OVIPOSITION BEHAVIOUR AND VULNERABILITY OF EGGS TO PREDATION IN FOUR NEWT SPECIES (GENUS *TRITURUS*)

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Most animals develop some kind of parental care in order to protect eggs or offspring from predation. Female newts (genus *Triturus*) protect eggs from predators by wrapping them individually in plant leaves. We studied oviposition characteristics of four newt species inhabiting the northern Iberian Peninsula (marbled newt, *Triturus marmoratus*; alpine newt, *T. alpestris*; palmate newt, *T. helveticus* and Bosca's newt, *T. boscai*). All of these species are able to wrap their eggs in aquatic plants in laboratory experiments, but – whereas *T. marmoratus*, *T. alpestris* and *T. helveticus* wrapped more than 90% of their eggs – *T. boscai* covered only half of the eggs completely with leaves. *T. boscai* is found in running waters more frequently than the other species, and lays larger eggs relative to female size, as is typical of running water urodeles. A parallel experiment exposing newt eggs to predation by larvae of the dragonfly *Aeshna cyanea*, demonstrated the protective value of wrapping behaviour. About half of the unwrapped eggs were consumed, whereas protected eggs remained almost unattacked.

Key words: Aeshnidae, egg-laying, Odonata, parental care, Urodela

INTRODUCTION

Amphibians show a great diversity of reproductive modes ranging from the deposition of masses of eggs in ponds to viviparity (Duellman & Trueb, 1986). The development of each reproductive mode is associated with different degrees of exposure of eggs and larvae to environmental factors such as predation, competition and desiccation. Amphibian larvae are well known to develop antipredator strategies (Sih, 1987; Alford, 1999), but eggs are more vulnerable to predation and other potential risks due to their immobility. In contrast to those of other salamanders, newt eggs (genus *Triturus*) are not known to contain unpalatable or toxic substances (Ward & Sexton, 1981), but female newts attach eggs individually to aquatic plants, wrapping these eggs by means of adhesive substances on the egg membranes, as reported by Díaz-Paniagua (1989) for *Triturus* (*marmoratus*) *pygmaeus,* and Miaud (1994*b*) for *T. alpestris* and *T. helveticus*. Wrapped eggs are inaccessible to the majority of predators, such as aquatic invertebrates or adult newts, and therefore experience reduced predation rates (Miaud, 1993).

The present study examines oviposition behaviour in four newt species inhabiting the northern Iberian Peninsula (marbled newt, *Triturus marmoratus*; alpine newt, *T. alpestris*; palmate newt, *T. helveticus* and Bosca's newt, *T. boscai*), and also experimentally tests for the effect of wrapping behaviour on egg survival in the presence of dragonfly larvae, *Aeshna cyanea*, one of the top predators found in ponds and other temporal aquatic habitats in the study area. Previous studies with other dragonfly species (Richards & Bull, 1990) indicated that predatory larvae detect the eggs by tactile cues, so wrapping eggs in leaves could prevent dragonflies detecting their presence. *A. cyanea* has also been reported to alter the behaviour and development of newt larvae (Van Buskirk & Schmidt, 2000; Schmidt & Van Buskirk, 2001).

MATERIALS AND METHODS

During the 2000 and 2001 reproductive seasons, adult females of four newt species (*T. marmoratus*, n=32; *T. alpestris*, n=13; *T. helveticus*, n=44; *T. boscai*, n=55) were collected in several pools and cattle-watering tanks in Asturias (northern Spain) and transferred to the laboratory. Females were placed for five days at 17°C and LD 12:12 photoperiod in individual plastic containers (18 cm in diameter) that held one artificial oviposition support consisting of nine cloth strips (10 x 0.8 cm) suspended from a float of polystyrene foam plate (8 × 5 × 1 cm). Eggs were removed from the strips twice a day and placed for 24 hr in a drying chamber at 50°C until the mass remained constant. Dry egg mass was recorded to the nearest 0.0001g (number of eggs: *T. marmoratus*, n=1379; *T. alpestris*, n=269; *T. helveticus*, n=1961; *T. boscai*, n=609). We recorded female wrapping behaviour by placing newts in the same type of plastic containers filled with tap water and *Glyceria* sp. leaves taken from nearby ponds. This type of vegetation was present in the localities of capture and was frequently used by newts (*T. cristatus, T. alpestris* and *T. marmoratus*) in experiments developed by Miaud (1995) studying the selection of plants during newt oviposition. Natural vegetation was used to develop the experiments in realistic conditions. Oviposition was controlled every two days for a total of ten days per female. Only females that laid at least 15 eggs were considered in the experiment (numbers of females: *T.*

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marmoratus, *n*=18; *T. alpestris*, *n*=20; *T. helveticus*, *n*=21; *T. boscai*, *n*=13). Eggs were classified as wrapped if they were completely covered by folds of the leaves or unwrapped if they were uncovered (number of eggs: *T. marmoratus*, *n*=683; *T. alpestris*, *n*=897; *T. helveticus*, *n*=898; *T. boscai*, *n*=260). The effect of wrapping behaviour on egg predation by dragonfly larvae was investigated in a 24 hr experiment conducted in plastic containers (18 cm in diameter) filled with 0.4 litre of water. Half of the containers received ten eggs wrapped with naturally vegetation, whereas the other half received ten unwrapped eggs. Wrapped eggs were selected from large samples of eggs so that the degree of wrapping was the same between species, i.e. only eggs completely covered with leaves were used. Unwrapped eggs were obtained from naturally unwrapped eggs if possible, and in the other cases by the careful removal of the vegetation covering wrapped eggs. This egg manipulation has been described by Miaud (1994a) as not affecting the survival of the embryos. Late instar *Aeshna cyanea* dragonfly larvae were collected from local water bodies and allowed to acclimatize to laboratory conditions for three days. Dragonflies used in the trials were starved for three days before the experiments to minimize the effect of satiation on predator performance. Each predator was used only once during the experiments. Trials for both treatments were repeated 15 times for each species. Total egg-mass consumption by *Aeshna* larvae in 'unwrapped' trials was calculated by multiplying the number of eggs consumed in each trial by the dry egg mass for each species, obtained as stated above. After the experiments all the dragonflies, newt females and the remainder of the eggs were released in their original locations.

One-way ANOVA's and Scheffé post-hoc tests were used to test for differences in female size (snout-vent length) and in dry egg mass between the four newt spe-

cies. Differences in oviposition tactics (mean percentage of wrapped or unwrapped eggs per female) and unwrapped egg survival between species were analysed using a Kruskal-Wallis test and Mann-Whitney tests for post-hoc paired comparisons. The effect of oviposition mode on egg predation by *Aeshna* was tested using a Mann-Whitney test. Differences in total egg mass consumption were tested with a Kruskal-Wallis test. Deviation from normality was tested with a Shapiro-Wilk test and homogeneity of variance with a Bartlett-Box test.

RESULTS

There were significant differences in female SVL among species (ANOVA: $F_{3,140}$=958.95, P<0.001; Scheffé test: *T. marmoratus* > *T. alpestris* > *T. helveticus* = *T. boscai*; Table 1). Dry egg mass differs significantly among species ($F_{3,140}$=176.71, P<0.001), revealing Scheffé post-hoc test differences between all the species except for *T. alpestris* and *T. boscai* (*T. marmoratus* > *T. boscai* = *T. alpestris* > *T. helveticus*; Table 1). Oviposition tactics differed between species (Kruskal-Wallis test: H_3=32.153, P<0.001; Table 1), with *T. alpestris* having a significantly higher percentage of eggs wrapped with the vegetation than the other species (Mann-Whitney tests: *T.mar-T.alp*: $U_{18,20}$=89, P=0.007; *T.alp-T.helv*: $U_{20,21}$=73, P<0.001; *T.alp-T.bosc*: $U_{20,13}$=17, P<0.001). No differences in percentage of wrapped eggs were found between *T. marmoratus* and *T. helveticus* ($U_{18,21}$=158.5, P=0.389), whereas *T. boscai* differed from the other species and had only a half of their eggs wrapped (*T.mar-T.bosc*: $U_{18,13}$=19.5, P<0.001; *T.helv-T.bosc*: $U_{21,13}$=25, P<0.001). When newt eggs were exposed to *Aeshna* larvae predation, egg survival was positively influenced by wrapping ($U_{60,60}$=557.5, P<0.001; Fig. 1). Differences in unwrapped eggs survival were found only between *T.*

TABLE 1. Summary of female size and characteristics of the eggs laid by Triturus species. All values are presented as mean ± SE, with range in parentheses.

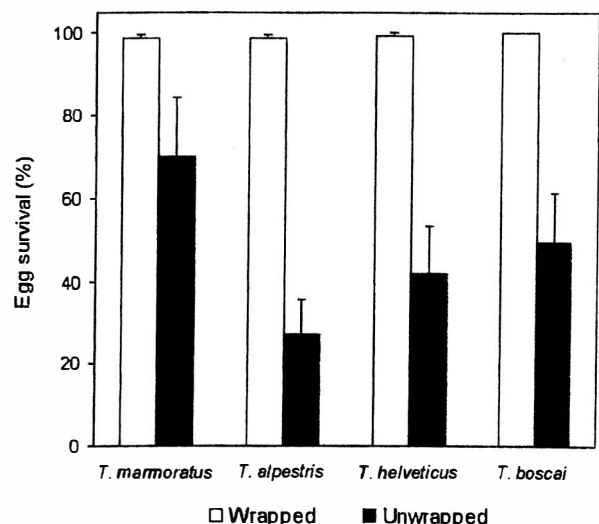| Female SVL (mm)	Egg dry mass (mg)	% wrapped eggs
T. marmoratus		
82.95±1.07	2.1±0.05	92.47±1.53
(68.28-96.31)	(1.5-2.7)	(78.57-100)
T. alpestris		
56.40±0.68	1.6±0.04	96.81±0.72
(53.03-62.02)	(1.4-2.0)	(88.88-100)
T. helveticus		
42.91±0.37	0.9±0.02	91.25±1.37
(38.01-47.98)	(0.6-1.2)	(75.47-100)
T. boscai		
42.81±0.38	1.7±0.03	45.81±7.55
(38.00-51.01)	(1.2-2.4)	(5.26-100)

FIG. 1. Effect of oviposition mode (wrapped or unwrapped egg) on the percentage of surviving eggs (mean + SE) after 24 hrs of exposition to larval dragonfly, *Aeshna cyanea*, predation.

marmoratus and *T. alpestris* ($U_{15,15}$=43, P=0.003). Total egg mass consumption by *Aeshna* presented marginally significant differences between species (H_3=7.62, P=0.054), being higher for *T. alpestris* (11.62±1.37 mg). Differences using Mann-Whitney tests were obtained only between *T. alpestris* and the two species with lower egg mass (*T. marmoratus* $U_{15,15}$=64, P=0.041 and *T. helveticus* $U_{15,15}$=35, P=0.001).

DISCUSSION

The results of this study revealed that wrapping behaviour is common in all the species considered. To our knowledge, this study provides the first data on wrapping behaviour in *T. boscai*, which – contrary to other species – wraps only a half of eggs in leaves. This species also lays eggs that are twice the size of those laid by a similar-sized species (*T. helveticus*), and is the only species frequently found in streams during the reproductive season. This reflects the pattern found in 74 salamander species by Kaplan & Salthe (1979), who observed that egg size was smaller in relation to female size in pond breeders than in those species breeding in running waters. A relatively large egg size could present difficulties in terms of egg manipulation in *T. boscai* females, affecting the wrapping sequence of eggs with plant leaves and consequently the percentage of eggs protected. *T. boscai* eggs can be found in natural habitats unwrapped or adhering to the underside of rocks, behaviour rarely observed in the other three species. Percentages of wrapped eggs observed for *T. alpestris* and *T. helveticus* are higher than those reported by Miaud (1994a) in previous experiments in which only 75% of the eggs were wrapped with the leaves.

Wrapping behaviour reduces algal infection and UV-B-damaging effects in *Triturus* eggs (Marco *et al.*, 2001), but the most frequently reported function was related to protecting eggs from predators (Miaud, 1993, 1994a). Both vertebrate and invertebrate predators have been reported to attack and consume amphibian eggs causing a significant reduction in reproductive success (Henrikson, 1990; Miaud, 1993; Axelsson *et al.*, 1997; Richter, 2000; Monello & Wright, 2001). Our results showed that the wrapping behaviour developed by female newts is highly effective in protecting eggs from *Aeshna* larvae predation. *Aeshna* larvae eat about a half of the unwrapped eggs whereas wrapped eggs did not suffer noticeable mortality. *T. marmoratus*, the species that laid bigger eggs, experienced the lowest percentage of egg consumption, but this is unlikely to be an effect of predator satiation because *A. cyanea* larvae consumed higher amounts (total dry egg mass) of *T. alpestris* and *T. boscai* eggs. Differential egg consumption could be due to differences between species in egg characteristics such as presence of toxic compounds or variation in chemical cues emitted by eggs. Experiments developed by Miaud (1993; 1994a) in order to study the protective effect of newt egg-wrapping behaviour found that 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. Furthermore, after one week in natural habitats no unwrapped eggs survived, whereas 20-54% of the wrapped eggs were still alive (Miaud, 1994a). In this study, predatory larvae were observed moving along the leaves where eggs are attached, searching for and identifying eggs using their antennae before eating them. So, wrapped eggs seem to be undetectable by the tactile sensory capacities of *A. cyanea*.

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