Reproductive and feeding biology of the pitviper *Rhinocerophis alternatus* from subtropical Brazil

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Dissection of preserved specimens of *Rhinocerophis* (previously *Bothrops*) *alternatus*, combined with data on captive individuals, provided information on the reproductive biology, sexual dimorphism and feeding habits of this viperid snake, forming the first comprehensive study on the natural history of this species in subtropical Brazil. Females were longer than males in snout–vent length (SVL), averaging 992 mm. Males averaged 664 mm in SVL and had relatively longer tails. Mating was observed in July under conditions of captivity. Mature males were found throughout the year, as were females with enlarged follicles. Nevertheless spermatogenesis, inferred by an increase in testicular volume, occurred during the early mating period and its preceding months. Oviductal embryos were recorded only from November to January and parturition occurred from February to August, characteristics of a seasonal reproductive cycle, a recurrent pattern for snakes from the subtropical domain. We observed asynchrony in the timing of mating and parturition, indicating long-term sperm storage after mating by females. In addition, our observations of muscular contraction and the presence of spermatozoids in the posterior portion of the uterus of mature females allowed us to suggest utero-muscular twisting as a possible mechanism used by *R. alternatus* for controlling the timing of its reproductive cycle. The number of newborns per litter varied from five to 20 (mean = 12). Feeding frequency was 29.7%, and the analysis of gut contents indicated a highly specialized diet, which is restricted to rodents (Muridae and Caviidae) and marsupials (Didelphidae). Most of the prey was ingested head-first.

Key words: Bothrops, diet, morphology, reproduction, Serpentes, Viperidae

INTRODUCTION

Rhinocerophis Garman 1881 (Serpentes, Viperidae, Crotalinae) has a wide distribution in South America, occurring in central and southern Bolivia, Paraguay, Uruguay, Argentina and southeastern Brazil (Campbell & Lamar, 2004). The genus was recently resurrected by Fenwick et al. (2009), who revised the neotropical pitviper genus *Bothrops* Wagler 1824 through the most taxon- and character-comprehensive study to date on this group. These authors suggested the recognition of the six major lineages of *Bothrops* as distinct genera. At present, the resurrected genus *Rhinocerophis* includes *R. alternatus*, *R. ammodytoides*, *R. cotiara*, *R. fonsecai*, *R. itapetinigae* and *R. jonathani*. Despite its wide distribution, aspects of the natural history – notably the reproductive biology – of *Rhinocerophis* are still poorly understood for many species.

Among the assemblage of about 36 species of bothropoid snakes, *Rhinocerophis alternatus* Duméril, Bibron & Duméril 1854 is a stout snake of exclusively terrestrial habits that occurs in Paraguay, Uruguay, Argentina and Brazil, in both tropical and subtropical domains (Martins et al., 2002). This species inhabits mainly wet habitats such as swamps and riparian zones, and is also found in tropical forests and open formations, including agricultural, suburban and disturbed areas (Lema, 2002; Campbell & Lamar, 2004; Giraudo et al., 2008; Sawaya et al., 2008). *R. alternatus* is a taxon of medical importance, given that it is involved in a considerable proportion of the cases of human snakebite throughout its distribution (Araújo et al., 2003; Giraudo et al., 2008).

Some studies on the reproductive biology of bothropoid snakes based on preserved specimens have been conducted in Brazil (e.g. Hartmann et al., 2004; Monteiro et al., 2006), but information on *R. alternatus* reproduction consists of occasional observations or reports from captive individuals (Amaral, 1927; Perkins, 1943; Silva Junior, 1956; Leitão de Araújo & Perazzolo, 1974; Pezzano, 1986; Sawaya et al., 2008), with the exception of a study by Giraudo et al. (2008) in the subtropical Santa Fé province, northeastern Argentina. A controversial aspect of the reproductive biology of viperids, including bothropoid genera (Hartmann et al., 2004), is the occurrence of morphological changes in the uterus, which have been suggested to be responsible for sperm storage (Almeida-Santos & Salomão, 1997; Almeida-Santos & Salomão, 2002; Sieger & Sevel, 2006).

Although studies on the natural history of bothropoid species are focused on dietary aspects, there is still disagreement about the degree of mammal specialization in the case of *R. alternatus*. Norman (1994) suggested that this species might feed mainly on mammals, whereas Yanosky et al. (1996) showed that it also feeds on frogs. However, other studies did not find any amphibians in the gut contents of *R. alternatus* (Martins et al., 2002; Giraudo et al., 2008; Zanella & Cechin, 2009). In addition, few provided information on the mammal families con-
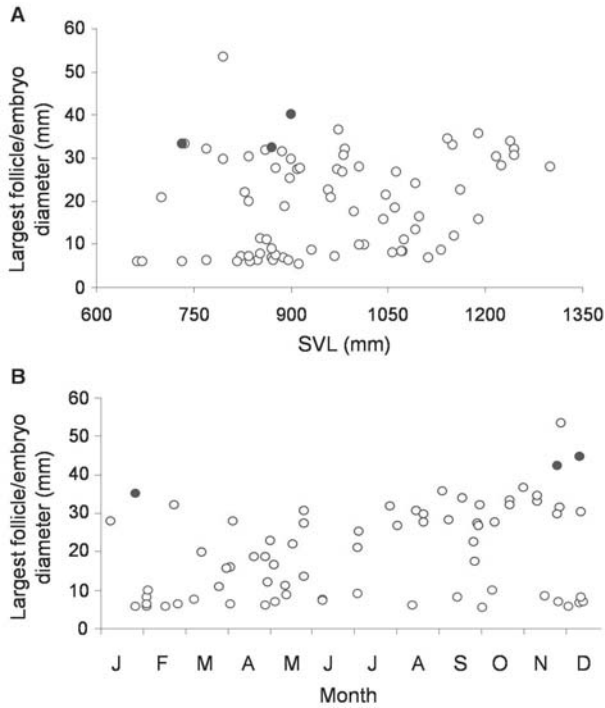
Fig. 1. Body size variation (A) and seasonal distribution (B) in the diameter of the largest follicle or oviductal embryo in mature females of *Rhinocerophis alternatus* from southern Brazil. Open circles represent vitellogenic follicles, and solid circles represent embryos.

sumed by *R. alternatus*. Natural history data are important for a better understanding of phylogenetic relationships among the group of taxa formerly known as the *Bothrops* species complex (Martins et al., 2002; Fenwick et al., 2009).

Based on specimens preserved in museum collections, together with data on captive individuals, here we report new information on the reproductive biology and feeding habits of *R. alternatus* in southern Brazil. This study provides the first comprehensive data set on the natural history of this conspicuous component of snake assemblages from the subtropical portion of the country. Moreover, it adds to a growing body of information on the ecology of the morphologically and ecologically diverse clade of the bothropoid pitvipers.

MATERIALS AND METHODS

A total of 182 specimens were examined from the following institutions: Universidade Federal de Santa Maria (ZUFSM/UFSM), Universidade de Passo Fundo (CRUPF/ UPF), Pontifícia Universidade Católica do Rio Grande do Sul (MCP/PUCRS) and Museu de História Natural Capão da Imbuia (MHNCI). The sample included only specimens from the southern states of Rio Grande do Sul (RS) and Paraná (PR) (see Appendix). These states form most of the subtropical region in Brazil, which is characterized by a highly seasonal temperature regime and precipitation evenly distributed throughout the year. The vegetation

includes several physiognomies such as savannahs, steppes, dense and mixed rain forests, and seasonal deciduous and semi-deciduous forests (Leite, 2002). Only individuals that were wild-caught and preserved soon after collection were included in the reproductive analyses. For the dietary analyses, we excluded any specimens that were previously maintained in captivity.

The following data were taken from each specimen: snout–vent length (SVL, in mm), tail length (TL, in mm), sex and stage of reproductive maturity. We also recorded, for males, the length, width and thickness of both testes and diameter of the deferent duct close to the cloaca, and for females, the number of ovarian follicles or oviductal embryos and the diameter of the largest vitellogenic follicles (>10 mm) and embryos. Males were considered reproductively mature if they had opaque and convolute deferent ducts (Shine, 1982); females if they had follicles in secondary vitellogenesis (hereafter FSV; larger than 10 mm) and/or embryos in the oviduct (Shine, 1977). Volume of testes was calculated by the ellipsoid formula $4/3\pi abc$, in which a = length/2, b = width/2 and c = thickness/2. Females larger than the smallest mature individual recorded that did not have FSV were regarded as non-reproductive mature. Immature individuals were considered juveniles, and those that showed an umbilical-cord scar were referred to as newborns. We used captivity data from the UPF and PUCRS serpentariums on mating, recruitment period and size of newborns (SVL and TL). Captive individuals were taken from the field and maintained at natural temperature in sand-filled glass terraria, with water supply, and were fed on mice.

We verified the presence of sperm storage in the posterior region of the female uterus through observation of uterine musculature and microscopic analyses. Tissue from the posterior portion of the uterus of 28 mature females was embedded in paraffin resin for histological analyses. Sections of 10 μm were cut with microtomes, mounted on glass slides, and stained with standard haematoxylin–eosin and fuchsin/toluidine blue techniques.

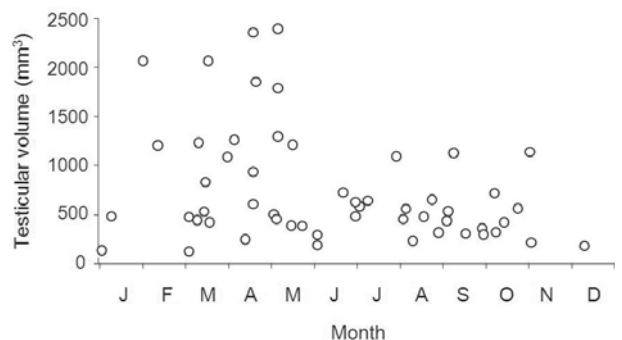We used the Mann–Whitney U test in order to evaluate sexual dimorphism in SVL. Because reproductive

Fig. 2. Seasonal variation in the volume of the testes in mature males of *Rhinocerophis alternatus* from southern Brazil.
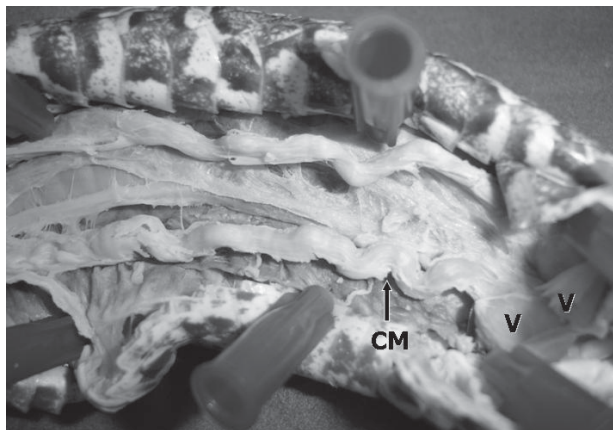
Fig. 3. Contracted musculature in the posterior uterus of a mature *Rhinocerophis alternatus*, indicating a possible sperm-storage mechanism. CM = contracted musculature; V = vaginal pouch.

organs are related to body length, we used analysis of covariance (ANCOVA) using SVL as a covariate to test relative testicular volume and deferent duct diameter among the seasons of the year. We also compared TL of males and females with ANCOVA using SVL as a covariate. The index of sexual size dimorphism (SSD) was calculated as follows: (mean SVL of the larger sex)/(mean SVL of the smaller sex) – 1. SSD is expressed as positive if females are the larger sex and negative if males are the larger (Gibbons & Lovich, 1990).

The digestive tract of each specimen was dissected for gut contents and number of prey types. Whenever possible, we recorded the direction of ingestion of prey. The food items were identified to the lowest possible taxonomic level and deposited in collections of the institutions mentioned above. We used Fisher's exact test to verify differences in feeding frequency (percentage of individuals with stomach contents) of *R. alternatus* between sexes and developmental stages.

Statistical analyses were performed with the program Statistica, version 6.0. A significance level of α=0.05 was assumed for all analyses (Zar, 1999).

RESULTS

Body size and sexual dimorphism

Newborn SVL ranged from 146 to 252 mm (mean=210.50; SD=22.26; *n*=28), ranging from 146 to 230 mm (mean=207.23; SD=21.77; *n*=13) in males and from 152 to 252 mm (mean=213.33; SD=23.05; *n*=15) in females. Newborn TL ranged from 18 to 45 mm (mean=32.61; SD=5.98; *n*=28), ranging from 30 to 45 mm in males (mean=36.54; SD=4.89; *n*=13) and from 18 to 36 mm in females (mean=29.20; SD=4.66; *n*=15).

Mature male SVL ranged from 438 to 884 mm (mean=664.73; SD=102.35; *n*=60) and TL ranged from 72 to 131 mm (mean=99.23; SD=12.91; *n*=60). Mature female SVL ranged from 701 to 1300 mm (mean=992.06;

SD=156.73; *n*=49) and TL ranged from 44 to 145 mm (mean=101.82; SD=17.92; *n*=49).

Females were longer than males in snout–vent length (U=89.0; *P*<0.001), with a degree of sexual size dimorphism of 0.49. However, males had a longer relative tail length (ANCOVA; *F*=43.41; *P*<0.0001; *n*=109).

Reproductive cycle and recruitment period

Mature males were found throughout the year, as were females with FSV (see Fig. 1 for vitellogenic follicles according to female body size and month collected). There was a marginally significant difference in the relative volume of the testes among seasons (ANCOVA; *F*=2.62; *P*=0.060; *n*=53), while no difference in the diameter of the deferent duct was observed (ANCOVA; *F*=1.85; *P*=0.149; *n*=53). Volume of the testes was greater between February and May (Fig. 2). Copulation (*n*=8) was observed in July under conditions of captivity. Oviductal embryos were recorded from November to January, and parturition occurred in February (*n*=5), March (*n*=10), April (*n*=3), June (*n*=2) and August (*n*=1). We observed muscular contraction in the posterior portion of the uterus of 28 mature females (Fig. 3). Through histological analyses, spermatozoids were observed in the posterior portion of the uterus of these individuals (Fig. 4). Among the females larger than the smallest mature individual (potentially reproductive females), 63.6% were reproductive. All males larger than 438 mm showed opaque and convolute deferent ducts.

Fecundity

The number of vitellogenic follicles per *R. alternatus* female ranged from four to 48 (mean=17.04; SD=9.58; *n*=47) and the number of embryos ranged from 10 to 13 (mean=11.33 SD=1.53; *n*=3). The number of newborns per litter ranged from five to 20 (mean 12.17; SD=6.11; *n*=6).
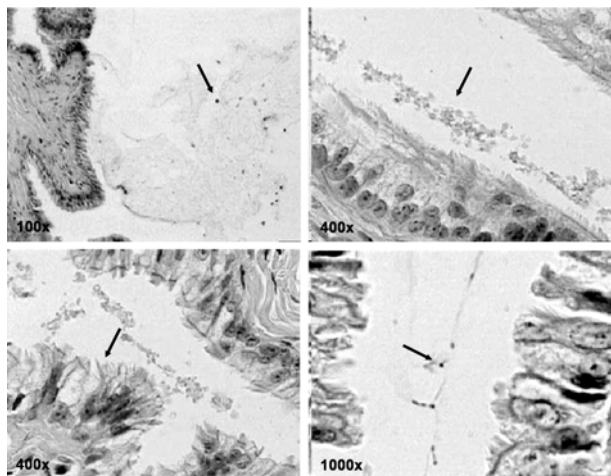

Fig. 4. Transverse sections (10 μm thick) of the contracted muscle of posterior uterus from *Rhinocerophis alternatus.* Arrows show spermatozoids in the lumen. Stained with haematoxylin–eosin and fuchsin/toluidine blue.

Table 1. Feeding frequency by sex and developmental stage in *Rhinocerophis alternatus* from southern Brazil. Males: juveniles <438 mm SVL, mature ≥438 mm SVL; females: juveniles <701 mm SVL, mature ≥701 mm SVL.

	With food items n (%)	Without food items n (%)	Total (n)
Male			
Juveniles	6 (37.5)	10 (62.5)	16
Mature	11 (18.3)	49 (81.7)	60
Female			
Juveniles	12 (41.4)	17 (58.6)	29
Non-reproductive mature	13 (46.5)	15 (53.5)	28
Reproductive mature	12 (24.5)	37 (75.5)	49
Total	54	128	182

Diet

Of 182 specimens examined, 54 (29.7%) contained prey in their guts. The diet was composed exclusively of mammals, and the relative frequency of prey families was as follows: Muridae (80.8%; n=42); Caviidae (9.6%; n=5) and Didelphidae (9.6%; n=5). We were not able to determine prey orientation when they were found in a transverse position or in late stages of digestion inside the digestive tract of the snake. Fourteen (93.4%) of 15 prey for which the direction of ingestion could be detected had been swallowed head-first.

The frequency of recently-fed individuals did not differ between juvenile and mature individuals for the entire sample (Fisher's exact test; P=0.092) or between juvenile and mature males (Fisher's exact test; P=0.172) and females (Fisher's exact test; P=0.493). Feeding frequency differed only marginally between reproductive and non-reproductive mature females (Fisher's exact test; P=0.075) and between sexes for the entire sample (Fisher's exact test; P=0.075) (Table 1).

DISCUSSION

Larger females were observed in this study and also in *R. alternatus* from Santa Fe, Argentina (Giraudo et al. 2008), besides other bothropoid species, such as *Rhinocerophis fonsecai* (Sazima & Manzani, 1998), *Bothrops asper* (Solórzano & Cerdas, 1989), *Bothrops atrox* (Oliveira, 2003), *Bothropoides jararaca* (Sazima, 1992) and *Bothropoides pubescens* (Hartmann et al., 2004). The larger body size of females may be a result of size-dependent fecundity, providing more space for reproductive organs and embryos within the body cavity (Seigel & Fitch, 1984). According to Shine (1994), the evolution of viviparity in snakes resulted in increased female body size in relation to conspecific males. In males, smaller body sizes confer a greater capacity for mobility and travelling, which may increase their mating opportunities (Shine, 1978). The difference in relative TL between the sexes observed in this study is most probably a result

of morphological constraints of the hemipenis and its retractor muscles (King, 1989). Tail length is also believed to influence male reproductive success, acting as a sexual selection factor (Shine et al., 1999).

Male *R. alternatus* can reach sexual maturity at smaller sizes than females, as has been reported for the viperids *B. jararaca* (Sazima, 1992) and *B. pubescens* (Hartmann et al., 2004) and several other snake species (Parker & Plummer, 1987). Because juvenile growth rates are frequently similar for both sexes (Beaupre et al., 1998, but see Furtado et al., 2006), differences in size are normally attributed to earlier maturation of males than conspecific females (Parker & Plummer, 1987; Shine et al., 1998). This is because sexual maturity, in females, can be delayed as a function of the high reproductive costs involved (Shine, 1978), given that they must attain larger body sizes in order to produce larger egg masses and offspring (Seigel & Ford, 1987; Luiselli et al., 1996).

Male *R. alternatus* were found to be reproductive throughout the year and showed low seasonal variation in testes volume and diameter of deferent ducts among the seasons. However, visual inspection of Figure 2 reveals that testicular volume was greater between February and May, a period that comprises the early mating period and its preceding months according to Giraudo et al. (2008) and this study. Given that increase in testicular volume reflects spermatogenesis (Volsøe, 1944), we can infer that male *R. alternatus* have a seasonal sperm production and consequently a seasonal reproductive cycle, as has been observed in other bothropoid species in Brazil (Pizzatto et al., 2007).

Reproductive cycles influenced by temperature and a prolonged time between copulation and parturition have been reported for other bothropoid species (e.g. Nogueira et al., 2003; Hartmann et al., 2004). Observations of captive individuals suggest different gestation periods for *R. alternatus*, such as two (Silva Junior, 1956), four to five (Amaral, 1927), five to six (Perkins, 1943; Pezzano, 1986) and nine months (Leitão de Araújo & Perazzolo, 1974). According to our data, the maximum period between *R. alternatus* copulation and parturition was seven months. Although other authors have reported similar periods for other bothropoid snakes in captivity (Leloup, 1975; Cruz et al., 1989; Alves et al., 1998), this cannot be taken as the gestation period for these species, since we acknowledge that captivity can distort reproductive patterns in snakes, mainly because of increased food availability under artificial conditions (Seigel & Ford, 1987).

The asynchrony in the timing of mating and parturition, together with our observations of muscular contraction and the presence of spermatozoids in the posterior portion of the uterus of mature females, allows us to suggest the occurrence of sperm storage in the oviduct of *R. alternatus*. The mechanism responsible for sperm retention may be a female morphological change termed uterine muscular twisting (hereafter UMT), which is reported to occur after copulation in the viperids *Crotalus durissus terrificus* (Almeida-Santos & Salomão, 1997), *B. jararaca* (Almeida-Santos & Salomão, 2002) and *B. pubescens* (Hartmann et al., 2004). Our analysis of the posterior uterus of *R. alternatus* showed signs of UMT as

well as the presence of spermatozoids. However, these observations should be interpreted with caution, given that the validity of UMT for sperm storage has been severely questioned (Siegel & Sever, 2006). These authors stated that even if some kind of utero-muscular contraction occurs in snakes, this mechanism can have no effect on sperm retention in the posterior uterus because spermatozoids can be found simultaneously in the anterior uterus and infundibular sperm-storage tubules, structures that were not investigated in this study. Therefore, further anatomical and physiological investigation is needed in order to assess the significance of uterine contraction and mechanisms of sperm storage in the Viperidae (Siegel & Sever, 2006).

Parturition occurred from February to August, with a peak in March. Recruitment restricted to a part of the year is a recurrent pattern for bothropoid species (see Sazima, 1992; Almeida-Santos & Salomão, 2002; Marques & Sazima, 2003; Hartmann et al., 2004), including *R. alternatus* from southeastern Brazil (Sawaya et al., 2008) and northeastern Argentina (Giraudo et al., 2008). In the case of *R. alternatus*, this phenomenon may be possible through long-term sperm storage after mating, which takes place during the coldest months of the year. As reported for the viperid *Crotalus d. terrificus* (Almeida-Santos & Salomão, 1997), spring ovulation in *R. alternatus* probably stimulates the relaxation of the uterine musculature, allowing spermatozoa to ascend the oviduct and consequently fertilize the eggs.

Although we found reproductive individuals of *R. alternatus* throughout the year, embryonic development occurred only in spring and summer, as is observed for snakes in temperate zones (Gregory, 2009). This period allows females to maintain constant and higher body temperatures, which can increase embryonic developmental rates and reduce the incidence of abnormalities (Burger et al., 1987). Therefore, the presence of oviductal embryos and parturition were restricted to a particular period of the year, characteristic of a seasonal reproductive cycle, as was reported for *R. alternatus* in Argentina (Giraudo et al., 2008) and for all snake species studied in subtropical Brazil, independently of the phylogenetic lineage to which they belong (Di-Bernardo et al., 2007).

Data regarding the fecundity of *R. alternatus* indicate that this species can produce between 12 and 13 newborns per litter (Leitão de Araújo & Perazzolo, 1974), while Giraudo et al. (2008) found a mean clutch size of 18.7 newborns. These values are in agreement with our fecundity data of a mean offspring number of 12 individuals.

Among the females larger than the smallest mature individual, a high percentage (63.6%) were reproductive. Viperid snakes are known to show a lower reproductive frequency, reproducing every two years, as observed for several species (e.g. Sazima, 1992; Nogueira et al., 2003; Hartmann et al., 2004). Reproduction in consecutive years also occurs in viperids, as observed in *Crotalus viridis* (Diller & Wallace, 1984). Reproductive frequency is controlled by food availability and population structure (Diller & Wallace, 1984), which can lead to a high variation in the proportion of reproductive females among years (Andren & Nilson, 1983). Body size also influences fat

storage and reproduction, affecting the reproductive frequency of snakes (Seigel & Ford, 1987; Naulleau & Bonnet, 1996). Given that gravid females could spend more time basking or otherwise out in the open in cooler climates because of their reduced displacement ability (Gregory, 2009), there might be a sampling bias toward gravid females and a consequent overrepresentation in collections compared to their frequency in the actual population.

Martins et al. (2002) noted that the recent accumulation of data on bothropoid feeding habits allowed the recognition of two common patterns: 1) generalist diet, where individuals feed on a variety of prey types, such as chilopods, amphibians, lizards, snakes, birds and mammals; and 2) presence of ontogenetic diet shifts, where endothermic prey is incorporated into the diet with increasing body size. Therefore, we can conclude that, in relation to feeding habits, *R. alternatus* figures as an exception together with *R. fonsecai*, *R. cotiara* and *Bothropoides neuwiedi*, which are known to have the specialized characteristic of feeding exclusively on mammals.

This specialization in mammals may be related to characteristics observed during *R. alternatus* ontogeny, such as 1) absence of a contrastingly coloured tail tip, as well as caudal luring behaviour, which is used to attract ectothermic prey (Sazima, 1991); 2) larger size of newborns (Martins et al., 2002; this study); and 3) the high toxicity of juvenile venom (Furtado et al., 1991). The last two characteristics may be associated with tactics employed by these snakes for the subjugation of small rodents (Martins et al., 2002). The head-first swallowing behaviour observed in this study and reported for other crotalines (Oliveira, 2003; Hartmann et al., 2005), although widespread among snakes, can also be considered a mechanism of reducing resistance by the hair and appendages of mammals during ingestion, and any consequent injury or energy expenditure during the feeding sequence.

Our finding of a marginal difference in feeding frequency between reproductive and non-reproductive mature females may be related to reduced feeding in females during the reproductive stage. Although *R. alternatus* is a sit-and-wait predator, a reduction in its ability to catch active prey may be partly related to the reduced locomotor performance that is seen in gravid/reproductive snakes (Gregory, 2009).

Information on habitat occupancy by *R. alternatus* is consistent with the prey recorded by this study, given that this snake can travel between open, cultivated and forested areas to feed (Lema, 2002). Similarly to *R. alternatus*, members of the rodent families Muridae and Caviidae can occupy a wide range of habitats: murids are common in open areas, croplands and peridomestic areas (Silva, 1994), while caviids inhabit forest edges, shrublands and other low and closed vegetation, also adapting well to human-disturbed areas (Silva, 1994; Cimardi, 1996). Although Martins et al. (2002) did not specify the mammal groups consumed by *R. alternatus*, another study (Zanella & Cechin, 2009) reported exclusively rodents as gut contents in the individuals that they

analysed. Our finding of five didelphids as prey of *B. alternatus* is congruent with the habitat in which these marsupials live (forests, fields and swampy areas, as well as cultivated and urbanized regions) (Cimardi, 1996) and is probably due to our large sample size in relation to the study of Zanella & Cechin (2009). Given that the availability of food items, together with phylogenetic constraints, is one of the main factors influencing the diet of snakes (Pough et al., 2004), we believe that the predominance of murids in the diet of *R. alternatus* is associated with a greater availability of this prey type in a wide range of environments in southern Brazil (Silva, 1994; Cimardi, 1996; Eisenberg & Redford, 1999).

In a study on a snake community in the Rio Grande do Sul Planalto Médio region, Zanella & Cechin (2009) found that *R. alternatus* was the most abundant species, while at the same time observing a wide local diversity of rodents and marsupials. In addition, Cechin (1999), in the Depressão Central region, also in Rio Grande do Sul, recorded the generalist *B. pubescens* as the most abundant bothropoid species in a snake community. In this area, a lower rodent availability was accompanied by a low capture frequency of *R. alternatus*. Therefore, prey availability may be an important factor in the geographical distribution of this specialist snake in southern Brazil. Nevertheless, we suggest further investigation of this hypothesis.

ACKNOWLEDGEMENTS

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APPENDIX: SPECIMENS EXAMINED

CRUPF 201 (Pulador-RS); CRUPF 220 (Pontão-RS); CRUPF 324 (Passo Fundo-RS); CRUPF 716 (Passo Fundo-RS); CRUPF 792 (Passo Fundo-RS); CRUPF 826 (Passo Fundo-RS); CRUPF 827 (Passo Fundo-RS); CRUPF 840 (Passo Fundo-RS); CRUPF 863 (Passo Fundo-RS); CRUPF 951 (Passo Fundo-RS); CRUPF 1001 (Passo Fundo-RS); CRUPF 1016 (Passo Fundo-RS); CRUPF 1034 (Passo Fundo-RS); CRUPF 1036 (Passo Fundo-RS); CRUPF 1046 (Passo Fundo-RS); CRUPF 1068 (Passo Fundo-RS); CRUPF 1084 (Passo Fundo-RS); CRUPF 1184 (Passo Fundo-RS); CRUPF 1201 (Nicolau Vergueiro-RS); CRUPF 1211 (Mato Castelhano-RS); CRUPF 1213 (Passo Fundo-RS); CRUPF 1299 (Passo Fundo-RS); CRUPF 1300 (Passo Fundo-RS); CRUPF 1301 (Lagoa Vermelha-RS); CRUPF 1349 (Nicolau Vergueiro-RS) ; CRUPF 1372 (Nicolau Vergueiro-RS); CRUPF 1375 (Passo Fundo-RS); CRUPF 1401 (Carazinho-RS); CRUPF 1437 (Passo Fundo-RS); CRUPF 1459 (Passo Fundo-RS); CRUPF 1460 (Passo Fundo-RS); CRUPF 1461 (Passo Fundo-RS); CRUPF 1467 (Passo Fundo-RS); CRUPF 1468 (Passo Fundo-RS); CRUPF 1469 (Passo Fundo-RS); CRUPF 1470 (Nicolau Vergueiro-RS); CRUPF 1471 (Passo Fundo-RS); CRUPF 1485 (Passo Fundo-RS); CRUPF 1486(Passo Fundo-RS); CRUPF 1487 (Passo Fundo-RS); MCP 224 (Lagoa Vermelha-RS); MCP 1194 (Cambará do Sul-RS); MCP 1195 (Gravataí-RS); MCP 1228 (Novo Hamburgo-RS); MCP 1914 (Campo Bom-RS); MCP 1917 (Campo Bom-RS); MCP 1918 (Campo Bom-RS); MCP 1929 (Estância Velha-RS); MCP 1938 (Campo Bom-RS); MCP 2043 (Ivoti-RS); MCP 3299 (Porto Xavier-RS); MCP 3659 (Canoas-RS); MCP 5153 (Viamão-RS); MCP 5588 (Gravataí-RS); MCP 5716 (Cachoeira do Sul-RS); MCP 5717 (Cachoeira do Sul-RS); MCP 5719 (Cachoeira do Sul-RS); MCP 5731 (Caxias do Sul-RS); MCP 5858 (Cachoeira do Sul-RS); MCP 5860 (Cachoeira do Sul-RS); MCP 5964 (Cachoeira do Sul-RS); MCP 6019 (Cachoeira do Sul-RS); MCP 6186 (Cachoeira do Sul-RS); MCP 6447 (Balneário Pinhal-RS); MCP 6616 (Cachoeira do Sul-RS); MCP 6617 (Cachoeira do Sul-RS); MCP 6618 (Cachoeira do Sul-RS); MCP 6619 (Cachoeira do Sul-RS); MCP 6620 (Cachoeira do Sul-RS); MCP 6699 (São Francisco de Paula-RS); MCP 7111 (Pontão-RS); MCP 7420 (Cachoeira do Sul-RS); MCP 7441 (Cachoeira do Sul-RS); MCP 7486 (São Francisco de Paula-RS); MCP 7582 (Tramandaí-RS); MCP 8862 (Cachoeira do Sul-RS); MCP 8870 Tapes-RS); MCP 9002 (Guaíba-RS); MCP 9042 (Campo Bom-RS); MCP 9043 (Sapiranga-RS); MCP 9073 (Glorinha-RS); MCP 9076 (Viamão-RS); MCP 9091(Gravataí-RS); MCP 9161 (São Francisco de Paula-RS); MCP 9269 (Viamão-RS); MCP 9424 (Cachoeira do Sul-RS); MCP 10473 (São Francisco de Paula-RS); MCP 10779 (Cachoeira do Sul-RS); MCP 10816 (Cachoeira do Sul-RS); MCP 10818 (Santana da Boa Vista-RS); MCP 10819 (Cachoeira do Sul-RS); MCP 10842 (Cachoeira do Sul-RS); MCP 10843 (Cachoeira do Sul-RS); MCP 10935 (São Francisco de Paula-RS); MCP 11215 (Formigueiro-RS); MCP 11347 (Arroio do Sal-RS); MCP 11370 (Taquara-RS); MCP 11383 (São Francisco de Paula-RS); MCP 11504 (Cachoeira do Sul-RS); MCP 11513 (Cerro Branco-RS); MCP 11524 (Cachoeira do Sul-RS); MCP 11739 (Taquara-RS); MCP 11873 (Nova Bassano-RS); MCP 11959 (General Câmara-RS); MCP 11964 (Cachoeirinha-RS); MCP 12162 (Taquara-RS); MCP 12422 (Balneário Pinhal-RS); MCP 12436 Cambará do Sul-RS); MCP 12437 (São Francisco de Paula-RS); MCP 12520 (Palmares do Sul-RS); MCP 12553 (São Borja-RS); MCP 12560 (Cachoeira do Sul-RS); MCP 12567 (Novo Cabrais-RS); MCP 12572 (Cachoeira do Sul-RS); MCP 12642 (Paraíso do Sul-RS); MCP 12643 (Cachoeira do Sul-RS); MCP 12711 (São Gabriel-RS); MCP 12727 (Balneário Pinhal-RS); MCP 12737 (Balneário Pinhal-RS); MCP 12766 (Candiota-RS); MCP 12777 (Balneário Pinhal-RS); MCP 12780 (Balneário Pinhal-RS); MCP 12785 (Rosário do Sul-RS); MCP 12903 (Palmares do Sul-RS); MCP 13182 (Balneário Pinhal-RS); MCP 13183 (Balneário Pinhal-RS); MCP 13208 (Cachoeira do Sul-RS); MCP 13265 (Cerro Branco-RS); MCP 13307 (Balneário Pinhal-RS); MCP 13344 (São Vicente do Sul-RS); MCP 13350 (São Vicente do Sul-RS); MCP 13367 (São Vicente do Sul-RS); MCP 13526 (Balneário Pinhal-RS); MCP 13707 (Pantano Grande-RS); MCP 13904 (Balneário Pinhal-RS); MCP 14147 (Cachoeira do Sul-RS); MCP 14331 (Palmares do Sul-RS); MCP 14414 (Campo Bom-RS); MCP 14571 (Balneário Pinhal-RS); MCP 14653(Cachoeira do Sul-RS); MCP 14659 (Cachoeira do Sul-RS); MCP 14719 (Balneário

Pinhal-RS); MCP 14846 (Canoas-RS); MCP 15004 (RS); MCP 15075 (RS); MCP 15076 (RS); MHNCI 244 (Piraraquara-PR); MHNCI 705 (Ponta Grossa-PR); MHNCI 870 (Curitiba-PR); MHNCI 3553 (Jaguariaíva-PR); MHNCI 5087 (Pinhão-PR); MHNCI 6063 (São José do Norte-RS); MHNCI 8167 (Reserva do Iguaçu-PR); MHNCI 8232 (Reserva do Iguaçu-PR); MHNCI 8934 (Reserva do Iguaçu-PR); MHNCI 9265 (Jaguariaíva-PR); MHNCI 9419 (Reserva do Iguaçu-PR); MHNCI 9804 (Reserva do Iguaçu-PR); MHNCI 10021 (Lapa-PR); MHNCI 10300 (Lapa-PR); MHNCI 11421 (Parai do Sul-PR); ZUFSM 217 (Santa Maria-RS); ZUFSM 878 (Santa Maria-RS); ZUFSM 950 (Santa Maria-RS); ZUFSM 1120 (Restinga Seca-RS); ZUFSM 1132 (Santa Maria-RS); ZUFSM 1325 (Santa Maria-RS); ZUFSM 1373 (São Sepé-RS); ZUFSM 1592 (Santa Maria-RS); ZUFSM 1637 (São Sepé-RS); ZUFSM 1975 (Manoel Viana-RS); ZUFSM 1986 (Santa Maria-RS); ZUFSM 1987(Santa Maria-RS); ZUFSM 1988 (Santa Maria-RS); ZUFSM 2218 (RS); ZUFSM 2394 (Santa Maria-RS); ZUFSM 2624 (Novo Hamburgo-RS); ZUFSM 2625 (Novo Hamburgo-RS).