

INTRASPECIFIC VARIATION IN THE AVOIDANCE RESPONSE OF STREAM FROG (*MANNOPHRYNE TRINITATIS*) TADPOLES TO FISH AND PRAWN PREDATORS

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The stream frog, *Mannophryne trinitatis*, lives in and beside steep mountain streams of Trinidad's Northern and Central ranges. Male frogs have strong anti-predator behaviour and prefer to deposit tadpoles in pools that lack predators (particularly the fish *Rivulus hartii* and the freshwater prawn *Macrobrachium carcinus*). The two predators are rarely found in the same streams and different *M. trinitatis* populations may show specific anti-predator behaviour to the predators they encounter in the wild. To assess tadpole spatial avoidance of predators, we presented small and larger tadpoles from four *M. trinitatis* populations to each predator. Three tadpole sources were from the Northern Range: Mount Saint Benedict, Lopinot (where *R. hartii* is abundant), and the Maracas Bay area (where *M. carcinus* is present); the fourth was from Tamana cave, Central Range, where neither predator occurs. To determine predator detection mechanisms employed by the tadpoles, we presented the predators in three container types: a mesh cage (for chemical and visual detection), an opaque container with holes (chemical but no visual detection), and a transparent container (visual but no chemical detection). Different sized tadpoles (large and small) showed the same response to predators, and tadpoles principally used chemical cues to detect predators. All populations showed a stronger response to the presence of *R. hartii* than to *M. carcinus*. We attribute this latter difference to the restricted distribution of *M. carcinus* and to the few sympatric zones between the tadpoles and these predators. Thus tadpoles lacked a specific anti-predator response to *M. carcinus*. Naïve tadpoles from Mount Saint Benedict and Tamana that had never previously encountered either of the predators showed strong anti-predator responses, suggesting that the anti-predator response is likely to be inherited.

Key words: anti-predator behaviour, *Macrobrachium*, *Rivulus*, Trinidad

INTRODUCTION

Amphibian larvae under strong predation pressures have evolved a variety of anti-predator strategies such as changes in levels of activity, release of alarm substances, schooling, shifts in microhabitat, chemical secretion and spatial avoidance, to increase their chances of survival (Huey, 1980; Hews, 1988; Petranka *et al.*, 1987; Lawler, 1989; Magnusson & Hero, 1991; Watt *et al.*, 1997; Laurila, 2000; Thiemann & Wassersug, 2000; Pearl *et al.*, 2003). Chemoreception has been well documented in amphibian larvae and is thought to be the primary mechanism by which they detect predators (Petranka *et al.*, 1987; Kats *et al.*, 1988; Skelly & Werner, 1990; Bridges & Gutzke, 1997; Brönmark & Hansson, 2000; Eklöv & Werner, 2000; Van Buskirk, 2001). Tadpoles that have co-evolved with predators or adapted to continuous predator presence react to chemical cues from actual predators (Kats *et al.*, 1988; Petranka *et al.*, 1994; Laurila *et al.*, 1997; Petranka & Hayes, 1998; Relyea & Werner, 1999; Relyea, 2001) and those that do not nor-

mally encounter predators have a minimal response if any to the chemical cues released by predators in experimental conditions (Semlitsch & Reyer, 1992; Lefcort, 1996; Kiesecker *et al.*, 1996; Schmidt & Amézquita, 2001; Pearl *et al.*, 2003).

In addition to the anti-predator responses exhibited by larvae, adult amphibians can assess the presence of predators so as to avoid oviposition or deposition of tadpoles in predator-containing environments (Resatarits & Wilbur, 1989; Bradford, 1989; Kats & Sih, 1992; Petranka *et al.*, 1994; Downie *et al.*, 2001). A lack of adult anti-predator behaviour has a significant negative impact on amphibian larval success (Laurila & Aho, 1997). Habitat selection or choice for tadpole deposition is predator density dependent and influences tadpole densities in pools, which in turn has immediate consequences for other physiological, developmental and behavioural traits of larvae, hence shaping prey communities and habitat choice for breeding (Smith, 1983; Alford, 1986; Skelly, 1992; Hopey & Petranka, 1994; Lardner, 2000).

As with many dendrobatid species, male *Mannophryne trinitatis* (Garman, 1888) (see Murphy, 1997 for species nomenclature) transport tadpoles on their backs after hatching and deposit them in predator-free pools, which are part of or near a stream. They show

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strong anti-predator selective behaviour when releasing larvae into pools, with tadpoles commonly deposited in pools which lack the freshwater fish *Rivulus hartii* (Boulenger, 1890) or the freshwater prawn *Macrobrachium carcinus* (Linnaeus, 1758) (Cummins & Swan, 1995; Downie *et al.*, 2001). Finding a suitable deposition site may take several days and may be costly either to the transporting male or the tadpoles (Downie *et al.*, 2005). Therefore, tadpoles should not encounter predators (except for opportunistic species) during development and might therefore be expected to show weak predator spatial avoidance responses in the presence of *R. hartii* or *M. carcinus* (Hopey & Petranka, 1994). On the other hand, if the predators are able to migrate extensively, deposition-selectivity may not result in predator avoidance for the lifetime of the larvae, so predator-avoidance behaviour may still be adaptive.

This study had six aims: to determine whether: (1) *M. trinitatis* tadpoles show anti-predator behaviour when presented with either *Rivulus hartii* or *Macrobrachium carcinus*; (2) *M. trinitatis* tadpoles detect predators primarily by chemical or visual cues or by both; (3) *M. trinitatis* tadpoles from different regions show stronger spatial predator avoidance to predators that are found in the same region; (4) tadpoles have different levels of spatial avoidance to different predators; (5) larval anti-predator behaviour is inherited or acquired through conditioning or experience; and (6) the predator avoidance response is dependent on tadpole size and/or age.

MATERIAL AND METHODS

STUDY SITES AND TAXA

Tadpole and predator collection (collected using handnets) and experimental trials took place during the 2002 rainy season, July - August in Trinidad, West Indies. Four populations of *M. trinitatis* tadpoles were collected from three sites in the Northern Range and one in the Central Range. The Northern Range sites were: (1) Lopinot (61° 20' W–10° 40' N), which has few *R. hartii* and no *M. carcinus*; (2) Mount Saint Benedict (61° 23' W–10° 41' N), which has many *R. hartii*, and no *M. carcinus*. The collection of small tadpoles at this site was from direct depositions by male frogs into tubs containing stream water positioned near the stream; (3) East Maracas Bay, three streams (61° 27' W–10° 46' N), one of which has many *M. carcinus* but no *R. hartii*; the others lack both predators. See Downie *et al.* (2001) for site

descriptions. The Central Range site was Tamana cave (61° 11' W–10° 29' N) whose stream has neither predator. See Kenny (1978-79) for site description. *R. hartii* were collected from Mount Saint Benedict and *M. carcinus* from East Maracas Bay.

TADPOLE AND PREDATOR MAINTENANCE

After collection, tadpoles were separated by eye into two size classes, small and large, and then maintained in separate tanks with constantly aerated, dechlorinated tap water. A random sample from each size class and site was measured: total body length to 0.1 mm using callipers; wet weight after removing surface water, measured using an electronic balance to 0.001 g. The ambient temperature in the laboratory varied little and kept the water at 27.5°C. Because *R. hartii* are capable of jumping, their tank was covered with muslin. Tadpoles were fed daily with tropical fish food flakes. *R. hartii* were fed fish food flakes daily and both predators were fed (non experimental) *M. trinitatis* tadpoles every other day. Tadpoles were kept in stock tanks for up to ten days, then released and a new tadpole stock was captured.

EXPERIMENTAL DESIGN

Batches of fifteen tadpoles from each of the four *M. trinitatis* populations were observed in glass tanks (90.5 cm × 35 cm × 35 cm) in the presence of each of the predators (*R. hartii* or *M. carcinus*), or in a control situation (no predator) using three types of containers. To test whether tadpoles reacted to visual or chemical cues, the three different predator containers were: a cage of green plastic mesh (8 cm × 8 cm × 5 cm; tadpoles could see the predator and detect it by any chemicals released); a white plastic opaque container with randomly perforated holes (6.5 cm in height × 13 cm in diameter; tadpoles could not see the predator but any chemicals released could be detected); a transparent plastic container with no openings (5 cm in diameter × 9 cm in height; tadpoles could see the predator but no predator chemicals could reach the tadpoles). Because *M. trinitatis* tadpole Gosner (1960) stages are difficult to assess, tadpoles were separated by size measurements (Table 1). Each treatment was repeated with small and large tadpoles from each population. To avoid conditioning of tadpoles to any one predator or container, the order of trials and controls was randomized with a maximum of four trials per day for each population. In

TABLE 1. Small and large *M. trinitatis* tadpole sizes: mean wet weight (g) and total body length (cm) (\pm SD) of all four populations (Mount Saint Benedict, Tamana, Maracas and Lopinot).

Populations	Large		Small	
	Mean weight	Mean length	Mean weight	Mean length
Benedict ($n=20$)	0.201 \pm 0.051	2.615 \pm 0.304	0.072 \pm 0.012	1.843 \pm 0.258
Maracas ($n=20$)	0.258 \pm 0.065	2.922 \pm 0.295	0.085 \pm 0.021	1.946 \pm 0.182
Tamana ($n=20$)	0.391 \pm 0.098	3.420 \pm 0.240	0.074 \pm 0.025	1.930 \pm 0.228
Lopinot ($n=20$)	0.201 \pm 0.033	2.74 \pm 0.197	0.075 \pm 0.015	1.831 \pm 0.277

TABLE 2. Summary of univariate analysis of variance (ANOVA) for all factors: containers, populations, predators (without controls), tadpole sizes (large and small) and interaction between the significant variables.

Source	SOS	Error	df	F	P
Predator	1.518	2.908 (df 288)	1	137.3	$P < 0.001$
Container	1.781	2.908 (df 288)	2	80.5	$P < 0.001$
Population	0.153	2.908 (df 288)	3	4.6	$P < 0.01$
Size	0.009	2.908 (df 288)	1	0.8	NS
Predator \times Container	0.289	2.908 (df 288)	2	13.0	$P < 0.001$
Container \times Population	0.447	2.908 (df 288)	6	6.7	$P < 0.001$
Population \times Predator	0.092	2.908 (df 288)	3	2.8	$P < 0.05$
Predator \times Container \times Population	0.142	2.908 (df 288)	6	2.1	$P < 0.05$

addition, the stock population from which each trial group was randomly selected was around 100 tadpoles, minimising the chance that any one tadpole would repeatedly take part in similar trials. The tank rear glass was divided into nine 10 cm sections by white tape stuck to the outside and marked A, 1, 2, 3, 4, 5, 6, 7, 8, where A marked the position of the predator container. To decrease tadpole disturbance, the tank sides were screened with muslin except at one side for observations. The tanks contained approximately 17 litres of de-chlorinated tap water which was aerated prior to the experiments, but not during them.

For each trial, we selected 15 tadpoles randomly from the required stock tank, using a handnet to introduce them all at once into section 4. They were left for 30 min to habituate with an empty predator container at A. One predator (either *Rivulus* or *Macrobrachium*) was placed in the container after the habituation period. To avoid differences in the experimental manipulation between predator and predator-free (control) trials, the containers were similarly opened and closed for control trials. Tadpole distribution in each tank section was recorded every 10 min for one hour following introduction of the predator. After each trial, the water was changed, containers were washed and predators and tadpoles were returned to stock before preparing the next trial. Each trial was repeated three times, giving a total of 216 trials.

DATA ANALYSIS

The number of tadpoles recorded in each tank section after each 10 min period was multiplied by the distance (in cm) from the predator container (beginning of section A) and divided by the total number of tadpoles in all three trials ($n=45$ tadpoles, three repeats of each trial with 15 tadpoles in each trial). This generated a data point representing the mean distance between tadpoles and predator container after each 10 minute period, with a minimum value of 10 (all tadpoles in section A) and a maximum value of 90 (all tadpoles in section 8). Because normal probability plots revealed highly right skewed data, we transformed each data point using the fourth root to correct for deviations from normality (Kittlitz, 1999), giving a possible range between 1.77 and 3.08.

We used parametric univariate analysis of variance (ANOVA) and Bonferroni corrections to compare differences between populations, tadpole sizes, predators, and type of predator container. All analyses were performed with the statistical package SPSS v10.

RESULTS

The size distributions of the tadpoles from the four sites are shown in Table 1. There were no significant differences in positional distribution between the two tadpole size classes (data not shown for individual experiments: Table 2 shows the lack of significance of size as a factor explaining tadpole positional distribution in all experiments). Because of this, all analyses were performed using the data from the two size classes combined.

The positional distribution of tadpoles did not change significantly with time in control experiments (with mesh container, ANOVA: $F_{5,39}=2.02$, $P=0.98$; with opaque container, ANOVA: $F_{5,39}=0.96$, $P=0.455$; with transparent container, ANOVA: $F_{5,39}=0.45$, $P=0.447$). However, the positional distribution of tadpoles did change significantly with time in some experimental situations. When any chemicals from *R. hartii* could be detected by tadpoles, tadpoles moved further away from the predator with time (predator in mesh container, ANOVA: $F_{5,39}=5.24$, $P < 0.001$; predator in opaque container, ANOVA: $F_{5,39}=3.84$, $P=0.006$), suggesting that the tadpoles sensed and attempted to avoid *R. hartii*. When *R. hartii* could only be detected visually by the tadpoles, they did not move significantly further from the predator with time (predator in transparent container, ANOVA: $F_{5,39}=1.80$, $P=0.137$). In experiments with *M. carcinus*, there were no significant changes in tadpole distribution with time in any of the experimental situations (predator in mesh container, ANOVA: $F_{5,39}=0.39$, $P=0.855$; predator in opaque container, ANOVA: $F_{5,39}=0.13$, $P=0.984$; predator in transparent container, ANOVA: $F_{5,39}=0.65$, $P=0.666$).

The predator and the type of container used in the trials were the two most significant variables in explaining the positional distribution of the tadpoles (Tables 2-4, Fig. 1). All *M. trinitatis* tadpole populations showed significant movement away from the two predators when the tadpoles used visual and chemical cues com-

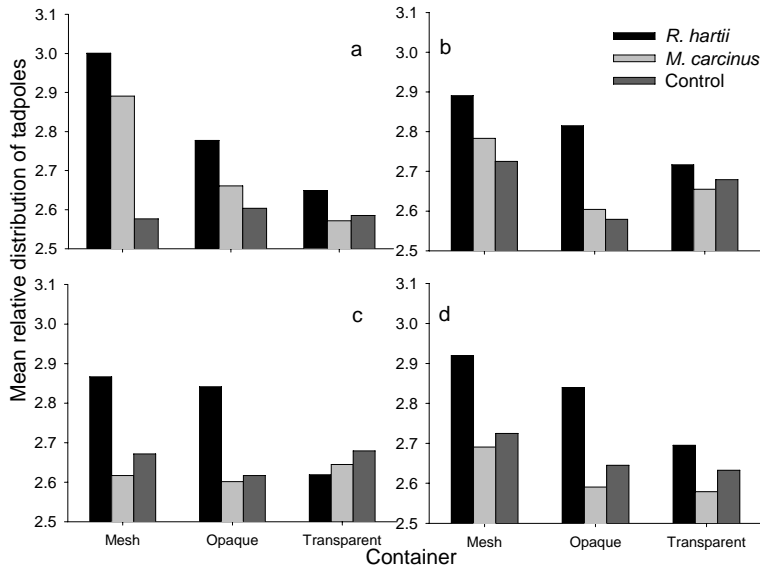


FIG. 1. *M. trinitatis* tadpole avoidance response to predators and in control experiments using the three predator containers for each population. A: Mount Saint Benedict; B: Tamana Cave; C: Maracas Bay; D: Lopinot. Calculation of the avoidance index is explained in Materials and Methods – Data Analysis. High values indicate that tadpoles were distributed further away from the container than experiments with low value results.

TABLE 3. Analysis of differences in tadpole distributions: comparisons between container types (mesh, opaque, transparent) for each population and each predator-control pairing. Where the mean value is positive, tadpoles moved further from the first factor in the comparison with time; when negative, they moved closer to the first factor. Non-significant values shown as NS.

(A) UNIVARIATE ANOVAS

Population	Container	SOS	Error	df	F	P
Benedict	Mesh	1.163	0.292 (33 df)	2	65.8	<i>P</i> <0.001
	Opaque	0.189	0.505 (33 df)	2	6.2	<i>P</i> <0.01
	Transparent	0.041	0.177 (33 df)	2	3.8	<i>P</i> <0.05
Tamana	Mesh	0.17	0.181 (33 df)	2	15.4	<i>P</i> <0.001
	Opaque	0.403	0.416 (33 df)	2	16.0	<i>P</i> <0.001
	Transparent	0.023	0.185 (33 df)	2	2.1	NS
Maracas	Mesh	0.415	0.355 (33 df)	2	19.3	<i>P</i> <0.001
	Opaque	0.433	0.386 (33 df)	2	18.5	<i>P</i> <0.001
	Transparent	0.018	0.278 (33 df)	2	1.1	NS
Lopinot	Mesh	0.356	0.308 (33 df)	2	19.1	<i>P</i> <0.001
	Opaque	0.417	0.301 (33 df)	2	22.9	<i>P</i> <0.001
	Transparent	0.08	0.426 (33 df)	2	3.1	NS

(B) PAIRED COMPARISONS WITH BONFERRONI CORRECTIONS

Population	Predator comparison	Mesh		Opaque		Transparent	
		Mean	<i>P</i>	Mean	<i>P</i>	Mean	<i>P</i>
Benedict	<i>R. hartii</i> vs <i>M. carcinus</i>	0.11	<i>P</i> <0.05	0.117	NS	0.077	<i>P</i> <0.05
	<i>R. hartii</i> vs Control	0.424	<i>P</i> <0.001	0.174	<i>P</i> =0.005	0.064	NS
	<i>M. carcinus</i> vs Control	0.314	<i>P</i> <0.001	0.057	NS	-0.013	NS
Tamana	<i>R. hartii</i> vs <i>M. carcinus</i>	0.108	<i>P</i> <0.01	0.211	<i>P</i> <0.001	0.061	NS
	<i>R. hartii</i> vs Control	0.166	<i>P</i> <0.001	0.236	<i>P</i> <0.001	0.037	NS
	<i>M. carcinus</i> vs Control	0.058	NS	0.025	NS	-0.024	NS
Maracas	<i>R. hartii</i> vs <i>M. carcinus</i>	0.25	<i>P</i> <0.001	0.24	<i>P</i> <0.001	-0.025	NS
	<i>R. hartii</i> vs Control	0.196	<i>P</i> <0.001	0.225	<i>P</i> <0.001	-0.055	NS
	<i>M. carcinus</i> vs Control	0.054	NS	-0.015	NS	-0.029	NS
Lopinot	<i>R. hartii</i> vs <i>M. carcinus</i>	0.229	<i>P</i> <0.001	0.249	<i>P</i> <0.001	0.116	NS
	<i>R. hartii</i> vs Control	0.186	<i>P</i> <0.001	0.197	<i>P</i> <0.001	0.062	NS
	<i>M. carcinus</i> vs Control	-0.042	NS	-0.052	NS	-0.053	NS

TABLE 4. Analysis of differences in tadpole distributions: comparisons between predator types (*R. hartii*, *M. carcinus*, control) for each population and each container type. Where the mean value is positive, tadpoles moved further from the first factor in the comparison with time. When negative, they moved closer to the first factor. Non-significant values shown as NS.

(A) UNIVARIATE ANOVAS

Population	Predator	SOS	Error	df	F	P
Benedict	<i>R. hartii</i>	0.76	0.366 (33 df)	2	34.3	$P < 0.001$
	<i>M. carcinus</i>	0.651	0.326 (33 df)	2	32.9	$P < 0.001$
	Control	0.004	0.282 (33 df)	2	0.3	NS
Tamana	<i>R. hartii</i>	0.183	0.200 (33 df)	2	15.1	$P < 0.001$
	<i>M. carcinus</i>	0.205	0.256 (33 df)	2	13.2	$P < 0.001$
	Control	0.133	0.326 (33 df)	2	6.8	$P < 0.01$
Maracas	<i>R. hartii</i>	0.446	0.476 (33 df)	2	15.5	$P < 0.001$
	<i>M. carcinus</i>	0.011	0.325 (33 df)	2	0.6	NS
	Control	0.046	0.219 (33 df)	2	1.9	NS
Lopinot	<i>R. hartii</i>	0.312	0.508 (33 df)	2	10.1	$P < 0.001$
	<i>M. carcinus</i>	0.094	0.460 (33 df)	2	3.2	NS
	Control	0.074	0.066 (33 df)	2	18.4	$P < 0.001$

(B) PAIRED COMPARISONS WITH BONFERRONI CORRECTIONS

Population	Container comparison	<i>R. hartii</i>		<i>M. carcinus</i>		Control	
		Mean	P	Mean	P	Mean	P
Benedict	Mesh vs Opaque	0.223	$P < 0.001$	0.23	$P < 0.001$	-0.026	NS
	Mesh vs Transparent	0.352	$P < 0.001$	0.319	$P < 0.001$	-0.008	NS
	Opaque vs Transparent	0.128	$P < 0.05$	0.089	NS	0.0183	NS
Tamana	Mesh vs Opaque	0.075	NS	0.179	$P < 0.001$	0.146	$P < 0.01$
	Mesh vs Transparent	0.174	$P < 0.001$	0.128	$P < 0.01$	0.045	NS
	Opaque vs Transparent	0.098	$P < 0.05$	-0.05	NS	-0.099	NS
Maracas	Mesh vs Opaque	0.025	NS	0.015	NS	0.053	NS
	Mesh vs Transparent	0.248	$P < 0.001$	-0.028	NS	-0.003	NS
	Opaque vs Transparent	0.223	$P < 0.001$	-0.043	NS	-0.057	NS
Lopinot	Mesh vs Opaque	0.08	NS	0.1	NS	0.09	$P < 0.001$
	Mesh vs Transparent	0.225	$P < 0.001$	0.112	NS	0.101	$P < 0.001$
	Opaque vs Transparent	0.145	$P < 0.05$	0.011	NS	0.01	NS

bined or independently to detect the predators (predators in mesh and in opaque containers (Tables 3-4; Fig. 1). Tadpoles that were able to use these cues to detect the predators avoided *R. hartii* significantly more than *M. carcinus* (Table 3, Fig. 1). Overall, when chemicals could not be detected (predators in transparent containers), tadpoles showed non-significant spatial avoidance differences between the two types of predator (Table 3) or between populations (Table 5). All tested *M. trinitatis* populations reacted significantly differently to the presence of predators and controls in mesh and transparent containers (Table 5). However, there were no significant differences in distribution between the four different *M. trinitatis* populations to the two predators when using only chemical detection to detect the predators (opaque container, Tables 5, Fig. 1). The Mount Saint Benedict tadpoles showed the strongest avoidance to *R. hartii* when visual and chemical cues were employed, being significantly greater than all other *M. trinitatis* populations (Table 5, Fig. 1).

DISCUSSION

TADPOLE SIZE EFFECTS

Responses of tadpoles of different size classes (representing different times since deposition) to predator cues might have been expected for two reasons. First, many predators hunt selectively on prey according to their size. However, both the predators used in this study range considerably in size, so it is likely to be adaptive for tadpoles to be able to detect predators as soon as they enter the stream. Second, predator detection could be partly a learned response, improving with time. The lack of any significant difference in response to predator cues between the two tadpole size classes suggests that predator detection is essentially an inherent ability of tadpoles.

PREDATOR DETECTION MECHANISM

Our observations support the earlier results showing that amphibian larvae detect predators primarily by

TABLE 5. Analysis of differences in tadpole distributions. Comparisons between populations (Benedict, Maracas, Lopinot, Tamana) for each container type and each predator. Where the mean value is positive, tadpoles moved further from the first factor in the comparison with time. When negative, they moved closer to the first factor. Non-significant values shown as NS.

(A) UNIVARIATE ANOVAS

Predator	Container	SOS	Error	df	<i>F</i>	<i>P</i>
<i>R. hartii</i>	Mesh	0.123	0.202 (44 df)	3	8.9	<i>P</i> <0.001
	Opaque	0.032	0.878 (44 df)	3	0.5	NS
	Transparent	0.069	0.470 (44 df)	3	2.2	NS
<i>M. carcinus</i>	Mesh	0.506	0.631 (44 df)	3	11.8	<i>P</i> <0.001
	Opaque	0.035	0.388 (44 df)	3	1.4	NS
	Transparent	0.067	0.348 (44 df)	3	2.8	<i>P</i> <0.05
Control	Mesh	0.187	0.304 (44 df)	3	9.1	<i>P</i> <0.001
	Opaque	0.023	0.342 (44 df)	3	1.0	NS
	Transparent	0.069	0.247 (44 df)	3	4.1	<i>P</i> <0.05

(B) PAIRED COMPARISONS WITH BONFERRONI CORRECTIONS

Predator	Population comparison	Mesh		Opaque		Transparent	
		Mean	<i>P</i>	Mean	<i>P</i>	Mean	<i>P</i>
<i>R. hartii</i>	Benedict vs Tamana	0.11	<i>P</i> <0.01	-0.037	NS	-0.067	NS
	Benedict vs Maracas	0.134	<i>P</i> <0.001	-0.064	NS	0.03	NS
	Benedict vs Lopinot	0.08	<i>P</i> <0.05	-0.062	NS	-0.045	NS
	Tamana vs Maracas	0.024	NS	-0.026	NS	0.097	NS
	Maracas vs Lopinot	-0.053	NS	0.001	NS	-0.075	NS
	Lopinot vs Tamana	0.029	NS	-0.024	NS	0.021	NS
<i>M. carcinus</i>	Benedict vs Tamana	0.108	NS	0.056	NS	-0.083	NS
	Benedict vs Maracas	0.274	<i>P</i> <0.001	0.059	NS	-0.073	NS
	Benedict vs Lopinot	0.2	<i>P</i> =0.001	0.07	NS	-0.007	NS
	Tamana vs Maracas	0.167	<i>P</i> <0.01	0.002	NS	0.01	NS
	Maracas vs Lopinot	-0.074	NS	0.01	NS	0.065	NS
	Lopinot vs Tamana	-0.092	NS	-0.018	NS	-0.075	NS
Control	Benedict vs Tamana	-0.148	<i>P</i> <0.001	0.024	NS	-0.094	<i>P</i> <0.05
	Benedict vs Maracas	-0.094	<i>P</i> <0.05	-0.01	NS	-0.089	<i>P</i> <0.05
	Benedict vs Lopinot	-0.157	<i>P</i> <0.001	-0.039	NS	-0.047	NS
	Tamana vs Maracas	-0.054	NS	-0.037	NS	0.004	NS
	Maracas vs Lopinot	-0.062	NS	-0.025	NS	0.041	NS
	Lopinot vs Tamana	0.008	NS	0.063	NS	-0.046	NS

chemicals released in the water. Stauffer & Semlitsch (1997), testing the responses of *Rana esculenta* and *Rana lessonae* tadpoles to fish by three possible mechanisms of predator detection (visual, tactile and chemical), determined that chemical cues produced the strongest response, while a combination of chemical and tactile cues resulted in a significantly stronger response than visual and tactile. They suggested that predator movements produced directional waves of chemical cues that could alert tadpoles to predator distance. When visual information is not available (at night, in turbid waters, with dense aquatic vegetation, with conspicuous or ambush predators) chemical and

tactile cues may be critical to the assessment of predation risks (Kiesecker *et al.*, 1996). Small underwater currents produced by predators in mesh cages may explain the strong response to predators in these containers and why the tadpoles' responses were stronger to *R. hartii* than to *M. carcinus* (the former predator moving more often than the latter one: personal observations).

SPECIFIC ANTI-PREDATOR RESPONSE

All tadpole populations reacted to *R. hartii* significantly more than to *M. carcinus*. These findings are similar to other studies where responses to crayfish

were not as strong as to predatory fish (Lefcort, 1996; Bridges & Gutzke, 1997; Pearl *et al.*, 2003). Kurzava & Morin (1998) suggested that fish are functionally distinct from other kinds of aquatic predators, being more efficient and exerting greater pressure on anuran prey populations. However, Gascon (1992) showed that dragonfly larvae chemicals were more of a deterrent to anuran larvae (*Osteocephalus taurinus*, *Epipedobates femoralis*, *Phyllomedusa tomoptera*) than chemicals from *Rivulus* species.

Tadpoles at high densities are known to react more strongly to predation threat, increasing the accuracy of predation risk assessment (MacNamara & Houston, 1992). *M. trinitatis* tadpoles are often found in large numbers (even thousands; Kenny, 1969) in small pools, and therefore the spatial avoidance response we observed when tadpoles were presented to predators may under-represent the possible response, due to the small number of tadpoles used in our trials. The two populations (Benedict and Lopinot) that naturally share habitat with *R. hartii* exhibited the greatest avoidance to this predator. Surprisingly, tadpoles from Maracas showed a significantly higher response to *M. carcinus* when chemical detection was impeded (transparent container) and Tamana tadpoles showed a greater response when this predator was in a transparent container than when enclosed in an opaque container, indicating that visual cues may also be used for predator detection.

Tadpoles are known to assess predation risk and respond to multiple predators (Semlitsch & Reyer, 1992). This behaviour is likely to be dependent on the amount of single cues emitted from a predator, the greater the amount emitted, the greater the tadpole response (Semlitsch & Reyer, 1992; Lefcort, 1996; Manteifel & Zhushhev, 1998; Van Buskirk, 2001). This may suggest that the strong avoidance response behaviour to *R. hartii* is a consequence of the larger amount of cues emitted by this predator. Differences in predator avoidance may reflect local environmental adaptations to predators but may be a disadvantage for survival when both predators are present in the same habitat. Kurzava & Morin (1988) showed that very few metamorphs of several anuran species survived when two predators (*Notophthalmus viridescens* and *Enneacanthus obesus*) were combined in artificial ponds, suggesting that different anuran species had specific anti-predator behaviours to different predators. In our experiments, lack of strong anti-predator response to *M. carcinus* could imply that if both predators were present in the same stream, *M. trinitatis* tadpoles would detect *R. hartii* cues but not *M. carcinus* cues.

PREDATOR DISTRIBUTION

Schmidt & Amézquita (2001) found that *Phyllomedusa tarsi* tadpoles showed an anti-predator behavioural response to a widely-distributed aeshnid dragonfly nymph species, but not to a more dangerous belostomatid bug. They concluded that the tadpoles encountered bugs in their natural environment too

rarely for an anti-predator response to have evolved. Different levels of spatial avoidance response to the two predators in our experiments may be explained by the current distribution of *R. hartii* and *M. carcinus* in Trinidad. *M. carcinus* are much less widespread than *R. hartii*, in streams utilised by *M. trinitatis*. Of all the streams surveyed at Maracas Bay, only one had *M. carcinus*. *M. carcinus* distribution is heavily constrained by abiotic factors and its need for a larval stage amphidromous migration (March *et al.*, 1998; Chung, 2001). Many of the north coast streams which support *M. trinitatis* populations are too steep to allow such migrations and the southern slopes of the Northern Range lack immediate access to the sea, hence have no *Macrobrachium* populations. *R. hartii* is an efficient colonizer and adapts to a variety of habitats, with the ability to leave streams and search over several metres to locate more suitable pools (even up steep stream slopes; Seghers, 1978). The distribution patterns of the two predators suggest that *M. trinitatis* tadpoles are unlikely to encounter *M. carcinus* as often as *R. hartii* and therefore may not have evolved specific anti-predator responses to these shrimps.

ADAPTATION OR INHERITANCE?

Tadpoles from the Mount Saint Benedict site were collected from containers placed at the site. These tadpoles were naïve (they had never been exposed to chemical cues from, or presence of *R. hartii* or *M. carcinus*). However, this population showed the strongest response to predators. Therefore this behaviour cannot reflect experience in the natural environment and must be inherited rather than acquired. This conclusion is also supported by the behaviour of tadpoles from Tamana cave, which showed anti-predator response to both predators, despite the lack of these predators in the cave. Although *R. hartii* is absent from Tamana cave, it is common throughout the Central Range. Because the Tamana cave population is part of the Central Range meta-population (Jowers & Downie, 2004, and unpublished data) this may explain why *M. trinitatis* tadpoles from this population show anti-predator behaviour to *R. hartii*.

At some sites, *M. trinitatis* tadpoles were found in high numbers (hundreds or even thousands) in predator-free pools (Tamana cave). At other sites, tadpoles were hard to find and were deposited in lower numbers in small pools (Mount Saint Benedict and Lopinot). Predator colonization could have significant consequences for successive generations at these breeding sites. Therefore anti-predator behaviour is of extreme importance for these palatable tadpoles and an inherited response to predators is likely to be advantageous to their survival.

ADULT AND LARVAL DETECTION MECHANISM

Male *M. trinitatis* depositing tadpoles seem highly efficient at assessing predation pressure in pools. Therefore, larvae should not normally face predators

and might have been expected to lack anti-predator responses. Downie *et al.* (2001) demonstrated that males preferred predator-free pools to pools containing either normally encountered or not normally encountered predators. When males were presented with pools containing either *M. carcinus* or *R. hartii*, males from Lopinot, Mount Saint Benedict and Maracas Waterfall (preyed on by *R. hartii* in the wild) avoided *R. hartii* but deposited in containers with *M. carcinus*. The males from Maracas (preyed on by *M. carcinus* in the wild) deposited few tadpoles in the *R. hartii* containers and none in *M. carcinus* containers. It should be noted, however, that Downie *et al.* (2001) were unable to be certain whether deposition selectivity was due to pool selection by transporting males or detachment selection by tadpoles.

However, the ability of predators to migrate within the stream environment means that deposition selectivity may not be a complete anti-predator protection. It is, therefore, not so surprising that we were able to demonstrate predator avoidance behaviour by tadpoles.

The strengths of the tadpole anti-predator responses in tadpoles from different populations and to the two predators were somewhat different to the deposition selectivity differences found by Downie *et al.* (2001), but the overall difference, that the response was greater to *R. hartii* than to *M. carcinus*, fits with their differential distribution and migration abilities.

LIMITATIONS OF THE STUDY

In the field, *M. trinitatis* tadpole anti-predator behaviour strategies are likely to be affected by abiotic factors which may influence different populations to exhibit anti-predator behaviours that depend on the locality they inhabit. For example, in the Mount Saint Benedict stream and pools, the substrate is composed primarily of leaf litter and tadpoles use it as refuge, reducing their inactivity levels. In contrast, *M. trinitatis* tadpoles in Tamana cave, where leaf litter is absent, show high activity levels and although limited refuge is available under rock crevices, tadpoles rarely hide under them. Thus, our laboratory-based experimental design did not allow tadpoles to exhibit the variety of anti-predator behaviours that otherwise may have been observed, such as refuge use, differences in activity levels and diurnal rhythms (Sih *et al.*, 1992).

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