Food availability induces geographic variation in reproductive timing of an aquatic oviparous snake (*Natrix maura*)

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**Abstract.** The viperine snake *Natrix maura* is a common water snake, which forages on aquatic prey such as fish and frogs in Western Mediterranean water bodies. Female viperine snakes collected from three populations at the Iberian Peninsula during the vitellogenesis period were compared. Mean clutch size and range, as well as the slope of the regression between body size and clutch size, did not show differences between populations. In contrast, mean size of enlarged follicles of females collected in May from the Ebro Delta proved significantly smaller than those of females from the Matarranya River (50 km far) and the Granada Depression (500 km far). There were no differences in climatic conditions between areas. However, seasonal variation in food availability was significantly different in the Ebro Delta. In this area, the rice fields are dry in early spring as men control the water flow, and prey are not available. This energetic constraint induced female viperine snakes to adjust reproductive timing according to seasonal availability and to delay vitellogenesis for at least one month. By contrast, other Mediterranean populations exhibited a rather high prey availability from early spring (e.g. Matarranya River), females being in good condition in this season and consequently vitellogenesis starting earlier than in the Ebro Delta. This study illustrates a new case of reproductive plasticity in snakes.

**Introduction**

In most organisms reproduction exerts intensive demand of energy in a short period of time. According to a popular hypothesis, in vertebrates, two extreme strategies derive from this scenario: “income breeders” (mainly endotherms) invest exogenous resources for reproduction whereas “capital breeders” (mainly ectotherms) use endogenous resources previously stored in specialized body tissues (Drent and Daans, 1980). Capital breeders accumulate energy when resources are abundant for use when resources become scarce. Moreover, recent studies have demonstrated that these organisms adjust reproductive fitness under variation in food availability (Madsen and Shine, 1999), and food intake just before vitellogenesis has revealed important for at least the aspic viper, a typical capital breeder (Lourdais et al., 2003). Hence, in both income and capital breeders, food availability is a central factor to understand plasticity in the reproductive biology of different species (Reznick and Yang, 1993; Shine, 2003).

Snakes are suitable organisms to test reproductive plasticity both by experimental studies with food manipulation (Ford and Seigel, 1989, 1994; Seigel and Ford, 1991; Lourdais et al., 2003), and by field work studies with natural populations under contrasting environmental conditions (Shine and Madsen, 1997; Madsen and Shine, 1999, 2000). In the latter case, reproductive plasticity can be explored by two ways: monitoring reproductive output in long-term studies of snake populations (Shine and Madsen, 1997; Madsen and Shine, 2000; Lourdais et al., 2002), and analyzing geographic variation between conspecific populations submitted to different environmental constraints (Bronikowski and Arnold, 1999; Seigel and Ford, 2001).

The present study reports an example of intraspecific variation in the reproductive seasonality of the viperine snake *Natrix maura*. This is a medium-sized water snake, which forages on aquatic prey (mainly fish and amphibians;
Braña, 1998). Its diet is generally composed of the most common aquatic prey in the habitat (Pleguezuelos and Moreno, 1989; Santos and Llorente, 1998; Rugiero et al., 2000; Santos et al., 2000), and for this reason has been defined as an opportunistic feeder (Rugiero et al., 2000). The viperine snake is a very common species in the Mediterranean region (Santos et al., 2002), where climatic seasonality implies summer dry periods, great annual variation in the level of water bodies, and, consequently, seasonal changes in aquatic prey availability. For this reason, this species could be a good model to test reproductive plasticity derived from variation in food availability.

In the Ebro Delta, Santos and Llorente (2001) reported that vitellogenesis seemed delayed one month with respect to other conspecific populations in Western Mediterranean. The authors suggested low foraging opportunities in spring and consequently low fat bodies, revealed the key factor to explain this timing delay in the vitellogenesis. To analyze for thoroughly this preliminary result and to test intraspecific variation in the reproductive timing, we have compared reproductive traits for this species in three Iberian populations: Ebro Delta Natural Park and Matarranya River in the NE Iberian Peninsula, and the Granada Depression in the SE Iberian Peninsula. The former two are close together (50 km), whereas the latter is some 500 km to the south. Despite the distance between the sites, the climate is similar (see fig. 1). For this reason, we hypothesized that geographic differences in reproductive traits could be related to variation in aquatic food availability, which has been seasonally quantified at two of these localities.

Material and methods

Study sites

The first viperine snake population was found in rice fields of the Ebro Delta Natural Park (thereafter area E; fig. 1). In this wetland area, more than 50% of the surface area of the natural vegetation was replaced by rice fields in around the end of the XIX century. Although the main traits of the climate (rainfall and temperature) follow the typical Mediterranean seasonality (fig. 1), the water flow in the rice fields is controlled by man. Hence, rice fields and canals are dry from November to mid-April, when they overflow, following a water dynamic very different than that observed in other Mediterranean water-bodies. The second population was in the Matarranya River (thereafter area M), a 100-km-long tributary of the Ebro River, approximately 50 km far from the Ebro Delta (fig. 1). The region has Mediterranean climate seasonality, with periods of floods (during the rainy seasons, in spring and autumn) and droughts (in summer) when the river has the lowest water flow (Sostoa et al., 1990). The third locality was in the Granada Depression (thereafter area G), roughly 500 km south of the former two sites (fig. 1). Although it is a more heterogeneous area than the two others, climate is also Mediterranean, with hot and dry summers, and rainy seasons in spring and fall. Hence, the water dynamics of rivers in this region follow a pattern similar to that of the Matarranya River.

Food availability measurement

In the rice fields of area E, food availability was assessed in monthly sampling from May to December 1991. Five rice fields were sampled by sinking a square (60 cm per side) in the muddy soil, to collect all the aquatic organisms (more details in González-Solís et al., 1996). Fish availability in area M was quantified by electrofishing, the most common non-lethal methodology to estimate fish populations (Bohlin et al., 1989). Fish were collected in standardized surveys every two months and released to the river after measurement and identification at species level. The study was conducted from 1984 to 1986, in order to compare fish variation among years. We lack data on prey availability for area G. The methodology to quantify food availability was different in the two first areas described above. However, the goal of this study was not to compare prey abundance or biomass between different aquatic environments, but to detect seasonal variation in food availability. We assume that patterns of seasonal prey availability can be compared despite having been established by different methods.

Lab methodology

Adult female viperine snakes from area M were collected during a three-year study (from 1984 to 1986). Samples were frozen and stored in the collection of the Department of Animal Biology, University of Barcelona. Before necropsy, specimens were weighed (to the nearest 0.1 g) and measured (snout-vent length, SVL in mm). Fat bodies were removed and weighed (to the nearest 0.1 g), and sex and reproductive status were determined by examination of gonads. Enlarged follicles (larger than 7 mm) or oviductal eggs were counted to determine clutch size. Reproductive data from specimens collected at the area M were compared with published and unpublished data of conspecific populations from areas E and G (Feriche and Pleguezuelos, 1999; Santos and Llorente, 2001). Differences in reproductive seasonality between populations were tested by the analysis of
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Figure 1. Map of the Iberian Peninsula with location of the three study areas: M (Matarranya River), E (Ebro Delta), G (Granada Depression). Each study area includes an annual climogram.
follicle size (maximum diameter of enlarged follicles) in females collected in May, as most females had large follicles during this month (Braña, 1998; Feriche and Pleguezuelos, 1999). Follicle size was also controlled in females collected in August in the area M once reproduction finished for most populations of this species (Braña, 1998).

**Statistical analysis**

Normality (log transformation when necessary) and homogeneity of variances were tested in variables before statistical analysis. Tests of parallelism in the regression of clutch size vs. body size were performed between populations. Fat bodies mass was standardized to avoid bias because of snake length, by the residuals of the regression of fat bodies mass on SVL (both variables log transformed). Moreover, body condition was calculated by residuals of the regression of log body mass on log SVL (Jayne and Bennett, 1990). Geographic differences in the size of follicles were tested by two-factor nested ANOVA with female-factor (random effect) being nested in population-factor (fixed effect). In the text the means are followed by ± one SD.

**Results**

**Reproduction in the Matarranya River**

Twenty five adult female viperine snakes were collected in May \( (n = 16) \) and August \( (n = 9) \). In May, 69% adult females \( (11/16) \) had enlarged follicles and the smallest gravid female was 420 mm SVL. Mean clutch size was \( 14.0 \pm 2.4 \) (range = 2-27, \( n = 11 \)), in females averaging 580.6 ± 29.6 mm SVL (range = 420-770 mm).

Clutch size (CS) increased with SVL \( (\log CS = -21.8 + 3.82 \times \log \text{SVL}, r^2 = 0.71, n = 11, P = 0.001) \) (fig. 2). Mean size of follicles in gravid females collected in May was 16.4 ± 0.4 mm \( (n = 100 \text{ follicles in } 8 \text{ gravid females}) \). In August, all adult females had follicles smaller than 5 mm maximum diameter, indicating that the gravid period had finished. Despite the lack of data for other months, the variation in follicle size suggests that the gravid period for female viperine snake of the area M started in April and that females would have oviductal eggs in June and July. Females with enlarged follicles had stomach content, as occurred in females from the area E (Santos and Llorente, 2001) and the area G (Feriche and Pleguezuelos, 1999).

There were no differences either in the relative fat bodies mass (ANCOVA \( F_{1,22} = 0.7, P = 0.4, \text{SVL as covariate} \)) or in body condition (ANCOVA \( F_{1,22} = 3.3, P = 0.08, \text{SVL as covariate} \)) between females collected in May and August. When adult females collected in May were subdivided according to their reproductive status, non-gravid females showed smaller fat bodies residuals (fig. 3) and poorer condition (fig. 4) than did gravid females, but scores similar to those of females collected in August. However, differences in these traits were not significant (body condition: ANCOVA test, \( F_{2,22} = 1.91, P = 0.17 \); fat bodies mass, ANCOVA test, \( F_{2,22} = 1.89, P = 0.17 \); in both
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Figure 4. Mean and standard error in body condition (body mass scaled for body length) in female viperine snakes from area M grouped according reproductive status and capture date.

cases SVL as covariate), probably because of the small sample size.

**Geographic variation in reproductive traits**

Average clutch size and range were 8.3 eggs (2-12) and 9.7 eggs (2-19) in the areas E and G, respectively (Santos and Llorente, 2001; Feriche and Pleguezuelos, 1999). These mean values were lower than those of the area M (14.0 eggs) as the upper value in the clutch-size range was higher in the Matarranya population (27 eggs). Lower values were equal in the three populations, despite differences in body size of females with the minimum clutch size (two eggs: 312 mm, 325 mm and 420 mm SVL in the areas G, E, and M, respectively). Clutch size increased with body size in all three populations (fig. 2). After log transformation of the variables, conspecific populations did not differ either in clutch size scaled for SVL (ANCOVA $F_{3,39} = 0.77$, $P = 0.47$) or in the regression slopes (Parallelism test $F_{2,37} = 2.50$, $P = 0.10$). Female viperine snakes from the area M achieved larger body sizes and consequently exhibited a higher upper range in clutch size than did females from the other localities (fig. 2).

Geographic differences in the vitellogenesis timing were analyzed by variation in the size of the follicles for females collected in May from the three populations (table 1). There were differences in the size of follicle (two-factor nested ANOVA, $F = 94.3$, $P = 0.000001$). The population from area E registered lower sizes of follicles, suggesting a delay in the vitellogenesis with respect to the other two populations.

**Variation in prey availability**

The area M had high fish densities. Electric fishing registered 16 fish species as well as the green frog, *Rana perezi* (Sostoa and Lobón-Cerviá, 1989). There were some differences in the number and type of fish as well as in the density of fish per hectare along the river, but all the localities showed extremely high fish densities (Sostoa and Lobón-Cerviá, 1989). In fact, the Matarranya is one of the rivers with highest fish densities in Europe (Sostoa, 2001, 2002). The viperine snake mainly foraged on fish in the area M (95% of occurrence; Santos, 2004). Figure 5 presents the seasonal variation in fish abundance per hectare. Density was high throughout the year (more than 10,000 fish/ha in all samples), peaking in summer, as fish concentrated in the remaining water pools in the river and underwent recruitment $0+$ fish class occurred. Despite annual variation in fish availability in the area M, a three-years study revealed that seasonality follows a similar yearly pattern as in other Mediterranean rivers (Sostoa et al., 1990).

In the area E, canals and rice fields remain dry during winter and a significant part of spring. In mid-April, the flood gates of the canals are opened and water from the Ebro River fills the canal system and floods the fields. Then, aquatic organisms begin colonization (Forés and Comín, 1986; González-Solís et al., 1996) and productivity rapidly increases (fig. 5). In the area E, flooding was man-controlled and hence concentration of nutrients, abundance of zooplankton and phytoplankton, as well as available food for snakes did not change significantly among years (Forés and Comín, 1986). This scenario contrasts with that of most Mediterranean and subtropical water bodies, in which aquatic-prey abundance for snakes is driven by
Table 1. Size of enlarged follicles in female viperine snakes collected in May at the three localities considered. Clutch size (mean and dispersion of data) for females of the same localities and other populations from the Western Mediterranean.

<table>
<thead>
<tr>
<th>Localities</th>
<th>Size of follicles</th>
<th>Clutch size</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± se (follicles)</td>
<td>n</td>
<td>mean ± se (snakes)</td>
</tr>
<tr>
<td>Matarranya</td>
<td>16.36 ± 0.36</td>
<td>100</td>
<td>14.0 ± 2.4</td>
</tr>
<tr>
<td>(area M)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ebro Delta</td>
<td>12.60 ± 0.59</td>
<td>53</td>
<td>8.3 ± 1.0</td>
</tr>
<tr>
<td>(area E)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Granada</td>
<td>15.44 ± 0.50</td>
<td>78</td>
<td>9.6 ± 1.2</td>
</tr>
<tr>
<td>(area G)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alicante</td>
<td></td>
<td>7.1</td>
<td>3-12</td>
</tr>
<tr>
<td>France</td>
<td></td>
<td>7.3 ± 0.8</td>
<td>4-16</td>
</tr>
<tr>
<td>Salamanca</td>
<td>12.7</td>
<td>9-17</td>
<td>7</td>
</tr>
<tr>
<td>Sardinia</td>
<td>8.8 ± 2.7</td>
<td>4-15</td>
<td>15</td>
</tr>
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</table>

Figure 5. Seasonal variation in prey availability for viperine snakes collected in areas M (squares) and E (circles). Rectangles in the top of the graph indicated the period when females with enlarged follicles or oviductal eggs were found in both populations.

Discussion

Mean clutch size showed geographic variation among populations (table 1) but differences occurred due to the range of clutch size found. Hence, the unusual upper limit for population of the area M (27 eggs, this work) and lower limit in Salamanca (9 eggs, Jaén, 1988) could have biased the mean clutch size in these populations with respect to the others. A general finding in all the studies (see table 1 for references) was the increasing clutch size with female body size. In the three populations studied here, body size consistently explained more than 60% of the variance in CS. When clutch size was compared using SVL as a covariate, the three populations did not differ. Despite differences in the CS range, the regression slopes of CS on SVL exhibited no geographic variation, either. In summary, there were no geographic differences in fecundity when data were scaled for female body size.

The proportion of females with enlarged follicles in the area M suggested an annual frequency of reproduction. This is a general finding in conspecific populations of *Natrix maura* throughout its range of distribution, such as Alicante (Hailey and Davies, 1987a), central and western France (Duguy and Saint Girons, 1966), the area G (Feriche and Pleguezuelos, 1999), the area E (Santos and Llorente, 2001), Salamanca (Jaén, 1988), and Sardinia (Rugiero et al., 2000). Female annual reproductive frequency has been also reported for populations of other *Natrix* species (Madsen, 1987; Luiselli et al., 1997; Luiselli and Zimmermann, 1997) with few exceptions (e.g. Luiselli and Zimmermann, 1997). Likewise, an annual frequency in female reproduction is a widespread characteristic in oviparous Mediterranean snakes (Agrimi and Luiselli, 1994; Capizzi et al., 1996; Feriche, 1998). In contrast, viviparous and some rainfall patterns (e.g. Madsen and Shine, 2000; Tierno and Luzón-Ortega, 2002).
oviparous species breed only every two or more years (Capula and Luiselli, 1994; Luiselli et al., 1996; Pleguezuelos and Feriche, 1998). The most plausible constraint to annual breeding is that many female snakes do not initiate reproduction until their body reserves exceed a threshold value (Naulleau and Bonnet, 1996; Bonnet et al., 2002). Two results reported in the present study reinforce this theory: 1) in the area M, comparisons in fat body mass (fig. 3) and condition (fig. 4) between adult non-reproductive and reproductive female viperine snakes collected in May suggest that the former did not attain the minimum fat stores to start vitellogenesis. This result has been previously observed in other snake species (Madsen and Shine, 1996, 1999; Naulleau and Bonnet, 1996) and it is indirect evidence of an energetic threshold for breeding in *Natrix maura*. This result has been previously observed in other snake species (Madsen and Shine, 1996, 1999; Naulleau and Bonnet, 1996) and it is indirect evidence of an energetic threshold for breeding in *Natrix maura*. 2) In the area E, all viperine snakes exhibited low condition and fat bodies in early spring (Santos and Llorente, 2001, 2004) because of the absence of available prey as a response of drastic fluctuations in the water level (Santos et al., 2000). Viperine snakes solved this trouble by delaying their reproductive period until females achieved enough reserves. Hence, females from the area E had smaller follicles in May and consequently bred later than did females from the other two populations. In support of this hypothesis, females from area M, only 50 km away from the area E population, showed a pattern more similar to that of a population located 500 km away in the area G (fig. 1). Delayed reproduction in Mediterranean populations of snakes has recently been reported by Filippi and Luiselli (2003) in Cocullo (central Italy). Local people collect non-venomous snakes for procession rituals in woodlands around this locality immediately before the onset of mating and vitellogenesis, and set them free by mid May after the rituals. Hence, human hunting prevents normal reproduction, resulting in a delayed timing. Filippi and Luiselli (2003) demonstrated that microclimatic differences did not explain this delay when reproductive seasonality was compared with respect to other Italian conspecific populations, this implying remarkable plasticity in Mediterranean snakes in response to human activity. In *Natrix maura*, variation among Iberian populations could not be explained by climatic differences, either. By contrast, food availability revealed the key to understanding geographic variation in reproductive timing. Aquatic environments in Mediterranean climates exhibit high productivity to ensure sufficient food for such predators as *Natrix maura*, thus enabling high snake densities (e.g. Hailey and Davies, 1987b). In early spring, fish and other aquatic prey are available for viperine snakes in natural habitats, and a significant portion of the female population is in good condition to initiate vitellogenesis. Furthermore, in these environments, it is not unusual to observe gravid females foraging for prey (unpub. data of the authors). However, the energetic constraint and disturbance caused by humans in the water dynamic of the area E for at least one hundred years (rice cultivation began in the area E towards the end of the XIX century), can induce organisms to modify reproductive timing in an evolutionary period short enough as one hundred years. Hence, delayed vitellogenesis appears to be an adaptive mechanism of *Natrix maura* to adjust its reproductive timing to prey-availability peaks, illustrating a new case of reproductive plasticity in snakes.

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