

## DO PREDATOR CHEMICAL CUES AFFECT OVIPOSITION SITE SELECTION IN NEWTS?

GERMÁN ORIZAOLA AND FLORENTINO BRAÑA

*Departamento de Biología de Organismos y Sistemas, Universidad de Oviedo, c/ Catedrático Rodrigo Uria s/n, 33071 Oviedo, Spain*

Predation on larval stages has been reported to play an important role in structuring amphibian communities, and for this reason the choice of suitable oviposition places is likely to influence newt fitness. In this study, we assessed whether females of four newt species – marbled newt (*Triturus marmoratus*), alpine newt (*T. alpestris*), palmate newt (*T. helveticus*) and Bosca's newt (*T. boscai*) – avoid chemical cues of predatory brown trout (*Salmo trutta*) in selecting their oviposition site. In laboratory tests, individual females were allowed to choose their oviposition site between places with water conditioned by fish chemicals and others with unconditioned water. *T. marmoratus* females selected preferentially tubs without predator cues as oviposition sites, whereas the other three species did not show significant preference under these conditions. Absence of chemical recognition capabilities, strong philopatry towards oviposition site or predator avoidance based in habitat characteristics are suggested as possible causes of the lack of chemical predator avoidance detected in this experiment.

*Key words:* amphibians, egg-laying, predation, *Triturus*, trout

### INTRODUCTION

Amphibians should choose a breeding habitat that maximizes their fitness by increasing offspring survival, growth and development (Kats & Sih, 1992). Considering the large qualitative differences among breeding ponds and the consequences of this variation for reproductive success, the choice of oviposition habitats is often a much more important factor for fitness than reproductive investment or mate choice (Resetarits, 1996). The value of a potential breeding site in aquatic habitats depends on biotic and abiotic factors, such as pond age (Fegraus & Marsh, 2000), temperature (Seale, 1982), vegetation cover (Wells, 1977), and presence of competitors or predators (Morin, 1983). Some amphibian species have developed toxic or unpalatable substances at egg, larval or adult stages as antipredator protection (Kats *et al.*, 1988; Kiesecker *et al.*, 1996), but in addition to these defences, the capacity to evaluate predator presence can be used to minimize contact with predators, thus increasing survival (Sih, 1987; Kats & Sih, 1992; Hopley & Petranka, 1994). Recognition and behavioural responses to predator cues have been extensively studied in larval amphibians (review in Kats & Dill, 1998; Alford, 1999), but the effects on adult stage responses are less well known (e.g. Joly & Miaud, 1993; Aragón *et al.*, 2000; review in Blaustein, 1999).

Fish predation is possibly the most likely biotic factor influencing the suitability of aquatic sites for use by amphibians and other aquatic organisms with complex life cycles (Kats *et al.*, 1988). Besides, fish introductions are widespread and have been reported as one of the factors associated with severe amphibian declines

(Hecnar & McLoskey, 1997; Tyler *et al.*, 1998; Knapp & Matthews, 2000; Gillespie, 2001; Pilliod & Peterson, 2001). For this reason, the aim of this study was to evaluate, in a laboratory experiment, the effect of chemical cues from predatory brown trout (*Salmo trutta*) on the oviposition site selection of adult females of four newt species (*Triturus marmoratus*, *T. alpestris*, *T. helveticus* and *T. boscai*). Chemical cues are more persistent than visual or mechanical cues, so can allow detection of cryptic predators and provide information of past predator presence (Kats & Dill, 1998). In addition, the ability to recognize predators by chemical cues should be advantageous for aquatic prey since it makes predator detection possible in darkness, turbid conditions, physically complex environments or high water volume habitats such as lakes (i.e. in situations in which visual detection is often difficult; Kiesecker *et al.*, 1996; Kats & Dill, 1998). Most previous studies on oviposition site selection were performed with direct presence of free or caged predators (but see Angelon & Petranka, 2002), so that the possible effect of visual or mechanical signals produced by predators could interact with chemical cues.

### MATERIALS AND METHODS

#### STUDY ANIMALS

Experiments were conducted with adult females of four newt species (marbled newt, *Triturus marmoratus*,  $n=56$ , snout-vent length, SVL, size range: 74.7-96.3 mm; alpine newt, *T. alpestris*,  $n=33$ , 53.1-62.0 mm; palmate newt, *T. helveticus*,  $n=66$ , 37.0-47.9 mm and Bosca's newt, *T. boscai*,  $n=64$ , 38.0-51.0 mm). *T. marmoratus* is usually found in well-vegetated ponds in lowland areas, *T. alpestris* is a species characteristic of high elevation areas and cool waters, *T. helveticus* appears to be widespread throughout the study area and *T.*

*Correspondence:* G. Orizaola, Departamento de Biología de Organismos y Sistemas, Universidad de Oviedo, c/ Catedrático Rodrigo Uria s/n, 33071 Oviedo, Spain. E-mail: orizaola@correo.uniovi.es

*boscai* is found more often than other newt species in running waters and small first-order reaches where brown trout are sometimes present (Griffiths, 1995; Barbadillo *et al.*, 1999). Female newts were captured by netting in temporary ponds and cattle-watering tanks of central Asturias (northern Spain) during the 1999 and 2000 breeding seasons. Newts were caught in ponds located in all cases more than 500 m away from watercourses, and had probably not experienced predatory fish previously. Females were maintained for a maximum of one week in the laboratory, and then released in their localities of capture, together with the eggs laid during the experimental period. The predator used in the production of chemical cues was the brown trout (*Salmo trutta*), obtained from first generation hatchery fish originating from a local wild stock.

#### EXPERIMENTAL PROCEDURES

Pairs of large brown trout (*Salmo trutta*; 25.0 to 35.0 cm fork length, mean $\pm$ SE = 28.8 $\pm$ 1.0 cm) were maintained in a 90 litre tank with dechlorinated and aerated water, and provided the conditioned water (*Predator treatment*). Predators were replaced several times during the experiments and returned to the fish hatchery after use. Trout were not fed to avoid contamination of the water with faeces, and the water was used only after trout had had time to clear their guts. Another 90 litre tank with dechlorinated and aerated water was used to provide unconditioned water (*Predator-free treatment*). Both tanks were located in a room held at constant temperature (10°C). Oviposition responses to fish chemical cues were tested in plastic containers (55 x 40 x 17 cm) each with two plastic tubs (2 litre, 18 cm in diameter) inserted in polystyrene foam. In each container, one randomly selected tub was filled every day with conditioned water (with fish chemicals) and the other was filled with unconditioned water. Water was changed daily to maintain potentially detectable levels of fish chemical cues (Petranka *et al.*, 1987). An artificial oviposition support composed of nine strips of cloth (10 x 0.8 cm) suspended from a float of polystyrene (8 x 5 x 1 cm) was placed in each tub. The experimental design was completed by installing access ramps to the tubs and a refuge outside the water filled with damp moss to prevent the desiccation of the females that did not enter the water. The containers, covered with plastic mesh to avoid the escape of females, were located in a room held at a constant 17°C, and illuminated with fluorescent lights with a LD 12:12 photoperiod. All the containers were checked twice a day (at 0800 and 2000 hrs GMT) for the presence of eggs, which were removed. Females that laid eggs in the first three days were maintained for two days more, and those that did not were excluded from the experiment.

#### STATISTICAL ANALYSES

We used binomial tests to test the null hypothesis that the oviposition sites were evenly distributed be-

tween treatments (50:50), both for females which laid eggs in only one tub type (with or without fish chemicals; hereafter "selective females"), and for the place of first oviposition for the females which laid eggs in the two tub types ("non-selective females"). Differences between species in the proportion of selective females were tested by a Chi-squared test. We used one-way ANOVAs (with oviposition site as factor) to test for differences in the size of selective females that laid eggs in each tub type. The effect of oviposition site on the number of eggs laid was analysed using a two-way ANCOVA (with species and oviposition site as factors and female SVL as covariate) for selective females, and a repeated-measures ANCOVA for the non-selective females (with SVL as covariate). In all cases, Scheffé tests were used as post-hoc comparison tests. Deviation from normality was tested with Shapiro-Wilk tests and homogeneity of variance with the Bartlett-Box test. We transformed data (square root) when parametric assumptions of normality and homogeneity of variances were not met.

#### RESULTS

Most selective *T. marmoratus* females selected the tubs without predator chemical cues to lay their eggs (binomial test, proportion 20:8,  $P=0.018$ , marginally significant even after applying the Bonferroni correction at  $P=0.072$ ). Selective females of the other species did not select either of the tub types (*T. alpestris* proportion 5:7,  $P=0.387$ ; *T. helveticus* 18:15,  $P=0.364$ ; *T. boscai* 17:20,  $P=0.371$ ; Fig. 1). We did not find significant differences in the selection of the place of first oviposition for the non-selective females of any of the four species (*T. marmoratus* proportion 4:4,  $P=0.636$ ; *T. alpestris* 1:5,  $P=0.109$ ; *T. helveticus* 11:9,  $P=0.412$ ; and *T. boscai* 9:7,  $P=0.402$ ). No differences were found in the proportion of selective females between species ( $\chi^2=2.5$ ,  $df=3$ ,  $P=0.481$ ). The size of the selective females was not significantly different between those that laid eggs in

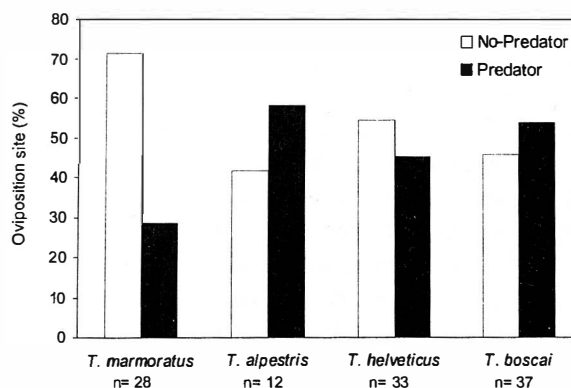


FIG. 1. Oviposition site selection in newts, expressed as the percentage of selective females that used the predator-conditioned or the predator-free tub to oviposit. Total sample size appears under species name.

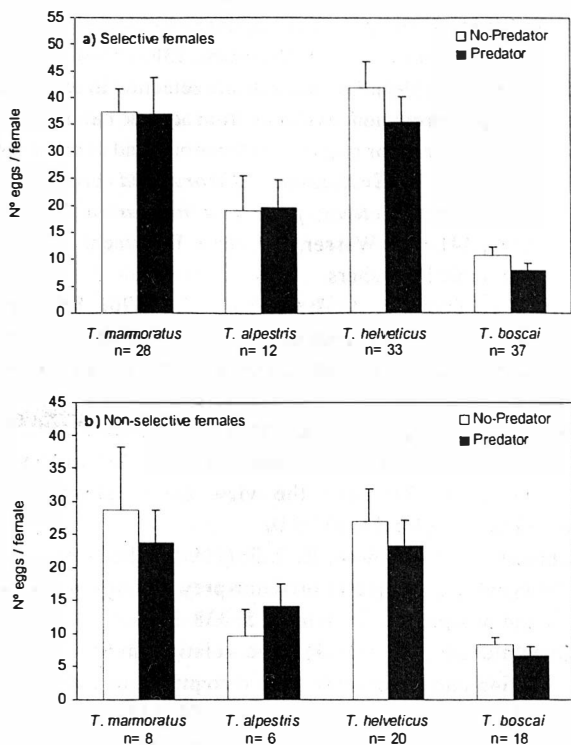


FIG. 2. Number of eggs laid per female newt (mean +SE) in fish-conditioned and unconditioned tubs: (a) females that laid eggs in just one tub type (selective); and (b) females that laid eggs in both tub types (non-selective females). Number of females considered in each case appears under species name.

predator tubs and in non-predator tubs (ANOVAs,  $F_{1,24} = 0.158$ ,  $P = 0.694$  for *T. marmoratus*;  $F_{1,6} = 2.652$ ,  $P = 0.154$  for *T. alpestris*;  $F_{1,28} = 0.640$ ,  $P = 0.430$  for *T. helveticus*;  $F_{1,35} = 3.841$ ,  $P = 0.058$  for *T. boscai*). The average number of eggs laid per female in the experimental period was significantly different between species, even considering size-corrected values (ANCOVA: selective:  $F_{3,92} = 31.5$ ,  $P < 0.001$ ; non-selective:  $F_{3,43} = 13.1$ ,  $P < 0.001$ ; in both cases  $T. marmoratus = T. helveticus > T. alpestris = T. boscai$ ), but not significantly different between tub types either in the case of selective females (Oviposition place:  $F_{1,100} = 0.6$ ,  $P = 0.449$ ; Fig. 2a), or non-selective females (Oviposition place:  $F_{1,44} = 0.04$ ,  $P = 0.831$ ; Fig. 2b).

## DISCUSSION

Newts frequently use fishless locations as reproductive habitats (e. g. low volume and temporary ponds), avoiding running waters typically occupied by fish (Griffiths, 1995). In the mountain lakes of the study area we have previously reported a strong negative effect of fish presence on amphibian distribution and abundance (Braña *et al.*, 1996). This could be a consequence of direct predation, habitat selection or detection of predators (visual, mechanical or chemical). Chemical senses of olfaction and taste are well developed in *Triturus* species and are important in sexual and feeding behaviours (Cogalniceanu, 1994; Aragón *et al.*, 2000), and in searching aquatic habitats suitable for reproduc-

tion (Joly & Miaud, 1993). The results of our experiments indicate that *T. marmoratus* females use fish-predator chemical cues to avoid oviposition in potentially risky situations, selecting non-conditioned tubs in which to lay their eggs. In the case of *T. helveticus*, all the responses were also developed in the direction of predator avoidance but they were not statistically significant. The other two species did not show any preferences between tub types. The results obtained for *T. marmoratus* are consistent with some previous studies that showed modifications of behaviour in prey exposed to predator chemical cues (revision in Kats & Dill, 1998). In particular, predator avoidance in oviposition site selection has been reported for several amphibian species by Resetarits & Wilbur (1989: *Hyla regilla*), Kats & Sih (1992: *Ambystoma barbouri*), Hopey & Petranka (1994: *Rana sylvatica*), Spieler & Linsenmair (1997: *Hoplobatrachus occipitalis*) and Binckley & Resetarits (2002: *Hyla squirella*). In these cases predator avoidance could provide an alternative mechanism to explain the negative spatial association between predatory fish and several amphibian species, which are usually attributed to contemporary predation.

In our experiment, *T. marmoratus* exhibited strong avoidance of predator chemical cues, whereas the other three species did not show any preference. The low number of species used in the experiment prevents us from testing potential ecological and evolutionary correlates of predator avoidance behaviour within a phylogenetic framework. The *cristatus-marmoratus* species group has been signalled as an assemblage of close-related species apart from other newt species, such as *T. alpestris*, *T. helveticus* and *T. boscai* (Busack *et al.* 1988; Zajc & Arntzen, 1999). Because of this separation, *T. marmoratus* exhibit several morphological, physiological and behavioural particularities such as a greater size and a greater use of vegetated habitats (Griffiths, 1995, and personal observations). These characteristics could be associated with a greater use of chemical cues for predator detection, but more studies should be developed to understand the real causes of these differences in antipredator behaviour.

The lack of antipredator avoidance with respect to oviposition site selection and number of eggs laid in other *Triturus* species could also indicate that female newts do not activate antipredator responses using chemical cues associated only with predator presence. This activation could also depend on the simultaneous perception of other cues associated with prey alarm or predator feeding activity (Laurila *et al.*, 1997; Chivers & Smith, 1998). Besides, cues other than chemicals, – such as visual or tactile signals (or some combination of cues) – could be needed to trigger defensive responses. In this regard, Stauffer & Semlitsch (1993) showed that tadpoles of *Rana lessonae* and *R. esculenta* exhibit enhanced responses to fish predator chemical cues, when combined with tactile signals. Otherwise, newt larvae reach metamorphosis only in suitable places (i.e. those with reduced risk of desiccation, competition and preda-

tion), so they could have evolved strong philopatry with respect to oviposition places instead of developing or maintaining detection mechanisms for fish predators that are naturally scarce in their environment (McPeck, 1989; Laurila & Aho, 1997). Also, the avoidance of habitats favourable to fish as oviposition sites could have evolved as the mechanism of predator avoidance, rather than detecting and avoiding the fish itself. In some instances, habitat avoidance could be more effective than predator detection, which is highly dependent on predator temporal and local presence, density or stimulus dilution (Anholt *et al.*, 2000; Van Buskirk & Arioli, 2002). For the species used in this work, this kind of habitat avoidance could be responsible for the use by newts of temporary ponds or water bodies unconnected to streams, as fish populations may not be viable in such habitats. In this scenario fish introductions in naturally fishless habitats (i.e. mountain lakes) can have severe effects on amphibian populations, as has been previously suggested (Terrero 1951; Braña *et al.* 1996). However, further studies are needed to understand the responses of newts to fish presence in more natural situations or when exposed to other types of predator cues.

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