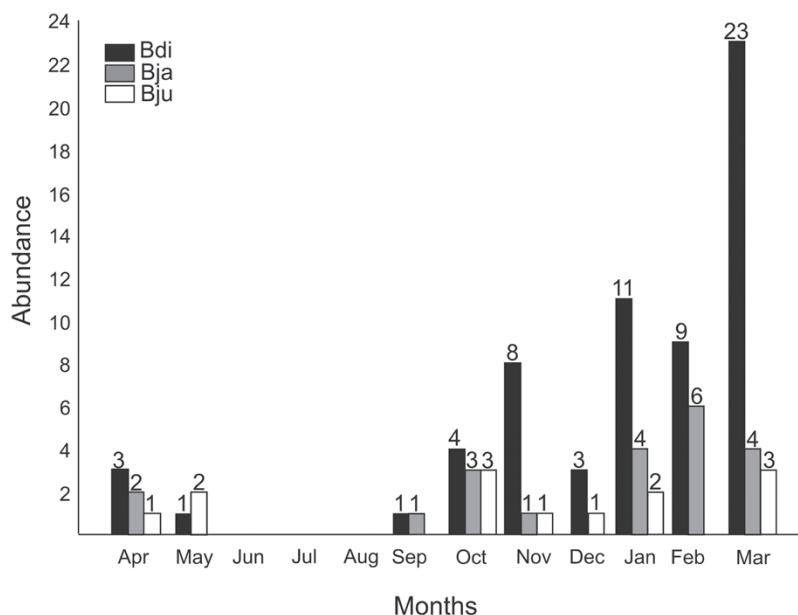


Figure 1 - Number of monthly records for the three viperid species found in Turvo State Park (Parque Estadual do Turvo), Rio Grande do Sul, Brazil, from April 2009 to March 2010, considering all sampling methods. Bdi = *Bothrops diporus*; Bja = *B. jararaca*; Bju = *B. jararacussu*.

Among the measures of temperature recorded for the study area, we chose the minimum temperature due to the higher coefficient of variation associated with this (CV = 29.91%). Therefore, we compared the number of snakes encounters with minimum monthly average temperature, monthly

rainfall, and monthly insolation (Total: $R^2 = 0.76$, $R^2_{\text{aj}} = 0.68$, $F(3,8)=8.65$ and $p < 0.05$). There was a positive and significant relationship between the occurrence of snakes and minimum temperature of PET ($\beta_{T_{\text{min}}} = 0.84$, $p < 0.05$; $\beta_{\text{Precipitation}} = -0.07$, $p = 0.77$; $\beta_{\text{Insolation}} = 0.10$, $p = 0.72$) (Figures 2 and 3).

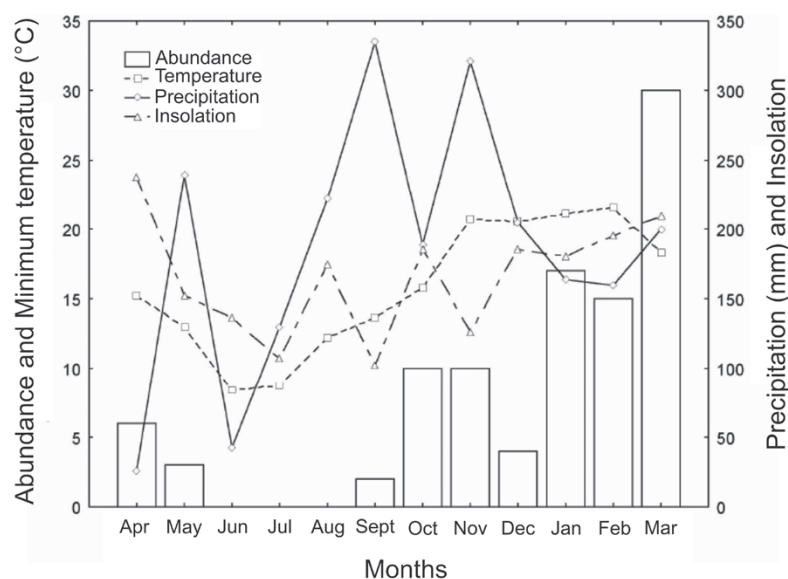


Figure 2 - Abundance of viperid snakes in Turvo State Park (Parque Estadual do Turvo), Rio Grande do Sul, Brazil, and environmental variables recorded during the present study (from April 2009 to March 2010).

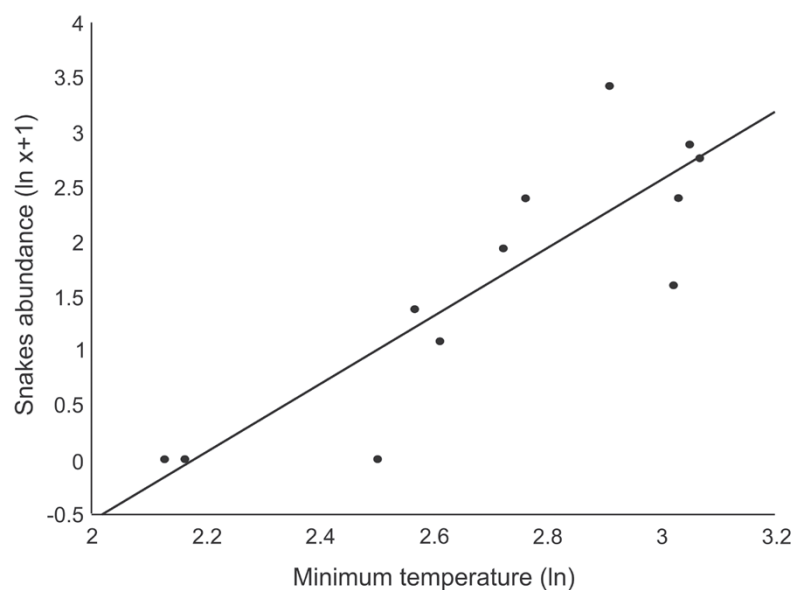


Figure 3 - Simple linear regression between abundance of snakes and the minimum environmental temperature, for viperids found in Turvo State Park (Parque Estadual do Turvo), Rio Grande do Sul, Brazil, from April 2009 to March 2010. All data were transformed into logarithm (ln).

Of the 97 viperid records, it was possible to determine if the snake was active or inactive in 86 encounters. In general, viperid snakes presented lower activity during the period between 12:00 and

15:00 (Figure 4A). *Bothrops diporus* was not found in just one of the sampled periods (between 13:00 and 14:00; Figure 4B); *B. jararaca* had the highest number of sightings in the afternoon and evening

(Figure 4C); whereas *B. jararacussu* comparatively were more frequently found at night (Figure 4D). The activity rate of viperids during daytime was 0.086 snakes/minute, and 0.144 snakes/minute overnight. *Bothrops diporus* and *B. jararaca* were found mainly in degraded areas, whereas *B. jararacussu* was found almost exclusively in forest interior (Figure 5). The use of mesohabitat was significantly different among viperids (Table I).

TABLE I

Results from variance analysis via data randomization to test the mesohabitat used by viperid snakes in Turvo State Park, southern Brazil, from April 2009 to March 2010.

Sources of variation	Sum of squares	p
Factor species		
Among species	9.47	0.0001
Contrasts		
Bdi Vs. Bja	1.54	0.003
Bdi Vs. Bju	9.06	0.0001
Bja Vs. Bju	3.21	0.0007

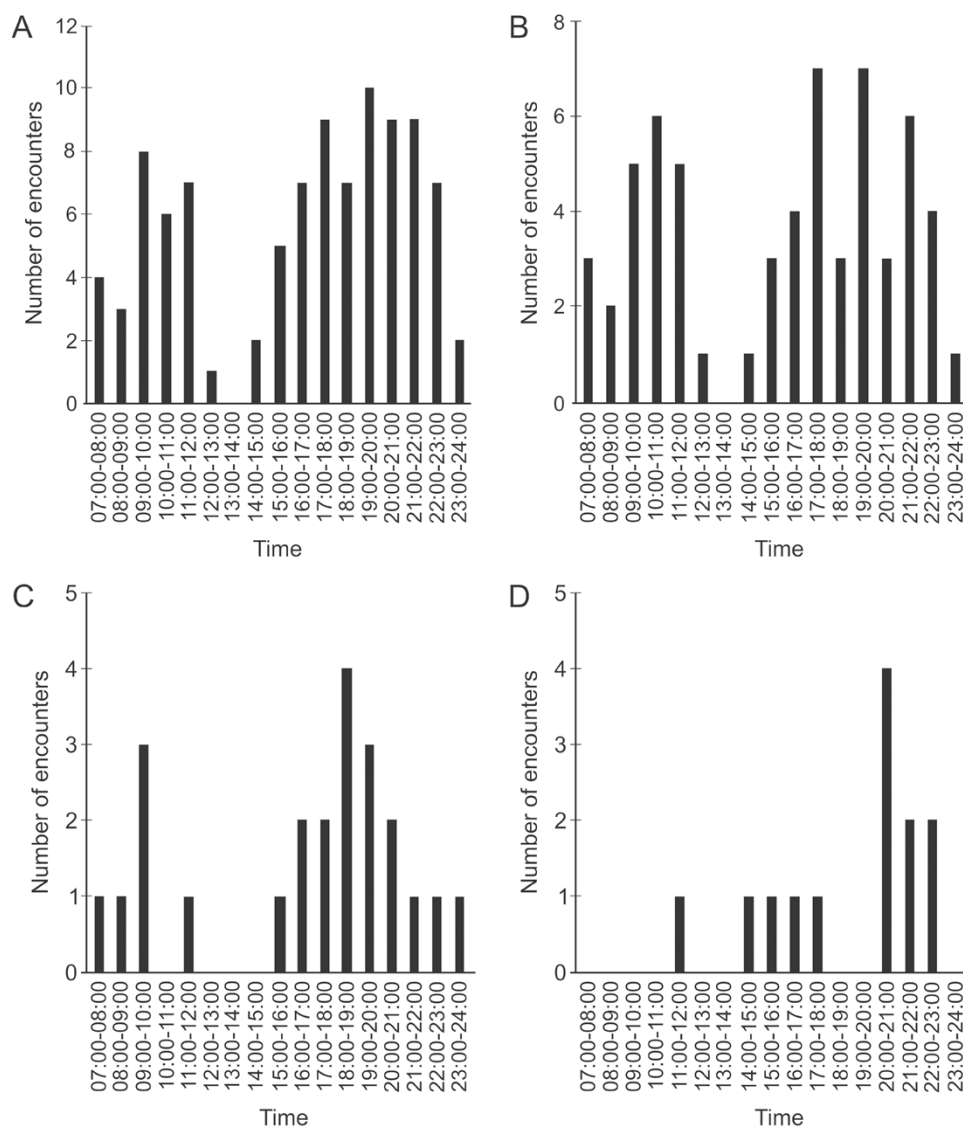


Figure 4 - Daily activity of recorded pit viper snakes, sampled from April 2009 to March 2010, in Turvo State Park (Parque Estadual do Turvo), Rio Grande do Sul, Brazil. A= all species; B= *Bothrops diporus*; C= *B. jararaca*; D= *B. jararacussu*.

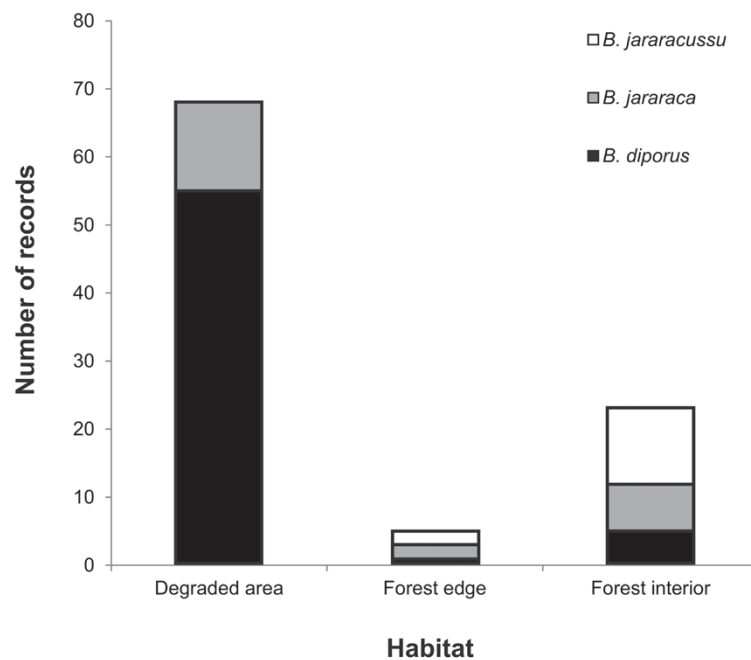


Figure 5 - Habitat use by viperid snakes in Turvo State Park (Parque Estadual do Turvo), Rio Grande do Sul, Brazil, during the present study (from April 2009 to March 2010).

DISCUSSION

The data indicated that the activity of *Bothrops diporus*, *B. jararaca*, and *B. jararacussu* reflected environmental seasonality, with reduced activity in the months of lower temperatures and higher activity in warmer months. Biotic and abiotic factors may influence the activity patterns of snakes, such as rainfall and temperature, prey availability, and reproductive partners (e.g., Henderson et al. 1978, Vitt 1987, Gibbons and Semlitsch 1987, Martins and Oliveira 1998, Bernarde and Abe 2006). However, snakes in general tend to vary their activity according to precipitation and temperature (see Gibbons and Semlitsch 1987). In Brazil, the variation in seasonal activity was recorded for other snakes in the southeastern Atlantic Forest (Marques et al. 2001, 2006, Pontes et al. 2009) and Amazon (Oliveira and Martins 2001). In these locations, the increased activity of snakes was usually observed in periods with greater incidence of rainfall (e.g., Martins and Oliveira 1998, Valdujo et al. 2003, Bernarde

and Abe 2006, Pontes et al. 2009). In the southern region, the seasonality is influenced by constancy of precipitation and the variation of temperature (see Maluf 2000, Mantovani and Silva 2002). Some studies indicate a trend on seasonal activity of snakes: at the subtropical region of Brazil, it may be related to the environmental temperature (e.g., Maciel et al. 2003, Winck et al. 2007).

Our data indicate that the temperature in the area influenced the seasonal activity of the viperid species, observed mainly by the lack of active snakes during the months of lower temperatures. Over the years, studies indicated low temperatures as a limiting factor for the activity of most snakes, because their metabolism is influenced by the thermal regime of the environment in which they live in (Gibbons and Semlitsch 1987). Previous studies with snakes in sites with marked temperature variation suggested the temperature as being the main factor limiting the activity of snakes in subtropical South America (Maciel et al. 2003, Hartmann et al. 2004, Winck et

al. 2007, Giraudo et al. 2008, Hartmann et al. 2009b). The environmental effects of seasonality may act in the reproductive activity of snakes (Sun et al. 2001, Brown et al. 2002), probably due to the higher probability of survival of the offspring in warm climate (see Gibbons and Semmlitsch 1987). Viperids from PET seem to present these features, since the main prey for these species (small mammals) was found throughout the entire sampled year.

Overall, our data suggest that the daily activity of the studied viperids tends to vary, with lower rates of encounter during periods with higher temperatures. Many Squamata (including snakes) adjust their activity patterns in response to changes in temperature (Nelson and Gregory 2000). Marques et al. (2001) showed the activity of *B. jararaca* and *B. jararacussu* to be mainly nocturnal. Sazima (1992) also classified *B. jararaca*, as a nocturnal snake. Hartmann et al. (2009b) found *B. jararaca* and *B. jararacussu* active during day and night; however, these species appeared to be more active during the night. Marques and Araújo (2011) observed an individual of *B. jararacussu* feeding during the day on a prey with predominantly diurnal activity. Oliveira et al. (2001) categorized *B. atrox* as a nocturnal species with occasional daytime activity. Valdujo et al. (2003) observed prevalence of nocturnal activity in *B. pauloensis*. Low rates of nocturnal snake encounters during the day could be related to the fact that these snakes could be sheltered during the day to avoid visual predators (Martins and Oliveira 1998). Our study confirmed previous data, since the proportion of encounters *B. diporus*, *B. jararaca* and *B. jararacussu* during the night was higher than the daytime.

In this study, our data indicate exclusive occurrence of *B. jararacussu* in forested areas of PET, and the probable inability of this species to use the open vegetation and/or crops around PET. The close relation between the species and forest areas with some degree of conservation was recognized in previous studies (e.g., Hartmann et

al. 2009b). However, the use of matrix areas in the vicinity of the fragments is not yet elucidated. Although *B. jararacussu* is not in the Brazilian List of Threatened Fauna (IBAMA 2003, Fundação Biodiversitas 2002), and in the category "Least Concern" in the IUCN Red List (IUCN 2012), the threat to Atlantic Forest environments logically consists of a direct risk to this species. The lack of knowledge on the status of populations (IUCN 2012) allied to the apparent limitation that the open areas exert on *B. jararacussu*, and to the high rates of habitat loss and fragmentation generate deep concern about the conservation and persistence of this species in natural environments.

The data presented in this study confirmed the trends from previous studies conducted in tropical climate in Brazil (e.g., Martins and Oliveira 1998, Marques et al. 2001, 2006, Oliveira and Martins 2001). Due to the large territory, Brazil has a great diversity of biomes, each with its own environmental characteristics. However, despite the great latitudinal range of viperids, the trend observed in the daily and seasonal activity remains very similar, although apparently influenced by different factors (temperature or precipitation, mostly). This trend has probably been shaped along the evolutionary time by snakes of the family Viperidae, reflecting on a similar adaptive response against different environments, with different environmental characteristics. Also, it is important to ensure the conservation of Atlantic Forest areas, to guarantee the preservation of the species reliant on forested areas (*B. jararacussu*).

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RESUMO

Serpentes viperídeas são amplamente distribuídas pela América do Sul, com maior radiação ocorrendo na subfamília Crotalinae. Apesar da aparente abundância dessas serpentes ao longo da sua distribuição geográfica, alguns aspectos ecológicos permanecem desconhecidos, principalmente em áreas subtropicais. No presente estudo, avaliamos a atividade (diária e sazonal) e o uso do ambiente por *Bothrops diporus*, *B. jararaca* e *B. jararacussu*, em uma área de Mata Atlântica, no sul do Brasil. Observamos maior incidência de viperídeos durante os meses com temperaturas mais altas, enquanto nenhuma serpente foi encontrada durante os meses de menores temperaturas. Os dados sugerem a temperatura mínima como a variável ambiental com maior influência na atividade sazonal dessas espécies. Considerando a atividade diária, observamos uma tendência das serpentes evitarem os horários com temperaturas mais altas. *Bothrops jararacussu* tendeu a evitar áreas mais abertas, sendo registrada apenas dentro e na borda da mata. Comparamos nossos resultados com estudos prévios realizados em áreas tropicais, e sugerimos a atividade sazonal observada como uma resposta evolutiva, apesar de influenciada por diferentes variáveis ambientais, de acordo com a região de ocorrência.

Palavras-chave: Parque Estadual do Turvo, Rio Grande do Sul, Mata Atlântica, *Bothrops*, padrão de atividade.

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