

Reproductive behaviour of the Alpine newt *Triturus alpestris*: Mating and oviposition preferences

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Like other newts, the alpine newt *Triturus alpestris* uses a combination of olfactory, vibrational and visual cues in courtship. The high degree of sexual dimorphism in this species suggests that colours and other visual cues are important in mate choice. The aims of this study were to understand the role of body coloration and morphometry in mating, and to understand females' preferences for substrates for oviposition. To this end, 12 adult newts were captured, measured, and characterized for morphological features. The early phases of the courtship were observed, and indexes were calculated for Attractiveness and Promiscuity. Females were exposed to different egg-laying choices, and the resulting number of eggs was counted. The Attractiveness index was higher in females than in males, with the most cryptic females being positively selected. On the other hand, females responded positively to displaying males that had low body mass, small tail, small eyes, small spots on the body, and narrow heads. Promiscuity data show that females were significantly more selective than males in mate choice. Among males, the more promiscuous were the larger ones. There were no significant differences in the choices of the width of substrate for oviposition. However, the females preferred to lay their eggs on lighter coloured strips, and at low rather than high density of aquatic plants.

KEY WORDS: *Mating behaviour, Oviposition, Visual cues, Triturus alpestris*

INTRODUCTION

Sexual selection in newts (genus *Triturus*) has been explored within the context of the traditional model (Halliday, 1977); all species use some combination of olfactory, vibrational and visual cues in courtship. In *T. alpestris*, courtship consists of four phases: Orientation phase, Fanning display phase, Cat-buckle with lean-in display phase, and Sperm-Transfer phase (Denoël, 2002). Visual cues must be very important in the displays made by the male during the fanning and Cat-Buckle phases: the position of the male, which is stationary in front of the female with an angle of 90° gives the female a particularly good view of the male's morphological characters. *T. alpestris* develop relatively small and invariant crests during the breeding season, and exhibit courtship behaviours that lack many of the strong vigour signals used by other newt species, such as *T. vulgaris* and *T. cristatus* (Arntzen & Sparreboom 1989).

Females judge male quality via a trait or traits, such as body size, physical vigour, or elaborate and costly ornaments, and a male that possesses the optimal traits represents the best choice for every female (Andersson, 1994). Casual observations in the genus *Triturus* suggest that males are fairly indiscriminate in choosing females, courting with equal intensity any female who happens to be in the vicinity, even those of a different species (Griffiths, 1995). Laboratory observations have shown that male *T. vulgaris* are capable of being selective, and given the choice, will pick females that are relatively larger and fatter, and therefore likely to be carrying more eggs (Verrel, 1986). However, the role of colours can also be important for male newts for finding mates in *T. alpestris* (Himstedt, 1979).

T. alpestris from northern and central Greece show a high degree of morphological and

genetic variation (Kyriakopoulou-Sklavounou, 1997, 2000). Dorsal surface colour is greyish-blue in males, sometimes almost black. Females never show such pronounced coloration: the dorsum is usually brownish-beige or olive. Indistinct light spots are often present, sometimes forming a blotched pattern; white spots can be present on the flanks. The ventral surface is bright yellow or orange. During the breeding season, males show a low crest with light and dark spots or bars. The crest extends from the back of the head to the tip of the tail. The male also has bright blue bands on the flanks and white or silver longitudinal bands with dark spots on the body flanks. However the colour can vary depending on the substrate or the mood and health of the newt. There are also significant sexual differences in the morphometrics of body and tail length: the male is usually slightly smaller and thinner than the female (Garcia, 1985; Kuzmin, 2000; Spallone, 2003).

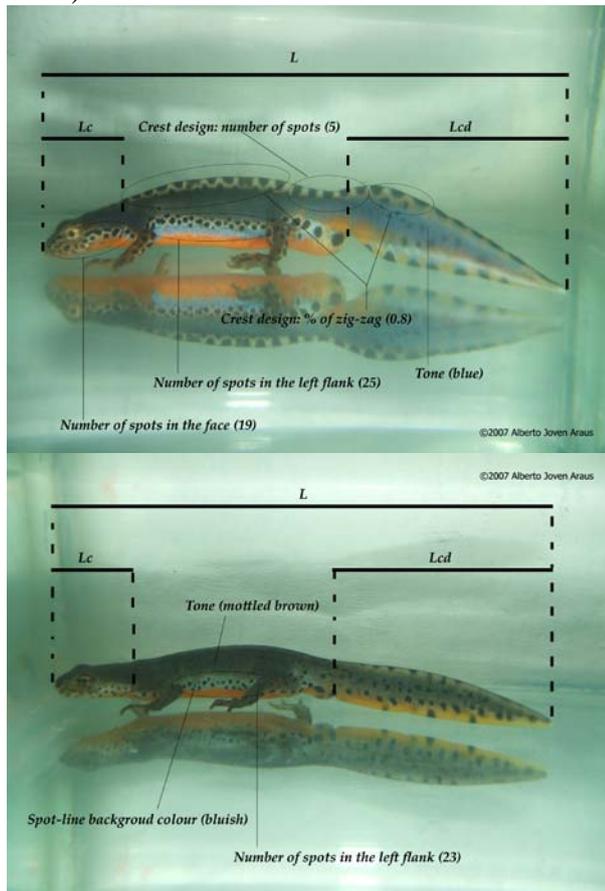


FIGURE 1: Some of the measurements and morphological characters studied (Upper: male *M4*; lower: female *F1*).

Female alpine newts attach eggs individually to aquatic plants, wrapping these eggs by means of adhesive on the egg membranes (Miaud 1994b). Wrapped eggs are unseen by the majority of predators, such as aquatic invertebrates or adult newts, and therefore experience reduced predation. In predation studies, only adult *Dytiscus marginalis* beetles consumed all the wrapped eggs, whereas the addition of other predators (six arthropods, one gastropod, and three amphibian species) did not affect egg survival. (Miaud, 1993, 1994a; Orizaola & Brana, 2003). The rate of egg development is influenced by a wide range of environmental factors, of which temperature is probably the most important (Griffiths, 1995).

The aim of this study was to understand the visual cues and preferences in mate-choice in *T. alpestris* (role of colours, morphometry, and pattern of spots) as well as preferences for the material of oviposition support of the females (different colours, sizes, and densities).

MATERIALS AND METHODS

Sampling and Laboratory Maintenance

Twelve adult alpine newts (6 males, 6 females) were caught in Pertouli (39°33'00.55" N, 21°29'52.14" E), central Greece, at the start of the breeding season (early April 2007). They were in a slow-moving stream at 1150 m altitude, mostly in areas next to the banks where there was some vegetation. Only newts involved in breeding interactions were taken. They were caught using a handled net. Newts were chosen which had noticeably different morphological features (colour, spots, and size) in order to facilitate identification and to study morphological preferences in mating. A sample of aquatic plants was also taken in order to recreate the natural habitat of the newts in the aquarium. The aquarium used was 40 x 50 x 60 cm, and the bottom was covered with gravel, pots, and stones irregularly distributed, generating hides for the newts. All

newts were maintained in a single aquarium throughout the experiments. The water temperature was measured in the sampling location, and the newts were maintained at this temperature (10-11°C). The photoperiod was 14 h light/10 h dark, the same as the conditions experienced by newts during reproductive season (Denoël *et al.*, 2005). The newts were fed 4 times per week with dried *Chironomus* larvae. All newts and resulting larvae were released back into their natural river after the experiment. The small number of animals used in the study was chosen in order to minimize any possible impact on the wild population.

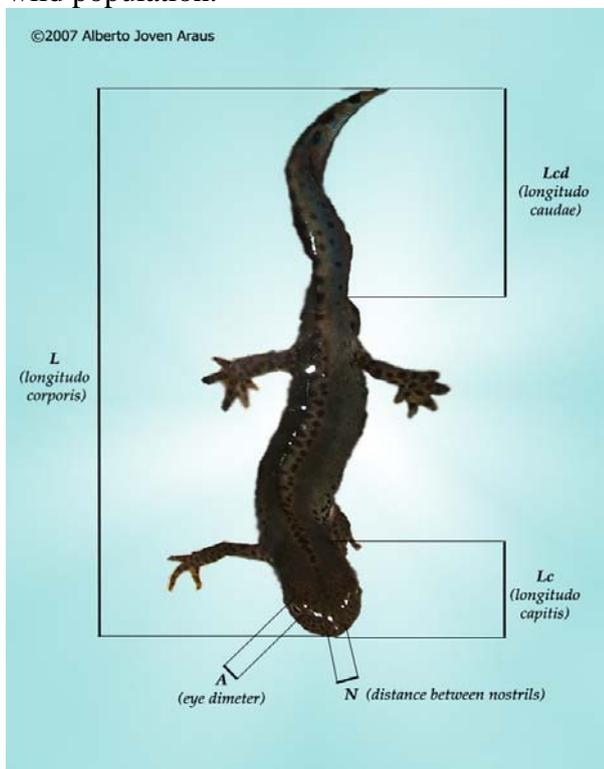


FIGURE 2: Morphometric measures used.

Experimental Procedures

No marking technique was used, as the sample was small. Each newt was photographed from both sides, and could be identified by morphological features such as body colour or spot locations (Figure 1). Each newt was assigned a number. All individuals were weighed and measured, and morphometrical distances were measured based on Nöllert & Nöllert, 1992 and Terent'ev, 1949 (Figure 2). The precision was ± 1 mm for lengths and ± 0.1

g for mass. Using the pictures, other morphological characteristics were also recorded, such as tone, number of spots, and design of the male's crest (Figure 1, Tables 2 & 3). To describe the relative shape of the head and tail, some quotients were made: Lc/L , N/Lc , A/Lc and Lcd/L . For example, a high value of N/Lc would indicate a wide head, while a low value would indicate a narrow head.

The newts were observed for 1 hour each day between 10:30 and 18:00 for 15 days. Mating behaviours were recorded during the experimental periods, based on the total encounters observed: *Positive male action* (males initiating Orientation or Display phases), *Positive female response* (receptive female moves her head toward the displaying male), *Negative male response* (a male approaches with his head toward a female but does not develop any mating behaviour such as following, fanning, or cat-buckle with lean-in) and *Negative female response* (a female ignores the displaying male). Success in sperm transfer was not recorded because the sexes were not isolated, and trustworthy data could not be recorded. Two mating frequencies were calculated for each individual: *Attractiveness* (positive responses received divided by positive action trials for males, and positive action trials tolerated divided by total encounters for females) and *Promiscuity* (proportion of positive actions/responses divided by total encounters). These values are shown in Figure 3.

To test egg-laying preferences, the females were provided 3 different sets of conditions, each for a period of 5-10 days. These experiments began on the same day as the breeding behaviour study. In each case, specific egg laying substrates were offered in one area of the tank. The 3 sets of conditions used were: (1) For 7 days, the newts were provided with natural plants collected from their habitat, including various aquatic plants, dead fern leaves (*Pteridophyta*), and aquatic moss (*Bryophyta*). Yellow plastic strips were also offered during this trial.

Code	Males: tone		Females: tone		Females: spot-line background colour	
1	Greyish	M3, M5	Smooth brown	F2, F4, F6	White	F4, F5
2	Blue-greyish	M1	Mottled brown	F1, F3	White-bluish	F2, F6
3	Blue	M2, M4, M6	Very mottled brown	F5	Bluish	F1, F3

TABLE 1: Qualitative data under study

	Weight (g)	L (mm)	Lc (mm)	Lcd (mm)	N (mm)	A (mm)
Males	2.83±0.40	812.17±38.26	131.83±41.83	359.33±41.83	28.33±4.08	27.66±0.98
Females	4.22±0.84	927.33±68.28	134.66±3.01	473.67±49.69	33.17±2.56	33.33±2.25

TABLE 2: Means and standard deviations of quantitative data measured in the laboratory

	Number of spots on the right flank	Number of spots on the left flank	Number of spots on the face	Crest design: % of zigzag	Crest design: number of spots
Males	36.33±12.38	37.83±12.08	22.83±4.70	0.69±0.12	6.67±2.80
Females	18.33±6.65	17.67±4.08	-	-	-

TABLE 3: Means and standard deviations of morphological features measured from photographs

(2) For 10 days, the newts were provided only with artificial oviposition substrate consisting of 10 different types of plastic strips (15 x 0.5 cm and 15 x 1 cm in 5 different colours: transparent, white, yellow, blue and red). (3) For 5 days, the newts were provided a single species of aquatic plant, with some plants placed singly (low density), and some in clumps (high density). After the exposure period for each set of conditions, all eggs in the tank were removed and divided according to the type of substrate or density of substrate. The number of eggs on each type of substrate was counted.

Statistical Procedures

Qualitative data for various tones and body colours were coded for use in the correlation analysis (Table 1). Because the data did not fit a normal distribution, non-parametric tests were used. Correlation coefficients were done in order to find relationships between mating preferences and morphological characters (1-tailed Spearman's rho). Other comparisons were made using Chi-square test in contingency tables between observed and expected values for the oviposition experiments and to compare the Indexes calculated between sexes.

RESULTS

Attractiveness and Promiscuity values (Figure 3) were significant different between sexes. Attractiveness values were higher in females than in males ($\chi^2=0.971^*$, 5 df, $P<0.05$). However, males were significantly more promiscuous than females in mate choice ($\chi^2=0.564^*$, 5 df, $P<0.05$).

The Spearman correlation shows which morphological characters under study are related with the Indexes calculated from the data. The attractiveness in males is significantly related to low values for weight (Correlation coefficient = -0.736*), Lcd (Correlation coefficient = -0.883**), N (Correlation coefficient = -0.806*), A (Correlation coefficient = -0.806*), Spot width (Correlation coefficient = -0.804*), Lcd/L (Correlation coefficient = -0.912**) and N/Lc (Correlation coefficient = -0.883**). These results suggest that females give positive responses to displaying males that have low body mass, small tail, small eyes, small points and narrow heads. In females, the attractiveness is correlated to a low number of spots on the flanks (Correlation coefficient = -0.783*), low values of spot-line background (Correlation coefficient = -0.883*) and High

values of A/Lc. In other words, the males prefer to display to females with few spots, spot-line background colour whiter than bluish, and big eyes in relation to the head size.

The males promiscuity is positively related with N/Lc (Correlation coefficient = 0.886**), spot width (Correlation coefficient = 0.878*), Lcd (Correlation coefficient = 0.771*), W (Correlation coefficient = 0.829*) and L (Correlation coefficient = 0.829*). These correlations show that old males (large in length and weight) with wide heads are more promiscuous than the young ones. The promiscuity among females is correlated to high values of Lc/L (Correlation coefficient = 0.928**), and low values of Lcd (Correlation coefficient = -0.841*), N (Correlation coefficient = -0.824*), A (Correlation coefficient = -0.794*), Lcd/L (Correlation coefficient = -0.899**), N/Lc (Correlation coefficient = -0.812*) and A/Lc (Correlation coefficient = -0.754*). This data means that females with relative large heads, short tails, small eyes, and sharpened heads are more promiscuous than those that have opposite characteristics.

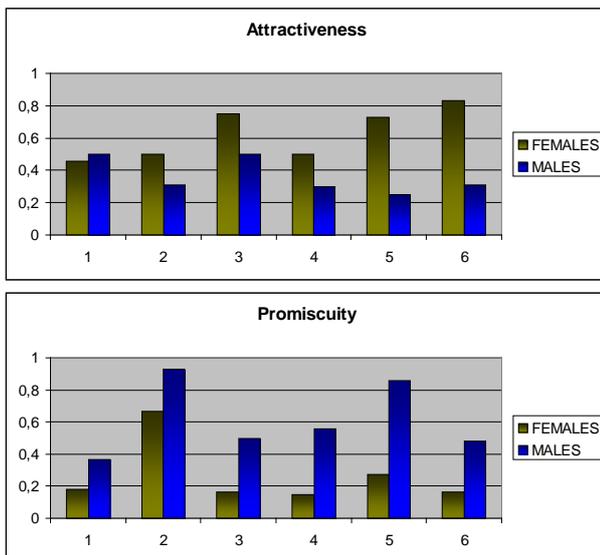


FIGURE 3: Attractiveness (upper) and Promiscuity (lower) calculated for each individual.

There were no significant differences between the kind of substrate for oviposition, neither in natural conditions ($\chi^2=0.909$, 4 df, $P<0.05$) nor

with different widths ($\chi^2=0.139$, 1 df, $P<0.05$). However, there were significant differences in choice between colours in artificial support ($\chi^2=0.508^*$, 4 df, $P<0.05$) and between different densities ($\chi^2=0.001$, 1 df, $P<0.005^{**}$). The females' choice for the colour of the strips were (in order of preference): yellow, white, transparent, blue, and red. They also preferred to hide their eggs in a low rather than high density of aquatic plants.

DISCUSSION

The fact that the *Attractiveness* values are higher in females than in males suggests that, after an encounter happens, it is more probable that a male starts displaying than that a female makes a positive response once the male is displaying her. In other words, females have more probability of finishing the spermatophore transfer phase once the encounter happens. The morphological characteristics that provoke a positive action or response are different in males and females. Males prefer to display to females with a low number of spots, a spot-line background colour whiter rather than bluish, and big eyes in relation to head size. The data suggest that the most cryptic females are positively selected by the males. It can be speculated that this choice confers an advantage in minimizing the risk of predation on the female, and thereby conferring an advantage on the future offspring. The absence of correlation with parameters such as weight and length contrast with the results of Verrel in 1986 for *T. vulgaris*, in which males chose larger females. This is probably due to the small size of our sample or to differences in mating preferences between *T. alpestris* and *T. vulgaris*. On the other hand, females give positive responses to displaying males that have low body mass, small tail, small eyes, small points and narrow heads. These parameters probably explain the conspicuous sexual dimorphism in body size and weight among this species, but also can be related with the age of the males: it is possible that

females tend to choose young males, perhaps due to better sperm quality.

Males in *Triturus* are not very selective in the mating processes (Griffiths, 1995). *Promiscuity* data show that females are significantly more selective than males in mate-choice, especially those that have relative small heads, long tails, big eyes and wide heads. Among males, the more promiscuous are the old ones: big size and weight values, and those that have a wide head. These correlations may be due to the inexperience of the young newts in the first steps of the courtship (recognition of the female and orientation phase).

There were no significant differences between kind of support in the oviposition neither in natural conditions nor with different widths. However, there were significant differences between colours in artificial support and between different densities. The females prefer to lay their eggs on light-coloured substrate that allows light to pass through (yellow, white, transparent) rather than opaque colours (blue, red). It can be related to the light that can reach the egg across the wrapped leaves heating it, and allowing a faster development. It would minimize the risk of egg predation. Although wrapped eggs are inaccessible to the majority of predators, such as aquatic invertebrates or adult newts, and therefore experience reduced predation rates, adult *Dytiscus marginalis* beetles consume all the wrapped eggs (Miaud 1994a). Among densities of submerged plants they prefer to hide their eggs at low than at high density of aquatic plants, probably related also with the increase of light and thereby temperature, that makes the embryo grow faster and avoid the predation.

In conclusion, mating preferences are different between males and females of *T. alpestris*: males are more selective when they are young and prefer to display to well camouflaged females, while females prefer small (and consequently young) males. About egg laying preferences, females choose places where the

light has more intensity, perhaps because such places are associated with higher temperatures and faster development. However, because of the small size of the sample, these results are preliminary and more research must be done.

Acknowledgements. I thank the teaching staff of Aristotle University of Thessaloniki, especially Paschalina Kyriakopoulou-Sklavounou for directing this research. I thank Vasilios Michaelidis and Alexandra Staikou for sharing their laboratory facilities, and Maria Lazaridou for helping to choose the statistical methods. This study was also made possible by Jesús Lizarbe and Joana Branco, who helped with the sampling trip to Pertouli.

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