

AVOIDANCE RESPONSE OF JUVENILE PACIFIC TREEFROGS TO CHEMICAL CUES OF INTRODUCED PREDATORY BULLFROGS

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Abstract—Bullfrogs (*Rana catesbeiana*), native to eastern North America, were introduced into Oregon in the 1930's. Bullfrogs are highly efficient predators that are known to eat a variety of prey including other amphibians. In laboratory experiments, we investigated whether juvenile Pacific treefrogs (*Hyla regilla*) recognize adult bullfrogs as a predatory threat. The ability of prey animals to acquire recognition of an introduced predator has important implications for survival of the prey. We found that treefrogs from a population that co-occurred with bullfrogs showed a strong avoidance of chemical cues of bullfrogs. In contrast, treefrogs from a population that did not co-occur with bullfrogs, did not respond to the bullfrog cues. Additional experiments showed that both populations of treefrogs use chemical cues to mediate predation risk. Treefrogs from both populations avoided chemical alarm cues from injured conspecifics.

Key Words—Predator recognition, introduced predators, chemical cues, alarm signals, Pacific treefrogs, bullfrogs, *Hyla regilla*, *Rana catesbeiana*.

INTRODUCTION

The failure of a prey animal to recognize and respond to a predator increases the probability that it will be captured during an encounter with a predator. As a result, there should be strong selective pressure for prey to identify potential predators (Sih, 1987; Lima and Dill, 1990). Studying the responses of prey

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to recently introduced predators provides an ideal system for testing the acquisition of predator recognition by prey populations (Kiesecker and Blaustein, 1997a).

Previous studies have demonstrated that in populations where larval amphibians have a long evolutionary history with a particular predator, they often recognize and respond to the predator without any prior experience. For example, Kats et al. (1988) demonstrated that larval amphibians, collected as eggs from a pond with predatory fish, responded to chemical cues of the fish predators. Similar results have been reported by Sih and Kats (1994). Kiesecker and Blaustein (1997a) documented that larval red-legged frogs (*Rana aurora*), collected as eggs from populations that co-occur with predatory bullfrogs (*Rana catesbeiana*), exhibit an antipredator response to the bullfrogs. In this case the response occurs even through red-legged frogs and bullfrogs do not share a long evolutionary history; they have co-occurred for less than 70 years (Nussbaum et al., 1983).

The first objective of this study was to examine the responses of Pacific treefrogs (*Hyla regilla*) from west central Oregon to chemical cues of introduced adult bullfrogs. We tested two different populations of treefrogs, one of which is syntopic with bullfrogs and one that is allotopic from bullfrogs. Bullfrogs were introduced into Oregon in the 1930's (Nussbaum et al., 1983). The adults are predators that are known to feed on a variety of invertebrate and vertebrate prey including other amphibians (Bury and Whelan, 1986; Werner et al., 1995; Kiesecker and Blaustein, 1997a, 1998). In our study, we tested the response of post-metamorphic (hereafter juvenile) treefrogs. Although studies of larval amphibians to chemical cues of predators are widespread (e.g., Kiesecker et al., 1996; Kats and Dill, 1998), few studies have examined responses of post-metamorphic amphibians to predators (but see Flowers and Graves, 1997; Chivers et al., 1999). It is unknown whether chemosensory responses of post-metamorphic anurans are widespread.

A second objective of our study was to examine the responses of juvenile Pacific treefrogs from both populations to chemical alarm signals released from injured conspecifics. A wide variety of larval amphibians, including those of the Pacific treefrogs (Adams and Claeson, 1998), exhibit an avoidance response to chemical alarm cues (see review by Chivers and Smith, 1998). However, to our knowledge only one study (Chivers et al., 1999) has documented responses of post-metamorphic anurans to chemical alarm cues. In that study, western toads (*Bufo boreas*, Family Bufonidae) and red-legged frogs (Family Ranidae) responded to cues of injured conspecifics while those of Cascades frogs (*Rana cascadae*) did not. The absence of a response in juvenile Cascades frogs is particularly noteworthy because tadpoles are thought to respond to cues of injured conspecifics (Hews and Blaustein, 1985). Our study is the first to test whether metamorphic frogs from the Family Hylidae respond to cues of injured conspecifics.

MATERIALS AND METHODS

We tested juvenile treefrogs from each of two populations for a response to both cues of injured conspecifics and cues of introduced bullfrogs. Recently metamorphosed treefrogs from the first population were collected in the Willamette Valley at the E. E. Wilson Wildlife Refuge (16 km north of Corvallis, Benton County, Oregon). Bullfrogs are common in the Willamette Valley. The specific pond where the treefrogs were collected contains a breeding population of bullfrogs (pers. obs.). Treefrogs from the second population were collected from a pond in the Oregon Cascade Mountains (89 km east of Albany, Linn County, Oregon). Bullfrogs are absent from this location. The closest bullfrog population is approximately 25 km away from this site.

Treefrogs were collected in the summer and fall of 1996 and transported to Oregon State University for testing. We maintained the treefrogs individually in Petri dishes (150 mm diameter $\times$ 25 mm high) lined with damp paper towels. The frogs were kept on a 14 hr light:10 hr dark photoperiod at approximately 16°C for a minimum of two weeks prior to testing. Treefrogs were fed *ad libitum* with crickets.

Responses of Treefrogs to Cues of Bullfrogs. In these experiments we tested the avoidance response of individual treefrogs exposed to chemical cues from: 1) predatory adult bullfrogs and 2) non-predatory adult conspecifics. Testing the response of treefrogs for a response to non-predatory treefrogs was done as a control to determine whether an avoidance of the predator stimulus could be simply a general response to any amphibian cue. Experiments on the two populations were completed separately.

Bullfrogs spend considerable periods of time sitting on the banks of ponds, lakes, slow moving streams, and rivers. As a result they are in a good position to encounter treefrogs that are undergoing metamorphosis and leaving the water. Many amphibians, including treefrogs, may spend several days at the water/land interface before moving to land. Bullfrogs that spend time at the water's edge may also be in a good position to prey on adult treefrogs that return to breed. To prepare the bullfrog cue we placed a single bullfrog into a container that was filled with 3 l of dechlorinated tap water. The bullfrog was removed after 24 hr at which time the experiments began. We used two different bullfrogs (mass = 27.6 and 33.3 g) as alternative donors in our experiments. We prepared the treefrog stimulus in the same manner as the bullfrog stimulus. However, to prepare the treefrog stimulus we added either 7 or 8 treefrogs to the stimulus collection chamber to match the mass of the particular bullfrog that we used. Prior to collecting stimuli, the bullfrogs and treefrogs were maintained on a diet of crickets for at least 2 wk.

For each trial we lined half of a rectangular plastic container (32 $\times$ 18 $\times$ 8 cm) with paper towel that was moistened with dechlorinated tap water (control side). The treatment side was lined with paper towel that contained 5 ml of the bullfrog

or treefrog cue. The two paper towel substrates in each container were separated by approximately 2 cm to reduce contamination of chemicals between the sides. After the appropriate stimuli were added to each side of the test containers, we used a spray bottle containing dechlorinated tap water to saturate the paper towels. This ensured that any observed avoidance of the control or treatment sides was not attributable to differences in moisture level.

At the start of each trial, we introduced a single test animal into the center of the test container. Every 30 min for 2.5 hr we recorded whether the test animal was located on the control or treatment side of the container. In the event the treefrog was positioned across the middle of the container, we used the position of the snout to assign location. We rotated the containers 180° every half hour during the experiment to control for the possibility of a bias in the treefrogs' orientation in the room. Our observation schedule followed the design of Chivers et al. (1999). We made observations only every half hour because juvenile anurans will not move if they are disturbed by an observer.

For the Cascade Mountain population we tested 30 frogs in the control treatment and 30 in the experimental treatment. Our sample size for the Willamette Valley population was reduced to 27 and 25 in the bullfrog (experimental) and treefrog (control) treatments respectively. Individual treefrogs were used in only one test. For each trial, we summed the number of times each animal was located on the treatment side of the container out of a possible 5 observations (one observation every 30 min for 150 min = 5 observations). For both of the treatments, we compared whether treefrogs spent significantly less time than expected on the treatment side of the containers using a Wilcoxon Signed Rank test (Siegel and Castellan, 1988). Several researchers (e.g., Lutterschmidt et al., 1994; Chivers et al., 1996a, 1997, 1999) have used a similar bioassay for testing responses of terrestrial amphibians to chemical cues.

Responses of Treefrogs to Cues of Injured Conspecifics. In these experiments we tested the avoidance response of individual treefrogs exposed to chemical stimuli from: (1) injured juvenile conspecifics, and (2) non-injured juvenile conspecifics. Experiments on the two populations were completed separately. Testing the response of treefrogs to non-injured conspecifics was done as a control to determine whether any avoidance of the injured stimulus was a general response to any conspecific stimulus (Chivers et al., 1996a, 1997).

Treefrogs are subject to predation by many different predators, including frogs, snakes, birds and mammals (Nussbaum et al., 1983). The amount of alarm cues released onto the ground and surrounding vegetation during a predation event must be highly variable. In designing this experiment, we followed the methodology of Chivers et al. (1999) to prepare the injured treefrog stimulus. We dispatched ten animals by decapitation and removed their viscera, spinal column and legs. The tissue (approximately 4.5 g, which contained mostly skin, but also some muscle tissue) was homogenized with 150 ml of dechlorinated tap water in a blender. The

homogenate was filtered through a 0.5 mm mesh net. We used 5 ml of injured treefrog stimulus per trial. The stimulus was used within 30 min of preparation. For a source of chemical stimuli from non-injured treefrogs, we used moistened paper towels that had housed a single conspecific for 48 hr.

For each trial, we lined half of a rectangular plastic container (32 × 18 × 8 cm) with a paper towel that was moistened with dechlorinated tap water (control side). The treatment side was lined with a paper towel that contained cues from injured treefrogs or cues from non-injured treefrogs. We used the same experimental protocol and statistical analyses as in the previous experiments. For both populations of treefrogs, we tested the response of 30 individuals to cues of injured conspecifics and 30 individuals to cues of non-injured conspecifics. Individual treefrogs were used in only one test.

RESULTS

Treefrogs from both the Willamette Valley and Cascade Mountain populations showed an avoidance response to cues from injured conspecifics (Willamette Valley: $Z = 3.94$, $P < 0.001$; Cascade Mountain: $Z = 3.87$, $P < 0.001$; Figure 1), but not cues of non-injured conspecifics (Willamette Valley: $Z = 0.39$, $P = 0.70$; Cascade Mountain: $Z = 1.13$, $P = 0.26$; Figure 1).

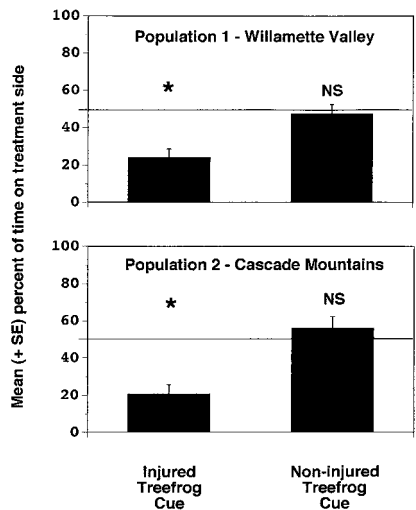


FIG. 1. Mean (+SE) percent time juvenile treefrogs from the Willamette Valley and Cascade Mountain populations spent on the treatment side of test chambers containing cues of injured conspecifics or non-injured conspecifics (NS indicates $P > 0.25$, * indicates $P < 0.001$).

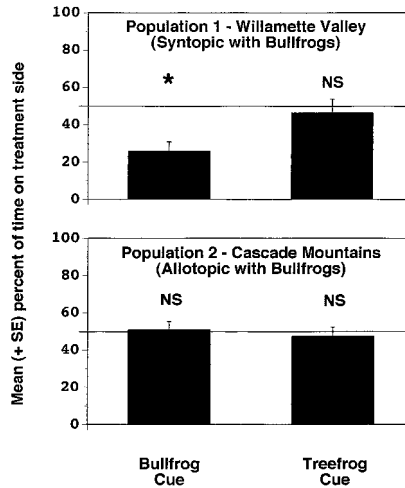


FIG. 2. Mean (+SE) percent time juvenile treefrogs from the Willamette Valley and Cascade Mountain populations spent on the treatment side of test chambers containing cues of predatory bullfrogs or non-predatory treefrogs (NS indicates $P > 0.50$, * indicates $P < 0.001$).

Treefrogs that co-occurred with bullfrogs in the Willamette Valley showed an avoidance response to cues of bullfrogs ($Z = 3.52$, $P < 0.001$, Figure 2), but did not show an avoidance response to cues of treefrogs ($Z = 0.58$, $P = 0.56$, Figure 2). In contrast, treefrogs from the Cascade Mountains did not show an avoidance response to cues of either bullfrogs ($Z = 0.02$, $P > 0.95$, Figure 2), or treefrogs ($Z = 0.57$, $P = 0.57$, Figure 2).

DISCUSSION

Our results show that juvenile treefrogs from two different populations use chemical cues to mediate predation risk. We observed avoidance responses of chemical alarm cues by treefrogs from both populations and avoidance of the bullfrog cues by treefrogs from the population syntopic with bullfrogs.

Numerous species of larval amphibians are known to exhibit antipredator behavior to chemical alarm cues (review Chivers and Smith, 1998). However, the responses of post-metamorphic anurans are less well known. Our results with Pacific treefrogs (Family Hylidae) show that the response to alarm cues is retained after metamorphosis. In our study, juvenile treefrogs from two distantly separated populations both exhibited avoidance of cues from injured conspecifics. Future studies should determine the amount of alarm cues released during predation events

and determine the threshold levels of responses exhibited by prey animals. This information would allow us to determine the scope of potential responses under natural conditions.

We did not have access to heterospecific frogs at the time of our study and hence could not test whether the avoidance of treefrogs to injured conspecific cues was a specific response to injured conspecifics or was a general response to injured amphibian cues. The avoidance of injured cues did not represent avoidance of a general amphibian cue. Treefrogs did not avoid cues of intact treefrogs. From a predation perspective it is of little importance if the avoidance of injured cues represents avoidance of injured conspecifics specifically or avoidance of injured amphibians in general. The important point is that by avoiding cues of injured conspecifics, treefrogs will decrease their probability of being captured. Chivers et al. (1999) used a similar approach to examine responses of juvenile red-legged frogs to alarm cues.

In our study, we observed that juvenile Pacific treefrogs from a population that co-occurred with bullfrogs exhibited an avoidance response to chemical cues of bullfrogs. The other population of treefrogs that had no contact with bullfrogs did not avoid the bullfrog cues. Inter-population differences in antipredator responses, whereby individuals from populations in which predators are common exhibit stronger antipredator responses than individuals from populations that experience lower levels of predation, have been reported in a variety of taxa (e.g., spiders: Riechert and Hedrick, 1990; salamanders: Ducey and Broodie, 1991; fishes: Seghers, 1974). However, few studies have documented population differences based on responses to chemical cues (but see Mathis et al., 1993). Our results most likely reflect that individuals from one population did not recognize the bullfrog cues, while individuals from the other population did recognize the bullfrog cues. However, our results could also reflect a population difference in the concentration threshold needed to elicit a response.

Population differences in antipredator responses may result from learning by prey in the high predation population (Mathis et al., 1993; Chivers and Smith, 1994). Several recent studies have documented that naïve prey animals can learn the identity of unknown predators by detecting conspecific alarm cues in the diet of the predator (Mathis and Smith, 1993; Chivers et al., 1996b). Population differences in anti-predator responses may also be genetically determined (Kiesecker and Blaustein, 1997a). The importance of genetics should increase if the predator and prey have a long evolutionary history together. Genetic changes can occur rapidly under natural conditions. For example, Reznick et al. (1990) documented changes in genetically determined life history traits in 30–60 generations. Pacific treefrogs and bullfrogs have co-occurred together in Oregon for only about 70 years (Nussbaum et al., 1983). For our experiments, we collected juvenile treefrogs.

Consequently, we do not know whether treefrogs from the population syntopic with bullfrogs have to learn that bullfrogs are a threat or whether this recognition is genetically determined. Future studies should differentiate these alternatives. We should also examine many populations of treefrogs with different densities of bullfrogs in order to determine whether responses vary with the intensity of predation or other site specific characteristics.

Many amphibian populations have been declining and undergoing range reductions over the last few decades (Blaustein and Wake, 1990; Stebbins and Cohen, 1995). The reasons for these declines are complex (Blaustein and Wake, 1995). Some studies have documented the decline of native frogs following the introduction of bullfrogs (Moyle, 1973; Kiesecker and Blaustein, 1998; Kupferburg, 1997). Pacific treefrogs appear to be one species that is not declining (Kiesecker and Blaustein, 1997a,b). This may be in part due to its success in recognizing and avoiding introduced predators such as bullfrogs. Responses of other amphibians to introduced bullfrogs remains mostly unknown (but see Kiesecker and Blaustein, 1997a). The success of treefrogs in regions now occupied by bullfrogs may stem from other factors besides their ability to recognize bullfrogs as predators. For example, treefrogs breed in a variety of habitats, including temporary ponds, where bullfrogs do not occur (Nussbaum et al., 1983).

The effects of introduced predators on native species are complex (Kiesecker and Blaustein 1997a, 1998). The ability of prey to recognize an introduced predator should minimize the prey's risk of capture. However, recognition of the predator does not by itself imply that there will not be significant predator effects. For example, Kiesecker and Blaustein (1997a) showed that red-legged frogs shift their microhabitat use in the presence of adult bullfrogs, and that this has a substantial cost in terms of decreasing growth rates and altering metamorphic characteristics, including time to and size at metamorphosis.

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