Predation Risk Avoidance by Terrestrial Amphibians: The Role of Prey Experience and Vulnerability to Native and Exotic Predators

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Abstract

We studied avoidance, by four amphibian prey species (Rana luteiventris, Ambystoma macrodactylum, Pseudacris regilla, Taricha granulosa), of chemical cues associated with native garter snake (Thamnophis elegans) or exotic bullfrog (R. catesbeiana) predators. We predicted that avoidance of native predators would be most pronounced, and that prey species would differ in the intensity of their avoidance based on relative levels of vulnerability to predators in the wild. Adult R. luteiventris (presumably high vulnerability to predation) showed significant avoidance of chemical cues from both predators, A. macrodactylum (intermediate vulnerability to predation) avoided T. elegans only, while P. regilla (intermediate vulnerability to predation) and T. granulosa (low vulnerability to predation) showed no avoidance of either predator. We assessed if predator avoidance was innate and/or learned by testing responses of prey having disparate levels of prior exposure to predators. Wild-caught (presumably predator-exposed) post-metamorphic juvenile R. luteiventris and P. regilla avoided T. elegans cues, while laboratory-reared (predator-naive) conspecifics did not; prior exposure to R. catesbeiana was not related to behavioural avoidance among adult or post-metamorphic juvenile wild-reared A. macrodactylum and P. regilla. These results imply that (i) some but not all species of amphibian prey avoid perceived risk from garter snake and bullfrog predators, (ii) the magnitude of this response probably differs according to prey vulnerability to predation in the wild, and (iii) avoidance tends to be largely learned rather than innate. Yet, the limited prevalence and intensity of amphibian responses to predation risk observed herein may be indicative of either a relatively weak predator–prey relationship and/or the limited importance of predator chemical cues in this particular system.

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Introduction

Prey animals may exhibit a suite of behavioural responses following exposure to predators, including direct displacement and alterations in movement or activity patterns (e.g. Lima & Dill 1990; Sih et al. 1990). Recent research has shown that the expression of such behaviours can be mediated by chemical cues associated with a predator; in systems where predator chemical cues can be used to forecast a predatory encounter, adaptive responses by prey to such cues should be favoured from an evolutionary standpoint (Vermeij 1994; Abrams 2000). Moreover, such responses should be related to the magnitude of predation risk faced by the prey species, as well as the length of the prey’s exposure to predation risk. It follows that in the case of predator–prey relationships where either predation risk is presently high or where predator-induced selection has existed for a long time, prey behavioural responses to perceived predation risk should be substantive.

Prey learning also may shape prey responses to predation risk, thereby further complicating the dynamic interaction between predator and prey. In theory, the extent of predation upon the youngest age classes and the likelihood of prey surviving an initial predator encounter, and responding adaptively following subsequent encounters, should in large part determine whether a particular anti-predator response is innate or learned. Because many predator–prey relationships are both complex and variable, for some prey species predator avoidance may possess both innate and learned components. It follows that the disparity in both the ecology and evolution of predator–prey interactions across taxonomic groups may lead to differential ontogeny and intensity of anti-predator behaviour across predator and prey species (e.g. Dickman 1992; Jedrzejewski et al. 1993).

Many aquatic amphibian species exhibit predator avoidance that is mediated by chemical cues (e.g. Peterson et al. 1992; Kats & Dill 1998), likely because predators and prey are often found in close proximity and exposure to predator cues tends to be intense. Limited evidence exists for similar patterns of recognition in terrestrial environments (e.g. Cupp 1994; Madison et al. 1999; Murray & Jenkins 1999). In the northwestern US, pond amphibians are preyed upon extensively by native garter snake predators (Thamnophis elegans, T. sirtalis) (Nussbaum et al. 1983); garter snakes (or their chemical cues) are known to elicit a variety of avoidance behaviours among amphibian prey (Madison et al. 1999; Murray & Jenkins 1999; Belden et al. 2000). The bullfrog (Rana catesbeiana), introduced recently (last 50 yr) from the eastern US to many western ponds (Nussbaum et al. 1983), is also known to prey on juvenile and adult forms of many species of western frogs and salamanders (Bury & Whelan 1984; Schwalbe & Rosen 1988), and also may compete with native amphibians for space or food (Bury & Whelan 1984). (Herein we refer to bullfrogs as amphibian ‘predators’, recognizing that if they also serve as competitors, amphibian avoidance behaviour should be accentuated.) Both adult and larval bullfrogs have been shown to elicit avoidance in a variety of aquatic amphibians.
Post-metamorphic amphibians may be less susceptible to predation by garter snakes and bullfrogs than their larval counterparts because they avoid aquatic/riparian habitats during most of the year. Thus, while aquatic amphibian larvae should avoid chemical cues from these two predators, expression of such responses may be absent among some adult amphibians because they confer little selective advantage (i.e. in cases where sympatry between adult amphibians and their predators is limited). Similarly, predator avoidance may not be beneficial in toxic amphibian species.

We assessed behavioural responses of spotted frogs (*R. luteiventris*), long-toed salamanders (*Ambystoma macrodactylum*), Pacific treefrogs (*Pseudacris* (or *Hyla* *regilla*)), and rough-skinned newts (*Taricha granulosa*) to chemical cues associated with native (garter snake) vs. exotic (bullfrog) predators. We hypothesized that predator avoidance would be associated with relative vulnerability to predation by different prey species. We predicted that (1) adult spotted frogs, which not only occupy riparian habitat throughout the spring–fall (Nussbaum et al. 1983) and are therefore in close proximity to both garter snakes and bullfrogs for much of the year, but also have been observed by us being consumed by both predators, should avoid chemical cues from both predator species. Adult salamanders and treefrogs should not avoid such predator cues as strongly because they occupy subterranean (salamanders) or arboreal (treefrogs) habitats (i.e. spatial refugia) during most of the spring–fall. Newts are toxic to both garter snakes and bullfrogs (see Brodie 1968; Macartney & Gregory 1981), and thus should gain the least benefit from any predator avoidance. Thus, the four amphibian species should exhibit a range in prey avoidance behaviour that is based on their susceptibility to snake and bullfrog predation. (2) The disparity in past exposure of western larval amphibians to the two predators suggests that (i) avoidance should be more intensive towards snake cues, and that (ii) predator avoidance should be strongest among post-metamorphic juvenile stages than adults (i.e. largely innate).

**Methods**

**Collection and Maintenance of Study Animals**

All animals were handled in accordance with protocol number 9053, which was approved by the University of Idaho Animal Care and Use Committee. Free-ranging adult salamanders, newts, and frogs were captured by hand, dipnet, or minnow trap from breeding ponds near Moscow, Idaho (49°N, 117°W). Adult salamanders and treefrogs were captured during the breeding season (March–May), spotted frogs were captured during April–August, while newts were captured during August–September. Post-metamorphic juvenile
Frogs, salamanders, and treefrogs were obtained near pond edges a few days before or after metamorphosis; newly transformed juvenile newts were not collected. Overall, amphibians were obtained from 10 different ponds, only two that are also known to be occupied by resident populations of bullfrogs (separate groups of long-toed salamanders and treefrogs were captured from ponds with and without bullfrogs; spotted frogs and newts were available from ponds lacking bullfrogs only); garter snakes were observed in/near all ponds. Because ponds with bullfrogs were separated by a minimum of 8 km from those without bullfrogs, we considered all amphibian populations to be discrete with respect to exposure to bullfrogs. All amphibians were housed in plastic containers in a coldroom (5°C) for 1–3 d prior to the behavioural trials; animals were allowed to acclimate to room temperature for several hours prior to the start of trials.

To evaluate the extent to which amphibian responses to garter snake predation risk were innate and/or learned, we raised frog tadpoles and salamander larvae in the laboratory and subjected them to snake chemical cues in behavioural trials, post-metamorphosis. Gastrula-stage amphibian egg masses were removed from ponds in spring and housed in 2-gallon plastic containers containing pond water and receiving continuous aeration. Hatched larvae were fed ad lib until metamorphosis (salamanders were fed brine shrimp and oligochaete worms; frogs were fed tropical fish food). Post-metamorphic juveniles were removed from containers after transformation and used within 3 d in behavioural trials (July–September). To evaluate the effect of prior exposure on amphibian avoidance of bullfrog cues, post-metamorphic juvenile and adult salamanders and treefrogs from ponds with vs. without bullfrogs were captured and subjected to behavioural trials.

Wandering garter snakes (n = 23) were captured by hand and housed individually in 10-gallon tanks, while bullfrogs (n = 12) were either captured from ponds or obtained commercially, and housed in 50-gallon plastic storage bins. In addition, to test the possibility that newts avoided cues from snakes that were toxin-resistant (see below), five common garter snakes (T. sirtalis) were captured and housed in conditions similar to that of T. elegans. Snakes and bullfrogs were kept in the laboratory year-round, and fed earthworms (Lumbricus spp.) or chicken hearts, every 3–4 d. Predators were fed earthworms rather than conspecific prey specifically to provide a neutral diet that would not bias interspecific comparisons of prey responses; because prey avoidance tends to be accentuated when conspecifics are fed to predators, the intensity of prey responses reported herein may be considered as conservative. All animals were maintained on a 12.00 : 12.00 hours (light : dark) photoperiod at approximately 20°C. Prior to behavioural trials, paper towels were left in the predator tanks/storage bins for 24–36 h, and then used as substrate in experiments. Predators were made to fast for a minimum of 4 d prior to being used in experiments; however, any faecal material that accumulated on paper towels during the treatment period was removed prior to behavioural trials.
Behavioural Trials

Our methods for evaluating anti-predator behaviour were similar to those used by Cupp (1994) and Murray & Jenkins (1999). Amphibians were placed in 24 cm × 45 cm behavioural arenas with a central line drawn on the bottom and one paper towel (substrate) on each side of the arena. Most trials were conducted under a fume hood to prevent cross-contamination of predator cues. Amphibian behaviour was observed for 120 consecutive minutes in a laboratory under fluorescent lighting (e.g. Chivers et al. 1995), and all behavioural trials were conducted at room temperature between 10.00 and 15.00 hours. The two paper towel substrates in each arena were separated by 2 cm to avoid diffusion of chemical cues between them. At the start of each trial, amphibians were released on the centre line; every 2 min their location in the experimental chamber (side A or side B) was recorded. After 30, 60 and 90 min, we rotated the arena 180°. To minimize the potential for amphibian dehydration during trials, we misted paper towels with dechlorinated water and covered experimental chambers with plexiglass.

Wild-caught adult and post-metamorphic juvenile amphibians and laboratory-reared juvenile amphibians were subject to predation risk treatments from wandering garter snakes and bullfrogs vs. untreated (control) substrates. Because we were unable to obtain newt eggs or juveniles, this species was excluded from such trials. To evaluate the ability of newts to discern between toxin-resistant vs. potentially non-resistant snake predators, we tested adult newts in behavioural trials consisting of common garter snake chemical cues (common garter snakes exhibit greater resistance to newt toxin than do wandering garter snakes, see Brodie & Brodie 1999). Baseline information on amphibian movement patterns in the absence of predator cues was determined in trials consisting of control substrate on both sides of the arena.

Statistical Analysis

In sum, we conducted a total of 520 behavioural trials evaluating amphibian anti-predator responses, with 160 trials being undertaken on salamanders, 160 on treefrogs, 120 on spotted frogs, and 80 on newts. An individual amphibian was considered as the experimental unit and thus was used in a single trial, except that 11 adult treefrogs and 20 adult newts were used in two trials each. Because for each individual used in multiple trials the second trial constituted exposure to control vs. control substrate (to determine baseline activity patterns), and given the relatively large number of predators used to condition the substrate, we felt that this level of pseudoreplication was unlikely to have confounded our results (see also Ramírez et al. 2000). While our experimental lighting regime and intensity of predator cues were comparable with those used in previous studies, we determined if our results were influenced by these procedures by comparing the behaviour of adult salamanders (subject to snake vs. control treatments) when (i) fluorescent lights were on vs. off, and (ii) paper towels had been left in snake
tanks for 1 vs. 3 d. These tests involved conducting an additional 40 behavioural trials (20 with lights off, 20 with 3-d-old paper towels) and comparing the results with a set of 20 trials using standard experimental protocols (i.e. lights on and 1-d-old paper towels) and involving snake cues. We found that neither lighting level ($F_{1,38} = 0.985, p = 0.32$), nor duration of exposure to paper towels ($F_{1,38} = 0.084, p = 0.77$) affected salamander behavioural responses to predator cues, implying that our results are unlikely to have been confounded specifically by our experimental protocol.

We sampled 20 individuals for each permutation of our experiment; this standardized approach eliminated potential effects of sample size variability on statistical power. Murray & Jenkins (1999) showed that a similar sample size was adequate to detect avoidance of garter snake cues by red-backed salamanders (*Plethodon cineru*s) under a variety of experimental conditions, including a standard earthworm diet for snakes. Considering that a 60–65% use of the control substrate (contrary to the null of 50%) might represent biologically significant avoidance of predator cues (keeping in mind that the neutral predator diet likely reduced the level of anti-predator stimulus), we conducted a power analysis using our sample size ($n = 20$) and the mean standard deviation observed across our main trials ($SD = 0.218$; see Table 1). We found that for $\alpha = 0.05$, statistical power for a single-sample t-test was 0.50 for a population mean of 60%, and 0.83 for a mean of 65%. Thus, we considered that our experimental protocol provided reasonable statistical power for detecting predator avoidance in the species under study.

We used overall percent time that subjects spent on the control side of the arena to measure antipredator behaviour. Because all of our sample means for use

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Percentage</th>
<th>SD</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. luteiventris</em></td>
<td>Control</td>
<td>0.506</td>
<td>0.151</td>
<td>0.176</td>
<td>0.86</td>
</tr>
<tr>
<td><em>A. macrodactylum</em></td>
<td>Control</td>
<td>0.482</td>
<td>0.187</td>
<td>0.481</td>
<td>0.63</td>
</tr>
<tr>
<td><em>P. regilla</em></td>
<td>Control</td>
<td>0.506</td>
<td>0.071</td>
<td>0.347</td>
<td>0.73</td>
</tr>
<tr>
<td><em>T. granulosa</em></td>
<td>Control</td>
<td>0.466</td>
<td>0.257</td>
<td>0.591</td>
<td>0.56</td>
</tr>
<tr>
<td><em>R. luteiventris</em></td>
<td><em>T. elegans</em></td>
<td>0.576</td>
<td>0.100</td>
<td>3.392</td>
<td>0.003</td>
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<td><em>P. regilla</em></td>
<td><em>T. elegans</em></td>
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<td>0.221</td>
<td>0.960</td>
<td>0.35</td>
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<tr>
<td><em>T. granulosa</em></td>
<td><em>T. elegans</em></td>
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<td>0.148</td>
<td>0.88</td>
</tr>
<tr>
<td><em>R. luteiventris</em></td>
<td><em>R. catesbeiana</em></td>
<td>0.640</td>
<td>0.116</td>
<td>5.370</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><em>A. macrodactylum</em></td>
<td><em>R. catesbeiana</em></td>
<td>0.505</td>
<td>0.270</td>
<td>0.078</td>
<td>0.90</td>
</tr>
<tr>
<td><em>P. regilla</em></td>
<td><em>R. catesbeiana</em></td>
<td>0.426</td>
<td>0.252</td>
<td>1.316</td>
<td>0.20</td>
</tr>
<tr>
<td><em>T. granulosa</em></td>
<td><em>R. catesbeiana</em></td>
<td>0.549</td>
<td>0.233</td>
<td>0.942</td>
<td>0.36</td>
</tr>
<tr>
<td><em>T. granulosa</em></td>
<td><em>T. sirtalis</em></td>
<td>0.475</td>
<td>0.206</td>
<td>0.539</td>
<td>0.60</td>
</tr>
</tbody>
</table>

All prey animals used in trials involving bullfrog cues were captured from ponds lacking bullfrogs. Each trial involved 20 prey animals and analyses were performed using one-sample t-tests, which evaluated the hypothesis of random (i.e. 50 : 50) use of substrates.

Table 1: Percent use of control substrates by four amphibian prey species during experiments evaluating the effect of predator chemical cues on avoidance behaviour.
of control substrate ranged between 25–75% and the distribution of data from all trials approached normality, percentage data were left untransformed and parametric tests were used in analyses. Percent avoidance of predator chemical cues was analysed using a one-sample t-test (Zar 1984) against the null hypothesis of no avoidance (i.e. 50% use of each side). ANOVA was used to compare the effect of specific treatments on amphibian avoidance. While we recognize that multiple tests can inflate the chances of type I statistical error, we reject the concern that Bonferonni adjustments should be used to correct our p-values in the above tests. Each statistical test that used previously analysed data addressed a unique hypothesis pertaining to predator species (exotic vs. native) or response type (innate vs. learned), while each data set used in such analyses was a composite of those used in previous tests.

Results

Avoidance of Predator Cues by Adult Amphibians

In control vs. control trials, the four prey species showed no selection for specific sides of the experimental chamber, indicating that there were no directional biases inherent in our experimental procedure (Table 1). We tested for amphibian avoidance of snake and bullfrog chemical cues, where all individuals tested against bullfrogs were from ponds lacking these predators. Consistent with our first prediction, spotted frogs were the only prey species where adults avoided chemical cues from both predators, with subjects spending on average 58% of the trial away from wandering garter snake cues, and 64% from bullfrog cues (Table 1). The intensity of avoidance manifest by adult spotted frogs did not differ significantly between garter snake and bullfrog predator cues ($F_{1,38} = 3.396, p = 0.073$). In contrast, adult long-toed salamanders were found to avoid wandering garter snake cues but not those of bullfrogs (Table 1), but intensity of salamander avoidance did not differ relative to the two species of predators ($F_{1,38} = 0.202, p = 0.65$). Neither adult treefrogs nor newts avoided chemical cues from either of the two main predator species (Table 1). Furthermore, newts did not avoid cues from common garter snakes (Table 1), and the pattern of substrate usage was similar for trials with both snake species ($F_{1,38} = 0.064, p = 0.80$).

Innate vs. Learned Responses

We compared the responses of laboratory-reared (predator naive) vs. wild-caught (ostensibly predator-exposed) spotted frogs, salamanders, and treefrogs, with wandering garter snake cues. Wild-caught juvenile spotted frogs and treefrogs exhibited snake avoidance, while laboratory-reared conspecifics did not (Table 2). However, we found that for both spotted frogs ($F_{1,38} = 0.002, p = 0.99$) and treefrogs ($F_{1,38} = 0.542, p = 0.47$), the intensity of avoidance did not differ between laboratory-reared vs. wild-caught recent metamorphs. Moreover,
The significant predator avoidance demonstrated by juveniles did not differ from the behavioural patterns reported for adults of either anuran species (spotted frog: $F_{1,38} = 0.375$, $p = 0.55$; treefrog: $F_{1,38} = 1.008$, $p = 0.32$). In contrast, neither laboratory- nor wild-reared juvenile salamanders exhibited predator avoidance when subject to snake cues (Table 2).

Bullfrog-naive adult spotted frogs (no spotted frogs were available from bullfrog ponds) avoided bullfrog cues, but we failed to detect a similar response among recent metamorphs (Table 2). Next, we compared behavioural responses of adult and juvenile salamanders and treefrogs captured from ponds with and without bullfrogs. For adults of both species, we found that individuals failed to avoid bullfrog cues, irrespective of whether they were from bullfrog or non-bullfrog ponds (Table 2). Intensity of avoidance did not differ between bullfrog-exposed and -naive adult salamanders ($F_{1,38} = 1.162$, $p = 0.29$) and treefrogs ($F_{1,38} = 2.957$, $p = 0.094$). Similarly, we failed to detect avoidance among recent metamorphs of either species from bullfrog or non-bullfrog ponds (Table 2), and the intensity of any potential avoidance behaviour also was similar between bullfrog/non-bullfrog capture sites (salamander: $F_{1,38} = 0.323$, $p = 0.57$; treefrog: $F_{1,38} = 1.018$, $p = 0.32$).

**Table 2:** Percent use of control substrates by three amphibian prey species in experiments evaluating the effect of prey experience on avoidance of predator chemical cues

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Age</th>
<th>Origin</th>
<th>Percentage</th>
<th>SD</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. luteiventris</em></td>
<td><em>T. elegans</em></td>
<td>J</td>
<td>Lab</td>
<td>0.556</td>
<td>0.190</td>
<td>1.315</td>
<td>0.20</td>
</tr>
<tr>
<td><em>R. luteiventris</em></td>
<td><em>T. elegans</em></td>
<td>J</td>
<td>Wild</td>
<td>0.556</td>
<td>0.112</td>
<td>2.238</td>
<td>0.032</td>
</tr>
<tr>
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<td><em>T. elegans</em></td>
<td>J</td>
<td>Lab</td>
<td>0.553</td>
<td>0.253</td>
<td>0.923</td>
<td>0.37</td>
</tr>
<tr>
<td><em>A. macrodactylum</em></td>
<td><em>T. elegans</em></td>
<td>J</td>
<td>Wild</td>
<td>0.522</td>
<td>0.277</td>
<td>0.358</td>
<td>0.72</td>
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<tr>
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<td><em>T. elegans</em></td>
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<td>Lab</td>
<td>0.561</td>
<td>0.256</td>
<td>1.070</td>
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<td>A</td>
<td>Non-BF</td>
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<td>5.370</td>
<td>0.001</td>
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<tr>
<td><em>A. macrodactylum</em></td>
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<td><em>A. macrodactylum</em></td>
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<td><em>P. regilla</em></td>
<td><em>B. catesbeiana</em></td>
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<tr>
<td><em>P. regilla</em></td>
<td><em>B. catesbeiana</em></td>
<td>A</td>
<td>BF</td>
<td>0.564</td>
<td>0.257</td>
<td>1.118</td>
<td>0.28</td>
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</table>

Each trial involved 20 prey animals and analyses were performed using one-sample t-tests, which tested the hypothesis of random (i.e. 50 : 50) use of substrates. Adult and newly transformed (juvenile) animals served as test subjects. Laboratory-reared and wild-caught juveniles were used in tests involving snake cues, whereas prey animals from bullfrog (BF) vs. non-bullfrog (non-BF) ponds were used in bullfrog trials. Spotted frogs were unavailable from non-bullfrog ponds and thus not tested.
Discussion

The results of this study generally support the contention that predator avoidance behaviour is shaped by vulnerability to predation by a given predator species and, in at least some cases, prey experience. Adult spotted frogs (high predation risk) avoided chemical cues associated with both garter snake and bullfrog predators, long-toed salamanders (intermediate predation risk) avoided snake cues only, while treefrogs (intermediate predation risk) and newts (low predation risk) did not avoid predator cues (Table 1). For juvenile spotted frogs and treefrogs, avoidance of snake cues appeared to be largely learned (but lost among adult treefrogs), whereas no positive effects of prior exposure to bullfrogs were observed for salamanders or treefrogs (Table 2).

Predation risk avoidance by terrestrial amphibians has been demonstrated across a wide range of taxa (see Kats & Dill 1998 for review), and a variety of evolutionary arguments have been used to explain the genesis of such behaviour. The ability to recognize potential predators through chemical cues clearly has adaptive value insofar as it may decrease the probability of encounter with, and subsequent capture by, a predator. However, if such behaviour is so generalized that it is elicited by a wide range of heterospecifics (including non-predators or opportunistic predators), then avoidance could be less effective from the cost–benefit standpoint (Lima & Dill 1990; Kiesecker et al. 1996). It follows that prey behavioural responses should be largely suited to offset risk elicited from a particular predator only where such risk is both predictable and associated with a high probability of mortality. Accordingly, prey species should exhibit predator-specific variability in their response to perceived predation risk. However, if the predator–prey relationship was shaped principally by past evolutionary history, or if adaptive response to predation risk is constrained by conflicting selective forces opposing such a response, then the relationship between predator avoidance and perceived predation risk should be weakened.

Spotted frogs were the only prey species where adults avoided cues from both snakes and bullfrogs. This observation is consistent with our first prediction, given that (1) spotted frogs and both predators have broadly overlapping habitat use patterns in the wild (i.e. riparian areas, Nussbaum et al. 1983), and (2) this species is known to be a palatable prey item for garter snakes and bullfrogs in our study area. The more limited anti-predator response of long-toed salamanders may stem from the tendency of this species to occupy subterranean habitat for much of the year, where the risk of predation by garter snakes and bullfrogs is likely reduced. The avoidance of snake cues only in this species could be related to the higher importance of snakes as a salamander predator and/or a longer-term interaction between salamanders and snakes than between salamanders and bullfrogs. Regrettably, these latter scenarios cannot be assessed thoroughly, for while bullfrogs are suspected predators of several western anuran species (Ehrlich 1979; Kiesecker & Blaustein 1997; Kupferberg 1997), studies comparing the efficacy of the bullfrog as an amphibian predator relative to the garter snake in the western United States have yet to be conducted (see Ferguson 1961; Nussbaum
et al. 1983; Beneski et al. 1986). For treefrogs, minimal habitat overlap with the predators in question (and thus low predation risk) probably contributed to their failure to avoid snake and bullfrog cues. However, it is notable that Chivers et al. (2001) observed that predator-exposed treefrogs avoided cues associated with bullfrogs, and the disparity between their research findings and ours may be related to spatial differences in bullfrog predation risk or other local factors affecting trade-offs in the predator–prey interaction. Finally, we would argue that the lack of antipredatory behaviour among the newts tested suggests that the toxicity, and consequent low palatability of this species, has removed predator-mediated selective pressure to avoid predator chemical cues.

Predation risk avoidance has been shown to possess both innate and learned components depending on a variety of factors related to predator and prey life history. Studies using laboratory-reared prey residing in aquatic environments have shown that anti-predatory behaviour has a strong genetic basis (e.g. Sih & Kats 1994), but that aspects of the response may be learned (Semlitsch & Reyer 1992; Chivers et al. 1995; Woody & Mathis 1998; Wildy & Blaustein 2001). We found that predator-naive juvenile amphibians failed to show significant avoidance of snakes, while predator-exposed juveniles from two species did exhibit such avoidance. While this finding suggests that amphibian responses to predation risk are shaped by prior exposure and thus are learned, it is notable that the actual intensity of snake avoidance was modest and that laboratory-reared individuals also tended to avoid snakes, albeit only qualitatively (Table 2). Accordingly, it remains possible that to some extent predator avoidance was of innate origin and perhaps refined or intensified following amphibian maturation and/or direct exposure to predation risk. This result is corroborated by the observed avoidance of bullfrog cues by bullfrog-naive adult spotted frogs, and the fact that a similar significant response was absent among newly transformed juveniles. Yet, because in the present study there was no clear effect of prior exposure to bullfrogs on amphibian responses, the short duration of bullfrog interactions with native amphibians, and/or the modest mortality risk caused by bullfrogs in the wild, could have precluded the significant expression of predator avoidance. Alternatively, amphibian responses to perceived risk may simply differ fundamentally between snake and bullfrog predators.

The idea that prey animals avoid perceived predation risk is widespread in ecology, and the vast majority of studies addressing this topic have demonstrated such avoidance (Lima & Dill 1990; Kats & Dill 1998). However, several published studies have failed to demonstrate a substantive prey response (e.g. Heinen 1994; Wolff & Davis-Born 1997; Kullberg & Lind 2002), and others have recently shown that such responses are more variable than previously thought (e.g. Hazlett & Schoolmaster 1998; Brown & Warburton 1999; Martín et al. 2003; Mathis et al. 2003). Thus, while the frequency with which predator avoidance has been demonstrated may reflect the actual prevalence of this phenomenon across taxa, this trend may instead be symptomatic of a publication bias favouring significant results (see Csada et al. 1996). Here, having invoked a robust study design likely to furnish non-significant results for species showing no response, we report a low
intensity of predator avoidance, even in comparison with studies on similar species also involving a 'neutral' predator diet (e.g. Cupp 1994; Madison et al. 1999; Murray & Jenkins 1999). By implication, we suggest that prey responses to perceived predation risk are subject to considerable taxonomic, geographic, and/or environmental variation that may be related to habitat-specific risk levels (Krupa & Sih 1999), and that the limited prevalence and intensity of behavioural responses reported herein may be indicative of either relatively weak predator–prey relationships and/or the limited importance of predator chemical cues in this system. However, because most attempts to evaluate prey responses to predator cues have occurred in the laboratory, where the intensity of predator stimuli may be unnaturally high and the potential for abnormal prey behaviour is substantive, it remains necessary to further assess how predator cues affect the fitness of free-ranging prey. Future studies need to assess the linkage between mortality risk incurred by a predator, how such risk affects prey behaviour, and how behavioural changes influence prey life history, survival, and production (e.g. Chivers et al. 1999; Maerz et al. 2001; Mirza & Chivers 2001; Gazdewich & Chivers 2002; Kiesecker et al. 2002), and ultimately, predator–prey population dynamics (see Vet 1999). Such studies are particularly needed in relation to varying predator diets and instances (such as ours) where avoidance responses appear to be biologically modest. Our results lead to the prediction that for most species, perceived predation risk will be linked to prey avoidance behaviour, but that such a linkage will be adjusted by other factors related to the life history of the prey species.

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