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Modification of anti-predator behaviour in tadpoles by environmental conditioning

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Summary

1. We examined the anti-predator behaviour of two closely related species of tadpoles (*Rana lessonae* and *Rana esculenta*). Eggs were hatched in the laboratory and tadpoles were conditioned for 30 days to fish, newts, odonates and *Bufo* tadpoles before testing tadpole responses to predator and control stimuli.

2. *Rana esculenta* tadpoles spent more time swimming than did *R. lessonae* tadpoles, but species did not differ in refuge use.

3. Conditioning treatment affected both the percentage of time spent swimming and use of refuge. Time spent swimming was highest in tadpoles conditioned to *Bufo* tadpoles and lowest in those conditioned to the three predators. Time spent in the refuge was highest in tadpoles conditioned to fish, newts, and odonates and lowest in those conditioned to tadpoles. Species of tadpoles differed in their responses to conditioning, *R. esculenta* spent more time swimming when conditioned to tadpoles whereas *R. lessonae* spent less time swimming when conditioned to odonates than the other species.

4. In predator treatments, tadpoles exposed to fish predators increased time spent in the refuge compared to odonates, newts, and the tadpole and water controls. Exposure to fish, newts, and odonates decreased the percentage of time spent swimming. In addition, there was a significant conditioning × predator interaction on swimming and open-water use.

5. Our results show that *R. lessonae* and *R. esculenta* tadpoles possess inherited differences in anti-predator behaviour which may be interpreted as general adaptations to aquatic environments, but that conditioning to predators can modify or 'fine-tune' their response to specific environments.

Key-words: amphibian, anti-predator behaviour, microhabitat, *Rana*, tadpole.

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Introduction

Predator–prey interactions can be mediated by characteristics of prey that act to reduce vulnerability to predators. In amphibian larvae, behaviours such as schooling, shifts in use of microhabitats, level of activity, and release of alarm substances can affect the survival of prey in the presence of predators (Wassersug 1973; Petranka, Kats & Sih 1987; Hews 1988; Kats, Petranka & Sih 1988; Lawler 1989). Kats, Petranka & Sih (1988) found that amphibian larvae of almost all species surveyed from permanent aquatic habitats had at least one defence against predatory fish: either chemically mediated use of refuges or unpalatability. Larvae from temporary aquatic habitats usually lacked any defence mechanism against predatory fish (Kats, Petranka & Sih 1988). It has also been shown that activity level (e.g. swimming) varies among species of tadpoles and that activity level is negatively correlated to survival with predators such as adult newts (Lawler 1989). Similarly, several studies on fish have shown that anti-predator behaviour varies significantly among populations and is positively associated with the level of natural predation (Seghers 1974; Giles & Huntingford 1984; Magurran 1986). Such variation in anti-predator behaviours is especially interesting because of its importance to survival and natural selection as well as its potential conflict with other behaviours (e.g. searching for mates, courtship, or foraging), and consequently, its trade-off in fitness (Sih 1980).

Surprisingly, we know little about the origin and maintenance of this variation in anti-predator behaviour. Magurran (1990) found that in some fish, anti-predator behaviour was found to be present in laboratory-reared offspring and assumed to be inherited. Yet, it also appeared to be modified according to the history of exposure to predators. Fish exposed to predators early in life increased inspection rate of potential predators, were more likely to form schools, and were less likely to hide than naïve fish (Magurran 1990).

Similar information on individual and population variation for amphibian larvae is scant. If variation in anti-predator behaviour exists, is it environmentally determined by exposure to predators, is it genetically determined and inherited, or can environmental exposure alter genetically based responses? Consequently, we addressed three primary questions.

1. Are anti-predator responses of tadpoles inherited or are they determined by environmental conditioning to predators?
2. Can inherited anti-predator responses of tadpoles be further modified by exposure to predators?
3. Do species of tadpoles differ in their responses to conditioning or to type of predator?

To answer these questions we reared tadpoles of two closely related species (*Rana lessonae* Camerano and *Rana esculenta* Linnaeus), with and without predators and then tested their behavioural responses to an array of vertebrate and invertebrate predators found in breeding ponds. *Rana esculenta* is an interspecific hybrid between *Rana lessonae* and *Rana ridibunda* Pallas (Berger 1968; Günther 1973) with a reproductive mode known as 'hybridogenesis' (Schultz 1969; Tunner 1973; Graf & Polls-Pelaz 1989). In central Europe, where *R. ridibunda* no longer occurs sympatrically (including our study sites in Switzerland), *R. esculenta* populations can only persist through sexual parasitism. Adults mate with *R. lessonae*, their sexual host, to regain one of the parental genomes lost during gametogenesis (Berger 1977, 1983; Graf & Polls-Pelaz 1989). Contrary to most other clonal vertebrate hybrids, the L–E system contains both sexes which in mixed populations can result in four different mating combinations and offspring L♀ × L♂, L♀ × E♂, E♀ × L♂, E♀ × E♂). The two heterogametic matings (L × E and E × L), produce highly heterozygous *R. esculenta* offspring while the two homogametic matings (L × L and E × E) produce *R. lessonae* and *R. ridibunda* offspring, respectively. This anuran system offers a unique opportunity to study the behaviour of genetically related species, without the potential confounding of morphological traits and behavioural abilities that can occur in distantly related species.

Methods

CONDITIONING OF TADPOLES TO PREDATORS

Controlled natural matings of both species were made from 10 pairs of adults collected on 25 May 1990 from a small pond near the Katzensee, Zürich, Switzerland. The species of each individual was determined before mating by electrophoretic analysis of albumin from lymph (Tunner 1973, 1979). Four clutches of the cross L × L and three clutches of L × E were obtained. Eggs were hatched and tadpoles reared in the laboratory. Ten days after hatching, tadpoles from the clutches of each species were mixed and then counted into eight groups of 40 individuals each. Two replicate groups of each species were randomly assigned to one of four rearing conditions that exposed tadpoles to three predators (juvenile *Cyprinus carpio* Linnaeus fish, $\bar{x} = 34{\cdot}2$ mm total length; *Triturus alpestris* (Laurenti) larva, $\bar{x} = 45{\cdot}3$ mm total length; *Anax imperator* (Leach) larva, $\bar{x} = 46{\cdot}0$ mm total length) and one control (*Bufo calamita* Laurenti tadpoles). All predators occur in local ponds with *R. lessonae* and *R. esculenta*.

Tadpoles were then reared in plastic dishpans (31 × 21 × 11 cm) containing 3·0 l of aged tapwater. The predators and control *Bufo* tadpoles were placed into a small plastic screen enclosure (10 cm diameter, 10 cm high, 2 mm mesh) centred in the dishpan. Predators were released into the dishpan after 20 days when tadpoles were large enough to escape gape-limited predation. Therefore, tadpoles were exposed to chemical, visual and tactile (i.e. water movement) cues of predators. Water in dishpans was changed every 3 days and tadpoles were fed a standard tadpole ration every 2 days (3:1 ground mixture of rabbit pellets and Tetra-Min fish flakes). All predators were fed chironomid larvae to satiation every 2 days. Tadpoles were tested after a total of 30 days of conditioning with predators and the control. The tadpoles that we tested measured 8–12 mm in body length (SVL) and were at developmental stage 28–31 (Gosner 1960).

TESTING SYSTEM AND PROCEDURES

Predator and control stimuli were manipulated in partitioned test chambers. We used 20 white plastic dishpans (31 cm long, 21 cm wide, 11 cm deep) with a partition 10 cm from one end. The partition consisted of two pieces of plexiglass, one removable solid opaque partition and one non-removable clear perforated partition. Plexiglass was perforated with 80–100 3-mm holes along the side and bottom edges, the centre was left intact allowing a clear view of the tadpoles. A piece of green plastic aquarium plant (*Limnophila*, 14 cm long) was placed at the opposite end from the partition as a refuge for the tadpole. Dishpans were arranged into two groups of 10 each and placed on tables next to each other (for the convenience of observers). Dishpans were filled with 3·0 l of aged tapwater at the start of each trial.

Each trial consisted of testing one species of tadpole from all four rearing conditions with each of five treatments:

1. one *Triturus alpestris* larva;

2. one juvenile *Cyprinus carpio* fish;
3. one *Anax imperator* larva;
4. three *Bufo calamita* tadpoles; and
5. aged tapwater.

Each trial was started by placing one *R. lessonae* or one *R. esculenta* tadpole from each conditioning treatment into the larger chamber of each test dishpan. Tadpoles for each trial were selected from the conditioning pans and matched for similarity in body size. Extremely small or large individuals were not used. Predators and controls were simultaneously added to the smaller partitioned area of the dishpans. After a 1-h 40-min acclimation period, pre-treatment responses of all 20 tadpoles were recorded at 1-min intervals for 20 min. We recorded six responses of tadpoles in the dishpans: (1) resting at the edge (i.e. any side of the dishpan), (2) resting in the open water (i.e. away from the sides), (3) resting under the refuge, (4) swimming along the edge, (5) swimming in the open water, and (6) swimming under the refuge. These responses provided measures of both microhabitat use and activity level, responses known to be correlated with survival in amphibian larvae. After recording pre-treatment responses the solid partition was removed and after a 5-min reacclimation period we recorded post-treatment responses at 1-min intervals for an additional 20 min. After each trial all dishpans were carefully rinsed with fresh tapwater and the position of each rotated to the left to control for positional bias. However, the same dishpan always was used for the same predetermined predator or control treatment. Each individual was tested only once in eight independent trials using 160 *R. lessonae* and 160 *R. esculenta* tadpoles. In the first trial for *R. lessonae* only, observations on resting and swimming were inadvertently not recorded and, therefore, were excluded from the analysis. All trials were conducted between 7 and 14 August 1990.

STATISTICAL ANALYSIS

Behavioural responses of the tadpoles were analysed by three-way analysis of variance for the fixed effects of tadpole species, predator conditioning, predator exposure, and three possible two-way interactions. Pairwise differences within significant treatment effects were analysed by Scheffe's multiple comparison procedure. For each tadpole the percentage of time spent in three microhabitats (i.e. open-water, edge, and refuge) and time spent in two activities (i.e. resting and swimming) were calculated for the 20 1-min pre-treatment and 20 1-min post-treatment observations. Pre- and post-treatment observations were analysed separately. The analyses were only applied to data on use of two microhabitats (open-water and refuge) and one activity (swimming) as the time spent in the other microhabitat (i.e. edge) or activity (i.e. resting) was uniquely determined by the other responses. Also, tadpoles would be most vulnerable to predation in the open-water microhabitat or if swimming, and least vulnerable in the refuge. All percentages were angularly transformed by the arcsine of the square-root before analysis to reduce skewness (Snedecor & Cochran 1980).

Results

RESPONSES OF SPECIES

Species were significantly different in the percentage of time spent swimming in pre- and post-treatment tests (Tables 1 & 2; Fig. 1). *Rana esculenta* tadpoles spent more time swimming in both pre- and post-treatment observations (24·8% and 24·3%) than did *R. lessonae* tadpoles (20·6% and 18·8%). Species were not significantly different in their use of either open-water or refuge microhabitats in either pre- or post-treatment observations (Tables 1 & 2; Fig. 1).

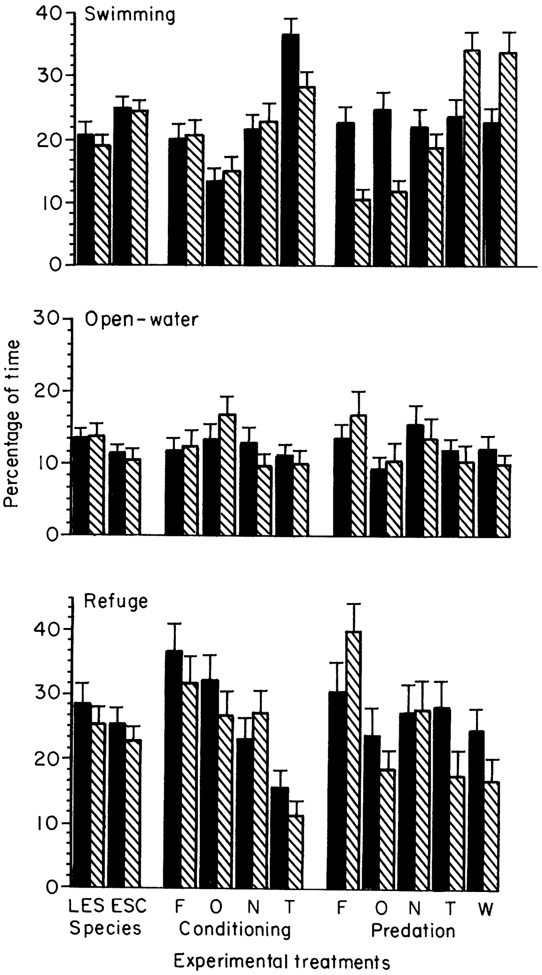

Fig. 1. Histograms of mean pre- (■) and post-treatment (▨) responses of all tadpoles shown by two species (ESC, *R. esculenta*; LES, *R. lessonae*), four conditioning treatments, and five predator treatments (F fish; O, odonate; N, newt; T, tadpole; W, water). Values plotted represent mean (± 1 SE) proportions calculated from the eight trials.

Table 1. Summary of analysis of variance for pre-treatment responses of microhabitat use and activity of tadpoles

Source of variation	df	MS	*F*-value	*P*-value
Refuge use				
Species	1	0·0409	0·20	0·6567
Conditioning	3	1·4654	7·31	0·0001
Predator	4	0·0616	0·31	0·8730
Sp. × Cond.	3	0·1484	0·74	0·5288
Sp. × Pred.	4	0·3315	1·65	0·1612
Cond. × Pred.	12	0·2628	1·31	0·2118
Residual	272	0·2005		
Open-water use				
Species	1	0·0835	1·36	0·2455
Conditioning	3	0·0148	0·24	0·8682
Predator	4	0·0866	1·40	0·2324
Sp. × Cond.	3	0·0027	0·04	0·9880
Sp. × Pred.	4	0·1142	1·85	0·1190
Cond. × Pred.	12	0·0648	1·05	0·4014
Residual	272	0·0616		
Swimming				
Species	1	0·4628	6·94	0·0089
Conditioning	3	1·5859	23·78	<0·0001
Predator	4	0·0186	0·28	0·8912
Sp. × Cond.	3	0·1684	2·52	0·0580
Sp. × Pred.	4	0·0524	0·78	0·5357
Cond. × Pred.	12	0·0838	1·26	0·2443
Residual	272	0·0667		

Table 2. Summary of analysis of variance for post-treatment responses of microhabitat use and activity of tadpoles

Source of variation	df	MS	*F*-value	*P*-value
Refuge use				
Species	1	0·1400	0·77	0·3892
Conditioning	3	1·6786	9·28	<0·0001
Predator	4	1·4236	7·87	<0·0001
Sp. × Cond.	3	0·0997	0·55	0·6480
Sp. × Pred.	4	0·2528	1·40	0.2351
Cond. × Pred.	12	0·2509	1·39	0·1716
Residual	282	0·1810		
Open-water use				
Species	1	0·2169	2·55	0·1116
Conditioning	3	0·1442	1·69	0·1687
Predator	4	0·1245	1·46	0·2140
Sp. × Cond.	3	0·1276	1·49	0·2151
Sp. × Pred.	4	0·1238	1·45	0·2166
Cond. × Pred.	12	0·1547	1·82	0·0452
Residual	282	0·0852		
Swimming				
Species	1	0·5444	8·10	0·0048
Conditioning	3	0·4276	6·36	0·0003
Predator	4	1·6799	24·98	<0·0001
Sp. × Cond.	3	0·1070	1·59	0·1916
Sp. × Pred.	4	0·0713	1·06	0·3766
Cond. × Pred.	12	0·1224	1·82	0·0447
Residual	282	0·0672		

RESPONSES TO PREDATOR CONDITIONING

Conditioning of tadpoles to predators had a significant effect on the percentage of time spent swimming and in the refuge in pre- and post-treatment observations (Tables 1 & 2; Fig. 1). Pre-treatment swimming time was highest in tadpoles conditioned to tadpoles (36·9%), significantly lower for those conditioned to newts (21·6%), and significantly lowest for those conditioned to fish and odonates (19·9% and 13·1%). Time swimming did not differ between tadpoles conditioned to fish and odonates. Post-treatment swimming time was also higher for tadpoles conditioned to tadpoles (28·1%) than for those conditioned to any of the three predators. Tadpoles conditioned to odonates spent the least percentage of time swimming (15·0%). Tadpoles conditioned to newts (22·8%) and fish (20·5%) were not significantly different from each other, but differed from tadpoles conditioned to either odonates or *Bufo* tadpoles.

Pre-treatment use of the refuge was significantly higher in tadpoles conditioned with fish (36·8%) and odonates (32·3%) than those conditioned with newts (23·2%) or tadpoles (15·7%). Pre-treatment refuge use did not differ between fish and odonates or between newts and tadpoles. Post-treatment use of the refuge was also higher when tadpoles were conditioned to fish (31·8%), newts (27·2%), and odonates (27·0%) compared to conditioning with tadpoles (11·4%). Post-treatment refuge use did not differ among tadpoles conditioned to the three predators (fish, newts or odonates), only between the three predators and the *Bufo* tadpole treatment. Conditioning of tadpoles had no significant effect on pre- or post-treatment use of open-water microhabitat (Tables 1 & 2; Fig. 1).

RESPONSES TO PREDATOR EXPOSURE

There were no significant responses of tadpoles in use of microhabitat or activity to predators in pre-treatment observations (Table 1; Fig. 1). This was important because it indicated both a lack of contamination of test dishpans from previous use and a lack of any significant leakage in the partition between predator treatments and test tadpoles. Confounding effects on pre- and post-treatment observations were therefore eliminated.

Exposure of tadpoles to predators significantly affected the percentage of time tadpoles spent swimming and in the refuge for post-treatment observations (Table 2; Fig. 1). Tadpoles exposed to *Bufo* tadpoles (34·0%) and the water control (33·6%) did not differ between each other, but spent significantly more time swimming than tadpoles exposed to the three predators (fish 10·1%, newts 18·4%, or odonates 11·4%). The percentage of time tadpoles spent swimming did not differ among the three predators (fish, newts, odonate). Exposure to the predator treatments did not significantly affect use of the open-water microhabitat in pre- or post-treatment observations (Tables 1 & 2; Fig. 1). However, tadpoles exposed to fish spent significantly more time in the refuge (39·9%) compared to tadpoles exposed to newts (28·1%), odonates (18·5%), *Bufo* tadpoles (17·7%), or the water control (16·8%). Refuge use in tadpoles did not differ among newt, odonate, *Bufo* tadpole, or water control treatments.

INTERACTION RESPONSES TO TREATMENTS

In pre-treatment observations the interaction between species and conditioning marginally affected ($P =$ 0·058) the percentage of time that tadpoles spent swimming (Table 1; Fig. 2). This meant that species responded differentially to the conditioning treatments. *Rana esculenta* spent more time swimming in response to conditioning with *Bufo* tadpoles (41·9%) and odonates (17·8%) than did *R. lessonae* that were conditioned to *Bufo* tadpoles (31·3%) and odonates (7·8%; Fig. 2). Both species, however, spent the same amount of time swimming when conditioned to fish and newts (Fig. 2). There were no other significant interactions with species in either pre- or post-treatment observations (Tables 1 & 2).

In post-treatment observations the interaction between tadpole conditioning and predator exposure significantly affected both the percentage of time spent in open-water and time swimming by tadpoles (Table 2; Fig. 3). This meant that conditioning had a differential effect on the responses to predators, dependent on the specific combination of treatments (i.e. responses were not parallel among conditioning treatments, Fig. 3). Use of open-water by tadpoles exposed to water, *Bufo* tadpoles, and odonates was similar irrespective of conditioning (Fig. 3). However, conditioning had strong effects on tadpoles exposed to fish and newts. Conditioning to *Bufo* tadpoles did not affect responses of tadpoles exposed to fish or newts differently to the water control (Fig. 3). Conditioning to odonates increased use of open-water in tadpoles exposed to fish and newts. Conditioning to fish only increased this response in tadpoles exposed to fish but not newts. Conditioning to newts increased use of open-water in tadpoles exposed to newts but decreased the response in those exposed to fish (Fig. 3). Percentage of time tadpoles spent swimming when exposed to *Bufo* tadpoles and the water control was altered most by conditioning (Fig. 4). Time spent swimming was lowest but similar among tadpoles exposed to the

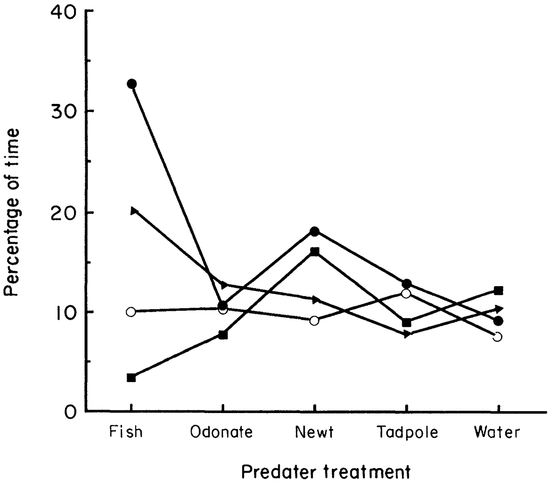

Fig. 3. Interaction between conditioning [odonate (●), fish (▲), tadpole (○) and newt (■)] and predator treatments in the proportion of time tadpoles spent in open-water. Each point plotted represents the mean calculated from eight trials and both species ($n = 16$).

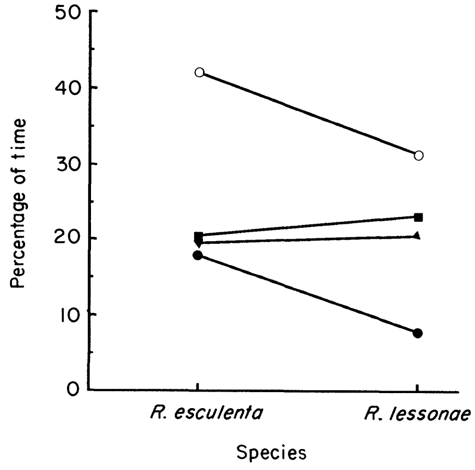

Fig. 2. Interaction between species and conditioning treatments [tadpole (○), newt (■), fish (▲) and odonate (●)] in the proportion of time tadpoles spent swimming. Each point plotted represents the mean calculated from eight trials and five predator treatments ($n = 40$).

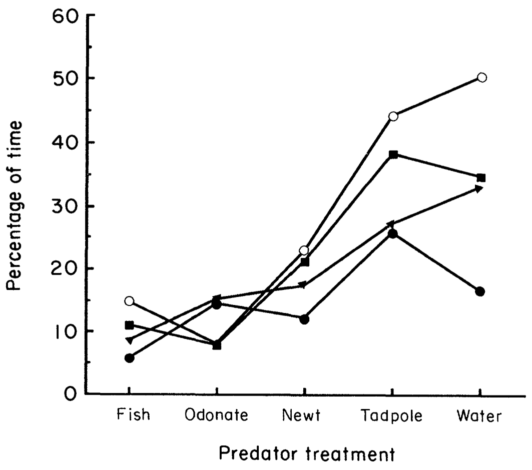

Fig. 4. Interaction between conditioning [tadpole (○), newt (■), fish (▲) and odonate (●)] and predator treatments in the proportion of time tadpoles spent swimming. Each point plotted represents the mean calculated from eight trials and both species ($n = 16$).

three predators (fish, newt, odonate), irrespective of conditioning. Conditioning to *Bufo* tadpoles, in general, increased the time spent swimming in tadpoles exposed to all treatments, but especially in the water control or tadpole treatment (Fig. 4). Conditioning to odonates decreased the time spent swimming in four of five treatments, but most in tadpoles exposed to the water control treatment. Conditioning to fish and newts increased the time spent swimming in the water control and tadpole treatments (Fig. 4).

Discussion

Our results clearly show an interaction between genetic and environmental factors on the anti-predator responses of tadpoles. The higher swimming activity of *R. esculenta* was maintained irrespective of conditioning or exposure to different predators. Because eggs were hatched and tadpoles were reared in the laboratory, any differences cannot reflect experience in natural habitats, exposure to predators, or environmental cues, and thus are assumed to reflect genetic differences. The interaction of species with conditioning also suggests that species are differentially sensitive to predators (Fig. 2). *Rana lessonae* responded more strongly (i.e. decreased swimming relative to *R. esculenta*) to conditioning by at least one predator (odonates) because of presumed sensitivity to the cues of that predator. Conversely, the responses of *R. esculenta* appeared unaffected by conditioning to odonates. *Rana esculenta* responded more strongly (i.e. increased swimming relative to *R. lessonae*) to conditioning by *Bufo* tadpoles and again was assumed to reflect sensitivity to the cues of other tadpole species not expressed by *R. lessonae*.

These species differences in anti-predator behaviour may be products of natural selection in their aquatic habitats. The adaptive significance of the observed behavioural responses is difficult to evaluate because comparative information on the larval ecology of *R. lessonae* and *R. esculenta* is lacking. We suggest, however, that tadpoles of the two species might occupy different ecological niches, even though they are syntopic in local ponds, due to the hybridogenetic mating system. This suggestion arises from including ecological information on *R. ridibunda*, the other parental species of *R. esculenta*.

Rana ridibunda typically lives in large, deep and permanent aquatic habitats, such as lakes and rivers, that contain predatory fish (Berger 1970, 1977; Nevo 1975; Tunner & Dobrowsky 1976). Its tadpoles are primarily benthic and feed on algae and vascular plants (Degani 1986). In contrast, *R. lessonae* typically inhabit smaller, shallower temporary ponds and its tadpoles are more pelagic. *Rana esculenta* appears to be intermediate (Blankenhorn 1977). We suggest that tadpoles of the two species we tested use different microhabitats in natural ponds, *R. lessonae* the shallow edges and *R. esculenta* the deeper, benthic habitats. If this is true they would be exposed to different selective pressures from predators found in these microhabitats. For example, a survey of 10 of our study ponds showed that insect predators (e.g. *Anax*, *Lestes*, *Ranatra*, *Dytiscus*) were more diverse and abundant in vegetation along the edges of ponds than in open-water or benthic microhabitats. Therefore, the higher sensitivity of *R. lessonae* to odonates we observed (Fig. 2) may be related to potential selection in its natural habitat. Likewise, the increased swimming activity of *R. esculenta* conditioned to *B. calamita* tadpoles could be a result of their past association in similar pond types (e.g. newly created or disturbed ponds) or similar microhabitats within a pond. The description of relative activity and use of microhabitats by species in natural ponds would identify potential mechanisms responsible for the maintainence of differential behaviours.

Experience accumulated through conditioning as well as actual exposure to predators also had clear effects on anti-predator behaviour. In general, swimming activity was lower and refuge use was higher after conditioning with and during exposure to predators than after conditioning with and exposure to controls (i.e. tadpoles or tapwater; Table 3, Fig. 1). This indicates that past and present predator experience interacts to make larvae more cautious. Caution appears manifested by decreasing activity or hiding and thereby reducing exposure to predator attacks. To illustrate the general effect of predators we ranked mean responses to each treatment from 1 to 5 (high to low, Table 3). Among the predators, the overall effect decreased from fish (average rank = 1·33), through odonates (2·0), to newts (2·67). More specifically, comparisons of the four conditioning treatments within any one predator exposure treatment show differences in both microhabitat use (i.e. open-water, Fig. 3) and activity (i.e. swimming, Fig. 4). These differences are assumed to reflect how well tadpoles assimilate cues of past predator conditioning (i.e. chemical, visual and tactile) and use them to modify current responses. Presumably, the degree of danger from each predator as well as the strength of the predator cues determines the total response. The consistently strong response to fish illustrates the additive effect of high danger and strong chemical cues. Our predator conditioning treatment, however, did not in all cases cause tadpoles to be more sensitive to predator cues (e.g. increased refuge use related to increased caution), our expectation of an adaptive response. There are at least two possible explanations for these results. Because predators in the conditioning treatment were satiated and did not have continuous access to tadpoles as prey, the connection between predator cues and realized danger was less than in natural

Table 3. Summary of responses of conditioning and predator treatments from Tables 1 & 2 and Fig. 2. Ranks were determined from treatment means. Significant differences from the Scheffé's tests are given by > or < and non-significant differences by =. Level of response decreases from left to right with rank order. Only results with at least one significant difference are shown. Predators: F = fish, O = odonate, N = newt. Controls: T = *Bufo* tadpoles, W = tapwater

Treatment	Response	Observation	Rank order 1 2 3 4 5
Conditioning	Swimming	Pre-treatment	O = F < N < T
	Swimming	Post-treatment	O < F = N < T
	Refuge use	Pre-treatment	F = O > N = T
	Refuge use	Post-treatment	F = N = O > T
Predator	Swimming	Post-treatment	F = O = N < T = W
	Refuge use	Post-treatment	F > N = O = T = W
			Predators Controls

habitats. An increase in open-water use by tadpoles exposed to fish and conditioned by fish may reflect a decrease in their perception of danger. Alternatively, tadpoles may have learned to actively assess their risk of danger in order to balance their other activities such as foraging. Hiding in the refuge would reduce the immediate risk of danger but not provide prey with vital cues concerning the predator activity as well as reduce the ability of prey to function normally in the habitat. In other studies, exposure to predators similarly modified the activity of fish. Repeated exposure to predators resulted in habituation as reflected by an increase in foraging of minnows (Magurran & Girling 1986). In another example, early exposure to a predator also caused minnows to increase their inspection rate of predators which presumably provided information on behaviour of the predator (i.e. risk assessment) and allowed prey to adjust their behaviour (Magurran 1990).

The general significance of the differences we found lies in the fact that environmental experience can modify innate behavioural responses. Breeding adult amphibians can non-randomly select oviposition sites (Resetarits & Wilbur 1989), thus minimizing the risk of predation to their larvae. Despite some degree of adult choice in relation to predation, larvae face an array of unpredictable aquatic environments in which they must successfully develop and metamorphose. Reproductive success of adults depends on the ability of its larvae to survive in whatever environment adults choose, favourable or not. Anti-predator behaviours are therefore important for small, fragile organisms such as tadpoles that are vulnerable to attack and injury from a wide range of predators. A genetically 'fixed' response to predators (*sensu* Lawler 1989), reflecting past adaptation to specific environments, however, may not allow tadpoles to adjust to varying levels of predation in the current environment and carry out other life-history functions related to growth and development (e.g. foraging). Nevertheless, if a 'fixed' innate response to low level predation is coupled with the inherited ability to modify the magnitude of the response, according to the current predator environment, then tadpoles can minimize the risk of predation in a wide range of aquatic environments chosen by adults while balancing other life-history functions. Elucidation of the range of anti-predator responses that are possible and how or when they can be modified by environmental conditions will help us understand more about the processes leading to coexistence in predator–prey systems.

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