Body size and reproductive characteristics of paedomorphic and metamorphic individuals of the northern banded newt (*Ommatotriton ophryticus*)

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Abstract. Paedomorphs and metamorphs of the northern banded newt (*Ommatotriton ophryticus*) were compared with respect to body size and some reproductive traits. Paedomorphic females are smaller than metamorphic individuals of the same sex. Both morphs have a similar reproduction pathway in terms of egg number and incubation period, while hatching success and egg diameter appear to be different between morphs. Survival rates of two morphs were significantly different and calculated as 66% for paedomorphs, and 81% for metamorphs. On the other hand, both forms shared a common allometric slope of the SVL vs. number of eggs and egg diameter, and larger females tend to produce more and larger eggs.

Keywords. Facultative paedomorphosis, reproductive output, fecundity, body size.

In many urodele species, including European newts (especially in *Mesotriton* sp., *Lissotriton* spp.) there are populations with alternative life-history pathways: metamorphosis vs. paedomorphosis (e.g. Duellman and Trueb, 1994). Most larvae transform into immature individuals which remain more or less terrestrial before reaching sexual maturity; however, some larvae attain sexual maturity with larval morphology. Such a discrete life-history polymorphism has both genetic and environmental components, and is usually viewed as an adaptation to contrasted environments in time and space (e.g. Denoël et al., 2005). Environmental conditions causing paedomorphosis are multifactorial and vary among species, but are often related to unsuitable terrestrial conditions, such that an aquatic lifestyle would be more beneficial.

Paedomorphic forms of northern banded newt *Ommatotriton ophryticus* (Berthold, 1846) were described in 2008 by Kaya et al. (2008) near Karasu in the vicinity of Sakarya, Turkey. This species ranges from the western Caucasus in southern Russia and Georgia,

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through northwestern Armenia and northern Turkey west to the Bosphorus Strait. It has been recorded from near sea level (Tabbaria Lake) up to around 2,750m asl (in Turkey). It is found in coniferous, mixed and deciduous forests up to sub-alpine meadows. Reproduction of this species occurs in lakes, ponds, large puddles, drainage canals, roadside ditches in meadows, slow-flowing streams and stream pools in open areas near or within forests. *O. ophryticus* is listed as Near Threatened (NT) by IUCN (2009). For conserving any target species, firstly, we have to understand its life-history.

Although, breeding biology is an important part of life-histories, it has not been well documented for *O. ophryticus* until now. For this reason, we aimed to describe some aspects of the reproductive biology of paedomorphic and metamorphic *O. ophryticus* specimens by quantifying clutch size, egg size, hatching success and female size-reproductive output relationships.

Location of the study site and its characteristics were described in Kaya et al. (2008). To collect paedomorphic individuals, the field studies were conducted between late February and early March. These months were previously reported as the breeding season of *O. ophryticus* (Andren, 1997; Kutrup et al., 2005). Both forms were monitored for three years (2007-2009), and each year they were sampled at least three times. Individuals were captured by dip nets. Even though, a total of 146 individuals were captured in 2007, there were no paedomorphic individuals. In 2008, 191 individuals were captured of which seven paedomorphic specimens, while in 2009 of 280 captured individuals six were paedomorphic individuals. Capture probability of paedomorphic individuals was estimated as 0 in 2007, 0.04 in 2008 and 0.02 in 2009 breeding seasons for this population. All captured paedomorphic individuals and randomly selected metamorphic newts were transported alive to our amphibia laboratory in covered plastic boxes. To compare reproductive output, 21 pairs of metamorphic females and males (16 pairs in 2008 and five pairs in 2009) randomly selected, were transported to our laboratory. The temperature of the laboratory was maintained at a constant temperature of 20±1 °C with a light/dark schedule of 14/10 hours.

Each paedomorphic and metamorphic female was weighed with an UFO TECH precision balance and their snout-vent lengths (SVL) were measured with digital calipers following Başoğlu and Özeti (1973). Then we placed them with a metamorphic male in separate 20 liter glass aquariums and some small-leaved aquatic plants were provided for egg deposition. Newts were fed three times a week with tubifex worms and earthworms. We changed the water of each aquarium every other day. Paedomorphs and metamorphs in captivity displayed courtship behaviour and then breeding activity. The metamorphic and paedomorphic forms generally started to lay eggs on the 3rd day of captivity. Eggs were collected and counted four times a day, over the course of the entire oviposition period. As many species of newts and salamanders are especially fond of consuming their own eggs (Dasgupta, 1996; Kuzmin, 1991), we moved and tried to rear these eggs separately from the parents' aquaria. Eggs which were transferred in a separate container were checked three or four times a day. Instead of the evaporating water during the incubation period, clean water was added to aquaria. Newly hatched larvae were counted and moved in a separate aquarium, and the experiment was lasted.

The mean ovum diameter was calculated by measuring 20 randomly chosen eggs (in 1st cleavages) from each individual under a stereomicroscope with an ocular micrometer. Hatching success (Survival Rate) was defined as the number of hatched larvae divided by the number of eggs and multiplied by 100. Confidence interval of survival rates was calculated as CI = 2 SQRT [$q(1-q)/n$], where q = 1-SR, SR is the survival rate and n is number of eggs at the beginning of the experiment (Miaud, 1994).

Body size measurements and all reproductive output parameters were normally distributed (Kolmogorov–Smirnov D test, all $P > 0.05$), thus allowing comparisons using parametric tests. Firstly, to explore the level of variation among females within morphs, analyses of one-way ANOVA were performed using SPSS 13.0 Version for Windows. To analyze the relationships between female body size and egg size, we used standardized major axis (SMA) analysis (Warton et al., 2006). The mean of egg diameter for each female was regressed to the SVL in SMA analysis. SMA analysis provide a better estimate of the line summarizing the relationship between two variables (i.e., the main axis along which two variables are correlated) to that of ordinary linear regression, because the residual variance is minimized in both "x" and "y" dimensions. The analysis also determined the differences between the slopes obtained for each species or for each maternal tree, so that a significant P indicated differences between the slopes of the groups studied. We used the free software statistics package SMART vers. 2.0 (Falster et al. 2006). There was a strong positive correlation between female SVL and body mass in paedomorphic (Pearson's, n = 13; $r^2 = 0.820$; $P < 0.01$), and metamorphic forms (Pearson's, n = 21; $r^2 = 0.600$; $P < 0.01$). Paedomorphic females were significantly smaller and lighter than metamorphic females (Table 1 and Fig. 1). Body size can be varied between paedomorphs and metamorphs depending on the population source (Denoël et al., 2009). For example, in some populations of newts (smooth, palmate and alpine newt) paedomorphic forms can be smaller than metamorphs or vice versa as well as both forms have approximately same size (Denoël et al., 2009; Kalezić et al., 1996).

The most notable results of our study that the mean number of eggs of the paedomorphs lesser than metamorphs, although, there are not significant differences the number of eggs between paedomorphs and metamorphs. However, egg diameters showed statistically difference between paedomorphs and metamorphs (Table 1 and Fig. 1). Generally, fecundity parameters and mating success are related to body size (Semlitsch, 1985), but the relation appears to be different in different cases. Paedomorphic females produced lesser eggs than metamorphic counterparts (Semlitsch, 1985; Kalezić et al., 1996), but,

Table 1. Descriptive statistics of female size and reproductive outputs shown in two forms of *O. ophryticus*. Results of One way Anova indicate the differences between paeodomorphic and metamorphic forms.

	Form	N	Min	Max	Mean	Std. Dev.	df	F	Sig.
SVL (mm)	P	13	31.88	43.08	37.47	3.495	1	117.82	< 0.0001
	M	21	44.96	64.34	52.68	4.232			
Body mass (g)	P	13	1.7	3.2	2.35	0.427	1	21.67	< 0.0001
	M	21	2.4	6.4	3.70	0.985			
Number of eggs	P	13	16	189	83.46	55.611	1	3.59	0.067
	M	21	25	296	122.90	60.965			
Egg diameter (mm)	P	116	0.60	0.98	0.78	0.068	1	314.41	< 0.0001
	M	105	0.79	1.02	0.92	0.049			

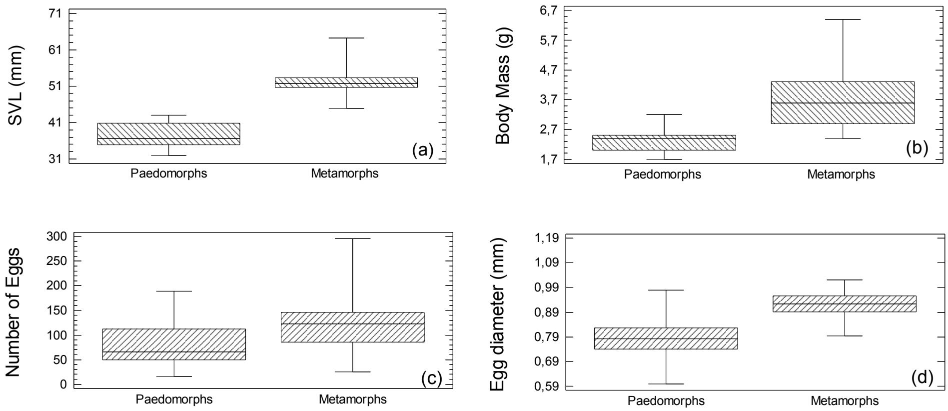

Fig. 1. Variations of SVL (a) and body mass (b), number of eggs (c) and egg diameter (d) in females of paedomorphic and metamorphic forms.

in some cases, the reverse situation also exists or they produced approximately the same number of eggs (Semlitsch, 1985; Kalezić et al., 1996; Rot-Nikcevic, et al., 2000).

Totals of 1085 eggs of paedomorphic forms and 2581 eggs of metamorphic forms were layed, 717 and 2092 larvae of which developed, respectively. Photographs of an egg and a resulting larva are shown in Fig. 2. On the side, survival rates of 0.66 (66%), CI 0.63-0.69 for paedomorphs, and 0.81 (81%), CI 0.80-0.83 for metamorphs. These results indicate that survival rates of two morphs are significantly different each other. The growth and development rates of amphibians are influenced by a number of intrinsic (body size or egg size and yolk reserves) and extrinsic factors (temperature, density and competition, food supply and quality, predation, breeding habitat and inhibitory compounds) (Crump, 1974, 1984; Kuramoto, 1975; Berven et al., 1979; Kaplan, 1980; Berven and Chadra, 1988; Duelman and Trueb 1994). Most likely, low survival rate in paedomorphic forms is underlined by intrinsic factors mostly because of constant laboratory conditions.

The linear regression analysis indicated that female SVL showed a strong positive relationship with egg diameter (metamorphs: $r^2 = 0.858$; $P = 0.024$, paedomorphs: $r^2 = 0.823$; $P = 0.013$), as well as with the number of eggs (metamorphs: $r^2 = 0.446$; $F = 15.232$; $P = 0.001$, paedomorphs: $r^2 = 0.653$; $F = 20.713$; $P = 0.001$). SMA analysis showed that both forms shared a common allometric slope of the SVL vs number of eggs and egg diameter. Pairwise combinations of SVL and number of eggs/egg diameters showed significant differences in elevation shift (Table 2). Consequently, our results indicate that larger individuals of both forms tend to produce more and larger eggs. Similarly, Furtula et al. (2008) found conformable relationships between the female SVL and vitellus volume in four crested newt species. In this sense, larger females produce larger eggs, and they have more yolk for larval development and/or thicker mucoid capsules which provide better protection (Semlitsch, l985; Semlitsch and Gibbons, 1990; Kalezić et al., 1994). For this reason, female body size and/or egg size can affect the low survival rates in paedomorphic forms in contrast to metamorphic counterparts.

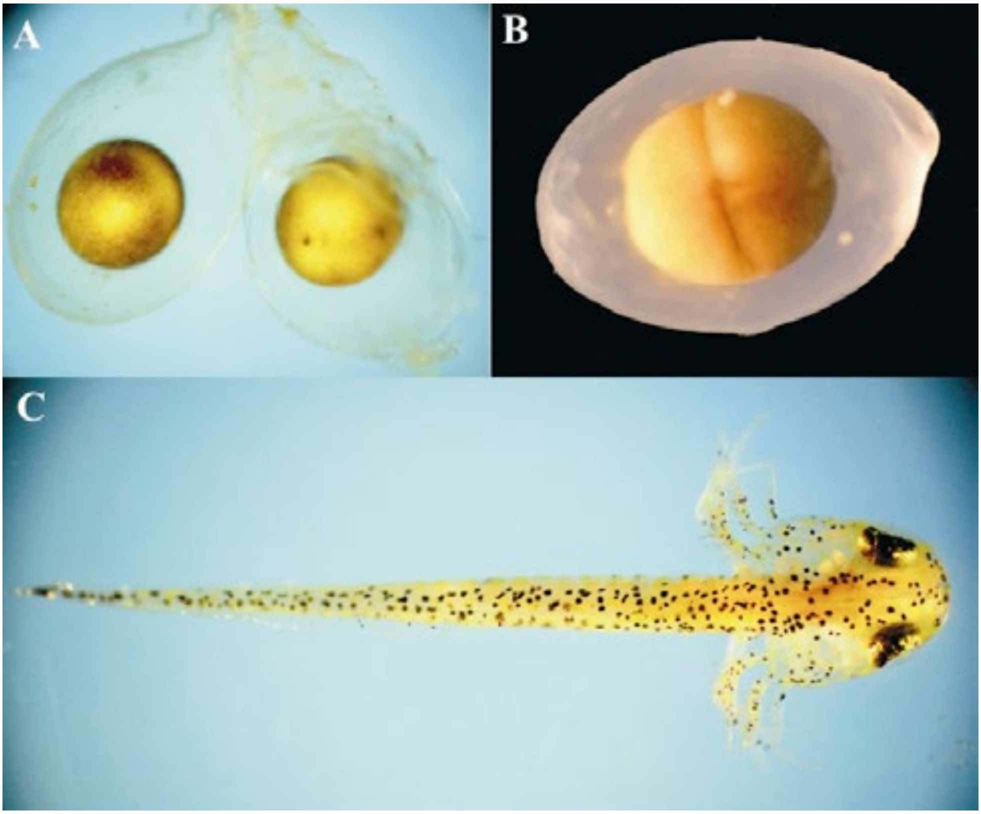

Fig. 2. The eggs: fertilized (A), cleaved (B), and a resulting larva of paedomorphic form (C).

Table 2. Results of standardized major axis (SMA) regression analysis for all pairwise combinations of snout-vent length (SVL) and reproduction traits of both forms.

Trait pair (X and Y)	Forms	n	r^2	P	Slope	Intercept	Slope homogeneity (P)	Shift in elevation (P)	Shift along slope (P)
SVL and egg number	P	13	0.592	0.002	0.1286	1.333	0.877	0.000	< 0.0001
	M	21	0.187	0.050	0.1340	1.454			
SVL and Egg diameter	P	6	0.342	0.223	18.47	4.288	0.409	0.004	0.009
	M	5	0.062	0.687	34.57	2.589			

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