Response of predator-naive newt larvae to food and predator presence

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Abstract: For naive prey, the ability to recognize predators is advantageous at the time of first predator encounter. After predator detection, prey could avoid the risk of being eaten by modifying the patterns of activity and use of habitat. The use of refuges is considered one of the most widespread antipredator tactics. In laboratory experiments, we tested the effects of food (cladocerans, Daphnia sp.) and predator (brown trout, Salmo trutta) presence on larval newt (Triturus helveticus and Triturus alpestris) refuging behaviour, and the effect of refuge use on larval survival. Newt larvae came from lakes without fish and were reared in the laboratory from the egg stage so that they lacked any previous experience with brown trout. Larvae of both species increased refuge use and reduced significantly their emergence rate in the presence of predators. Food had no effect on refuge use or activity. For both species, refuge availability increased larval survival by more than twofold when the predator was present. These results suggest that naive newt larvae are able to detect predators and show an innate antipredator response.

Résumé : Chez les proies inexpérimentées, la capacité de reconnaître les prédateurs constitue un avantage lors de la première rencontre avec un prédateur. Une fois le prédateur détecté, la proie peut éviter le risque d’être mangée en modifiant ses patterns d’activité et son utilisation de l’habitat. L’utilisation de refuges est reconnue comme l’une des tactiques anti-prédateurs les plus répandues. Des expériences de laboratoire nous ont permis de vérifier les effets de la présence de nourriture (cladocères, Daphnia sp.) et de prédateurs (truite brune, Salmo trutta) sur le comportement de dissimulation de larves de tritons (Triturus helveticus et Triturus alpestris), ainsi que les effets de l’utilisation de refuges sur la survie des larves. Les larves proviennent de lacs sans poissons et elles ont été élevées en laboratoire depuis l’œuf; elles n’ont donc aucune expérience de la présence de la truite brune. En présence de prédateurs, les larves des deux espèces utilisent plus les refuges et réduisent leur taux d’émergence. La présence de nourriture reste sans effet sur l’utilisation des refuges ou sur l’activité. Chez les deux espèces, la disponibilité de refuges accroit la survie des larves en présence d’un prédateur par un facteur de plus de deux. Ces données indiquent que les larves inexpérimentées de tritons sont capables de reconnaître les prédateurs et qu’elles possèdent une réaction anti-prédateurs innée.

[Intaduit par la Rédaction]

Introduction

Prey species should be able to detect predator presence to diminish the risk of being eaten. In aquatic ectotherms, predator detection can be based on visual, mechanical, or chemical predator cues or be mediated by alarm or injury signals emitted by prey (Wilson and Lefcort 1993; Chivers and Smith 1998; Kats and Dill 1998). One crucial point for prey survival is established at the time of first predator encounter when naive prey should be able to react to the predator presence in a way that increases its survival possibilities (Magurran 1990). Several studies have signalled the requirement of previous predation experience and learning to develop antipredator decisions (Magurran 1989; Mathis and Smith 1993; Chivers and Smith 1994, 1995). Some naive organisms can also recognize predation risk without direct exposure by using alarm cues released by injured conspecifics or feeding substances released by predators (Wilson and Lefcort 1993; Chivers and Smith 1998; Chivers and Mirza 2001). Antipredator responses in naive animals not previously exposed to any predation cue have been reported in a few cases (e.g., for fathead minnows, Phoxinus phoxinus, Magurran 1990; for roach, Rutilus rutilus, Jachner 2001). Previous studies on the role of experience on the development of amphibian antipredator behaviour have not shown conclusive results: some species modify their defensive strategies with learning (e.g., Semlitsch and Reyer 1992), whereas others develop protective responses without any experience (e.g., Kats et al. 1998; Mathis et al. 2003).

One of the most widespread behaviours developed by prey to avoid predators is the use of refuges (Sih et al. 1985, 1988). An increase in refuge use is associated with reductions of food intake, of mating opportunities, or some physiological costs (Lawler 1989; Horat and Semlitsch 1994; Martín and López 1999). Therefore, prey is expected to evolve flexible strategies to manage the use of refuges, minimizing the negative costs associated with inactive periods (Martín and López 1999). In amphibians, activity reduction associated with refuge use may have, in addition to other costs, a negative effect on growth and developmental rates, increasing the time necessary to reach metamorphosis (Sih...
et al. 1988; Jackson and Semlitsch 1993), and therefore increasing the time that larvae are exposed to aquatic predators and to the risk of habitat desiccation (Laurila and Kujasalo 1999). The development of this kind of behavioural antipredator responses has been well studied in anuran larvae (see Lawler 1989; Alford 1999; Van Buskirk 2000), but less so in the case of tailed amphibians (Holomuzki 1986; Semlitsch 1987; Sih et al. 1992; Van Buskirk and Schmidt 2000).

The aim of this paper was to investigate the capability of predator-naïve newt larvae to alter their behaviour under a disturbing situation associated with predator presence. In the first experiment, we used larvae of two newt species, palmarine newt (Triturus helveticus Razoumowskii, 1789) and alpine newt (Triturus alpestris Laurenti, 1768), and tested the effect of food (Daphnia sp.) and predator presence (nonlethal brown trout, Salmo trutta) on overall refuge use (time in refuges and refuge emergence rate), activity, and food consumption. In a second experiment, we quantified the differences in survival when larvae were exposed to lethal predatory fish in the presence or absence of refuges.

**Materials and methods**

**Study animals**

Gravid *T. alpestris* (*n* = 31) and *T. helveticus* (*n* = 13) females were collected in June and July 2000 from two small mountain lakes of Asturias, northern Spain (Veneros and La Sobia, elevation 1300 and 1200 m, respectively), that have no fish. Females were allowed to lay eggs in natural vegetation arranged in laboratory aquaria and then returned to the lake of origin. At hatching, each newt larva was introduced into an individual 50-mL plastic flask. The larvae were held at constant temperature (17 °C) at a 12 h light : 12 h dark photoperiod and were fed live zooplankton (cladocerans and copepods) and frozen chironomid (Chironomidae) larvae every 2 days. Only larvae with well-developed limbs were used in the experiment. Larvae were measured 2 days before the experiments and assigned to each treatment to avoid size differences among groups. Total length (mean ± SE) of the larvae used in the trials was 16.94 ± 0.11 mm for *T. helveticus* and 17.80 ± 0.09 mm for *T. alpestris*. To standardize hunger level, the newt larvae were starved for 2 days before the experiments. After the experiments, all the larvae were released to their places of origin. Brown trout predators came from the spawning of wild parents from the Color River, Asturias (northern Spain). Brown trout is the most common fish species in rivers of the area (Reyes-Gavilán et al. 1995) and is known to be an active predator of newts (Brñăa et al. 1996). Brown trout used in the trials (fork length 116.80 ± 3.90 mm, mean ± SE) were starved for 3 days before the experiments to minimize the effect of satiation on predator performance and to avoid faecal contamination of the water. Between trials, brown trout were fed commercial pellets and chironomid larvae. The experiments were carried out in November and December 2000, in a room held at constant 17 °C.

**Experimental procedures**

The effect of food and predator presence on newt behaviour was studied in white plastic containers (40 cm long, 31 cm wide), which were filled with about 9 L of dechlorinated tap water to a depth of 7.5 cm. A transparent acetate sheet divided the container into two compartments, a smaller one (12 cm × 31 cm) to lodge the brown trout predator and a larger one (28 cm × 31 cm) for the prey. The partition was perforated to allow water diffusion but not animal movement between compartments. The bottom of the prey compartment was marked with lines dividing it into four quadrants. To provide refuges to the newts, we placed in the centre of each quadrant a piece of mesh (8 cm × 8 cm) elevated 1 cm above the bottom by four polystyrene cubes on the corners of the mesh. The water was aerated continuously by a single airstone located in the predator compartment. Two predator treatments (one brown trout or no predator) and two food treatments (15 Daphnia sp. added to the newt compartment or no food) were tested. A total of 14 brown trout were used in the trials, introducing one brown trout in the container 5 min before introducing the newt to allow the fish to acclimate and to allow the predator odour to diffuse throughout the container. Then a newt larva was transferred to the centre of the prey compartment and was left to acclimate for 5 min. After the acclimation period, the trial started and we recorded the position of the larva every minute for 30 min. From these recordings, we calculated the percentage of time that the larvae spent under refuge, the number of times that the larvae left the refuge (for larvae that use the refuge at least once), and larval activity (the number of times that larval changed quadrants). The number of *Daphnia* sp. remaining at the end of the trial was also recorded for the food treatments. Between trials the containers were thoroughly rinsed. In total 68 *T. alpestris* and 56 *T. helveticus* larvae were used in this experiment. There were no within-species differences in larval length among the four treatment combinations (ANOVA; *T. helveticus*: \( F_{[3,39]} = 0.474, P = 0.701; T. alpestris: F_{[3,39]} = 0.175, P = 0.912 \)).

We studied the effect of refuge availability (four refuges or no refuge) on the survival of the two newt species in white plastic containers (60 cm long, 40 cm wide). A removable plastic mesh partition divided the containers into two compartments, a smaller one to hold the predator and a larger one where pieces of plastic similar to those used in the previous experiment served as refuges for the newts. The containers were filled to a depth of 7.5 cm with 20 L of dechlorinated tap water that was continuously aerated with one airstone. The order of the trials was completely randomized, rinsing the containers after each trial. One brown trout was used as the predator and it was allowed to acclimate for 5 min before introducing the newts. Because only nine brown trout were available, they were evenly assigned to the different trials. Time between trials was at least 12 h, and satiation problems were not detected. Because of the time elapsed between the trials and the small size of prey available, it is unlikely that feeding cues emitted by brown trout could have had any effect on newt larval behaviour. Five minutes before a trial started, three newt larvae were transferred to the centre of the larger compartment and the trial started when the partition was removed. Because of the different availability of individuals from the two species, treatments involving *T. alpestris* were replicated 11 times, whereas treatments with *T. helveticus* were replicated 9 times.
Fig. 1. Refuge use expressed as the percentage of time (mean + SE) that newt larvae spent under refuge in relation to predator presence and food availability. Treatments were replicated 14 times for *Triturus helveticus* and 17 times for *Triturus alpestris*.

There were no within-species differences in larval body length among treatments (ANOVA; *T. helveticus*: $F_{[1,41]} = 0.025$, $P = 0.873$; *T. alpestris*: $F_{[1,64]} = 0.337$, $P = 0.563$) or among the groups of three larvae in each treatment (ANOVA; *T. helveticus*: $F_{[17,36]} = 1.110$, $P = 0.381$; *T. alpestris*: $F_{[17,23]} = 0.433$, $P = 0.961$).

Both newt species are abundant and not endangered in the study area, inhabiting the majority of ponds and lakes. In addition, special considerations have been given to reduce the pain of prey animals. The first experiment involved no physical contact between predators and prey, whereas in the second experiment, when prey consumption was necessary to accomplish the objectives of the study, trials were performed using the minimum number of replicates.

**Statistical analyses**

We used a three-way ANOVA (newt species, predator presence, and food as factors) to test for differences in the percentage of time spent in refuge and number of refuge emergences, and a three-way ANCOVA (with the number of times that each larva was outside the refuge as covariate) to analyse larval activity. A two-way ANOVA was used to examine the effect of predator presence on the number of *Daphnia* sp. eaten by the two newt species. Differences in larval survival in relation to refuge availability were analysed using a $G$ test. The level of significance was set at $\alpha = 0.05$ for all tests. Percent data were arcsine square-root transformed, and the square-root transformation was used in the case of the number of *Daphnia* sp. eaten.

**Results**

*Triturus helveticus* used the refuges more than *T. alpestris* ($F_{[1,116]} = 40.427$, $P < 0.001$) and both species increased refuge use with predator presence ($F_{[1,116]} = 13.036$, $P < 0.001$), but no effect of food was detected ($F_{[1,116]} = 0.057$, $P = 0.812$; Fig. 1). Refuge use increased during the experiment for both species. At the end of the trials (30 min), the percentage of *T. helveticus* under refuge in the treatment with no predator was 57% lower than in treatments with a predator, and it was 23% lower in the case of *T. alpestris*. A significant interaction was detected between newt species and predator presence ($F_{[1,116]} = 40.427$, $P < 0.001$), revealing a stronger response in *T. helveticus* larvae than in *T. alpestris* larvae. Newt larvae not exposed to predators moved more actively in and out of the refuge ($F_{[1,97]} = 10.169$, $P = 0.002$; Fig. 2). Food had no effect on the number of refuge emergence ($F_{[1,97]} = 0.0086$, $P = 0.981$). *Triturus alpestris* showed a higher rate of refuge emergence than *T. helveticus* ($F_{[1,97]} = 8.275$, $P = 0.005$). Neither food nor predator presence had a significant effect on the activity of newt larvae outside refuges (predator: $F_{[1,110]} = 2.238$, $P = 0.137$; food: $F_{[1,110]} = 2.369$, $P = 0.127$). There was, however, a significant interaction effect between predator and food ($F_{[1,110]} = 8.617$, $P = 0.004$) that was related to higher activity in trials with food and no predators ($P = 0.033$). The number of *Daphnia* sp. consumed by newt larvae did not differ between species ($F_{[1,120]} = 0.156$, $P = 0.693$) or predator treatments ($F_{[1,120]} = 0.054$, $P = 0.815$).

When the newts were exposed to a lethal predator, larval survival was positively influenced by the presence of refuges ($G = 4.37$, $P < 0.05$ for *T. alpestris*; $G = 5.384$, $P < 0.025$ for *T. helveticus*), which were highly used throughout the trials (mean percentage of larvae under refuge was 79% for *T. alpestris* and 96% for *T. helveticus*). The number of newt larvae surviving when refuges were available was, on average, 44% higher for *T. helveticus* and 33% for *T. alpestris* (Fig. 3).

**Discussion**

Predator recognition has an obvious associated survival value for prey, and the earlier the recognition is developed, the greater is the benefit. The results of this study show the capacity of naïve newt larvae to respond to predatory fish presence; the presence of brown trout increases the use of
Fig. 3. Effect of refuge presence on $T. helveticus$ and $T. alpestris$ survival. Data are expressed as the percentage of surviving larvae (mean ± SE) at the end of the experimental period (30 min). For each treatment, sample size (n) is 27 for $T. helveticus$ and 33 for $T. alpestris$.

refuges and reduces the emergence rates of naïve newt larvae. In the experiments, we used laboratory-reared newt larvae from lakes without fish and exposed them to brown trout that had not previously eaten newts. Also, the newt larvae were maintained individually isolated during their development and were individually exposed to predators so that results were free from the effects of previous experience or from interactive effects produced by alarm or feeding cues released by conspecifics. Therefore, the results of our experiment suggest the existence of innate defensive behaviour. These responses could be fine-tuned with learning in situations of coexistence between predators and prey (Magurran 1990; Semlitsch and Reyer 1992; Chivers and Smith 1994).

The observed increase in refuge use and the consequent reduction in activity have been described for different prey species as typical responses to predator presence (Sih et al. 1988, 1992; Lima 1998; Van Buskirk and Schmidt 2000). An increase in refuge use implies a reduction in the time that the animal is exposed to predators and, for this reason, it has been associated with an increase in survival (Sih et al. 1988; Lawler 1989; Skelly 1994). In this study, refuge availability increased newt survival by more than twofold in relation to trials without refuges. Refuge presence also contributed to increased habitat complexity, which could reduce the predator–prey encounter rate, especially in visually oriented predators such as brown trout, and could also impair predator visual capabilities and movements (Werner et al. 1983; Sredl and Collins 1992; Babbitt and Jordan 1996; Babbitt and Tanner 1997). However, refuge use also reduces the opportunities for social interactions or foraging (Sih 1987; Martín and López 2000). Therefore, prey should be able to evaluate predator persistence to reinitiate normal activity (Martín and López 1999, 2000). In this experiment, we have shown that newt larvae under a risky situation had low emergence rates from refuges, whereas larvae in predator-free treatments moved frequently in and out of the refuges. Permanence in refuges could be associated with periods of prey latency after detecting predator cues or with prey detecting the persistence of risk (Sih 1992).

Defensive behaviour of newt larvae exposed to brown trout could not be strictly considered a specific antipredator behaviour but could be interpreted as a response to a general disturbance or a conservative response to a potential predator (see Mathis et al. 2003). Additional experiments are necessary to address such specificity. In any case, the development of protective behaviours could be advantageous for prey exposed to a wide range of potential predators, as occurs in the breeding habitats of newts (Van Buskirk and Schmidt 2000; G. Orizaola, personal observations). Behavioural responses such as reduction in activity and increase in refuge use have been reported for many amphibian species exposed to predation risk (Sih et al. 1988, 1992; Kats et al. 1998; Van Buskirk and Schmidt 2000; Teplitsky et al. 2003). On the other hand, several studies have shown that different amphibian species (e.g., $Rana sylvatica$, $Rana temporaria$, $T. alpestris$, $T. helveticus$) exposed to different predators are also able to develop similar morphological modifications (deep tails, small bodies) that increase their capacity to escape from predators (Van Buskirk and Relyea 1998; Van Buskirk and Schmidt 2000; Relyea 2001; Van Buskirk 2002).

Our results showed that newts recognize risky situations and display innate responses, and such capacity should favour the maintenance of fish–newt communities in habitats where they meet in natural conditions. However, in our study area, a definite decrease in newt abundance has been reported in fish-inhabited lakes (Braña et al. 1996; G. Orizaola and F. Braña, unpublished data). The responses observed in this study were stronger for $T. helveticus$ larvae than for $T. alpestris$ larvae, paralleling the results obtained by Van Buskirk and Schmidt (2000) for the same two species exposed to dragonfly ($Aeshna cyanea$) predators. Several studies have reported that the costs associated with avoiding predators (reduced activity and increased refuge use) could be as important as the direct effects of predation and might affect population viability (Abrams 1991; McPeek and Peckarsky 1998; Tyler et al. 1998; Peacor and Werner 2001). Therefore, it is possible that those indirect effects, as well as direct predation on adults and larvae, could be contributing to the reduction of newt populations in our study area (Braña et al. 1996).

Acknowledgements

We are especially grateful to Carlos Rodríguez del Valle for his assistance and friendship. Felipe G. Reyes-Gavilán improved the manuscript with his suggestions and English-style advice. All the animals utilized in this study were used under collection and experimental permissions provided by the regional authorities (Dirección General de Recursos Naturales y Protección Ambiental, Principado de Asturias). A Ministry of Science and Technology research project (BOOS2000-0452) supported this research. A Spanish Ministry of Education and Culture doctoral fellowship and a University of Oviedo grant provided financial support to G. Orizaola.

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