REPRODUCTIVE ECOLOGY OF A MEDITERRANEAN RAT SNAKE, THE LADDER SNAKE RHINECHIS SCALARIS (SCHINZ, 1822)

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Organisms that produce more than one newborn at every reproductive event must choose between two options with respect to their reproductive output: to produce a few large or many small young. The decision will be influenced by the spectrum of prey sizes available to young. The ladder snake (Rhinechis scalaris), a heavy-bodied Mediterranean colubrid, is well suited for the study of its reproductive ecology under this cue: the species consumes only endothermic prey and, thereafter, hatchlings of this gape-size-limited predator must be large enough to prey on small mammals. We analysed the reproductive ecology of this species, a quasi-endemic to the Iberian Peninsula, by studying a large sample of specimens collected in the south-eastern Iberian Peninsula. Most adult females (83.3%) reproduced in sequential years, with vitellogenesis beginning in early spring, and oviposition occurring in the first half of July. In contrast to the general rule for most temperate snakes, no depletion in fat bodies was observed during the period of vitellogenesis, females exhibiting a very high level of fat-body reserves throughout all months of the activity period. When compared with other Mediterranean species, hatching occurred very late in the activity season (October), newborns were rather heavy bodied, with very high fat-body levels, and apparently did not feed until the following spring. From our dataset, we suggest that female R. scalaris produce hatchlings large enough to enter hibernation without feeding, perhaps increasing in this way the survival rate of juveniles in their first calendar year. They probably need to devote their reserves at hatching to growth and to better face their first, bulky prey.

Key words: colubrid, fat bodies, hatching time, hatchling size

INTRODUCTION

Snakes have begun to attract increasing attention from many researchers as model organisms in ecological studies (Shine & Bonnet, 2000). Reproductive ecology is among the topics most addressed by researchers, as snakes exhibit wide diversity in reproductive strategies, in terms of frequency of reproduction, strategies to fuel reproductive energy cost (“capital” versus “income” breeding, as proposed by Drent & Daans, 1980), and clutch size or hatchling size (Ford & Seigel, 1989). Moreover, noteworthy links can be found between the reproductive ecology of a snake and other traits of its natural history, such as feeding ecology (Shine, 2003). Certainly, food intake determines the reproductive output of snakes (Ford & Seigel, 1989).

The reproductive output of organisms, in the simplest analysis, can be split into two possibilities: to produce a few large young, or to produce many small young (Roff, 1992; Gregory & Larsen, 1993). The choice would be mediated by the spectrum of prey sizes available to the hatchlings (Nussbaum, 1981). With a given amount of energy supplied for reproductive output, a species that preys on a wide range of prey types and size would choose between the two possibilities. However, species with a narrower spectrum in prey type and/or size, particularly if available prey for neonates are large, would find the choice of producing a few large hatchlings to be more adaptive (Sun et al., 2002). The ladder snake, Rhinechis scalaris, is a rather large, heavy-bodied and oviparous colubrid, that inhabits the Iberian Peninsula, south-eastern France, and the westernmost part of Italy (Pleguezuelos & Cheylan, 1997), and is a suitable organism for analysing links between reproductive and feeding ecology. This colubrid has the peculiarity of feeding only on endotherms, mostly small mammals (Cheylan & Guillaume, 1993). As opposed to other medium-sized Mediterranean predators, whether snakes (Diaz-Paniagua, 1976; Saint Girons, 1980) or other animals (Jacksic et al., 1982), R. scalaris does not undergo a typical ontogenetic shift in dietary habits. While young individuals of many snake species prey on small-sized, slender-bodied ectotherms, and large individuals prey on bulky prey such as small mammals, both young and adult R. scalaris prey almost exclusively on small mammals (Pleguezuelos, 1998). Thus, we would expect R. scalaris, a gape-limited predator, to produce few large hatchlings to minimize predator/prey size constraints in the first period of individual development (King, 1993).

Here we study a large sample of free-ranging animals and museum specimens from the south-eastern Iberian Peninsula to determine the reproductive ecology of this snake; individuals were also checked for stomach contents to obtain an index of the feeding rate of the species. We address the following main questions: (1) Do male and female reproduce in sequential years? (2) Is there fat-body cycling in sexually mature individuals, and if so, is cycling tied to vitellogenesis in females? (3) Is clutch size related to maternal size? Does the female produce large clutches of small
hatchlings, or small clutches with large hatchlings? 4) Do newborns feed in their first calendar year, or rely on the vitellogenic reserves at the beginning, delaying feeding until next calendar year?

This species is remarkably differentiated from other ratsnakes in morphology, plasma protein and mitochondrial-DNA sequence (Helfenberger, 2001; Lenk et al., 2001; Utiger et al., 2002). In this sense, there is evidence for a separate position of this taxon within the genus *Elaphe*, derived in the revalidation of the monotypic genus *Rhinechis* Michaelsae, 1833 (Helfenberger, 2001; Utiger et al., 2002). For this reason, we adopt the new taxonomical proposal and use the combination *Rhinechis scalaris* for the species.

**MATERIAL AND METHODS**

The field study was conducted in the Granada Depression and closely surrounding areas, a region of approx. 3000 km² in the south-eastern Iberian Peninsula (36° 55'–37° 20' N, 3° 30’–4° 15' W), which spans altitudes between 450 and 1200 m asl. Climate is typically Mediterranean; mean minimum temperature ranged from 1.9 to 4.4 °C in winter (January), the mean maximum temperature from 31.0 to 35.6 °C in summer (July). The mean annual temperature ranged from 12.5 to 14.3 °C, and average yearly rainfall ranged from 355.4 to 448.0 mm (data from the Cartuja weather station [37° 12' N, 3º 36' W], representative of the study area).

Field sampling was conducted from 1993 to 2000, within the framework of a larger study on the snake fauna of the region (details in Feriche, 1998). We made searches 3–4 field days per month (c. 6 hr each), throughout all months of the year. Specimens were collected from among those killed by local people and by traffic. A total of 320 specimens were collected in this way, with vouchers preserved in alcohol and deposited in the University of Granada (DBAG). Although the species is hard to find and difficult to catch, we hand-captured specimens alive when possible (25 individuals), although they provided little information on reproduction. We also analysed some specimens from the collections of the Estación Biológica de Doñana, Seville (EBD; n=20), and Museo Nacional de Ciencias Naturales, Madrid (MNCN; n=3), collected in the study area. In total, we examined 368 specimens (202 males, 166 females). Because this study spanned nine years, we assumed that the reproductive cycle of the species remained stable in the study area over the years.

In all specimens, snout-vent length (SVL) and tail-length (TL) were measured with a cord (± 1 mm). Live snakes were sexed by the SVL/TL relationship and the shape of the base of the tail (Feriche et al., 1993), and females were gently palpated in the rear abdomen to check for pregnancy. All specimens from collections were dissected, and we took the following measurements: longest, medium and shortest axes of the right testis (± 0.1 mm) in males; diameter of the largest follicle or oviductal egg (± 0.1 mm) in females; and fat-body size in both sexes. In males, testicular volume (TV) was estimated using the formula for the volume of a flattened ellipsoid (Mayhew, 1963). In that sex, size at maturity and the spermatogenic cycle were determined by relating TV with spermatogenic activity (Seigel & Ford, 1987). To calculate size at maturity and reproductive cycle in males, we used the right testis, always larger and in front of the left one (Feriche, 1998). Because TV increases significantly with SVL, TV was standardized to avoid bias by the residuals of the regression of TV (log transformed) on SVL. To calculate size at maturity in females, we observed the shift in follicle size with respect to body length (SVL) in the whole sample of females. Because we were unable to weigh fat bodies accurately from some road-killed specimens, or to remove fat bodies from museum specimens, we scored the fat-body level in five visual categories: 0: no traces of fat; 1: small traces of fat among the intestine loops; 2: fat bodies covering less than half of the intestinal surface; 3: fat bodies covering more than half of the intestinal surface; and 4: a continuous fat layer in the ventral zone of the abdominal cavity (Pleguezuelos & Feriche, 1999). We also checked for stomach content to obtain an index of feeding frequency, and correlate it to some traits of the reproductive ecology of the species: in live snakes by gentle palpation of the fore abdomen to force regurgitation in the case of recently ingested food, and in voucher specimens by an incision in the stomach. Measurements were taken only from well-preserved specimens and/or organs, resulting in different sample sizes for various measurements. Mean values are followed by ± 1 SD.

**RESULTS**

The SVL of *R. scalaris* in the study area ranged from 233 to 1385 mm (mean=719.3±206.9 mm, n=368), and weight from 11.2 to 1720.2 g (mean=239.9±233.0 g, n=288). Testicular volume was lower than average in specimens under 400 mm SVL, while males larger than 500 mm SVL had higher than average TV, indicating a testicular recrudescence (Fig. 1A). Thus, we classified males > 450 mm SVL as sexually mature. The smallest female that showed signs of reproductive activity (follicles >13 mm in length) was 660 mm SVL (Fig. 2A), and this was tentatively taken as the size at which females attained sexual maturity.

With respect to the reproductive cycle, in adult males testicular recrudescence began in mid-May, peaked in July and August, and decreased through autumn, testes volume reaching its smallest size during winter (Fig. 1B). The beginning of the mating period was deduced from the observation of a mating ball in the wild, which occurred at the end of March. The annual TV and the mating period of this species indicates that males have a seasonal and aestival spermatogenic cycle (*sensu* Saint Girons, 1982). From Fig. 1B, we also deduced that most adult males underwent spermatogenesis in sequential years. In females, the reproductive cycle was also sea-
Vitellogenesis begins in April, and oviductal eggs were present during June and the first half of July (Fig. 2B). Neither of the adult females from the second half of July and August contained oviductal eggs (Fig. 2B). Therefore, we estimated oviposition in the field to occur in the first half of July.

In mature males and females, abdominal fat bodies were rather large and homogeneous over the year (K-W test; males, $H_{6,81}=2.22$, $P=0.3$; females, $H_{7,55}=5.49$, $P=0.6$; in both analyses, winter months pooled because of small sample size; Fig. 3). Mature females had larger fat bodies than mature males (M-W $U$ test, $U=1922.5$, $P<0.003$) or immature females ($U=429.5$, $P<0.0001$).

In mature females, frequency of specimens with prey in the stomach did not differ when those carrying enlarged follicles were compared with those outside the vitellogenesis period (2 x 2 table, $\chi^2=0.10$, $n=98$, $P=0.9$). Thus, females apparently continued to feed during the vitellogenesis period.

Clutch size ranged from 4 to 14 eggs (mean $=7.4 \pm 2.8$, $n=19$), with a significant positive relationship between body length and clutch size ($r=0.690$, $n=19$, $P<0.001$; clutch size $=0.0099 \times$ SVL$-1.605$). Of the 27 sexually mature females in the reproductive period, 20 (74%) contained large vitellogenic follicles or oviductal eggs. The percentage of annual reproductive females became higher (83.3%) when discounting three of the seven non-reproductive females, with body size close to the minimum size to be sexually mature, this size (660 mm SVL) being an approximation more than a thresh-
old value. Hence, most of the sexually mature females reproduced in sequential years.

In the study area, the first hatchlings were recorded in the field on 5 October after intensive field searching. Taking into account the oviposition date and that newborns remain in the nest for 7–13 days, until first ecdysis (Blázquez, 1994), we deduced the incubation period to be approximately 65 days. Offspring size, calculated from hatchlings found in the field in their first calendar year, ranged from 240 to 310 mm SVL (mean=277.0±28.3, n=11), and offspring weight ranged from 11.2 to 17.5 g (mean=15.1±1.2, n=6). The fat-body level of hatchlings was rather high (mean 3.4±0.84, range 2–4, n=10). None of the 11 newborns found in the field had gut content. Thus, we suggest that newborns enter their first hibernation period relying only on their vitellogenic reserves. In the spring of their second calendar year, young exhibited a fat-body level (mean=2.0±1.1, range 0–4, n=12) lower than in the hatching period (M-W U test, Z=1.94, P=0.052). Although the comparison did not reach significance, it suggests depletion in fat-body level during the first hibernation period.

**DISCUSSION**

As observed in other large oviparous colubrids (Parker & Plummer, 1987), or in other species of the former genus *Elaphe* (Fitch, 1963), male *R. scalaris* matured at shorter absolute and relative SVL (SVL at maturity as a percentage of maximum size) than did females. In snakes with no sexual dimorphism in body size, the different size at maturity has been interpreted as the result of females requiring a high threshold level of energy stores to initiate reproduction (Duvall et al., 1992). The finding in the present study that mature females exhibited higher fat-body levels than immature specimens of both sexes and adult males supports this interpretation.

Males exhibited an aestival cycle, in which sperm overwinters in the ducts deferent until the mating period, in spring of the next year. The aestival cycle is typical of snakes from the Temperate Zone (Seigel & Ford, 1987), and fits well with the northern Mediterranean distribution of *R. scalaris*. The aestival spermatogenic cycle allows this species to mate early the next year, immediately after emerging from winter dormancy, as confirmed by our observation of mating activity.

Females exhibit a seasonal reproductive cycle, as is general in snakes from the temperate zones (Saint Girons, 1982). This highly seasonal pattern observed in the reproduction of many snakes should be guided by seasonal variation in available resource levels, hatching survival rates (Shine, 2003), or thermal requirements during embryogenesis (Saint Girons, 1982). In *R. scalaris*, vitellogenesis occurs in late spring, when resource availability peaks in terrestrial Mediterranean habitats, with oviposition in early summer, and embryogenesis in the warmer months of the year (Feriche, 1998). Since no specimen was found with gut content in their first calendar year (autumn season), reproduction in this species is most likely not timed to favour the access of newborns to feeding resources.

In reptiles, ectothermic vertebrates with relatively low metabolisms, the accumulation of body reserves to fuel reproductive processes is a common phenomenon (Bonnet et al., 1998b). Thus, a major issue in studying snakes that inhabit temperate regions, with a seasonal reproductive cycle, is to understand the seasonal variation in energy related to reproduction (Congdon, 1989; Santos & Llorente, 2004). In most temperate snakes fat bodies are large in spring, reach a low point in late spring to early summer, and then increase gradually until hibernation (Seigel & Ford, 1987; Pleguezuelos & Feriche, 1999; Santos & Llorente, 2001). However, surprisingly, fat-body cycling in female *R. scalaris* was not tied to the timing of the reproductive cycle. In this species, stored lipids must contribute to the energy needed for follicular maturation and egg yolkling, but not to the degree of depleting fat-body levels during the reproductive period. This pattern mirrors that observed in some tropical snakes (Seigel & Ford, 1987), for which it has been suggested that foraging success is high enough to preclude the necessity of resorting to fat reserves for reproduction (Berry & Lim, 1967). Though *R. scalaris* is not tropical, the cue for the lack of fat-body cycling in tropical snakes (high foraging success) would apply to this species (see also Valverde, 1967; authors’ unpublished data). Mature *R. scalaris* females during vitellogenesis continue feeding (but not in the late pregnancy stage; M. C. Blázquez, pers. comm.), an uncommon phenomenon in snakes (Agrimi & Luiselli, 1994; Gregory et al., 1999; Rohr, 2003). Thus, the high fat-body level of mature females at the beginning of vitellogenesis suggests that the species functions as a capital breeder for fuelling the breeding process, as occurs in most ectotherms (Bonnet et al., 1998a). Taking into account that females continue to feed during vitellogenesis, we should consider that the species is an income breeder to some degree. However, this major issue in the reproductive ecology of ectothermic vertebrates is in reality far from a simple dichotomy (capital vs. income). Many species must rely on intermediate possibilities, or simply be “facultative income” breeders, as has been recently found for some colubrids and vipers (Lourais et al., 2002; Reading, 2004; Santos & Llorente, 2004); *R. scalaris* would be another example.

The ladder snake exhibited a low relative fecundity (mean clutch size vs. female body length), as is normal in terrestrial colubrids when compared with aquatic ones (Seigel & Ford, 1987). At least the mean and range for the clutch size found here for this species were within the values recorded for other Palaearctic species of the former genus *Elaphe* (Schulz, 1996; Rugiero, 1998; Rugiero et al., 1998). However, clutch size is not the sole measure of snake reproductive investment (Seigel et al., 1986). In *R. scalaris*, egg size is rather
large (Cheylan & Guillaume, 1993; see also Fig. 2B), and relative clutch mass is considerable, representing between 31 and 47% of female body mass (Blázquez, 1994; Cheylan & Guillaume, 1993; data for comparison in Seigel et al., 1986). A reproductive strategy in female snakes that invest substantial energy in reproduction is to lower the frequency of reproduction (Shine, 2003). Nevertheless, our results, supported by a large sample size, indicate that most mature, good-sized females reproduce in sequential years, as is general in active searcher snakes (such as the species analysed here; Pleguezuelos, 1998), compared to ambush foragers (Webb et al., 2003).

Besides reproducing in sequential years, females of R. scalaris lay large eggs that give birth to large, heavy-bodied hatchlings (average hatching body weight as a percentage of average adult body weight scored 5.1% in R. scalaris, and 2.5–4.1% in other western Mediterranean medium-sized colubrids; data from Feriche, 1998 and Fahd, 2001).

In temperate snakes, late-born hatchlings may have fewer opportunities to feed before winter, but normally they benefit from larger energy reserves (Sun et al., 2002). According to our data, R. scalaris agrees with this strategy, which combines hatching date, newborn fat reserves and feeding rate. Hatchlings of R. scalaris born later in the year than hatchlings or offspring of any other western Mediterranean snake (see reviews in Böhme, 1993, 1999) exhibit very high fat-body levels, and apparently do not feed in their first calendar year (present study). The large amount of vitellogenic reserves attached to the gut in hatchlings must fuel growth until spring, in the next calendar year. This has been defined as extended parental care in the form of yolk reserves that remain in the hatchling after it leaves the egg (Congdon, 1989). In any case, this conclusion should be regarded with caution, because of the small sample size for newborns, and more information is needed on hatching survival and growth in order to draw definitive conclusions concerning the adaptive significance of delayed hatching dates in snakes.

Although the interrelationships among clutch size, hatching size, maternal body size and maternal body condition are complex and should be addressed by a multivariate analysis (King, 1993; Sun et al., 2002), we can draw certain conclusions from our dataset on R. scalaris: (1) most mature, large-sized females reproduce in sequential years; (2) reproductive output seems to be focused on a few but large-sized and fatty hatchlings; (3) these hatchlings appear to enter their first hibernation period without feeding, probably enhancing in this way their survivorship. Meanwhile, they devote time and reserves to growth, in order to face their first bulky prey (small mammals).

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