

would result in it breeding later. Clearly, these conclusions are based on limited information and await confirmation by more detailed studies.

*Conclusions:* *P. clarkorum* and *P. ornatus* are sand-dependent lizards with preferred habitats of firm sand and sand-strewn alluvium. *P. clarkorum* is intimately connected to the main desert sand formations. *P. ornatus* ranges more widely following migratory sand accumulations. Where sympatric both occur in stable and compatible populations neither showing dominance but are less abundant than in areas where either one or the other species is found. Behaviour, escape methods and detection avoidance can be related to morphological differences. *P. clarkorum* seems to breed later than *P. ornatus* and is probably more sensitive to lower temperatures.

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HERPETOLOGICAL JOURNAL, Vol. 2, pp. 142-144 (1992)

### VARIATION IN VIABILITY DURING DEVELOPMENT AND HATCHING SUCCESS IN EMBRYOS OF THE TOAD *BUFO CALAMITA*

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*(Accepted 14.6.91)*

Many authors have dealt with embryo hatching success in anurans as failures in fertilization efficiency (Davies & Halliday, 1977; Gerhardt *et al.*, 1987; Ryan, 1985; Robertson, 1990). However, other sources may contribute to additional embryo mortality throughout development: these include

failures or abnormalities in development, fungal infestations prior to hatching, etc. (Herreid & Kinney, 1966; Woodruff, 1976; Seigel, 1983; Travis *et al.*, 1987; Banks & Beebe, 1988). Therefore, any attempt to separate fertilization efficiency from later factors governing mortality is an important matter that ultimately relates to sexual selection and fitness, since Licht (1976) proposed that an optimal male/female body size ratio might enhance fertilization rate.

Little information is available regarding embryonic development and viability of the next generation, and non-proximate factors such as female body size and age. Other factors such as egg size may affect some components of offspring fitness such as embryo size at hatching (Kaplan, 1980; Crump, 1984). However, no information is available concerning embryo survival and egg size. Finally, external fertilization in anurans may result in a lower rate of successful insemination at higher clutch sizes.

The present study examines the variation in embryo viability during development from fertilization to hatching in the natterjack toad, *Bufo calamita*. Fertilization success is analyzed as a function of body size ratio (defined as relative male body length/female body length). Likewise, both fertilization efficiency and hatching success are studied as a function of female body size, age, and clutch and egg size.

The study was conducted in central Sierra Morena, Córdoba Province, Spain, from January to April of 1987 and 1988. Naturally-occurring pairs of natterjack toads in amplexus were captured in the breeding area; 71 in 1987 and 44 in 1988. The pairs were isolated in glass aquaria (30 x 20 x 20 cm) filled with 3 l of pond water and some vegetation. The aquaria were placed at the site where the pair had been captured until oviposition occurred. Then the individuals were measured for body length and toe-clipped. Toes were frozen for skeletochronological study in order to determine successive resting lines, providing an estimate of the age of each individual (Tejedo, 1989). All clutches were photographed to estimate clutch size and were then carefully released into the pond. The absolute number of eggs for each clutch was counted from the resulting photographs. Average egg size was determined by measuring the diameter of ten eggs from samples randomly selected in each clutch, to the nearest 0.02 mm, using an ocular micrometer. Estimates of embryo viability were obtained from samples taken sequentially from individual clutches. Embryos were staged according to Gosner (1960). In 1987, four samples were taken at 3-4 day intervals: (1) late cleavage, stages 9-10, taken about 12 hr after oviposition. This sample was used as an estimate of fertilization efficiency; (2) late gastrula or early neurula, stages 12-13; (3) neurulation, stages 14-16; and, (4) hatching, stages 17-19. During 1988, only two samples were taken, spaced about 10 days apart: (1) stages 9-10, taken about 12 hr after oviposition (fertilization success estimate); and (2) stages, 16-18. The final samples from both years were used to estimate total number of viable embryos and percent of hatching success. Additionally, collections were taken from non-manipulated clutches, those laid freely, not in aquaria ( $N = 10$  in 1987 and  $N = 6$  in 1988), to ascertain if manipulation affected total number of viable embryos. Manipulated clutches did not differ from control ones in clutch size or average egg size (Mann-Whitney  $U$ -test,  $P > 0.05$ ). The average sample size ( $\bar{x} \pm SD$ ) was  $71.2 \pm 33.2$  embryos,  $n = 246$ , in 1987, and  $85.4 \pm 37.4$  embryos,  $n = 100$ , in 1988. Each sample was immediately examined with a dissecting microscope. Embryos were counted and scored as non-viable or dead when they were grey and swollen. Dead

SAMPLE	1	2	3	4
1		3.732 **	4.307 ***	3.361 *
2			0.348 NS	0.181 NS
3				0.521 NS

TABLE 1. Non-parametric Tukey multiple comparisons analysis (q) of percent of viable embryos of successive samples taken from clutches during the 1987 breeding season.  $\bar{x} \pm SD$  of survival rate in each sample were: 1st sample,  $95.18 \pm 10.38\%$ ,  $N = 71$ ; 2nd sample,  $84.30 \pm 18.14\%$ ,  $N = 41$ ; 3rd sample,  $81.05 \pm 20.22\%$ ,  $N = 43$ ; 4th sample,  $80.44 \pm 27.59$ ,  $N = 71$ . \*  $P < 0.005$ ; \*\*  $P < 0.002$ ; \*\*\*  $P < 0.001$

embryos were quickly covered by fungi, hence estimates of embryo survival in the later samples were made by examining the proportion of embryos either infected by fungus hyphae or decomposed.

A comparison of percent of embryo surviving during 1987 revealed significant differences between successive samples (Kruskal-Wallis test,  $H = 24.34$ ,  $df = 3$ ,  $P < 0.0001$ , Fig. 1). A *posteriori* non-parametric Tukey multiple comparisons analysis revealed particular differences (Table 1). Embryos in Gosner stages 9-10 had a significantly higher mean survival than subsequent samples. These subsequent samples showed no significant differences when compared to each other (Table 1). Comparison between the two samples taken in 1988, however, failed to reveal any significant difference (1st sample  $\bar{x} = 94.72\%$ ,  $SD = 14.9$ ,  $N = 44$ ; 2nd sample  $\bar{x} = 93.71\%$ ,  $SD = 13.6$ ,  $N = 44$ , Mann-Whitney  $U$ -test,  $Z = 1.22$ ,  $P = 0.11$ ). Some authors have found that most embryo mortality in amphibians occurs during gastrulation and neurulation (Anderson *et al.*, 1971; Smith, 1974 in Cooke, 1975; Woodruff, 1976, Travis *et al.*, 1987). A possible explanation of the differential mortality recorded during this period may be that gastrulation is a critical process in development which involves important interaction between the nuclear genome and cytoplasmic proteins initiating differentiation in embryos (Sargent & Dawid, 1983). This process may give rise to strong sensitivity in embryos at this stage. All dead embryos were quickly covered by fungi but these may have been saprophytic. Some authors have observed fungal contamination of dead amphibian embryos (Herreid & Kinney, 1966; Tilley, 1972; Cooke, 1975; Woodruff, 1976; Banks & Beebe, 1986, 1988) but only Villa (1979) could find clear evidence of the parasitic role of fungi on developing embryos.

Fertilization success was similar in both years of study (1987,  $\bar{x} = 95.2 \pm 10.4\%$ ,  $N = 71$ ; 1988,  $\bar{x} = 94.7 \pm 14.9$ ,  $N = 44$ , Mann-Whitney  $U$ -test,  $Z = 0.85$ ,  $P > 0.20$ ). Manipulated clutches did not differ from control clutches in average fertilization success (Mann-Whitney  $U$ -test, 1987,  $Z = 0.98$ ,  $P > 0.10$ ; 1988,  $Z = 0.32$ ,  $P > 0.20$ ). This suggests that the confinement of eggs and toads to an aquarium did not result in an unnaturally high fertilization success. Male/female body length ratio was not correlated with fertilization success either in 1987 ( $r_s = 0.084$ ,  $P > 0.20$ ,  $N = 71$ ) or in 1988 ( $r_s = 0.045$ ,  $P > 0.20$ ,  $N = 44$ ). This result does not support Licht's (1976) prediction of an optimal male/female size ratio which would enhance fertilization efficiency. Some authors have verified this prediction (Davies & Halliday, 1977; Ryan, 1985; Gibbons & McCarthy, 1986; Robertson, 1990); however others have shown that no such relationship exists (Kruse, 1981; Gerhardt *et al.*, 1987; Höglund & Robertson, 1987; Krupa, 1988). The differences observed among these species seem to be unclear.

Female body size ranged between 55.5 and 93.5 mm, with an average value of  $72.95 \pm 8.49$  mm,  $N = 115$ . Female size was not related to fertilization success (1987,  $r_s = 0.034$ ,  $P > 0.20$ ,  $N = 71$ ; 1988,  $r_s = 0.123$ ,  $P > 0.20$ ,  $N = 44$ ). Age was estimated only in 1987 from a sample of  $N = 41$  mated females. Averaged age value was  $4.49 \pm 2.3$  resting lines, range 2-10. Female age variation was also independent of fertilization success ( $r_s = 0.17$ ,  $P > 0.20$ ,  $N = 41$ ). The average egg size ranged between 1.37 and 1.93 mm,  $\bar{x} = 1.69 \pm 0.11$  mm,  $N = 95$  clutches. Egg diameter was also unrelated to fertilization success (1987,  $r_s = 0.084$ ,  $P > 0.20$ ,  $N = 51$ ; 1988,  $r_s = -0.08$ ,  $P > 0.20$ ,  $N = 44$ ). The average number of eggs per clutch was  $3818.9 \pm 1373.5$  eggs, (range 1234-8840,  $N = 115$ ). Likewise, clutch size was unrelated to fertilization success (1987,  $r_s = 0.084$ ,  $P > 0.20$ ,  $N = 71$ ; 1988,  $r_s = 0.11$ ,  $P > 0.20$ ,  $N = 44$ ). The average hatching success was significantly lower in 1987 ( $\bar{x} = 80.4 \pm 27.6\%$ ,  $N = 71$  clutches) than in 1988 ( $\bar{x} = 94.3 \pm 8.97$ ,  $N = 44$  clutches; Mann-Whitney  $U$ -test,  $Z = 2.92$ ,  $P = 0.002$ ). Environmental factors may induce the observed variability. However, it is not possible to account for the differences in hatching success observed between years. Manipulated clutches did not differ from undisturbed control clutches in mean hatching success during either year (Mann-Whitney  $U$ -test, 1987,  $Z = 0.28$ ,  $P > 0.20$ ; 1988,  $Z = 0.002$ ,  $P > 0.20$ ). Embryo hatching success was unrelated with female body size (1987,  $r_s = 0.097$ ,  $P > 0.20$ ,  $N = 71$ ; 1988,  $r_s = -0.129$ ,  $P > 0.20$ ,  $N = 44$ ). Variation in female age was also independent of hatching success ( $r_s = 0.146$ ,  $P > 0.20$ ,  $N = 41$ ). Mean egg size and clutch size was not correlated with hatching success (egg size, 1987,  $r_s = 0.167$ ,  $P > 0.20$ ,  $N = 51$ ; 1988,  $r_s = 0.025$ ,  $P > 0.20$ ,  $N = 44$ . Clutch size, 1987,  $r_s = -0.126$ ,  $P > 0.20$ ,  $N = 71$ ; 1988,  $r_s = 0.130$ ,  $P > 0.20$ ,  $N = 44$ ).

Fertilization success recorded in this study was similar to that found in other studies in which average values ranged from 78-96%, with the exception of notably lower values found in *Bufo calamita* by Banks & Beebe (1986). The difference may lie in the fact that the senility effect, responsible for lower efficiencies in older individuals from the British population, was not observed in this study. Female body size or age influenced neither embryo survival at early stages nor hatching success. Moreover, reproductive traits, such as egg size, did not affect embryo survival. This is not consistent with models which predict a correlation between egg size and offspring fitness (e.g. Smith & Fretwell, 1974). Larger clutches did not present a lower fertilization success, hence sperm cost does not seem to limit fertilization potential as has been suggested in other species (Robertson, 1990). Variation in embryo survival and hatching success in *Bufo calamita* may be mediated by random mechanisms or perhaps by environmental factors not measured in this work.

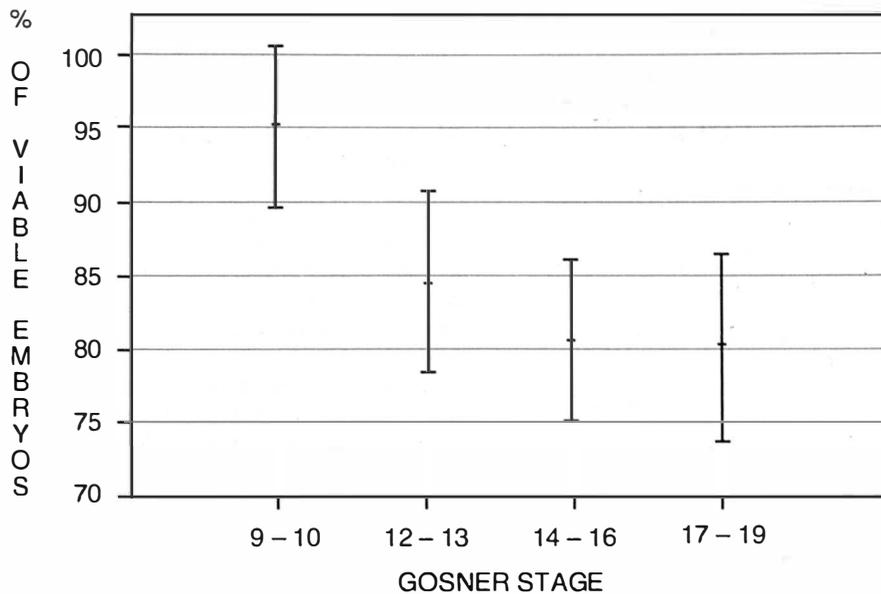


Fig. 1. Percent of embryos alive in four successive samples taken from clutches of *Bufo calamita* during the 1987 breeding season. Embryos from each sample were in the Gosner stages indicated. Mean values are indicated and bars represent 95% confidence intervals.

*Acknowledgments:* I am grateful to Carmen Díaz-Paniagua and one anonymous referee for their comments on an earlier draft of this manuscript. This work was partially supported by a grant from the Spanish Research Training Program (P.F.P.I.).

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