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## **Threat-sensitive Predator Avoidance by Larval Pacific Treefrogs (Amphibia, Hylidae)**

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### **Abstract**

According to the threat-sensitive predator avoidance hypothesis, the intensity of a prey animal's antipredator response should reflect its vulnerability to a specific predator. In laboratory experiments, we observed the intensity of antipredator responses of Pacific treefrog (*Hyla regilla*) tadpoles to stimuli from caged larval northwestern salamander (*Ambystoma gracile*) predators. We varied the sizes of the tadpoles relative to the salamanders in an attempt to create differences in vulnerability of tadpoles to the salamander predators. After documenting the response of the tadpoles to the caged predator, we tested the tadpole's vulnerability to the predator by releasing the tadpole with the predator. We observed that as the relative size of the tadpoles to the caged salamanders increased, the antipredator response of the tadpoles decreased. These changes in behaviour closely mirrored changes in actual vulnerability to the predator. Our results provide experimental support for the threat-sensitive predator avoidance hypothesis.

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### **Introduction**

Prey species may respond to potential predators with a diversity of flexible antipredator responses (Lima & Dill 1990). Such defences may be behavioural (e.g. Kats et al. 1988; Kiesecker et al. 1996; Chivers & Smith 1998), morphological (Bronmark & Miner 1992; McCollum & Van Buskirk 1996), or life historical (Crowl & Covich 1990; Skelly & Werner 1990). For an individual prey animal, the costs and benefits of responding to a predator probably vary over the lifetime of the individual. For example, if the prey's probability of capture changes over time

because it is more difficult to capture (Tejedo 1993; Brown & Taylor 1995) or because it is less preferred by predators (Formanowicz 1986), then the intensity of antipredator responses should reflect this differential vulnerability. The hypothesis that prey species assess and behave flexibly towards different degrees of predation threat is known as the threat-sensitive predator avoidance hypothesis. Threat-sensitive predator avoidance has been documented in several groups of aquatic organisms including lobsters (Wahle 1992), freshwater isopods (Holomuzki & Short 1990), fishes (Helfman 1989; Williams & Brown 1991; Bishop & Brown 1992) and amphibians (Petranka 1989; Kats et al. 1994; Horat & Semlitsch 1994).

There is good reason to believe that threat-sensitive predator avoidance may be widespread among larval amphibians. For example, several empirical studies of larval amphibians indicate size- or stage-specific vulnerability to predators. Formanowicz (1986) demonstrated that as tadpoles grew in size they became less vulnerable to insect predators. Similar results documenting size-specific vulnerability are provided by Cronin & Travis (1986), Tejedo (1993), Richards & Bull (1990) and Semlitsch (1990). It is also known that some tadpoles have stage-specific predation vulnerability. For example, Crump (1984) found that tadpoles in earlier and later developmental stages were more vulnerable to predation than those in middle stages. Given that tadpoles experience differential vulnerability to predators through ontogeny, we may expect that tadpoles may be able to assess their risk of predation and adjust their antipredator response accordingly.

Most studies of threat-sensitive predator avoidance have shown that prey animals exhibit stronger antipredator responses as the perceived level of threat increases. However, few studies have addressed whether the change in behavioural responses of prey to predators accurately reflects changes in vulnerability. Live predation tests are required in order to assess whether changes in behaviour mirror changes in actual vulnerability.

In this study, we tested threat-sensitive predator avoidance in a larval amphibian predator/prey system. We observed the antipredator response of Pacific treefrog (*Hyla regilla*) tadpoles to caged larval northwestern salamanders (*Ambystoma gracile*).

We used activity level as the assay of antipredator behaviour of tadpoles. Reduced activity is a common behavioural response of tadpoles to predators (e.g. Hokit & Blaustein 1995; Kiesecker et al. 1996). Pacific treefrog tadpoles and northwestern salamanders co-occur in many ponds throughout the Pacific Northwest and larval salamanders readily consume treefrog tadpoles (Peterson & Blaustein 1991). Larval salamanders may overwinter prior to metamorphosis (Nussbaum et al. 1983). Thus, under natural conditions treefrog tadpoles may encounter a large variety of sizes of northwestern salamander larvae. In our experiment, we varied the size of the tadpoles relative to that of the salamanders in an attempt to create differences in the vulnerability of the tadpoles to the salamanders. After observing the behavioural response of tadpoles to the caged salamander, we tested the tadpole's vulnerability to the salamander by releasing the tadpole with it. This allowed us to specifically test whether changes in behaviour in response to the predator mirrored actual changes in vulnerability.

## Methods

### Collection and Maintenance

Pacific treefrog eggs were collected from a pond at the E. E. Wilson Wildlife Refuge (16 km north of Corvallis, Benton County, Oregon, USA). All clutches were transported to our laboratory and kept in separate 850 ml plastic cups in dechlorinated tap water, until the eggs hatched. One day after all eggs completed hatching, the tadpoles were placed into individual 850 ml cups and maintained between 15 and 20°C on a 14 h light:10 h dark photoperiod. All tadpoles were fed *ad libitum* with ground rabbit chow. Water was changed weekly.

Northwestern salamanders were collected as eggs from a pond located  $\approx 10$  km west of Corvallis. The eggs were housed in 38 l glass aquaria and kept there after hatching. Four weeks before the trials began, the larval salamanders were moved into individual 850 ml plastic cups. Salamanders were maintained between 15 and 20°C on a 14 h light:10 h dark photoperiod and were fed *ad libitum* with tadpoles every 3 d. The water was changed weekly.

### Experimental Procedure

The antipredator response of treefrog tadpoles to larval salamanders was tested in a plastic container ( $31 \times 14 \times 9$  cm) filled to a depth of 5 cm with dechlorinated tap water. There were three mesh chambers in the testing container, two at each end ( $9 \times 9 \times 4.5$  cm) and one ( $10 \times 10 \times 7$  cm) placed in the centre of the testing container. The bottom of the centre chamber was removed so that the chamber could easily be removed from the water. Before each trial, the container and chambers were rinsed thoroughly with tap water. For each trial a single tadpole was tested. In experimental trials, a single larval salamander was placed in one of the end chambers and the other chamber was left empty. The end chamber that received the salamander was determined randomly for each trial. Salamanders were allowed to acclimate for 5 min before testing began. During control trials no salamander predators were placed in either end chamber. Individual test tadpoles were placed in the centre chamber and allowed to acclimate for 30 s, at which time the centre chamber was removed releasing the tadpole. Tadpole activity was recorded for 5 min after the tadpole was released. Reduced activity is a common behavioural response of tadpoles to predators (e.g. Hokit & Blaustein 1995; Kiesecker et al. 1996). Activity was measured by recording every 15 s whether or not the test tadpole was moving. The tadpole was considered moving if its tail was in motion regardless of whether the tadpole was progressing through the water column. At the end of each trial, the weights of the tadpoles and salamanders were recorded.

Immediately after being weighed, both the tadpole and the larval salamander were placed together in a 850 ml cup. The cups were scanned every 15 min to determine if the larval salamander had captured the tadpole. We ended each trial after 2 h regardless of whether the salamander was able to capture the tadpole. We completed a total of 200 trials in this experiment. Starting on day 7 posthatching,

we completed 10 control and 10 experimental trials every 3rd day for a total of 10 different testing days. Tadpoles were not used in more than one trial.

We standardized the hunger level of the salamanders in our experiment. Three days prior to conducting the trials, all salamanders were fed ad libitum with tadpoles and then not fed until the trials. Each day that tests were completed we randomly chose 10 salamanders from our laboratory population of 45 individuals. Salamanders used on one test day were not used on the subsequent test day. There was a minimum of 6 d between tests using the same salamander predator.

A two-way ANOVA was used to test for differences in the effect of the independent factors treatment (control and experimental) and mass at the time of testing (Tabachnick & Fidell 1989). Animals were assigned to three mass categories for the analysis: < 0.15 g; 0.15–0.30 g; and > 0.30 g. The mass at the time of testing was used in the analysis to examine the interaction between the treatment effect and how the behavioural responses may have changed during the growth of the prey. To test whether the susceptibility of the prey changed as the prey’s size increased relative to that of the predator, we compared the time taken for the predator to capture the prey with the ratio of prey to predator mass using a linear regression. To conduct this analysis, animals not captured during the 2 h trials were assigned the maximum capture time of 2 h. To further understand the relationship between the prey to predator size ratio and the susceptibility to predation, we used a t-test to compare the prey to predator size ratio of tadpoles that were consumed with those that were not consumed. To understand how susceptibility to predation influences a prey’s behavioural response, we determined which prey were vulnerable to the predator (i.e. captured by the predator during the staged encounters) and which were not, and used a t-test to compare whether animals in these two groups initially responded differentially to the predator during the behavioural tests. For all statistical tests, parametric assumptions were met and no data transformations were required.

Results

A two-way ANOVA revealed a strong difference in activity between control and experimental animals (Table 1). However, the difference in activity between the control and experimental treatments changed as tadpole mass changed (Table 1,

Table 1: ANOVA results for prey activity with the main effects treatment (control and experimental) and tadpole mass at the time of testing

Source	df	MS	F	
Treatment	1	74.793	7.207	= 0.008
Mass	2	20.598	1.985	= 0.140
Treatment × mass	2	46.248	4.456	= 0.013
Error	190	10.379		

Fig. 1). When the tadpoles were relatively small, there was a strong behavioural response to the predators. When small, the tadpoles were less active in the presence of the predator (experimental treatment) than in the absence of the predator (control treatment, Fig. 1). In contrast, when large, the tadpole's response to the predator resembled the response exhibited to the control (Fig. 1).

There was a strong correlation between the time taken for the predator to capture the prey and the ratio of prey to predator mass ( $r = 0.626$ ,  $p < 0.001$ ). As tadpole size increased, the time to capture also increased (Fig. 2).

A t-test revealed that the ratio of prey to predator mass of tadpoles that were eaten was significantly lower than that of tadpoles that were not eaten ( $T_{96} = 8.70$ ,  $p < 0.001$ , Fig. 3). A t-test also showed that tadpoles that were susceptible to predation (i.e. were eaten by the predator) showed a stronger antipredator response during the initial behavioural tests than those that were not susceptible to predation ( $T_{96} = 3.187$ ,  $p = 0.002$ , Fig. 3).

### Discussion

The results of this study show that Pacific treefrog tadpoles are able to assess their individual vulnerability to northwestern salamander larvae and adjust their antipredator response according to their level of risk. Tadpoles that were shown to be vulnerable to capture during predation trials showed a stronger antipredator response during the initial behavioural tests than those that were not susceptible to predation. Our results provide empirical support for the threat-sensitive predator avoidance hypothesis.

A few other studies provide evidence of threat-sensitive predator avoidance

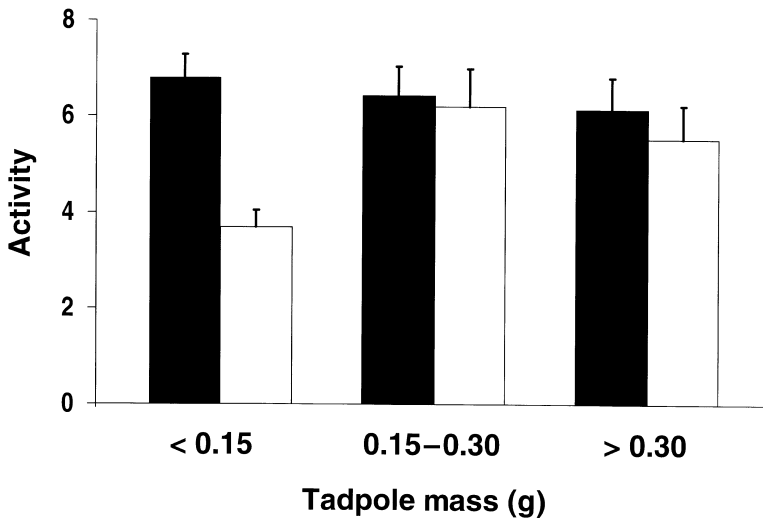


Fig. 1: Mean ( $\pm$  SE) activity levels of tadpoles in the predator absent (control, solid bars) and predator present (experimental, open bars) treatments

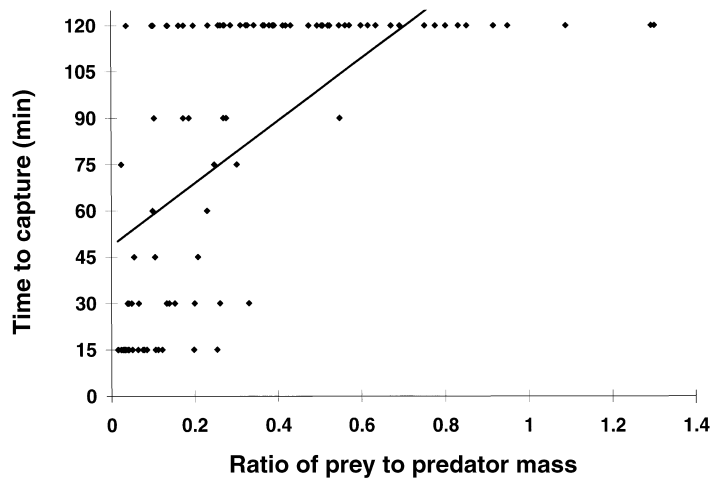


Fig. 2: Linear regression showing time to capture (min) vs. ratio of prey to predator mass

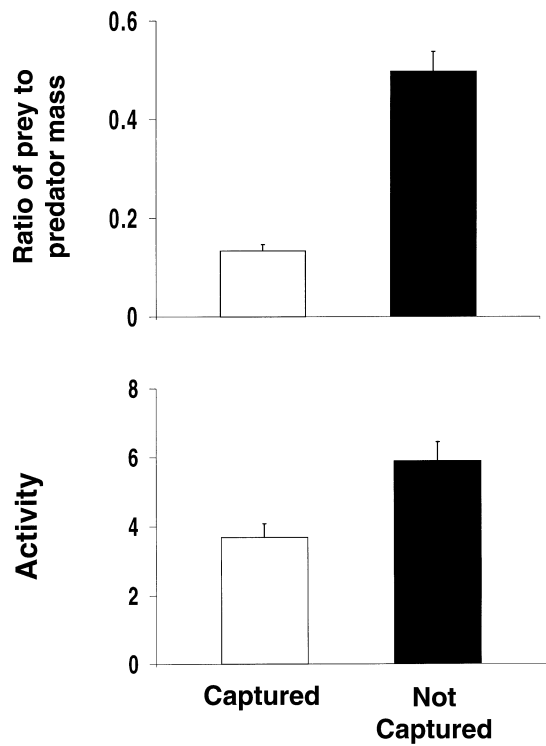


Fig. 3: Mean ( $\pm$  SE) ratio of prey to predator mass of tadpoles eaten and not eaten during predation trials (top panel) and mean ( $\pm$  SE) activity levels of tadpoles during initial behavioural trials that were subsequently captured or not captured during predation trials (lower panel)

by larval amphibians. For example, Horat & Semlitsch (1994) demonstrated that two species of frogs (*Rana lessonae* and *R. esculenta*) showed greater reductions in activity as they were exposed to a greater concentration of chemical cues of a predatory pike (*Esox lucius*). Similarly, Petranka (1989) demonstrated that American toad (*Bufo americanus*) tadpoles showed a greater avoidance response to conspecific chemical alarm cues as the concentration of chemical cues increased. Anholt et al. (1996) demonstrated that American toad tadpoles showed a stronger response to larval dragonflies (*Anax junius*) as predator density and food availability increased. These studies document an increase in intensity of antipredator behaviour with an increase in the perceived level of threat.

Our design differed from most other studies of threat-sensitive predator avoidance. In our experiment we conducted live predation trials to specifically test the vulnerability of the tadpoles to the salamander predators. Testing vulnerability enabled us to document that individual treefrog tadpoles adjusted the intensity of their antipredator response to match their level of risk to a specific predator. We suggest that future studies should make direct comparisons between a prey animal's vulnerability and the intensity of its antipredator behaviour. This is needed in order to determine whether changes in behaviour mirror changes in actual vulnerability. Numerous factors can potentially affect vulnerability and, hence, may influence the intensity of antipredator responses. For example, over time a prey animal's probability of capture may change because it is more difficult to capture or because it is less preferred by the predator.

The ability of prey animals to assess and behave flexibly towards different degrees of predation threat has important implications. The failure of prey to respond to a predator increases the probability that it will be captured during an interaction with a predator. However, prey that exhibit antipredator behaviour upon encountering a nonthreatening predator waste valuable time and energy that would otherwise be available for other activities (Lima & Dill 1990). Thus, there should be strong selection pressure on prey to distinguish between predators that pose a risk and those that do not.

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