Predator, but not conspecific, chemical cues influence pond selection by recently metamorphosed Iberian green frogs, *Rana perezi*

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Abstract: In amphibians, adults and larvae have different ecological requirements that could force recently metamorphosed individuals to disperse. The presence of chemical cues of conspecifics and predators could provide information about habitat quality, which might influence the juveniles' settlement decisions. We examined in the laboratory whether pond choice by recently metamorphosed Iberian green frogs (*Rana perezi* Seoane, 1885) is influenced by the presence of chemical cues from conspecifics and (or) from predators in the water. Our results suggest that frogs were able to detect the presence of chemical cues of snake predators in the water and that they avoided entering such ponds. However, frogs did not show either attraction or avoidance of ponds with conspecific chemical cues. Thus, juvenile frogs may select their postmetamorphic territories based on the lack of predation risk, and possibly on some habitat features, but not on the presence of conspecifics.

Résumé : Chez les amphibiens, les adultes et les larves ont des besoins écologiques différents, ce qui peut forcer les individus nouvellement métamorphosés à se disperser. La présence de signaux chimiques émis par des individus de même espèce ou par des prédateurs fournissent peut-être des renseignements sur la qualité des habitats, ce qui peut influencer les décisions des jeunes concernant le choix de leur site d'établissement. Nous avons examiné en laboratoire si le choix d'un étang par des grenouilles de Pérez (*Rana perezi* Seoane, 1885) récemment métamorphosées est influencé par la présence dans l'eau de signaux chimiques provenant d'individus de même espèce et (ou) de prédateurs. Nos résultats indiquent que les grenouilles sont capables de détecter la présence de signaux chimiques de couleuvres prédatrices dans l'eau d'étang et qu'ils évitent d'aller dans de tels étangs. Cependant, les grenouilles ne montrent ni attrait, ni évitement des étangs qui contiennent des signaux chimiques d'individus de même espèce. Les jeunes grenouilles choisissent donc, peut-être, les territoires qu'ils habiteront après la métamorphose en se fiant à l'absence de risque de prédation et possiblement aussi d'après certaines caractéristiques de l'habitat, mais non d'après la présence d'individus de même espèce.

[Traduit par la Rédaction]

Introduction

In amphibians, changes in life style are especially obvious in the transition from aquatic larvae to terrestrial postmetamorphic and adult stages (Duellman and Trueb 1986). Adults and larvae often have different ecological requirements that could force recently metamorphosed individuals to disperse when they complete their growth as larvae. During the transient phase of dispersal, selection of an appropriate future home range is very important (Clobert et al. 2001). In amphibians, the dispersal distance is generally small and juveniles may occupy home ranges in vacant spots among adults or in peripheral locations (Zug et al. 2001). Thus, recently metamorphosed juveniles may need to search for potential home ranges in peripheral locations, which require an evaluation of habitat quality and predation risk at these locations.

In selecting a good home range where to establish, a dispersing individual has to consider such factors as temperature and humidity, as well as the presence of conspecifics or predators (Stamps and Tanaka 1981; Clobert et al. 2001). Some amphibians may detect conspecific chemical cues (e.g., Jaeger et al. 1986; Aragón et al. 2000; Waldman and Bishop 2004). Conspecific cues could provide information about habitat quality, because they may reflect the presence of food, appropiate environmental conditions, and low predation risk (Woody and Mathis 1997). Individuals may, then, settle in clusters because they are attracted to conspecifics, rather than to habitat features (Stamps 1988; Graves et al. 1993; Muller et al. 1997; Quinn and Graves 1999). Nevertheless, the presence of conspecifics might not be desirable, as it may increase competition for food or potential mates (Griffiths et al. 1991).

Additionally, the detection of predators is important. Prey

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often respond to the threat of predation by decreasing the frequency or efficiency of behaviour associated with other activities (Lima and Dill 1990). Minimizing the negative effects of such trade-offs requires prey to discriminate threat levels and to adjust their behaviour accordingly. Animals that fail to respond appropriately to predator stimuli have a decreased probability of survival. In aquatic vertebrates, both visual cues (Karplus and Algom 1981) and chemical cues (Chivers and Smith 1993) may be important for predator recognition. However, chemical detection of predators is clearly advantageous to prey when the use of other sensory mechanisms is made difficult by environmental characteristics such as turbid water or abundant vegetation (Kats and Dill 1998). Whereas many frog tadpoles detect predators using chemical cues, from predators or conspecific alarm cues (Kats and Dill 1998), adult frogs mainly use vision and hearing to detect predators, which could be advantageous when a terrestrial predator approaches. However, many frogs escape predators by jumping into the water (Williams et al. 2000). Thus, the ability of tadpoles to detect chemical cues from predators could be advantageous, especially when assessing a potential habitat. Many studies have tested the mechanisms of predator chemical detection in amphibian larvae (e.g., Petranka et al. 1987; Manteifel 1995; Griffiths et al. 1998; Petranka and Hayes 1998; see review in Kats and Dill 1998). However, only a few studies have examined the prevalence of this mechanism in metamorphosed frogs or toads (Heinen 1994; Flowers and Graves 1997; Schley and Griffiths 1998; Belden et al. 2000; Chivers et al. 2001).

In this paper, we examined in the laboratory whether pond choice by recently metamorphosed Iberian green frogs (*Rana perez* Seoane, 1885) is influenced by the presence of chemical cues from conspecifics and (or) from predators in the water. We hypothesized that if frogs are attracted to territories near conspecifics, then they should select ponds containing conspecific cues and avoid ponds containing predator cues.

Methods

Study animals

During August 2003, we collected 20 recently metamorphosed Iberian green frogs (snout–vent length 20.7 ± 0.2 mm, mean ± SE) at several small ponds in Collado Mediano (Madrid, central Spain). Frogs in this habitat had the opportunity of choosing from among small nearby ponds with abundant aquatic vegetation. Frogs were maintained under two experimental conditions at the “El Ventorrillo” field station (5 km from the capture site). Twelve frogs were housed individually in aquaria (18 cm × 25 cm × 10 cm), with water at ambient temperature and under a natural photoperiod. The other eight frogs were kept in groups in two aquaria (20 cm × 20 cm × 15 cm), to be used as conspecific scent donors. We fed frogs tenebrio (*Tenebrio molitor* L., 1758) larvae twice a week.

We also captured in nearby larger ponds two viperine snakes, *Natrix maura* (L., 1758), to be used as predator scent donors. *Natrix maura* is predominantly aquatic and feeds mainly on insects, amphibians (both larvae and adults), and fishes (Haley and Davies 1986; Braña 1998). The snakes were housed individually in cages (36 cm × 25 cm × 13 cm) containing sawdust and tree barks for cover and a pond with water. The caged snakes were placed in rooms separate from the frogs to avoid contact with the scent and visual stimuli from the frogs. The snakes were captive for 2 weeks and were fed small pieces of commercial freshwater fishes obtained from a fish market. Frogs were not fed to the snakes to avoid the potential effect of the test frogs responding to alarm cues from conspecifics rather than to the chemical cues from the snakes alone (Chivers and Mirza 2001). All animals appeared healthy during the trials and were returned to their exact capture sites at the end of trials.

Experimental procedure

We conducted the experiments in glass terraria (50 cm × 40 cm × 50 cm). Two plastic rectangular trays (18 cm × 40 cm × 2 cm) filled with water were placed at the opposite ends of the terrarium. A plate (14 cm × 40 cm) of Porespan was placed between the two trays to simulate two ponds separated by a central dry area.

We planned a repeated-measures design where each frog was tested in each of three treatments in a randomized sequence, but participated in only one test per day. In the “control” treatment, the two ponds were filled with “clean” water from a nearby mountain stream that contained neither frogs nor snakes. In the “conspecific” treatment, we used clean water in one pond and water conditioned with conspecific chemical stimuli in the other pond to test for attraction or avoidance to conspecific cues. In the “snake predator” treatment, we used clean water in one pond and water conditioned with viperine snake chemical stimuli in the other pond to test for possible recognition and avoidance of water used by potential predators. We randomly altered the spatial position of the ponds in each trial. Water conditioned with the conspecific chemical stimuli was taken from the aquaria that housed the groups of frogs for at least 3 days. Water conditioned with the snake chemical stimuli was prepared by placing the snakes in aquaria with clean water for 48 h, after which 200 mL of the conditioned water was used to fill each pond for the respective choice tests 15 min prior to the start of each test.

Tests were conducted between the hours of 1100 and 1300, when frogs were fully active. We gently placed a single juvenile frog covered with a small glass cage in the centre of experimental aquarium and waited 5 min for habituation. Each test began when we lifted the cage to release the frog. We subsequently stood as motionless as possible to record the frog’s behavior from a hidden point 4 m away from the aquarium. Preliminary trials showed that frog behavior did not differ between situations when there was a motionless observer present in the room and those filmed with video without any observer present in the room. Typically, frogs stayed for a few minutes in the middle of the aquarium before jumping into the water of one pond, and occasionally they would changed from one pond to the other.

We recorded the location of the frog every 5 min, the first pond selected, and the number of times that the frog changed between ponds. A frog was designated as having chosen a specific pond if it was located in that pond, whereas a frog was designated as having made no choice if it was located out of the water. We also recorded the "total time" that the frog spent in each pond and calculated the "relative time" spent in each pond as the time spent in a particular pond / total time spent in any pond. Each trial lasted 2 h, after which the aquarium, the trays, and the Porespan were carefully washed with unscented detergent and drained to avoid odour contamination.

A frog's preferred pond was the one in which it spent >50% of its time (excluding the time spent in the no-choice area). To assess whether frogs chose one of the ponds, we calculated the number of frogs that spent >50% of their time in a particular stimulus pond and compared it with an expected binomial distribution, assuming frequencies to be equiprobable on each pond (for a similar procedure see Chivers et al. 1997 and Aragón et al. 2000). To compare the total time and relative time spent in the pond with chemical cues, and the number of changes between ponds across treatments, we used nonparametric Friedman's two-way ANOVAs. To include the control (no cue) treatment in these analyses, we used the time spent in one of the two ponds with clean water selected at random. Pairwise comparisons of means were conducted using nonparametric multiple comparison procedures described in Sokal and Rohlf (1995).

Results

In their first choice, frogs did not significantly select any pond in any of the treatments; based on the null hypothesis that the likelihood of entering one pond was equal for any of the ponds (clean water or water with chemical cues), the probability that one type of pond was chosen first was not significantly different from the expected in any of the three treatments (two-tailed binomial tests; clean water/control (no cue): 5/7, $P = 0.77$; clean water/conspecific cue: 4/8, $P = 0.39$; clean water/predator cue: 7/5, $P = 0.77$).

Total time spent in the pond containing the water conditioned with chemical cues differed significantly between treatments (Friedman's ANOVA, $\chi^2_{[2,12]} = 9.30$, $P < 0.01$; Table 1). Thus, frogs spent significantly less time in the pond with chemical cues in the predator treatment than in the other treatments (nonparametric multiple comparisons, $P < 0.02$ in both cases), but there were no significant differences between the conspecifics and the control treatments ($P = 0.18$). Similarly, relative time spent in the pond containing water conditioned with chemical cues differed significantly between treatments (Friedman's ANOVA, $\chi^2_{[2,12]} = 13.31$, $P < 0.002$; Table 1). Thus, frogs spent significantly relatively less time in the pond with scent in the predator treatment than in the other treatments (nonparametric multiple comparisons, $P < 0.02$ in both cases), but there were no significant differences between the conspecifics and the control treatments ($P = 0.48$). The number of changes between ponds did not differ significantly among treatments (Friedman's ANOVA, $\chi^2_{[2,12]} = 3.80$, $P = 0.15$; Table 1).

When we estimated the pond selected by frogs from the proportion of time spent in each pond, frogs in the control and conspecific treatments did not significantly select any pond (Table 1). However, in the predator treatment, frogs clearly avoided ponds containing water conditioned with viperine snake chemical cues. Similar results were noted when

Table 1. Summary statistics of total and relative times spent by recently metamorphosed green frogs, *Rana perezi*, in ponds containing water from the three treatments (control, snake predator, conspecific).

	Total time (min)				Relative time (%)				Number of changes between ponds	
Treatment	Mean ± 1 SE (range)	Median	Scent/clean*	$P^{\dagger}$	Mean ± 1 SE (range)	Median	Scent/clean*	$P^{\dagger}$	Mean ± 1 SD (range)	Median
No cue	54±6 (25–100)	50	6/6	1	45±5 (20–83)	41	6/6	1	3.1±0.7 (1–7)	2.0
Predator cue	30±5 (0–65)	35	1/11	0.006	25±4 (0–54)	29	1/11	0.006	3.3±0.6 (0–6)	3.5
Conspecific cue	62±8 (15–115)	60	7/5	0.77	52±6 (12–95)	50	6/6	1	1.9±0.4 (0–5)	1.5

*Ratio of the number of frogs that spent >50% of their time in each pond containing water conditioned with either predator or conspecific chemical cues to those in ponds containing clean (no cue) water.

[†]Calculated from binomial tests.

we considered the relative time spent in each pond to assess which pond was chosen (Table 1).

Discussion

Our results suggest that recently metamorphosed Iberian green frogs were able to detect the presence of chemical cues from snake predators in the water and that they left the pond as soon as they detected the predator cues. Visual cues are important in eliciting an antipredator response in anurans (e.g., Williams et al. 2000), but the use of more than one sensory stimulus could be advantageous in habitat selection. The detection of chemical cues may inform the prey animal about the continuous presence of a predator in a certain area even if it is hidden (Kats and Dill 1998), whereas visual cues only inform the prey animal about the current presence of the predator. Thus, when frogs select a pond, the combined use of visual and chemical detections could be more advantageous than the use of visual cues alone. Although visual detection could be useful in avoiding active foraging predators, chemical cues are more useful in detecting ambush predators, especially in complex habitats where visual detection might be restricted (Van Damme et al. 1995; Mathis and Vincent 2000). Thus, chemical cues could be especially important in the detection of viperine snakes because these snakes typically remain hidden in aquatic vegetation while ambushing frogs (Haley and Davies 1986).

Although previous studies on amphibians have demonstrated the ability of anurans to avoid chemical cues of predators, most were performed with tadpoles (see review in Kats and Dill 1998) and only a few demonstrated the prevalence of this mechanism in postmetamorphic anurans. These studies were performed on terrestrial bufonids (Heinen 1994; Flowers and Graves 1997; Belden et al. 2000), discoglossids (Schley and Griffiths 1998), hylids (Chivers et al. 2001), and ranids (Chivers et al. 1999). Our study shows the presence of the ability to detect snake chemical cues in recently metamorphosed ranid juveniles and suggests its importance in habitat selection. This mechanism may be present in other anuran groups because anurans have well-developed vomeronasal organs (Scalia 1976).

Metamorph Iberian green frogs are often forced to disperse from breeding ponds, which provide suitable habitat for the adults, to small nearby swallow temporal ponds (Lizana et al. 1989), because adults compete for the best spots (Schneider and Steinwarz 1990), with larger adults being found in central locations in the pond. Thus, there is some degree of spatial segregation between adults and juveniles (Lizana et al. 1989). Moreover, adult frogs eat any prey smaller than themselves, including metamorphs (J. Martín, personal observation). Juveniles can either select territories with similar-sized conspecifics (Stamps 1988; Graves et al. 1993) or avoid them altogether (Léna and Fraipont 1998). The detection of conspecific chemicals could be advantageous in selecting a habitat either because they provide information about the habitat quality (Woody and Mathis 1997) or because they reflect a high potential local competition for food (Griffiths et al. 1991). The detection of conspecific chemical cues is common in many newts and salamanders (e.g., Jaeger et al. 1986; Aragón et al. 2000), but is more rare in anurans (Waldman and Bishop 2004). Our results suggest that recently metamorphosed Iberian green frogs do not show attraction or avoidance to ponds with conspecific chemical cues. Thus, Iberian green frogs do not appear to be able to detect chemical cues of conspecifics. However, it is possible that the frogs could detect conspecifics and be attracted to low densities of conspecifics, but not to high densities of conspecifics (as might be the case for water with concentrated chemical cues from the small aquaria containing the four conspecifics used as donors in our experiment), because competition may be too high. If this was the case, then it is possible that the lack of a response to conspecific cues might be a density issue. Alternatively, the presence of conspecifics may not be important when selecting a pond. Food could be available to all individuals in all ponds and not be a restricted factor for juveniles, or not be as important as other factors. Adult male Iberian green frogs defend small territories against other males, but only during the mating season (Schneider and Steinwarz 1990). Also, because this frog occurs in relatively dense populations, the localization of ponds with low population densities of conspecifics might be difficult.

We conclude that postmetamorphic Iberian green frogs seemed to select ponds based on the potential threat of predation by water snakes, whereas conspecific cues did not seem to influence their decisions.

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