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EGG DEPOSITION STRATEGIES OF THE SMOOTH NEWT (*TRITURUS VULGARIS*) IN AN UNPREDICTABLE ENVIRONMENT

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Studies carried out in England on the reproduction of smooth newts have revealed that under relatively constant, favourable conditions, they spend a period of up to six months in the water, with oviposition lasting about 4-6 weeks. The reproductive strategies of two Romanian smooth newt populations inhabiting unpredictable, highly variable and hostile environments were analysed for comparison. Females in both populations had an average body weight at the beginning of the reproductive period of 1.09 and 0.96 g respectively, one third of the average body weight of adult females in England. The average snout-vent lengths were 34.9 and 33.8 mm, about 25% shorter than those from English populations. The average numbers of eggs deposited by female newts from the two populations were 74 and 51 respectively, compared to an average of 300 eggs in England. The average age of females from one of the populations studied was 4.2 years and age at first reproduction was estimated at three years, similar to another English population studied. At the end of the oviposition period females still contained yolked oocytes, suggesting that clutch size cannot be correctly estimated by counting the initial numbers of yolked ovarian oocytes. Their reproductive effort was reduced since, due to environmental hostility, body size was significantly diminished and this leads to a smaller clutch size.

Key words: *Triturus*, newts, reproductive strategies, variable environments

INTRODUCTION

Female newts of the genus *Triturus* deposit eggs individually over several weeks. Four to ten days after insemination (Diaz-Paniagua, 1989; Pecio, 1992), the females start depositing the eggs fertilized with the sperm stored in a special organ, the spermatheca (Sever, 1994). The oviposition period lasts from several days up to three months (Verrell, Halliday & Griffiths, 1986), during which time the female feeds actively and may be inseminated by several different males (Pecio, 1992; Gabor & Halliday, 1997).

Large differences between the number of eggs in the ovaries and the number of deposited eggs, and between the fertility estimates, have been reported by authors studying populations in different countries (Hagström, 1980; Baker, 1992). Estimates vary between a minimum of 25-80 eggs deposited (Verrell, 1986), up to 637 eggs (Baker, 1992), while oocyte counts in the ovaries can reach an average of 1000, ranging between 581-1573 (Hagström, 1980).

Studies carried out in England on the reproduction of smooth newts (*Triturus vulgaris*) have revealed that under relatively constant, favourable conditions, they spend a period of up to six months in water, with oviposition lasting on average 4-6 weeks (Verrell & Halliday, 1985; Verrell, Halliday & Griffiths, 1986; Baker, 1992). These results contrast with observations made in other European countries (Accordi, Massarek & Nobili, 1990; Fasola & Canova, 1992; Pecio, 1992; Kalezic, Cvetkovic, Djorovic & Dzukic, 1996) indicating that newts tend to spend shorter periods in water. I investigated the reproductive strategies of two Romanian smooth newt populations (*T. v. vulgaris*) inhabiting unpredictable, highly variable environments.

I wanted to test whether (1) the parameters describing the rate of oviposition are similar to the ones reported by Baker (1992) for an English smooth newt population; (2) the predicted correlation between body size and clutch size is valid for the studied populations; (3) ovarian oocyte numbers are reliable estimates of clutch size; and (4) clutch size and oviposition period are correlated.

MATERIALS AND METHODS

The two Romanian smooth newt populations studied, are exposed to different degrees of environmental stress. The first population studied inhabits a pond several hundred square metres in area, with a maximum water depth of less than 1 m. The pond is located in a forest consisting mainly of oak and lime trees (Baneasa forest, north of Bucharest). The pond is subjected to prolonged drought and lasts in spring for only four to six weeks. Other amphibian species reproducing in the pond are *Triturus cristatus, Rana dalmatina, Pelobates fuscus*, and *Hyla arborea.* Due to the brief hydroperiod, there are years when amphibians cannot reproduce, while in other years only *Rana dalmatina* - an early breeder - achieves metamorphosis. Only in rainy years are newt and spadefoot toad (*Pelobates fuscus*) larvae able to reach metamorphosis. The second population studied inhabits a temporarily flooded area on an island in the lower Danube floodplain (latitude 44°47'52", longitude 27°49'05"), frequently subjected to flooding

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TABLE 1. The main parameters (mean, standard deviation, and range) describing egg deposition in two smooth newt populations from Romania, compared with the population studied by Baker (1992) in England.

Locality	Number of eggs deposited	Oviposition period (days)	Female initial weight (g)	Female SVL (mm)	Average rate of oviposition (eggs/day)	Maximum no. eggs deposited per day
Banesa Forest	Mean=51.2	Mean=11.9	Mean=0.96	Mean=33.8	Mean=5.51	24
n=22	SD=30.8	SD=8.9	SD=0.1	SD=2.72		
	Range 15-105	Range 2-33	Range 0.7-1.4	Range 30.2-38.4	Range 1.7-17	
Danube	Mean=74.6	Mean=12.3	Mean=1.09	Mean=34.9	Mean=5.85	37
floodplain	SD=61.4	SD=6.4	SD=0.24	SD=2.34		
n=13	Range 13-195	Range 1-21	Range 0.76-1.64	Range 28-39.8	Range 1.6-10.6	
England	Mean=300	Mean=36.9	Mean=2.7	Mean=45.9	Mean=8.7	54
(Baker, 1992)	SD=189	SD=19.7	SD=0.8	SD=3.81		
n=10	Range 88-637	Range 11-74	Range 1.6-4.2	Range 40-52	Range 3.9-17.3	

(see Cogalniceanu, Cristofor & Vadineanu, 1997 for a detailed description of the site).

The Baneasa forest population was visited in 1996, four days after a warm spring rain had melted most of the remaining snow. Also present were large numbers of actively breeding *Pelobates fuscus*, *Triturus cristatus,* and freshly laid *Rana dalmatina* spawn. The floodplain population was visited in 1995, shortly after severe spring floods had covered large parts of the island and had filled the pond. In both populations, smooth newt pairs in courtship were frequently observed.

Twenty-two females from Baneasa forest and 17 from the floodplain population were captured and kept individually in aquaria. Identical experimental conditions were provided during both years. Newts show a high individual variability in their response to the stress induced by captivity. Captivity-induced stress is easily detectable because animals fail to feed and emaciate rapidly. Care was taken to avoid unnecessary stress that can induce the loss of reproductive condition.

The body weight of females was measured at the beginning and end of the experiment on an electronic Gibertini balance with a precision of 0.01 g. Snout-vent length (SVL) was measured with dial callipers at the beginning of the breeding season with a precision of 0.1 mm. Captive females were fed every other day with *Tubifex* sp. Plastic strips were provided for egg deposition, and the eggs were collected and counted daily according to Arntzen & Hedlund (1990). Males were added to each aquarium for at least two days/week to ensure that females were inseminated (Pecio, 1992).

Four females from the Danube floodplain population were sacrificed immediately after capture, before oviposition started (control group). Six of the remaining thirteen females oviposited for less than two weeks on average. After a week without any further eggs being laid they were also sacrificed (group 1). The remaining seven females oviposited for almost a month and were sacrificed after a week without deposition. Females were first anaesthetized in MS-222 (Sandoz), and then sacrificed. The ovaries were dissected, stored in Gilson's solution (Montori, 1989) and the remaining oocytes counted. Yolked oocytes with a diameter larger than one mm were counted as mature. The hind limb was removed and stored in alcohol. Age was assessed by skeletochronology following Miaud (1991).

RESULTS

Females in the two populations had, at the beginning of the reproductive period, body weights averaging 1.09 and 0.96 g respectively, representing about one third of the average body weight of adult females in England. Their average SVL's were 34.9 and 33.8 mm respectively, about 75% of the SVL of females in England. Both initial body weight and SVL of females did not differ significantly between the two populations (ANOVA: F=3.70, P>0.05, df=2,33). The average numbers of eggs deposited were low, 74 and 51 respectively, but differences were not significant (ANOVA: F=3.46, P=0.07, df=2,33). The oviposition rates and the maximum number of eggs deposited in a day were also lower (Table 1; Fig. 1). Oviposition rates did not differ significantly between the two populations (ANOVA: F=0.34, P>0.5, df=2,33).

Females from the Danube floodplain population decreased in body weight on average by 0.31±0.05 g

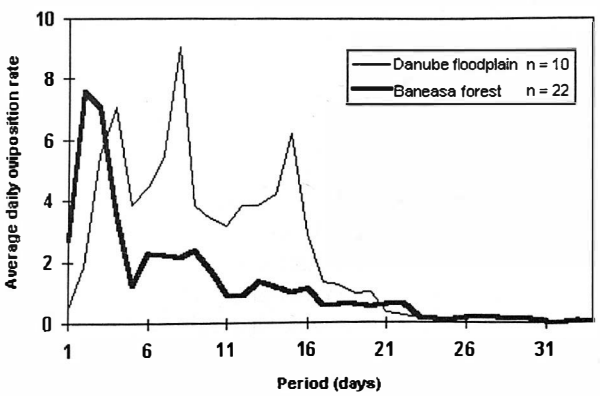

FIG. 1. Mean daily egg deposition rate of female smooth newts.

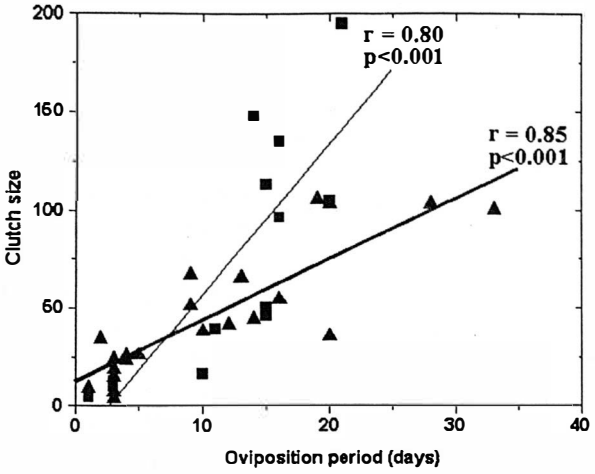

FIG. 2. Relationships between oviposition period and clutch size. Bold line, triangles, Banesa Forest (n=22); thin line, squares, Danube floodplain (n=13).

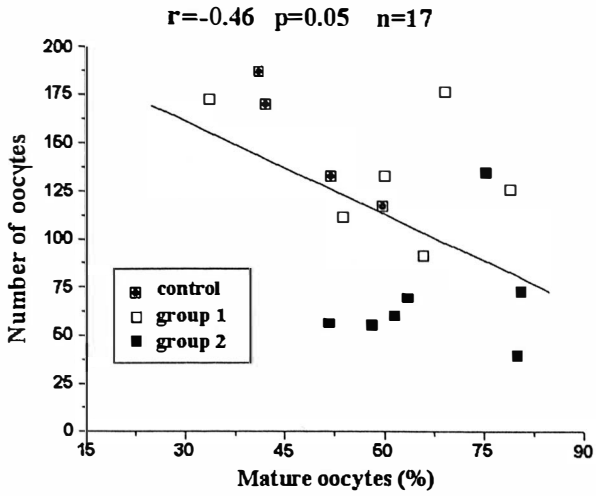

FIG. 3. Relationship between the total no. of ovarian oocytes present at the end of the experiment and the percentage of mature oocytes in the three groups from the Danube floodplain population. See text for details.

(ranging between 0.09 and 0.75, n=13) during the oviposition period, corresponding to an average body weight loss of 26.8% (ranging between 11.2 and 45.3%). Both weight loss and percentage weight loss were positively correlated with clutch size (r=0.63, P=0.02, n=13 and r=0.69, P=0.009, n=13 respectively).

Clutch size and oviposition period were significantly related in both Romanian populations studied, but the slopes of the regression lines differed significantly (t=2.59, df=33, P<0.01; Fig. 2).

The number of remaining ovarian oocytes was negatively correlated with clutch size in the Danube floodplain population (r=-0.79, P=0.001, n=13), suggesting that the number of ovarian oocytes before reproduction should only be used to estimate potential clutch size. They do not represent actual clutch size, since not all oocytes are deposited.

The females from the Danube floodplain population sacrificed at the beginning of reproduction (control group), had on average 151 oocytes in their ovaries. The females that oviposited for only a few days and were sacrificed ten days later (group 1) had on average 135 oocytes left, while the females which oviposited for almost a month (group 2) had on average only 70 oocytes left. The differences between the means of the three groups were significant (ANOVA: F=10.54, P=0.0016, df=2,16). A Tukey-Kramer multiple comparison test showed that the differences between the means were not significant for the control group and group one, but were highly significant for group 1 and group 2 (P<0.01), and for the control group and group 2 (P<0.01).

After summing the number of eggs deposited and the number of oocytes left in the ovaries, similar values were obtained for all three groups (mean±SE; control group 151.5±16.2; group 1 157.1±14.9; group 2 189.2±12.8). There was no significant variation in oocyte numbers between the groups (ANOVA: F=2.06, P=0.16, df=2,16).

When comparing the percentage of mature oocytes and the number of oocytes (mature and immature) present after deposition in the ovaries, it appears that the females in the control group and in group 1 had higher number of oocytes, but lower percentages of mature oocytes than the females in group 2. In females belonging to group 2 the percentage of mature ovarian oocytes increased to almost 80%, suggesting that vitellogenesis continued during the oviposition period (Fig. 3).

The mean age of 13 females from the Danube floodplain, estimated by skeletochronology, was 4.23±0.34 years (Cogalniceanu & Miaud, in prep.). The minimum age was three years (n=5), suggesting that this is the age of the first reproduction.

TABLE 2. Correlations between the main parameters describing oviposition in smooth newt females.* P< 0.05; ** P < 0.01; *** P < 0.001; NS - not significant.

Locality	Initial body weight and clutch size	Initial body weight and deposition rate	Clutch size and oviposition period
Baneasa forest	r = 0.07NS	r = - 0.17NS	r = 0.85***
Danube floodplain	r = 0.42NS	r = 0.40NS	r = 0.80***
England (Baker, 1992)	r = 0.61*	r = 0.89**	-

DISCUSSION

Severe environmental conditions affecting the two Romanian populations studied probably influenced their body size. Feeding in water lasts for only short periods while feeding opportunities on land are dis-

rupted by long unfavourable intervals (floods or drought respectively). The population studied by Baker (1992) inhabited a permanent pond, with a humid, more favourable surrounding habitat, allowing for prolonged feeding during both the aquatic and terrestrial phases. The weight lost during the aquatic phase is usually regained during the terrestrial phase (Verrell & Halliday, 1985; Fasola & Canova, 1992). Unfavourable conditions, mainly drought, can reduce this gain and impose smaller body dimensions. The two populations studied from Romania are both subjected to strong environmental pressures, either from drought or from high floods, with temperatures fluctuating between up to 40°C in the summer and as low as -20°C in the winter during most years. The Baneasa forest population inhabits an area where, due to the lowering of the underground water level during the last 20 years, the pond dries very rapidly, usually before metamorphosis of the larvae. Prolonged periods of inundation in the lower Danube floodplain were shown to cause a decrease in the body condition and fitness of green frog populations (Cogalniceanu, 1997). Inundation was also shown to cause increased mortality in two species of toads, especially in areas deprived of refuges from floods (Bosman, van Gelder & Strijbosch, 1997), and to increase the risk of predation from fish (Aronsson & Stenson, 1995). These factors might act in favour of an early breeding strategy, with all the potential advantages that it has (Nilsson & Svenson, 1996).

Although the data were collected during consecutive years, the parameters describing egg deposition do not differ significantly between the two populations. The only difference between the two populations studied appears to be that of larger clutches laid over shorter periods of time by females from the Danube population, compared to the Baneasa population (Fig. 2). This might be explained by the fact that females from the Danube population have a more diverse food supply (thus stimulating oviposition) but are also prone to stronger predation. Overall, despite the fact that environmental pressures are different, reproductive strategies appear similar in the two populations.

The body weights of the females in the populations studied, around one gramme, are extremely low. In Italy larger females were also reported with an average female body weight of 1.9 g (Fasola & Canova, 1992). Verrell & Halliday (1985) indicate an average female body mass of 2.6 g in another population from southern England.

These results compare favourably with the study by Kalezic *et al.* (1996) who found a similar number of oocytes in the ovaries, an average of 152 (ranging between 99 and 206), but no correlation between oocyte number and female size.

The hypothesis that actual clutch size can be measured by counting the ovarian oocyte number before reproduction, confirmed by Baker (1992), is not supported by this study, since not all oocytes are deposited. Similar results were obtained by Hagström (1980), who reported that after reproduction females still contained yolked oocytes. The small clutch size might also be caused by lack of further insemination. Pecio (1992) reported that females inseminated with only one spermatophore laid on average 52 eggs, while females further inseminated laid 137 eggs/season.

The average age of females from the floodplain population and the British population studied by Verrell & Francillon (1986) are similar (Mann-Whitney test, $z=1.44$, $P>0.05$). The range of age classes in smooth newt females is nevertheless different, three to six years in the Romanian floodplain population and two to five years in the British population. Similar results are reported by Kalezic *et al.* (1996), who estimate the time of attainment of sexual maturity for females at 3.2 years. Thus, reproduction at a younger age is not the cause for the large differences in body size.

The present results indicate great variability in the reproductive effort of populations that inhabit unpredictable and highly variable environments. In unfavourable conditions, *Triturus alpestris* females can stop depositing eggs or can reproduce only every other year (Vilter & Vilter, 1963). There seems to be a trade-off between clutch size and resource allocation. A smaller clutch size is preferred since resources also have to be allocated to somatic maintenance because of irregular feeding opportunities (Rosenheim, 1996). I suggest that female newts have a higher reproductive potential than previously estimated. When females terminate oviposition this is not a consequence of the depletion of mature oocytes but it is probably triggered by environmental factors.

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