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DIFFERENCES IN SIZE AT BIRTH AND BROOD SIZE AMONG PORTUGUESE POPULATIONS OF THE FIRE SALAMANDER, SALAMANDRA SALAMANDRA

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> Size at birth and brood size were studied in ovoviviparous Salamandra salamandra gallaica and S. s. crespoi from areas differing in hydrological regime. Gravid females were maintained in open-air terraria until parturition was completed. Sizes of offspring at birth tended to be less variable in populations from mesic areas, and brood sizes (numbers of offspring) were larger in a population from a xeric site. Large sizes at birth, close to those observed in viviparous S. s. bernardezi, could be attributed not to cold climate, the risk of larval drift or short pond duration, but perhaps to competition or predation by conspecific larvae. Large and small larvae differed in time taken to reach metamorphosis, but not in size at metamorphosis. Females from the xeric site gave birth to largenumbers of small larvae, mainly in small groups and on separate occasions. In the wild, this probably results in the dispersal of a female's offspring among several ponds.

Key words: fecundity, larval size, Mediterranean climate, metamorphosis, Urodela

INTRODUCTION

Size at birth is an important component of an individual's life history, as small initial differences in size can become large differences in several life history traits later in life (Roff, 1992; Stearns, 1992). As resources available for reproduction are limited, the investment in large offspring should be counterbalanced by selection favouring the production of the greatest number of progeny. This compromise between quality and quantity of offspring is still one of the central ideas in theories concerning the evolution of size at birth (Smith & Fretwell, 1974; Steams, 1992).

Among other factors, climatic conditions and food availability may select for increased variability in size at birth. However, if the function relating offspring size to survival probability is strong and highly repeatable from year to year, a population-specific optimal size at birth (OSB) may be selected (McGinley, 1989; Shine, 1989; Steams, 1992). If an OSB does exist, most variation in reproductive output due to environmental conditions will be in clutch size rather than offspring size. Thus, some models predict that clutch mass will be related to clutch size, but not to offspring size (Roff, 1992; Roosenburg & Dunham, 1997).

Reproductive characteristics such as egg size, size at birth and clutch size are traditionally seen as highly variable within – as well as between – populations of amphibians (Kaplan & Cooper, 1984). Much of that variability is regarded as an adaptation to unpredictable environments, such as vernal ponds and streams – the major larval habitats for temperate-zone amphibians. Such factors as within-clutch variation in offspring size, platykurtic distributions of egg sizes, or the absence of an OSB, have been seen as an adaptation to unpredictability (Crump, 1981; Kaplan & Cooper, 1984).

Some factors linking urodele larval size to survival have already been identified: (1) Larval drift - as small larvae are more prone to drift (Bruce, 1985), larger sizes at birth should be favoured in running waters (and smaller sizes in still waters). For example, stream-breeding females of Ambystoma texanum tend to lay larger eggs than pond-breeding females (Petranka, 1984); in northern Spain, larvae of stream-breeding Salamandra salamandra bernardezi are considerably larger than those of pond-breeding Salamandra s. almanzoris (Thiesmeier, 1994). (2) Pond or stream duration - ultimately, this determines the amount of time available to grow and metamorphose. Given that age at metamorphosis is usually inversely related to size at birth (Semlitsch, 1987; Rowe & Ludwig, 1991), the shorter the pond duration, the larger the neonates should be. For example, Ambystoma maculatum populations that lay in temporary ponds produce larger eggs than those that lay in permanent ponds (Woodward, 1982).

The fire salamander, Salamandra salamandra (L.), is a long-lived, iteroparous urodele, widespread in western and southern Europe (Thorn, 1968). Some subspecies or populations, usually from mountain habitats, reproduce biennially and give birth to fully metamorphosed juvewhile lowland subspecies are usually niles. ovoviviparous and reproduce annually, giving birth to aquatic larvae at different stages of development (Joly et al., 1994). In the Iberian Peninsula, fire salamanders occur from sea level to altitudes of more than 2000 m and from wet temperate forests to arid scrubland and steppe (Pleguezuelos, 1997). South of the river Tejo, the species withstands typical Mediterranean conditions at one of the dry limits of its distribution. The subspecies occurring in Portugal, Salamandra salamandra gallaica and S. s. crespoi (Fig. 1), are ovoviviparous, giving

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FIG. 1. Study areas: Gerês (1), Sintra (2), Monchique (3), and Grândola (4). Shaded area, *Salamandra salamandra gallaica* (adapted from Thorn, 1968); stippled area, *S. salamandra crespoi* (adapted from Malkmus, 1983).

birth to well-developed larvae in temporary ponds or streams between November and April. Reproduction is probably annual, as in southern France (Joly *et al.*, 1994). Larvae are born inside the transparent egg capsule and hatch immediately after contact with water (R. Rebelo, *pers. obs.*). The larval period may vary from one to six months (Barbadillo, 1987). Studies addressing the reproductive traits of *S. salamandra* in Iberia have focused on the viviparous subspecies, *S. s. bernardezi* and *S. s. fastuosa* (Dopazo & Alberch, 1994; Thiesmeier *et al.*, 1994).

We studied four populations of the fire salamander living in regions with marked differences in hydrological regimes. Our aim was to examine the habitat influences on size at birth. We investigated the following: (1) the influence of maternal body size, season and year on size at birth; (2) the trade-off, if any, between brood size and neonate size; (3) the existence of an optimal size at birth; (4) the relationship between size at birth and both size at metamorphosis and length of larval period.

STUDY AREAS AND METHODS

We sampled four areas arranged along a north-south axis (Fig. 1), which encompass much of the environmental variability withstood by the subspecies S. s. gallaica and S. s. crespoi. The extent and altitude of the mountain ranges on which the study areas are located decrease from area 1 to area 4: area 1 is subjected to more mesic conditions, while xeric conditions become dominant in area 4 (Table 1). Area 1 (Gerês) is a climax deciduous oak forest (mainly Quercus robur and Q. pyrenaica) on Gerês Mountain. Area 2 (Sintra) is on a plateau covered with introduced Acacia sp. Area 3, Monchique, is the highest mountain in southern Portugal, constituting an ecological island of mesic, wet climate in an otherwise Mediterranean, flat and dry region, as typified by area 4. In this area, salamanders were caught in valleys covered with O. canariensis, O. suber and introduced Acacia sp. Area 4 (Grândola), is a flat area located at the base of

TABLE 1. Physical and climatic parameters of the study areas. Data were obtained from the "Atlas do Ambiente" (C. N. A., 1983): Pond/stream duration from 1992/93 to 1997/98 as follows: pond filling - pond drying month (no. months with water). No visits were made during 1996/97.

	Gerês (1)	Sintra (2)	Monchique (3)	Grândola (4)
Altitude (m)	550	450	500	100
Maximum altitude (m)	1508	490	902	326
Slope (%)	>35	8-15	15-25	4-8
Mean annual temperature (°C)	7.5/10	12.5/15	15	17
Mean annual precipitation (mm)	>2800	1000/1200	1000/1200	600/800
Pond/stream duration 1992/93	Oct-May (8)	Oct-Mar (6)	Oct-Mar (6)	-
Pond/stream duration 1993/94	Oct-Apr (7)	Nov-Mar (5)	Nov-Feb (4)	Nov-Jan (3)
Pond/stream duration 1994/95	Oct-Apr (7)	Nov-Mar (5)	Nov-Mar (5)	Nov-Mar (5)
Pond/stream duration 1995/96	Oct-May (8)	Nov-Mar (5)	Nov-Mar (5)	Nov-Feb (4)
Pond/stream duration 1997/98	no data	Nov-Mar (5)	no data	Nov-Jan (3)

the Grândola Hills, covered with an open forest of cork oak (*Q. suber*).

As in other Mediterranean regions, the climate in Portugal is characterized by long, hot summers, during which almost all ponds and streams dry up. The first rains, usually in October, mark the beginning of the activity period for the fire salamander, which extends until April/May. From 1992 to 1996, the study areas were visited at least once a month during the salamander activity period. As part of another study, Sintra and Grândola were also visited until 1998. Ponds and streams were checked first for the presence of water and then for larvae or pre-metamorphs (characterized by the typical adult yellow-and-black pattern, together with reducted gills).

Gravid females were found crossing unpaved forest roads. Captures were made in early autumn (October), before the filling of ponds or streams. Gerês (1) was sampled in 1994/95, Sintra (2) in 1994/95 and 1995/96, and Monchique (3) and Grândola (4) in 1995/96. Altogether, 64 females were caught at the beginning of the rainy season. During 1995/96, 18 additional females from Sintra and Grândola were caught during the monthly visits, well after the beginning of the rains. Females were measured (snout-vent length - SVL), weighed and photographed dorsally in order to allow subsequent individual recognition. We also collected females that were found dead during the autumnal visits - mostly animals that had been run over by vehicles on paved roads. In the dead animals, we counted the number of ovarian follicles larger than 2 mm diameter (Greven & Guex, 1994)-roughly corresponding to stages V and VI of oocyte maturation, already described for another fire salamander subspecies, S. s. infraimmaculata (Sharon, et al., 2000).

The live females were kept at the Faculty of Sciences, Lisbon, in individual, open-air terraria $(50 \times 30 \times 30 \text{ cm})$ provided with small pools. Food (Tenebrio molitor larvae and earthworms) was provided ad libitum, as the parturition period can last up to one month. Terraria were checked every morning for larvae, which were measured to the nearest mm (total length and SVL), weighed to the nearest mg, and moved to a different container (one container per brood). Most of the data concern the larvae produced by the 64 females maintained from the beginning of the rainy season. For these animals it was possible to calculate relative brood mass (RBM), i.e. brood mass divided by female initial mass. Late-season females from Sintra and Grândola also gave birth to some larvae, which were considered as partial broods and used for some analyses if at least two larvae were obtained (this is the reason for some differences in sample sizes). Nine females were killed after their residence in the terraria, and their uterine contents were examined. Larvae and females were released at the place of capture at the end of the study.

In order to assess the effect of size at birth on length of the larval period and size at metamorphosis, seven similar-sized larvae from each of five broods from Sintra and four broods from Grândola were raised at the bioterium of the Faculty of Sciences. Larvae were kept under natural photoperiod in individual, 250 ml containers filled with pond water. Water temperatures fluctuated daily at 20 ± 2 °C. Every other day, water was changed and larvae were fed *ad libitum* with minced beef. At the completion of metamorphosis, individuals were measured and released. Due to the long larval periods, the experiment was concluded for each brood when four of the initial seven larvae completed metamorphosis. Larval period was recorded from the date of birth to that of metamorphosis. Mean growth rate during the larval period was calculated as [(size at metamorphosis – size at birth)/ larval period].

STATISTICAL ANALYSES

To avoid statistical non-independence of the data from siblings, we considered each brood as the sampling unit and used mean values for each trait within each brood.

The Chi-square test was used to compare absolute frequencies. After checking for normality and homogeneity of variances (Levene's test), a *t*-test or one-way ANOVA was used for the majority of comparisons; the Tukey HSD for unequal *N* was the *a posteriori* test used. Whenever ANOVA assumptions were not met, a nonparametric test was used (Kruskal-Wallis ANOVA). Correlations among variables were determined with Pearson's product-moment correlation coefficient. For correlations involving temporal parameters, the first of November was chosen as day 1, as no larvae were born before that day. Analyses were performed with STATISTICA for Windows, Release 5.0 (StatSoft, Inc. 1995).

RESULTS

STUDY AREAS

Gerês is crossed by many rapid streams, owing to the steep slopes and abundant rainfall. Larvae were found mainly in the quieter sections of the streams, but also in some temporary ponds. This was the only area that maintained free water in small streams during all the year. In Sintra and Monchique, the few streams dried quickly and larvae were mostly found in springs and wells, and in the water of underground mines. Ponds and slow-moving streams were abundant during the rainy season in Grândola, but dried quickly during the spring (with considerable annual variation). In two of the study years (1993/94 and 1997/98), the lack of January rains led to the early drying of all the water bodies and very few larvae were observed after the spring rains in March. Considering all the study years, and as would be expected, pond duration decreased from area 1 to area 4, while between-year variation increased (Table 1). Except for two single cases in Gerês, no larvae were found after May.

	Gerês (1)	Sintra (2)	Monchique (3)	Grândola (4)
Female SVL (mm)	96.16±6.27 ^a (86.5-107.5) <i>n</i> =24	101.91±7.73 ^{ac} (91.1-112.75) n=24	107.11±7.54 ^{bc} (92.4-116) <i>n</i> =17	111.97±10.31 ^b (93.5-128.4) <i>n</i> =11
Relative brood mass (%)	0.168±0.064 ^a *	0.159±0.049 ^a *	0.195±0.058	0.247±0.088 ^b
	(0.078-0.318)	(0.086-0.247)	(0.114-0.282)	(0.170-0.416)
	<i>n</i> =13	<i>n</i> =15	<i>n</i> =10	<i>n</i> =6
No. of parturition nights	3	2	3	8
	(1-7)	(1-10)	(2-7)	(2-12)
	<i>n</i> =1 1	<i>n</i> =14	<i>n</i> =9	<i>n</i> =5
Parturition period (days)	5	8	7	19
	(1-24)	(1-28)	(5-18)	(2-43)
	<i>n</i> =11	<i>n</i> =14	<i>n</i> =9	<i>n</i> =5

TABLE 2. Reproductive characteristics of female salamanders. Data in each case are: top row, mean \pm standard deviation, except for the number of parturition episodes and parturition period, where the median is presented; middle row, range; bottom row, sample size. P<0.05; P<0.01; P<0.01; P<0.001. Values in the same row with different superscripts are significantly different.

FEMALE CHARACTERISTICS

Female body size differed among the populations $(F_{3.72}=12.75, P<0.001)$, increasing from area 1 to area 4 (Table 2). Some females did not give birth to any larvae (1, 0, 3 and 2 females from areas 1 to 4, respectively), while others (4, 2, 6 and 2 females, respectively) produced only 1 to 5 larvae. The proportion of such females did not differ among populations (χ^2 =5.76, df=3, P>0.05) and they were excluded from some analyses.

Two out of the nine females that were killed at the end of the study had not given birth to any larvae, and their uteri were empty. Of the remaining seven females, five had empty uteri and the remaining two still had some larvae (1 and 4, respectively) in the uteri; the unborn larvae were smaller than the larvae that had been born previously.

There were significant differences in relative brood mass (RBM) among the populations ($F_{3,40}$ =3.10, P< 0.05), salamanders at Gerês and Sintra had smaller RBMs than those at Grândola (Table 2). There was no statistically significant correlation between RBM and female SVL at any site.

When giving birth, different strategies were employed by different females, even within each population: e.g. releasing the entire brood in 2-3 nights within one week and releasing just a few larvae at a time, over more than one month. Overall, both number of parturition episodes and parturition period were highest among salamanders from Grândola (Table 2); however, differences among populations were not statistically significant (Kruskal-Wallis tests: $H_{3,39}$ =5.56, *P*=0.13 for number of parturition nights; $H_{3,39}$ =5.43, *P*=0.14 for parturition period). Neither of these traits was correlated with either brood mass or brood size in any of the populations.

BROODS AND LARVAE

Log-transformed brood size and ovarian follicle counts differed among populations ($F_{3,40}$ =22.32, P<0.001; $F_{3,28}$ =8.76, P<0.01 respectively), and both were significantly larger in Grândola salamanders (Table 3). The smallest follicle counts and the smallest brood sizes were found at Sintra. In no case was logtransformed brood size correlated significantly with female SVL.

In total, 2205 larvae were born in captivity. Larval length and mass at birth were strongly correlated (r=0.907, P<0.0001). Within-brood larval size variation was small, regardless of the population (Table 3). In the majority of broods, whatever the population, larval body mass was normally distributed. In a few broods the distribution was leptokurtic (Table 3); the number of such broods did not differ among sites ($\chi^2=5.38$, df=7, P<0.61).



FIG. 2. Mean larval body mass at birth as a function of female body size in *Salamandra salamandra gallaica* and *S. crespoi* at four sites in Portugal: Gerês (1), Sintra (2), Monchique (3) and Grândola (4). Data concern all broods (complete and partial). Symbols as in Fig. 1.



FIG. 3. The relationship between mean larval body mass at birth and brood size in *Salamandra salamandra gallaica* and *S. salamandra crespoi* at four sites in Portugal: Gerês (1), Sintra (2), Monchique (3), Grândola (4).Symbols as in Fig. 1.

There were significant between-population differences in larval body mass ($F_{3.63}$ =8.76, P<0.001) and larval length ($F_{3.63}$ =18.32, P<0.001). Salamanders from Sintra had the largest larvae, while very small larvae were mainly found in salamanders from Gerês and Grândola (Table 3). None of the within-population correlations between mean larval mass and female SVL was significant (Fig. 2). A negative correlation between brood size and mean larval mass was found only at Monchique (r=0.620, n=10, P<0.05; Fig. 3).

To search for evidence of population-specific optimal size at birth, we compared the coefficients of variation for both brood size and larval size at birth, and looked for positive correlations between brood mass and brood size. For the northern populations (1 and 2), brood size varied almost twice as much as larval size at

TABLE 3. Brood and larval characteristics: top row, mean \pm standard deviation; middle row, range; bottom row, sample size. * P<0.05; " P<0.01; " P<0.001; NS, not significant. Sample sizes differ whenever data from partial broods were included and for wild SVL at metamorphosis. Values in the same row with different superscripts are significantly different.

	Gerês (1)	Sintra (2)	Monchique (3)	Grândola (4)
Brood size	33.5±9.7 ^a	26.5±7.2 ^a	35.7±9.4 ª	74.0±5.5 ^b ····
	(19-57)	(14-35)	(24-51)	(66-80)
	<i>n</i> =13	<i>n</i> =15	<i>n</i> =10	<i>n</i> =6
No. of ovarian follicles	52.1±11.5 ^a	33.5±12.1 ^a	50.0±11.6 °	89.6±43.3 ^b ***
	(37-75)	(18-53)	(34-62)	(43-143)
	<i>n</i> =11	<i>n</i> =9	<i>n</i> =7	<i>n</i> =5
Larval total length at birth (mm)	30.3±2.1 ^a	34.2±1.6 ^{b***}	31.3±2.7ª	29.3±2.6 ^a
	(25-31.96)	(31-35.5)	(27-38)	(23-32.4)
	<i>n</i> =15	<i>n</i> =23	<i>n</i> =13	<i>n</i> =16
Larval mass at birth (mg)	167±22 ^a	263±41 ^{b***}	198 ± 51^{a}	164± 34 °
	(120-190)	(164-337)	(143-331)	(105-238)
	<i>n</i> =15	<i>n</i> =23	n=13	n=16
Within-brood coefficient of variation of larval mass at birth	0.11±0.02	0.12±0.05	0.09 ± 0.03	0.10±0.03
	(0.06-0.15)	(0.05-0.17)	(0.06-0.12)	(0.08-0.16)
	<i>n</i> =13	<i>n</i> =15	n=10	<i>n</i> =6
Among-broods coefficient of variation of larval mass	13.17	15.59	25.76	20.73
	n=15	<i>n</i> =23	<i>n</i> =13	<i>n</i> =16
Coefficient of variation of brood size	28.92	27.05	26.41	7.5
	n=13	<i>n</i> =15	<i>n</i> =10	n=6
Correlation (r) between brood size and brood mass	0.85***	0.84***	0.37 NS	0.06 NS
No. of leptokurtic broods	1	3	3	4
	<i>n</i> =13	n=15	<i>n</i> =10	<i>n</i> =6
SVL at metamorphosis in the wild (mm)	25.2±6.5	25.7±2.0	27.2±2.9	26.5±1.8
	(15-37)	(22-28)	(23-32)	(24-30)
	<i>n</i> =21	<i>n</i> =23	<i>n</i> =13	<i>n</i> =22



FIG. 4. Mean larval mass at birth as a function of the parturition date of the first larva of each brood (day 1 = 1 November). Data were collected at Sintra (2) in 1994/95 (filled squares); and in 1995/96 (open squares), and at Grândola (4) in 1995-96 (lozenges).

birth and brood mass was correlated with brood size; for the southern populations (3 and 4), larval size at birth varied as much as, or even more than, brood size and there was no correlation between brood mass and brood size (Table 3).

SEASONAL AND ANNUAL VARIATION

Female SVL was not correlated with date of parturition in any area. Also, there was no significant correlation between mean larval mass and date of parturition, or between the coefficient of variation of larval mass and date of parturition, for any population (Fig. 4). Mean larval mass at Sintra did not differ between 1994/ 95 and 1995/96 (258±40 mg and 272±42 mg, respectively; t_{21} =-0.763, P=0.45; Fig. 4).

METAMORPHOSIS

The largest and smallest sizes at metamorphosis in the wild were observed at Gerês (Table 3). However, the metamorphic size range was generally narrow, and overall no significant difference was found among the populations (Kruskal-Wallis test, $H_{3,85}$ =2.35, P=0.50).

Larvae from Sintra and Grândola were reared in the laboratory and the results of the experiment are depicted in Table 4. Despite the significant size differences at the beginning of the experiment ($t_2=7.13$, P<0.001), SVL at metamorphosis was not different between the two populations $(t_2 = 0.826, P > 0.05)$. However, there was a very significant difference in length of larval period for the first larva to metamorphose (t_2 =-6.61, P<0.001), as well as for the fourth (t_2 =-8.4, P<0.001). Overall, there were no population differences in the mean daily growth rates $(t_2=0.916, P=0.11)$. Wild and laboratory-reared metamorphs from Sintra were similar in size $(t_{12}=0.465,$ P=0.65), but laboratory-reared animals from Grândola were somewhat smaller than wild metamorphs $(24.5\pm1.17 \text{ versus } 26.5\pm1.77, \text{ respectively; } t_{24}=2.151,$ P=0.046).

DISCUSSION

Maintaining animals in captivity until the completion of reproduction is time- and space-consuming, but is a non-invasive method for determining clutch size. This method has already been used in some urodeles (e.g. Baker, 1992), including other subspecies of the fire salamander (Dopazo & Alberch, 1994; Degani & Warburg, 1995). However, one must be sure that animals were caught at the beginning of the reproductive period. In fact, the small broods found for some animals may indicate that they had already begun giving birth when

TABLE 4. Results of the larval rearing experiment. Seven larvae from each of five broods from Sintra (2) and four broods from Grândola (4) were reared. The experiment was concluded with the metamorphosis of the fourth larva from each brood. Length of the larval period is provided for the first and fourth l. *** P < 0.001.

Broods	Mean SVL at birth (mm) (<i>n</i> =7)	Mean SVL at metamorphosis (mm) (n=4)	Larval period –1 st larva (days)	Larval period – 4 th larva (days)	Mean growth rate (µm/day) (n=4)
Sintra (2)					
1	21.71	26	50	71	65.5
2	20.91	23.75	35	69	53.4
3	20.37	23.75	49	61	60.5
4	19.85	26.5	51	89	104.8
5	19.38	26.25	67	93	81.2
Mean \pm SD	20.4±0.91***	25.3±1.38	50.4±11.35***	76.6±13.74***	73.1±20.46
Grândola (4)					
1	17.48	26	121	140	61.4
2	15.74	24.75	92	157	73.6
3	15.6	23.25	136	149	53.4
4	15.81	24	114	135	63.6
$Mean \pm SD$	16.2±0.89***	24.5±1.17	115.7±18.3***	142.3±9.74***	63.0±8.32

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captured (and this is more probable for Gerês, as it is the only area that maintains some free water throughout the year). It is also possible that not all females reproduce every year, as about 10% of the whole sample did not bear any larvae.

Reproduction in the fire salamander is notoriously variable. This may be explained by subspecific differences, and also by inter-population variations within subspecies (Dopazo & Alberch, 1994; Veith, 1994). The number and distribution of fire salamander subspecies in the Iberian Peninsula is still an unresolved question. Recent studies agree on some degree of genetic distinctiveness for south-western populations, including S. s. crespoi, but not for the population from Grândola (García-Paris et al., 1998; Steinfarz et al, 2000). However, reproductive traits from the Monchique population were not particularly different from those of S. s. gallaica. Overall, values of RBM, brood size, size at birth, number of parturition episodes and parturition period of the studied populations were not dissimilar to those of other ovoviviparous populations of the fire salamander from central Europe (Thiesmeier, 1992; Thiesmeier, 1994), the Iberian Peninsula (Barbadillo, 1987; Lizana et al., 1989) and Israel (Warburg, 1992; Degani & Warburg, 1995). There were some exceptions, particularly at Grandola, where brood sizes, RBM, parturition period and number of parturition episodes were relatively large. Larger brood sizes (up to 192) have been reported only in S. s. infraimmaculata in Israel (Degani & Warburg, 1995). Large sizes at birth at Sintra were also an exception, slightly overlapping those of the viviparous S. s. bernardezi (Thiesmeier et al., 1994).

Differences in reproductive traits may be related to population differences in female body size. Positive correlations between female mass and brood size or larval size were found for *S. s. terrestris* (Thiesmeier, 1992). However, we found no significant within-population correlations between brood characteristics and female traits. In small samples of long-lived, iteroparous animals, such correlations – if they do exist – may be masked by confounding age- or year-specific factors (Kaplan & Salthe, 1979). Female *S. s. gallaica* and *S. s. crespoi* begin reproducing at four years old and may live up to 18 and 12 years, respectively (Rebelo & Caetano, 1997).

The four populations can be arranged from those that invest more in many small larvae to those that invest less in few, large larvae (Fig. 3). When females from populations with different optimal sizes at birth (OSB) allocate the same energy to reproduction (like those from Sintra and Gerês), differences in brood size may emerge, with smaller OSB allowing the production of larger broods, and vice/versa (Roff, 1992).

Metamorphic size was virtually the same in our populations, whether for wild or laboratory-reared larvae. Differences in size at birth are thus probably related to selection acting on larvae in the aquatic environment. Gerês is the only area where strong negative effects of larval drift on survival could be expected, yet this is one of the sites where larval sizes at birth were smaller. Short pond durations could account for the larger larvae from Sintra, but do not explain the smaller larval sizes from Monchique, and especially from Grândola.

Larvae from Sintra were consistently born with a size close to that of viviparous populations from considerably colder climates in northern Spain. There are two historical records of viviparity for S. s. gallaica, both from populations located near Coimbra, roughly halfway between Sintra and Gerês (Hillenius, 1996), but this is not a common phenomenon. The Sintra population has some peculiar demographical traits, such as a high proportion of old animals (more than 10 and up to 21 years old) (Rebelo & Caetano, 1997), and a high adult density (from 170 to 500 individuals/ ha) (R. Rebelo, unpubl. data). Smaller broods of larger juveniles have been recorded for old individuals in a number of salamander species (Kaplan & Salthe, 1979). Furthermore, larval fire salamanders are cannibalistic and body size, largely through allometric effects on gape size, determines the possibility of cannibalizing other larvae (Warburg, 1992; Reques & Tejedo, 1996). Sibling cannibalism was observed within all the broods that were kept in captivity. So, perhaps the adoption of large birth sizes is the result of selection for large larvae, able to avoid predation by conspecifics in crowded ponds.

In the laboratory, length of the larval period varied much more than size at metamorphosis, even within each brood, and metamorph sizes were very close to those found in the wild, which suggests that metamorphosis in S. s. gallaica is probably more size-determined than age-determined. An initial size difference of half a centimetre corresponded to a two month difference in length of the larval period, but no differences in mean daily growth rate or size at metamorphosis, which is an indication that a small size at birth probably does result in a longer larval period. Given the generally short and unpredictable duration of ponds at Grândola, the striking absence of large birth sizes in this population is puzzling. Here, an interesting parallel may be established between Iberian and Israeli fire salamander populations, which represent the western and eastern limits of the species' distribution. In both cases a large adult body size and the birth of large broods of relatively small larvae were found to occur in dry climates where catastrophic mortality of the larvae due to pond drying occurs frequently. Similarly, in both cases smaller body sizes and smaller broods were found in nearby mesic areas (Degani & Warburg, 1995; this study). For the Israeli populations, Degani & Warburg (1980) found that salamanders from xeric habitats migrate yearly to winter ponds, while those from mesic habitats remain all year near the water sources. This study has found considerably extended parturition periods in Grândola. This may be due to sequential development of several egg cohorts (Dopazo & Alberch, 1994; Sharon et al., 2000). Nevertheless, if females in xeric habitats are mobile and visit different places on different nights, numerous small larvae may be dispersed among several ponds, which may be a good strategy in areas where such places are often abundant but of unpredictable duration. Similarly, female *Ambystoma annulatum* do not deposit a large proportion of the clutch in one specific location (Hutcherson *et al.*, 1989).

Salamandra s. infraimmaculata, the Middle Eastern subspecies of the fire salamander, was recently proposed as a distinct species, apparently having separated from S. salamandra between 5 and 13 million years ago (Steinfarz *et al.*, 2000), yet the same responses to mesic and xeric climates persist. Other examples of parallel evolution of reproductive traits are known for the genus Salamandra. For example, a viviparous mode of reproduction may have evolved separately at least three times (Steinfarz *et al.*, 2000; Veith, 1994), as an adaptation to high mountain habitats.

Ovoviviparity probably evolved in the fire salamander to counteract larval drift in permanent streams, the main larval habitat in central-European forests (Thiesmeier, 1994; Baumgartner et al., 1999). If that is the case, a return to pond-breeding habits occurred in some marginal habitats, such as mountain plateaus and the Mediterranean ecosystems. The populations we studied, where (with one exception) pond breeding occurs more frequently, still maintain some stream-associated traits - intra-brood coefficients of variation of larval size were very similar, all the broods had normal or leptokurtic size-distributions and there were characteristics consistent with an OSB in some populations. The unpredictable environment of the Mediterranean habitats may be the reason for the greater brood sizes, greater relative brood masses and absence of an OSB in the southern populations.

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