

AN ABSTRACT OF THE THESIS OF

David J. Paoletti for the degree of Master of Science in Environmental Science presented on June 29, 2009.

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

Abstract approved:

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The consequences of species introductions into non-native habitats are a major cause of concern in the U.S. An introduced species may alter native habitats, cause economic damage, compete with natives for resources or prey on them. Of particular interest are the effects of predation by introduced fishes on native amphibians.

Amphibians as a group have been declining worldwide due to a variety of factors, one of which being introduced species. In the Pacific Northwest of the U.S., one example of these declining amphibians is the foothill yellow-legged frog (*Rana boylei*). In Oregon, *R. boylei* has disappeared from more than half of its historical sites and is now listed as a state and federal Sensitive Species. Although specific causes have not been determined, declines may be partly attributed to the recent introduction of smallmouth bass (*Micropterus dolomieu*) to some of the river systems in which these frogs live. Although smallmouth bass have been implicated as a cause of losses, very little is known about interactions between these two species. Given the relatively short period of time these two species have been co-existing, we sought to determine whether *R. boylei* larvae could

even recognize bass as a predatory threat. Through a series of experiments, we examined the behavioral responses of larvae to a variety of stimuli including a native potential predator (rough-skinned newt, *Taricha granulosa*), introduced predator (smallmouth bass, *M. dolomieu*), and a native non-predatory fish (speckled dace, *Rhinichthys osculus*). Each experiment examined a different potential mode of detection: 1) chemical cues only; 2) visual cues only; or 3) a combination of chemical/visual/mechanical cues simultaneously. We predicted that in each experiment, larvae would respond to the native predator by exhibiting antipredator behaviors, whereas those exposed to cues of the non-native, unfamiliar predator would display activity levels similar to larvae exposed to controls. In addition, we tested amphibian larvae from two populations – one where they co-occur with *M. dolomieu*, and compared them to larvae from a location where *M. dolomieu* has not yet invaded – to determine whether any antipredator responses observed were recently developed behavioral adaptations. We analyzed initial and overall responses to stimuli. Our analyses of the initial responses of *R. boylii* larvae revealed an increase in activity when exposed to the visual cues of bass relative to controls. Furthermore, our results suggested that individual *R. boylii* larvae require multiple cues to facilitate predator detection. When exposed to multiple cues of their native predator, the rough-skinned newt, larvae responded with a significant reduction in activity levels. Those larvae exposed to cues of the non-native predator, smallmouth bass, displayed similar behaviors relative to control cues, supporting our prediction. Consequently, foothill yellow-legged frog larvae appear to be especially vulnerable to predation by non-native smallmouth bass.

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

By

David J. Paoletti

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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David J. Paoletti, Author

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## CHAPTER 1 INTRODUCTION

The consequences of species introductions into non-native habitats are a major cause for concern. An introduced species may alter native habitats, cause economic damage, compete with natives for resources or prey on them. From 1950 – 2000, the number of introduced species has more than tripled in the United States (USGS 2009). These introductions are particularly prevalent in aquatic systems. For instance, it is estimated that 360 non-native species have been introduced throughout California's coastal and estuary waters (Foss et al. 2007). Muir and Jenkins (2002) have documented up to 140 aquatic non-natives in the Great Lakes. According to the U.S. Geological Survey's Nonindigenous Aquatic Species Database, over 42% (n = 675) of introduced aquatic species are fishes (USGS 2009), with many introductions being intentional. At present, 3,072 fish introductions have been reported worldwide with over half of these non-natives now established (i.e., surviving and reproducing successfully) in the wild (Casal 2006).

In the Pacific Northwest, USA, non-native fishes have been stocked in a variety of habitats from rivers to sub-alpine lakes to backyard ponds. Schade and Bonar (2005) conducted surveys of 12 western states from 2000-2002 and found that of the most widely distributed and abundant non-native species (n = 15, Table 1), almost all were introduced for sport. Species such as rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), channel catfish (*Ictalurus punctatus*), and smallmouth bass (*Micropterus dolomieu*) have been, and continue to be, stocked in many areas of the western U.S. for recreational purposes.

**Table 1.1:** Most common introduced fishes in the fish-bearing streams of 12 states in the western USA. Adapted from Schade and Bonar (2005).

<b>Species</b>	<b>Purpose of Introduction</b>
Brook trout ( <i>Salvelinus fontinalis</i> )	Sport
Brown trout ( <i>Salmo trutta</i> )	Sport
Channel catfish ( <i>Ictalurus punctatus</i> )	Sport
Common carp ( <i>Cyprinus carpio</i> )	Food
Cutthroat trout ( <i>Oncorhynchus clarkii</i> )	Sport
Fathead minnow ( <i>Pimephales promelas</i> )	Bait
Golden shiner ( <i>Notemigonus crysoleucas</i> )	Bait
Green sunfish ( <i>Lepomis cyanellus</i> )	Sport
Largemouth bass ( <i>Micropterus salmoides</i> )	Sport
Western mosquitofish ( <i>Gambusia affinis</i> )	Biocontrol
Rainbow trout ( <i>O. mykiss</i> )	Sport
Red shiner ( <i>Cyprinella lutrensis</i> )	Bait
Smallmouth bass ( <i>M. dolomieu</i> )	Sport
Yellow bullhead ( <i>Ameiurus natalis</i> )	Sport
Yellow perch ( <i>Perca flavescens</i> )	Sport

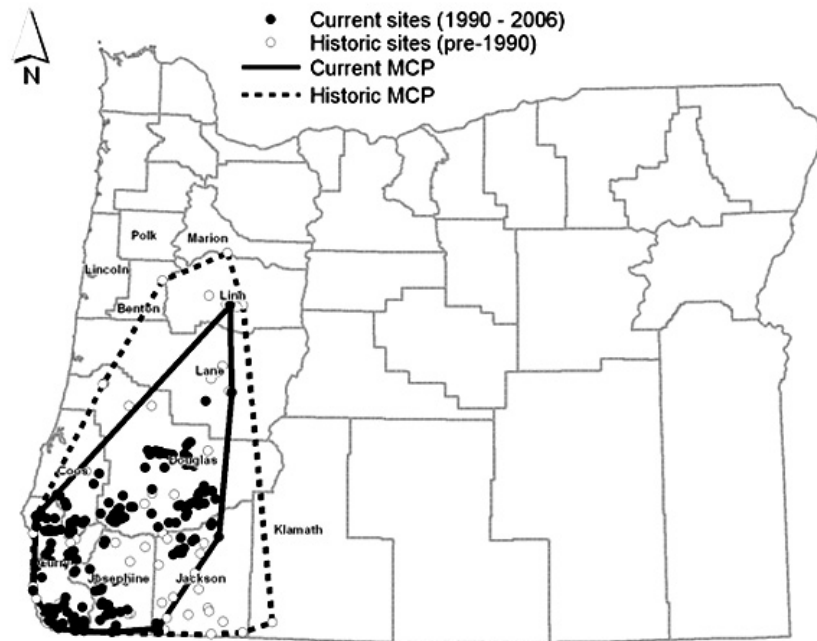
Of particular interest are the impacts on native amphibians when these introduced fishes act as predators. Amphibian populations have been declining worldwide due to a myriad of reasons including habitat loss, disease, environmental changes, pollutants, and introduced species (Alford and Richards 1999; Blaustein and Wake 1990; Pounds et al. 2006). One estimate suggests that of the world's 6,000+ amphibian species, 42% are in decline and 32% are threatened or extinct (IUCN 2008). Of these 6,000+ species, almost 500 amphibians are considered to be threatened by invasive species (IUCN 2008). Adams (1999) found that as a result of habitat degradation, the loss of ephemeral wetlands prompted the redistribution of native amphibians towards permanent water bodies occupied by non-native predatory fishes. Recent research also suggests that amphibian

population declines are a result of two or more of these factors working synergistically (Blaustein and Kiesecker 2002; Collins and Storfer 2003; Davidson and Knapp 2007).

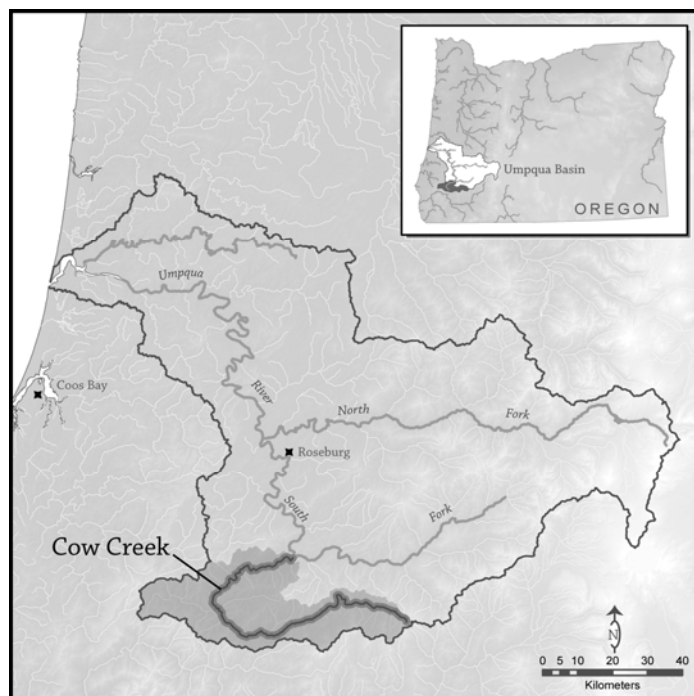
In the western U.S. introduced fishes have been implicated in affecting distributions of several amphibian species (e.g., cascades frogs, *Rana cascadae* [Fellers and Drost 1993]; mountain yellow-legged frogs, *Rana muscosa* [Bradford et al. 1993; Knapp and Matthews 2000]; and long-toed salamanders, *Ambystoma macrodactylum* [Tyler et al. 1998]). Due to the life history requirements of most amphibians, the potential threat that non-native fishes pose is of special concern. Whereas adult amphibians are often terrestrial, eggs and larvae of many species are completely dependent on water. Upon hatching, metamorphosis of amphibian larvae may take from two months to four years, depending on the species (Jones et al. 2005). As a result, pre-metamorphic amphibians may be in constant contact with fishes. Having few defenses other than crypsis or refuge use, palatable amphibian larvae are highly vulnerable to larger, faster fish predators. This underscores the importance of rapidly determining the extent of fish-amphibian interactions to mitigate potentially negative impacts.

In Oregon, one example of these interactions is that of the foothill yellow-legged frog (*Rana boylei*) and smallmouth bass (*Micropterus dolomieu*). *Rana boylei* is a native frog listed as a “Sensitive Species” in the state, having been reduced to only 43% of their historical locations (Figure 1) (Olson and Davis 2007). Conversely, *M. dolomieu* was introduced into the Umpqua River system in the mid-1960s, and their distribution is rapidly expanding (Simon and Markle 1999). This spread is particularly evident in Cow Creek, a major tributary of the South Umpqua River in Douglas County, Oregon (Figure 2). Recent distributional surveys in Cow Creek have found a strong negative correlation

**Figure 1.1:** Past and present *Rana boylei* distributions in Oregon, USA. Reprinted from Olson and Davis (2007). MCP = Minimum Convex Polygon of species range from known site locations.



**Figure 1.2:** Location of foothill yellow-legged frog (*Rana boylei*) study site at Cow Creek, Douglas County, Oregon.



between bass and frog presence. As *M. dolomieu* have spread upstream through Cow Creek, a coincident reduction of *R. boylei* has been observed (Borisenko and Hayes 1999; Rombough 2006). In the Conservation Assessment for *R. boylei*, Olson and Davis (2007) proposed a variety of reasons for the decline of this species, including habitat loss, pollutants, change in hydrologic regimes, and introduced species such as *M. dolomieu*. Unfortunately, there is a void in the literature regarding these threats, and specific causes of decline remain unexamined. More information is required before frog declines can be attributed to the invasion of smallmouth bass in Cow Creek, Oregon.

Amphibians are centrally nested in food webs, and consequently have developed a complex array of predator-detection and predator-avoidance adaptations. The principal mode of predator detection by many amphibian larvae is through waterborne chemical cues (Kats and Dill 1998; Kiesecker et al. 1996). Therefore, if larvae are unable to recognize and respond to a non-native predatory fish, it may result in disastrous consequences. Several studies have addressed this concern. For instance, Pearl et al. (2003) found that invasive bluegill (*Lepomis macrochirus*) elicited a defense response from the native northern red-legged frog (*Rana aurora*), but not from the Pacific treefrog (*Pseudacris regilla*). Another study found that *P. regilla* from both ephemeral and permanent water bodies responded to introduced brook trout (*S. fontinalis*) as a threat (Paoletti; *unpubl. data*). Recent research by Bosch et al. (2006) found that the stream-breeding Iberian frog (*Rana iberica*) responded to recently introduced brook trout (*S. fontinalis*) with a reduction in activity level. Therefore, it appears that some amphibian species have the plasticity to recognize and respond to a non-native fish predator. It is unknown whether *R. boylei* larvae possess this ability.

Surveys for *M. dolomieu* in Cow Creek show that juveniles favor the same habitat type as *R. boylei* larvae (Rombough 2006; Simon *pers. comm.*). Therefore, indications are that tadpole-bass interactions may be occurring on a regular basis. When faced with stressors such as predation and competition, larval growth and development in amphibians is negatively affected (Skelly 1992; Kiesecker and Blaustein 1998; Kupferberg 1997). Therefore, tadpole-bass encounters may be adversely affecting survival, growth and development of larval foothill yellow-legged frogs.

Here I investigate possible mechanisms behind *R. boylei* declines in Cow Creek, Oregon, by experimentally assessing the interactions of their larvae with *M. dolomieu*. Specifically, I examine whether *R. boylei* larvae have the ability to recognize *M. dolomieu*, a non-native predator. Absence of an appropriate anti-predator response by larvae may directly impact *R. boylei* populations in Cow Creek. Whereas this study focuses on species-specific effects, findings here also will provide clues to possible alterations in community structure. For example, *R. boylei* larvae and metamorphs are potential prey for native species such as garter snakes (*Thamnophis sp.*) (Fitch 1936; Lind 2004), aquatic invertebrates (Hayes et al. 2005), rough-skinned newts (*T. granulosa*), and the American dipper (*Cinclus mexicanus*) (Olson and Davis 2007). Consequently, a reduction in *R. boylei* larvae may result in a direct perturbation of the local food web. Other research has shown how tadpole foraging habits indirectly impact community structure. For example, Ranvestel et al. (2004) found that larval amphibians influence food web dynamics by altering sediment levels and algal abundances in tropical streams. Therefore, results of the current study will provide the foundation for a fruitful area of research in understanding the effects of smallmouth bass invasions.

## STUDY SYSTEM

### **Study Area:**

Lower Cow Creek includes the final 42 km of stream from the Middle Fork tributary, where it flows roughly northeast and drains into the South Umpqua River near the town of Riddle, Oregon (Figure 2) (Geyer 2003). In this area of steep topography, the predominant land use (91%) is timber harvest and forest management. Native fishes include winter steelhead (*Oncorhynchus mykiss*), coho (*O. kisutch*), cutthroat trout (*O. clarki*), fall chinook (*O. tshawytscha*), Umpqua chub (*Oregonichthys kalawatseti*), Redside shiner (*Richardsonius balteatus*), Speckled dace (*Rhinichthys osculus*), Umpqua longnose dace (*R. evermanni*), and Umpqua pikeminnow (*Ptychocheilus umpquae*) (Geyer 2003).

### **Smallmouth Bass (*Micropterus dolomieu*):**

The smallmouth bass is a warmwater fish native to the Mississippi valley and Great Lakes region of North America (Fuller, USGS 2009). They inhabit rocky, shallow areas of lakes as well as clear streams. *Micropterus dolomieu* reach reproductive status between 2-4 years and may attain sizes of 69 cm total length (TL) and 5.4 kg. Bass are found at a variety of depths, which is usually size-dependent. Larger bass ( $\geq 260\text{mm}$ ) are generally found in the deeper pools ( $> 2\text{m}$ ), while juveniles ( $< 50\text{mm}$ ) are found at much shallower depths (5-30 mm) around the edges of pools and cobble bars (Rombough 2006). Young feed predominantly on aquatic insects and plankton while adults are more opportunistic, with a diet consisting of crayfish, fishes and insects (Gray 2004). Temperature influences feeding activity, with bass being fairly inactive at temperatures

under 10°C, and not actively feeding again until stream temperatures rise above 15.6°C (Gray 2004). In the Umpqua basin, bass begin spawning in late April to May (Simon, *pers. comm.*)

It is believed that broad-scale introduction of *M. dolomieu* occurred in 1964 following a flooding event that washed them from stocked farm ponds into the Umpqua river system (Simon and Markle 1999). At present they are established and considered abundant in the South Umpqua River and lower portion of Cow Creek. In the John Day River of north-central Oregon, *M. dolomieu* were purposefully introduced for sportfishing where they maintain an abundant population. The precise impacts of these introductions on native communities in Oregon have yet to be determined. Recent surveys along Cow Creek have noted a significant, negative correlation between bass presences with that of the Umpqua chub (*O. kalawatseti*). These distributional patterns are similar to those of *M. dolomieu* and *R. boylei*. In fact, Rombough (2006) determined that bass were the best predictor of *R. boylei* presence in Cow Creek. If bass are acting to displace *R. boylei*, the exact mechanism has yet to be determined.

**Foothill Yellow-legged Frog (*Rana boylei*):**

The foothill yellow-legged frog is the Pacific Northwest's smallest ranid (Hayes et al. 2005). They are a stream amphibian that is highly tied to water, rarely venturing more than a meter or so from waters edge (*pers. observ.*). Adults and larvae prefer areas with ample sun and clear waters. Larvae are often found in slow-flow areas or side pools and prefer substrates comprised of bedrock, cobble and gravel. Areas that are predominantly sand or silt are avoided (*pers. observ.*). *Rana boylei* become sexually reproductive in 1-2

years and may live as many as 6 years. In this time they can reach a maximum size of 82 mm SVL (snout-vent length) and 48 g, with females being slightly larger than males (Hayes et al. 2005). In Oregon, this species begins breeding in early May, triggered by a simultaneous rise in ambient temperatures and a drop in water velocity (Rombough, *pers. comm.*). Breeding sites are generally located at the confluence of a tributary and main stem, in shallow reaches where the chances of eggs being scoured are reduced (Kupferberg 1996). Following breeding, oviposition occurs on the downstream surface of boulders, bedrock or large cobble. Eggs hatch in 2-3 weeks and in the areas around Cow Creek, larvae metamorphose in late July through early September (*pers. observ.*). *Rana boylei* larvae feed on algae and detritus, while adults prey mainly on invertebrates (Olson and Davis 2007). In the Umpqua basin, *R. boylei* face a variety of native predators. Snakes, birds, and some fish species may consume *R. boylei* in the adult stage, while dragonfly larvae, rough-skinned newts, fishes and garter snakes prey upon the larvae. Non-native predators such as smallmouth bass and bullfrogs may consume *R. boylei* of either stage.

The U.S. Fish and Wildlife Service list the foothill yellow-legged frog as a “Species of Concern,” while the Oregon Department of Fish and Wildlife list them as a “Sensitive” species. Surveys show that *R. boylei* has disappeared from 57% of their historic sites in Oregon (Fig. 1.1) (Borisenko and Hayes 1999). The Interagency Special Status/Sensitive Species Program (ISSSP) Conservation Assessment for *Rana boylei* by Olson and Davis (2007) cite introduced species, alteration of natural flow regimes (via dams), and agriculture as the primary threats to this species. At this time, little is known about the severity or extent of these threats.

Studies examining the effects of introduced species on native amphibians are numerous, but to my knowledge only one (Kupferberg 1997) has been conducted investigating these effects on *R. boylei*. This is the first attempt to determine empirically possible effects of *M. dolomieu* on *R. boylei* larvae. Combined with the information provided by survey efforts completed thus far, we can more accurately predict the consequences of bass and frog syntopic occurrences. Understanding these interactions is necessary for development of effective measures to reduce the potential adverse effects of these invasive species on native amphibians.

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CHAPTER 2  
RESPONSES OF FOOTHILL YELLOW-LEGGED FROG (*RANA BOYLI*) LARVAE  
TO AN INTRODUCED PREDATOR

ABSTRACT

The consequences of species introductions into non-native habitats are a major cause for concern in the U.S. An introduced species may alter native habitats, cause economic damage, compete with natives for resources or prey on them. Of particular interest are the effects of predation by introduced fishes on native amphibian communities. In Oregon, the foothill yellow-legged frog (*Rana boylei*) has disappeared from more than half of its historical range and is now listed as a state and federal Sensitive Species. These declines may be partly attributed to the recent introduction of smallmouth bass (*Micropterus dolomieu*) to some of the river systems in which these frogs live. Although smallmouth bass have been implicated as a cause of losses, very little is known about interactions between these two species. We sought to determine whether *R. boylei* larvae could recognize bass as a predatory threat. Through a series of experiments, we examined the behavioral responses of larvae to a variety of stimuli including a native predator (newts), introduced predator (bass), and a native non-predatory fish (dace). Each experiment examined a different potential mode of detection: 1) chemical cues; 2) visual cues; or 3) a combination of chemical/visual/mechanical cues. We hypothesized that in all experiments, larvae would respond to the native predator by exhibiting antipredator behaviors, whereas those exposed to cues of the non-native, unfamiliar predator would display activity levels similar to larvae exposed to controls. We analyzed both initial and

overall responses to stimuli. Analyses of initial response showed that *R. boylei* larvae responded with an increase in activity levels when exposed to the visual cues of bass relative to controls. Results also suggested that individual *R. boylei* larvae 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, supporting our hypothesis. Consequently, foothill yellow-legged frog larvae appeared to be especially vulnerable to predation by non-native smallmouth bass.

## INTRODUCTION

The consequences of species introductions into non-native habitats are a major cause of concern. An introduced species may alter native habitats, cause economic damage, compete with natives for resources or prey on them. These introductions are particularly prevalent in aquatic systems. From 1950 to present, the number of introduced aquatic species has more than tripled in the United States (USGS 2009). Muir and Jenkins (2002) have documented up to 140 aquatic non-native species in the Great Lakes alone. According to the U.S. Geological Survey's Nonindigenous Aquatic Species Database, over 42% ( $n = 675$ ) of introduced aquatic species are fishes (USGS 2009), with many of these introductions being intentional.

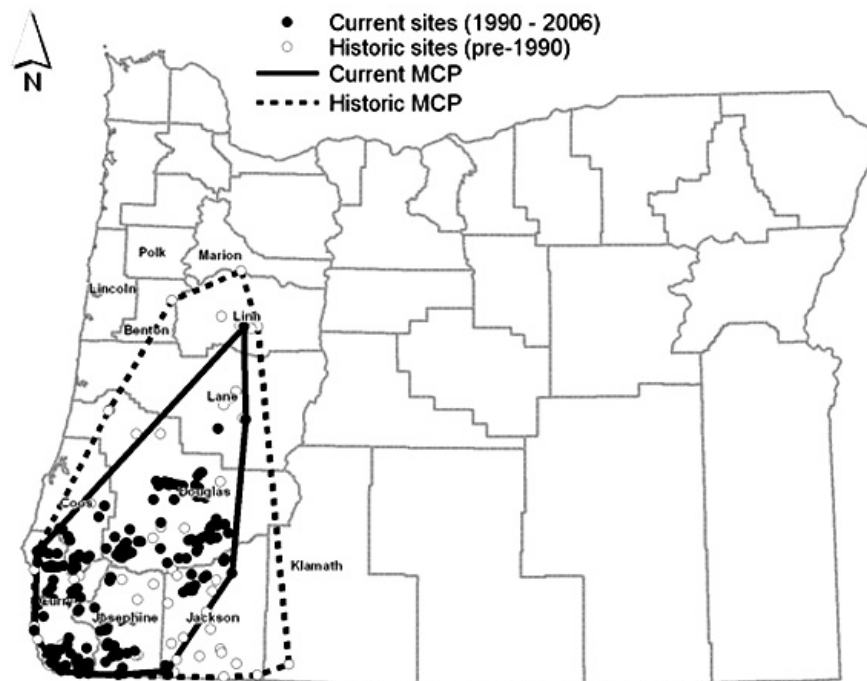
In the Pacific Northwest, USA, non-native fishes have been stocked in a variety of habitats from rivers to sub-alpine lakes to backyard ponds. Schade and Bonar (2005) conducted surveys of 12 western states from 2000-2002 and found that of the most widely distributed and abundant non-native species ( $n = 15$ ), almost all were introduced

for sport fisheries. Species such as rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), channel catfish (*Ictalurus punctatus*), and smallmouth bass (*Micropterus dolomieu*) have been, and continue to be, stocked in many areas of the western U.S. for recreational purposes.

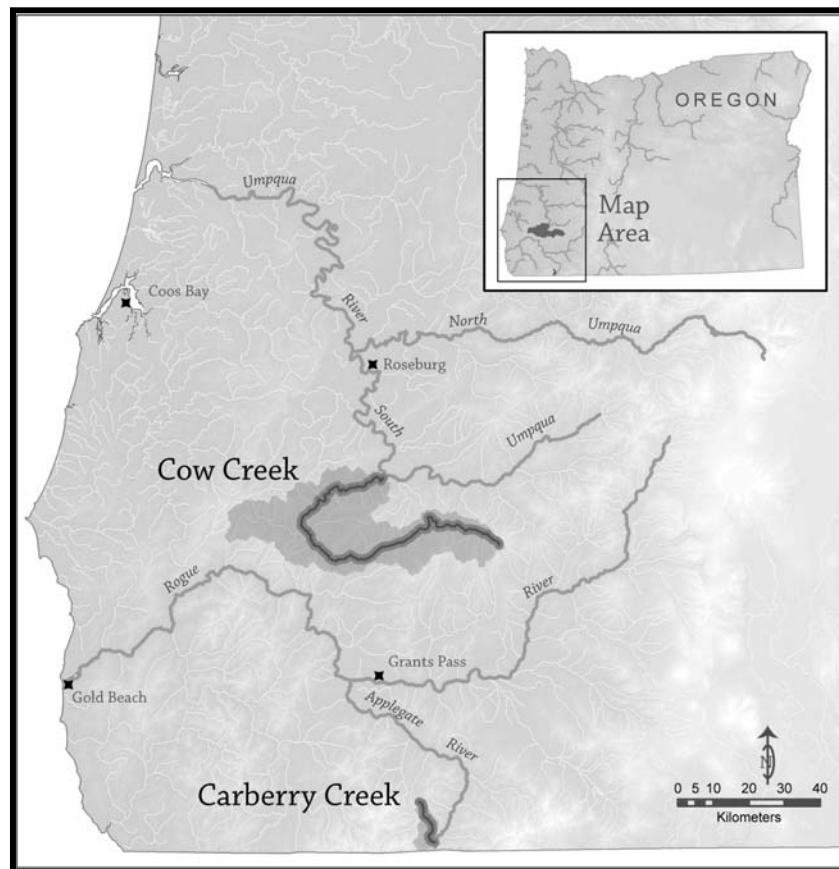
Of particular interest are the impacts on native amphibians when these introduced fishes act as predators. Amphibian populations have been declining worldwide due to a myriad of reasons including habitat loss, disease, environmental changes, pollutants, and introduced species (Alford and Richards 1999; Blaustein and Wake 1990; Pounds et al. 2006). Research suggests that declines are a probably a result of two or more of these factors working synergistically (Blaustein and Kiesecker 2002; Collins and Storfer 2003; Davidson and Knapp 2007). In the western United States, introduced fishes have been implicated in affecting distributions of several amphibian species (e.g., Cascades frogs, *Rana cascadae* [Fellers and Drost 1993]; mountain yellow-legged frogs, *Rana muscosa* [Bradford et al. 1993; Knapp and Matthews 2000]; and long-toed salamanders, *Ambystoma macrodactylum* [Tyler et al. 1998]). Due to the life history requirements of most amphibians, the potential threat that non-native fishes pose is of special concern. Whereas adult amphibians are often terrestrial, eggs and larvae of many species are completely dependent on water. Upon hatching, metamorphosis of amphibian larvae may take from two months to four years, depending on the species (Jones et al. 2005). As a result, pre-metamorphic amphibians may be in constant contact with fishes at their most susceptible stage. This underscores the importance of rapidly determining the extent of fish-amphibian interactions in order to mitigate potentially negative impacts.

In Oregon, one example of these interactions is that of the foothill yellow-legged frog (*Rana boylei*) and smallmouth bass (*Micropterus dolomieu*). *Rana boylei* is a native frog listed as a “Sensitive Species” in the state, having been reduced to only 43% of their historical locations (Fig. 2.1) (Olson and Davis 2007). Conversely, *M. dolomieu* has invaded the Umpqua River system as recently as the mid-1960s, and their distribution is rapidly expanding (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. 2.2). Recent distributional surveys in Cow Creek have found a strong negative correlation between bass and frog presence. As *M. dolomieu* have spread upstream through Cow Creek, a coincident reduction of *R. boylei* has been observed (Borisenko and Hayes 1999; Rombough 2006).

**Figure 2.1:** Past and present *Rana boylei* distributions in Oregon, USA. Reprinted from Olson and Davis (2007). MCP = Minimum Convex Polygon of species range from known site locations.



**Figure 2.2:** Location of foothill yellow-legged frog (*Rana boylei*) study site and egg-collection sites at Cow Creek, Douglas County, and Carberry Creek, Jackson County, Oregon, USA.



In the Conservation Assessment for *R. boylei*, Olson and Davis (2007) proposed a variety of reasons for the decline of this species, including habitat loss, pollutants, change in hydrologic regimes, and introduced species such as *M. dolomieu*. Unfortunately, there is a void in the literature regarding these threats, and specific causes of decline remain unexamined.

Here we investigate possible mechanisms behind *R. boylei* declines in Cow Creek, Oregon, by experimentally assessing the interactions of their larvae with *M. dolomieu*. Specifically, we examine whether *R. boylei* larvae have the ability to recognize *M.*

*dolomieu*, a non-native predator. This was done through a series of four correlated experiments. Predator recognition often occurs through waterborne chemical cues. Upon detection of a potential threat, larvae often respond with antipredator behaviors such as a reduction in activity levels or refuge use (Petranka et al. 1987; Kiesecker et al. 1996). Experiment I is conducted to determine if chemical cues alone are sufficient for *R. boylei* larvae 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 are evident in *R. boylei*, Experiment Ia is conducted using early-stage larvae, whereas Experiment Ib focuses on the responses of late-stage larvae. Because chemical cues may be insufficient to detect a potential threat in a lotic environment, Experiment II is designed to determine the role of visual cues in *R. boylei* larvae. In a lotic environment, amphibian larvae may need to use several modes of detection in tandem to identify a potential threat. Experiment III is designed to expose *R. boylei* larvae to chemical, visual, as well as mechanical cues of the stimulus animal concurrently.

We hypothesized that in all experiments, *R. boylei* larvae would respond to the 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 (*R. catesbeiana*) on *Rana boylei*. Our study is the first attempt to determine empirically the effects of

*M. dolomieu* on *R. boylei* larval behavior. Absence of an appropriate anti-predator response by larvae may directly impact *R. boylei* populations in Cow Creek, Oregon.

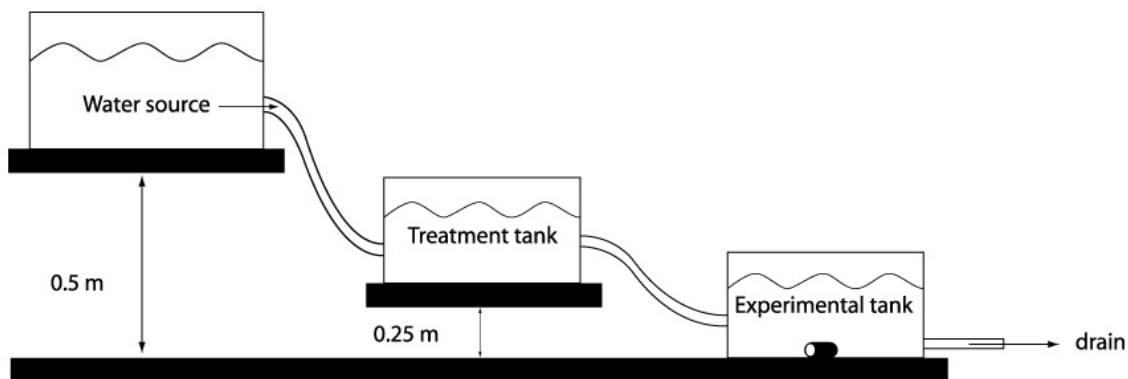
## MATERIALS AND METHODS

*Rana boylei* eggs were collected in June 2008 from an area populated by smallmouth bass (Cow Creek, Douglas Co., OR; 42°52'3"N, 123°34'32"W) and also from a bass-free area (Carberry Creek, Jackson Co., OR; 42°4'6"N, 123°10'7"W) (Fig. 2.2). Larvae from these populations will be referred to as BASS and BASSLESS hereafter. 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 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: rough-skinned newts (*Taricha granulosa*), speckled dace (*Rhinichthys osculus*), and smallmouth bass (*Micropterus dolomieu*). Newts are a native predator of frog larvae (Jones et al. 2005), which were used to compare responses with the non-native bass predator. Dace are a non-predatory native minnow which served as our 'positive fish control' in this study. All animals were brought back to Oregon State University and maintained in the conditions listed above. Dace were fed fish flakes and tubifex worms; newts and bass were fed earthworms and crickets *ad libitum*.

Experiment Ia: Detection via Chemical Cues by Early-stage larvae – The study was conducted in the laboratory once larvae reached Stage 20-24 (Gosner 1960). Larvae were

exposed to one of four different chemical cue treatments: Control (no cue); Positive Control (non-lethal, native speckled dace); Native Predator (rough-skinned newt); and Non-native Predator (smallmouth bass). Individual larvae (N=104) were randomly assigned 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. 2.3; modified from Petranka et al. 1987) to mimic 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 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. Food was withheld from all animals 24 h prior to conducting the experiment to avoid the effects of satiation. A blind was constructed around all tanks to prevent disturbing the animals during observations.

**Figure 2.3:** Design of flow-through tanks addressing the potential response of *Rana boylei* larvae 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.



Just prior to running a trial, animals supplying the cue were placed in the treatment tank. A randomly selected larva was then placed in the experimental tank and allowed to acclimate for 10 min. At this point the valve from the source tank was slowly opened, followed by the treatment tank valve. Observations began immediately and locations of larvae were recorded during “spot-checks” conducted every two min. for 10 min. A grid divided into six quadrants (10 x 10 cm each) was placed under each experimental tank to track movement. 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 10 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). There were 9 replicates for each population (9 replicates x 4 tanks = 36 animals tested per population = 72 total).

*Experiment II: 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 of the previous experiment to allow for maximum visibility. Beneath one of each pair was placed a 6-square grid (4.5 x 10.5 cm each) which allowed the observer to track tadpole movement. In addition, the treatment tank was divided into three sections (4.5 x 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 speckled dace); Native Predator (rough-skinned newt); and Non-native Predator (smallmouth bass). A total of 40 larvae were tested (10 replicates x 4 tank-pairs = 40 animals), none of which were re-used within the study. All feedings ceased two days prior to running the experiment to avoid the effects of satiation. Blinds were constructed around the tanks.

All animals were randomly assigned to a treatment, as well as their position within each of the 4 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 2 min. for 10 min. 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* – Four plastic tanks (50 x 32 x 14 cm) were divided in half width-wise by fiberglass mesh to separate the tadpole from treatment animal. A 5-cm (2.5 cm diameter) piece of black plastic pipe was affixed to the bottom of the tadpole half to provide potential refuge for larvae. A nine-square grid (8.3 x 10.7 cm) was positioned under the tadpole 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 8 replicates for each population (8 replicates x 4 tanks = 32 animals tested per population = 64 total). All feedings ceased two days prior to running the experiment to avoid effects of satiation. Blinds were constructed around the tanks to prevent the observer from startling animals.

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 10 min. acclimation period, larvae were gently placed in the center of their half and observations began immediately. Spot-checks were conducted every 2 min. for 10 min. I 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. A limited number of larvae used in this study were 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 in the first 4 min. of a trial; overall responses were data from all five observations over 10 min. The sum of the number of grid lines 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. The significance level for all analyses was  $\alpha \leq 0.05$ . Analyses were performed using S-Plus 8.0 statistical software.

## RESULTS

### *Initial Response*

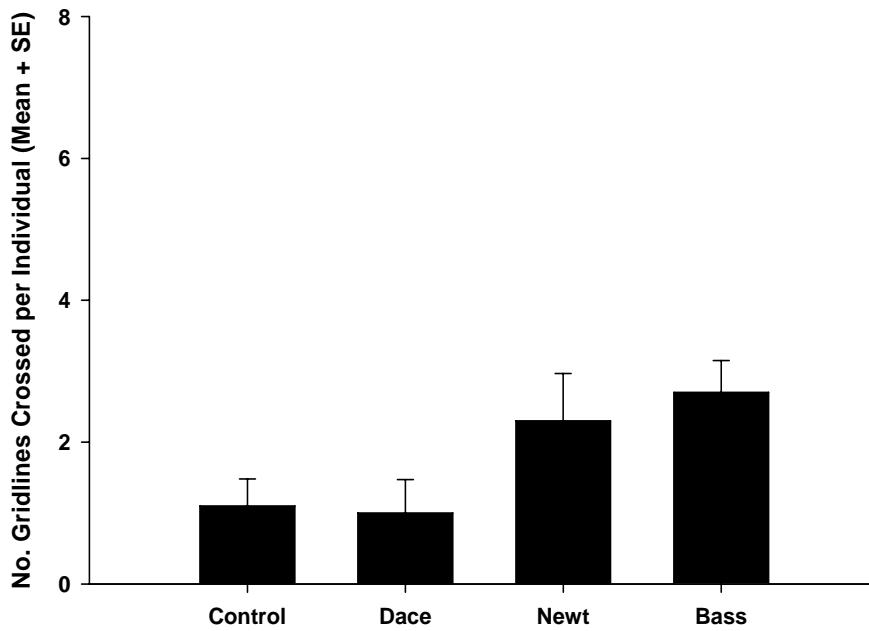
Our analyses of the initial responses of larvae revealed a difference among treatments only in Experiment II, in which we examined visual cues ( $df = 3$ ,  $p = 0.03$ ; Fig. 2.4). *Post hoc* t-tests revealed significant differences in number of gridlines crossed between larvae exposed to Bass (mean = 2.7) versus the Control (mean = 1.1;  $p = 0.01$ ), as well as Bass (mean = 2.7) versus Dace (mean = 1.0;  $p = 0.02$ ). Analyses of initial responses for the other three experiments revealed no differences when larvae were exposed to chemical cues only (Early-stage,  $p = 0.38$ ; Late-stage,  $p = 0.80$ ), or multiple cues ( $p = 0.55$ ).

### *Overall Response*

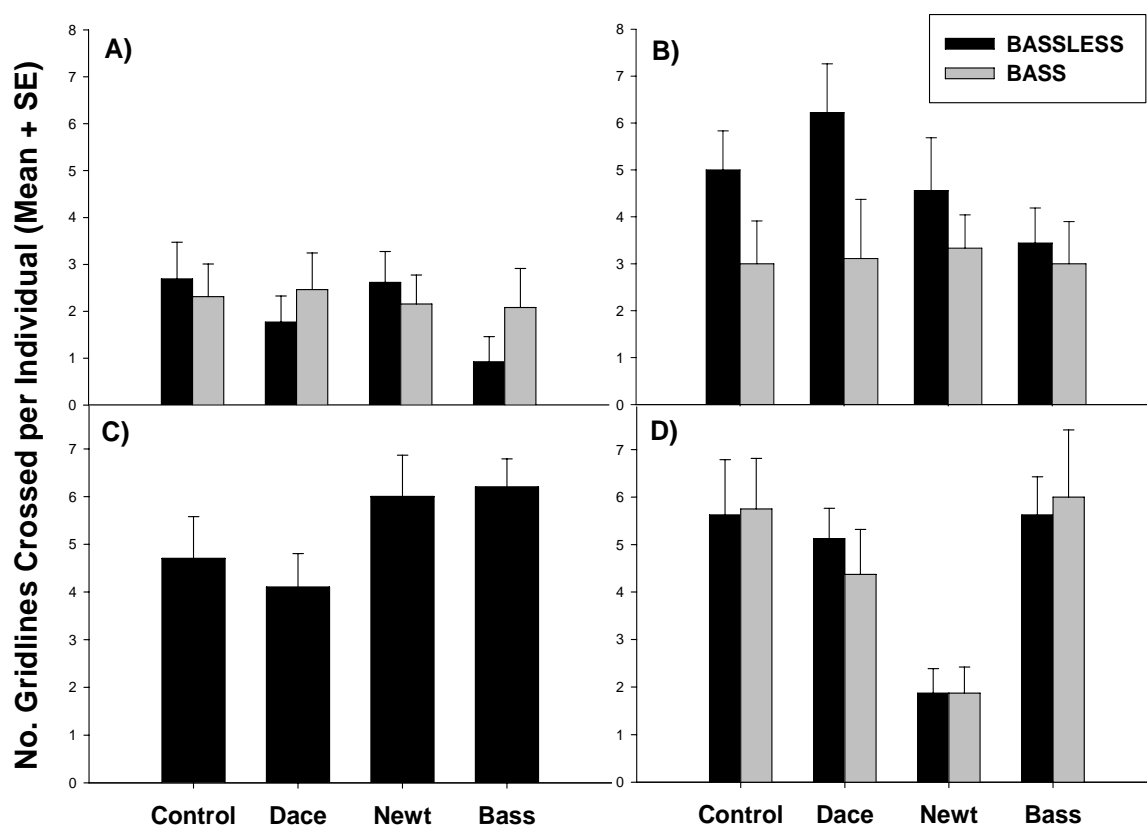
In Experiment Ia, in which we examined the overall responses of early-stage larvae to chemical cues, we found a near-significant interaction between population and treatment (Poisson distribution,  $df = 3$ ,  $p = 0.06$ ; Fig. 2.4). Follow-up one-way ANOVAs indicated some difference at the treatment level ( $p = 0.05$ ), but not between the two populations ( $p = 0.38$ ). However, *post hoc* Welch t-tests found no significant differences between pairs of treatments ( $p > 0.17$  for all). In Experiment Ib, in which we examined late-stage larvae, we found no differences in overall response among the four chemical cues ( $df = 3$ ,  $p = 0.47$ ). However, when all treatment data were combined, late-stage larvae from the BASSLESS population moved more often (Welch t-test,  $p = 0.01$ ), and travelled greater distances (Welch t-test,  $p = 0.01$ ), than individuals from the BASS population (Fig. 2.5). In Experiment II, in which we examined tadpole response to visual cues, we found no

significant differences in larval activity levels to any of the four treatments ( $df = 3$ ,  $p = 0.14$ ; Fig. 2.4). Compared to the Control treatment, larvae did not exhibit an overall avoidance response (i.e., preference in Section) when visually exposed to the other three treatments ( $\chi^2_{0.05,2}$ ,  $p > 0.25$  for all). Lastly, in Experiment III, in which we examined a combination of stimuli, we found that when *R. boyllii* larvae were exposed to Newts, a native predator, larvae from both BASS and BASSLESS populations responded strongly by decreasing both the frequency of movements ( $F_{3,60} = 6.02$ ,  $p < 0.01$ ) and the average distances travelled ( $F_{3,60} = 7.77$ ,  $p < 0.01$ ; Fig. 2.4). Larvae exposed to Control, Dace, and Bass cues responded similarly to one another, exhibiting higher activity levels relative to larvae exposed to Newts. Throughout all trials, only two individual larvae were observed utilizing refuge over the course of experiments; therefore data on refuge use were disregarded.

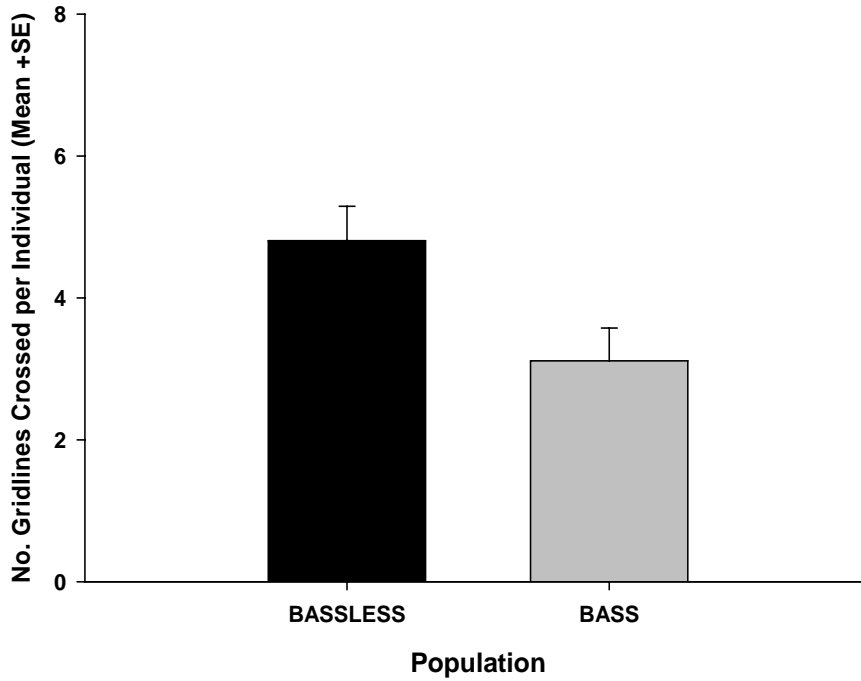
**Figure 2.4:** Initial response in average number of gridlines crossed by late-stage (Stage 33-40; Gosner 1960) *Rana boylei* larvae from Oregon, USA, to visual cues of potential native predators (rough-skinned newts, *Taricha granulosa*), non-native predators (smallmouth bass, *Micropterus dolomieu*), and non-predatory native fish (speckled dace, *Rhinichthys osculus*).



**Figure 2.5:** Average number of gridlines crossed per treatment for each experiment testing the overall response of *Rana boylei* larvae from Oregon, USA, to potential native predators (rough-skinned newts, *Taricha granulosa*), non-native predators (smallmouth bass, *Micropterus dolomieu*), and non-predatory native fish (speckled dace, *Rhinichthys osculus*). Larvae from areas with bass (BASS) and without bass (BASSLESS) were tested. (A) Experiment Ia: Detection via Chemical Cues by Early-stage larvae. (B) Experiment Ib: Detection via Chemical Cues by Late-stage larvae. (C) Experiment II: Detection via Visual Cues. (D) Experiment III: Detection via Chemical/Visual/Mechanical Cues. When exposed to all sensory cues, larvae reduced activity levels when exposed to newts.



**Figure 2.6:** Average number of gridlines crossed by late-stage *Rana boylei* larvae from populations with bass (BASS) and without bass (BASSLESS) in their overall response to chemical cue treatments in Experiment 1b (Welch modified two-sample t-test).



## DISCUSSION

We found no behavioral differences among treatments when *R. boylei* larvae were exposed to chemical cues only (Experiments Ia and Ib). The near-significant result in overall activity level in Experiment Ia was influenced by those larvae exposed to Bass cues. However, our follow-up analyses revealed no significant relationship. Studies have shown that chemical cues alone are often sufficient for predator detection in amphibians (e.g., 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 *R. boylei* larvae 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. This may be due to the fact that eggs from the BASSLESS population were collected from a much higher elevation (707 m) compared with those from the BASS population (273 m), which likely results in a shorter larval period. Since *R. boylei* larvae 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 over the course of 10 min. The initial response was reflected as an increased activity tendency of the Newt- and

Bass-exposed larvae. This is not surprising given that bass and newts were occasionally observed behaving aggressively towards larvae (e.g., newts and bass followed larvae and lunged at them). Over the longer course of the observation period, this response dampened, suggesting that visual cues alone are 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 (Hickman et al. 2004; Kiesecker et al. 1996; Stauffer and Semlitsch 1993).

Results of Experiment III, examining combined sensory cues, indicated that multiple cues may be necessary to elicit a sustained antipredator response in *R. boylei* larvae. Larvae from both BASS and BASSLESS populations significantly reduced their overall activity levels when placed in the same tank as their native predator, the rough-skinned newt. Smallmouth bass readily detect motion to capture prey (Sweka and Hartman 2003) and we observed bass lunging at the larva during several observations. However, those larvae placed in the same tank as smallmouth bass did not show any difference in activity levels relative to controls which supports our original hypothesis 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 in the presence of bass could increase predation events on *R. boylei* larvae.

The ability to adapt behaviorally to an unfamiliar predator is contingent upon a range of factors such as time spent in sympatry, experience, amount of predation pressure exerted, and the overall behavioral plasticity of the species. Our findings contribute to the growing body of work examining the defense capabilities of native amphibians to introduced predators. Analogous studies examining other native Pacific Northwestern (USA)

amphibians suggest that amphibians differ greatly in their predator recognition capabilities (Table 2.1). For instance, 10 of 21 studies reported no response to the chemical cues of an introduced predator. Our results suggest that after ~40 years of coexistence (approximately 20 generations; Hayes et al. 2005), *R. boylei* lack the ability to recognize smallmouth bass 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 (*R. aurora*) have developed the ability to detect and avoid non-native bullfrogs (*R. catesbeiana*), a potential predator. The behavioral responses witnessed in this study emphasize the difficulty in predicting how a naïve, native species might respond to an unfamiliar predator. Our results also may indicate that in the brief 40 years the two species have coexisted, smallmouth bass have not exerted selection pressure strong enough to modify *R. boylei* behavioral patterns. Given the chance, smallmouth bass will readily consume *R. boylei* larvae and adults (*pers. observ.*). However, we do not know whether *R. boylei* 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 (Chivers and Mirza 2001; Laurila et al. 1997). Like many ranids, *R. boylei* larvae are found in loose aggregations until metamorphosis (*pers. observ.*). It is possible that an individual cannot maintain the vigilance necessary to detect predators, so rely on the alarm cues produced by one or a few group members in order 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

**Table 2.1:** Studies examining behavioral responses of U.S., Pacific Northwest, amphibians to introduced predators. Stage of the study animal used: L=Larval; N=Neotene; J=Juvenile; A=Adult. Date of Predator Introduction refers to the location at which experiment was conducted and/or location the study animals were collected; this information is supplied by the authors of these studies, and may conflict with dates from other sources (INS=Information Not Supplied). Behavioral Response exhibited by study animal: R=Increased refuge use; LA=Lowered activity levels; A=Avoidance response (spatial or temporal); AO=Avoided ovipositing. Cue to which they were exposed: C=Chemical only; V=Visual only. *Syntopic* refers to animals that occur within the same microhabitat, whereas those that are *allotopic* do not co-occur with one another.

Native Species	Stage	Introduced Predator	Date of Predator Introduction	Behavioral Response/ Cue	Source
Red-legged frog ( <i>Rana aurora</i> )	L	Bullfrog (larvae) ( <i>R. catesbeiana</i> ) - syntopic	Early 1930s	R, LA (C)	Kiesecker & Blaustein (1997)
	L	Bullfrog (adult) ( <i>R. catesbeiana</i> ) - syntopic	Early 1930s	R, LA (C)	Kiesecker & Blaustein (1997)
	L	Bullfrog (larvae) ( <i>R. catesbeiana</i> ) - allotopic	Early 1930s	None (C)	Kiesecker & Blaustein (1997)
	L	Bullfrog (adult) ( <i>R. catesbeiana</i> ) - allotopic	Early 1930s	None (C)	Kiesecker & Blaustein (1997)
	L	Red Swamp Crayfish ( <i>Procambarus clarkii</i> )	INS	R (C)	Pearl et al. (2003)
	L	Bluegill Sunfish ( <i>Lepomis macrochirus</i> )	INS	R (C)	Pearl et al. (2003)
Columbia Spotted Frog ( <i>Rana luteiventris</i> )	A	Bullfrog (adult) ( <i>R. catesbeiana</i> )	1950s	A (C)	Murray et al. (2004)
	J	Bullfrog (adult) ( <i>R. catesbeiana</i> )	1950s	None (C)	Murray et al. (2004)
Mountain Yellow-legged Frog ( <i>Rana muscosa</i> )	A	Rainbow Trout ( <i>Oncorhynchus mykiss</i> ); Brook Trout ( <i>Salvelinus fontinalus</i> )	Late 1800s	AO	Vredenburg (2004)

Bullfrog* ( <i>Rana catesbeiana</i> )	L	Redside Shiner ( <i>Richardsonius balteatus</i> ); Northern Squawfish ( <i>Ptychocheilus oregonensis</i> )	INS	R (C); R (C)	Pearl et al. (2003)
Western Toad ( <i>Bufo boreas</i> )	L	Rainbow Trout ( <i>Oncorhynchus mykiss</i> )	INS	None (C, V)	Kiesecker et al. (1996)
Pacific Treefrog ( <i>Pseudacris regilla</i> )	L	Brook Trout ( <i>Salvelinus fontinalus</i> )	Late 1800s	LA (C)	Paoletti ( <i>Unpub. data</i> )
	L	Bluegill Sunfish ( <i>Lepomis macrochirus</i> )	INS	None (C)	Pearl et al. (2003)
	L	Red Swamp Crayfish ( <i>Procambarus clarkii</i> )	INS	None (C)	Pearl et al. (2003)
	J	Bullfrog (adult) ( <i>R. catesbeiana</i> )	Early 1930s	A (C)	Chivers et al. (2001)
	J	Bullfrog (adult) ( <i>R. catesbeiana</i> )	1950s	None (C)	Murray et al. (2004)
Northwestern Salamander ( <i>Ambystoma gracile</i> )	A	Bullfrog (adult) ( <i>R. catesbeiana</i> )	1950s	None (C)	Murray et al. (2004)
	L/N	Brook Trout ( <i>Salvelinus fontinalus</i> )	1940s	R, A (C)	Hoffman et al. (2004)
	A	Bullfrog (adult) ( <i>R. catesbeiana</i> )	1950's	None (C)	Murray et al. (2004)
Long-toed Salamander ( <i>Ambystoma macrodactylum</i> )	J	Bullfrog (adult) ( <i>R. catesbeiana</i> )	1950s	None (C)	Murray et al. (2004)

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\*In this study, *R. catesbeiana* was a non-native species responding to an unfamiliar, but native predator.

by predators. Therefore, the strength of the responses elicited by stimulus animals in the experiments reported herein should be considered highly conservative.

Because amphibian larvae are tied to water, the probability of tadpole-fish encounters are likely increased. When larvae are unable to avoid predation by an

unfamiliar fish, it may result in negative population-level consequences. Developing the ability to predict these responses prior to stocking a non-native species is therefore essential in order to mitigate negative impacts on the native community, particularly for sensitive species with known declines such as *Rana boylei*.

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CHAPTER 3  
FOOTHILL YELLOW-LEGGED FROG (*RANA BOYLI*) TADPOLE  
RESPONSE TO PREDATORS IN LABORATORY TRIALS:  
SUMMARY AND IMPLICATIONS

The ways in which a novel species may impact the native community are often difficult to predict. As the rate of species introductions increases, the urgency to predict the consequences also increases. Over the last 50 years, the number of introduced species in the United States has more than tripled, with a majority of these organisms being fishes (USGS 2009). Of particular concern are the impacts on native communities when these non-native fishes act as predators. When a novel predator is introduced into a system, native species may not recognize it as a threat.

The goal of this study was to determine whether foothill yellow-legged frog, *Rana boylei*, larvae would respond behaviorally when exposed to an unfamiliar, non-native predator - smallmouth bass (*Micropterus dolomieu*). Given their relatively short period (~40 years) of co-existence at our study population in Cow Creek, Oregon, we hypothesized that *R. boylei* larvae would not respond to *M. dolomieu*, but would exhibit antipredator behavior when exposed to a familiar native predator. To test our hypothesis, we developed a series of four experiments monitoring tadpole response to the stimuli from four different treatments – Control (no animal); Positive Control (speckled dace [*Rhinichthys osculus*]); Native Predator (rough-skinned newt [*Taricha granulosa*]); Non-native Predator (smallmouth bass [*M. dolomieu*]).

In Experiments Ia and Ib, we examined early-stage and late-stage (respectively) tadpole responses when exposed to chemical cues of stimulus animals. We found no

behavioral differences between treatments in either experiment when *R. boylei* larvae were exposed to chemical cues only. Many studies have shown that chemical cues alone are often sufficient for predator detection in amphibians (e.g., Petranka et al. 1987; Kiesecker et al. 1996). But a majority of these studies focused on amphibian larvae from lentic environments (but see Sih and Kats 1994; Jowers et al. 2006). It is likely that chemical cues disperse very differently in a lotic environment. Dahl et al. (1998) examined whether an amphipod (*Gammarus pulex*) could detect the chemical cues of a downstream fish predator (*Salmo trutta*). Their results showed that detection of a downstream predator was possible via small backflows created by substrate heterogeneity. However, in their study, predator detection occurred within millimeters of the source, which in our study context is likely insufficient for a tadpole to avoid predation by smallmouth bass. The stream environment in which *R. boylei* larvae live may limit their reliance on chemical cues alone to detect potential threats.

Although we found no difference in tadpole activity levels in experiments Ia and Ib, we found that late-stage larvae (Stage 31-37) from the BASSLESS population exhibited significantly higher activity levels overall relative to late-stage larvae (Stage 30-36) from the BASS population. This may be due to the fact that eggs from the BASSLESS population were collected from a much higher elevation (707 m) compared with those from the BASS population (273 m), which would therefore result in a shorter larval period. Since *R. boylei* larvae 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 possibly foraging behaviors that were previously unknown for the species.

Experiment II examined tadpole responses when exposed to visual cues of stimulus animals. Again, we found no differences between treatments in terms of activity levels or avoidance behavior. These results agree with those of similar studies examining the role of visual cues for predator detection by amphibian larvae (Hickman et al. 2004; Kiesecker et al. 1996; Stauffer and Semlitsch 1993).

Experiment III examined tadpole responses when exposed to a combination of cues of stimulus animals - chemical, visual and mechanical. We found that larvae from both BASS and BASSLESS populations significantly reduced their activity levels when exposed to their native predator, the rough-skinned newt. Larvae exposed to smallmouth bass did not show any difference in activity levels relative to controls which supported our hypothesis that larvae exposed to the cues of a non-native, unfamiliar predator would display activity levels similar to larvae exposed to control cues. Stauffer and Semlitsch (1993) explored the antipredator responses of larvae to chemical, visual, and tactile cues of a fish predator (independently and in combinations). They found that tadpole response was strongest when exposed to a combination of chemical and tactile cues. It may also be that the response seen here is due to a perceived higher degree of predation threat (i.e., the threat-sensitive predator avoidance hypothesis; Helfman 1989). Several studies show that amphibian larvae exhibit this tendency depending on the level of perceived threat (Ferrari and Chivers 2009; Puttlitz et al. 2001). We observed a significant antipredator response to rough-skinned newts only when *R. boylei* larvae were exposed to multiple

cues concurrently. Our results indicate that reliance on only one cue may be insufficient for predator detection in *R. boylei* larvae.

The ability to adapt behaviorally to an unfamiliar predator is contingent upon several factors such as time spent in sympatry, experience, amount of predation pressure exerted, and the overall plasticity of the species. Our findings contribute to the growing body of work examining the defense capabilities of native amphibians to introduced predators. Analogous studies have shown that amphibians differ greatly in their predator recognition capabilities (Appendix A). The behavioral responses witnessed in this study emphasize the difficulty in predicting how a naïve, native species might respond to an unfamiliar predator. For example, our results suggest that after ~40 years of coexistence (approximately 20 generations; Hayes et al. 2005), *R. boylei* lack the ability to recognize smallmouth bass as a threat. Contrast this with an analogous study by Kiesecker and Blaustein (1997), who found that in approximately 30 generations (McAllister and Leonard 2005), larvae of the red-legged frog (*R. aurora*) have developed the ability to detect and avoid non-native bullfrogs (*R. catesbeiana*), a potential predator.

Nevertheless, our results may indicate that in the brief 40 years the two species have coexisted, smallmouth bass have not exerted selection pressure strong enough to modify *R. boylei* behavioral patterns. This is interesting given that juvenile bass seem to prefer the same microhabitats as *R. boylei* larvae. Dauwalter (2007) determined relationships between smallmouth bass densities and stream habitat in eastern Oklahoma. He found that bass were often absent in stream units with fine sediment substrates, with the highest bass densities occurring in areas composed of gravel and bedrock. Surveys by Rombough (2006) found that juvenile bass (<50 mm) were often found at much

shallower depths (5-30 mm) around the edges of pools and cobble bars. These are the same habitats favored by larvae of *R. boylei* (pers. observ.). Not only do they share the same habitat preferences, but the incidence of fish-tadpole encounters may be increased by the feeding habits of smallmouth bass. Beamesderfer and Ward (1994) noted that in northern Oregon, bass preyed primarily on benthic organisms and made little use of pelagic items. Given the chance, smallmouth bass will eat *R. boylei* larvae and adults (pers. observ.). However, we do not know whether *R. boylei* is the preferred prey in Cow Creek, or simply an opportunistic food source.

This study focused on the response of an individual tadpole to the cues of a treatment animal, without the aid of group effects or supplemental dietary cues produced by predators. 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 (Chivers and Mirza 2001; Laurila et al. 1997). Like many ranids, *R. boylei* larvae are found in loose aggregations until metamorphosis (pers. observ.). It is possible that an individual cannot maintain the vigilance necessary to detect predators, and instead relies on the alarm cues produced by one or a few group members in order to detect a predatory threat.

Our results indicate that *R. boylei* populations in Cow Creek may be at risk due to the inability of larvae to recognize smallmouth bass as a potential threat. However, without more empirical evidence we cannot make any further inferences regarding *R. boylei* declines in and around Cow Creek. Studies from other systems may provide alternate explanations behind *R. boylei* declines. Nevertheless, there exists a large body of literature documenting the negative relationship between the presence of non-native

predatory fish and amphibian distributions. For example, Matthews et al. (2001) surveyed >1700 water bodies in the Sierra Nevada and found that the odds of finding Pacific treefrogs (*Pseudacris regilla*) in waters without introduced trout was 2.4 times greater than in those containing fish. Tyler et al. (1998) found a similar negative relationship between introduced trout and long-toed salamander (*Ambystoma macrodactylum*) presence in montane lakes. Vredenburg (2004) found that experimental removal of introduced predatory fish resulted in native mountain yellow-legged frog (*Rana muscosa*) abundances that were similar to those in naturally fish-free lakes. Therefore, it is possible that *R. boylei* populations in Cow Creek are not necessarily declining, but adults may be avoiding stream reaches containing predatory fish. This could be verified by conducting surveys during breeding season along Cow Creek. This information used in conjunction with previous survey efforts (e.g., Borisenko and Hayes 1999; Rombough 2006) may provide a clearer picture of the status of *R. boylei* populations in this watershed.

Finally, we must not overlook the impacts of anthropogenic disturbances on *R. boylei* populations, most notably dam construction. Water flow through Cow Creek is regulated by the Galesville Dam. Built in 1986, it is largest dam in the Umpqua Basin (Geyer 2003). Its main functions are to provide recreational opportunities, flood control, and to generate hydroelectric power (Geyer 2003). However, since the dam was constructed, water temperatures are cooler overall and flow regimes have been altered from their natural conditions. The intense winter floods are now reduced, and summer flows are now increased thereby eliminating the natural peaks and valleys in flow rate prior to dam construction (Kincaid 2002). Increased summer flows may scour egg masses from attachment sites. The elimination of scouring winter flows may allow streamside

vegetation to establish. This could result in a reduction of basking areas for adult *R. boylei*; over-hanging vegetation may decrease sun exposure for larvae; and a decreased bank width may allow for easier capture of *R. boylei* adults by hidden predators. Further study comparing *R. boylei* populations under natural and altered flow regimes would help describe the impact of dams on this species.

The continued introduction of non-native organisms is likely to result in a myriad of complex, unpredictable consequences for native species. Of particular interest are the effects of introduced predatory fishes on native amphibians. Because many amphibian larvae are tied to water, the probability they encounter fish is likely increased. When amphibian larvae are unable to respond appropriately to avoid predation by unfamiliar fish, the resulting cumulative mortality may result in negative population-level consequences. One study by Simon (2008) in Cow Creek may reveal similar consequences with native fish as prey of smallmouth bass. He conducted distributional surveys of smallmouth bass and native Umpqua chub (*Oregonichthys kalawatseti*) in Cow Creek which revealed very distinct negative relationships. These results, compared with similar survey efforts in 1987 and 1998, showed increasingly restricted chub distributions, with virtually no overlap between the two species. This is one example of the rapid impact bass can have on prey populations, reducing them before they develop the ability to adjust behaviorally to a novel predator. Developing the ability to predict these responses prior to stocking a non-native species, and considering those responses in species management decisions, would help to mitigate negative impacts on the native community, particularly for Sensitive species such as *Rana boylei*. Although removal of smallmouth bass from the Umpqua system is probably no longer feasible, our results

should prove useful in guiding educators and stocking programs regarding the effects of non-native fish introductions.

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