Reproductive life history of snakes in temperate regions: what are the differences between oviparous and viviparous species?

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Abstract. Studying life history (LH) allows a broader understanding of organisms and populations' responses to their environments. Snakes display an immense diversity in terms of reproductive traits, which is reflected in LH traits. The aim of this study is to compare reproductive biology and morphological variables in viviparous and oviparous snakes of a temperate South American community. We studied nearly 1000 specimens of eight oviparous and seven viviparous species pertaining to the four taxonomic families that inhabit the Paraná basin floodplain. Dimorphic variables did not show a different tendency between oviparous and viviparous species. Our results showed that the reproductive mode determined some reproductive traits of a snake's LH, such as reproductive frequency and reproductive potential. Oviparous snakes reproduce annually, while viviparous snakes reproduce biannually or multi-annually. All species showed seasonal reproductive cycles and no correlation between clutch size (fecundity) and maternal body size. The reproductive strategy of both oviparous and viviparous species of the Paraná River floodplain was to adjust their reproductive cycles to both hydrological cycle of the river and temperature regime. The reproductive traits under study are suggested to have been influenced by environmental factors as well as by genetic characteristics. The studied assemblage is the result of an admixture of evolutionarily distinct clades, each contributing a set of species with different reproductive traits. Although we do not ignore this fact, we emphasize the importance of studying reproductive LH as raw material for an integrative analysis.

Keywords: neotropical snakes, oviparity, Paraná basin floodplain, reproductive ecology, reproductive strategy, sexual dimorphism, viviparity.

Introduction

The life history (LH) of an organism consists of a set of coevolved traits that affect the survival and reproductive potential of an individual; however, LH studies examine populations (Vitt and Caldwell, 2013). Reproduction is a critical event in the life of an individual because it represents a substantial energetic cost, especially to ectotherms, animals that have low energy maintenance costs (Shine, 1994, 2003; Vitt and Caldwell, 2013). Life history strategies represent the ways in which organisms acquire and expend resources: some animals rely upon simultaneous energy acquisition and re-

productive expenditure, whereas others accumulate energy over long periods prior to its expenditure in reproduction (Bonnet, Bradshaw and Shine, 1998). Moreover, reproduction is a central aspect of LH studies; and information of a substantial amount of snake taxa is essential in order to investigate the evolution of reproductive traits, processes, and patterns (Almeida–Santos and Salomao, 2002). Reproductive life history (RLH) of snakes varies significantly among species and even among individuals of the same species due to the interaction of phylogenetic and environmental factors (Cadle and Greene, 1993; Almeida–Santos et al., 2014; Lind et al., 2016; Bellini, Arzamendia and Giraudo, 2017). Characters related to reproduction in Neotropical snakes seem to be relatively conserved in some phylogenetic lineages, although they may widely differ in the reproductive ecology of other groups and even in closely related species (Pizzatto et al., 2008b). The reproductive mode is usually phylogenetically constrained in snakes, which re-

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produce either by laying eggs (oviparity) or by living birth (viviparity) (Feldman et al., 2015). Viviparity is estimated to have evolved independently more than 100 times within squamates (Shine, 1985; Blackburn, 2006). Even though viviparous species are distributed worldwide, they constitute a larger proportion of the fauna in higher than in lower latitudes and elevations (Tinkle and Gibbons, 1977; Shine, 1985, 2005; Blackburn and Stewart, 2011). Considering this, the earliest and most widely accepted hypothesis about reptilian viviparity is that it evolved as a result of adaptation to cold climates (Shine, 1985). However, some warm regions, such as the tropics and temperate regions of South America, host more viviparous species but in smaller proportions than those found in some cold regions (Tinkle and Gibbons, 1977). Certainly, oviparity is the most common reproductive mode among reptiles in general; but viviparity could be favourable in certain variable environments (Shine, 1985). Snakes from temperate regions experience great environmental variability in terms of temperature and precipitation (Brown and Shine, 2002). However, Neotropical rivers, such as those in the Paraná Basin, present an additional factor that can be preponderant in reptiles' activity patterns: variation in the hydrological cycle (Junk, 1997; Giraudo, Arzamendia and López, 2007). The proportion of viviparous reptile species in the reptile community of the mid-Paraná River basin is twice as high as that for the whole Argentine territory (Giraudo, Arzamendia and López, 2007). This type of reproduction can provide an effective solution to the difficulty of egg-laying in dry environments and at variable temperatures, and avoiding the loss of eggs in floods (Giraudo, Arzamendia and López, 2007; Bellini, Arzamendia and Giraudo, 2013; Bellini, Giraudo and Arzamendia, 2014). Tinkle and Gibbons (1977) suggested that egg retention enables females to oviposit when environmental conditions are optimal. This is advantageous in both cold and warm environments, but is probably more important in unstable environments (Feldman et al., 2015).

Reproductive biology of snakes includes various factors, such as reproductive mode, reproductive cycles, fecundity, age and size at maturity, sexual dimorphism, mating systems, and reproductive behaviour (Almeida-Santos et al., 2014). Generally, the absence of variability in these traits among snake populations that live under different climatic conditions is commonly attributable to the influence of phylogenetic factors (Bellini et al., 2015; Bellini, Arzamendia and Giraudo, 2017). Among the morphological variables, however, there is a very common pattern in snakes in which males have longer tails – generally associated with the presence of hemipenes – while females have a longer body – associated with an advantage in fecundity (Shine, 2003). The increase in maternal body size is tightly related to an increased reproductive output, mostly due to greater litter sizes and – in a few snake species – also to increases in offspring size and relative clutch masses (Shine, 1994, 2003). As regards dimorphism in the body size, Fitch (1981) suggests that viviparous females of temperate zones are likely to be relatively longer than their male conspecifics when compared to oviparous species. That is to say, sexual dimorphism index (SSD) should be larger for viviparous species and for females in the region under study. Unfortunately, there are little empirical data of reproductive output, particularly for snake species of temperate regions in the southern hemisphere (Gallardo and Scrocchi, 2006; Bellini, Arzamendia and Giraudo, 2017). In South America, most studies on reproductive aspects of snakes have been conducted in different biomes of the tropics, while the assemblages of subtropical and temperate areas have received less attention (Gallardo and Scrocchi, 2006). Even though diversity of species in these areas is great, there are insufficient studies that provide qualitative data on reproduction (Gallardo and

Scrocchi, 2006). Such limited information impairs both an overview of species reproduction in temperate regions and our ability to draw broad comparisons among those species. As a contribution to filling this gap in knowledge, we combine information about RLH traits obtained from both museum and live specimens to describe sexual maturity, sexual dimorphism, reproductive timing of females (vitellogenesis, gravidity, egg-laying and parturition), and fecundity (mean fecundity, reproductive potential) of a South American temperate snake community. In this study, we compare the reproductive traits and the morphological variables within an assemblage of eight oviparous and seven viviparous snake species that inhabit the Paraná basin floodplain, and thus contribute to a better understanding of their LH.

Materials and methods

Study area and species

Reproductive data were obtained by analysing 1098 specimens of 15 snake species that together represent four taxonomic families: Viperidae, Elapidae, Colubridae, and Dipsadidae. We examined and compared seven viviparous species, namely *Bothrops alternatus*, *B. diporus*, *Helicops infrataeniatus*, *H. leopardinus*, *Thamnodynastes chaquensis*, *T. hypoconia*, *T. strigatus*, and eight oviparous species: *Hydrodynastes gigas*, *Erythrolamphrus semiaureus*, *Leptophis ahaetulla*, *Micrurus altirostris*, *Paraphimophis rusticus*, *Philodryas patagonienis*, *Xenodon dorbingyi*, *Xenodon merremii*. Our field study was carried out in eastern Argentina. Geomorphology and landscape of this region are strongly influenced by three large South American rivers of the Paraná Basin, namely Paraná, Paraguay, and Uruguay, which join to form La Plata River (Iriondo and Paira, 2007). These rivers, as well as their paleochannels and main tributaries, have extensive floodplains that give rise to and maintain several types of wetlands (Arzamendia and Giraudo, 2009). Vegetation there is a mosaic, ranging from wet savannahs and grasslands to subtropical dry forests, gallery forests and a wide variety of wetlands (e.g., rivers, streams, marshes, swamps). The climate is seasonal, with hot and rainy springs and summers (mean temperature: 25–27.5°C) and dry autumns and winters (mean temperature: 10–15°C). Precipitation decreases from northeast to southeast; and annual precipitation is 1000–1800 mm (Paoli, Iriondo and García, 2000). We sampled the study area from January 1991 to December 2016, recording 961 wild specimens mainly by means of road sampling in different habitats (Campbell and Christman, 1981; López and Giraudo, 2008). Only recently road-killed snakes, which were in good

condition, were preserved for collecting reproductive data. We deposited these specimens in the collections of Instituto Nacional de Limnología, Santa Fe, Argentina. Reproductive data were supplemented with original data from 137 specimens deposited in the following scientific collections: Museo Argentino de Ciencias Naturales "Bernadino Rivadavia" (MACN, Buenos Aires), Centro Nacional de Investigaciones Iológicas (currently deposited in MACN), Colección del Museo de La Plata (MLP, Buenos Aires), Museo Antonio Serrano (MAS, Entre Ríos), Universidad Nacional del Nordeste (UNNE, Corrientes).

Reproductive data collection and analysis

For each individual, we recorded the date of collection. In the laboratory, each individual was weighed to measure body mass (BM nearest 0.1 g) and was measured for their snout-vent length (SVL, in mm) and tail length (TL), using a measuring tape (accuracy of 1 mm). To collect reproductive data, females were dissected via an incision in the median ventral region, and the following information was recorded: number of oviductal eggs; diameter of the largest ovarian follicles (in primary or secondary vitellogenesis) or oviductal egg (length and width in mm), using a digital caliper. Females were considered mature if they had follicles in secondary vitellogenesis, oviductal eggs, or folded oviducts (see Pizzatto, Almeida-Santos and Shine, 2007; Pizzatto, Jordão and Marques, 2008; Leite et al., 2009). The aspect of the oviduct, either folded or loose (post-spawning period), was used as a secondary criterion for establishing female sexual maturity (Shine, 1977). Males were considered mature when they had turgid testes and opaque and convoluted deferent ducts (Shine, 1978; Pizzatto, Jordão and Marques, 2008; Leite et al., 2009). We recorded males and females with SVL values higher than those of the smallest mature specimen as adults (Hartmann and Marques, 2005). The reproductive cycle of females was characterised by the distribution of different stages of the reproductive cycle over the year (Shine, 1977, 1988). In those species that did not obtain records of some of the stages in the reproductive cycle, the data were inferred through the available records. For *Bothrops*, *Helicops*, *Thamnodynastes*, and *Xenodon*, the records of congeneric species were taken into account to infer different aspects of the reproductive cycle. We defined the reproductive season as lasting from secondary vitellogenesis to the bearing of the young in viviparous species, and to the laying of eggs in oviparous species, and counting months to calculate the length of this period. Reproductive frequency (RF) was estimated by the percentage of reproductive females (with ovarian follicles in secondary vitellogenesis and/or uterine eggs or embryos) (Pizzatto, 2005). Fifty percent or less of mature females with no vitellogenic follicles or eggs in a single reproductive season was considered as an evidence of a biannual or multiannual reproductive cycle (Bellini, Arzamendia and Giraudo, 2013). By contrast, an annual frequency is assumed when more than 50% of the population of mature females is reproductively active in a single breeding season. Oviductal eggs and offspring were counted to estimate mean fecundity (MF) (Pizzatto, 2005). Reproductive potential (RP), which shows the

number of potential neonates of one species per female per year, was estimated as MF × RF (Trauth, 1978). RP was considered in two ways: as a continuous variable for statistical analysis and as a category for comparison among species. Classification of categories was done as follows: low, when the value was lower than five; medium, when the value was higher than five and lower than ten; and high, when the value was higher than ten. We used an analysis of variance (ANOVA) to compare reproductive frequency and potential; mean fecundity; SSD; and size at sexual maturity among females of different reproductive modes. We performed a Spearman's Rank Correlation to test the relationship between SVL and clutch size in mature females.

To analyse sexual dimorphism, we compared SVL of both sexes using an analysis of variance (ANOVA). Since TL and BM vary with body length, we used SVL as a covariate in the analysis of covariance (ANCOVA) in order to compare these variables between sexes (Shine, 1994). A sexual size dimorphism index (SSD) was calculated according to Shine (1994): mean body size of the larger sex divided by mean body size of the smaller sex, minus one. By convention, the index is given a positive sign when females are larger than males and a negative sign when males are larger than females (Shine, 1994). We performed all statistical analyses with Infostat software version 5.1 (Di Rienzo et al., 2005), with $\alpha < 0.05$. All raw data were examined to determine whether the assumptions of parametric tests (homogeneity of variances, normality) were met prior to testing.

Results

When we compared the size at which females attained sexual maturity, we found no significant difference between oviparous and viviparous species (n = 15, F = 2.4, P = 0.14). Sexual body size dimorphism was evident in most mature individuals; however, *L. ahaetulla* and *M. altirostris* did not present differences between males and females for any of the analysed variables (Table 1). When sexual dimorphism existed in body length (SVL) or body mass (BM), females were always significantly larger (Table 1). The opposite was true for tail length (TL), with sexual dimorphism occurring in favour of males (Table 1). Sexual dimorphism index (SSD) did not show significant differences between the various reproductive modes (n = 15, F = 0.07, P = 0.80) (Table 2). The species with the highest SSD was viviparous *H. leopardinus* (0.52), showing that the mean SVL of mature females was 52% larger than that of mature males, followed by oviparous *E. semi-*

aureus with a very similar value of SSD (0.47). However, most species showed a low degree of SSD (between 0.29 and 0.08) (Table 2). Some species even had a value of SSD that could be considered null, e.g., *T. chaquensis* (0.0002), meaning that there was no sexual dimorphism in the SVL. Only two species (one of each reproductive mode) showed a negative SSD, which would indicate that males are larger than females, although only to a very small degree (Table 2).

All analysed species (both oviparous and viviparous) showed to have seasonal reproductive cycles, although these differed in length (Fig. 1). The reproductive season was longer in viviparous species (covering between 11 and 14 months) than in oviparous species (covering between 9 and 12 months) (Fig. 1). Most oviparous females had vitellogenic follicles from January to September or, in some cases, until November (Fig. 1). For viviparous species, however, vitellogenesis seemed to begin almost by the end of summer (March) and continued until September or December, depending on the species (Fig. 1). Vitellogenesis was inferred from records in previous or subsequent months. Oviductal eggs were found from July to January and from October to January in viviparous and oviparous species, respectively. The presence of oviductal eggs was inferred from data of them in previous or subsequent months, and taking into account records of egg-laying or parturitions. The reproductive output of snakes seemed to match the hydrological cycle of the Paraná River as well as the temperature regime (Fig. 2). Oviparous snakes laid their eggs from mid-spring (October) to the beginning of the summer (January) when the river level was low and temperatures started to rise. Hatching began in December when temperatures were high; and by February, when the river began to rise, most young had already hatched (Fig. 2). Newborns with umbilical scars of oviparous species were found from December to February. On the other hand, viviparous species began to give birth from the beginning of summer (Jan-

Table 1. Morphometric variation in mature individuals of 15 snake species of a community in a temperate South American region.

Species	Sex	Snout-to-Vent Length (cm)				
		N	X	SE	Range	Statistic
Erythrolamprus semiaureus	M	39	534.72	122.78	315-758	*** $F = 35.5$
	F	56	790.11	139.64	547-1147	
Hydrodynastes gigas	M	48	1307.15	338.58	853-2752	** $F = 5.8$
	F	58	1436.15	302.87	925-2360	
Leptophis ahaetulla	M	42	773.85	215.55	528-1852	NS
	F	27	675.07	96.18	560-977	
Micrurus altirostris	M	49	608.85	11.12	350-1150	NS
	F	5	634.20	2.39	570-664	
Paraphimophis rusticus	M	18	772.88	210.34	561-983	* $F = 4.4$
	F	10	899.50	110.47	554-1211	
Philodryas patagoniensis	M	61	671.72	116.78	411-960	*** $F = 10.9$
	F	87	883.43	133.77	635-1315	
Xenodon dorbingyi	M	36	348.27	74.2	221-487	NS
	F	30	372.32	89.08	273-590	
Xenodon merremii	M	26	546.46	142.83	276-950	*** $F = 41.2$
	F	23	689.59	123.13	503-968	
Bothrops alternatus	M	58	722.69	142.3	518-985	*** $F = 77.6$
	F	44	982.43	154.03	755-1360	
Bothrops diporus	M	13	610.46	65.43	516-751	** $F = 6.90$
	F	15	736.93	149.29	509-1020	
Helicops infrataeniatus	M	10	379.90	35.1	334-454	*** $F = 18.7$
	F	21	518.28	97.34	367-725	
Helicops leopardinus	M	71	335.58	60.08	208-480	*** $F = 155$
	F	51	509.63	93.88	368-755	
Thamnodynastes chaquensis	M	27	422.14	80.13	315-590	NS
	F	37	425.42	63.04	314-619	
Thamnodynastes hypoconia	M	67	399.37	66.75	256-537	NS
	F	35	395.34	32.87	341-475	
Thamnodynastes strigatus	M	25	477.80	102.55	341-685	NS
	F	8	517.44	153.73	403-795	

uary) to the beginning of autumn (April), coinciding with the maximum historical hydrometric values (Fig. 2). Most births and neonates of viviparous species were found in March. Newborns with umbilical scars of viviparous species were found from January to April. In some species, we could not obtain directly observed data, so egg-laying or parturitions were inferred by the presence of newborns with umbilical scars in those months. These inferences are shown using dashed line bars in Fig. 1.

All analysed oviparous snakes reproduce annually, while viviparous species did so biannually or multiannually. The finding of 50% or less of mature females with no vitellogenic follicles or eggs in the reproductive season was evidence of a biannual or multiannual reproductive cycle (Fig. 3A). In contrast, an annual frequency was confirmed when more than 50% of the population of mature females were reproductively active in the breeding season (Fig. 3B). Oviparous snakes had a shorter reproductive cycle than viviparous species, and therefore their reproductive frequency was higher (n = 15, $F = 9.67$, P = 0.008) (Table 2). Reproductive potential (RP) was categorized as high in most of the oviparous species (n = 4), while none of the viviparous species were found in this category (Table 2). Among oviparous snakes, half of the species showed low RP while the other half were found to have medium potential. Nevertheless, when we compared RP between oviparous and viviparous species, no significant differences were found (n = 15, $F = 2.73$, P = 0.12) (Table 2). Litter size was variable in all species, although there were no significant dif-

Table 1. (Continued.)

Species	Sex	Tail Length (cm)				
		N	X	SE	Range	Statistic
Erythrolamprus semiaureus	M	31	124.19	81.36	69-150	NS
	F	43	152.74	29.91	113-220	
Hydrodynastes gigas	M	43	429.26	79.67	255-565	*** $F = 27.1$
	F	48	357.73	67.46	194-510	
Leptophis ahaetulla	M	33	400.84	51.41	304-506	NS
	F	23	362.35	52.40	315-501	
Micrurus altirostris	M	40	38.67	200.51	16-63	NS
	F	5	41.21	8.52	32-54	
Paraphimophis rusticus	M	17	173.41	25.08	112-217	*** $F = 54.8$
	F	8	133.37	30.56	103-170	
Philodryas patagoniensis	M	49	316.0	48.31	207-342	*** $F = 49.1$
	F	56	268.02	31.57	173-380	
Xenodon dorbingyi	M	33	67.45	14.54	32-86	*** $F = 30.8$
	F	29	63.86	15.00	37-90	
Xenodon merremii	M	24	105.58	32.89	26-162	* $F = 5.6$
	F	22	101.54	20.99	65-138	
Bothrops alternatus	M	53	116.45	15.32	85-150	*** $F = 31.8$
	F	39	110.85	16.39	74-150	
Bothrops diporus	M	12	97.42	14.76	62-124	NS
	F	13	105.69	19.01	73-137	
Helicops infrataeniatus	M	10	172.20	27.30	132-220	*** $F = 12.8$
	F	20	164.60	36.29	70-219	
Helicops leopardinus	M	68	171.75	30.66	79-210	*** $F = 13.3$
	F	42	144.78	31.59	112-251	
Thamnodynastes chaquensis	M	25	124.48	24.91	83-174	*** $F = 29.4$
	F	35	106.88	21.08	69-166	
Thamnodynastes hypoconia	M	63	145.84	25.57	87-193	*** $F = 22.1$
	F	33	136.60	37.89	93-329	
Thamnodynastes strigatus	M	22	151.54	28.39	95-204	** $F = 9$
	F	9	132.25	15.40	112-158	

ferences in mean fecundity between the different reproductive modes ($n = 15$, $F = 0.29$, $P = 0.59$) (Table 2). The relationship between clutch size (fecundity) and SVL for any species was not significant either (Table 2).

Discussion

Sexual dimorphism

Reproductive success of an organism is determined by several aspects. Body size is one of them, but at the same time it is extraordinarily variable; mean size varies among congeneric species and even among sexes within the same species (Bonnet et al., 2000). On the other hand, body size sexual dimorphism may be correlated with fecundity selection since larger females produce larger clutches (Shine, 1994, 2003). Fe-

cundity selection involves the evolution of traits that enhance reproductive output directly, such as through an increase of body size to accommodate large clutches (Bonnet et al., 2000). Accordingly, females of our temperate snake community reached greater body size and weight than males, but the latter had longer tails, generally associated with the presence of hemipenes (Shine, 1994). This kind of dimorphism in body size, tail length, and body mass found here is a very common pattern in snakes (Shine, 1994, 2003). Nevertheless, two of the studied species presented no sexual dimorphism in any of the analysed variables. In *L. ahaetulla*, this could be due to the arboreal habit of the species. Pizzatto et al. (2007) suggest that the evolution of arboreality was accompanied by elongation of the tail, in relation to the body. This tendency

Table 1. (Continued.)

Species	Sex	Body Mass (g)				
		N	X	SE	Range	Statistic
Erythrolamprus semiaureus	M	39	83.53	51.26	37-156	NS
	F	49	195.57	86.51	99-411	
Hydrodynastes gigas	M	35	1924.31	732.93	551-3221	NS
	F	38	2040.36	869.39	512-4548	
Leptophis ahaetulla	M	37	92.52	46.69	22-260	NS
	F	19	74.47	39.37	35-140	
Micrurus altirostris	M	36	68.22	52.88	9-230	NS
	F	5	63.66	26.43	47-83	
Paraphimophis rusticus	M	17	203.61	165.93	26.5-372	NS
	F	9	290.11	110.03	56-538	
Philodryas patagoniensis	M	35	129.33	132.56	100-552	NS
	F	32	173.13	154.81	23-274	
Xenodon dorbingyi	M	27	42.03	26.57	7-99	NS
	F	25	73.06	55.55	29-228	
Xenodon merremii	M	24	133.81	75.88	17-320	$^{*}F = 4.4$
	F	21	286.33	129.47	89-530	
Bothrops alternatus	M	49	250.43	107.68	73-573	$^{***}F = 122$
	F	28	695.5	352.43	271-1551	
Bothrops diporus	M	12	120.00	47.94	45-224	$^{***}F = 106$
	F	7	355.57	142.43	161-527	
Helicops infrataeniatus	M	8	46.37	11.26	31-63	NS
	F	18	146.55	106.80	36-349	
Helicops leopardinus	M	52	37.18	20.02	9-101	$^{*}F = 4.01$
	F	42	144.96	76.11	13-373	
Thamnodynastes chaquensis	M	22	43.79	19.52	17-91	$^{**}F = 9.45$
	F	35	58.09	19.41	28.5-105	
Thamnodynastes hypoconia	M	62	21.66	9.00	7-52	$^{***}F = 16.7$
	F	33	25.27	7.96	9-45	
Thamnodynastes strigatus	M	19	62.27	30.45	21.75-125	$^{**}F = 8.3$
	F	7	90.57	33.53	32-134	

References: Shaded taxa correspond to oviparous species, taxa without shading correspond to viviparous species. Females (F); Males (M); Number of individuals (N); Mean (X); Standard Error (SE). Statistic: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, NS: Not significant.

of presenting longer tails is probably due to the adaptation of *L. ahaetulla* to arboreal habitats, which dilutes sexual dimorphism. On the other hand, the absence of dimorphism in SVL is associated with snake species in which males engage in combat or have antagonistic interactions during the nuptial period (Shine, 1994). This is a possible explanation for the absence of dimorphism in *M. altirostris*, since combats were recorded between males of this species (Almeida-Santos, Aguiar and Balestrin, 1998; Marques, Almeida-Santos and Rodrigues, 2006; Marques, Pizzatto and Almeida-Santos, 2013; Giraudo, pers. obs.).

Despite we find differences in sexual dimorphism between species, we did not find such differences among reproductive modes. Sexual dimorphism index (SSD) results in the studied species were not consistent with that hypothesis by Fitch (1981), since no significant differences were found for this index among species with different reproductive modes. Here, since it is a temperate zone, females of viviparous species should be larger than their male conspecifics, and relatively larger than females of oviparous species. Although SSD values for *Bothrops* and *Helicops* species were relatively high, those for *Thamnodynastes* species were among the lowest found. On the other hand, two of the oviparous species (*E. semiaureus*, *P. patagoniensis*) showed a relatively high SSD. In a review incorporating data from 374 snake

Table 2. Reproductive life history (RLH) traits of 15 snake species of a community in a temperate South American region.

Species	N		Fe		RP		RF	SSD	Correlation SVL/F(X)
	F	M	Total	X	Value	Category			
Erythrolamprus semiaureus	56	39	10-20	14	12	H	0.9	0.47	$\rho = 0.5$ NS
Hydrodynastes gigas	58	48	11-36	23	16.3	H	0.7	0.09	$\rho = 0.6$ NS
Leptophis ahaetulla	27	42	6-9	8	4.5	L	0.6	−0.14	$\rho = 0.9$ NS
Micrurus altirostris	5	49	4-6	5	3	L	0.6	0.04	
Paraphimophis rusticus	10	18	7-10	9	5.1	Me	0.6	0.16	
Philodryas patagoniensis	87	61	3-22	12	11	H	0.9	0.31	$\rho = 0.7$ NS
Xenodon dorbingyi	30	36	5-19	11	11	H	0.7	0.125	$\rho = 0.9$ NS
Xenodon merremii	23	26	3-20	10	8.3	Me	0.8	0.29	
Bothrops alternatus	44	58	3-37	19	4.9	L	0.3	0.36	$\rho = 0.3$ NS
Bothrops diporus	15	14	2-18	9	2.6	L	0.3	0.2	
Helicops infrataeniatus	21	10	7-26	17	8.2	Me	0.5	0.36	$\rho = 0.6$ NS
Helicops leopardinus	51	71	8-23	15	7.5	Me	0.5	0.52	$\rho = 0.7$ NS
Thamnodynastes chaquensis	37	27	2-18	11	5.8	Me	0.8	0.0002	$\rho = 0.3$ NS
Thamnodynastes hypoconia	35	67	4-13	8	3.2	L	0.5	−0.086	$\rho = -0.2$ NS
Thamnodynastes strigatus	8	25	8-16	11	8	Me	0.5	0.082	$\rho = 0.8$ NS

References: Shaded taxa correspond to oviparous species; taxa without shading correspond to viviparous species. Number of individuals (N); Females (F); Males (M); Fecundity (Fe); Reproductive Potential (RP); High (H); Low (L); Medium (Me); Reproductive Frequency (RF); Sexual Size Dimorphism Index (SSD); Statistic: Not significant (NS).

species, Shine (1994) did not find significant differences between SSD of oviparous species and SSD of viviparous species.

Reproductive traits: frequency, potential, and fecundity

While dimorphic variables did not show different tendencies between oviparous and viviparous species, some patterns were evident when comparing reproductive traits of females with different modes. Oviparous species had an annual frequency of reproduction, i.e., an individual could potentially reproduce every year. In contrast, females of viviparous species had a biannual or multi-annual reproductive frequency, meaning that it is practically impossible for the same female to reproduce in two consecutive years. Reproduction always entails costs in terms of survival or future fecundity, or both (Shine, 1980). Both these costs may be substantial in viviparous snakes because of the long period that females carry their developing offspring (Gregory, 2009). These species have their offspring in summer, between January and April, so the time available for energy accumulation before winter is very short. This means that, at the beginning of the next

reproductive event, females do not have the required energy to reproduce (Shine, 2003; Gregory, 2009; Bellini, Giraudo and Arzamendia, 2014). On the other hand, oviparous species have a longer period after oviposition for replenishing energy reserves and should be therefore less likely to omit reproduction the following year than sympatric viviparous species (Gregory, 2009). Egg-laying in our temperate snake community occurred in spring; however, the duration of the reproductive season of both oviparous and viviparous species, coupled with the climatic seasonality of the studied area, suggests that females produce, at most, only a single clutch per reproductive season. Indeed, this suggestion is corroborated by the absence of females containing follicles in secondary vitellogenesis at the same time as having oviductal eggs (Almeida-Santos et al., 2014).

Moreover, the relatively low number of embryos per female per year (RP), as well as the biennial reproductive frequency, is probably a consequence of viviparity (Seigel and Fitch, 1984). Fitch (1981) argues that larger SSD in favour of viviparous females would be a way of compensating for the difference in reproductive frequency between the two reproduc-
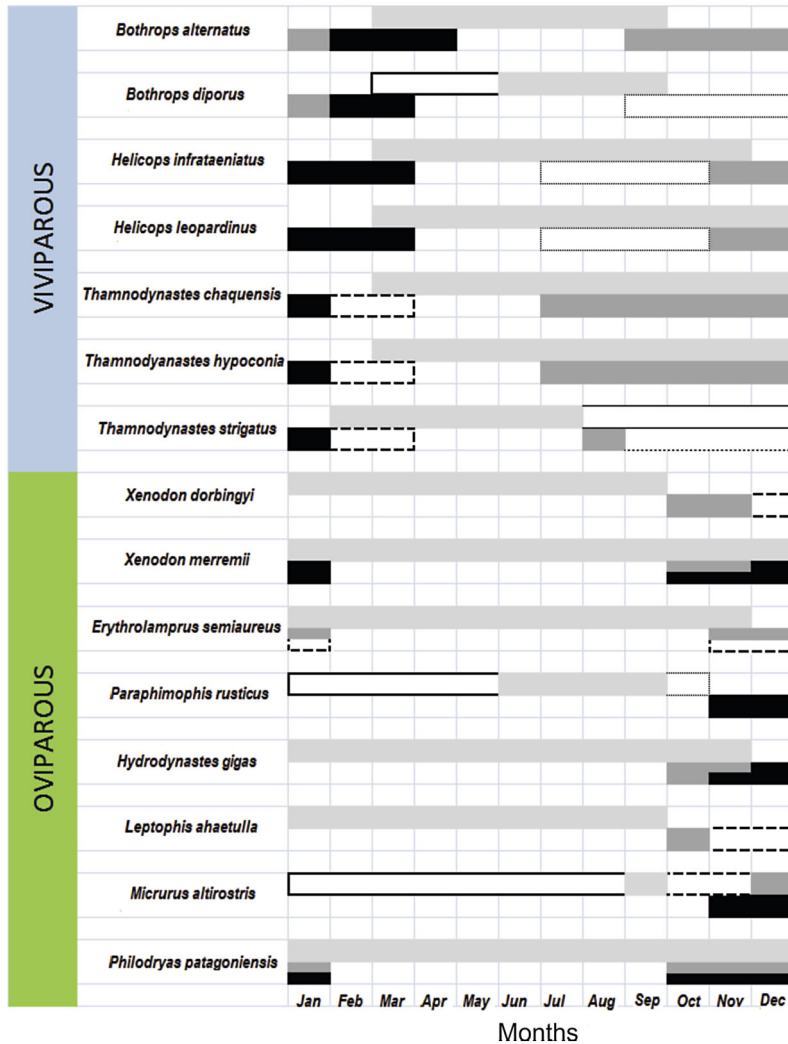
Figure 1. Snake annual reproductive cycles in temperate regions. Shaded bars: observed data; open bars: inferred data; light grey bars: vitellogenic follicles; dark grey bars: oviductal eggs; black bars: egg-laying or parturition; continuous line bars: inferred vitellogenesis; dotted line bars: inferred oviductal eggs; dashed line bars: inferred egg-laying or parturition.

tive modes. Thus, while viviparous species reproduce sporadically, they may harbour more offspring because of their larger size. As discussed above, however, the results obtained in this study did not support the hypothesis by Fitch (1981), since SSD did not differ between species with different reproductive modes, and there was a lack of correlation between greater female body lengths and a greater production of neonates. This lack of a relationship between clutch size and maternal SVL contradicts an

otherwise common pattern in viviparous snakes (Shine, 1994, 2003).The higher RP in oviparous species seemed logical, since these reproduce more frequently. However, mean fecundity did not differ among species with different reproductive modes. Despite the influence of environmental factors on reproduction, phylogenetically related snakes were found to have more similar reproductive traits among them than with those non-related species (Bellini, Arzamendia and Giraudo, 2017).
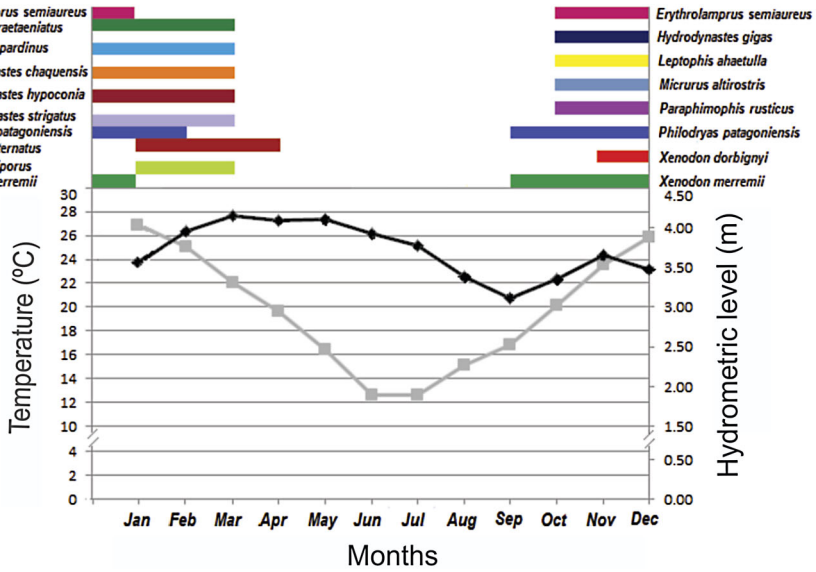
Figure 2. Egg-layings and births of snakes in relation to temperature and Paraná River level in the port of the city of Santa Fe between 1991 and 2016. Coloured bars show egg-layings of oviparous species and births of viviparous species. Each species is represented by a different colour. Grey line: temperature; black line: hydrometric level. (*) Viviparous species.
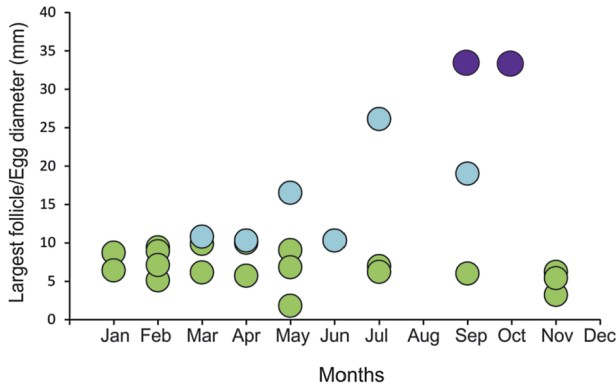

Figure 3A. Female reproductive cycle of *Bothrops alternatus* from eastern Argentina. Green circles: primary vitellogenesis; light blue circles: secondary vitellogenesis; purple circles: oviductal eggs.

The environment and the reproductive strategy of snakes

Another important difference between oviparous and viviparous snakes of our community is the reproductive strategy they adopt in response to environmental conditions. Tinkle and Gibbons (1977) argued that uterine retention of eggs might be selectively advantageous when a reproducing female reptile is unable to predict the conditions to which her eggs will be exposed during their incubation period. According to this, females could thus delay oviposition until environmental cues indicate that conditions for egg-laying are optimal (Gregory, 2009). The reproductive strategy of oviparous snakes of our assemblage was to lay their eggs when the river level was generally low and most offspring are already hatched by the time the river begins to rise. On the other hand, viviparous species gave birth when the level of Paraná River was
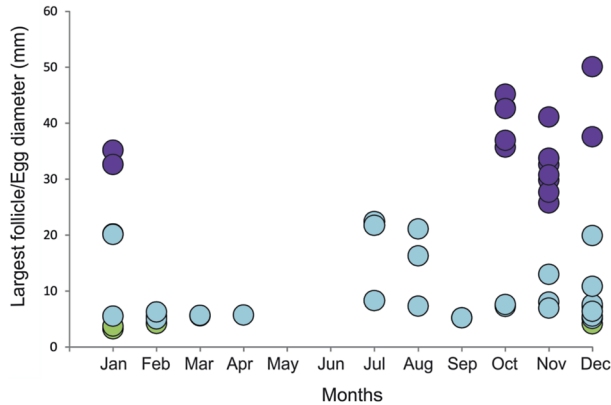
Figure 3B. Female reproductive cycle of *Philodryas patagoniensis* from eastern Argentina. Green circles: primary vitelloge-nesis; light blue circles: secondary vitellogenesis; blue circles: oviductal eggs.

high, coinciding even with the maximum histor-ical values of the hydrometric level (Ávila, Fer-reira and Arruda, 2006; Giraudo et al., 2007). In viviparous species, life history traits are affected both as a simple consequence of shorter activity seasons and as adaptations to different selective regimes (Adolph and Porter, 1993). This makes sense if we consider that five (*Thamnodynastes* sp. and *Helicops* sp.) out of the seven viviparous species in our assemblage are aquatic species; and even *Bothrops*, despite being terrestrial, easily moves through these wetlands. When the river rises to a high level, the floodplain be-comes a network, interconnecting many lakes and marshes (Iriondo and Paira, 2007). Under these conditions, species find a greater avail-ability of aquatic environments, optimizing the possibility of finding refuge and feeding areas (Ávila et al., 2006; Giraudo et al., 2007). In a previous study, we found a high correlation between reproductive modes and the presence or absence of an aquatic habitat (positive for viviparity and negative for oviparity) (Bellini, Arzamendia, and Giraudo, 2017).

Final considerations

Our results showed that, despite the reproduc-tive mode, snakes of the Paraná floodplain have reproductive cycles adjusted to both the hy-drological cycle of the river and temperature regime. The snake assemblage from Paraná

River is, to a significant extent, the result of an admixture of evolutionarily distinct clades, each contributing a set of species with different re-productive traits, giving the assemblage a parti-cular and complex phylogenetic structure (Ca-dle and Greene, 1993). The reproductive traits strongly depend on the phylogenetic history of each species, reflecting the clade to which they belong (Bellini, Arzamendia and Giraudo, 2017). Therefore, we find it relevant to highlight that plasticity in RLH traits of our Neotropi-cal assemblage of snakes was influenced by en-vironmental factors, such as hydrological cy-cle of Paraná River and temperature regime, as well as by evolutionary history. Nowadays, it is indisputable that ecological studies should be accompanied by phylogenetic and evolution-ary analyses in order to illustrate how historical contingencies can shape the ecology of organ-isms (Hernández Gallegos, Méndez de la Cruz and Méndez Sanchez, 2010). However, the eco-logical data that constitute the raw material of these analyses are still scarce for many taxa of Neotropical snakes (Gallardo and Scrocchi, 2006). This notion is what has led ecologists to consider the evolutionary history of organisms to help determine the underlying causes of the community structures currently observed (Vitt et al., 2003; Bellini et al., 2015; Bellini, Arza-mendia and Giraudo, 2017). Although we do not ignore the influence of evolutionary history

on reproductive traits, we emphasize the importance of studying ecology as a fundamental component of integrative analyses that explain in a holistic way the reproductive life history of snakes.

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