Responses of Foothill Yellow-legged Frog (*Rana boylii*) Larvae to an Introduced Predator

Author(s): David J. Paoletti, Deanna H. Olson, and Andrew R. Blaustein


Published By: The American Society of Ichthyologists and Herpetologists

DOI: 10.1643/CE-09-170

Responses of Foothill Yellow-legged Frog (Rana boylii) Larvae to an Introduced Predator

David J. Paoletti1, Deanna H. Olson2, and Andrew R. Blaustein1

The consequences of species introductions into non-native habitats are a major cause for concern in the U.S. Of particular interest are the effects of predation by introduced fishes on native amphibian communities. We sought to determine whether Foothill Yellow-legged Frog (Rana boylii) larvae could recognize non-native Smallmouth Bass (Micropterus dolomieu) as a predatory threat. Through a series of laboratory experiments, we examined the initial and overall behavioral responses of larvae to native predators (Rough-skinned newts, Taricha granulosa), introduced predators (M. dolomieu), and native non-predatory fish (Speckled Dace, Rhinichthys osculus). Each experiment examined a different potential mode of detection including chemical cues; visual cues; or a combination of chemical, visual, and mechanical cues. Initially, larvae of R. boylii responded with an increase in activity levels when exposed to visual cues of M. dolomieu. Analyses of overall responses suggested that individual larvae of R. boylii require multiple cues to facilitate predator detection. When exposed to multiple cues of their native predator, larvae responded with a significant reduction in activity levels. Those larvae exposed to cues of the non-native predator displayed similar behaviors relative to control cues. Consequently, larvae of R. boylii appear to be especially vulnerable to predation by non-native M. dolomieu.

In Oregon, the distribution of the Foothill Yellow-legged Frog (Rana boylii) appears to be influenced by introduced M. dolomieu in some river systems. Rana boylii is a native stream-dwelling frog listed as a “Sensitive Species” in the state, where its range has been reduced to 43% of historical locations (Fig. 1; Olson and Davis, 2007). In the Umpqua River system, M. dolomieu was introduced as recently as the mid-1960s, where their distribution has rapidly expanded (Simon and Markle, 1999). This spread is particularly evident in Cow Creek, a major tributary of the South Umpqua River in Douglas County, Oregon (Fig. 2). As M. dolomieu have spread upstream through Cow Creek, a coincident reduction of R. boylii has been observed (Borisenko and Hayes, unpubl.; Rombough, unpubl.). In the federal Conservation Assessment for R. boylii in Oregon, Olson and Davis (2007) proposed a variety of causes for the decline of this species, including habitat loss, pollutants, change in hydrologic regimes, and introduced species including M. dolomieu. However, these potential factors in the frog’s decline in Oregon remain unstudied.

We investigated possible mechanisms behind declines of R. boylii in Cow Creek, Oregon, by experimentally assessing their responses when exposed to M. dolomieu. We chose a laboratory setting for our experiments in order to maintain consistent environmental conditions between experimental treatments, including the chemical composition of the water, while eliminating a majority of the biotic and abiotic variation that might confound interpretations of larval behavior in the field. Amphibian larvae often “recognize” predators via waterborne chemical cues (Kats and Dill, 1998). Upon detection of a potential threat, larvae often respond with antipredator behaviors such as refuge use or a reduction in activity levels (Petranka et al., 1987; Kiesecker et al., 1998). Upon detection of a potential threat, larvae often respond with antipredator behaviors such as refuge use or a reduction in activity levels (Petranka et al., 1987; Kiesecker et al., 1998). Upon detection of a potential threat, larvae often respond with antipredator behaviors such as refuge use or a reduction in activity levels (Petranka et al., 1987; Kiesecker et al., 1998). Upon detection of a potential threat, larvae often respond with antipredator behaviors such as refuge use or a reduction in activity levels (Petranka et al., 1987; Kiesecker et al., 1998). Upon detection of a potential threat, larvae often respond with antipredator behaviors such as refuge use or a reduction in activity levels (Petranka et al., 1987; Kiesecker et al., 1998). Upon detection of a potential threat, larvae often respond with antipredator behaviors such as refuge use or a reduction in activity levels (Petranka et al., 1987; Kiesecker et al., 1998). Upon detection of a potential threat, larvae often respond with antipredator behaviors such as refuge use or a reduction in activity levels (Petranka et al., 1987; Kiesecker et al., 1998).

1 Environmental Science Graduate Program, 104 Wilkinson Hall, Oregon State University, Corvallis, Oregon 97331; E-mail: (DJP) paolettd@onid.orst.edu; and (ARB) blaustea@science.oregonstate.edu. Send reprint requests to ARB.

2 USDA Forest Service, Pacific Northwest Research Station, 3200 SW Jefferson Way, Corvallis, Oregon 97331; E-mail: dedeolson@fs.fed.us.


© 2011 by the American Society of Ichthyologists and Herpetologists DOI: 10.1643/CE-09-09-170
observed motionless when in close proximity to a native predator, the Rough-skinned Newt (Taricha granulosa; Jones et al., 2005). The newt appeared to be a stealthy predator, slowly stalking its prey, although no studies of their predation behavior under natural conditions are known to us. It is possible that the more rapid predatory behavior of introduced bass is novel to R. boylii.

We conducted Experiment I to determine if chemical cues alone are sufficient for larvae of R. boylii to detect a potential threat. Kats et al. (1994) showed that amphibian larvae may change antipredator behaviors based on their developmental stage. To determine if these ontogenetic differences were evident in R. boylii, Experiment 1a was conducted using early-stage larvae, whereas Experiment 1b focused on the responses of late-stage larvae. Because chemical cues may be insufficient to detect a potential threat in the stream environment in which larvae of R. boylii live, we designed Experiment II to determine the role visual cues play in response to predator stimuli. In a lotic environment, amphibian larvae may need to use several modes of detection in tandem to identify a potential threat. Experiment III was designed to expose larvae of R. boylii to chemical, visual, as well as mechanical (i.e., water movement) cues of the stimulus animal concurrently.

We hypothesized that larvae of R. boylii would respond to cues of a native predator by reducing activity levels and/or increasing refuge use, whereas those exposed to cues of the non-native, unfamiliar predator would display activity levels similar to larvae exposed to control cues. Studies examining the effects of introduced species on native amphibians are numerous, but to our knowledge only one (Kupferberg, 1997) has been conducted investigating the effects of non-native species (American Bullfrog, Lithobates catesbeianus, formerly Rana catesbeiana) on Rana boylii. Our study is the first attempt to determine empirically the effects of M. dolomieu on larval behavior of R. boylii. Absence of an appropriate antipredator response by larvae may directly impact R. boylii where their populations overlap with M. dolomieu.

MATERIALS AND METHODS

Eggs of R. boylii were collected on 13 June 2008 from an area populated by M. dolomieu (Cow Creek, Douglas Co., OR; 42°52′3″N, 123°34′32″W). On 24 June 2008, a second set of eggs was collected from a bass-free area (Carberry Creek, Jackson Co., OR; 42°4′6″N, 123°10′7″W; Fig. 2). Larvae from these populations will be referred to as BASS and BASSLESS.
Eggs were transported to Oregon State University and maintained in aerated, 38-L aquaria filled with dechlorinated water. Animals were exposed to a natural 14L:10D photoperiod at a constant temperature of 16°C. Upon hatching, larvae were fed a mixture of fish flakes (TetraFin) and ground rabbit chow ad libitum. Source animals for the stimulus cues were collected from Cow Creek and tributaries of the South Umpqua River. These included: T. granulosa, Speckled Dace (Rhinichthys osculus), and M. dolomieu. We compared larval responses between treatments, exposing them to native and non-native predators, and to R. osculus, a non-predatory native minnow which served as our “positive fish control.” All animals were maintained in the conditions listed above at Oregon State University. Rhinichthys osculus were fed fish flakes and tubifex worms; T. granulosa and M. dolomieu were fed earthworms and crickets ad libitum. Sated larvae may exhibit reduced activity levels, making it difficult to discern between inactivity due to satiation or the presence of a potential threat. In each experiment, food was withheld from all animals 24–48 h prior to experimental trials to avoid the effects of satiation. Blinds around test tanks prevented disturbance of animals during experimental observations.

Experiment Ia: Detection via chemical cues by early-stage larvae.—Early-stage larvae (Stage 20–24; Gosner, 1960) were exposed to one of four chemical cue treatments: Control (no cue); Positive Control (non-lethal, native R. osculus); Native Predator (T. granulosa); and Non-native Predator (M. dolomieu). Individual larvae (n = 104) were randomly assigned to one of the four treatments and were not re-used in trials to address this objective. In addition, larvae from BASS and BASSLESS populations were alternately tested. There were a total of 13 larvae for each population (13 replicates x 4 treatments = 52 animals tested per population = 104 total). Chemical cues were supplied through a series of gravitational “flow-through” tanks (Fig. 3; similar to those in Petranka et al., 1987) and were designed to simulate the stream environment in which these animals are found. Three clear plastic tanks were arranged in a linear fashion, in sequentially descending heights. Filtered, dechlorinated water was supplied via a 144-L source tank. Water was gravity-fed through 2.5 cm polyvinyl chloride (PVC) tubing into a “treatment tank” (23 x 37 x 16 cm). This tank held the animal responsible for supplying the chemical cue treatment. From here, water was gravity-fed to a third tank, the “experimental tank” (23 x 37 x 16 cm), which held a single frog larva. A 5-cm (2.5 cm diameter) piece of black plastic pipe was affixed to the bottom of the tank to provide a standardized potential refuge for larvae. A drain pipe was inserted to maintain a constant water height of 11 cm (mean flow rate = 2.0 L/min). Four of these systems ran concurrently, with each of the four treatment tanks supplying one of the four cues. The cue assigned to each treatment tank was randomly assigned. Experimental tanks were rinsed thoroughly between each replication.

Prior to running each trial, animals supplying the cue were placed in the treatment tank, and a randomly selected larva was placed in the experimental tank and allowed to acclimate for ten minutes. After this the valve from the source tank was slowly opened, followed by the treatment

**Fig. 2.** Location of foothill yellow-legged frog (*Rana boylii*) study site and egg-collection sites at Cow Creek, Douglas County, and Carberry Creek, Jackson County, Oregon, USA.

**Fig. 3.** Design of flow-through tanks addressing the potential response of larvae of *Rana boylii* from Cow Creek, Oregon, USA, to chemical cues of native and non-native predators, and native non-predators. Animals supplying chemical cues were placed in the treatment tank, and larvae were placed in the experimental tank.
tank valve. Observations began immediately and locations of larvae were recorded during “spot-checks” conducted every two minutes for ten minutes. A grid divided into six quadrants (10 × 10 cm each) was placed under each experimental tank to track movement (Garcia et al., 2009). Grids were designed to be large relative to larval body size to ensure that any movement captured was deliberate. For example, an early-stage larva would have to travel the equivalent of ten body-lengths in order to cross one gridline, therefore providing a highly conservative representation of activity levels. Furthermore, this grid size approximates larval movements observed in their natural environment. At each observation we recorded the location of each larva, whether there was movement, number of gridlines crossed following their previous position, and whether or not they were utilizing the refuge. The experiment was conducted over three days.

Experiment Ib: Detection via chemical cues by late-stage larvae.—Methods were identical to those listed above, using late-stage larvae (Stage 30–37; Gosner, 1960). Over 70% of these individuals were stages 34 to 36. There were nine replicates for each population (9 replicates × 4 tanks = 36 animals tested per population = 72 total).

Experiment Il: Detection via visual cues.—We used four pairs of 9.5-L glass tanks, with an opaque divider placed between each pair. Glass tanks were used instead of plastic tanks from the previous experiment to allow for maximum visibility. Beneath one of each pair was placed a six-square grid (4.5 × 10.5 cm each) which allowed the observer to track tadpole movement. In addition to these gridlines, the treatment tank was divided into three sections (4.5 × 21 cm each) situated relative to the treatment tank—Near Section, Middle Section, and Far Section. Late-stage larvae (stages 33–40; Gosner, 1960) from the BASSLESS population were exposed to one of four different visual cues: Control (no animal); Positive Control (native R. osculus); Native Predator (T. granulosa); and Non-native Predator (M. dolomieu). A total of 40 larvae were tested (10 replicates × 4 tank-pairs = 40 animals), none of which were re-used within the study.

All animals were randomly assigned to a treatment, as well as their position within each of the four pairs (i.e., left- or right-hand tank). Randomly selected treatment animals and larvae were placed into tanks and allowed 15 minutes to acclimate. Opaque dividers were then slowly removed from in between each pair of tanks to allow animals to see one another, and observations began immediately. Observations were taken every two minutes for ten minutes. During every observation, we recorded within which section each larva was located, whether there was movement, and number of gridlines crossed from their previous position. The experiment was conducted over one day.

Experiment III: Detection via chemical/visual/mechanical cues.—This experiment was designed to reflect the same exposure to cues larvae might receive in the side pools of streams in which they are often found. Four plastic tanks (50 × 32 × 14 cm) were divided in half width-wise by fiberglass mesh to separate larvae from the treatment animal. A 5-cm (2.5 cm diameter) piece of black plastic pipe was affixed to the bottom of the larva half to provide potential refuge. A nine-square grid (8.3 × 10.7 cm) was positioned under the larva half to monitor movement (or lack thereof). Late-stage larvae (Stage 31–37; Gosner, 1960) from BASS and BASSLESS populations were alternately tested by exposing them to one of four different treatments (Control, Dace, Newt, or Bass). There were eight replicates for each population (8 replicates × 4 tanks = 32 animals tested per population = 64 total).

Orientation of tanks was randomly assigned, as was the treatment. Treatment animals were randomly selected and placed in their pre-designated half of the tank. Following a ten-minute acclimation period, larvae were gently placed in the center of their half and observations began immediately every two minutes for ten minutes. We recorded the location of each larva, whether there was movement, number of gridlines crossed following their previous position, and whether or not they were utilizing the refuge. Ten individuals used in this study were randomly selected from larvae used in previous experiments. The experiment was run over two days.

Statistical analysis.—We examined initial and overall responses of larvae to treatments. Initial responses were activity data from the first two observations (the first four min of a trial); overall responses were data from all five observations over ten minutes. The sum of the number of gridlines crossed across observations for a single larva was used as an indicator of activity level in analyses. For all experiments, we tested for differences in activity levels between the four treatment groups using a full generalized linear model (GLM) examining the effects of population, treatment, and their interaction. A GLM was selected to account for non-normality of the observations and potential heteroscedasticity. A Poisson distribution was applied when data appeared non-normal; otherwise a Gaussian distribution was used. Drop in deviance Chi-square tests were used to test the statistical significance of population, treatment, and their interaction. One-way ANOVAs and Welch modified two-sample t-tests were applied to population and treatment means to assess which populations or treatments were responsible for significant main effects indicated by the GLM. In Experiment II, the proportion of the overall 10 min time spent in each section was analyzed using a Contingency Table Analysis, with the Control treatment used as our baseline for comparison. Tank size and grid size varied among experiments, and the chemical cue concentrations in Experiment I may have differed from those in Experiment III. Hence, each experiment should be considered independently. Analyses were performed using S-Plus 8.0 statistical software (Insightful Corp.; http://www.insightful.com).

RESULTS

In Experiment Ia, examining the responses of early-stage larvae to chemical cues, analyses of initial responses revealed no differences among treatments (df = 3, P = 0.38), but in analyses of overall responses, we found an interaction between population and treatment (Poisson distribution, df = 3, P = 0.06). Follow-up one-way ANOVAs indicated a difference at the treatment level (df = 3, P = 0.05; Fig. 4A) but not between the two populations (df = 1, P = 0.38). However, no significant differences between pairs of treatments were detected (post hoc Welch t-tests, 6 comparisons conducted, P > 0.17).

In Experiment Ib, where we examined late-stage larvae responses to chemical cues, we found no differences among treatments initially (df = 3, P = 0.80) or overall (df = 3, P = 0.47). However, in post hoc analyses, when all treatment data
We found no behavioral differences among treatments when larvae of *R. boylii* were exposed to chemical cues only (Experiments Ia and Ib). The overall activity level differences in Experiment Ia were influenced by larvae exposed to Bass...
cues. However, our follow-up analyses revealed no significant relationship. In some systems, chemical cues alone are often sufficient for predator detection in amphibians (Petranka et al., 1987; Kiesecker et al., 1996). However, many of these studies focused on amphibian larvae from lentic environments (but see Sih and Kats, 1994; Jowers et al., 2006). The stream environment in which larvae of R. boylii live could moderate their reliance on chemical cues alone to detect potential threats.

Although tadpole activity levels did not differ at the treatment level in Experiment Ib, late-stage larvae from the BASSLESS population exhibited higher activity levels overall relative to late-stage larvae from the BASS population. Eggs from the BASSLESS population were collected from a higher elevation (707 m) compared with those from the BASS population (273 m), which likely results in a shorter larval period. Since larvae of R. boylii do not overwinter, increased activity levels may be necessary at higher elevations to acquire the resources necessary to speed development and metamorphose prior to the onset of cooler weather. These results may reveal population-level differences in life history and behavior that were previously unknown for the species.

In Experiment II, larvae exhibited an initial response to visual cues within the first few minutes of the experiment, but not an overall response. The initial response was reflected as increased activity of the Newt- and Bass-exposed larvae. This is not surprising given that both M. dolomieu and T. granulosa were occasionally observed behaving aggressively towards larvae (for example, T. granulosa and M. dolomieu followed larvae and lunged at them). Over the longer course of the observation period, this response dampened, suggesting that visual cues alone were not a sustained stimulus for antipredator behaviors. Our results agree with those of similar studies examining the role of visual cues for predator detection by amphibian larvae (Stauffer and Semlitsch, 1993; Kiesecker et al., 1996; Hickman et al., 2004).

Results of Experiment III, examining combined sensory cues, indicated that multiple cues may be necessary to elicit a sustained antipredator response in larvae of R. boylii. Larvae from both BASS and BASSLESS populations significantly reduced their overall activity levels when placed in the same tank as their native predator, T. granulosa. Micropterus dolomieu readily detect motion to capture prey (Sweka and Hartman, 2003), and we observed M. dolomieu lunging at larvae during several observations. However, those larvae placed in the same tank as M. dolomieu did not show any difference in activity levels relative to controls which supports our original prediction that larvae exposed to the cues of a non-native, unfamiliar predator would display activity levels similar to larvae exposed to control cues. Therefore, the failure to reduce activity levels to avoid detection in the presence of M. dolomieu could increase predation events on larvae of R. boylii.

It is unclear why larvae did not utilize refuge during trials. In the field we regularly witnessed larvae of R. boylii retreating between stones and under cobble upon our approach. We can only surmise that larvae avoided artificial refugia used in laboratory trials due to some quality of the material used or the tube-design of the refugia. In future studies, the use of natural cover objects could be tested.

The ability to adapt behaviorally to an unfamiliar predator is contingent upon a range of factors such as time spent in sympathy, experience, amount of predation pressure, and the behavioral plasticity of the species. Our findings contribute to the growing body of work examining the responses of native amphibians to introduced predators. Analogous studies examining other native U.S. Pacific Northwest amphibians suggest that species differ greatly in their responses to predators. For instance, 10 of the 21 studies we reviewed (Paoletti, 2009) reported no response to the chemical cues of an introduced predator. Our results suggest that after approximately 40 years of coexistence (approx. 20 generations; Hayes et al., 2005), R. boylii lack the ability to respond to M. dolomieu as a threat. On the other hand, Kiesecker and Blaustein (1997), found that in approximately 30 generations (McAllister and Leonard, 2005), larvae of the Red-legged Frog (Rana aurora) developed the ability to detect and avoid non-native American Bullfrogs (L. catesbeianus), a potential predator. The behavioral responses witnessed in this study emphasize the difficulty in predicting how a naive, native species might respond to an unfamiliar predator. In the brief 40 years the two species have coexisted, M. dolomieu may have not exerted selection pressure strong enough to modify behavioral patterns of R. boylii. Given the chance, M. dolomieu will readily consume larvae and adults of R. boylii (pers. obs.). However, we do not know whether R. boylii is the preferred prey or simply an opportunistic food source.

Several studies have shown that amphibian larvae respond to the chemical cues of injured conspecifics, or those chemicals emitted from predators that have consumed conspecifics (Laurila et al., 1997; Chivers and Mirza, 2001). Like many ranids, larvae of R. boylii are found in loose aggregations until metamorphosis (pers. obs.). It is possible that an individual cannot maintain the vigilance necessary to detect predators, and so rely on the alarm cues produced by one or a few group members to detect a predatory threat. Our study focused on the response of an individual tadpole to the cues of the treatment animal, without the aid of group effects or supplemental dietary cues produced by predators. In addition, our study monitored larval behavior in an artificially homogeneous habitat. The transmission and detection of chemical, visual, and mechanical cues may be altered by the physically heterogeneous habitat in which larvae are normally found. Therefore, the strength of the responses elicited by stimulus animals in the experiments reported herein should be considered highly conservative.

Our results indicate that M. dolomieu are capable of directly affecting populations of R. boylii in areas where they overlap. Removal of non-native fish has led to recovery of local amphibian populations in some areas (Hoffman et al., 2004; Vredenburg, 2004). Despite being recognized as an introduced species by Pacific Northwestern state agencies, M. dolomieu remains an actively managed sport fishery. Because of this and the difficulty involved with removal, eradication is not a feasible option. If larvae of R. boylii are unable to avoid predation by an unfamiliar predator, as our findings show, then local populations may disappear before they are able to adapt. It is therefore critical to understand the behavioral responses of native wildlife when faced with a novel predator. Developing the ability to predict these responses could aid fisheries managers when developing stocking plans to mitigate amphibian declines at the regional level.

ACKNOWLEDGMENTS
We would like to thank D. Simon, D. Markle, C. Rombough, G. Weaver, C. Baldwin, M. Kluber, and T. Garcia for their
assistance. Financial support for this project was provided by the USDA Forest Service, Pacific Northwest Research Station (Corvallis, Oregon), and Oregon State University. These experiments comply with the current laws of the United States and with Oregon State University animal care regulations (ACUP #3732). Animals were collected according to Oregon Department of Fish and Wildlife regulations (permit #068-08 and permit #OR2008-4516).

LITERATURE CITED


Simon, D. C., and D. F. Markle. 1999. Evidence of a relationship between smallmouth bass (Micropterus dolo-


