Available online at www.sciencedirect.com

ScienceDirect

Zoology 110 (2007) 9–19

ZOOLOGY

www.elsevier.de/zool

Reproductive ecology of *Vipera latastei*, in the Iberian Peninsula: Implications for the conservation of a Mediterranean viper

Juan Manuel Pleguezuelos[a,*], Xavier Santos[a,b], José Carlos Brito[c], Xavier Parellada[d], Gustavo Adolfo Llorente[b], Soumia Fahd[e]

[a]*Dep. Biología Animal y Ecología, Fac. Ciencias, Univ. Granada, E-18071 Granada, Spain*
[b]*Departament Biología Animal (Vertebrats), Fac. Biología, Univ. Barcelona, Avda. Diagonal 645, E-08028 Barcelona, Spain*
[c]*CIBIO, Inst. Ciências Agrárias de Vairão, R. Padre Armando Quintas, Vairão P-4485-661, Portugal*
[d]*Servei Protecció Fauna, Dep. Medi Ambient, Generalitat Catalunya, E-08017 Barcelona, Spain*
[e]*Dép. Biologie, Fac. Sciencies, Univ. Abdelmalek Essaadi, Tetuan, Morocco*

Received 31 October 2005; received in revised form 2 May 2006; accepted 23 May 2006

Abstract

Eurosiberian vipers have been considered model organisms, and studies on their reproductive ecology have afforded much of the current knowledge concerning viviparity in snakes. However, such studies are biased towards northern species and there is little information on Mediterranean species and/or populations. The reproductive ecology of *Vipera latastei* in the Iberian Peninsula was studied by analysing a large sample of specimens from collections, to better understand the conservation status of this Mediterranean viper. Males and females matured at small and similar body sizes (240 and 265 mm snout–vent length, respectively) and reproductive cycles in both sexes were seasonal. Spermatogenesis peaked in August, vitellogenesis developed in spring and the timing of the mating period was puzzling, with populations mating in autumn, spring, or in both seasons. The most striking finding was that adult females reproduced triennially on average. Lataste's viper is currently in continuous decline in the IP, and most of its populations are isolated in Mediterranean mountains. We hypothesize that prey scarcity and the brevity of the activity period in mountain habitats diminishes the ability of vipers to recover over the short term the energy expended in reproduction. The species needs 2 years for the acquisition and storage of energy ("capital breeder"), and a third year for the expenditure of this energy (in vitellogenesis and embryogenesis), a year during which females feed consistently ("income breeder"). Thus, this viper combines both strategies to supply the reproductive energy cost. Current decline in population and distribution, together with a poor capacity to renew populations, renders Lataste's viper vulnerable to environmental stochasticity.

Keywords: Reproduction; Viperids; Spain; Portugal; Conservation

Introduction

Conservation theory predicts that the risk of extinction is high in species which have small population sizes (Pimm et al., 1988; Mace and Kershaw, 1997), small

---

[*]Corresponding author.
E-mail address: juanple@ugr.es (J.M. Pleguezuelos).

0944-2006/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.zool.2006.11.001

geographic range (Manne et al., 1999), or in species which inhabit areas with dense human population (Hawkins et al., 2000). Furthermore, certain biological characteristics increase vulnerability and proneness to extinction: low reproductive success, high habitat and dietary specialization, and low dispersal rates (Owens and Bennett, 2000; Purvis et al., 2000; Reed and Shine, 2002). Species with "slow" life history patterns are more prone to extinction because some of their reproductive traits (low reproductive frequency, small litters) limit recruitment rates and restrain population growth (MacArthur and Wilson, 1967). Snakes are good models to test the "slow" life-history hypothesis as they display great variability in reproductive patterns at both the intraspecific (Zuffi et al., 1999; Santos et al., 2005) and interspecific levels (Shine, 2003). In this sense, European vipers (genus *Vipera*) exhibit extremely slow life-history traits, as their reproductive mode is viviparity, they normally do not reproduce in sequential years, and are typical ambush foragers (Brito, 2003a). Members of this genus have been considered "model organisms", and studies on their reproductive ecology have afforded much of our current knowledge of viviparity in snakes (Saint Girons and Naulleau, 1981; Madsen and Shine, 1994; Bonnet et al., 1998). Such studies have been performed in species or populations from the Eurosiberian region (e.g. *V. berus* [Vølsoe, 1944; Andrén and Nilson, 1983], *V. aspis* [Bonnet et al., 2002]), and a few on Mediterranean species (*V. ammodytes* [Luiselli and Zuffi, 2002]), or Mediterranean populations of European species (*V. aspis* [Zuffi et al., 1999]). Since the breeding ecology of central and northern European vipers is well known, to study the same natural-history traits in Mediterranean vipers would be rewarding, from the standpoint of both ecology and conservation biology. The comparative study of the closely related European vipers (Eurosiberianan vs. Mediterranean) is of interest because it enables an understanding of the plasticity of these organisms in response to different environments. For instance, vipers are typical capital breeders (sensu Drent and Daans, 1980), i.e. organisms that store large fat reserves for reproduction (Bonnet et al., 1998). Female vipers from cold temperate regions, limited by short activity periods during the year, normally have difficulties acquiring enough reserves for reproduction in sequential years, and thus biennial or even triennial reproductive cycles are the most common (Andrén and Nilson, 1983; Naulleau and Bonnet, 1996). However, the more suitable climatic conditions of the temperate Mediterranean region, with an extended activity season and consequently a relatively long feeding period, should allow female vipers to accumulate reserves faster, and to reproduce in sequential years. At an intraspecific level, the shift from biennial and triennial reproductive frequency in central Europe to annual frequency in Mediterranean regions of the Italian Peninsula has been described for *V. aspis* (Zuffi et al., 1999).

Under this scenario, we studied the reproductive ecology of Lataste's viper (*V. latastei*), a small-sized viper (405 mm snout–vent length (SVL) on average) that occurs in the Mediterranean regions of the Iberian Peninsula (IP) and a few mountain regions of northwestern Africa. Despite its large distribution area in the IP (although with very scattered populations), *V. latastei* has until recently been the least-known European viper (Brito, 2003a). Although some studies treat its natural history (see reviews in Bea and Braña, 1998; Brito, 2003a), information on its reproductive ecology is rather sketchy (but see Parellada, 1995; Brito, 2003b; Brito and Rebelo, 2003). Such lack of information is probably a consequence of the population scarcity and discreet habits of this viper. The species is hard to find in the wild, which normally precludes the study of natural populations. The current conservation status (IUCN criterion) of the species in Spain, the country with more than half of the distribution area of the species, is "Near Threatened" and in Portugal "Vulnerable", being considered the most endangered snake in the IP (Pleguezuelos and Santos, 2002). Moreover, in a review of snake status worldwide, Dodd (1993) identified the European genus *Vipera* among the priorities for conservation.

The aim of this work is to infer the reproductive strategy of a Mediterranean viper. We compare its reproductive pattern with related viper species from the Eurosiberian climatic regions of Europe hitherto studied. We also seek to compile sound information that can be applied in conservation planning for the species. Following Dodd (1993), research into a snake life-history trait, such as its reproductive ecology, can lead to information that contributes substantially to the species' conservation.

Material and methods

A total of 268 individuals from the whole distribution area of *V. latastei* in the IP were examined. The sample was comprised of preserved specimens from the collections of the following institutions: Department Biología Animal, Granada Univ. ($n = 91$); Dept. Biología Animal, Barcelona Univ. ($n = 12$); Museo Nacional de Ciencias Naturales, Madrid ($n = 82$); Museu de Ciències Naturals, Barcelona ($n = 4$); Estación Biológica de Doñana, Seville ($n = 28$); Monestir Montserrat, Barcelona ($n = 1$); and the private collection of J. Brito ($n = 50$).

For all specimens, the date of collection and locality were recorded, and SVL measured with a cord (±1 mm). Individuals were dissected, and, for the analysis of reproductive characteristics, the following measurements were taken: in males, the long and medium axes of the right testis (±0.1 mm); in females, the diameter of the largest follicle or oviductal egg/folded embryo (±0.1 mm); and in both sexes the fat-body size. Given that fat bodies could not be removed from museum specimens, the fat-body level was ranked according to five visual categories following Pleguezuelos and Feriche (1999): zero, no traces of fat; one, small traces of fat among the intestine loops; two, fat bodies covering less than half of the intestinal surface; three, fat bodies covering more than half of the intestinal surface; and four, a continuous fat layer in the ventral zone of the abdominal cavity. Male reproductive cycle was inferred from the analysis of the testis surface area (TS, longest × medium axes of the testis), less subject to shrinkage by the effect of the preservation liquid than testis volume (personal observation of the authors). For males, size at maturity and the spermatogenic cycle were determined by relating TS with spermatogenic activity. TS was size-scaled using the scores of residuals from the regression between TS and SVL (both variables log transformed). This procedure has the drawback that in the estimation of body size at maturity, one of the variables (SVL) is included on both axes (see Fig. 1A). However, this methodology avoids biases due to the allometric development of TS and was performed only for graphic purposes, with no statistical treatment of the data in this graph. For females, the shift in follicle/egg size with respect to body length (SVL) was observed to determine size at maturity, vitellogenesis cycling, and embryogenesis period.

Smears from the testes and spermiducts were performed in a sub-sample of adult males ($n = 17$), stained with hematoxylin-eosin (Dift-Quick method), and observed at 400 × magnification. Because histological techniques applied to alcohol-preserved specimens do not work as well as in fresh specimens, the method only allowed verification of the presence/absence of spermatozoa in testes or spermiducts in

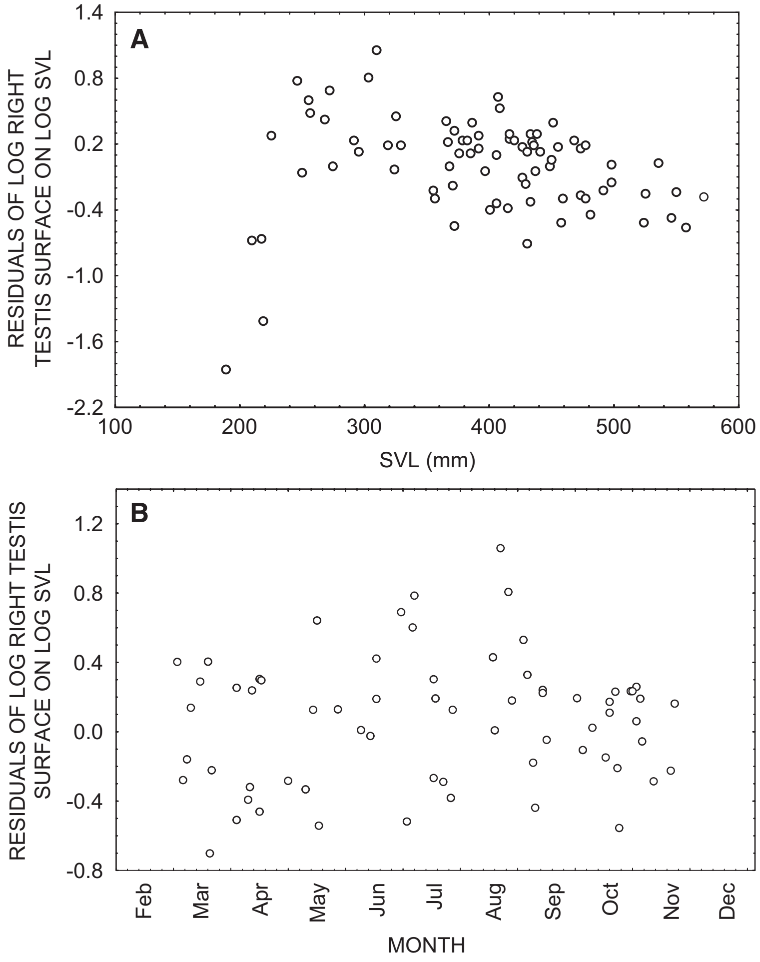

Fig. 1. Changes in testicular size for male Lataste's viper (*Vipera latastei*) from the Iberian Peninsula: residual scores for the regression of the testis surface area (long × medium axes of the right testes) on snout–vent length (SVL) vs. (A) body size (SVL, all individuals considered, $n = 80$), and (B) date of the activity period (only sexually mature individuals, SVL > 240 mm, $n = 75$). Each data point represents one individual.

order to define the spermiogenesis period or spermatozoa storage, but not to count the number or types of germinal cells.

The proportion of adult females with enlarged follicles in spring or embryos in summer was used as an estimation of reproductive frequency. However, catchability is greater in reproductive females than in non-reproductive ones, and thus a bias towards the overestimation of the breeding frequency should be expected (Bonnet and Naulleau, 1996). In this study, relative clutch mass (RCM) refers to wet clutch mass/post-partum female mass; this was determined from two pregnant females captured in mid-summer in the Garraf and Tortosa mountains (NE Spain), and placed in outdoor enclosures very close to the site of capture, under semi-natural conditions (detailed data in Parellada, 1995). Litter and female mass were recorded within 24 h after parturition, and the vipers were returned to the wild (in the same locality of capture) at the end of the study. Gut contents of preserved specimens were checked by making a mid-ventral incision in the stomach and in the anterior portion of the intestine, to relate feeding and fat-body cycling.

Assumptions of parametric tests (normality, homocedasticity of the variances) were tested for each variable before the analysis. Transformations were performed when variables of different dimension (length vs. surface) were correlated. Comparisons of body size were based on single-factor ANOVA, and comparisons of categorial variables (e.g. fat-body level) were performed by nonparametric tests: Mann–Whitney *U*-test (M–W *U*-test) or Kruskal–Wallis test. Observed versus expected frequencies were compared by χ^2-tests. Linear regression was used to identify relationships between pairs of variables (e.g. SVL vs. litter size). Mean values are followed by ±1 SD, and we set alpha equal to 0.05 for significance in all tests. Analyses were performed with STATISTICA (version 6.0).

Results

Sexual maturity

The TS (scaled by SVL) was low in males under 240 mm SVL and was high in larger males (Fig. 1A). Smears from testes and the *vasa deferentia* of specimens under this size did not have spermatozoa. Thus, males >240 mm were classified as sexually mature. Females larger than 263 mm SVL had ovarian follicles greater than 6 mm in length, oviductal eggs or embryos, or flaccid oviducts (an indication of recent embryos), whereas those smaller than this size exhibited none of these features; this was taken as the size at which females attained sexual maturity (Fig. 2A).

Reproductive cycle

According to the graphic interpretation of data in Fig. 1B, males showed seasonal variation in TS. In adult males, testicular growth began in March, peaked in August, and decreased abruptly in September. Testicular recrudescence could begin earlier than March, but not enough data were available for the time from December to February (hibernation period; Fig. 1B). In a statistical treatment of the data, residuals of log TS on log SVL were higher for males during the summer period (June–August) as compared to the spring (March–May; $F_{1,42} = 5.37$, $P<0.02$) or autumn period (September–-November; $F_{1,44} = 4.28$, $P<0.04$). Spermatozoa were found in testes of specimens from summer and autumn, and in the *vasa deferentia* of specimens from spring and autumn, suggesting the possibility of mating in both seasons. Copulations were observed in the north-western IP in autumn (15 September–10 October; Brito, 2003b), in the north-central IP in early spring (15 March), in the north-eastern IP in autumn (19 September–19 October; Parellada, 1995), and in the south-western IP both in autumn and spring (6–8 April, 19 October). This suggests an estival spermatogenic cycle in all individuals, and a post-nuptial cycle with sperm storage during autumn and winter in some areas of the IP.

In females, the reproductive cycle was also seasonal. Vitellogenesis began apparently in March, when some follicles attained 10 mm in length and continued during spring (Fig. 2B). Sample size was low during hibernation (November–February) which meant that the beginning of vitellogenesis during this period could not be verified. Sample size of reproductive females was also small at the beginning of summer (Fig. 2B). However, we observed that a female from the southern IP had oviductal eggs by mid-May, two specimens from the northern IP had still vitellogenic follicles at the beginning of June, and all reproductive females captured during July–September had embryos. Moreover, although we did not perform an histological analysis of ovaries, at least in four gravid females from late May to August there were medium sized and distinct follicles that we interpreted to be regressing *corpora lutea*. Thus, we suggest that ovulation occurs around May and the beginning of June. Females with embryos were observed until the first few days of October, and parturition was observed in the field on 20 and 21 September, and in outdoor enclosures (pregnant females captured in the wild) on 7 and 15 September, and 7 October. Thus, pregnancy spans about 4 months and the species exhibits an annual ovarian cycle (Fig. 2B).

Frequency of reproduction and litter size

During the vitellogenesis and pregnancy periods, 50 out of the 73 adult females had follicles <6 mm in

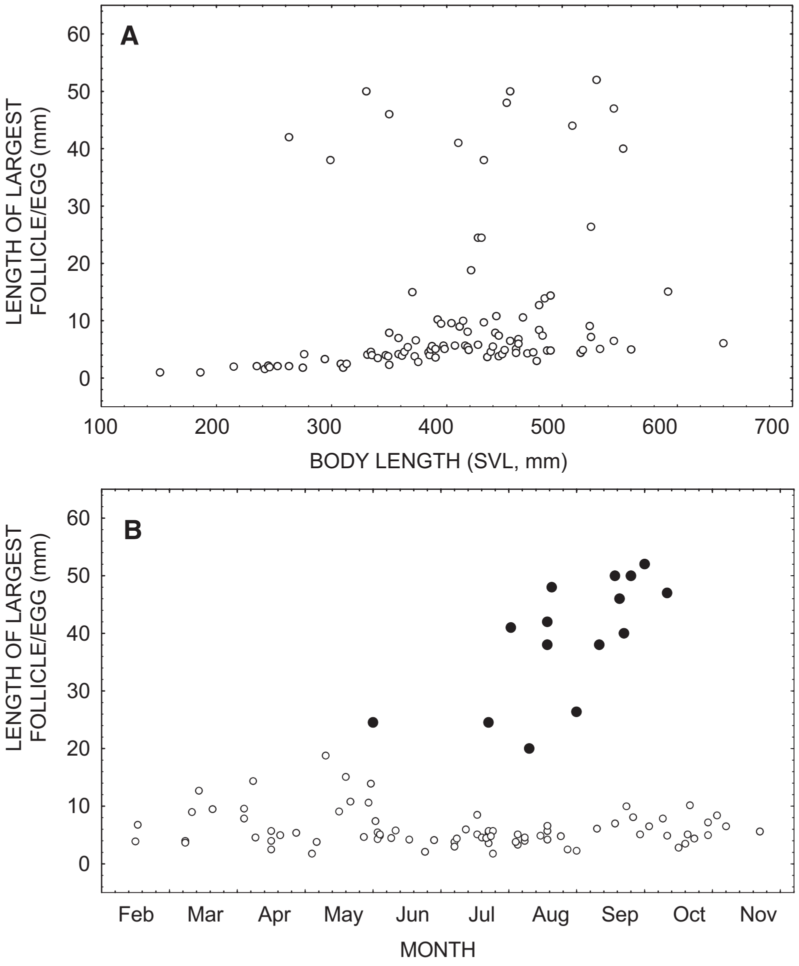

Fig. 2. Length of the largest follicle (open circle) and oviductal egg/embryo (full circle) in female Lataste's viper (*Vipera latastei*) from the Iberian Peninsula plotted against (A) body size (SVL, all individuals considered, $n = 131$), and (B) date of the activity period (only sexually mature individuals, SVL > 265 mm, $n = 71$). Each data point represents one individual.

length and depleted fat-body reserves, suggesting a non-reproductive condition (Fig. 2B). Therefore, female *V. latastei* has a less-than-annual breeding frequency, and data indicates that for the entire IP, on average, reproduction is triennial (when the observed frequency of reproduction is compared with an expected triennial frequency of reproduction, a 2 × 2 table leads to the following results: $\chi^2 = 0.06$, $P = 0.81$). No significant differences were found in SVL between reproductive and adult but non-reproductive females during the vitellogenesis and pregnancy periods (ANOVA, $F_{1,107}$: 1.36, $P = 0.24$), which rejects the possibility that the low breeding frequency was an artifact of including many small-bodied females within the non-reproductive group. Based on counts of oviductal eggs and two parturitions, litter size ranged from 2 to 13 (mean = 7.3 ± 3.0; $n = 20$), with a significant positive relationship between maternal body length and litter size ($r = 0.73$, $n = 20$, $P < 0.001$; litter size = 0.031*SVL + 5.836). The strength of this relationship is rather high ($R^2 = 0.54$), suggesting that body size is a proximate factor for fecundity.

Offspring size measured in newborns in collections, together with those from parturition in semi-natural conditions, ranged from 141 to 188 mm (SVL; mean = 158.8 ± 6.2 mm, $n = 7$; for the litters, only the mean data used), and new-born weight ranged from 3.0 to 7.5 g (mean = 5.3 ± 1.2 g, $n = 7$). RCM ranged from 48.3% to 69.0% ($n = 2$; Table 1).

Fat body and feeding cycle

In adult males, abdominal fat bodies did not differ from homogeneity throughout the months of the activity period (K–W test; $H_{8,97} = 9.86$, $P = 0.27$; Fig. 3A). In adult females, the fat-body level slightly declined during summer months, coinciding with the last

Table 1. Some reproductive traits for European species of the genus *Vipera*

Species	Study region	Gravid females (%)	Mean litter size	Mean newborn body length	Mean newborn body weight (g)	RCM (%)	Authorities
V. ammodytes	NW Italy	46	5.6				Luiselli and Zuffi (2002)
V. aspis	France		6.6	196 TL	5.4	43.9	Saint Girons and Naulleau (1981)
V. aspis	France	33	6.2				Naulleau and Bonnet (1995)
V. aspis	Central Italy	76		240 TL	6.9	57.5	Zuffi et al. (1999)
V. berus	France		7.1		4.7	45.6	Saint Girons and Naulleau (1981)
V. berus	Sweden	30–75	7.5–10.4				Andrén and Nilson (1983)
V. seoanei	NW Spain	50	5.9		5		Braña (1998)
V. ursini	France	50	4	136 SVL	3	39	Barón et al. (1996)
V. latastei	NW Portugal	59					Brito and Rebelo (2003)
V. latastei	Iberian Peninsula	31.5	7.3	159 SVL	5.3	55	This study

SVL: snout–vent length (mm); TL: total length (mm); RCM: relative clutch mass (clutch mass/post-partum female mass). Only examples are reported, as for some of the species, many additional cases are known.

vitellogenic period and pregnancy (K–W test; $H_{7,77} = 14.35$, $P = 0.04$; in both analyses, winter months pooled because of small sample size). The last analysis was performed with the whole sample of adult females and included many individuals (two-thirds) that did not reproduce (see above). The mixture of reproductive and non-reproductive females could mask the real effect of reproduction on fat-body reserves (Van Wyk, 1994). When reproductive and non-reproductive females were considered separately, a strong effect of vitellogenesis and pregnancy on the fat-body level was found: reproductive females exhibited higher fat-body levels than did non-reproductive ones in spring (March–June, M–W U-test, $Z = 2.22$, $P < 0.01$; Fig. 3B), sharply decreasing their fat-body reserves during advanced vitellogenesis, pregnancy and post-partum stages (July–October, M–W U-test, $Z = -3.33$, $P < 0.001$; Fig. 3B).

Nine out of 24 females in vitellogenesis, and eight out of 33 adult but non-reproductive females, had stomach contents during the same period of the year (March–-July; 2×2 table, $\chi^2 = 0.62$, $n = 57$, $P = 0.43$). No female with embryos had gut contents (July–September; $n = 11$); thus females apparently continued to feed consistently during vitellogenesis and ceased to feed during pregnancy.

Discussion

Size at maturity and reproductive cycle

Male *V. latastei* matured at shorter SVL than did females, although differences were small (7% of SVL). This is consistent with prior research indicating that *V. latastei* do not show sexual dimorphism in body size (Brito et al., 2006).

In ectothermic animals, climatic factors are important in determining the reproductive cycling (Duvall et al., 1982; Van Wyk, 1995). In male *V. latastei*, maximum spermatogenic activity coincides with the warmest period of the year (Fig. 1B). This cycle is the commonest for snakes in the temperate region (Duvall et al., 1982), and the cycle exhibited by all European vipers (Vølsoe, 1944; Saint Girons, 1992). In females, the most temperature-dependent process of the reproductive cycle, embryogenesis, is also well timed with the warmest month of the summer (Fig. 2B). Although the distribution of *V. latastei* is typically Mediterranean, a climatic zone where temperatures are generally mild, most of the current distribution of the species is restricted to mountain areas, with some populations at 3000 m a.s.l. (i.e. Sierra Nevada, south-eastern IP). The

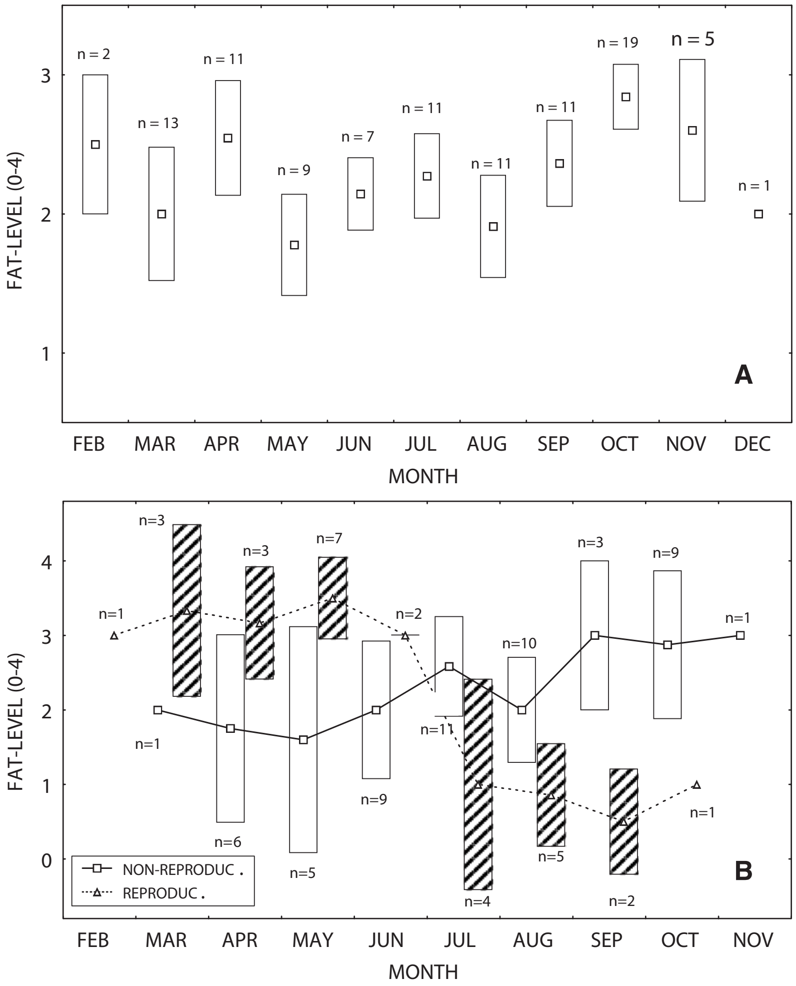

Fig. 3. Abdominal fat level of sexually mature individuals of Lataste's viper (*Vipera latastei*) from the Iberian Peninsula according to month of the activity period (box-plot with mean ± standard error): (A) males and (B) females. Between March and September, reproductive females can be recognized because of the presence of vitellogenic follicles or oviductal eggs/embryos. Thus, for that period, the abdominal fat level has been represented separately for the subsamples of reproductive and non-reproductive females. Fat-body level is scored in five categories, from zero to four (see the Methods section for more details).

observation that the reproductive time of both sexes corresponds to the warmest months of the year (together with viviparity as the reproductive mode) should contribute to the ecological ability of this species to inhabit such high altitudes in Mediterranean mountains.

In most male snakes with an estival spermatogenesis cycle, spermatozoids are ready by the end of summer, but overwinter in the deferent ducts until the following spring, when mating occurs (Saint Girons, 1992). However, in *V. latastei* copulation has been observed in different seasons, depending on the regions within the IP, without a clear biogeographic or climatic pattern: in early autumn in the rainy north-western IP and in typical Mediterranean areas of the north-eastern IP, and in spring in other mild Mediterranean areas of the south-western IP and in continental areas of the central IP. In the majority of temperate-zone snakes, the mating season is temporally dissociated from spermatogenesis, and reptiles are in general able to separate the mating period from fertilization (Crews, 1984; Aldridge and Duvall, 2002). When mating occurs in spring, it probably precedes (or is close to) ovulation in females, so that egg fertilization can occur; this process has been defined as short-term sperm storage. However, autumnal mating does not coincide with ovulation, implying that spermatozoids overwinter in the gonoducts of females for approximately 7 months until female ovulation the next spring; this has been defined as long-term sperm storage (Aldridge and Duvall, 2002). The dissociated timing of the male and female reproductive cycle in *V. latastei* requires that spermatozoids overwinter either in the male or in the female reproductive tracts, a situation that deserves future research, as well as the existence of a real transfer of spermatozoa in both mating periods. Evidently, mating in both seasons implies the need to have spermatozoids available in both seasons. We have found spermatozoids in spermiducts of *V. latastei* during spring as well as late summer; the same has been reported in *V. aspis*, a species which also mates in both seasons (Saint Girons, 1992), and in another European viviparous snake, *Coronella austriaca* (Capula et al., 1995).

Frequency of reproduction

Low frequency of reproduction is common in live-bearing snakes, particularly in viperids, mainly because they are ambush foragers with low foraging success, which precludes rapid energy recovery after a breeding event (Bull and Shine, 1979; see also results for fat-body cycling in *V. latastei*). Interspecific comparative data from European vipers indicates that every year about 50% of adult females reproduce (Table 1). In an intraspecific comparison, Zuffi et al. (1999) found that a population of *V. aspis* evolving under the mild conditions of a low-altitude Mediterranean climate (Italian Peninsula) showed an annual frequency of reproduction in contrast to the biennial or triennial breeding frequency of the northernmost populations. Translating this finding into an interspecific context, we would expect the related *V. latastei*, a strictly Mediterranean species, to exhibit an annual breeding frequency also. However, we found this viper to reproduce in a triennial mode on average, a frequency of reproduction characteristic of viper species and populations with northernmost distributions (Andrén and Nilson, 1983; Bonnet and Naulleau, 1996). The question arises why the Mediterranean *V. latastei* exhibits a reproduction frequency as low as northern European vipers, with the latter apparently living under worse climatic conditions for ectotherms. We hypothesize that this is the consequence of the current distribution of *V. latastei* in the IP. Most of the populations of this species are now restricted to mountains (hence most of the sample studied), because of human-induced habitat shrinkage and direct persecution (Brito, 2003a; Pleguezuelos and Santos, 2002; Santos et al., 2006, in press). Mediterranean mountain habitats generally imply prey scarcity (Pleguezuelos and Villafranca, 1997; Atauri and de Lucio, 2001) within a short daily and seasonal activity period. These environmental factors diminish the ability of snakes to recover, over the short term, the energy expended in reproduction. Populations of *V. latastei* from a small area in the north-western IP reproduce on a biennial basis on average (Brito and Rebelo, 2003), but they inhabit lowlands where availability of the most suitable prey for adult vipers (i.e. small mammals) is higher than in Mediterranean mountains (Barbosa and Benzal, 1996). In support of this contention, we found a smaller occurrence (close to significance) of small mammals as prey items in Mediterranean and mountain *V. latastei* (66.9%) in comparison to populations of the rainy lowlands of the north-western IP (83.9%; 2×2 table, $\chi^2 = 3.51$, $P = 0.06$; $n = 179$ prey items; unpub. data of the authors). This suggests an effect of prey abundance and prey suitability on the reproductive success of adult vipers, hence plasticity of reproductive traits. An island population of *V. berus* also showed a triennial frequency of reproduction after periods of prey scarcity (Andrén and Nilson, 1983). Data on snake reproduction within the context of geographic variation indicates a strong correlation between prey availability and some reproductive traits (Fitch, 1985), especially reproductive frequency (Shine, 2003). Under favorable conditions, female *V. latastei* exhibit a clear annual reproductive cycle, although they breed triennially on average.

Litter size

As known for many other snake species, larger mature females produce larger litters (Seigel and Ford, 1987). Mature female body size of *V. latastei* ranges between 263 and 640 mm SVL. The mean litter size of *V. latastei* is the modal clutch or litter size reported in a review of a wide sample of snakes by Seigel and Ford (1987). Comparative data on the reproductive ecology of the Mediterranean *V. latastei* (Table 1) showed that litter size and other reproductive traits fell within the limits for other European vipers (Eurosiberian and Mediterranean in distribution). Thus, European vipers are very similar in major reproductive characteristics (Naulleau and Saint Girons, 1981). However, considering that only one-third of adult female *V. latastei* reproduce every year, the mean annual reproductive output of an adult female is 2.4 newborns, suggesting a *K*-selected reproductive strategy (Lemen and Voris, 1981). Species with such strategies have limited capacity to recover from a population decline (because of environmental stochasticity or human-induced habitat loss), and are vulnerable from a conservation standpoint (MacArthur and Wilson, 1967).

Abdominal fat cycling

Vipers are low-metabolism snakes, with an ambush foraging strategy that normally precludes a high feeding rate (Greene, 1983). The most immediate explanation for the triennial reproductive frequency of female *V. latastei* is that individuals need 2 years for the acquisition and storage of energy, and a third year for the expenditure of this energy during the processes of vitellogenesis and embryogenesis. In fact, many female snakes do not initiate reproduction until their body reserves exceed a threshold value (e.g. Naulleau and Bonnet, 1996), and this seems to be the case for

V. latastei. According to our results, approximately two-thirds of adult females exhibit a medium to low level of fat bodies during all months of the activity period and thus do not engage in reproductive activity. The remaining one-third of adult females, which are in their reproductive year, undergoes a sharp plunge in fat-body level during late spring and summer (Fig. 3B). The same has been observed for other northern (*V. berus*; Nilson, 1981), central (see Fig. 1 in Bonnet et al., 1992 for a very similar finding in reproductive and non-reproductive *V. aspis*), and southern European populations of vipers (*V. aspis*; Luiselli and Zuffi, 2002). Lataste's viper would be classified as a "capital" breeder to fuel reproduction, temporally dissociating energy acquisition from expenditure. Capital breeding may be advantageous if the annual food level fluctuates or is insufficient for a successful breeding event. However, checking for gut contents in the museum specimens, we determined that reproductive females feed consistently during vitellogenesis, foregoing feeding opportunities in the reproductive year only during the months of gestation (see also Lourdais et al., 2002). Lataste's viper also acts as an "income" breeder (Bonnet et al., 1998), obtaining energy through frequent feeding during the year in which it breeds (see Luiselli and Zuffi, 2002 for a similar result in Mediterranean populations of *V. aspis*). Thus, *V. latastei* shows that energy for reproduction can be acquired on a long- and a short-term basis, and that capital and income acquisition of energy for reproduction are not alternative, but additive strategies. This would be the case for Mediterranean mountain populations of *V. latastei*, where suitable prey, small mammals, are usually scarce (Barbosa and Benzal, 1996), and adult vipers must consume a lot of suboptimal prey, such as lizards (unpublished data of the authors). Reproducing females of *V. aspis* also combine energy from capital and income to maximize their litter sizes to cope with fluctuating levels of prey abundance (Lourdais et al., 2003). In other organisms, when food is available during breeding, a capital breeder may resume foraging and turn into an income breeder (Jonsson, 1997).

Conservation issues

We can speculate that the phylogenetically constrained reproductive mode of most vipers, viviparity, would contribute in *V. latastei* to the maintenance of isolated and threatened populations in the mountains of the IP. Viviparity has selective advantages to confront two of the major problems currently facing *V. latastei*: (i) adaptation to the cold mountain environments (Shine, 1995) to which most of the Iberian populations are restricted; (ii) adaptation to variable environments (i.e. rainfall, prey availability; Tinkle and Gibbons, 1977), a characteristic of Mediterranean regions, probably magnified in mountain habitats. Lataste's viper must also cope with some of the disadvantages inherent to viviparity (Shine, 1985): (i) cessation of food intake during the rather long pregnancy period (approximately 3–4 months); (ii) long periods to restore energy reserves necessary for reproduction (2 years), as a consequence of the previous point and from the ambush foraging tactic; and (iii) a very low reproductive output (2.4 offspring/year on average).

In conclusion, *V. latastei* has some of the biological attributes of species prone to decline and to become vulnerable, such as insularity, small population size, heavy predation or killing, ambush foraging tactic (in the particular case of snakes), and a small rate of population recruitment (Mace and Kershaw, 1997; Manne et al., 1999; Purvis et al., 2000; Reed and Shine, 2002). The present Iberian populations, constrained to mountains because of direct or indirect human pressures, could be considered as distributed in mainland islands (Pleguezuelos and Santos, 2002; Brito, 2003a). In each one of these isolated areas, population size must be low, as deduced from data on its home range and habitat use (Brito, 2003b, c), and the paucity of new citations of individuals in the last 10 years for the whole IP (Santos et al., in press). With respect to heavy killing, in the study area most viper–human encounters end in a killed viper (Brito, 2003a). Ambush foraging snakes may be vulnerable because this strategy is associated with a suite of life-history traits that involve low rate of feeding, growth, and reproduction (Reed and Shine, 2002), and *V. latastei* is a typical sit-and-wait forager (Bea and Braña, 1998). Finally, the present study shows that *V. latastei* has a poor capacity to renew populations. We have arrived at this conclusion after studying a sample from a wide geographical area although, evidently, local environmental conditions and resource levels can significantly alter reproductive output.

Acknowledgements

We thank the curators of the collections with vipers we studied: J.E. González (MNCN, Madrid), J. Cabot (EBD, Seville), E. García (MZB, Barcelona), J.M. Baucet (MM, Montserrat), and people who kindly provided us with relevant information on the reproduction of Lataste's viper, including J.P. González de la Vega, A. Moreno, and F. Martínez. This study was partially funded by the research project REN2000-1376 GLO of the Spanish MCYT, in a grant to the senior author. X. Santos was supported by a post-doctoral Grant (EX2003/0253) from Ministerio de Educación, Cultura y Deporte (Spain) and J.C. Brito was supported by a post-doctoral Grant (SFRH/BPD/11542/2002) from Fundação para a Ciência e Tecnologia (FCT, Portugal).

References

Aldridge, R.D., Duvall, D., 2002. Evolution of the mating season in the pitvipers of North America. Herpetol. Monogr. 16, 1–25.

Andrén, C., Nilson, G., 1983. Reproductive tactics in an island population of adders, *Vipera berus* (L.), with a fluctuating food resource. Amph.-Rep. 4, 63–79.

Atauri, J.A., de Lucio, J.V., 2001. The role of landscape structure in species richness distribution of birds, amphibians, reptiles and lepidopterans in Mediterranean landscapes. Landscape Ecol. 16, 147–159.

Barbosa, A., Benzal, J., 1996. Diversity and abundance of small mammals in Iberia: Peninsular effect or habitat suitability. Z. Säugetierkd. 61, 236–241.

Barón, J.P., Ferrière, R., Clobert, J., Saint Girons, H., 1996. Stratégie démographique de *Vipera ursinii ursinii* au Mont-Ventoux (France). C. R. Acad. Sci. Paris 319, 57–69.

Bea, A., Braña, F., 1998. *Vipera latasti*. In: Salvador, A. (Ed.), Fauna Ibérica, vol. 10. MNCN, Madrid, pp. 480–488.

Bonnet, X., Naulleau, G., 1996. Catchability in snakes, consequences for estimates of breeding frequency. Can. J. Zool. 74, 233–239.

Bonnet, X., Naulleau, G., Mauget, R., 1992. Cycle sexuel de la femelle de *Vipera aspis* (Squamata, Viperidae); importance des réserves et aspects métaboliques. Bull. Soc. Zool. Fr. 117, 279–290.

Bonnet, X., Bradshaw, S.D., Shine, R., 1998. Capital versus income breeding: an ectothermic perspective. Oikos 83, 333–342.

Bonnet, X., Lourdais, O., Shine, R., Naulleau, G., 2002. Reproduction in a typical capital breeder: costs, currencies, and complications in the aspic viper. Ecology 83, 2124–2135.

Braña, F., 1998. *Vipera seoanei* Lataste, 1879. In: Salvador, A. (Ed.), Fauna Ibérica, vol. 10. MNCN, Madrid, pp. 489–497.

Brito, J.C., 2003a. Ecologia da víbora-cornuda (*Vipera latastei*, Boscá 1878) em Portugal e a problemática da sua conservaçao. Ph. D. Thesis, Lisbon Univerersity, Lisbon.

Brito, J.C., 2003b. Seasonal and daily activity patterns of *Vipera latastei* in northern Portugal. Amph.-Rep. 24, 497–508.

Brito, J.C., 2003c. Seasonal variation in movements, home range, and habitat use by male *Vipera latastei* in northern Portugal. J. Herpetol. 37, 155–160.

Brito, J.C., Rebelo, R., 2003. Differential growth and mortality affect sexual size dimorphism in *Vipera latastei*. Copeia, 865–871.

Brito, J.C., Santos, X., Pleguezuelos, J.M., Fahd, S., Llorente, G., Parellada, X., 2006. Morphological variability of the Lataste's viper (*Vipera latastei*) and the Atlas dwarf viper (*Vipera monticola*): patterns of biogeographical distribution and taxonomy. Amph.-Rep. 27, 219–240.

Bull, J.J., Shine, R., 1979. Iteroparous animals that skip opportunities for reproduction. Am. Nat. 114, 296–316.

Capula, M., Luiselli, L., Rugiero, L., 1995. Ecological correlates of reproductive mode in reproductively bimodal snakes of the genus *Coronella*. Vie Milieu 45, 167–175.

Crews, D., 1984. Gamete production, sex hormone secretion, and mating behaviour uncoupled. Horm. Behav. 18, 22–28.

Dodd, D.K., 1993. Strategies for snake conservation. In: Seigel, R.A., Collins, J.T. (Eds.), Snakes, Ecology and Behavior. McGraw-Hill, New York, pp. 363–393.

Drent, R.H., Daans, S., 1980. The prudent parent: energetic adjustment in avian breeding. Ardea 68, 225–252.

Duvall, D., Guillette Jr., L.J., Jones, R.E., 1982. Environmental control of reptilian reproductive cycles. In: Gans, C., Pough, H. (Eds.), Biology of the Reptilia, vol. 13. Academic Press, New York, pp. 201–231.

Fitch, H.S., 1985. Variation in clutch and litter size in New World Reptiles. Univ. Kansas Nat. Hist. Mus. Misc. Publ. 76, 1–76.

Greene, H.W., 1983. Dietary correlates of the origin and radiation of snakes. Am. Zool. 23, 431–441.

Hawkins, J.P., Roberts, C.M., Clark, V., 2000. The threatened status of restricted-range coral reef fish species. Anim. Conserv. 3, 81–88.

Jonsson, K.I., 1997. Capital and income breeding as alternative tactics of resource use in reproduction. Oikos 78, 57–66.

Lemen, C.A., Voris, H.K., 1981. A comparison of reproductive strategies among marine snakes. J. Anim. Ecol. 50, 89–101.

Lourdais, O., Bonnet, X., Doughty, P., 2002. Costs of anorexia during pregnancy in a viviparous snake (*Vipera aspis*). J. Exp. Zool. 292, 487–493.

Lourdais, O., Bonnet, X., Shine, R., Taylor, E.N., 2003. When does a reproducing female viper (*Vipera aspis*) "decide" on her litter size? J. Zool. 259, 123–129.

Luiselli, L.M., Zuffi, M., 2002. Female life history traits of the Aspic viper (*Vipera aspis*) and Sand viper (*Vipera ammodytes*) from the Mediterranean region. In: Schuett, G.W., Höggren, M., Douglas, M.E., Greene, H.W. (Eds.), Biology of the Vipers. Eagle Mountain Publishing, Salt Lake City, pp. 279–284.

MacArthur, R.H., Wilson, E.O., 1967. The Theory of Island Biogeography. Princeton University Press, Princeton.

Mace, G.M., Kershaw, M., 1997. Extinction risk and rarity on an ecological timescale. In: Kunin, W.E., Gaston, K.J. (Eds.), The Biology of Rarity: The Causes and Consequences of Rare–Common Differences. Chapman & Hall, London, pp. 131–149.

Madsen, T., Shine, R., 1994. Components of lifetime reproductive success in adders (*Vipera berus*). J. Anim. Ecol. 63, 561–568.

Manne, L.L., Brooks, T.M., Pimm, S.L., 1999. Relative risk of extinction of passerine birds on continents and islands. Nature 399, 258–261.

Naulleau, G., Bonnet, X., 1995. Reproductive ecology, body reserves and foraging mode in females of two contrasted snake species: *Vipera aspis* (terrestrial, viviparous) and *Elaphe longissima* (semi-arboreal, oviparous). Amph.-Rep. 16, 37–46.

Naulleau, G., Bonnet, X., 1996. Body condition threshold for breeding in a viviparous snake. Oecologia 107, 301–306.

Naulleau, G., Saint Girons, H., 1981. Poids des nouveau-nés et reproduction de *Vipera aspis* (Reptilia: Viperidae), dans des conditions naturelles et artificielles. Amph.-Rep. 2, 51–62.

Nilson, G., 1981. Ovarian cycle and reproductive dynamics in the female adder, *Vipera berus* (Reptilia, Viperidae). Amph.-Rep. 2, 63–82.

Owens, I.P.F., Bennett, P.M., 2000. Ecological basis of extinction risk in birds: habitat loss versus human persecution and introduced predators. PNAS 97, 12144–12148.

Parellada, X., 1995. About the apparent inexistence of a spring mating in the Catalan population of *Vipera latasti* (Reptilia: Viperidae), and note about the reproductive success. In: Llorente, G. (Ed.), Scientia Herpetologica. SEH-AHE, Barcelona, pp. 250–254.

Pimm, S.L., Jones, H.L., Diamond, J., 1988. On the risk of extinction. Am. Nat. 132, 757–785.

Pleguezuelos, J.M., Feriche, M., 1999. Reproductive ecology of the Hoseshoe Whip Snake, *Coluber hippocrepis*, in the Southeast of the Iberian Peninsula. J. Herpetol. 33, 202–207.

Pleguezuelos, J.M., Santos, X., 2002. Vipera latasti Boscá, 1878. In: Pleguezuelos, J.M., Márquez, R., Lizana, M. (Eds.), Atlas y Libro Rojo de los Anfibios y Reptiles de España. OAPN, Madrid, pp. 298–300.

Pleguezuelos, J.M., Villafranca, C., 1997. La distribución altitudinal de los anfibios y reptiles ibéricos. In: Pleguezuelos, J.M. (Ed.), Distribución y Biogeografía de los Anfibios y Reptiles de España y Portugal. Monogr. Herpetol., vol. 3, Granada, pp. 321–341.

Purvis, A., Gittleman, J.L., Cowlishaw, G., Mace, G.M., 2000. Predicting extinction risk in declining species. Proc. R. Soc. Lond. 267, 1947–1952.

Reed, R.N., Shine, R., 2002. Lying in wait for extinction: ecological correlates of conservation status among Australian elapid snakes. Conserv. Biol. 16, 451–461.

Saint Girons, H., 1992. Strategies reproductrices des Viperidae dans les zones tempérées fraiches et froides. Bull. Soc. Zool. Fr. 117, 267–278.

Saint Girons, H., Naulleau, G., 1981. Poids des nouveau-nés et stratégies reproductrices des vipères européennes. Terre Vie 35, 597–616.

Santos, X., Llorente, G., Feriche, M., Pleguezuelos, J.M., Casals, F., de Sostoa, A., 2005. Food availability induces geographic variation in reproductive timing of an aquatic oviparous snake. Amph.-Rep. 26, 183–191.

Santos, X., Brito, J.C., Sillero, N., Pleguezuelos, J.M., Llorente, G.A., Fahd, S., Parellada, X., 2006. Inferring habitat-suitability areas with ecological modelling techniques and GIS: a contribution to assess the conservation status of *Vipera latastei*. Biol. Conserv. 130, 416–425.

Santos, X., Brito, J.C., Pleguezuelos, J.M., Llorente, G.A., in press. Comparing Filippi and Luiselli's (2000) method with a cartographic approach to assess the conservation status of secretive species: the case of the Iberian snake-fauna. Amph.-Rep. 27.

Seigel, R.A., Ford, N.B., 1987. Reproductive ecology. In: Seigel, R.A., Collins, J.T., Novak, S.S. (Eds.), Snakes, Ecology and Evolutionary Biology. Macmillan, New York, pp. 210–252.

Shine, R., 1985. The evolution of viviparity in reptiles: an ecological analysis. In: Gans, C., Billet, F. (Eds.), Biology of the Reptilia, vol. 15. Wiley, New York, pp. 606–694.

Shine, R., 1995. A new hypothesis for the evolution of viviparity in reptiles. Am. Nat. 145, 809–823.

Shine, R., 2003. Reproductive strategies in snakes. Proc. R. Soc. Lond. B 270, 995–1004.

Tinkle, D.W., Gibbons, J.W., 1977. The distribution and evolution of viviparity in reptiles. Mis. Publ. Mus. Nat. Hist. Univ. Mich. 154, 1–55.

Van Wyk, J.H., 1994. Fat body and liver cycles in the large Girdled Lizard, *Cordylus giganteus*. Amph.-Rep. 15, 153–169.

Van Wyk, J.H., 1995. The male reproductive cycle of the lizard, *Cordylus giganteus* (Sauria: Cordylidae). J. Herpetol. 29, 522–535.

Vølsoe, H., 1944. Structure and seasonal variation of the male reproductive organs of *Vipera berus*. Spol. Zool. Mus. Haun. 5, 9–159.

Zuffi, M.A.L., Giudici, F., Ioalè, P., 1999. Frequency and effort of reproduction in female *Vipera aspis* from a southern population. Acta Oecol. 20, 633–638.