PALATABILITY AND ANTIPREDATOR RESPONSE OF YOSEMITE TOAD (*BUFO CANORUS*) TO NONNATIVE BROOK TROUT (*SALVELINUS FONTINALIS*) IN THE SIERRA NEVADA MOUNTAINS OF CALIFORNIA

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A Thesis

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Department of Biological Sciences

Abstract

of

PALATABILITY AND ANTIPREDATOR RESPONSE OF YOSEMITE TOAD (*BUFO CANORUS*) TO NONNATIVE BROOK TROUT (*SALVELINUS FONTINALIS*) IN THE SIERRA NEVADA MOUNTAINS OF CALIFORNIA

Statement of the Problem - The global phenomenon of unexplained amphibian declines has been met with an increase of investigations into the possible anthropogenic as well as natural causes. Alien or introduced predators such as fish will often compete, displace or prey upon native amphibians. Amphibians that have evolved in fishless habitats often lack the necessary chemical defenses and behavioral responses to avoid predation and are thus unable to resist or co-occur with introduced predators due to the lack of a shared history. Toads, however possess noxious chemicals that may be adequate to deter non-native predators which may allow co-occurrence with alien predators, even when the same introduced predator has already been implicated in the decline of other amphibian species. Yosemite toads have experienced population declines throughout their range in which trout have been widely introduced, but it is not clear whether trout are responsible for the decline through direct predation of larval life stages.

Sources of the Data - Research was conducted June, 2004 and August, 2005 at the University of California Santa Barbara's Sierra Aquatic Research Laboratory (SNARL), part of the Valentine Eastern Reserve System. Brook trout were collected via hook-and-line from two alpine lakes in Inyo National Forest. Yosemite toad eggs (2004) and recently metamorphosed toads (2005) were collected by hand from Edith Lake area and Glacier Bench (Mono Co., CA). Through a series of no-choice palatability trials, antipredator response experiments and choice experiments I determined the level of threat brook trout pose to larval Yosemite toad life stages.

Conclusions -Two major conclusions can be drawn from my research. Firstly, brook trout found all early life stages of Yosemite toads to be unpalatable and unlikely rely on these stages as a primary food source in aquatic montane environments. Secondly, even though Yosemite toads were sampled by trout the toads did not suffer any ill effects.

_____, Committee Chair

Dr. Ron Coleman

Dedication

This research is dedicated to the memory of my brother..friend

Thomas Vincent Grasso

1979 – 2005

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Global Amphibian Declines – Worldwide, amphibians are declining faster than any other group of vertebrates including mammals and birds and it is perceived to be largely attributable to anthropogenic causes such as habitat loss and over utilization. Of all the amphibians, the family Bufonidae (True Toads) faces the highest risk of extinction (Stuart et al. 2004). In light of the possible causes for decline exists the global phenomenon of unexplained amphibian declines in remote locations thought to be protected from human disturbance. These areas include the montane regions of Australia, Central and South America and the United States. The Yosemite toad (*Bufo canorus*) in California is one such case were a toad species is declining in protected areas of wilderness and national parks but the basis remains enigmatic. Studies in these locations are currently underway investigating a variety of potential causes including, infectious diseases such as chytridiomycosis caused by the fungal infection of *Batrachochytrium dendrobatidis* (Daszak et al. 2003), agrochemicals (Davidson 2004), alien predators (Kats and Ferrer 2003) and the synergistic effects of all possible causes for decline (Collins and Storfer 2003; Storfer 2003).

California Amphibian Declines – Amphibian declines in California are well documented and perceived causes include habitat alteration and loss, alien species, commercial exploitation and toxicants, (Hayes and Jennings 1986; Drost and Fellers 1996). Nonnative species, especially introduced fish, have been shown to have a strong negative effect on native amphibian populations in California (Bradford 1989; Fellers and Drost 1993; Lawler et al. 1999; Knapp and Matthews 2000; Matthews et al. 2001; Knapp

2005). Alien or introduced fish will often compete, displace or prey upon native amphibians. Petranka et al. (1987) identified fish predation as an important factor in the structuring of anuran larval communities and argued that fish could be responsible for complete extermination of tadpoles. Amphibians that have evolved in fishless habitats often lack the necessary chemical defenses and behavioral responses to avoid predation and are thus unable to resist or co-occur with introduced predators due to the lack of a shared history. Removal of fish predators in amphibian environments is promising for restoring populations (Knapp et al. 2001; Vredenburg 2004), but can potentially be costly and labor intensive. Toads which possess noxious chemicals may be able to deter non-native predators such as fish, allowing for some level of co-occurrence with alien predators, even when the same introduced predator has already been implicated in the decline of other amphibian species. Thus the need to immediately remove fish from areas occupied by native toads may not be warranted and resources should be focused on other possible causes for decline before expensive fish eradication methods are deployed.

History of Trout Stocking in California – The Sierra Nevada Mountains of California were historically fishless above 1 400m (Bradford 1989, Knapp 1996). This is attributable to the last glacial episode during the Pleistocene in which ice fields covered and scoured the range (Storer and Usinger 1969). As they receded, they left impermeable barriers (e.g., waterfalls) to fish migration upstream (Drost and Fellers 1996). Trout stocking in the Sierra Nevada began in the mid to late 1800's (Moyle 2002) and has since had negative effects on other native species of amphibians, most notably the mountain yellow - legged frog (*Rana muscosa*) (Grinnell and Storer 1925; Bradford 1989; Knapp

and Matthews 2000; Vredenburg 2004), which was once the most common vertebrate in the Sierra Nevada (Grinnell and Storer 1925). The introduction of nonnative brook trout (*Salvelinus fontinalis*) and the decline of other native Sierra Nevada amphibians prompted the need to experimentally test whether brook trout are a possible contributor to Yosemite toad decline through direct predation of larval life-stages.

Statement of Research Problem being Investigated – The Yosemite toad occurs mainly in the High Sierra of California (2 000 – 3 500 meters) and is believed to have experienced a 50 percent decline from historic locations range-wide (Jennings and Hayes 1994). The amount of scientific research addressing the decline of Yosemite toad populations is alarmingly low for a Federal candidate endangered species and State species of special concern. In December, 2002 the U.S. Fish and Wildlife Service (USFWS) published in the Federal Register a 12-month finding for a petition to list the Yosemite toad as an endangered species and found it to be "warranted but precluded by higher priority listing actions" (Federal Register 2002). Potential causes contributing to Yosemite toad decline identified by the USFWS included trout stocking, livestock grazing, roads and timber harvest, disease, chemical contaminants, pesticide drift, recreation, vegetation and fire management activities, dams and water diversion, and climate change. Chemical contaminants (e.g., pesticides) have been investigated with some evidence suggesting an association influencing Yosemite toad decline but it was not statistically significant (Davidson 2004). UV-B radiation has been implicated in the decline of the western toad (*Bufo boreas*) (Kiesecker and Blaustein 1995); however, Sadinski (2004) did not find any evidence of Yosemite toads succumbing to increased

UV-B during experimental exposure and Yosemite toads likely normally experience high UV-B levels at high altitude. Disease and effects of livestock grazing studies are currently underway by the University of California, Berkeley, University of California, Davis and the U.S. Forest Service. My objective was to evaluate the extent to which nonnative trout introductions may be contributing to Yosemite toad decline and also to contribute to the limited baseline of information currently available for the toad. Thus, my master's research has focused on the potential of trout stocking to be a factor contributing to the decline of the Yosemite toad and it was my goal to (1) test whether Yosemite toad eggs, tadpoles, and newly metamorphosed toads are a palatable food source for brook trout, and (2) assess the antipredator behavior of Yosemite toad tadpoles by testing their ability to chemically detect introduced brook trout. In addition to the main focus of my research, I also explored Yosemite toad tadpole's antipredator response to a native invertebrate predator, adult predaceous diving beetles (Order: Coleòptera: Suborder: Adéphaga: Family: Dytíscidae) (Borror et al. 1989), a vertebrate predator, western terrestrial garter snakes (*Thamnophis elegans elegans*), and response to injured conspecific tadpoles as well as non-conspecific (Pacific treefrog, *Hyla regilla*) tadpoles. Furthermore, as a control I tested the antipredator response of Pacific treefrog, a known palatable species, to brook trout chemical cues. I concluded my research by performing choice experiments testing the ability of brook trout to differentiate and select between palatable (treefrog) and non-palatable (toad) prey items. The information gained from my research is likely to be used in management decisions to consider future listing of the Yosemite toad as an endangered species.

Natural History of the Yosemite Toad – Etymology of *Bufo canorus*: *Bufo* comes from Bufonid for "without teeth" and *canorus* is Latin for “tuneful or melodious” referring to trilling by adult male toads during breeding. Originally described by Camp (1916), the Yosemite toad is morphologically similar to the western toad (*Bufo boreas*) but is slightly smaller, has proportionally larger parotoid glands, and adult toads exhibit sexual dichromism (Karlstrom 1962) (Figure 1). The Yosemite toad occurs mainly in the boreal zone of the Central Sierra (Karlstrom 1962), specifically, the Lower Canadian to the Upper Hudsonian Life - Zone (Grinnel and Sorer 1925) and occasionally the Arctic – Alpine Life Zone (Stebbins 1951). The Yosemite toad ranges in elevation from around 6 400 - 11 300 feet (2 000 – 3 500 m) (Kagarise Sherman and Morton 1984) and ranges within California, north from South Lake Tahoe (Ebbett’s Pass) south to Kings Canyon National Park (Evolution Valley) with the majority of populations occurring between 8 500 - 10 000 feet (Karlstrom 1962) (Figure 2). Adult toads are active soon after snow melt where males will congregate at breeding pools, streams or lake margins within meadows and begin trilling day and night to attract females. Male toads will remain in these meadow breeding areas and continue to call for females for up to four weeks. Female toads enter breeding areas only briefly to mate, depositing 1 500 – 2 000 eggs (Karlstrom, 1962). The presence of uneaten female skin, parotoid glands and undeposited eggs at breeding areas during my collections suggests that these entities are unpalatable to predators. While traveling to field survey sites I found that adult female Yosemite toads suffer from higher mortality than males in some breeding areas likely as a result of avian predators such as Clark’s nutcrackers (*Nucifraga columbiana*), common

ravens (*Corvus corax*) and California gulls (*Larus califonicus*) as witnessed by Kagarise Sherman and Morton (1993). This is possibly due to exposure on substrates that make adult female toads more conspicuous in unvegetated breeding environments than males who tend to be more cryptic. The preferred shallow breeding habitats also make eggs and tadpoles vulnerable to predators. Eggs hatch into developing free-swimming embryos in about one week. Although little has been documented on egg predation, secondary infection of freezing resulting from eggs being laid to close to the surface has been observed (Kagarise Sherman and Morton 1993). Due to the short duration of Sierra summers, developing toad tadpoles only have a limited opportunity to successfully reach metamorphosis. I believe desiccation and predation are likely the most significant factors governing Yosemite toad population success at high elevations. Winter snowpack, which provides run-off for breeding habitats in meadows, can vary greatly year to year, and in drought years I have witnessed tadpole desiccation likely as a result of below-average snowpacks. Thus the importance of toad populations that rely on and breed in more permanent bodies of water, such as lakes with trout, is apparent. Drying and shallower habitat may also increase the rate of predation by native predators such as the western terrestrial garter snakes, a known predator of Yosemite toads as well as mountain yellow – legged frogs (*Rana muscosa*) (Mullally 1953). In addition, post metamorphic toads also likely suffer from high predation rates. I have personally witnessed recently metamorphosed toads being preyed heavily upon by Western terrestrial garter snakes (*Thamnophis elegans elegans*) and an undergraduate student also observed Brewer's blackbirds (*Euphagus cyanocephalus*) feeding heavily on this life-stage (Mike Cane,

personal communication). Young-of-the-year toads will forage into the fall and like the adults will over-winter in rodent burrows in and around meadows. Yosemite toads also have a number of invertebrate predators throughout their life history. I have observed tadpoles and sub-adult toads being preyed upon by predaceous diving beetle larvae and adults (Family: Dytiscidae) as well as Dragonfly larvae (Family: Odonata). For a complete review of Yosemite toad natural history review Grinnel and Storer 1925; Mullally 1953; Karlstrom 1962; Kagarise Sherman and Morton 1984.

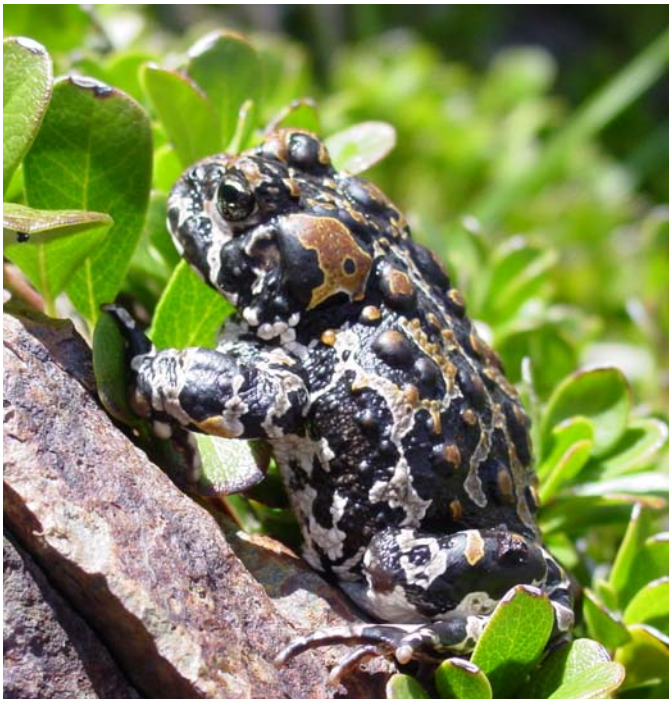

Figure 1. Adult Yosemite toads in amplexus (above); adult female Yosemite toad (below). Photos by Rob Grasso.

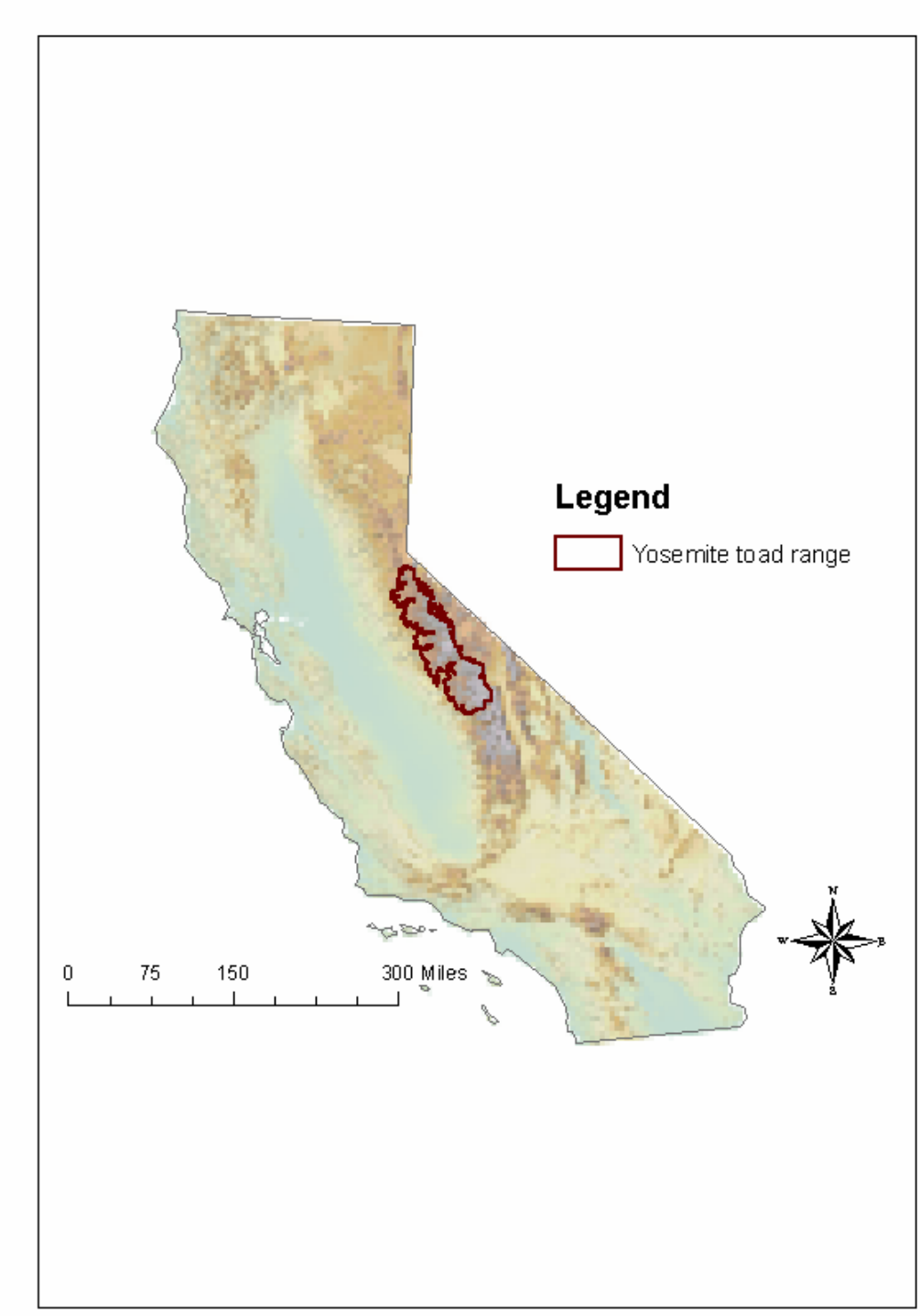

Figure 2. Range of the Yosemite toad. Map created in ArcGIS 9.0 by Rob Grasso.

Natural History of Brook Trout in California – Etymology of *Salvelinus fontinalis*: *Salvelinus* means “little salmon” and *fontinalis* is Latin for “breeds in springs.” Brook trout are actually a true char, not a trout, native to the Eastern United States and Canada (Moyle 2002) (Figure 3.). Brook trout have been widely introduced into the High Sierra (> 2 500m) using pack stock and aerial stocking techniques into permanent water bodies necessary for over-wintering and are the most persistent trout in lakes between 2 500-3 500m (Knapp 1996). The ability of brook trout to spawn in lakes lacking stream inlets made them an attractive species to plant in California (Dill and Cordone 1997). It is estimated that prior to the 1980’s, [1, 700, 000] brook trout were stocked annually statewide. This number decreased to about [850, 000] annually in the 1980s and 1990s (Dill and Cordone 1997). Brook trout are also favored because they can withstand warm water temperatures up to 26 °C and are also extremely cold tolerant, suitable to high elevation lakes (Moyle 2002). Spawning for brook trout occurs in the fall starting in mid – September through January (Moyle 2002). Brook trout rarely exceed 30 cm TL (340 g) in California (Moyle 2002). In California, brook trout inhabit over 1 000 lakes and 2 200 km of streams (Moyle 2002). According to Moyle (2002), trout in the Sierra Nevada are the most significant factor responsible for native amphibian declines. For further review of brook trout introductions and life history in California see (Dill and Cordone1997; Moyle 2002).

Figure 3. Brook trout illustrating convoluted back pattern and red spots surrounded by blue halos. Photo by Rob Grasso

Field Observations between Yosemite Toads and Brook Trout – There is limited information about the interactions between Yosemite toad larvae and brook trout. Drost and Fellers (1994) suspect Yosemite toads might be less susceptible to predation from trout for the reason that Yosemite toad larval stages are usually found in ephemeral water bodies that lack trout. However, there are many observations of brook trout and Yosemite toad larvae presence in the same water body, both temporary and permanent, but observational data conducted by field researchers in these environments can sometimes be confounded. Brook trout have been observed to pick at Yosemite toad egg masses and tadpoles (Dave Martin, personal communication 2003) while in other areas brook trout have been observed actively avoiding Yosemite toad tadpoles in (Roland Knapp, personal communication 2003). I have personally observed brook trout in ephemeral water bodies containing Yosemite toad eggs and tadpoles likely stranded from migrating during early season snow melt and run-off. Stomach content analyses from trout I captured in these ephemeral water bodies did not produce any larval Yosemite toads while other prey items (e.g., invertebrates) were present. Kats et al. (1988) found that amphibians that breed in permanent habitats occupied by fish often have unpalatable larvae that strongly respond to fish chemical cues while those that breed in fishless or ephemeral habitats possessed larvae that were more palatable and did not respond to fish chemical cues. Thus, assessing the effects of brook trout predation on Yosemite toad larval stages, in both permanent water bodies containing trout and ephemeral water bodies absent of trout are necessary to our understanding of how introductions may have contributed to the extirpation of the Yosemite toad.

Possible Relationships between Yosemite Toads and Brook Trout – To better understand the association between Yosemite toads and brook trout, I designed my research around three scenarios that may be describing this relationship. Scenario (1): brook trout actively sample aquatic larval stages of Yosemite toad (e.g., egg masses, tadpoles and recently metamorphosed toads) and either consume or cause mortality of these stages as a result of sampling. Scenario (2): brook trout do not prey upon larval stages of Yosemite toads either because alternative food sources are available or these toad life stages are simply unpalatable to brook trout. Tadpoles are presumably the most vulnerable stage since this is the longest stage and exposure to fish is prolonged, Scenario (3): Yosemite toad tadpoles have the ability to detect brook trout as predators and respond through antipredator behavior reducing the risk of predation.

Scenario 1. Trout Predation – Brook trout are known to selectively feed on the most abundant prey organism (Moyle 2002). According to Karlstrom (1962), a single adult Yosemite toad female may lay up to 2 000 eggs in one breeding event. Given that there will likely be multiple breeding pairs in a single water body, Yosemite toad offspring can be viewed as candidate prey items for brook trout. Brook trout also spawn in the fall when feeding slows or stops (Moyle 2002) resulting in increased predation risk on Yosemite toad egg masses and tadpoles that are laid in late-spring when trout are actively feeding (Moyle 2002). There is evidence that fish do recognize and learn to avoid unpalatable toad tadpoles (Kruse and Stone 1984; Lawler and Hero 1997) but this has not been tested in trout using Yosemite toads. Furthermore, negative effects of naïve trout sampling larval Yosemite toad life-stages have also not been investigated. Licht

(1969) reported that sticklebacks (*Gasterosteus aculeatus*) and cutthroat trout (*Salmo clarkii*) "mouthed" western toad eggs but rejected them instantaneously. In a study by Kruse and Stone (1984) the authors found that largemouth bass (*Micropterus salmoides*) learned to avoid and ceased feeding on *Bufo* tadpoles but continued to engulf them, often resulting in mortality to the tadpoles. Voris and Bacon (1966) also reported that *Bufo* tadpoles were vigorously and repeatedly attacked by fish followed by forceful ejection. Lawler and Hero (1997) also reported mortality from sampling but not consumption of marine toad tadpoles to barramundi fish (*Lates calcarifer*). Thus, there is ample evidence that naïve brook trout could still negatively affect Yosemite toads through sampling and subsequent rejection of larval stages. There is evidence in other toad species (e.g., marine toad *Bufo marinus*) that palatability changes ontogenetically, with older, larger stages becoming less palatable (Lawler and Hero 1997; Crossland 1998). However, Brodie and Formanowicz (1987) found intermediate tadpole stages of American toads to be more palatable to vertebrate and invertebrate predators than early or late stage tadpoles. Furthermore, Peterson and Blaustein (1992) did not find any evidence for palatability differences in newly hatched or recently metamorphosed western toads when offered to invertebrate predators. For my palatability trials, I controlled for possible ontogenetic variation by testing eggs, tadpoles (at two stages: newly-hatched and intermediate) and recently metamorphosed toads. The objective of experiments for Scenario (1) was not only to test the palatability of Yosemite toad larval stages to brook trout, but also assess whether naïve brook trout inflict harm on these stages and can subsequently learn to avoid Yosemite toad stages that are, in fact, unpalatable. I chose to

test recently metamorphosed toads because a trade-off might exist between energy being invested toward physiological changes for the terrestrial phase compromising toxic defense mechanisms. Metamorphosing toads are often cryptic and leave breeding areas synchronously likely as an alternate defense strategy of satiating land predators (Arnold and Wassersug 1978). Stomach content analysis on golden trout (*Oncorhynchus mykiss aquabonita*) revealed that these trout consume post-metamorphic stages of western toads (Roland Knapp, personal communication, 2004). Thus, it is possible that these stages are vulnerable to predation do lack of chemical defenses and could hold true for Yosemite toads and brook trout.

Scenario 2. Trout Avoidance – Research has shown that many tadpole species of the genus *Bufo* are unpalatable to a host of fish predators (Voris and Bacon 1966; Kruse and Stone 1984; Kats et al. 1988; Kiesecker et al.1996; Lawler and Hero 1997) as well as oocytes and eggs (Licht 1968, 1969). Furthermore, the closely related western toad is reported as being unpalatable to some salmonids including rainbow trout (A.R. Blaustein in Kiesecker et al. 1996). Amphibian larvae demonstrate a variety of plasticity in traits maintained for changing environmental conditions, which have been shown to play a role in the development of antipredator responses (Lardner 2000). It is plausible that Yosemite toad tadpoles have maintained some level of plasticity in possessing traits for chemical toxins stemming from the necessity of post metamorphic stages to deter land predators. Benard and Fordyce (2003) theorize that metamorphosing western toads could benefit from increased larval investment of chemical toxins. Yosemite toad tadpoles do have native aquatic invertebrate predators (e.g., Belastomatidae: giant water bugs,

Dytiscidae: predaceous diving beetles, Notonectidae: backswimmers) all of which consume prey by piercing the skin and sucking out body fluids. Predators that ingest prey items whole or pierce the skin are not as susceptible to tadpole toxins as those that masticate, chew or sample tadpoles (Kruse and Stone 1984; Peterson and Blaustein 1992). Therefore, the likelihood that Yosemite toad tadpoles possess chemical defenses that would be ineffective against piercing predators may be reduced due to the energetic costs associated. Induced chemical defenses triggered by brook trout may be costly to Yosemite toad larvae leading to reduced growth rates that could delay metamorphosis (see Benard and Fordyce 2003). Given that Yosemite toad tadpoles co-occur with trout in certain water bodies suggests that selection or plasticity has resulted in tadpoles incurring the cost of chemical defense strategies earlier in their life history. Alternatively, there may be sufficient primary and secondary prey items (e.g., invertebrates) available to brook trout such that Yosemite toads are less profitable in terms of energy costs than other prey items and hence are not preyed upon.

Scenario 3. Tadpole Avoidance – There is extensive evidence of amphibians using chemical stimuli to detect predators, especially the antipredator response of tadpoles to chemical cues of fish (Hews and Blaustein 1985; Brodie and Formanowicz 1987; Petranka et al. 1987; Kats et al. 1988; Lawler 1989; Kiesecker et al. 1996; Lefcort 1996 and 1998; Chivers et al. 1999; Chivers et al. 2000; Laurila 2000; Nyström and Åbjörnsson 2000). Kulzer (1954) was the first to suspect that the chemical bufotoxin in toads was responsible for deterring predators as well as an alarm signal to warn conspecifics of predatory threat. The ability to detect predators and warn conspecifics

should allow Yosemite toad tadpoles to reduce predation risk by seeking refuge in substrate, vegetation or other cover. Animals considered prey are more likely to avoid predators when there is a shared evolutionary history (Vredenburg 2004). However, Chivers et al. (2001) found that juvenile Pacific treefrogs responded to chemical cues of introduced predatory bullfrogs (*Rana catesbeiana*) where there was only a recent history of co-occurrence while treefrog juveniles from areas historically absent of bullfrogs did not respond to bullfrog chemical cues. Kiesecker et al. (1996) concluded that predator recognition in western toad tadpoles was more dependent on chemical cues rather than visual cues. The detection of chemical cues over visual detection may be a prey response to ambush predators as well as response to predators that locate their prey by movement (Chivers 2001). Lawler (1989) however, demonstrated that Fowler's toad (*Bufo woodhousei*) exhibited antipredator behavior to black-banded sunfish (*Enneacanthus obsesus*) even though these fish find the toad unpalatable. Lefcort (1998) also detected that southern toads (*Bufo terrestris*) reduced activity levels in the presence of warmouth sunfish (*Lepomis gulosus*), which find the toads unpalatable. Such alterations in behavior to a non-predatory threat could lead to reduced foraging time as resulting in increased developmental rates (Lawler 1989; Lefcort 1998).

Hypotheses tested – Based on the previous scenarios describing the interactions between Yosemite toads and brook trout, I evaluated the following hypotheses. For clarity, I will refer to Yosemite toad larvae and brook trout that are known to co-occur and come into contact in the same water body as "experienced" while Yosemite toad larvae and brook trout from separate water bodies where there is no known historical contact are referred to as "naïve." Since preliminary experimentation revealed that brook trout did not find Yosemite toad larvae to be palatable, hypotheses (**H_{O5}**) and (**H_{O6}**) were added.

H_{O1}: There *is* a difference in the sampling rate (palatability) between experienced and naïve Yosemite toad eggs offered to naïve brook trout.

H_{O2}: There *is* a difference in the sampling rate (palatability) between experienced and naïve Yosemite toad tadpoles offered to naïve brook trout.

H_{O3}: There *is* a difference in the sampling rate (palatability) between experienced and naïve recently metamorphosed Yosemite toads offered to naïve brook trout.

H_{O4}: There *is* a difference in antipredator response behavior between experienced and naïve Yosemite toad tadpoles to chemical cues of brook trout.

H_{O5}: There *is* a difference in antipredator response behavior between Yosemite toad and Pacific treefrog tadpoles to chemical cues of brook trout.

H_{O6}: There *is* a difference in the sampling rate (palatability) of Yosemite toad and Pacific treefrog tadpoles to naïve brook trout.

MATERIALS AND METHODS

Brook trout and Yosemite toads used in this study were collected from water bodies in Mono and Inyo County within Inyo National Forest. Research animals were collected following take provisions listed under California Department of Fish & Game (CDFG) Permit #: SC-5130. All experiments were conducted during the summer in 2004 and 2005 in the field and at the Sierra Nevada Aquatic Research Laboratory (SNARL) near Mammoth Lakes, CA administered by the University of California, Santa Barbara.

Sample Collection – Brook Trout – A total of 100 adult naïve brook trout (< 300mm FL; < 60 g) were collected by hook-and-line from Grass Lake, Inyo County, CA in 2004 and 2005. Grass Lake is a known location of non co-occurrence with Yosemite toad. A total of 20 adult (< 300mm FL) experienced brook trout were collected from Edith Lake, Inyo County, CA in 2004 while Yosemite toad egg masses were being collected. Thus, Edith Lake is a known location of co-occurrence between brook trout and Yosemite toad.

Sample Collection – Yosemite Toad – A total of 200 naïve Yosemite toad eggs were collected by hand from a single egg mass in 2004 and 50 newly metamorphosed toads (Avg: 10.5mm SVL; 0.14g) were collected in 2005 from Glacier Bench, Mono County, CA. Glacier Bench is a known location of non co-occurrence with brook trout. A total of 200 experienced Yosemite toad eggs were collected by hand from a single egg mass from Edith Lake, Inyo County, CA. Edith Lake is a known location of co-occurrence with brook trout and, in 2005, 50 recently metamorphosed toads (Avg: 10.5mm SVL; 0.14g) were collected from Cloverleaf Lake, Inyo County, CA located 500

meters upstream of Edith Lake. All animals held at SNARL were maintained on a 14L : 10D photoperiod. Developing Yosemite toad larvae were held in plastic dissecting pans and placed in a heated (Avg: 23°C) water bath under full-spectrum lighting (Zoo Med Flora Sun®). Tadpoles were offered a combination of Tetramin Tropical Flakes®, ground rabbit chow (Kaytee Supreme Rabbit Daily Blend®) and NascoTadpole Brittle® *ad libitum*. Brook trout did not attempt to feed when offered a non-natural food item (trout pellets) acquired from a hatchery and subsequently were not fed while held at SNARL. Yosemite toads used in all experiments were staged under a dissecting microscope using the Gosner (1960) staging system. Trout were measured (Fork Length) and weighed post experiment.

Palatability Experiments – The palatability of Yosemite toad eggs (experiments 1, 2, and 3), newly hatched tadpoles (experiments 4 and 5), mid-developmental tadpoles (experiment 6) and recently metamorphosed toads (experiments 7 and 8) was evaluated by using a randomized block design adapted from Crossland (1998) (Table 1), which consisted of two treatments, namely (1) one trout per container to act as control treatment, and (2) one trout offered 10 eggs, 10 tadpoles or four metamorphosed toads to act as palatability treatment. Single eggs – not eggs connected in a string – were used when offered to trout. Containers were arranged in a 2 x 5 array for experiments 1 – 6, and a 4 x 5 array for experiments 7 and 8. In experiments 1 – 6 treatments were replicated five times while experiments 7 and 8 treatments were replicated 10 times. Plastic dividers were placed between containers so that trout could not observe each other. Prior to experiments, brook trout were starved for a minimum of 36 hr. and placed

separately into 57 L Rubbermaid Latch Toppers ® filled with 40 L of stream water from Convict Creek (average temperature 18 °C), which were then placed into a water bath in egg incubators (Figure 4). Trout in each array were randomly selected using a random number generator (http://www.random.org) to (1) receive Yosemite toads or (2) act as captive environment controls. This experimental design allowed for the separation between the possible lethal effects of trout consuming toxic tadpoles (see Licht 1968) and the potential deleterious effects that might exist from starved trout being confined to experimental containers for long periods since water was not exchanged or aerated during trials. Consumption or sampling rate for all experiments was observed and recorded every 0.5, 1, 2, 4 and 8 hr., following Lawler and Hero (1997). Additional observations at 16 and 24 hr. were recorded during experiments 4, 5 and 6. Upon completion of each experiment, control trout were offered a piece of worm or Pacific treefrog tadpole for one hour to test for appetite and willingness of trout to accept prey items in a captive setting.

Experiment 1. Naïve Brook Trout offered Experienced Yosemite Toad Eggs – The palatability of experienced Yosemite toad eggs [Edith Lake] (Stage 1 – 12) characterized by pre-neural fold formation (Gosner 1960) was assessed by performing no-choice experiments using naïve[Grass Lake] brook trout ($N = 5$) starved for 36 hr. A worm piece was offered to each control trout ($N = 5$) for 1 hr. at the end of the 8 hr. experiment.

Experiment 2. Experienced Brook Trout offered Experienced Yosemite Toad Eggs – Although this experiment was not originally planned I took the opportunity of collecting experienced trout while collecting Yosemite toad eggs at Edith Lake and tested whether or not experienced trout consume experienced Yosemite toad eggs (Stage 1 – 12;

pre-neural fold formation). Brook trout (N = 5) in this experiment were starved for 36 hr. A worm piece was offered to each control trout (N = 5) for 1 hr. at the end of the 8 hr. experiment. Eggs offered during this trial were monitored until 48 hr. post-experiment.

Experiment 3. Naïve Brook Trout offered Naïve Yosemite Toad Eggs – The palatability of naïve Yosemite toad eggs [Glacier Bench] (Stage 1 – 12; pre-neural fold formation) was assessed by performing no-choice experiments using naïve brook trout (N = 5) starved for 120 hr. A worm piece was offered to each control trout (N = 5) for 1 hr. at the end of the 8 hr. experiment.

Experiment 4. Naïve Brook Trout offered Experienced Yosemite Toad Tadpoles – The palatability of experienced newly hatched Yosemite toad tadpoles (Stage 24-25) was assessed by offering them to naïve brook trout (N = 5) starved for 48 hr. This trial ran for 24 hr. at which time control trout (N = 5) were offered both a worm piece and Pacific treefrog tadpole (Stage 25) for 1 hr.

Experiment 5. Naïve Brook Trout offered Naïve Yosemite Toad Tadpoles – The palatability of naïve newly hatched Yosemite toad tadpoles (Stage 23-25) was assessed by offering them to naïve brook trout (N = 5) starved for 312 hr. (13 days). This trial ran for 24 hr. at which time control trout (N = 5) were offered both a worm piece and Pacific treefrog tadpole (Stage 25) for 1 hr.

Experiment 6. Naïve Brook Trout offered Experienced Yosemite Toad Tadpoles – The palatability of experienced mid-developmental Yosemite toad tadpoles (Stage 29-35) was assessed by offering them to naïve brook trout (N = 5) starved for 384 hr. (16 days).

This trial ran for 24 hr. at which time five Yosemite toad tadpoles were removed from each container in which they were offered to trout and replaced with five Pacific treefrog tadpoles of similar stages. Similarly, five Yosemite toad tadpoles and five Pacific treefrog tadpoles were offered to each control trout (N = 5) and monitored for an additional 24 hr.

Experiment 7. Naïve Brook Trout offered Experienced Metamorphosed Yosemite Toads – The palatability of experienced recently metamorphosed Yosemite toads (Stage 46) characterized by complete absorption of the tail (Gosner 1960) was assessed by offering them to naïve brook trout (N = 10) starved for 96 hr. This trial ran for 8 hr. at which time control trout (N = 10) were offered a worm piece for 1 hr.

Experiment 8. Naïve Brook Trout offered Naïve Metamorphosed Yosemite Toads – The palatability of naive recently metamorphosed Yosemite toads (Stage 46) was assessed by offering them to naïve brook trout (N = 10) starved for 168 hr. (7 days). This trial ran for 8 hr. at which time control trout (N = 10) were offered both a worm piece and Pacific treefrog tadpole (Stage 25) for 1 hr.

Table 1. Summary of palatability experiments using Yosemite toad life-stages.

Exp	Life-stage	Relationship with trout	Stage of development	Trout relationship with toads	Time trout starved	Avg. length of trout (mm)	Duration of experiment
1	egg	experienced	1 - 12	naïve	36 hr.	162	8.0 hr.
2	egg	experienced	1 - 12	experienced	36 hr.	193	8.0 hr.
3	egg	naïve	1 - 12	naïve	120 hr.	144	8.0 hr.
4	tadpole	experienced	24 - 25	naïve	48 hr.	152	24 hr.
5	tadpole	naïve	23 - 25	naïve	312 hr.	149	24 hr.
6	tadpole	experienced	29 - 35	naïve	384 hr.	155	24 hr.
7	metamorph	experienced	46	naïve	96 hr.	162	8.0 hr.
8	metamorph	naïve	46	naïve	168 hr.	163	8.0 hr.

Figure 4. Palatability array with lids on, white cups indicate trout that received Yosemite toads (above) and lids off showing trout in containers (below).

Antipredator Response Experiments – According to Lima and Dill (1990) any time away from necessary functions such as foraging due to predator presence will likely induce trade-offs (e.g., increased developmental rates as a result of reduced foraging time). To test if Yosemite toad tadpoles respond to brook trout chemical cues through changes in activity I tested several scenarios. I originally hypothesized that if Yosemite toad tadpoles are palatable to brook trout it is likely that experienced toad tadpoles (Edith Lake) with trout would exhibit antipredator behavior to reduce the risk of predation. Similarly, naïve Yosemite toad tadpoles (Glacier Bench) would not display antipredator behavior to brook trout due to the lack of experience with trout. I also tested Yosemite toad tadpole response to a native vertebrate predator, western terrestrial garter snakes, an invertebrate predator, predaceous diving beetle adults. I had originally planned to test toad tadpoles response to brook trout fed a diet of Yosemite toad tadpoles, but palatability experiments revealed that brook trout were not willing to accept Yosemite toad larval stages as prey. As a surrogate, I decided to test Yosemite toad tadpole response to chemical stimuli of trout fed a diet of a non-conspecific tadpole (*Pacific treefrog*). To test for an antipredator response of Yosemite toad tadpoles to different chemical stimuli I used gravitational flow-though systems modified from Petranka et al. (1987) to pass water containing brook trout chemical cues into a container containing eight Yosemite toad tadpoles (Figure 5). Three plastic 25 L Rubbermaid SnapTopper® containers were positioned at different heights and filled with untreated well water (Average temperature)for experiments 9 – 12, 14 and 15 and with nearby stream water in the field for experiment 13. Containers were then connected with aquarium airline tubing

so that water flowed from one to the next at a rate of 0.5 – 0.6 L/min. I measured activity as the number of times tadpoles cross a centerline and the number of tadpoles hiding in the refuge after each minute for a 20 min. period (Petranka et al.1987). Each experiment consisted of two treatments, namely (1) one trout in a container to act as a predator chemical stimulus, and (2) one trout free container to act as a treatment control. Containers were arranged in a 2 x 3 array for all antipredator experiments. Experiments testing Yosemite toad tadpole response to brook trout only cues consisted of trout starved a minimum of 36 hr. to control for secondary alarm signal (e.g., ammonium) releases in effluent that may have influenced tadpole behavior.

Experiment 9. Experienced Yosemite Toad Tadpoles exposed to Brook Trout Chemical Cues – The antipredator response of experienced Yosemite toad tadpoles (Stage 24) was assessed by exposing tadpoles (N = 8) to naïve brook trout chemical cues along with a control group (N = 8) exposed to water only. This experiment tested the response of experienced Yosemite toad tadpoles, collected as eggs from a lake with brook trout, to trout chemical cues in order to explore a possible recent evolutionary relationship.

Experiment 10. Naïve Yosemite Toad Tadpoles exposed to Brook Trout Chemical Cues – The antipredator response of naïve Yosemite toad tadpoles (Stage 23-25) was assessed by exposing tadpoles (N = 8) to naïve brook trout chemical cues along with a control group (N = 8) exposed to water only. This experiment tested the response of naïve Yosemite toad tadpoles, collected as eggs from a lake without brook trout, to trout

chemical cues in order to confirm the naiveté of tadpoles due to the lack of a shared evolutionary history.

Experiment 11. Pacific Treefrog Tadpoles exposed to Brook Trout Chemical Cues – The antipredator response of Pacific treefrog tadpoles (Stage 25) was assessed by exposing tadpoles (N = 8) to naïve brook trout chemical cues. This experiment acted as a control to test the response of a palatable species to trout chemical cues.

Experiment 12. Yosemite Toad Tadpoles exposed to Chemical Cues of Brook Trout fed a diet of Pacific Treefrog Tadpoles – The antipredator response of Yosemite toad tadpoles (Stage 26-27) to non-conspecific predation (Pacific treefrog) was assessed by exposing toad tadpoles (N = 8) to chemical cues of brook trout fed a diet of Pacific treefrog tadpoles (Stage 25). This experiment compensated for the inability to test the response of Yosemite toad tadpoles to brook trout fed a diet of conspecific tadpoles.

Experiment 13. Yosemite Toad Tadpoles exposed to Chemical Cues of Western Terrestrial Garter Snakes – The antipredator response of Yosemite toad tadpoles to a native vertebrate predator, western terrestrial garter snakes, was assessed by exposing tadpoles (N = 8) to chemical cues of garter snakes. Garter snakes for these experiments were captured from a location where Yosemite toads were not present and starved for approximately 12 hr. to control for any possible alarm substances that may have been emitted through effluent.

Experiment 14 Yosemite Toad Tadpoles exposed to Adult Predaceous Diving Beetles fed a diet of Yosemite Toad Tadpoles (stimulus) and starved Adult Predaceous Diving beetles (control) – The antipredator response of Yosemite toad tadpoles to a native invertebrate predator, predaceous diving beetles, was assessed by exposing tadpoles ($N = 8$) to chemical cues of adult beetles. Adult beetles for these experiments were captured from a location where Yosemite toads were present and accepted toad tadpoles as prey items so it was possible that alarm substances may have been emitted through effluent even though beetles were starved approximately 12 hr. before experiment.

Experiment 15. Yosemite Toad Tadpoles exposed to Extract of Macerated Yosemite Toad Tadpoles – The antipredator response of Yosemite toad tadpoles to alarm substances from injured conspecifics was assessed by exposing tadpoles ($N = 8$) to chemical cues made from an extract of dispatched and macerated Yosemite toad tadpoles.

Figure 5. Gravitational flow-through system used in antipredator response experiments

Additional Experiments – Subsequent choice experiments were performed simultaneously with palatability trials to determine if adult predaceous diving beetles and brook trout were able to distinguish between palatable and nonpalatable tadpole species and test for preferences. Ten beetles were placed in covered 15 cm glass containers filled with 5 cm of water. Choice experiments for trout were carried out in 10 gallon (38 L) glass aquaria. Ten naïve brook trout that had not been used in previous experiments were randomly chosen and placed in 10 gallon aquaria. Each trout or beetle was offered five Yosemite toad and five Pacific treefrog tadpoles offered in pairs every 2 min. for 10 min. for trout experiments and in pairs until one tadpole was consumed in beetle experiments. Following Kruse and Stone (1984) for the trout choice experiments, I recorded (A) total number of tadpoles taken into the mouth (engulfed); (B) total number of each tadpole eaten; and (C) total number of times a tadpole was sampled and rejected.

Experiment 16. Predaceous Diving Beetle Choice Experiments – To test for palatability and prey species preference in a native invertebrate predator, I offered a Yosemite toad and Pacific treefrog tadpole pair of similar life-stage (Stage 25) to starved beetles. After the first tadpole was consumed, the second tadpole was removed and the beetle was offered a new pair. This was repeated five times over a 24 hr. period until the beetle consumed or fatally injured 10 tadpoles. Beetles were not subject to time constraints between tadpole pair offerings.

Experiment 17. Brook Trout Choice Experiments – To test for observable tadpole palatability and prey species preference in brook trout, I offered a Yosemite toad and Pacific treefrog tadpole pair of similar life-stage (Stage 25) to starved brook trout. Tadpole pairs were offered every 2 min. for the first 10 min. Experimental trials lasted for one hour and uneaten tadpoles were not removed as trout were too timid in the presence of an observer. In the event a trout consumed all five treefrog tadpoles within the first 10 min. an additional treefrog tadpole was added every 15 min. to test if trout would continue to sample and recognize between palatable and non-palatable prey.

Experiment 18 Brook Trout Choice Experiments using Recently Metamorphosed Yosemite Toads vs. Pacific Treefrog Tadpoles – To test for observable recently metamorphosed toad palatability and prey species preference in brook trout, I offered a Yosemite toad metamorph (Stage 46) and Pacific treefrog tadpole (Stage 36 – 41) pair to starved brook trout. Recently metamorphosed Pacific treefrog tadpoles could not be used in these experiments because treefrogs were adept at climbing out of the water making them unavailable to trout. Two Stage 46 Yosemite toads and two Pacific treefrog tadpoles were offered to brook trout for one hour and subsequent prey items were not added. To control for the timid nature of trout all experiments ($N = 6$) were video recorded.

RESULTS

Palatability Experiments – I designed my experiments so that I would be able to test for differences between and among groups of experienced and naïve Yosemite toads offered to trout using ANOVA. Additionally, I was prepared to compare mortality rates of trout if they experienced fatality as a result of consuming Yosemite toads using a Fisher's exact test, which would have allowed me to compare the proportion of trout that died as a result of sampling versus the proportion of trout surviving. However, statistical analyses were not performed for palatability trials because not a single Yosemite toad life stage offered to trout were consumed during the experiments. Though there were a few instances in which Yosemite toad eggs, tadpoles and recently metamorphosed toads appeared to have suffered injury as a result of trout sampling, such instances were rare. Eggs appeared to be damaged in experiments 2 and 3, but. eggs from experiment 1 and 2 (N = 100) were monitored for 48 hr. post-experiment for development rates and 48 out 100 (48%) appeared to be developing normally. The percentage of originally fertilized eggs was not known and thus eggs that were monitored post experiment that did not develop and succumbed to fungal infection could not be attributed to trout sampling alone.

Experiment 1. Naïve Brook Trout offered Experienced Yosemite Toad Eggs – No experienced Yosemite toad eggs (N = 50) were consumed by any naïve brook trout (Avg. FL 162mm) to which they were offered (N = 5) nor did any eggs appear to be damaged from trout sampling. Four of five control trout consumed a worm piece offered for 1 hr. at the end of the experiment.

Experiment 2. Experienced Brook Trout offered Experienced Yosemite Toad Eggs – No experienced Yosemite toad eggs (*N* = 50) were consumed by any experienced brook trout (Avg. FL 193mm) to which they were offered (*N* = 5). One egg appeared to be damaged possibly from trout sampling. Three of five control trout consumed worm piece offered for 1 hr. at the end of the experiment.

Experiment 3. Naïve Brook Trout offered Naïve Yosemite Toad Eggs – No naïve Yosemite toad eggs (*N* = 50) were consumed by any naïve brook trout (Avg. FL 144mm) to which they were offered (*N* = 5). Four eggs appear to be damaged, possibly from trout sampling. Three of five control trout consumed a worm piece offered for 1 hr. at the end of the experiment.

Experiment 4. Naïve Brook Trout offered Experienced Yosemite Toad Tadpoles – No experienced Yosemite toad tadpoles (*N* = 50) were consumed by any naïve brook trout (Avg. FL 152mm) to which they were offered (*N* = 5). However, one tadpole was eviscerated, indicating trout did sample tadpoles. The eviscerated tadpole was still alive 48 hr. post-experimental monitoring, but tadpoles were not monitored further so its fate is unknown. Control trout in this experiment were offered both a worm piece and Pacific treefrog tadpole. Five of five control trout consumed the worm piece while three of five control trout consumed both a worm piece and Pacific treefrog tadpole offered for 1 hr. at the end of the experiment.

Experiment 5. Naïve Brook Trout offered Naïve Yosemite Toad Tadpoles – No naïve Yosemite toad tadpoles (*N* = 50) were consumed by any naïve brook trout (Avg. FL 149mm) to which they were offered (*N* = 5) nor did any tadpoles appear to be injured

from trout sampling. Control trout in this experiment were offered both a worm piece and Pacific treefrog tadpole. Five of five control trout consumed both the worm piece and Pacific treefrog tadpole offered for 1 hr. at the end of the experiment.

Experiment 6. Naïve Brook Trout offered Experienced Yosemite Toad Tadpoles – No experienced Yosemite toad eggs (N = 50) were consumed by any naïve brook trout (Avg. FL 155mm) to which they were offered (N = 5) nor did any tadpoles appear to be injured from trout sampling. Four of five control trout consumed a worm piece offered for 1 hr. at the end of the experiment.

Experiment 7. Naïve Brook Trout offered Experienced Metamorphosed Yosemite toads – No experienced recently metamorphosed Yosemite toads (N = 40) were consumed by any naïve brook trout (Avg. FL 162mm) to which they were offered (N = 10). However, one metamorphosed toad was dead and eviscerated during experiments and replaced. Two of 10 control trout consumed the worm piece offered for 1 hr. at the end of the experiment.

Experiment 8. Naïve Brook Trout offered Naïve Metamorphosed Yosemite Toads – No naïve recently metamorphosed Yosemite toads (N = 40) were consumed by any naïve brook trout (Avg. FL 163mm) to which they were offered (N = 10) nor did any metamorphosed toads appear to be injured from trout sampling. Control trout were offered both a worm piece and Pacific treefrog tadpole. Seven of 10 control trout consumed both the worm piece and the Pacific treefrog tadpole offered for 1 hr. at the end of the experiment.

Antipredator Response Experiments – Paired *t*-tests were performed in SigmaStat with two-tail criteria because I was uncertain whether Yosemite toad tadpoles would increase or decrease activity in response to brook trout chemical cues. Most literature regarding antipredator behavior in larval amphibians leads to a reduction in activity (Lawler 1989). Hews and Blaustein (1985) experimenting with western toad tadpoles exposed to extract of injured conspecific tadpoles actually increased activity. All box plot graphs were created in SigmaPlot and bar graphs created in Microsoft Excel.

Experiment 9. Experienced Yosemite Toad Tadpoles exposed to Brook Trout Chemical Cues – A paired *t*-test revealed that experienced Yosemite toad tadpoles did not show a significant difference in activity levels between tadpoles exposed to brook trout chemical cues and controls (Figure 6,. $t = 0.349$, $P = 0.735$, $df = 9$). Similar results were found for refuge use of tadpoles (Figure 7. $t = 0.533$, $P = 0.607$, $df = 9$).

Experiment 10. Naïve Yosemite toad tadpoles exposed to brook trout chemical cues – A paired *t*-test revealed that experienced Yosemite toad tadpoles did not show a significant difference in activity levels between tadpoles exposed to brook trout chemical cues and controls (Figure 8, $t = 1.432$, $P = 0.186$, $df = 9$). Similar results were found for refuge use of tadpoles (Figure 9, $t = -0.790$, $P = 0.186$, $df = 9$).

Experiment 11.Pacific Treefrog Tadpoles exposed to Brook Trout Chemical Cues – A paired *t*-test revealed that Pacific treefrog tadpoles showed a significant difference in activity levels between tadpoles exposed to brook trout chemical cues and controls

through reduced activity (Figure 10, $t = 3.737$, $P = 0.005$, $df = 9$). Similar results were not found for refuge use (Figure 11, $t = 0.419$, $P = 0.685$, $df = 9$)

Experiment 12. Yosemite Toad Tadpoles exposed to Chemical Cues of Brook Trout fed a diet of Pacific Treefrog Tadpoles – A paired t-test revealed that Yosemite toad tadpoles did not show a significant difference in activity levels between tadpoles exposed to chemical cues of brook trout fed a diet of Pacific treefrog tadpoles and controls (Figure 12, $t = -0.0169$, $P = 0.987$, $df = 9$). Similar results were found for refuge use (Figure 13, $t = 0.539$, $P = 0.603$, $df = 9$).

Experiment 13. Yosemite Toad Tadpoles exposed to Chemical Cues of Western Terrestrial Garter Snakes – A paired t-test revealed that Yosemite toad tadpoles did not show a significant difference in activity levels between tadpoles exposed to chemical cues of western terrestrial garter snakes and controls (Figure 14, $t = 1.196$, $P = 0.262$, $df = 9$). Refuge use was only recorded for 6 trials due to missing data (Figure 15, $t = 0.458$, $P = 0.666$, $df = 5$).

Experiment 14 Yosemite Toad Tadpoles exposed to Adult Predaceous Diving Beetles fed a diet of Yosemite Toad Tadpoles (stimulus) and starved Adult Predaceous Diving Beetles (control) – No statistical analysis was performed because this experiment was terminated after one trial because of unusually high activity levels among tadpoles. This made recording accurate data difficult. For the single trial, average number of crossings for the 20 min. experiment was 9.7 crossings/min. whereas the average number

of crossings for experienced and naïve Yosemite toad tadpoles exposed to trout chemical cues was 2.1 crossings/min.

Experiment 15. Yosemite Toad Tadpoles exposed to Extract of Macerated Yosemite Toad Tadpoles – No statistical analysis was performed because this experiment was also terminated after one trial because of unusually high activity levels among tadpoles resulting in the inability to record data accurately. For the single trial, average number of crossings for the 20 min. experiment was 11.8 crossings/min. whereas the average number of crossings for experienced and naïve Yosemite toad tadpoles exposed to trout chemical cues was 2.1 crossings/min.

Additional Experiments: Experiment 16 Predaceous Diving Beetle Choice Experiments – Choice experiments (N = 10) using predaceous diving beetle adults revealed that adult beetles would consume Yosemite toad tadpoles without ill-effect. Interestingly, once a beetle consumed a Yosemite toad tadpole it proceeded to only consume treefrog tadpoles and in all four cases in which a Yosemite toad was consumed was during the first offered pair (Figure 16).

Experiment 17. Brook Trout Choice Experiments – Choice experiments (N = 10) using trout were met with similar results to palatability trials but revealed that brook trout would at least engulf or sample Yosemite toad tadpoles without any apparent ill-effect. During trials, 100% of Yosemite toad tadpoles engulfed were rejected; 68% of Pacific treefrog tadpoles engulfed were consumed, while 32% engulfed were rejected (Figure 17). However, it is worth noting that the remaining 32% of treefrogs rejected by trout

were eviscerated and immobile. So, this result could be interpreted as 100% mortality for Pacific treefrog tadpoles sampled by trout.

Experiment 18 Brook trout choice experiments using recently metamorphosed toads vs. Pacific treefrogs – Choice experiment ($N = 6$) results for brook trout with recently metamorphosed Yosemite toads in 2005 were quite different than those conducted with toad tadpoles in 2004. These experiments with metamorphosed toads were shortened to 0.5 hr. and video recorded. However, even the absence of an observer trout activity appeared to be low. Trout remained on the bottom of the tank immobile for the first 10 – 15 min. Only two trout sampled and rejected metamorphosed toads and did so only once. Interestingly, Pacific treefrogs were also not as readily sampled by trout in 2005 as in 2004 with only four tadpoles being consumed. This suggests that trout could either (1) detect Yosemite toad noxiousness through smell and thus do not sample (i.e., take into the mouth) or (2) brook trout are in a reduced feeding state due to the proximity of the spawning season or both.

Table 2. Summary of antipredator experiments and predatory stimulus.

Exp	Species	Statistically significant	Predator stimulus	Average # of crossings stimulus	Average # of crossings (control)	Average # refuge use stimulus	Average # refuge use (control)	# of replicates
9	Toad	No	Brook trout	1.25	1.37	4.84	5.21	10
10	Toad	No	Brook trout	3.00	3.82	5.56	5.24	10
11	Treefrog	Yes[1], No[2]	Brook trout	3.19[1]	5.80[1]	7.16[2]	7.27[2]	10
12	Toad	No	Brook trout fed treefrogs	15.85	15.82	2.26	2.38	10
13	Toad	No	Garter Snake	6.25[1]	5.09[1]	4.51[2]	4.63[2]	10[1], 6[2]
14	Toad	Unknown	Predaceous Diving Beetle	9.7	N/A	4.3	N/A	1
15	Toad	Unknown	Injured conspecific	11.8	N/A	6.25	N/A	1

N/A = not available, data was not collected.

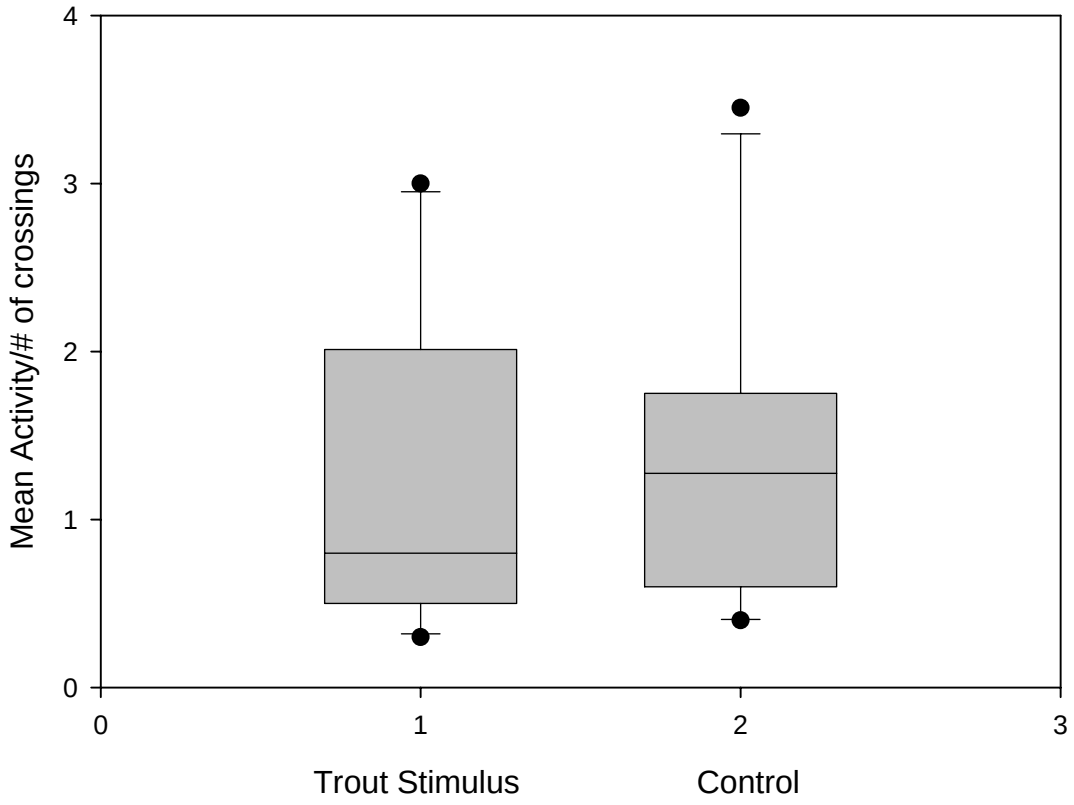

Figure 6. Experiment #9: Antipredator response (Activity: mean+ s.e.) of experienced Yosemite toad tadpoles (Stage 25) exposed to trout chemical stimuli based on 10 replicates for each treatment (t = 0.349, P = 0.735, df = 9). The edge of the box nearest zero depicts the 25th percentile, the line within the box indicates the median, and the edge of the box farthest away from zero depicts the 75th percentile. The 90th and 10th percentiles are represented by whiskers above and below the box edges.

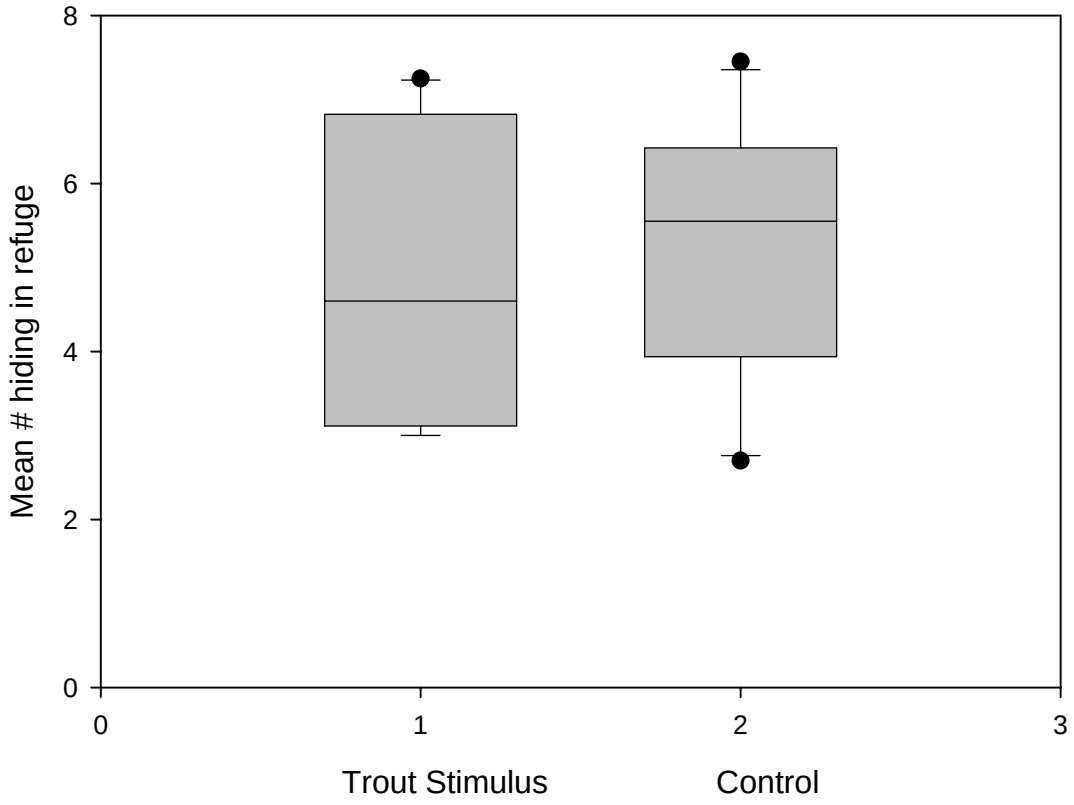

Figure 7. Experiment #9: Antipredator response (Refuge use: mean+ s.e.) of experienced Yosemite toad tadpoles (Stage 25) exposed to trout chemical stimuli based on 10 replicates for each treatment (t = 0.533, P = 0.607, df = 9). The edge of the box nearest zero depicts the 25th percentile, the line within the box indicates the median, and the edge of the box farthest away from zero depicts the 75th percentile. The 90th and 10th percentiles are represented by whiskers above and below the box edges.

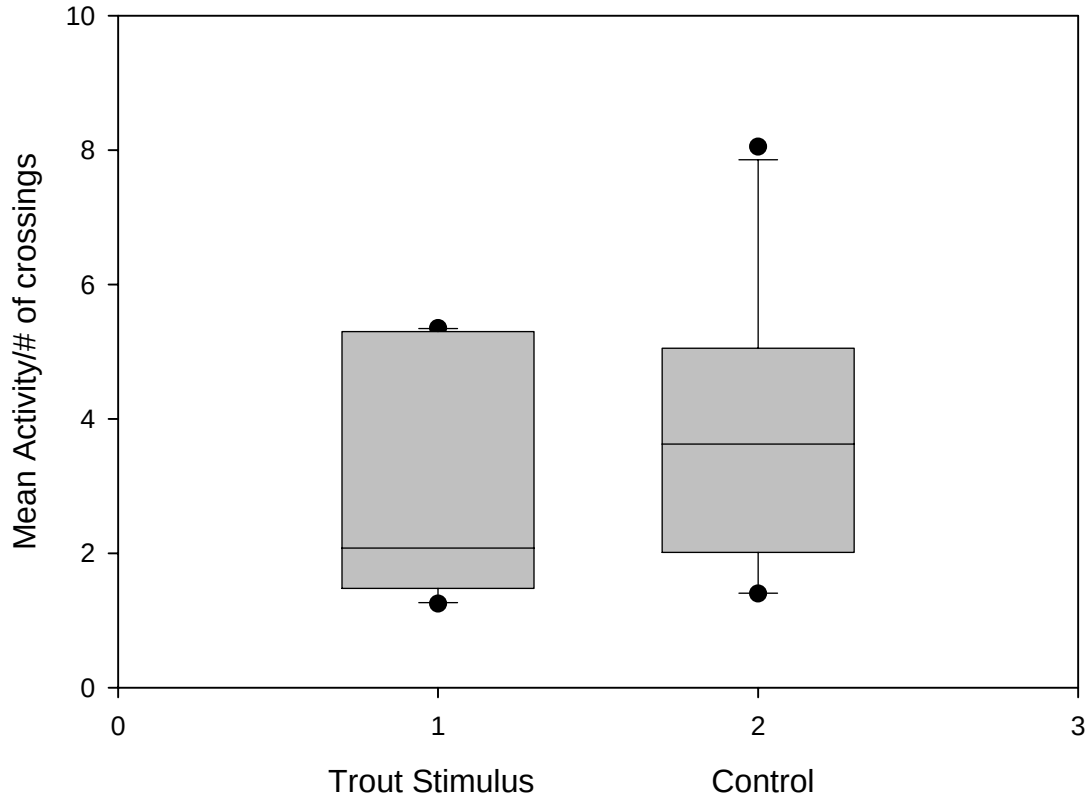

Figure 8. Experiment #10: Antipredator response (Activity: mean + s.e.) of naïve Yosemite toad tadpoles (Stage 25) exposed to trout chemical stimuli based on 10 replicates for each treatment (t = 1.432, P = .186, df = 9).

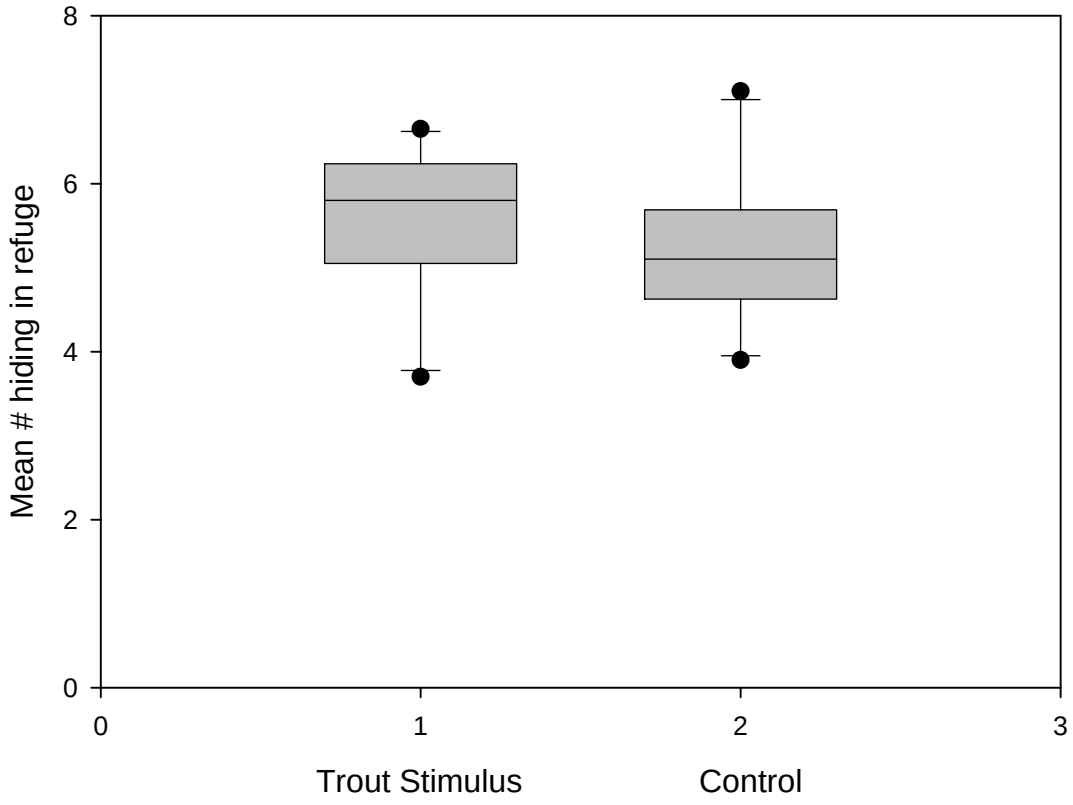

Figure 9. Experiment #10: Antipredator response (Refuge use: mean + s.e.) of naïve Yosemite toad tadpoles (Stage 25) exposed to trout chemical stimuli based on 10 replicates for each treatment (t = -0.790, P = 0.450, df = 9).

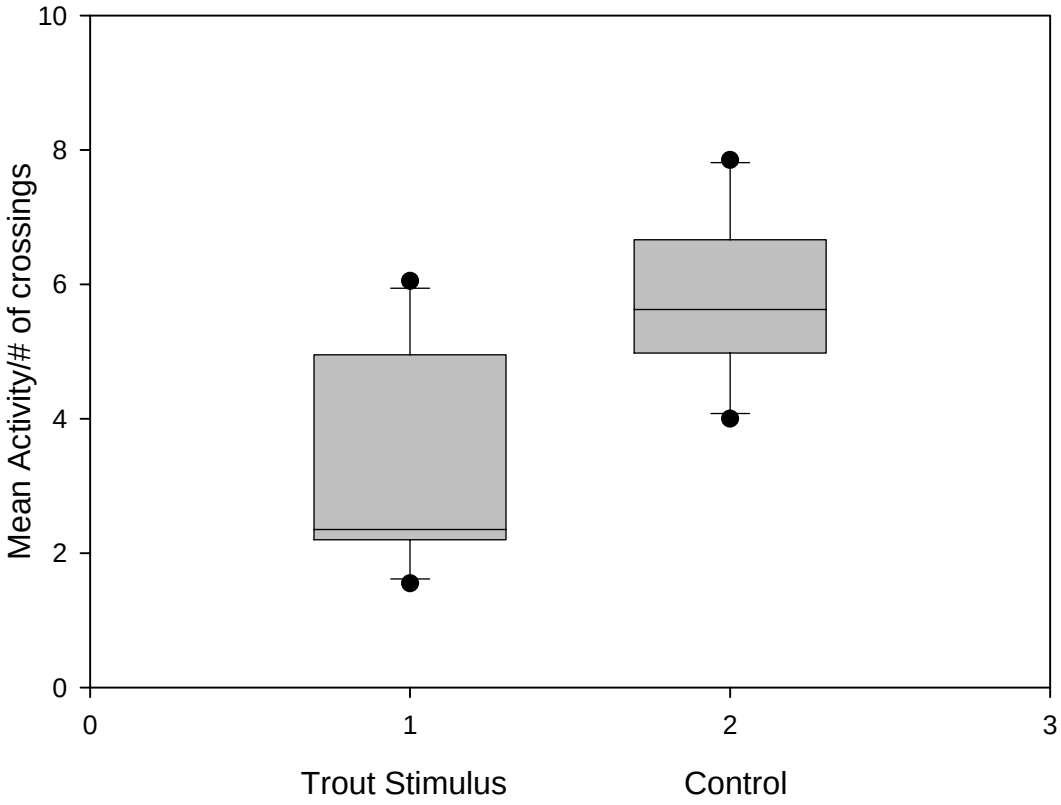

Figure 10. Experiment #11: Antipredator response (Activity: mean + s.e.) of Pacific treefrog tadpoles (Stage 25) exposed to trout chemical stimuli based on 10 replicates for each treatment (t = 3.737, P = 0.005, df = 9).

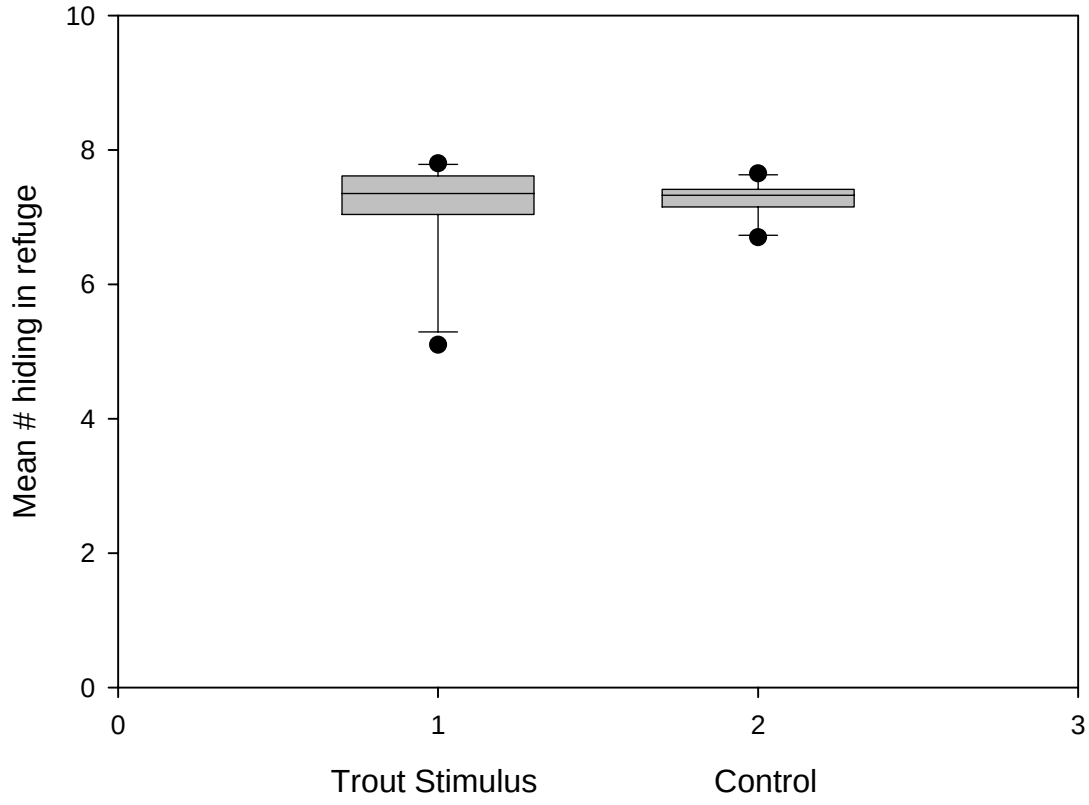

Figure 11. Experiment #11: Antipredator response (Refuge use: mean + s.e.) of Pacific treefrog tadpoles (Stage 25) exposed to trout chemical stimuli based on 10 replicates for each treatment (t = 0.419, P = 0.685, df = 9).

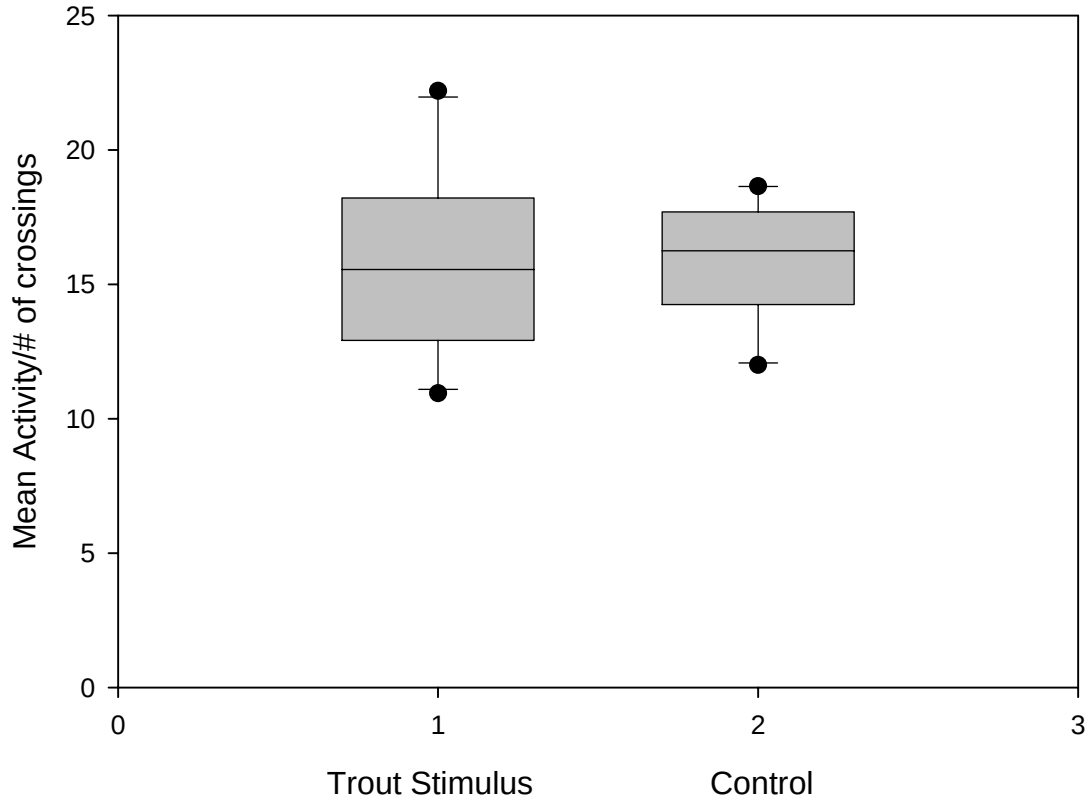

Figure 12. Experiment #12: Antipredator response (Activity: mean + s.e.) of experienced Yosemite toad tadpoles (Stage 25) exposed to chemical stimuli of trout fed a diet of Pacific treefrogs based on 10 replicates for each treatment (t = -0.0168, P = 0.987, df = 9).

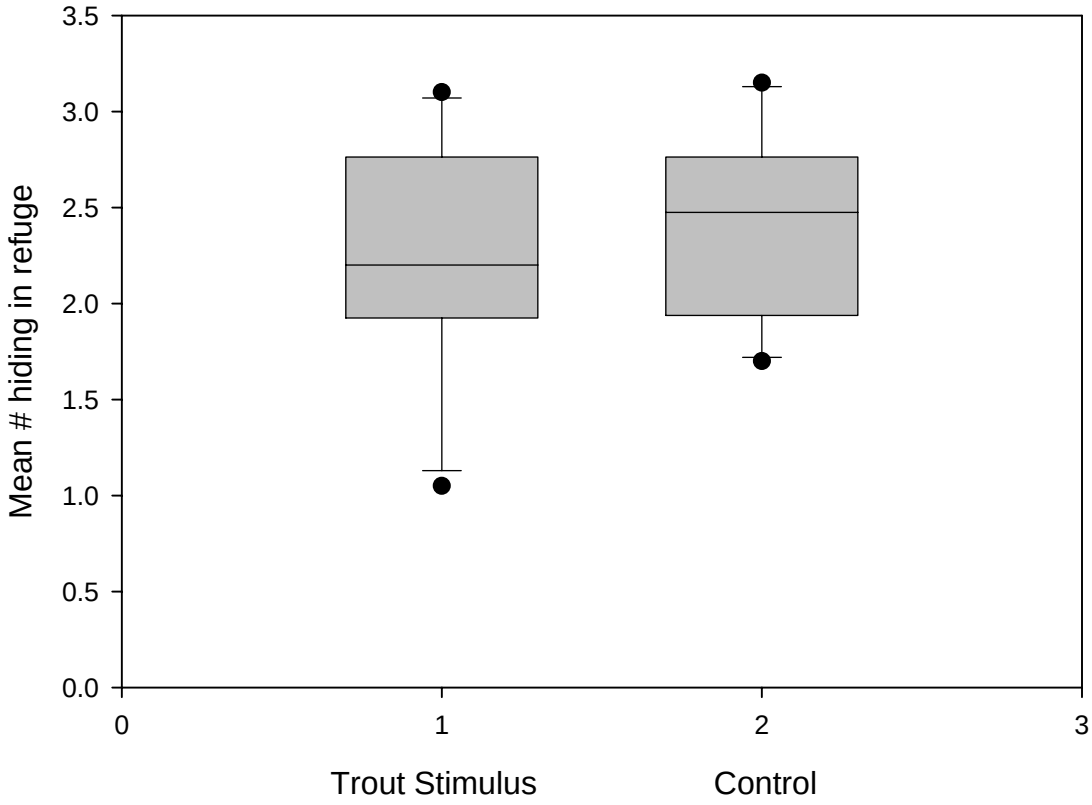

Figure 13. Experiment #12: Antipredator response (Refuge use: mean + s.e.) of experienced Yosemite toad tadpoles (Stage 25) exposed to chemical cues of trout fed a diet of Pacific treefrogs based on 10 replicates for each treatment (t = 0.539, P = 0.603, df = 9).

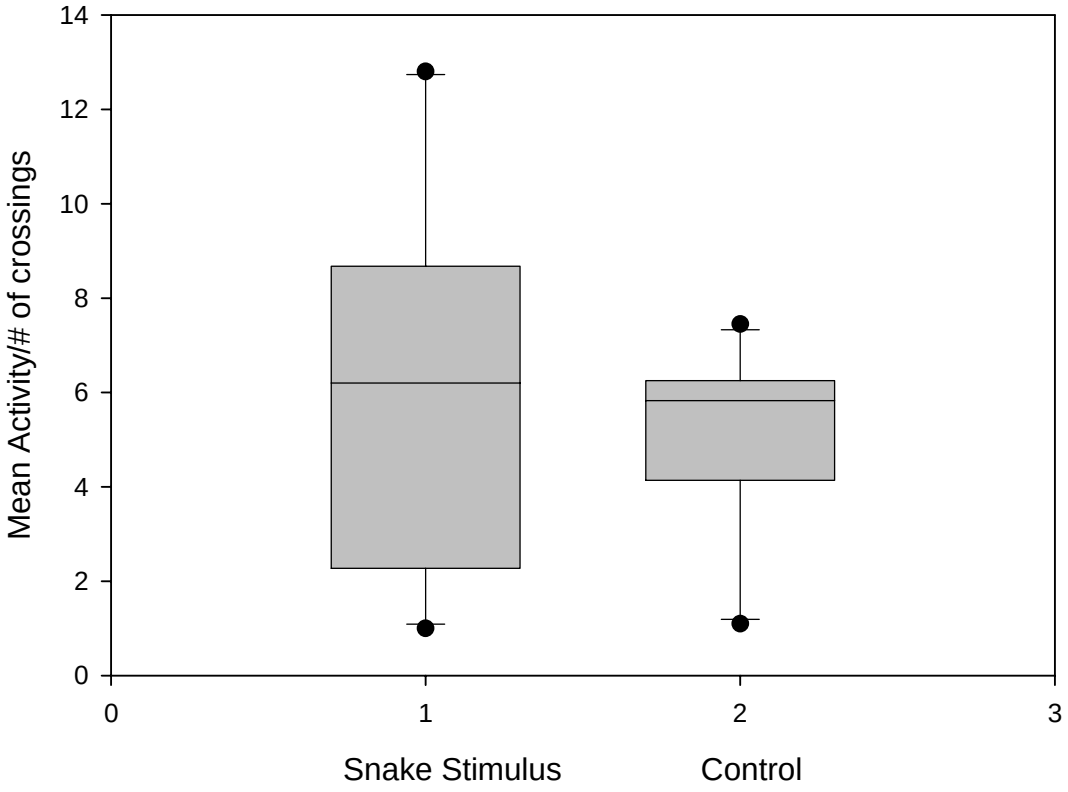

Figure 14. Experiment #13 Antipredator response (Activity: mean + s.e.) of Yosemite toad tadpoles (Stage 36-41) exposed to chemical stimuli of western terrestrial garter snakes based on 10 replicates for each treatment (t = 1.196, P = 0.262, df = 9).

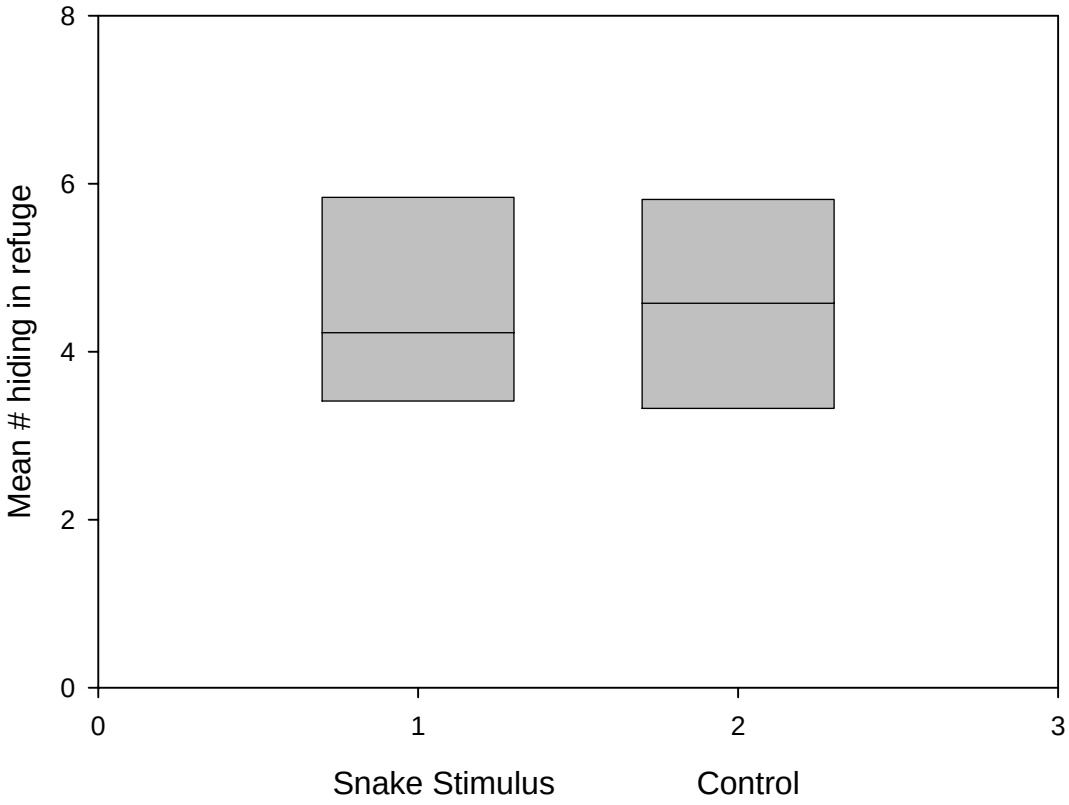

Figure 15. Experiment #13 Antipredator response (Refuge use: mean + s.e.) of Yosemite toad tadpoles (Stage 36-41) exposed to chemical stimuli of western terrestrial garter snakes based on 6 replicates for each treatment (t = 0.458, P = 0.666, df = 5).

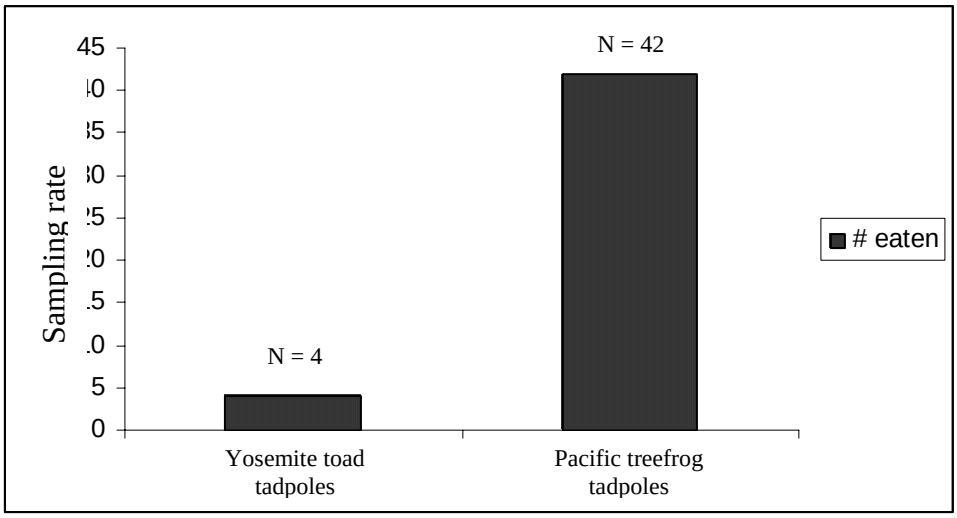

Figure 16. Experiment #16: Predaceous diving beetle choice experiments (*N* = 10): Yosemite toad tadpoles (Stage 25) vs. Pacific treefrog tadpoles (Stage 25). Total number eaten shown.

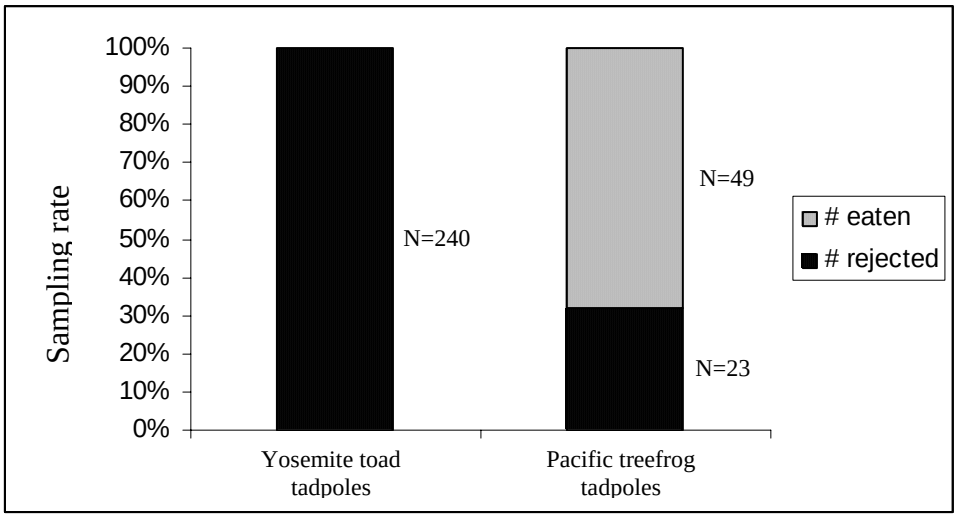

Figure 17. Experiment #17: Brook trout choice experiments (*N* = 10): Yosemite toad tadpoles (Stage 25) vs. Pacific treefrog tadpoles (Stage 25) shown as percentage of total.

DISCUSSION

My research demonstrates that Yosemite toad eggs, tadpoles, and recently metamorphosed toads are completely unpalatable to nonnative brook trout in the Sierra Nevada. Similarly, I found no differences in palatability of these life-stages between experienced and naïve Yosemite toads offered to trout. These results coincide with other studies that have shown larval stages of the genus *Bufo* to be less palatable to fish predators than other anurans including *Rana* and *Hyla* (Voris and Bacon 1966; Licht 1968, 1969; Kruse and Stone 1984; Kats et al. 1988; Kiesecker et al.1996; Lawler and Hero 1997). The fact that not a single Yosemite toad egg (N = 150), tadpole (N = 150), or recently metamorphosed toad (N = 80) was consumed in any experiment is striking especially since some trout were starved up to 16 days (384 hr.), far longer than they are likely to experience in nature during the timeframe experiments were conducted. This supports the idea that Yosemite toads, like most other toads possess toxic properties in their skin throughout their life history rendering them unpalatable to trout. Flier et al. (1980) found that a class of cardiac glycosides (bufadienolides) may be responsible for unpalatable properties. However, Benard and Fordyce (2003) did not find bufadienolides present in the closely related western toad tadpoles, but did find bufadienolides in recently metamorphosed western toads, suggesting that another noxious chemical likely exists in tadpoles causing unpalatability. Similar properties have been found in the western toad by Kiesecker et al (1996), and are currently being investigated in the Yosemite toad by a fellow graduate student (Jeff Weaver, California State University, Sacramento). Although brook trout are not negatively affecting Yosemite toads through

direct predation of eggs, tadpoles or recently metamorphosed toads, sub-lethal effects from engulfing or sampling can potentially have an effect on development and survival rates. A total of five damaged toad eggs (4% of total eggs offered) were observed in four trout containers (27% of trout offered eggs) during palatability trials likely as a result of trout sampling. One toad egg was consumed post-experiment by a trout that ate a piece of worm was presumed accidental since no other eggs were eaten. A total of 100 eggs from two palatability trials were maintained separately for 48 hr. at which time half (50%) appeared to be developing normally. The remainder of the eggs succumbed to infection from fungus, likely Saprolengia, which according to Sadinski (2004) is highly devastating to Yosemite toad eggs once established. Since I was not certain if all eggs used in palatability were fertilized, I could not make the distinction that non-developing embryos were unsuccessful as a result of trout sampling or due to natural attrition from fungal infection. Palatability trials involving Yosemite toad tadpoles resulted in only one potential case for mortality as a naïve trout, presumably through sampling, eviscerated a single toad tadpole, however this tadpole was still alive 48 hr. post-experimental monitoring. Furthermore, a single recently metamorphosed toad was eviscerated likely as result of trout sampling and euthanized. To minimize observer effects on timid trout I did not continually observe or video record individual palatability trials. Since I did not know if trout were able to differentiate between palatable and unpalatable tadpole prey items, I decided to compensate for this by conducting choice experiments using brook trout offered Yosemite toad and Pacific treefrog tadpoles as well as recently metamorphosed individuals. Results of these choice experiments did reveal that brook

trout were actively sampling Yosemite toads but not willing to consume them. Treefrogs were always preferred over toads in tests and trout did not seem to be able to distinguish between tadpoles species by sight or smell, only through taste. Another revealing aspect was that toads which were repeatedly sampled (i.e., tasted) by trout did not succumb to any ill effects such as evisceration. For example, during one test five Yosemite toad tadpoles were engulfed and rejected over 125 times without apparent harm. Although these palatability experiments illustrated that Yosemite toads appear to be protected from trout predation, such toxins do not seem to provide complete protection against native vertebrate and invertebrate predators. It has been confirmed that many garter snakes of the genus (*Thamnophis*) are immune to toad toxins (Licht 1969; Arnold and Wassersug 1978) and I have personally observed western terrestrial garter snakes consuming all but the egg stages of Yosemite toads during field surveys. Invertebrate predators such as predaceous diving beetle larvae that pierce the skin of their prey and suck out fluids likely circumvent the Yosemite toad's chemical defense system without impairment (see Peterson and Blaustein 1992). However, adult predaceous diving beetles that do not pierce skin, but rather masticated tadpoles as witnessed during my experiments, also do not appear to suffer from any negative effects from the ingestion of these toxins. Through choice experiments, I demonstrated that adult predaceous diving beetles preferred palatable Pacific treefrog than Yosemite toad tadpoles. And for my own satisfaction, I followed Wassersug (1971) in which I held a Yosemite toad tadpole in my mouth ($N = 2$) without chewing or biting for 30 seconds. Although I did not experience the bitterness described by Wassersug or his test subjects I did experience a tingling or

numbing sensation which may suggest morphine – like properties also effective against predators (Daly 1994).

I found that Yosemite toad tadpoles did not exhibit a significant difference in behavior when exposed to water containing trout chemical cues nor was there any difference in activity or refuge use between experienced and naïve tadpoles. This corresponds to the trade-off theory by Lima and Dill (1990) that prey animals should not alter their behavior in the presence of a non-predator. However, Nyström and Åbjörnsson (2000) found that the common toad (*Bufo bufo*) also did not respond to chemical cues of rainbow trout even though trout did occasionally consume toad tadpoles. Nonetheless, there is ample evidence that tadpoles, usually palatable species (but see Lawler 1989, Lefcort 1998), do respond to the presence of predator chemical cues (Hews and Blaustein 1985; Brodie and Formanowicz 1987; Petranka et al. 1987; Kats et al. 1988; Lawler 1989; Kiesecker et al. 1996; Lefcort 1996 and 1998; Chivers et al. 1999; Chivers et al. 2000; Laurila 2000; Nyström and Åbjörnsson 2000). There are two potential reasons why Yosemite toads may not have responded to chemical cues of brook trout, (1) it is possible that Yosemite toads did in fact recognize trout presence through chemical cues but did not respond to such cues because they are unpalatable to trout and alterations in behavior are unnecessary and energetically costly, (2) Yosemite toads are simply unable to detect brook trout from the lack of a shared history with trout or fish in general and are thus unable to respond to brook trout chemical cues but negated by the fact that they are unpalatable. To clarify the first scenario I had originally decided to test the ability of Yosemite toad tadpoles to detect trout that had consumed conspecific

tadpoles, but palatability trials revealed that brook trout were unwilling to consume any early life-stages of Yosemite toads. I compensated by conducting antipredator trials using naïve Pacific treefrog tadpoles, a known palatable species from a lake without trout. Pacific treefrog tadpoles strongly responded to brook trout chemical cues through a reduction in activity levels, which makes sense for a palatable species. However, it is not exactly clear why they exhibited such behavior since they were collected as eggs from a water body void of trout. Kats et al. (1988) found that four families of amphibians (Ranidae, Hylidae, Ambystomatidae, and Plethodontidae), which include treefrogs, but not toads, increased their refuge use in the presence of rainbow trout (*Oncorhynchus mykiss*) chemical cues even though the amphibians used in their experiments had never encountered fish. One possible explanation is that Pacific treefrogs possess a genetic component that allows them to detect fish predators. Trout and other fish species are native to other parts of the Pacific treefrog range and hence overlapping populations with fish likely maintain gene flow with populations lacking fish. Secondly, it could be that palatable species are more sensitive to predatory threats regardless of the type of predator. Since treefrog tadpoles were readily consumed by brook trout I tested whether Yosemite toads would respond to non-conspecific alarm cues. It has been suggested by Kiesecker et al. (1999) that frog tadpoles that appear to be responding to alarm substances may actually be responding to increased ammonium concentrations from effluent. I theorized that Yosemite toad tadpoles might respond to this type of predation if the similar signaling is occurring in toad tadpoles. However, I did not witness a statistically significant result in toad tadpole activity in response from exposure to

chemical cues of brook trout fed a diet of treefrog tadpoles. These results for Yosemite toad tadpoles were consistent with other literature in which tadpoles did not respond to chemical stimuli from non-conspecifics (Hews 1988; Chivers et al. 1999a, 1999b). Given that antipredator experiments using conspecifics was not possible coupled with the fact that Yosemite toads did not respond to non-conspecific predation, I decided to test the ability of Yosemite toad tadpoles to detect native vertebrate and invertebrate predators. My field surveys confirmed that western terrestrial garter snakes and predaceous diving beetles consume Yosemite toad tadpoles as well as recently metamorphosed toads without ill effect. Arnold and Wassersug (1978) found stage 40-46 western toad tadpoles to be most susceptible to garter snake predation. These stages are characteristic of front leg emergence just prior to metamorphosis. Kiesecker et al. (1996) established that western toad tadpoles will more often than not respond to predators through chemical and not visual cues, however western toad tadpoles (Stage 25) during their experiments, did reduce activity levels in response to visual cues of active garter snakes. I tested the ability of Yosemite toad tadpoles that were nearing metamorphosis (Stage 36 – 41) to chemically detect garter snakes. Surprisingly, I did not detect a significant result between tadpoles exposed to garter snake stimuli and controls. This result did not coincide with other studies which found a significant reduction in activity of western toad tadpoles and post-metamorphic toads exposed to garter snake chemical cues (Chivers et al. 1999b; Kiesecker et al. 1996). There was however, a high rate of variation in activity levels among groups suggesting that some tadpoles were indeed responding to chemical cues of garter snakes. One major difference in my study and the

two aforementioned studies is that I collected garter snakes from an area where Yosemite toads were not present and hence garter snakes were not preying upon toads, while Kiesecker et al. (1996) collected garter snakes from an area that contained western toad tadpoles and Chivers et al. (1999b) maintained experimental garter snakes on a diet of western toads for several weeks prior to experimentation. So it is possible that these authors witnessed toad tadpoles responding to alarm substances from conspecifics in garter snake feces and not garter snake cues alone. The variation between my experiments and those of Kiesecker et al. (1996) and Chivers et al. (1999b) may be explained as follows. In 1989, Hayes examined the antipredator response of recently metamorphosed American toads to eastern garter snakes (*Thamnophis sirtalis sirtalis*) and found that the toads did not exhibit any behavioral changes to garter snake chemical cues but did respond to visual cues. Similar results were found by Heinen (1994) in which recently metamorphosed American toads did not respond to odors of eastern garter snakes, but visual cues. This the variation witnessed during my experiments may be best explained by the fact that toad tadpoles close to metamorphosis which are more susceptible to aquatic predators respond favorably to chemical cues and that post metamorphic toads which have increased susceptibility to land predators depend more on visual cues. For the reason that I observed predaceous diving beetles preying on Yosemite toad tadpoles in the field and during choice experiments, I tested the ability of toad tadpoles to respond to these native invertebrate predators. Chivers et al. (1999) states “Chemical cues are of prime importance in recognition of insect predators by western toad tadpoles” and Kiesecker et al. (1996), demonstrated that western toad

tadpoles responded only to chemical and not visual cues of predatory invertebrates. Yosemite toad tadpoles did appear to respond through increased activity when exposed to adult predaceous diving beetles fed a diet of Yosemite toad tadpoles, however this experimental trial was cancelled and only replicated once because of the high amount of tadpole activity observed could not be measured accurately. Seeing as it was becoming more and more evident from my experiments that Yosemite toads may only respond to chemical cues of injured conspecifics or effluent from predators feeding on conspecifics, I tested the ability of Yosemite toad tadpoles to respond to conspecific alarm substances alone. I dispatched and macerated a small group of Yosemite toad tadpoles and exposed them to other conspecifics. Similarly, these experiments were also cancelled after one trial due to high rates of tadpole activity which could not be accurately measured without video equipment. In light of all this, my preliminary experiments testing alarm substance response were insightful. For instance, activity level averages per minute for this experiment as well as the antipredator experiments involving predaceous diving beetles were 4 – 5 times higher than the average of all other antipredator trials using Yosemite toad tadpoles. This result is consistent with Hews and Blaustein (1985) who demonstrated an increase in activity of western toad tadpole exposed to tincture made from injured conspecifics. Similar to my experiments exposing Yosemite toad tadpoles to predaceous diving beetles, these authors could not make the distinction of whether western toad tadpoles responded to predator only cues or to predators feeding on conspecifics that released an alarm signal. Analogous results using an odanate or dragonfly predator (*Anax junius*) were reported by Skelly and Werner (1990). However,

there is at least some evidence that toad tadpoles respond to chemical cues of predators alone and not in conjunction with conspecific alarm substances. Anholt et al (1996) thought that American toad tadpoles did reduce their activity when exposed to unfed Anax predator cues except the result was not statistically significant between toad tadpole groups exposed to Anax only cues and Anax fed a diet of toad tadpoles. It seems likely that tadpoles would gain the most protection if they did not rely on chemical or visual cues alone for predator detection, but on the alarm substances released from injured conspecifics (Hews and Blaustein 1985; Hews 1988; Chivers et al. 1999), but see Lefcort (1998). Thus in any case, more investigation is needed in this area.

In summary, brook trout found all early stages (egg, tadpole and metamorph) of Yosemite toads to be unpalatable. Furthermore, these Yosemite toad life-stages were sampled by trout without harm with only a few exceptions. Yosemite toad tadpoles, a nonpalatable species, did not respond to trout presence in the same manner as a palatable species, the Pacific treefrog. Treefrogs exposed to trout chemical cues exhibited significantly reduced activity levels even though tadpoles were collected from a pond without trout. This likely allows Yosemite toad tadpoles to maintain regular activity levels in the presence of even potential predators. In addition, Yosemite toad tadpoles nearing metamorphosis did not exhibit antipredator behavior toward garter snakes, a known native predator possibly because visual cues might be more important for detecting land predators. Yosemite toad tadpoles did not exhibit antipredator behavior in response to non-conspecific predation from stimuli of brook trout fed a diet of Pacific treefrog tadpoles. Preliminary analysis from antipredator response to adult predaceous

diving beetles however, was promising. Yosemite toad tadpole activity increased sharply when (1) tadpoles were exposed to adult predaceous diving beetles fed a diet of Yosemite toad tadpoles and (2) when toad tadpoles were exposed to extract of macerated conspecifics. Although informative, these results are a little confounding because predaceous diving beetles used in antipredator experiments were allowed to consume Yosemite toad tadpoles and thus it is not clear if the beetles or their effluent is triggering the observed response. However, it is worth noting that these preliminary trials were terminated because tadpole activity was so high it could not be accurately measured. Yosemite toads in the High Sierra likely have already adapted necessary trade-offs for species survival. The short duration of Sierra summers, relatively small female body and clutch sizes, and native predation pressures such as the western terrestrial garter snake and adult predaceous diving beetles protects Yosemite toads from trout predation and may allow the two species to co-occur even though trout have been implicated in the decline of the mountain yellow-legged frog and Pacific treefrog. Still, trout may be in competition with Yosemite toads for invertebrate food sources or brook trout may displace native invertebrate predators from lakes with trout, concentrating them into ephemeral habitats and increasing predation on larval toads.

Since this scenario might hold true for other amphibians the need for direct evidence is critical to assess non-native predatory threat.

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