PREDATOR-PREY INTERACTIONS BETWEEN HELLBENDERS

(*CRYPTOBRANCHUS ALLEGANIENSIS ALLEGANIENSIS* AND *C. A. BISHOPI*)

AND NATIVE AND NONNATIVE FISHES

A Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Biology

By

Brian G. Gall

August 2008

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ABSTRACT

The introduction of nonnative fishes often results in the local extinction of native amphibians due to a lack of evolutionary history and therefore, minimally-adapted antipredator behaviors toward the introduced fishes. Populations of hellbenders (*Cryptobranchus alleganiensis*) in Missouri have declined considerably since the 1980's, coinciding with a rapid increase in trout introductions for recreational angling. I examined hellbender and fish predator-prey interactions by: (1) examining the foraging behavior of predatory fishes in response to a hellbender secretion; (2) comparing the number of secretion and control-soaked food pellets consumed by trout; and (3) comparing the response of larval hellbenders to chemical stimuli from introduced (trout) and native fish predators. Brown trout, walleye and large banded sculpin respond to hellbender secretions with increased activity while small banded sculpin responded by decreasing activity. In addition, brown trout ingested more hellbender secretion-soaked food pellets than control pellets, while rainbow trout expelled secretion-soaked food pellets. Finally, larval hellbenders exhibited weak fright behavior in response to chemical stimuli from nonnative trout relative to their responses to native predatory fish stimuli. These combinations of responses indicate that predation by nonnative fishes may be a plausible hypothesis for the decline of hellbender populations in Missouri.

KEYWORDS: Predator-prey interactions; Amphibian declines; Introduced trout; *Cryptobranchus alleganiensis*; hellbender

This abstract is approved as to form and content

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Alicia Mathis
Chairperson, Advisory Committee
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CHAPTER I. OVERVIEW

Fish Predation

Predation has a profound influence on the evolution of life history characteristics (Lima and Dill 1990). Nothing impacts future reproductive success as much as being eaten, and this extreme pressure has favored adaptations in prey species that have resulted in predation avoidance (Lima and Dill 1990). Some adaptations, such as cryptic coloration and immobility, prevent predators from ever detecting prey (Wassersug 1971; Woodward 1983). Some prey species have developed unpalatability or toxicity and can advertise their unprofitabilty via aposematic behaviors (Voris and Bacon 1966; Wassersug 1971; Brodie et al. 1978). When a predator detects or approaches prey, flight and protean movements can be utilized to allow escape from the predator's perceptual field (Taylor 1983). For a prey species to efficiently evade predation, it must first be capable of detecting predators. For aquatic species, chemically-mediated predator detection is a common antipredator mechanism (reviewed in Kats and Dill 1998). Prey can use chemical cues to distinguish between predators and nonpredators, assess the level of predation risk (i.e. high/low), determine predator diet and assess predation risk from the safety of refugia (reviewed in Kats and Dill 1998).

In aquatic habitats, prey are particularly vulnerable to predation from a wide array of potential predators. Predators such as birds and mammals have the ability to strike from terrestrial habitats, while aquatic invertebrates, fishes, amphibians, reptiles and mammals may strike from within the aquatic habitat. Predatory fishes are often the primary predator in many aquatic habitats, and are considered the most destructive

predators of amphibians (Petranka et al. 1987; Stebbins and Cohen 1995), including the aquatic larval stage that is highly susceptible to fish predation (Duellman and Trueb 1986). Numerous species have escaped fish predation by breeding in ephemeral or fishless pools (Kats et al. 1988). Other species have adapted to cohabitate with fishes via a host of antipredator mechanisms (Petranka et al. 1987).

Fishes have been deliberately introduced to countless ephemeral and permanent aquatic habitats for various biological and economic reasons (Fausch 1988; Goodsell and Kats 1999). Trout (Family: Salmonidae) have been a particularly common choice for human introductions because of their popularity for recreational fishing; in some cases trout have been implicated in the decline of local amphibian assemblages (Burger 1950; Macan 1966; Bradford 1989; Braña et al. 1996; Drost and Fellers 1996; Tyler et al. 1998; Bosch et al. 2000; Knapp and Matthews 2000; Gillespie 2001; Vredenburg 2004; Welsh et al. 2006). Introduced trout have had a profound effect on historically fishless montane lakes and streams (Macan 1966; Bradford 1989; Braña et al. 1996; Drost and Fellers 1996; Tyler et al. 1998; Knapp and Matthews 2000; Vredenburg 2004; Welsh et al. 2006). Antipredator adaptations typically evolve only when the predator and prey have co-occurred over evolutionary time (Kats and Ferrer 2003), and amphibians in fishless habitats have not evolved antipredator mechanisms to fish predation (Gillespie 2001; Kats and Ferrer 2003).

When amphibians have co-evolved with fishes, predator recognition is often innate and species-specific (Sih and Kats 1994; Kiesecker et al. 1996; Lefcort 1996; Laurila et al. 1997; Whitham and Mathis 2000; Gallie et al. 2001). In these habitats, amphibians may be exposed to a wide array of predatory and nonpredatory fishes, and the

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ability to discriminate between fishes that are dangerous and those that are not could have substantial fitness consequences. For example, an amphibian that hides every time that it detects a minnow would lose valuable foraging time. Trout have also been stocked in permanent aquatic habitats containing predatory fishes (Bosch et al. 2000; Gillespie 2001), and could potentially influence amphibian populations in these habitats. Although amphibians have evolved antipredator responses to predation by native fishes, they may be ill-equipped to avoid trout predation due to the lack of evolutionary history with this introduced predator (Bosch et al. 2000; Gillespie 2001).

Hellbender Declines

The hellbender (*Cryptobranchus alleganiensis*) is a fully aquatic salamander native to the eastern United States. They inhabit cold, spring-fed streams with high dissolved oxygen concentrations and low turbidity (Smith 1907; Bishop 1941; Nickerson and Mays 1973). The eastern subspecies (*C. a. alleganiensis*) ranges from southwestern New York southward to northern Georgia and Alabama and west to Missouri (Nickerson and Mays 1973). The Ozark subspecies (*C. a. bishopi*) can only be found in the Black River and White River drainages in southern Missouri and northern Arkansas (Firschein 1951; Nickerson and Mays 1973).

Adult hellbenders are the largest salamanders in North America, reaching 70 cm (Nickerson and Mays 1973). They are primarily nocturnal, remaining under large flat rocks during the day and foraging on crayfish, other invertebrates and small fishes at night (Nickerson and Mays 1973). Hellbenders are long-lived, reaching sexual maturity

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after 4–6 yrs and living 30 yrs or more (Smith 1907; Bishop 1941; Nickerson and Mays 1973).

Unfortunately, global amphibian populations have declined in recent years (Houlahan et al. 2000; Kiesecker et al. 2001; Semlitch 2003), and Missouri's hellbender populations have not remained immune to this trend. Hellbender population data were collected in Missouri during the 1970's, 1980's, and late 1990's. By the late 1990's, all eastern and Ozark hellbender populations in Missouri declined by 75% to 85% from their early 1970's levels (Wheeler et al. 2003). In addition, a shift in age structure occurred, with fewer young individuals captured in the late 1990's (Wheeler et al. 2003). The decline in numbers and apparent recruitment failure could result from higher than historical adult mortality, reproductive failure, high egg mortality, low larvae survivorship or some combination of these factors. The cause of decline is likely complex, but could include siltification and habitat destruction, agricultural and livestock pollution, toxin exposure, introduced disease (e.g. chytridiomycosis), climactic changes, and the introduction of exotic species (Stebbins and Cohen 1995; Semlitsch 2003; Wheeler et al. 2003).

Trout have similar habitat requirements as hellbenders and have been introduced into most of the hellbenders' range in the United States (MacCrimmon 1971). In Missouri, trout either reproduce naturally or are stocked in almost all rivers historically occupied by hellbenders (Alsup 2005; Missouri Department of Conservation 2006a). Missouri's hellbenders have no native trout predators, and the impact trout have had on the recent hellbender decline is unknown.

General Approach

Due to the current population status of Missouri's hellbenders, direct examination of the behavioral interactions between hellbenders and fishes is inadvisable because such interactions could lead to injury or death of the hellbenders. I used behavioral bioassays to examine possible predator-prey interactions between hellbenders and fishes indirectly. Because both predator and prey play a vital role in the success or failure of a predation event, I examined hellbender predation from both the predator (predatory fishes) and prey's (hellbenders) perspective. An examination of both perspectives is necessary to determine whether introduced trout may have had a role in the decline of hellbender populations, and to determine at what stage (i.e. larvae, juvenile or adult) predation is most likely to occur. The following three chapters will address questions concerning the potential for trout predation on hellbenders from the perspective of both the predator and prey.

Predation from the Predator's Perspective. Chemical cues are utilized extensively by aquatic organisms (reviewed in Kats and Dill 1998), and convey a large range of information to predators and prey alike. For example, many species of fishes produce chemical alarm cues (when the skin is abraded) that warn conspecifics of immediate danger (reviewed in Chivers and Smith 1998). However, predators sometimes respond to these same cues with increased foraging behavior (Mathis et al. 1995, Harvey and Brown 2004). In Chapter II, I examined the foraging behavior of predatory fishes in the presence of a secretion (potential chemical alarm cue) produced by stressed hellbenders. A change in fish foraging behavior in the presence of this secretion would indicate avoidance or attraction to hellbenders. Even if fish predators are initially

attracted to the hellbender secretion, they still might find it distasteful once taken in to the mouth. To determine palatability of the hellbender secretion to fishes, I soaked food items (pellets) in the hellbender secretion and fed them to trout (Chapter III). My goal was to determine whether the hellbender stress secretion functions as an antipredator mechanism by either acting as a general repellent or by being unpalatable.

Predation from the Prey's Perspective. Innate predator recognition via olfactory cues is common among amphibians (Sih and Kats 1994; Laurila et al. 1997; Gallie et al. 2001), but it is unknown whether predator-naïve hellbenders can utilize olfactory cues to detect potential predators. In addition, because hellbenders lack an evolutionary history with trout, they may be unable to recognize trout as predators even if they recognize native fish predators. In Chapter IV, I exposed larval hellbenders to various fish stimuli to determine: (1) if predator recognition is innate; (2) whether larval hellbenders can distinguish between predatory and nonpredatory fishes; and (3) between native and nonnative predatory fishes.

CHAPTER II. FISH FORAGING BEHAVIOR

Introduction

The hellbender (*Cryptobranchus alleganiensis*) is a giant salamander native to the continental United States. At a maximum length of more than 70 cm, it is North America's largest salamander. This fully aquatic species lacks gills and relies on cold spring-fed waters with high dissolved oxygen concentrations for cutaneous gas exchange. Of the two sub-species, the eastern hellbender (*C. a. alleganiensis*) has a larger geographic distribution, ranging north from Georgia to southern New York and west to Missouri. The Ozark hellbender (*C. a. bishopi*) is geographically limited to southern Missouri and northern Arkansas in the Ozark Mountains.

The apparent global decline of amphibian populations has exposed a lack of long-term population studies vital to conservation. A review of 936 amphibian population studies revealed an average study duration of 6 years with a maximum of 20 years (Houlahan et al. 2000). Fortunately, hellbender population data were collected in Missouri during the 1970's, 1980's, and late 1990's. Populations of both eastern and Ozark hellbenders in Missouri declined by 70% between the early 1980's and late 1990's (Wheeler et al. 2003). A shift in age structure was also observed for all populations, with samples from the late 1990's yielding significantly fewer young individuals (Wheeler et al. 2003). This decline in recruitment could indicate reproductive failure, high egg mortality, or low larvae survivorship. Each of these potential causes could be attributed to a number of environmental and anthropogenic factors.

Factors potentially contributing to the decline of hellbender populations include increased sedimentation and eutrophication, UV-B radiation exposure, agricultural pollution (i.e. pesticides and fertilizers), livestock waste runoff, disease, parasites, climate shifts and introduced or invasive species (Stebbins and Cohen 1995; Semlitsch 2003; Wheeler et al. 2003). The decline in hellbenders can likely be attributed to multiple and interactive factors. For example, habitat alteration such as siltification from land development and agricultural practices puts pressure on remaining habitat. Agriculture and livestock production are prominent features in the mountains surrounding Ozark streams and can contribute large amounts of pesticides, fertilizers and fecal coliform bacteria into stream water. Some of these contaminants have been shown to disrupt reproductive hormones, such as estrogen, in amphibians and other species and could be negatively affecting hellbender populations (Hayes et al. 2002; Gregor et al. 2004; Solis et al. 2007). An increase in UV-B radiation and changing climate are linked to increases in the prevalence of diseases such as the fungal disease Chytridiomycosis, which has been linked to multiple amphibian population declines (Carey et al. 1999; Kiesecker et al. 2001; Middleton et al. 2001; Pounds et al. 2006). Introduction of nonnative predatory fishes have also been linked to population declines for some species. For example, introduction of nonnative trout into mountain lakes in the western United States has led to population extinctions for the yellow-legged frog (*Rana muscosa*) throughout much of its natural range (Knapp and Matthews 2000; Vredenburg 2004). More complex effects could be present if, for example, individuals that are weakened due to toxicity effect are more vulnerable to predation (Relyea and Mills 2001).

For hellbenders in Missouri, one hypothesis is that predation from fishes has played a role in population declines (Briggler et al. 2007). Introductions of game trout in Missouri may have increased predation pressure on larval, juvenile or adult hellbenders; trout may have been a particularly detrimental influence because there are no native trout in Missouri, and hellbenders would not have evolved defenses against these predators. Predation by native fishes would most likely have played a role in population declines if (1) populations of native fishes have substantially increased during the period of the decline (there is no evidence that this has occurred) or (2) negative effects caused by other environmental factors (e.g., Unger 2003) led to a decrease in condition, making hellbenders more vulnerable to predators (e.g., Relyea and Mills 2001).

Hellbenders are among the small number of amphibians that co-exist with fishes. Amphibians that co-exist with fish predators often are unpalatable to fishes (e.g., Hero et al. 2001), but there is substantial variation among fish species (e.g., Manteifel and Reshetnikov 2002; Gunzburger and Travis 2005). It is unknown whether hellbenders are palatable to either native or nonnative fishes. Adult, juvenile and larval hellbenders produce a white secretion when agitated (Nickerson and Mays 1973; personal observation). I tested the hypothesis that this secretion influences foraging by fishes. Possible responses are that presence of the secretion: (1) stimulates foraging behavior; (2) depresses foraging behavior; or (3) is neutral with respect to foraging behavior.

Methods

Collection of Secretions. Secretions were collected from four wild-caught Ozark hellbenders [mean snout-vent length (SVL) $\pm$ SE = 27.6 $\pm$ 1.4 cm] on 21 June, 9 and 19

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July 2007. Each hellbender was placed in a plastic container (38 cm L × 25 cm W × 23 cm D) with approximately 4.0 l of river water. The hellbender was agitated by being restrained in a net until the milky secretion was produced (approximately 1 min). This process was repeated four times or until a copious quantity of secretion was present in the container; the entire process usually took about 5 min. The secretion was collected in plastic jugs, stored on ice, and taken to Missouri Department of Conservation's Shepherd of the Hills Fish Hatchery (SHFH) in Branson, MO. Most of the secretion solution was then transferred to 50-ml centrifuge tubes (Fisher Scientific, Hampton NH) in 30-ml units and frozen: dechlorinated tap water was also frozen in the same manner to serve as a control stimulus. The remaining secretion (about 4 l) was left in the plastic jugs and frozen for use in another experiment (Chapter 3).

Collection of Test Fishes. I tested five species of predatory native fishes, two species of predatory nonnative fishes, and one species of nonpredatory fish (Table 1). I use "predatory" to describe species that are known to consume vertebrate prey. Most test fishes were collected in the summer of 2007 (Table 1) from the North Fork of the White River (North Fork) and were transferred immediately to SHFH. The exception was walleye, which were rare during sampling of the North Fork, and so were collected from Bull Shoals Lake. All fishes were collected by boat or backpack electro-fishing.

For the nonnative species (rainbow and brown trout), I also tested responses of hatchery reared individuals. Hatchery raised rainbow and brown trout were collected with a net from an outdoor raceway at SHFH that was somewhat larger but otherwise similar to the raceway used for the other species.

Table 1. Species of fishes tested, including assignment to predator/nonpredatory and native/nonnative categories, range of lengths for tested fishes and date collected.

Species	Predator/ nonpredator	Native/ Nonnative	Length (cm)		Date Collected
			Min	Max	
Redhorse spp. *Moxostoma* spp.	Nonpredator	Native	22.9	39.0	7 & 14 June 2007
Rainbow trout (hatchery) *Oncorhynchus mykiss*	Predator	Nonnative	19.2	29.2	3 September 2007
Rainbow trout *Oncorhynchus mykiss*	Predator	Nonnative	25.2	36.4	7 & 14 June 2007
Brown trout (hatchery) *Salmo trutta*	Predator	Nonnative	21.7	29.0	3 September 2007
Brown trout *Salmo trutta*	Predator	Nonnative	24.6	42.0	7 & 14 June 2007
Smallmouth bass *Micropterus dolomieu*	Predator	Native	15.6	35.0	7 & 14 June 2007
Ozark bass *Ambloplites constellatus*	Predator	Native	16.9	24.6	7 & 14 June 2007
Spotted bass *Micropterus punctulatus*	Predator	Native	11.5	34.0	7 & 14 June 2007
Walleye *Sander vitreus*	Predator	Native	17.5	25.6	24 Oct 2007
Banded sculpin *Cottus carolinae*	Predator	Native	8.1	16.4	14 June 2007 20 Nov 2007

All fishes (except sculpin) were placed together into an open-air outdoor raceway (15.24 m L × 1.52 m W × 0.45 m D) that was supplied with a constant inflow of well water and water from Table Rock Lake; water continually drained from the raceway (a "flow-through" system), so there was no filtration. Water temperatures ranged from 11–12°C. Fishes were fed hatchery-collected rainbow trout [mean total length (TL) = 4.8 cm, range: 4.3–5.2 cm] and floating trout pellets ad libitum; sinking pellets were provided for redhorse. Banded sculpin were placed in a 76-l aquarium with flow-through well water and were fed hatchery-collected rainbow trout (mean TL = 3.0 cm, range: 2.8–3.2 cm) ad libitum. This research was conducted under animal use protocol 2007H.

Rationale for the Use of Trout as Prey. The overall goal of this study was to gain insight into whether secretions by hellbenders provide protection from fish predation. Although the use of larval or juvenile hellbenders as prey in this study would have provided the most direct test of this hypothesis, potentially lethal trials for large numbers of a threatened species would violate ethical concerns (see Animal Behavior Society, 1996). Note that this study required in excess of 1000 prey individuals.

We considered several alternatives to using hellbenders as prey in the study. First, we attempted to use salamander-shaped prey models; we were able to control the movement of the models in a way that mimicked the benthic movements of small hellbenders. Unfortunately, during our preliminary studies predatory fishes never approached or struck at the salamander model. We also considered using other aquatic salamanders as prey, but discarded this option because the behavior of these salamanders might be affected by exposure to secretions from other salamanders (hellbenders), which would potentially be a confounding variable. In addition, some amphibians produce

alarm secretions that can warn both conspecific and heterospecific amphibians that

predators are in the area (Chivers et al. 1997). We decided to use hatchery-reared fish as

prey because they should not respond to hellbender secretions (note: we confirmed this

assumption; see Results) and because the predatory fish were willing to strike at fish prey

in the confines of the testing tanks during our preliminary observations. We chose trout

as prey because they do not historically co-occur with hellbenders, and thus should not

have evolved responses to hellbender alarm secretions, and because they were readily

available in large numbers.

Testing Protocol: Predatory Fishes. Individual predatory fishes (N = 5-17;

Appendix 2) were exposed to one of two treatments: (1) a dechlorinated water blank

(control); or (2) milky-white secretion from agitated hellbenders, and then their foraging

behavior was quantified. Testing tanks were 76-l aquaria (73 cm L × 29 cm W × 45 cm

D) lined with black plastic on three sides to reduce external visual stressors and to

minimize visual inconsistencies among tanks. The front wall of the tank was left clear

for observations. Tanks had natural rock substrate and flow-through well water (13.3

l/min). When testing was complete, all fish were measured and placed in the holding

raceway or aquarium separate from untested fishes. Individual test fishes were placed

into test tanks and acclimated for a minimum of 48 hrs prior to testing. Two small

rainbow trout (prey, 2.8–3.2 cm) were added to the tank at the same time as the test fish

to ensure the predator would feed in the test tanks. A predatory fish was eliminated from

testing if it failed to eat both initial prey. Immediately prior to testing, the flow-through

water system was turned off and the test stimulus was thawed in a warm water bath. At

the start of a trial, the lid covering the tank was removed, and a clear plastic barrier was

placed in front of the focal test fish, separating the tank into two halves with the test fish

at the back of the tank. For most fish species, five prey (mean TL = 4.8 cm, range: 4.3–

5.2 cm) were introduced into the front of the tank with a dip net. Ten prey (mean TL =

3.0 cm, range: 2.8–3.2 cm) were used during sculpin trials. A randomly chosen test

solution (30 ml of blank or hellbender secretion) was poured into the back of the tank.

After 30 sec, the barrier was removed and the following quantitative response variables

were recorded during the 20-min observation period: (1) latency to first approach

(approach = tip of snout within 2 cm of prey); (2) latency to first strike (strike = rapid

open and close of mouth within 1 cm of a prey fish); (3) number of approaches; and (4)

number of strikes. These response variables are similar to those recorded in other studies

of fish feeding behavior (e.g., Wahl and Stein 1988, Eklov and Hamrin 1989, Harvey and

Brown 2004). Individual fishes were tested only once.

Because brown trout were extremely agitated by the introduction of the clear

barrier, it was not used in tests of this species. At the beginning of a trial, the test

stimulus was poured into the back of the tank. After 30 sec, the front of the tank's lid

was raised, and five prey, which had been placed in a 1-l beaker with approximately 200

ml of well water, were gently poured into the front of the tank. For this species,

observations were completed behind a black plastic blind 0.5 m from the front of the test

tank. Data were recorded as for the other species.

Hellbender Secretion's Effect on Prey Behavior. Because the hellbender

secretion may have altered the behavior of the prey, the hellbender secretions effect on

the prey's behavior was assessed by comparing the activity of prey exposed to

dechlorinated water blank (control) and hellbender secretion (N = 10 groups of 5 prey for

each treatment). Groups of prey fishes (juvenile rainbow trout) were tested in the same

testing tanks as used in the other trials. To assess activity of prey fishes, we drew a grid

across the front of the testing tank to indicate the top, middle and bottom thirds of the

tank and the left and right sides. A randomly-chosen stimulus solution (blank versus

hellbender secretion) was poured into the test tank in the same manner as in the other

trials, and the number of lines crossed by a randomly-chosen focal fish was recorded

during a 3-minute observation period. Activity was measured as number of lines

crossed. There was no difference in the number of lines crossed between control [mean

number of lines crossed (LC) $\pm$ SE = 24.6 $\pm$ 5.6] and hellbender secretion (mean LC =

29.7 $\pm$ 4.9) treatments (t-test; t = -0.68, P = 0.503).

Testing Protocol: Nonpredatory Fish (Redhorse). Because redhorse typically

do not consume vertebrates, their testing protocols were modified from those of the

predatory species. Redhorse were transferred to individual testing tanks, and given 100

sinking pellets (Silver Cup 1/8 inch sinking trout pellets; Nelson & Sons, Inc., Murray,

Utah). Testing occurred after a 21-day acclimation period. One week prior to testing, the

redhorse were fed 20 sinking trout pellets. Five min before testing, 20 sinking pellets

were soaked for five minutes in either dechlorinated water blank (control) or hellbender

secretion. Excess liquid was drained, soaked pellets were placed at the front of each test

tank and observations were made for 1 hr. None of the fish consumed any pellets during

these initial observations, so the pellets were left in the tank and checked the following

morning. The number of pellets consumed during the night was recorded.

Statistical Methods. For each species, differences between treatments were

assessed using the nonparametric Mann-Whitney U-test because data were not normally

distributed. Two-sample t-tests were performed to ensure fish length did not differ

between treatments of any species (Appendix 1).

Results

Smallmouth bass, spotted bass, Ozark bass, wild-caught rainbow trout and

hatchery-collected rainbow trout were neither stimulated nor inhibited to forage in the

presence of the hellbender secretion (Fig. 1–4; Appendix 2). The presence of hellbender

secretions also did not affect the number of pellets consumed by redhorse (control: mean

± SE, 13.1 ± 3.28, N = 9; hellbender: 17.1 ± 2.86, N = 7; Appendix 2).

The behavior of two species appeared to be stimulated by the presence of the

hellbender secretion. Walleye approached prey significantly faster and more often in the

hellbender treatment compared to control ($P < 0.01$, Fig. 1 and $P < 0.01$, Fig. 3,

respectively; Appendix 2). Similarly, hatchery-collected brown trout also approached

prey significantly faster and tended to approach more often (although this variable was

not statistically significant) in the presence of hellbender secretion compared to control (P

< 0.01, Fig 1 and $P = 0.06$, Fig. 3, respectively; Appendix 2). A similar trend, although

not statistically significant, occurred within wild brown trout ($P = 0.22$, Fig. 1 and $P =$

0.24, Fig. 3, respectively; Appendix 2).

Behavior of sculpin was highly variable and qualitatively appeared to vary with

body size, which also showed a high degree of variability (range = 8.1–16.4 cm).

Because of this variation, I assigned sculpin to two size classes (small: 8.1–9.6 cm; large:

12.1–16.4 cm) and performed a 2-Way ANOVA, with body size (small or large) and

treatment (hellbender secretion or control) as the two factors; assumptions for parametric
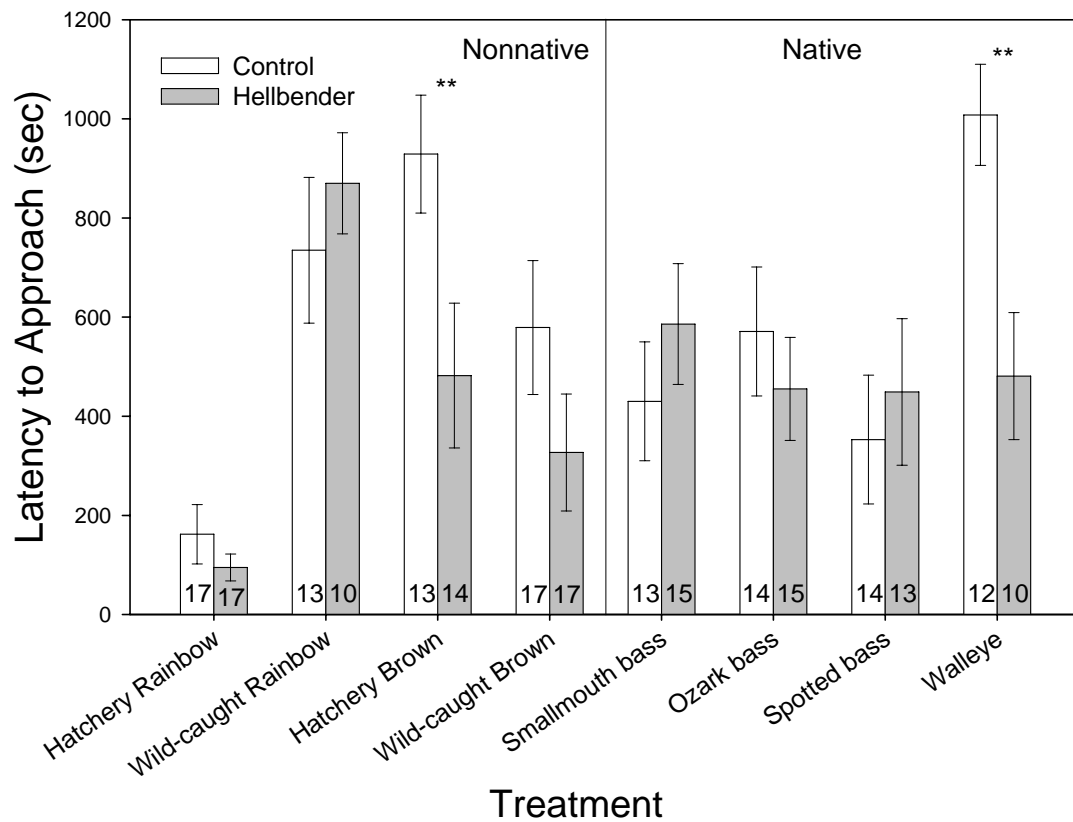
Figure 1. Mean (± SE) latency to first approach for eight predatory fish treatments exposed to dechlorinated water (control) and hellbender secretions. Number inside bar is N, **P < 0.05, all others P > 0.10.
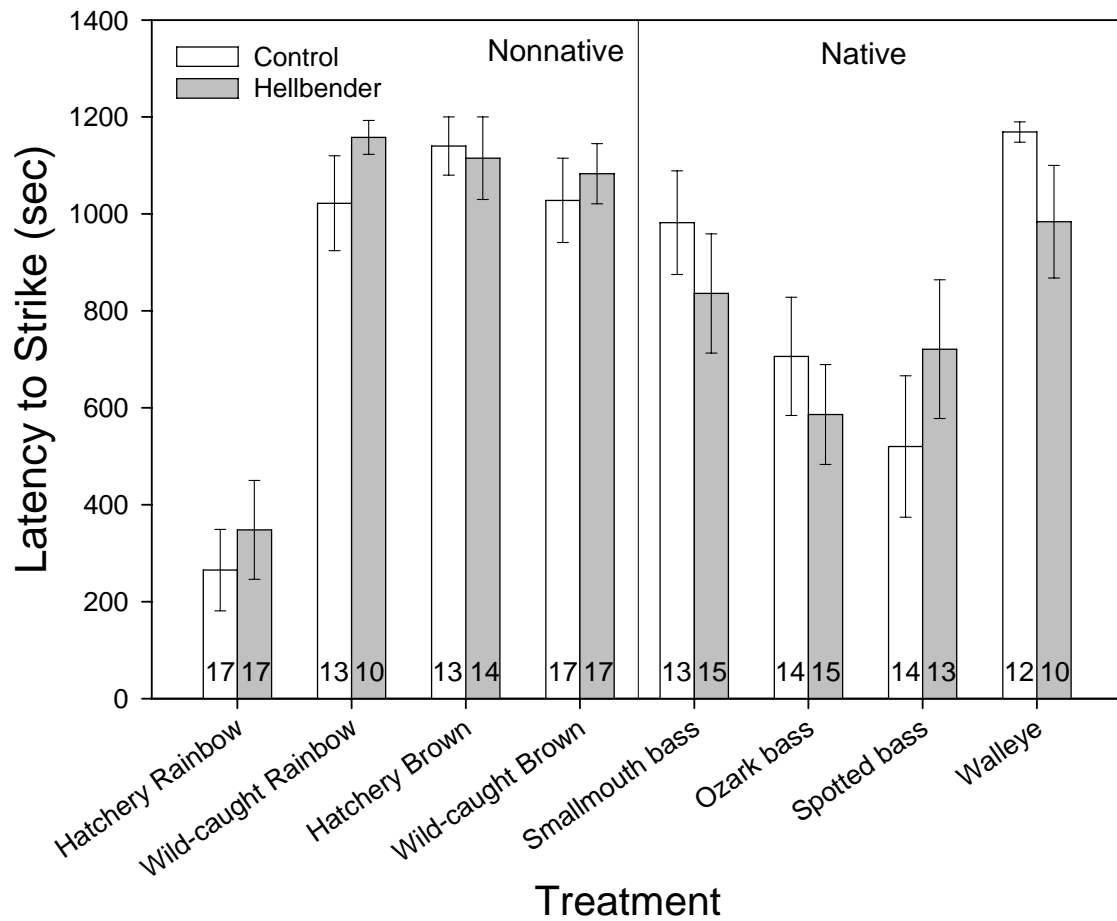
Figure 2. Mean (± SE) latency to first strike for eight predatory fish treatments exposed to dechlorinated water (control) and hellbender secretions. Number inside bar is N, all P > 0.10.
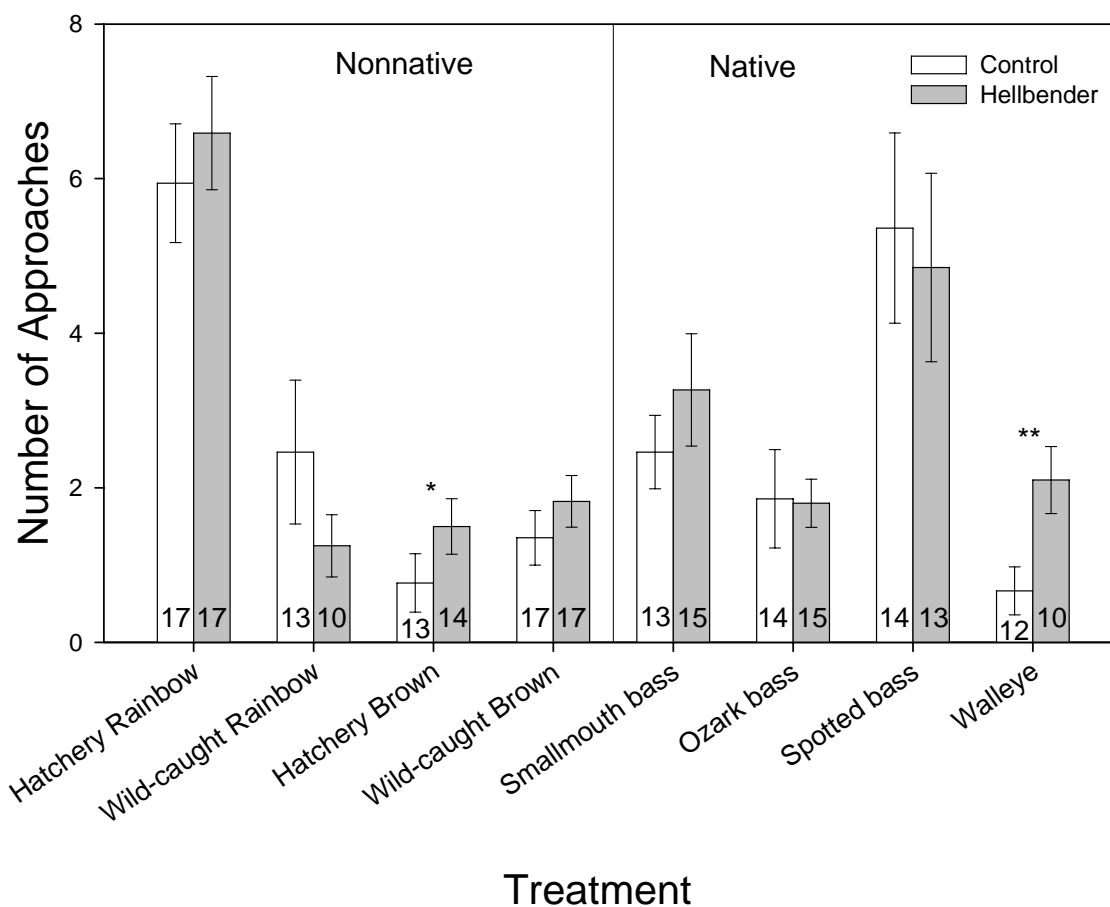
Figure 3. Mean (± SE) number of approaches in 20 minutes for eight predatory fish treatments exposed to dechlorinated water (control) and hellbender secretions. Number inside bar is N, **P < 0.05, *P < 0.10; all others P > 0.10.
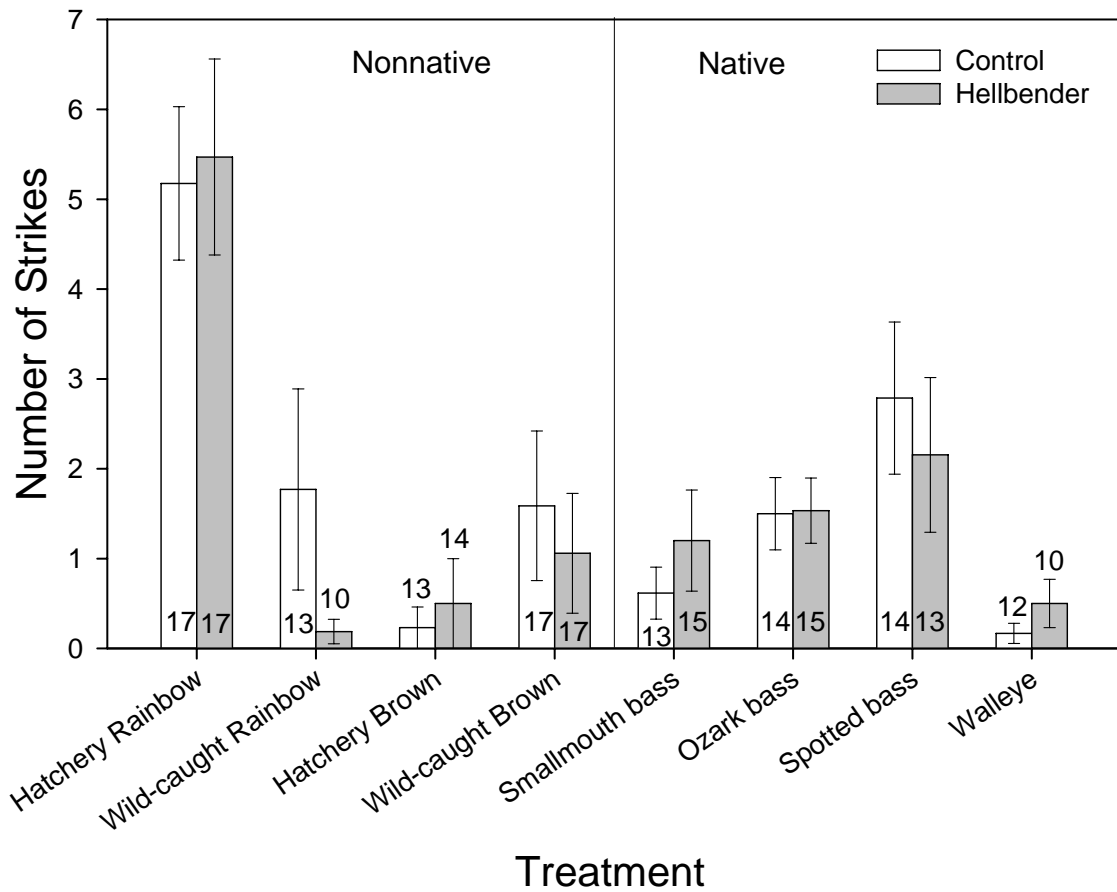
Figure 4. Mean (± SE) number of strikes in 20 minutes for eight predatory fish treatments exposed to dechlorinated water (control) and hellbender secretions. Number inside or above bar is N, all P > 0.10.

tests were met by the data. There were no significant main effects of treatment or size for any response variable (all $P > 0.17$). However, there were significant or near-significant interactions between treatment and size for latency to approach ($P = 0.07$, Fig. 5; Appendix 2), latency to strike ($P = 0.05$, Fig. 6; Appendix 2) and number of strikes ($P = 0.07$, Fig. 7; Appendix 2). In all these cases the foraging of small sculpin was inhibited in the presence of hellbender secretion, whereas large sculpin were stimulated to forage.

Discussion

  Attraction to Prey in the Presence of Hellbender Secretions. Two species were stimulated to approach prey in the presence of the hellbender secretions: brown trout and walleye. Attraction to prey is the first step in a successful predation event (Lima and Dill, 1990). Although "approach" did not always lead to predation attempts in the confined areas of the testing tanks, increased levels of approach would likely lead to increased predation in natural habitats. It is not clear whether the fishes are attracted to the chemicals associated with the hellbender secretion specifically or if they are responding to a novel scent. However, in either case, attraction to the secretions should increase detection of hellbenders and, thus, potentially lead to predation.

  Brown trout are opportunistic and vary their diets according to the availability of food resources within a stream (Cada et al. 1987, Bridcut and Giller 1995, Pflieger 1997). The hypothesis that brown trout recognized hellbender scent, per se, seems unlikely

because both wild-caught and hatchery-collected trout were attracted (stimulated to

approach) to prey in presence of the hellbender secretions (although the data for wild-
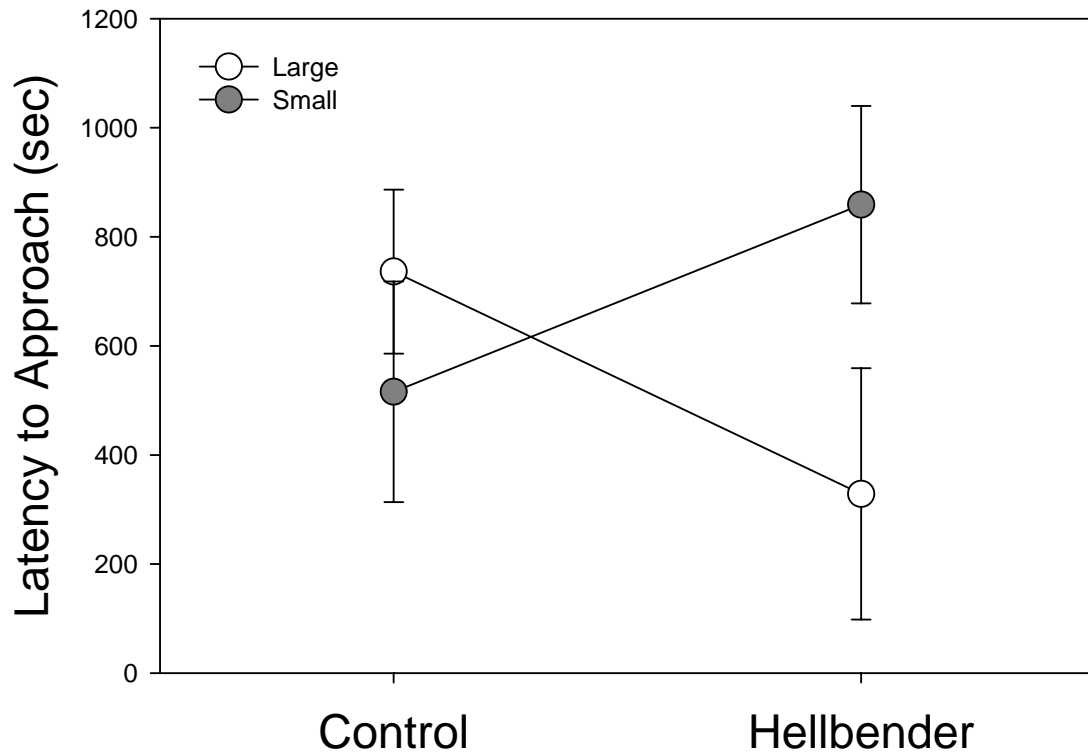
Figure 5. Mean (± SE) latency to first approach for large and small sculpin exposed to dechlorinated water (control) and hellbender secretions. Interaction effect: P = 0.07. Connecting lines are included to indicate trends
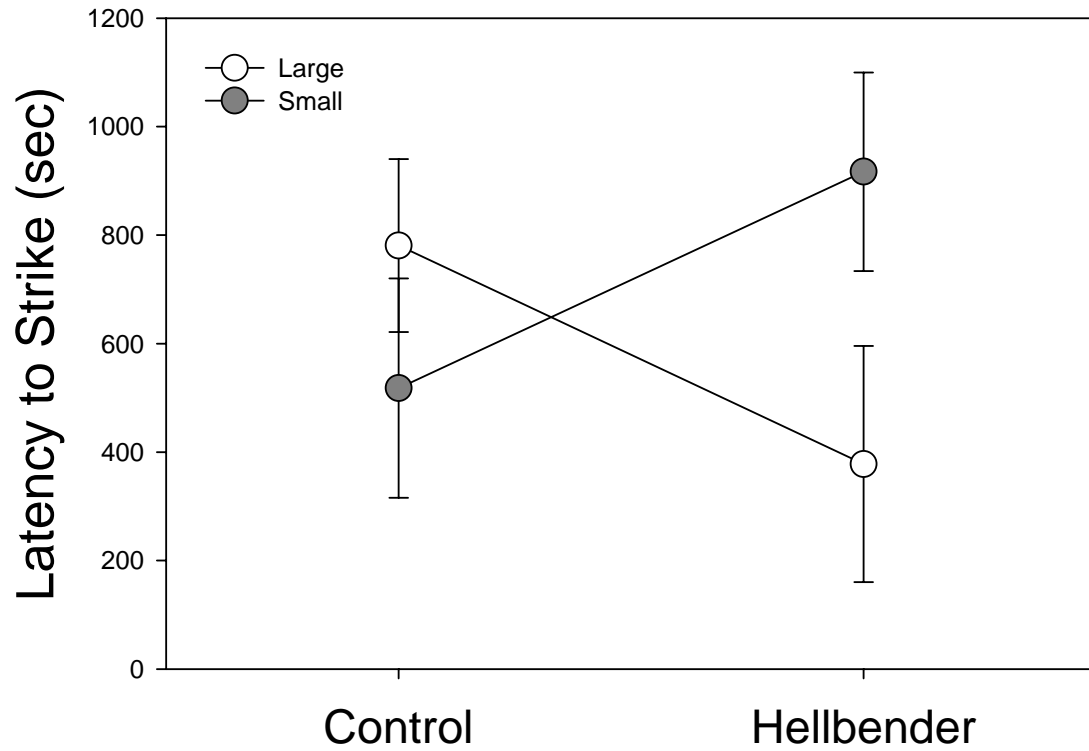
Figure 6. Mean (± SE) latency to first strike for large and small sculpin exposed to dechlorinated water (control) and hellbender secretions. Interaction effect: P = 0.05. Connecting lines are included to indicate trends
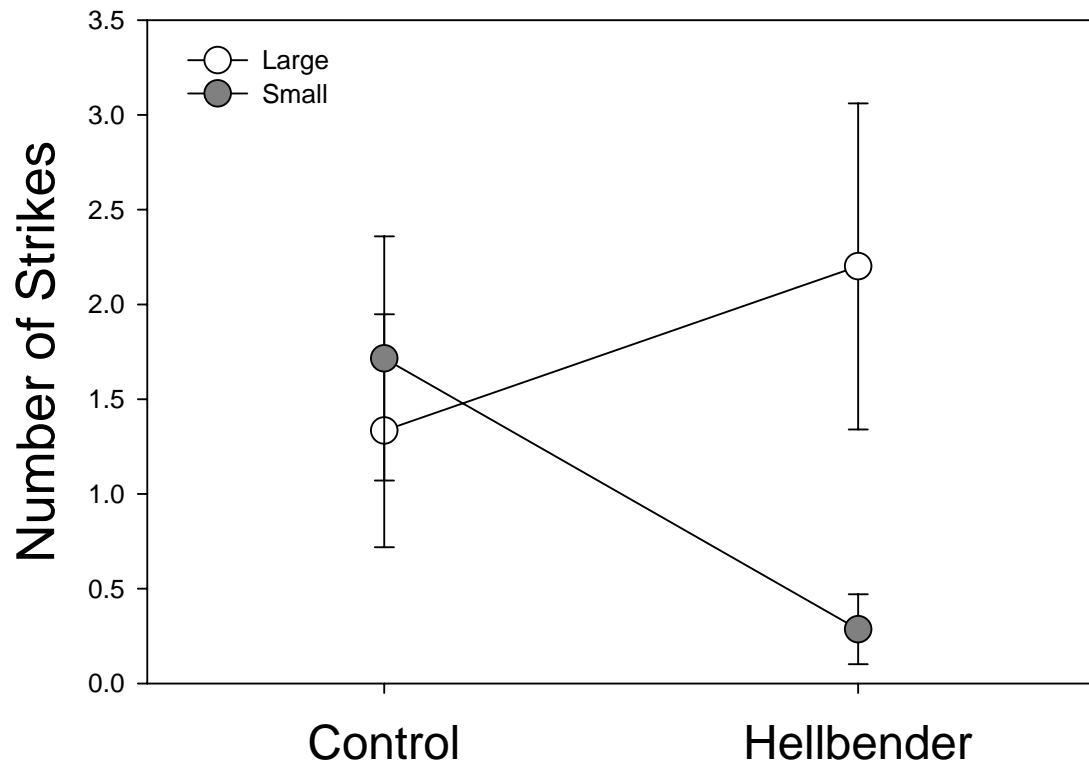
Figure 7. Mean (± SE) number of strikes for large and small sculpin exposed to dechlorinated water (control) and hellbender secretions. Interaction effect: P = 0.07. Connecting lines are included to indicate trends

caught trout showed only a nonsignificant trend). In general, trout do use chemical cues

(scents) to detect prey (e.g., Olsén et al 1986, Bres 1989). A new odor may indicate a

potential new food resource that should be investigated. Brown trout and hellbenders are

primarily nocturnal predators (Elliot 1973, Nickerson and Mays 1973, Pflieger 1997,

Young 1999), so there is strong potential for interactions to occur between these two

species. Introduced brown trout have been shown to reduce populations of riverine

amphibians, whereas predation by native fishes showed little effect (Gillespie 2001).

Although most studies of effects of trout on amphibian populations have been studies of

anurans (see references in Kats and Ferrer 2003), predation by trout has also been shown

to influence salamander populations. In New England streams, salamander densities

were higher above waterfalls where brook trout (a native predator) were absent than in

area's containing trout; artificial addition of brook trout to these areas reduced

salamander densities after just one year (Barr and Babbitt 2007). Responses of wild-

caught trout were weaker than those of hatchery-collected trout; more research is needed

to explain differences in the behavior of wild-caught and hatchery-collected brown trout.

Walleye also approached prey faster and more often in the presence of hellbender

secretions. Like brown trout, walleye also are primarily nocturnal predators (Kelso 1978)

and so share activity periods with hellbenders. However, opportunities for interactions

between hellbenders and walleye may be limited because hellbenders tend to occupy

riffle areas in the streams (Nickerson and Mays 1973), and walleye tend to prefer deeper

pools (Pflieger 1997). At the time of the study, density of walleye in the North Fork River appeared to be relatively low (2 captures in two all-day sampling events).

Size-Dependent Responses of Sculpin. Given that sculpin are ambush predators that occur in benthic microhabitats where they are extremely likely to come into contact with hellbenders, responses of sculpin were of particular interest. Preliminary examination of the data indicated that sculpin responses to the hellbender secretions appeared to be dependent on body size, so I made a post-hoc decision to consider body size in the analysis for this species. I found significant or near significant size-dependent effects on three of the response variables: latency to approach, latency to strike, and number of strikes. Small sculpin showed decreased foraging activity in the presence of the hellbender secretions: reduced activity is a common antipredator response of sculpin (Chivers et al. 2001). Although the primary prey of hellbenders is crayfish, they also occasionally consume small fishes including sculpin (Nickerson and Mays 1973, personal observation). Field data from an extensive study (Cooper 1975) of sculpin stomach contents support the hypothesis that small sculpin are not likely to be significant predators of hellbenders. Cooper (1975) examined the stomach contents of 521 "small" (2.6-10.0 cm) mottled sculpin (*Cottus bairdi*) and 18 "small" (4.0-12.6 cm) banded sculpin (*Cottus carolinae*) from the North Fork River between July 1970 and May 1971 (including a time period and area of extensive hellbender reproductive activity). She found no evidence of consumption of hellbender eggs or larvae.

In contrast, large sculpin showed increased foraging activity in the presence of hellbender secretions. Hellbenders are gape-limited predators, and so large sculpin may be relatively safe from predation by hellbenders. The presence of a hellbender (as

indicated by the secretions), might indicate an opportunity to consume hellbender eggs, larvae or juveniles. Similar size-based ontogenetic shifts in response to chemical cues have been shown for other species of fishes, including largemouth bass (Brown et al. 2001) and pumpkinseed sunfish (Marcus and Brown 2003).

Neutral Responses to Hellbender Secretions. None of the native predators besides walleye and sculpin altered their activity or feeding behavior in the presence of hellbender secretions. Hellbender secretions also did not affect the behavior of either hatchery-collected or wild-caught rainbow trout. However, there was one noticeable difference in behavior between hatchery-collected rainbow trout and other predatory fishes: hatchery-collected rainbow trout were much quicker to approach and strike, and struck more frequently than wild-caught rainbow trout or any of the other species that we tested. The reason for this difference is not clear, but we hypothesize that being hatchery-reared resulted in their being more strongly habituated to foraging in captivity. However, long-term captivity did not lead to increased foraging activity by brown trout (Fig.'s 1-4).

Interestingly, neither wild-caught nor hatchery-collected rainbow trout altered their foraging behavior in the presence of hellbender secretions. The reason for the difference between the response of rainbow and brown trout are not known. Both species eat a variable diet that can include amphibians. Unlike brown trout, benthic feeding by rainbow trout typically occurs diurnally (Sánchez-Vázquez and Tabata 1998), so they may rely more heavily on visual cues than on chemical cues for benthic feeding.

CHAPTER III: HELLBENDER PALATABILITY

Introduction

Of the potential antipredator mechanisms exhibited by amphibians, noxious skin secretions are the most effective against potential predators (Brodie 1978; Brodie et al. 1979). Many species produce toxic and irritating skin secretions as adults (Brodie et al. 1979), but the larvae of many salamanders are palatable to various predators (Formanowicz and Brodie 1982, Petranka 1983, Kats et al. 1988). The amphibian integument undergoes complex changes during metamorphosis (Duellman and Trueb 1986), and although most larval salamanders produce skin secretions (Duellman and Trueb 1986), toxic and distasteful secretions generally do not develop until after metamorphosis (Formanowicz and Brodie 1982).

Adult hellbenders are large amphibians and probably have few predators due to their large body size (Nickerson and Mays 1973). In addition, when stressed or being captured, metamorphosed hellbenders often produce a milky secretion that is distasteful when applied to the tongue (Brodie 1971; personal observation), and the secretion may be unpalatable to predators. In contrast, larval and juvenile hellbenders are probably highly vulnerable to predation due to their small size and slow developmental rate (Nickerson and Mays 1973). It is unknown exactly when the ability to produce secretion develops in hellbenders. During my laboratory observations, young hellbender larvae (0–9 weeks post hatching) did not appear to produce the secretion (personal observation), and may be incapable of doing so. However, 25-week-old larvae produced copious amounts of a milky secretion that was similar in appearance and tasted similar to the adult secretion

(Adam Crane, personal communication; personal observation). Hellbenders, including

larvae, typically produce the secretion as soon as they are handled or stressed (personal

observation), and it is likely that they would also produce the secretion during an actual

predation event. Because hellbenders are completely aquatic, fishes are probably the

most important predators of hellbenders and their larvae; however, the relative

palatability of the hellbender secretion to various fishes has not been studied.

Hellbender populations have declined over the past several decades (Wheeler et

al. 2003). Both the eastern hellbender (*C. a. alleganiensis*) and Ozark hellbender (*C. a.

bishopi*) are listed as state endangered in Missouri (Missouri Department of Conservation

2006b), and the Ozark hellbender is a candidate for federal listing (USFWS 2007).

Because feeding larval or juvenile hellbenders to potential predators is unethical due to

their current population status, I tested the palatability of the hellbender secretion to fish

predators by soaking food pellets in the secretion. I chose rainbow trout (*Oncorhynchus

mykiss*) and brown trout (*Salmo trutta*) because they have been introduced into many

streams where hellbenders live and because they feed readily on food pellets in captivity.

Methods

At the conclusion of testing the foraging behavior of rainbow and brown trout in

the presence of hellbender secretion (Chapter 2), all remaining prey were removed from

test tanks and the predatory fish were acclimated for an additional 24 h. Prior to testing,

Silver Cup floating trout pellets (1/8 inch or 0.31 cm; Nelson & Sons, Inc., Murray, Utah)

were soaked in 2 l of hellbender secretion or 2 l of dechlorinated water blank (control) for

1 h. At the completion of soaking, pellets were removed from the treatment solution, transferred to plastic containers and frozen at -6°C.

Immediately prior to testing, the inflow of well water was shut off and treatment pellets were thawed by placing the containers in a warm-water bath. To ensure that the test fish in the secretion-soaked pellet treatment had no prior experience with hellbender secretion during previous trials, each test fish was assigned the opposite treatment it had been assigned during the live-prey foraging study (Chapter 2). Ten pellets from the appropriate treatment were introduced into the middle of the test tank, and the following quantitative response variables were recorded during a 5-min observation period: (1) the number of pellets consumed; and (2) the number of pellets expelled from the mouth. Individual fishes were tested only once. This research was conducted under animal use protocol 2007H. Data for each species were analyzed using Kruskal-Wallis nonparametric ANOVAs, because parametric assumptions were not met.

Results

Rainbow trout consumed significantly more control pellets than hellbender pellets ($H = 5.21$, $P = 0.022$; Fig. 8) and expelled more hellbender pellets than control pellets ($H = 5.60$, $P = 0.018$, Fig. 9). In contrast, brown trout did not differ between hellbender and control treatments in terms of either number of pellets consumed ($H = 0.17$, $P = 0.677$, Fig. 8) or expelled ($H = 2.80$, $P = 0.094$; Fig. 9).
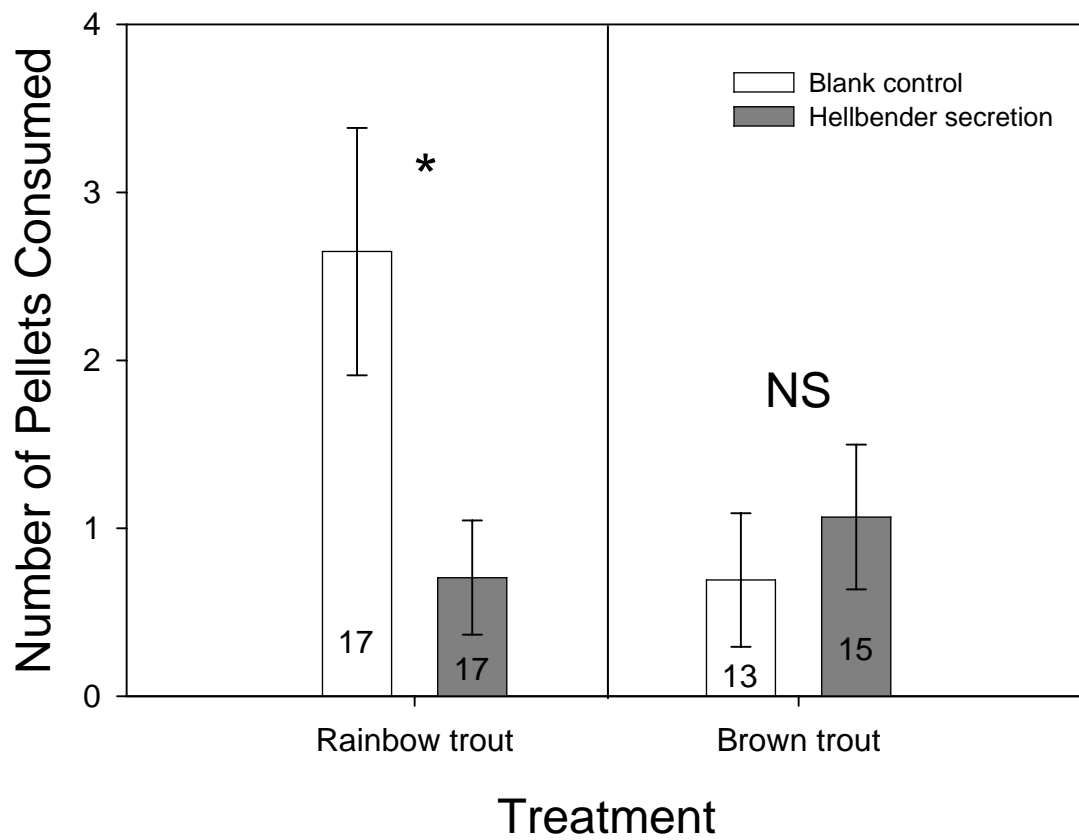
Figure 8. Mean (± SE) number of pellets consumed by rainbow and brown trout in blank control (white bars) and hellbender secretion (gray bars) treatments. Number on bar is N. Kruskal-Wallis ANOVA: *: P = 0.022; NS: P = 0.677.
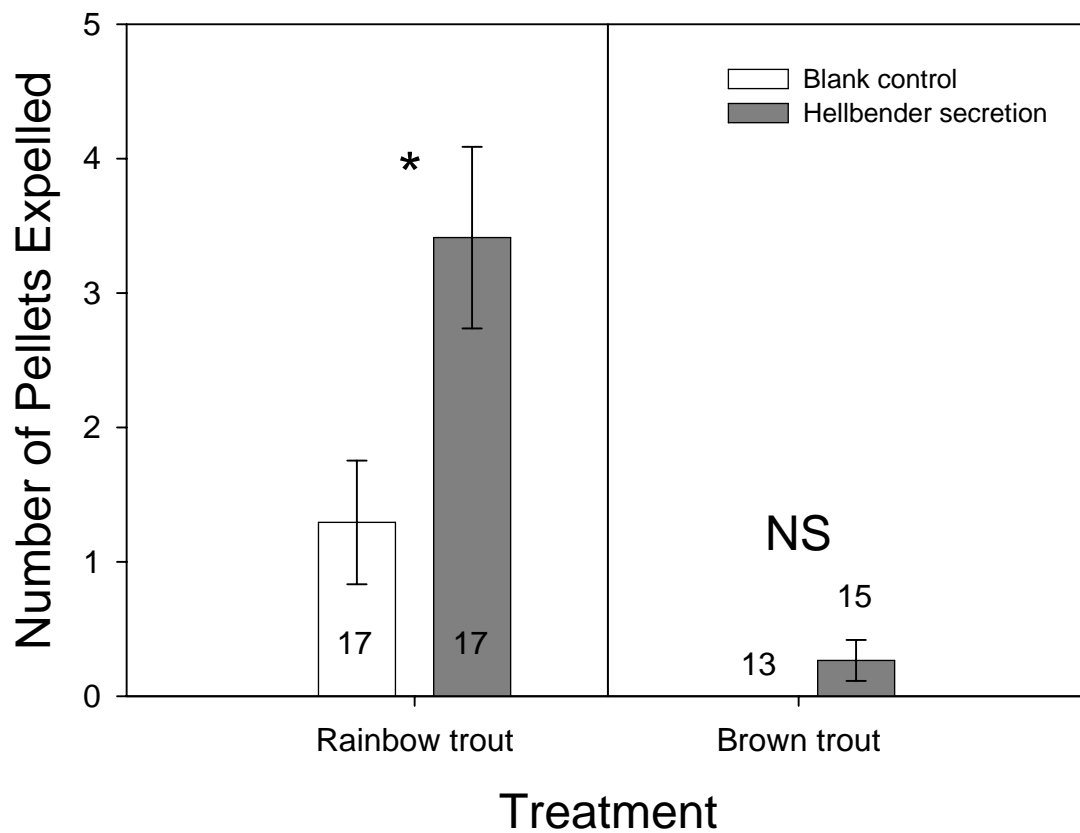
Figure 9. Mean (± SE) number of pellets expelled from the mouth by rainbow and brown trout in blank control (white bars) and hellbender secretion (gray bars) treatments. Number on or above bar is N. Kruskal-Wallis ANOVA: *: P = 0.018; NS: P = 0.094.

Discussion

Rainbow trout and brown trout differed in their response to pellets soaked with hellbender secretion versus the control-soaked pellets. Rainbow trout responded by expelling more pellets and consuming fewer pellets when they were soaked in hellbender secretion. These data indicate that rainbow trout found the hellbender secretion to be distasteful. However, brown trout did not distinguish significantly between the two types of pellets and actually consumed more pellets soaked in the hellbender secretion than in the control stimulus (although this trend was nonsignificant). Therefore, brown trout do not appear to be deterred from consuming pellets soaked with the hellbender secretion, and thus, may not find hellbenders to be distasteful.

Although noxious secretions are a potent antipredator mechanism, different predators can respond differently to the noxious secretions of amphibians. For example, adult rough-skinned newts (*Taricha granulose*) maintain great quantities of the neurotoxin tetrodotoxin (TTX) in their skin (Brodie 1968). The neurotoxin is potent enough to kill various avian, mammalian, reptile and amphibian predators within minutes following injection or ingestion (Brodie 1968). Nevertheless, several species of garter snakes (*Thamnophis* spp.) have developed TTX resistance, and will readily consume adult newts with little or no locomotory effects (Brodie 1968; Brodie and Brodie 1990; Hanifin et al. 2008).

Differential predation of prey by native fishes and introduced trout has also been observed. In Australia, tadpoles of two stream-breeding frogs have apparently developed unpalatability as an antipredator mechanism. Two native fishes, the mountain galaxias (*Galaxias olidus*) and two-spined blackfish (*Gadopsis bispinosus*), preyed heavily on a

lotic tadpole species, but ate few or none of the lentic tadpoles (Gillespie 2001). In
contrast, nonnative trout did not find the riverine tadpoles unpalatable, and preyed upon
all three species (Gillespie 2001).

If rainbow trout find hellbenders to be unpalatable, one or two encounters
between these species may be sufficient for avoidance behavior to become established.
On the other hand, if hellbenders are palatable to brown trout, then predation on
hellbenders would not decline with experience. Increased predation on this long-lived
species following introduction of nonnative trout cannot be ruled out as a cause of the
hellbender population declines in Missouri.

CHAPTER IV. BEHAVIOR OF LARVAL HELLBENDERS

Introduction

Many plant and animal species have drastically expanded their ranges in recent decades, coinciding with the global dispersion of humans (Mooney and Cleland 2001). Species are often purposely transported for human use (Goodsell and Kats 1999; Mooney and Cleland 2001), or accidentally moved to new regions (Fritts and Rodda 1998). These "introduced" species often cause extensive damage to native flora and fauna in a variety of ways including, transfer of novel pathogens, competition for resources (i.e. food, nesting sites, etc.), hybridization and predation (Meffe and Carroll 1997). Introduced species that negatively impact native biota become "invasive" species (Richardson et al. 2000). Some species have specific characteristics making them well suited to invasion. These characteristics include long life span, high dispersal rate, large genetic variability, broad native range, generalist diets and habitat, and high reproductive potential (Meffe and Carroll 1997).

Some terrestrial ecosystems have seen their native fauna decimated by invasive species. The domesticated cat (*Felis catus*) is a particularly destructive invasive species and has been introduced throughout the world (Nogales et al. 2004). Feral cats are opportunistic foragers, quickly establishing themselves as dominant predators and consuming native species across many taxa (Kirkpatrick & Rauzon 1986; Paltridge et al. 1997; Courchamp and Sugihara 1999). Nowhere is the negative impact of introduced predators on native fauna so evident than island ecosystems where a lack of predators and competitors often contributes to the success of invaders (Meffee and Carroll 1997). The

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brown treesnake (*Boiga irregularis*) was accidentally introduced to Guam in the 1950's

and resulted in the extinction of 10 bird, 2 mammal and 6 reptile species (Fritts and

Rodda 1998).

Aquatic ecosystems have also been heavily impacted by introduced species.

Crayfish, for example, are often used as live prey for sport fishing and many species have

been introduced by anglers releasing unused bait (Ludwig and Leitch 1996). Many

aspects of introduced crayfish ecology, including their generalist lifestyle, high

reproductive potential and aggressive behavior, make them good invaders (Kats and

Ferrer 2003). Bullfrogs (*Rana catesbeiana*) have been translocated to many regions of

the globe as a food source for humans (Hayes and Jennings 1986; Kats and Ferrer 2003).

Bullfrogs are dietary generalists and negatively impact native organisms by competing

with them for food or via direct predation (Moyle 1973; Hayes and Jennings 1986).

Amphibian communities in particular have been devastated by bullfrog introductions. In

the western United States, several species of amphibians have been extirpated from much

of their natural ranges primarily due to bullfrog introductions (Moyle 1973; Kiesecker

and Blaustein 1997; Kiesecker and Blaustein 1998; Lawler et al. 1999).

Of all taxonomic groups of invaders, fishes have proven to be the most deadly to

amphibians (Stebbins and Cohen 1995). Fishes are common predators of amphibian

eggs, larvae and adults (Duellman and Trueb 1986) and are considered the most

destructive predators of amphibian larvae (Petranka et al. 1987). Amphibians that co-

exist with fishes have evolved a range of antipredator defenses including cryptic

coloration (Wassersug 1971), chemical repellents (Voris and Bacon 1966; Wassersug

1971; Brodie et al. 1978), reduced activity (Woodward 1983), changes in activity patterns

(Taylor 1983), and the ability to detect fishes through chemical cues (Petranka et al. 1987; Kats 1988; Stauffer and Semlitsch 1993). Predation avoidance mechanisms only evolve between predators and prey that co-exist over evolutionary time, and amphibians often have no evolutionary history with introduced fish predators because they frequently occur in fishless habitats (Kats and Ferrer 2003). Fishes are introduced for a variety of reasons, including serving as biological controls, providing increased food resources for humans and providing recreational fishing opportunities for anglers (Fausch 1988; Goodsell and Kats 1999). Many species of salmonids, such as trout, have been extensively stocked for recreational fishing, with little consideration of their potential effects on native species (Fausch 1988).

Trout are effective predators and can profoundly affect food webs in habitats where they have been introduced (Powers 1990; Flecker and Townsend 1994; Townsend 1996, 2003). Trout are stocked throughout the world and have been reported to negatively impact amphibian communities. For instance, introduction of brown trout (*Salmo trutta*) to a British lake drastically decreased the number of ranid and bufonid tadpoles (Macan 1966). In Spain, introduction of both rainbow trout (*Oncorhynchus mykiss*) and brown trout inhibited amphibian recruitment and reduced amphibian abundance and density, resulting in a regional decline of amphibians (Braña et al. 1996). Trout introductions in North America have negatively impacted numerous amphibian species. For example, in Colorado, tiger salamander (*Ambystoma tigrinum*) larvae were extirpated from ponds stocked with trout (Burger 1950). Several lines of evidence indicate that trout have dramatically decreased populations of mountain yellow-legged frogs (*Rana muscosa*) in the Pacific northwest. These frogs were found to be present in

fishless lakes in the Sierra Nevada of California, but absent from lakes containing

introduced trout (Bradford 1989); an in-depth survey of 1700 mountain lakes found that

introduced trout distributions were strongly negatively correlated with the distribution of

the mountain yellow-legged frog (Knapp and Matthews 2000). In addition, experimental

removal of trout from five lakes resulted in the recovery of frog populations within three

years (Vredenburg 2004).

Many amphibians rely heavily on chemical cues to detect predators in aquatic

environments (Kats and Dill 1998) where predators are often visually cryptic and

visibility can be obscured by obstructions, such as rocks, aquatic plants or sedimentation.

The majority of cases implicating trout in amphibian declines have occurred in

historically fishless habitats, and the subsequent decline of amphibian populations are

likely due to an absence of effective antipredator mechanisms against fishes among local

amphibians (Kats and Ferrer 2003). In at least some species, amphibian larvae from

fishless habitats, such as ephemeral ponds, do not alter their behavior in response to

chemical stimuli from predatory fishes (Kats et al. 1988; Storfer and Sih 1998),

presumably because they have no evolutionary history with fish predators (Gillespie

2001; Kats and Ferrer 2003). In contrast, in permanent aquatic habitats, amphibian larvae

often come into contact with a wide range of fish species, and some species are able to

distinguish between chemical cues of predatory and nonpredatory fish species (Kiesecker

et al. 1996; Lefcort 1996; Whitham and Mathis 2000). Recognition of predators is often

innate for larval amphibians (Sih and Kats 1994; Laurila et al. 1997; Gallie et al. 2001).

Therefore, even amphibians that co-exist with fishes may be unable to recognize a

recently introduced predatory fish due to the short time frame in which they have co-

occurred (Gillespie 2001). The effect that introduced salmonids have on amphibians that have historically co-existed with other fish taxa has not been well studied. In addition, previous studies have focused on interactions between salmonids and the larvae of terrestrial amphibians. To my knowledge, no study has been conducted examining the possible impact of salmonids on a completely aquatic amphibian.

The hellbender (*Cryptobranchus alleganiensis*) is a giant salamander native to the continental United States. It is the third largest salamander in the world, attaining a maximum length of 60 to 70 cm (Nickerson and Mays 1973). This fully aquatic species lacks gills as an adult and relies on cold spring-fed waters with high dissolved oxygen concentrations for cutaneous gas exchange (Nickerson and Mays 1973). Hellbenders are primarily nocturnal, exiting depressions under rocks and logs in the early evening to forage on crayfish, aquatic invertebrates and small fishes (Bishop 1941; Nickerson and Mays 1973). Of the two sub-species, the eastern hellbender (*C. a. alleganiensis*) has a larger geographic distribution, ranging south from New York to northern Georgia and west to Illinois and Missouri (Nickerson and Mays 1973). The Ozark hellbender (*C. a. bishopi*) is geographically limited to the White and Black River drainages of southern Missouri and northern Arkansas (Nickerson and Mays 1973).

Parental care is widespread among amphibians but the mode varies greatly among taxa (Deullman and Trueb 1986). Egg guarding is the only known mode of parental care among salamanders, and the female is usually the attendant (Deullman and Trueb 1986). The hellbender exhibits egg guarding, but it is the male that attends and defends the eggs (Smith 1912a; Alexander 1927). Male hellbenders excavate nests under large flat rocks and lead receptive females into the nest prior to oviposition (Smith 1912a; Alexander

1927; Nickerson and Mays 1973). The eggs are fertilized externally (Smith 1907; Smith 1912a) and the female is subsequently chased from the nest (Alexander 1927). As the eggs develop, the male guards them from potential predators including other hellbenders (Smith 1907; Bishop 1941) and ensures that they stay well oxygenated by rocking his body and forcing fresh water around them (Bishop 1941). The larvae hatch approximately six weeks after fertilization and are between 23 and 30 mm in total length (Smith 1912b; Grenell 1939).

Very little is known about the life history of larval hellbenders. It is hypothesized that upon hatching, larval hellbenders may bury under cobble, move under small flat stones or remain in the nest. Pitt and Nickerson (2006) conducted a study of the stomach content of larval hellbenders from the Little River in Tennessee and found a single larva contained remains of pupae and adults of several species of aquatic insects, including Megaloptera, Ephemeroptera and Diptera. In captivity, larval hellbenders readily accept brine shrimp, black worms, stonefly and mayfly larvae and various isopods and amphipods (Pitt and Nickerson 2006; James Civiello, personal communication). Larval hellbenders metamorphose 1.5–2 yrs after hatching (Grenell 1939) at 100–130 mm total length (Smith 1912b; Bishop 1941).

Due to their small size and slow developmental rate, larval hellbenders are particularly vulnerable to predation. Turtles and water snakes have been reported to prey upon hellbenders (Nickerson and Mays 1973). In addition, hellbenders are cannibalistic, often consuming eggs, larvae or juveniles (Smith 1907; Nickerson and Mays 1973; Humphries et al. 2005). Because hellbenders are unusual among amphibians by occupying permanent streams, they are vulnerable to predation by fishes, which are

common predators of amphibians (Duellman and Trueb 1986). Fishes that have been documented preying upon hellbenders include northern pike (*Esox lucius*), muskellunge (*Esox masquinongy*), smallmouth bass (*Micropterus dolomieui*) and various catfish species (Nickerson and Mays 1973).

Over the past three decades, amphibian declines have been reported on every continent on which they occur (Macan 1966; Braña et al. 1996; Lips 1999; Houlahan et al. 2000; Knapp and Matthews 2000; Gillespie 2001; Ron et al. 2003). Missouri's hellbender populations have unfortunately not escaped this trend, declining by an average of 77% between the early 1980's and late 1990's (Wheeler et al. 2003). Potential causative factors include habitat destruction, increased UV-B radiation exposure, pesticide and fertilizer pollution, toxins and endocrine disruptors, livestock waste pollution, disease and parasites, climate changes and introduced or invasive species (Stebbins and Cohen 1995; Semlitsch 2003; Wheeler et al. 2003). Hellbenders are long lived, attaining sexual maturity after 4–6 years (Smith 1907; Bishop 1941). In addition to declining numbers, a shift in age structure also was observed for all populations in Missouri, with significantly fewer young individuals sampled in the late 1990's (Wheeler et al. 2003). The apparent decline in recruitment is likely attributable to either depressed reproduction (Unger 2003) or low egg/larval survivorship. This study addresses the hypothesis that an increase in larval hellbender predation by introduced fishes has played a role in the decline. Specifically, I tested whether the response of larval hellbenders to chemical stimuli from trout is similar to their responses to native fish predators.

State-run stocking programs and private trout farms have introduced rainbow and brown trout into many streams and rivers in Missouri. Introduced trout can be found in

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all hellbender-occupied rivers, except the Jacks Fork River (Alsup 2005; Missouri

Department of Conservation 2006a; Anthony Pratt, personal communication; Nick

Girondo, personal communication). Rainbow trout were introduced to Missouri in 1882

and were naturally reproducing by 1887 (Maynard 1887). Brown trout were introduced

around 1890 and were haphazardly stocked until termination in 1936, and fingerlings

were again introduced in 1966 and stocked until 1980 (Alsup 2005). Stocking of adult

(20–30.5 cm in length) brown trout began in 1980 and resulted in the introduction of

almost 400,000 fish to hellbender populated rivers by 1999 (Alsup 2005). In 2007,

36,200 brown trout and 1,604,719 rainbow trout were stocked in all of Missouri's trout

waters (James Civiello, personal communication).

Clearly, the number of trout stocked and the density of trout per km of stream has

risen dramatically over the last 40 years. The extent of the role that these introduced

fishes have played in recent hellbender declines is unknown. Although hellbenders have

successfully co-existed with many species of predatory fish for thousands of years, there

are no native trout species in Missouri, and so Missouri populations of hellbenders have

no evolutionary history with trout. The purpose of this study was to determine whether

larval hellbenders raised in captivity can distinguish between chemical cues from (1)

predatory and nonpredatory species of native fishes, and (2) predatory native and

nonnative (trout) fishes.

Methods

Egg Collection and Larval Maintenance. Two clutches (690 eggs) of *C. a.*

alleganiensis and two clutches (136 eggs) of *C. a. bishopi* were collected from the Big

Piney and North Fork Rivers between September and November 2007 at developmental stages ranging from 17–23 (Smith 1912a, Table 2). Clutches were kept separate, and transported in aerated water to the Shepherd of the Hills Fish Hatchery (SHFH) in Branson, Missouri where they were maintained in fish egg incubation trays. Larvae of eastern hellbenders were supplied with a constant inflow of well water (16°C) that continually drained from the trays (a "flow-through" system), so there was no filtration. Upon hatching, eastern hellbender larvae (N = 466, Table 2) were transferred to covered aluminum raceways with well-water flow-through systems and no substrate. Due to possible exposure to the chytrid fungus (*Batrachochytrium dendrobatidis*) (Bd), eggs and larvae (N = 61, Table 2) of Ozark hellbenders were maintained in a 170-l polyethylene tank with a closed filtration system and no substrate. Larvae of both subspecies were not fed until the majority of the yolk sac was absorbed (approximately 4 weeks). Larvae were then fed a mixture of isopods and amphipods (collected from trout hatchery effluent) and black worms at 1000 h, Monday through Friday.

Fish Collection. Eastern hellbender larvae were tested with the following treatments: a dechlorinated water blank (control), and water from tanks containing redhorse (nonpredator control), smallmouth bass, rock bass, largemouth bass, large banded sculpin (see Chapter 2), wild-caught rainbow trout, wild-caught brown trout, hatchery-collected rainbow trout and hatchery-collected brown trout. Because few Ozark hellbender larvae hatched (Table 2), fewer treatments were used. The treatments selected for Ozark hellbenders included a blank control, and water from tanks containing wild-caught brown trout, wild-caught rainbow trout, smallmouth bass and walleye. All tests were conducted with stimulus water from fish from the larvae's river of origin.

Table 2. Larval hellbender clutches (BP = Big Piney River, NF = North Fork of the White River), date and number of eggs received at SHFH, developmental stage at collection, hatching date and number of eggs that successfully hatched.

Clutch	Date Received	Number Received	Dev stage at collection (Smith 1912b)	Hatch date	Number Hatched
BP1	17 Sept 2007	243	17	17 Oct 2007	58
BP2A	16 Oct 2007	447	23	17 Oct 2007	192
BP2B	16 Oct 2007		20-21	3 Nov 2007	216
NF1	1 Nov 2007	23	19-20	17 Nov 2007	8
NF2	1 Nov 2007	113	19-20	18 Nov 2007	53

Brown trout, rainbow trout and smallmouth bass were collected by boat electro-fishing on 13 December 2007 between 1200 h and 1600 h from the North Fork of the White River. Smallmouth bass, largemouth bass, rock bass, redhorse, banded sculpin and rainbow trout were collected on 17 December 2007 between 1100 h and 1430 h by boat electro-fishing from the Big Piney River. Brown trout were collected on 19 December 2007 between 0900 h and 1200 h by boat electro-fishing in the Rubidoux stream, a tributary to the Big Piney River. Because walleye densities were low in the selected river systems, they were collected from Lake Tanycomo on 24 October 2007 by boat electro-fishing between 1800 h and 2200 h, maintained in an outdoor raceway at SHFH at 11ºC and fed small rainbow trout ad libitum until stimulus collection on 20 December 2007. Hatchery-collected rainbow and brown trout, fed exclusively floating trout feed (Silver Cup; Nelson & Sons, Inc., Murray, Utah) from hatching to collection, were obtained from large outdoor raceways at SHFH on 11 February 2008. All fish were transported in oxygenated water to Missouri State University (MSU) where they were transferred to water-filled ice chests (95cm L × 38cm W × 38cm D) at approximately 8°C.

Justification for use of Redhorse as a Nonpredator Control. Redhorse were selected as the nonpredator control due to their relatively large size (which makes them similar in size to at least some of the predatory species in this study) and because they apparently do not prey on vertebrates. Another stream-dwelling amphibian (graybelly salamanders, *Eurycea tynerensis*) has been shown to respond neutrally to redhorse stimuli (Whitham and Mathis 2000). Graybelly salamanders are similar to larval hellbenders in that they inhabit the gravel substrate of streams, which are commonly utilized by feeding redhorse. Although these salamanders exhibited fright responses when exposed to

45

stimuli from predatory sculpin, they did not differentiate between chemical cues of redhorse and leopard frog tadpoles, a definitive nonpredatory species.

Redhorse suckers have dorsally positioned mouths and forage by suctioning substrate and extracting various food particles. In the Ozark region of Missouri, the most common redhorse are the black redhorse (*Moxostoma duquesnei*) and golden redhorse (*Moxostoma erythrurum*). The silver redhorse (*Moxostoma anisurum*) and river redhorse (*Moxostoma carinatum*) are less common and do not inhabit the North Fork of the White River. In the Ozark region of Missouri, black redhorse prefer pools throughout the year but move to deeper holes during the winter (Bowman 1970). Juveniles feed on sandy bottom pools while adults feed immediately above or below riffles (Bowman 1970). Bowman (1970) examined the stomach contents of 216 black redhorse and found that the food items consisted of aquatic insect larvae and other aquatic invertebrates. He found no evidence of fish egg consumption in 775 stomachs. On several occasions, he also observed redhorse foraging near a centrarchid nest; when the guarding male left the nest to drive an intruder away, the redhorse did not invade the nest to feed. Golden and silver redhorse feed on similar prey, including chironomids, ephemeroptera and trichoptera (Meyer 1962). In Georgia, robust redhorse diets consisted primarily of bivalves (Freeman 2003). The failure to find even small vertebrates (larval fishes, amphibians) in redhorse diets may be due to the feeding behavior of suckers; while feeding, suckers tend to stir up the substrate (Bowman 1970, COSEWIC 2005), which would likely provide sufficient warning for vertebrate prey to swim away or to burrow deeper in the substrate.

Stimulus Collection. Each treatment stimulus (except banded sculpin) was collected by placing an individual fish (no fish in blank control) in a 19-l container (34

cm L × 21 cm W × 23 cm H) with an aerator for 24 h. A ratio of 25 ml of dechlorinated

tap water per 1 g of fish mass was used to standardize the concentration of fish stimulus

among different-sized fish. Partitions were placed between each container to minimize

visual inconsistencies among containers. Methods were identical for the banded sculpin

stimulus collection except that a 1.9-l container (19 cm L × 13.3 cm W × 8.9 cm D) was

used. For each treatment, stimuli were collected from three individuals (Table 3).

Within each eastern hellbender treatment (i.e. species), responses to stimuli were

generally consistent; only one of 11 treatments resulted in a significant difference among

individual donor fish (Appendix 3).

After 24 h, each fish's stimulus water was collected by filtering it through aqua-

pure filter fiber (100% polyester) to remove solid particles and transferring it to 15 plastic

bottles (89 ml HDPE cylinder, Price Leader Packaging, Vacaville, CA) in 60-ml aliquots.

The process was repeated for all 11 treatments. All stimuli were taken to SHFH on ice

and frozen at -6°C. This research was conducted under animal use protocol 2007H.

General Testing Protocol. The test chamber was a plastic 5.7-l container (31 cm

L × 17.5 cm W × 10.5 cm D) filled with 1 kg of stone substrate [brand: Estes'

Spectrastone, size: #25, color: walnut (Clifford W Estes co., inc. Totowa NJ)]. Four lines

were drawn across the bottom and sides of the container at 5.5 cm intervals. The

container was surrounded on all sides with black plastic to minimize external visual

influences and inconsistencies among test containers. A clear plastic cylinder (half a

tennis ball container, 8 cm L × 7.5 cm Diameter) that was open at both ends was used as

an acclimation chamber for the test larva. The cylinder was drilled with 24 holes, 0.278

cm (7/64 inch) in diameter, to allow water and stimulus to flow into and out of the

Table 3. Treatments tested for eastern hellbender larvae, including assignment to predator/nonpredatory and native/nonnative categories, and assigned stimulus fish and corresponding length. For rainbow trout, brown trout, and smallmouth bass, and walleye, second length is the length of stimulus fish taken from the North Fork River for testing responses of Ozark hellbender larvae.

Treatment	Native/Nonnative	Predator/Nonpredator	Fish No.	Fish Length (cm)
Control			1	n/a
			2	n/a
			3	n/a
Redhorse	Native	Nonpredator	1	25.5
			2	24.7
			3	25.8
Rainbow trout (H)	Nonnative	Predator	1	30.8
			2	25.4
			3	26.3
Rainbow trout	Nonnative	Predator	1	24.7/31.0
			2	28.3/27.3
			3	26.5/30.3
Brown trout (H)	Nonnative	Predator	1	23.5
			2	26.0
			3	26.5
Brown trout	Nonnative	Predator	1	27.0/34.1
			2	23.2/28.8
			3	25.0/29.0
Largemouth bass	Native	Predator	1	31.1
			2	28.2
			3	26.5
Smallmouth bass	Native	Predator	1	21.3/22.8
			2	26.0/19.8
			3	30.3/21.1
Rock bass	Native	Predator	1	23.0
			2	20.6
			3	20.4
Walleye	Native	Predator	1	23.3/23.2
			2	22.2/22.2
			3	22.5/22.5
Banded sculpin	Native	Predator	1	14.0
			2	12.8
			3	16.2

cylinder.

The test chamber and substrate were rinsed with well water for 1 min; the

substrate was stirred and rinsed an additional 1 min. The acclimation cylinder was rinsed

with well water for 30 sec. The test chamber was drained and the substrate was leveled.

Well water (1.8 l) was poured into the test chamber and the acclimation cylinder was

placed upright in the center of the test chamber. Immediately prior to testing, a treatment

stimulus and one of the three replicate samples within that treatment were randomly

selected and thawed in a warm water bath. Treatments were coded prior to testing so that

the observer was blind to treatment selection. During testing, test chambers were

illuminated by one row of 4 incandescent lights. Water temperatures were 15.1-17.4°C

for eastern hellbender trials and 16.7-19.3°C for Ozark hellbender trials. At the time of

testing, eastern hellbender larvae were 6.05 ± 0.04 cm (mean TL ± SE) and Ozark

hellbender larvae were 5.83 ± 0.07 cm (mean TL ± SE).

Testing Protocol: Eastern Hellbenders. For each trial, a larval hellbender was

randomly chosen from a selected clutch and placed inside the acclimation cylinder using

a small aquarium net (Aqua Culture, Bentonville AR). After 90 sec, the randomly-

chosen test stimulus was gently shaken. At 120 sec, the stimulus was poured between the

acclimation cylinder and one wall of the container. In a previous trial using dye, I

determined that 90 sec was sufficient for the stimulus to disperse throughout the

container and into the acclimation cylinder containing the hellbender. Therefore, after

240 sec, the acclimation cylinder was slowly lifted and the following quantitative

response variables were recorded during the 10-min observation period: (1) latency to

move; (2) latency to cross the first line (recorded when half of the larva's body crossed

the line); (3) number of lines crossed; (4) latency to jerk head (rapid, lateral movement of the head, often accompanied with a mouth gape); and (5) number of head jerks.

The holding raceway was partitioned into three sections, with the middle section remaining empty to prevent tested and untested larvae from coming into contact. After testing, larvae were placed in the downstream portion of the raceway. With one exception, individual larvae were tested only once. The treatments of hatchery-collected rainbow and brown trout were added after the experiment was in progress; to maintain equivalent representation of clutches, I re-tested 12 eastern hellbender individuals from the BP1 and BP2A clutches. Additional replicates for wild-caught trout were added so that random assignment of treatment and testing order could be maintained for the new treatments.

Testing Protocol: Ozark Hellbenders. Because Bd has been found to occur on hellbenders captured from the North Fork of the White River, I used a slightly different testing protocol for Ozark hellbender larvae then for eastern larvae to prevent potential Bd contamination in uncontaminated areas. Methods for the two subspecies were identical except for the following details. The test chamber, substrate and acclimation cylinder were rinsed with cold tap water prior to rinsing with well water. Test water (1.8 l) was taken from the Ozark hellbender closed-system tank. Upon completion of each trial, tested larvae were transferred to a mesh container placed inside the closed-system tank. Individual larvae were tested only once.

Statistical Analyses. Data were analyzed by Kruskal-Wallis tests (Minitab 15) followed by nonparametric multiple comparisons (WINKS SDA 6.0). The two subspecies were analyzed separately for latency to move, latency to cross a line, and

number of lines crossed. Head jerks were relatively rare, and so data for both subspecies were combined for analysis of this behavior.

Results

General Levels of Activity: Eastern Hellbender Larvae. Statistical results are presented in Table 4. Larvae were most active following exposure to the blank control, but hellbenders did not differentiate statistically between the blank stimulus and the stimulus from the nonpredatory redhorse in terms of latency to move (Fig. 10), latency to cross a line (Fig. 11) and number of lines crossed (Fig. 12). When compared to the nonpredator control, all native species had significantly longer latencies to move (Fig. 10) and cross a line (Fig. 11) and crossed significantly fewer lines (Fig. 12). In contrast, responses to stimuli from the nonnative species appeared intermediate between that of the controls and the predatory fishes (Fig. 10-12). Responses to the nonnative stimuli were not significantly different from the nonpredator control for any response variable. In comparison to the blank control, all predatory treatments except the hatchery-collected rainbow trout had a significantly longer latency to move than the blank control (Fig. 10), and all predatory treatments had a significantly longer latency to cross a line (Fig. 11) and crossed significantly fewer lines (Fig. 12).

General Levels of Activity: Ozark Hellbender Larvae. Larvae exhibited significantly stronger responses to stimuli from wild-caught brown trout, walleye and smallmouth bass than to the blank control in at least one of the response variables (Table 5; Fig. 13-15). In contrast, larval responses to stimuli from rainbow trout did not differ significantly from the blank control in for any response variable (Table 5; Fig. 13-15).

Table 4. (a) One-way Kruskal-Wallis ANOVA results for three response variables for eastern hellbender larvae exposed to predatory and nonpredatory stimuli. (b) Nonparametric multiple comparisons of predatory treatments versus the blank control and the nonpredatory control for three response variables.

4a

Response Variable	KW Test Statistic (H)	d.f.	P-value	Figure Number
Latency to Move	128.00	10	$P < 0.001$	Fig. 10
Latency to Cross Line	122.99	10	$P < 0.001$	Fig. 11
Number Lines Crossed	113.81	10	$P < 0.001$	Fig. 12

4b

Treatment	Response Variable	Comparison to Blank Control		Comparison to Nonpredator	
		Test Stat	P-value	Test Stat	P-value
Redhorse	Latency to Move	1.872	>0.5	n/a	n/a
	Latency to Cross Line	1.732	>0.5	n/a	n/a
	Number Lines Crossed	1.58	>0.5	n/a	n/a
Rainbow trout (H)	Latency to Move	2.972	>0.1	1.263	>0.5
	Latency to Cross Line	3.637	<0.02	2.056	>0.5
	Number Lines Crossed	3.503	<0.05	2.06	>0.5
Rainbow trout	Latency to Move	4.559	<0.001	2.481	>0.5
	Latency to Cross Line	4.557	<0.001	2.634	>0.2
	Number Lines Crossed	4.237	<0.002	2.482	>0.5
Brown trout (H)	Latency to Move	3.799	<0.01	2.089	>0.5
	Latency to Cross Line	4.087	<0.005	2.506	>0.5
	Number Lines Crossed	3.865	<0.01	2.422	>0.5
Brown trout	Latency to Move	4.924	<0.001	2.845	>0.2
	Latency to Cross Line	4.875	<0.001	2.952	>0.1
	Number Lines Crossed	4.739	<0.001	2.984	>0.1
Largemouth bass	Latency to Move	6.584	<0.001	4.696	<0.001
	Latency to Cross Line	6.896	<0.001	5.149	<0.001
	Number Lines Crossed	6.43	<0.001	4.836	<0.001
Smallmouth bass	Latency to Move	6.939	<0.001	5.102	<0.001
	Latency to Cross Line	6.475	<0.001	4.775	<0.001
	Number Lines Crossed	6.286	<0.001	4.735	<0.001
Rock bass	Latency to Move	6.906	<0.001	5.051	<0.001
	Latency to Cross Line	6.41	<0.001	4.694	<0.001
	Number Lines Crossed	5.722	<0.001	4.156	<0.002
Walleye	Latency to Move	6.953	<0.001	5.081	<0.001
	Latency to Cross Line	6.563	<0.001	4.831	<0.001
	Number Lines Crossed	6.336	<0.001	4.756	<0.001
Banded sculpin	Latency to Move	7.274	<0.001	5.42	<0.001
	Latency to Cross Line	7.314	<0.001	5.598	<0.001
	Number Lines Crossed	6.985	<0.001	5.419	<0.001
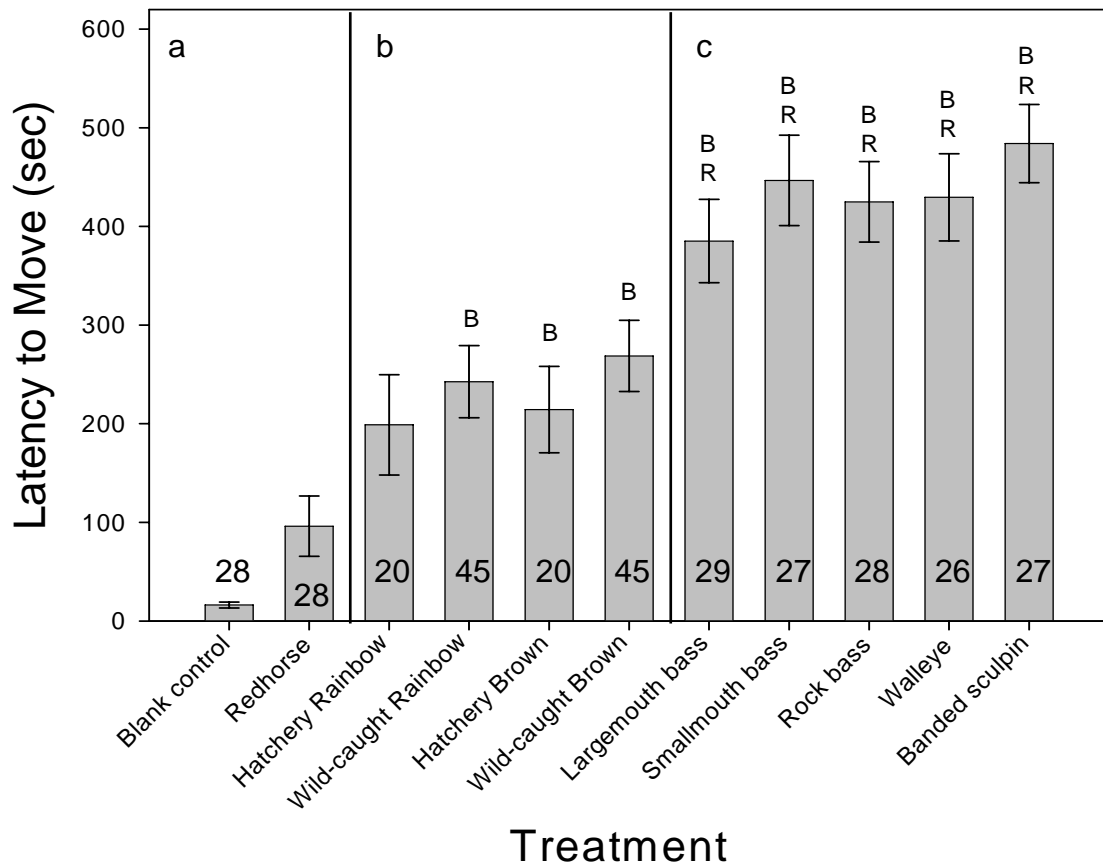
Figure 10. Mean (± SE) latency to move (sec) for eastern hellbender larvae exposed to (a) controls of dechlorinated tap water (blank) and to chemical stimuli from a nonpredatory fish (redhorse), (b) hatchery-collected and wild-caught introduced fish (rainbow and brown trout), and (c) five species of native predatory fish. Different letters indicate significant differences ($P < 0.05$) from blank (B) and nonpredator control (R). Number inside or above bar is N.
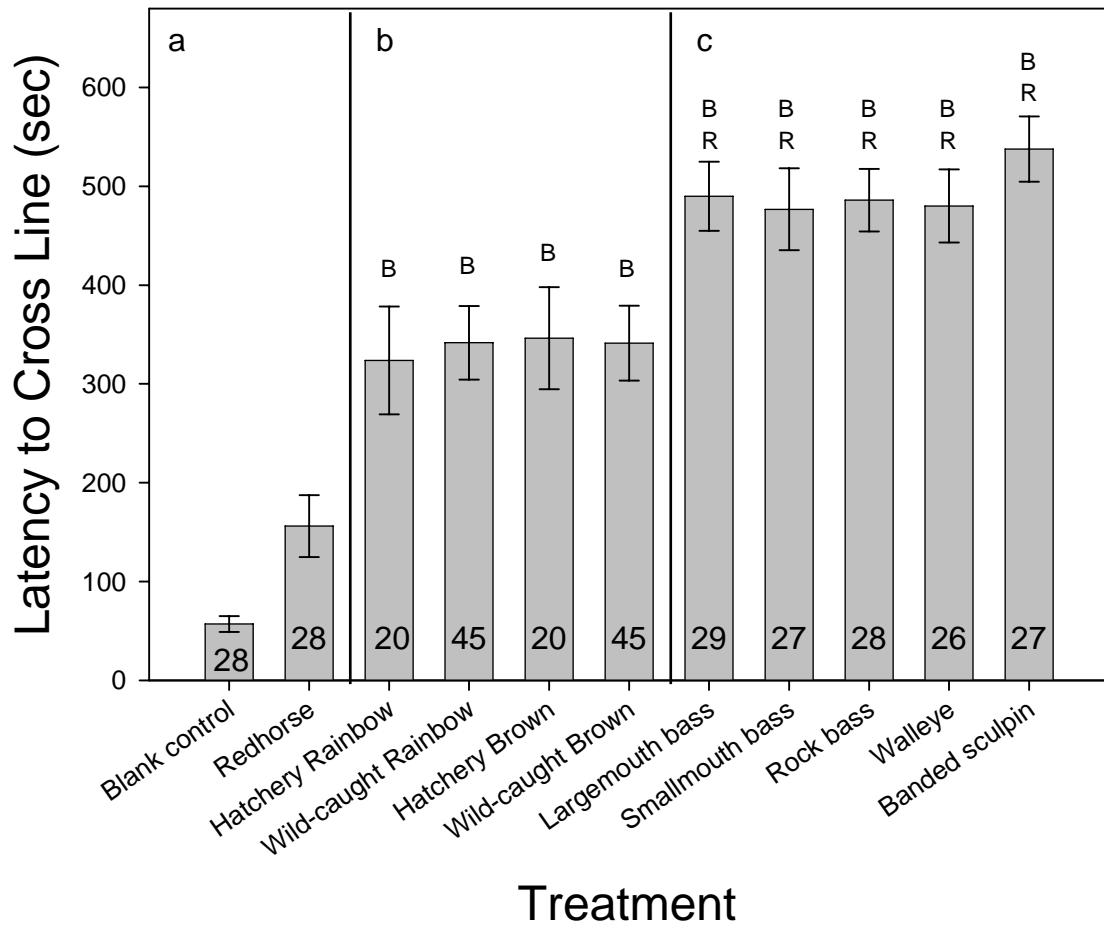
Figure 11. Mean (± SE) latency to cross the first line (sec) for eastern hellbender larvae exposed to (a) controls of dechlorinated tap water (blank) and to chemical stimuli from a nonpredatory fish (redhorse), (b) hatchery-collected and wild-caught introduced fish (rainbow and brown trout), and (c) five species of native predatory fish. Different letters indicate significant differences ($P < 0.05$) from blank (B) and nonpredator control (R). Number inside bar is N.
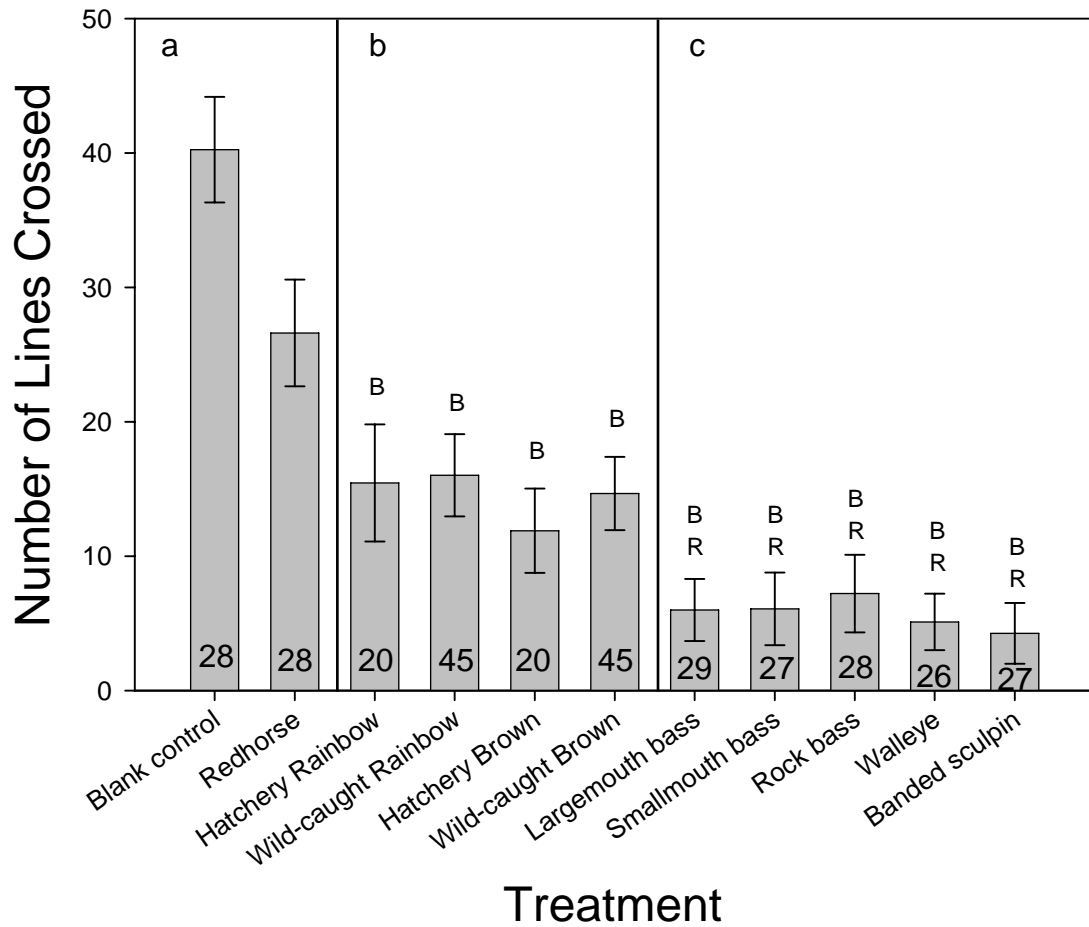
Figure 12. Mean (± SE) number of lines crossed for eastern hellbender larvae exposed to (a) controls of dechlorinated tap water (blank) and to chemical stimuli from a nonpredatory fish (redhorse), (b) hatchery-collected and wild-caught introduced fish (rainbow and brown trout), and (c) five species of native predatory fish. Different letters indicate significant differences ($P < 0.05$) from blank (B) and nonpredator control (R). Number inside bar is N.

Table 5. (a) One-way Kruskal-Wallis ANOVA results for three response variables for Ozark hellbender larvae exposed to predatory stimuli and a blank control. (b) Nonparametric multiple comparisons of predatory treatments versus the blank control for three response variables.

5a

Response Variable	Test Statistic (H)	d.f.	P-value	Figure No.
Latency to Move	128.00	10	P < 0.002	Fig. 13
Latency to Cross Line	122.99	10	P < 0.001	Fig. 14
Number Lines Crossed	113.81	10	P < 0.001	Fig. 15

5b

| Treatment	Response Variable	Test Stat (Q)	P-value
Rainbow trout	Latency to Move	1.089	>0.5
	Latency to Cross Line	0.69	>0.5
	Number Lines Crossed	0.871	>0.5
Brown trout	Latency to Move	2.835	<0.05
	Latency to Cross Line	2.999	<0.05
	Number Lines Crossed	3.104	<0.02
Smallmouth bass	Latency to Move	2.269	>0.2
	Latency to Cross Line	2.751	>0.05
	Number Lines Crossed	2.916	<0.05
Walleye	Latency to Move	3.613	<0.005
	Latency to Cross Line	3.778	<0.002
	Number Lines Crossed	3.71	<0.005

Figure 13. Mean (± SE) latency to move (sec) for Ozark hellbender larvae exposed to dechlorinated tap water (blank), and to chemical stimuli from wild-caught rainbow trout, wild-caught brown trout, smallmouth bass, and walleye. Significant differences from dechlorinated water (blank) indicated by asterisk. Number inside or above bar is N, *P < 0.05; **P < 0.005.
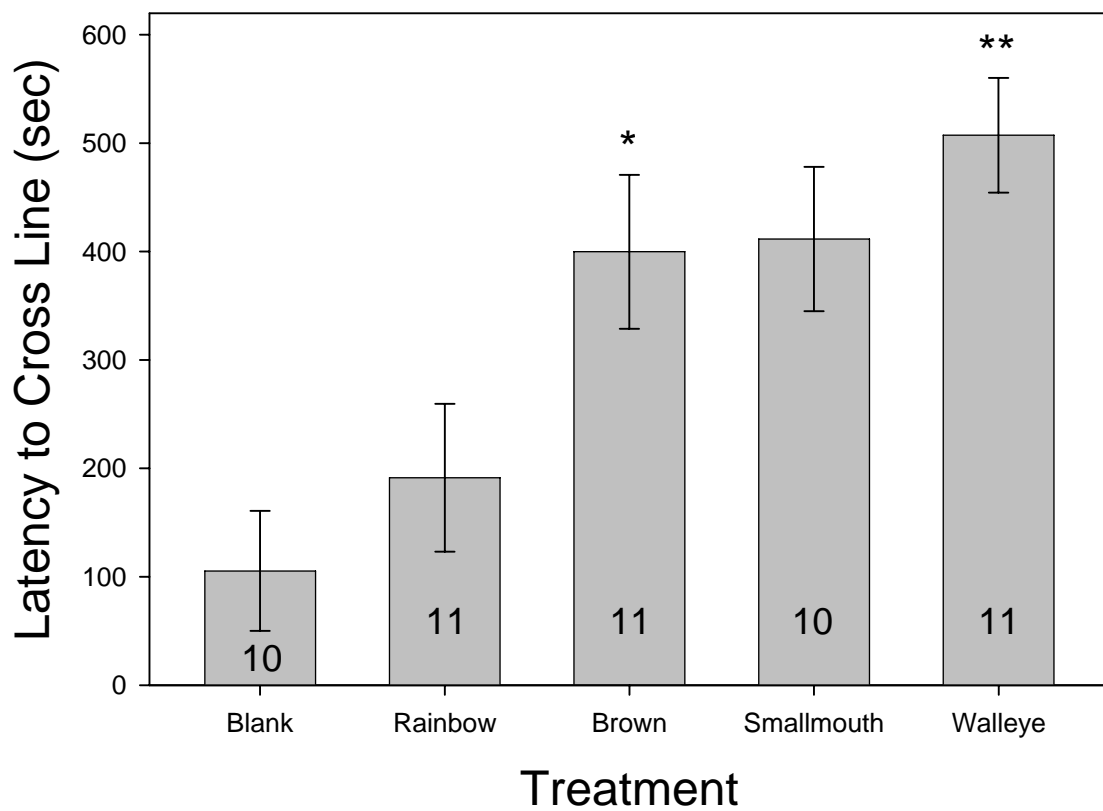
Figure 14. Mean (± SE) latency to cross the first line (sec) for Ozark hellbender larvae exposed to dechlorinated tap water (blank), and to chemical stimuli from wild-caught rainbow trout, wild-caught brown trout, smallmouth bass, and walleye. Significant differences from dechlorinated water (blank) indicated by asterisk. Number inside bar is N, *P < 0.05; **P < 0.005.
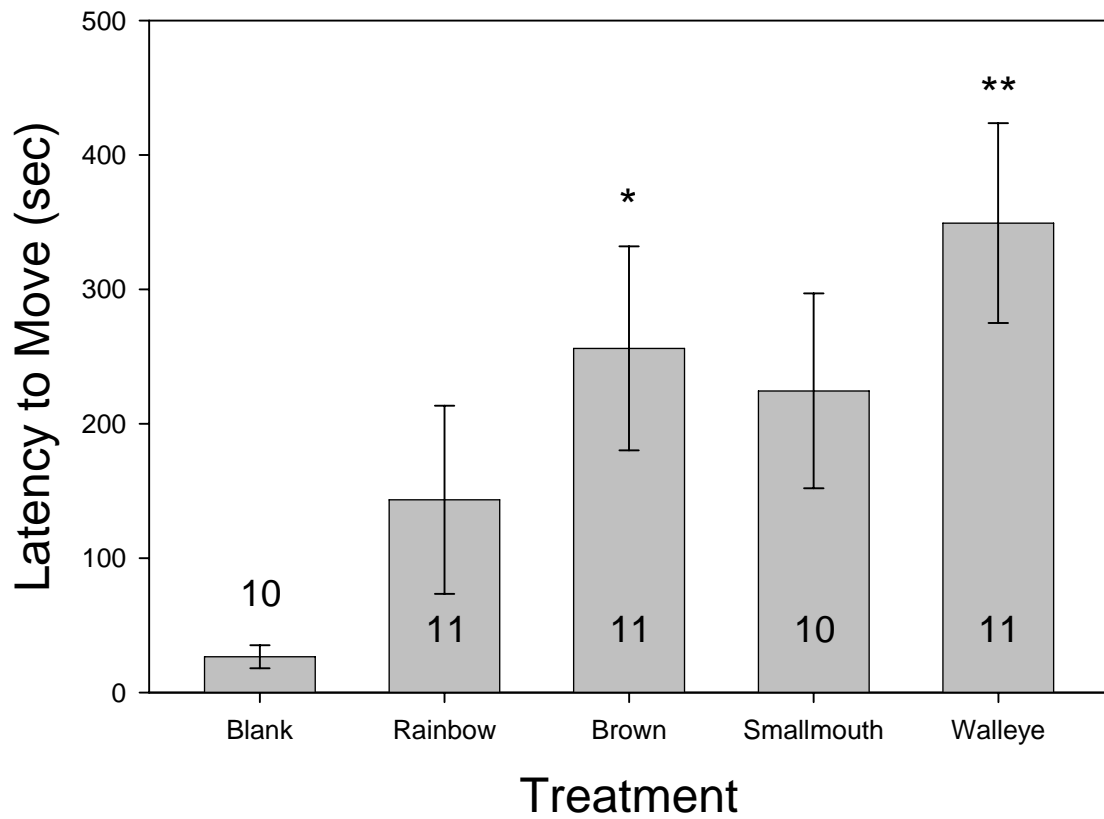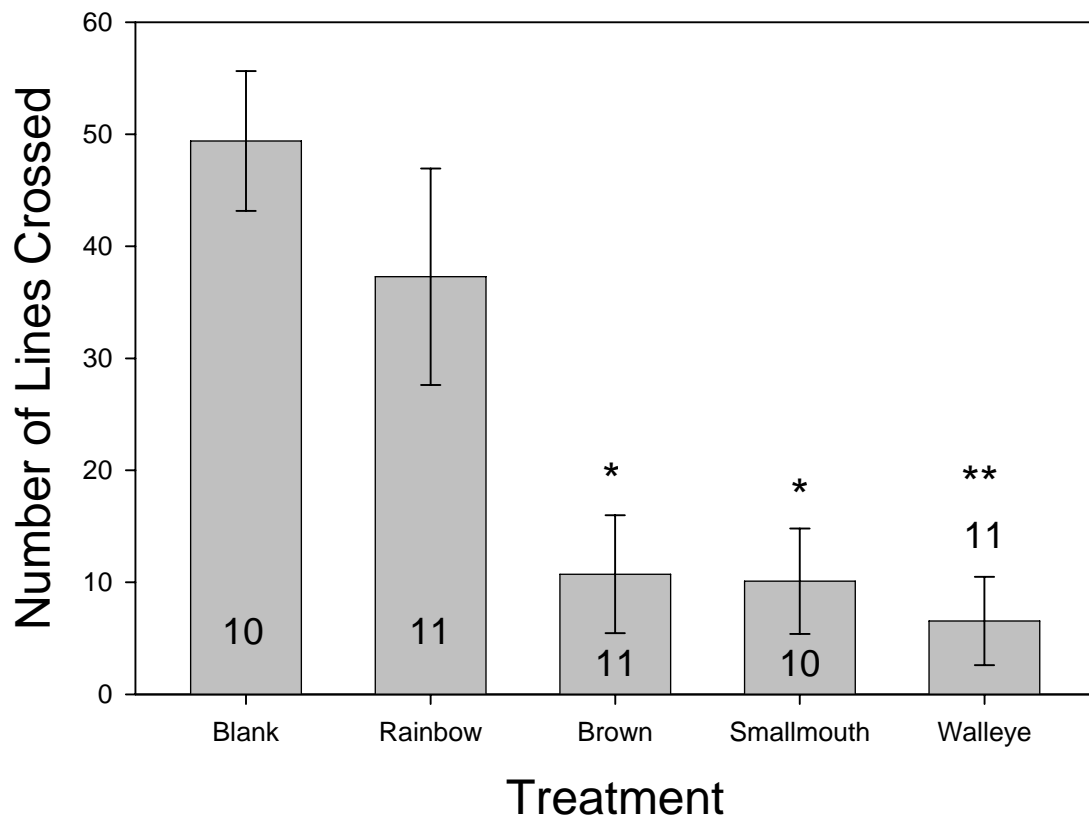
Figure 15. Mean (± SE) number of lines crossed for Ozark hellbender larvae exposed to dechlorinated tap water (blank), and to chemical stimuli from wild-caught rainbow trout, wild-caught brown trout, smallmouth bass, and walleye. Significant differences from dechlorinated water (blank) indicated by asterisk. Number inside or above bar is N, *P < 0.05; **P < 0.005.

Head Jerks: Eastern and Ozark Hellbender Larvae. Head jerks were only performed in the presence of nonpredatory stimuli and stimuli from rainbow trout (hatchery-collected and wild-caught) and hatchery-collected brown trout. Latency to perform head jerks was not significantly longer for the nonpredatory redhorse [mean latency to head jerk (HJ) ± SE = 522 ± 33.4 sec], wild-caught rainbow trout (mean HJ = 580 ± 13.4 sec), hatchery-collected rainbow trout (mean HJ = 524 ± 33.5 sec) and for the hatchery-collected brown trout (mean HJ = 564 ± 21.5 sec) than in the blank treatment (mean HJ = 482 ± 35.5 sec).

Discussion

Larval hellbenders exposed to chemical cues from native species responded by decreasing overall activity compared to blank and nonpredator controls (Fig. 10-12). Prey typically respond to the threat of predation in one of two ways: (1) by decreasing activity, and subsequently decreasing the probability of being detected by a predator; or (2) exhibiting escape or avoidance behavior by increasing activity (Lima and Dill 1990). Decreased activity, or freezing, is a common antipredator mechanism among aquatic amphibians (Woodward 1983; Feminella and Hawkins 1992; Kiesecker et al. 1996; Kats and Dill 1998; Bosch et al. 2000; Mathis et al. 2003). Cryptic species are more likely to reduce movement in the presence of predators than conspicuous species (Lima and Dill 1990). Hellbenders are extremely cryptic and often remain motionless after their hide rock is overturned (Nickerson and Mays 1971; Personal observation), and I therefore conclude that reduced activity was an antipredator response of larval hellbenders in this study. Because the larvae in this study were lab-reared, with no experience with fish

predators, these data confirm that recognition of at least some native predators is innate, which is consistent with results from other studies of amphibians (Sih and Kats 1994; Laurila et al. 1997; Gallie et al. 2001).

In contrast, although larval hellbenders often recognized a difference between a blank stimulus and stimuli from the nonnative fishes, they did not significantly discriminate between chemical cues from nonnative fishes and the nonpredatory redhorse (Fig. 10-12). This difference indicates larval eastern hellbenders do not fully recognize brown and rainbow trout as predators, which could lead to increased larval predation in the wild. For antipredator mechanisms to evolve, predator and prey must co-occur over evolutionary time (Brodie et al. 1991; Kats and Ferrer 2003). Trout have only recently been introduced to hellbender habitat (reviewed by Alsup, 2005); the relatively low-level of response of hellbender larvae to trout is likely due to the limited time in which these species have co-occurred.

In addition to changes in activity levels, I also observed that larval hellbenders of both subspecies perform a behavior that I term "head jerks". This behavior is similar to that observed when hellbender larvae subjugate and then consume prey; therefore, I hypothesize that head jerks represent feeding behavior. Although I did not make prey available during the trials, larvae may have perceived small pieces of sediment as potential prey. Head jerk behavior may be important for two reasons: (1) it represents a form of activity and, as such, would be expected to decrease when larvae are frightened; and (2) it may indicate a potential trade-off for hellbender larvae. Lifetime reproductive success is not based solely on avoiding predation, and prey must balance the risk of predation with other activities such as territorial defense, mating and foraging (Lima and

Dill 1990). Prey must therefore be capable of adjusting antipredator behaviors to the

immediate level of predation risk (Lima and Dill 1990). For example, an organism

should forgo foraging when the risk of predation is high, but resume if the threat of

predation decreases. Larval hellbenders did not perform the head-jerk behavior (i.e.,

foraging) when exposed to stimuli from native fish predators, presumably because they

perceived a high-risk environment; like other forms of activity, head jerks would likely

draw the attention of nearby predators. However, larval hellbenders did perform head

jerks when exposed to stimuli from nonnative predatory fishes, presumably because they

did not perceive that these stimuli represented danger. Increased foraging activity (as

indicated by head jerks) by larvae in the presence of trout (i.e. high predation risk) should

lead to increased levels of predation.

Several other species of amphibians have been shown to be differentially affected

by native and nonnative fish predators. In simple field enclosures, Gillespie (2001) found

that tadpoles of the stream-breeding amphibians *Litoria spenceri* and *L. phyllochroa* were

preyed upon heavily by introduced trout, but few or none were eaten by two native fish

predators. Additionally, in more natural stream enclosures incorporating alternative prey

and natural refugia, trout reduced survivorship of these same species. The lack of

evolutionary history with trout apparently is responsible for poor survival of these two

species in the face of trout predators (Gillespie 2001). Responses to introduced fishes

can be inefficient even if the introduced predator is closely related to a native species

(Kats and Ferrer 2003). Bosch et al. (2000) found that Iberian frog (*Rana iberica*)

tadpoles responded to native brown trout by decreasing activity, but they did not respond

as strongly to the exotic brook trout (*Salvelinus fontinalis*). There are no native salmonid

fishes in Missouri, so hellbenders from the populations in this study not only lack an evolutionary history with trout per se, but also with any closely-related fish species.

In general, larvae of the two subspecies (Ozark and eastern) responded similarly to the predatory stimuli. However, there was one notable difference in their responses: Ozark hellbenders responded to brown trout in a similar manner as their responses to native fishes. Because the trout stimulus for Ozark hellbenders was collected from wild-caught trout, I hypothesize that this difference may be due to dietary differences between the two trout species in the wild. Invertebrates are important dietary components for all size classes of rainbow trout (Maciolek and Needham 1952; Tippets and Moyle 1978), as well as for small brown trout (Ellis and Gowing 1957; Brynildson et al. 1963; Pflieger 1997). However, as fish length increases (approximately 22 cm), brown trout shift to foraging on fishes (Evans 1952; Brynildson et al. 1963). In the Current River in Missouri, sculpin make up a large percentage of the diet of adult brown trout (Pflieger 1997). The level of response to predatory chemical cues is often influenced by the predator's diet, with prey responding more strongly to chemical stimuli from predators whose diet is composed of conspecifics or other members of the same prey guild (Mathis and Smith 1993; Wilson and Lefcort 1993; Chivers et al. 1996; Laurila et al. 1997; Laurila et al. 1998; Murray and Jenkins 1999). If the wild-caught brown trout in this study had been consuming primarily fish prey, then the larvae may have perceived them as more dangerous than rainbow trout that had been consuming only invertebrate prey; small fishes are in the same prey guild as larval amphibians. It is also possible that the wild-caught trout in this study had consumed larval hellbenders, which also would account for a dietary effect. However, the question remains as to why the two larvae

from the two subspecies differed in their responses to stimuli from the wild-caught brown trout. Note that for at least one response variable (Fig. 10), there was a nonsignificant trend for eastern hellbender larvae to respond more strongly to wild-caught trout (both rainbow and brown) than hatchery-collected trout.

The quantity of trout stocked and the density of trout per km of river has increased dramatically in the past 40 years (see review in Alsup 2005). For example, special management regimes for the North Fork River have increased the total number of rainbow trout by 87% to 335 per km since the 1980's (Missouri Department of Conservation 1999). The number of rainbow trout greater than 15 and 18 inches has increased by 725 and 800%, respectively (Missouri Department of Conservation 1999). Compared to the early 1990's, the density of brown trout has increased to 287% (350 per km), and the number greater than 15 and 18 inches has increased by 52 and 30%, respectively (Missouri Department of Conservation 1999). Now, more than ever, there is a greater potential for trout and hellbenders to interact and consequently, a greater probability that hellbenders will be predated by introduced trout.

The evolutionary history of predators and prey is largely responsible for the development of antipredator behaviors, and an absence of predation avoidance mechanisms can lead to increased predation of a prey species (Lima and Dill 1990; Brodie et al. 1991). The lack of a co-evolutionary history between Missouri's hellbenders and trout may explain the limited antipredator responses to trout for these aquatic salamanders. Although additional research is warranted, it is possible that nonnative trout have been negatively impacting hellbender populations since their

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introduction, and to some extent are responsible for the dramatic hellbender population

declines in Missouri.

CHAPTER V. GENERAL DISCUSSION

Predator-Prey Interactions between Introduced Trout and Hellbenders

In this study, I found evidence suggesting that hellbender predation by introduced trout cannot be ruled out as a hypothesis to explain the declines of hellbender populations in Missouri. Other studies have shown that negative impacts of trout on amphibian communities primarily result from (1) a lack of evolutionary history between predator and prey, (2) an absence of antipredator behaviors toward the introduced predator and consequently (3) increased predation on the amphibian communities (Knapp and Matthews 2000; Gillespie 2001; Kats and Ferrer 2003). In Missouri, hellbenders evolved in permanent streams and rivers where the threat of predation from a variety of fishes was ever present. Therefore, in addition to general antipredator mechanisms that deter fish predation (e.g., crypsis, shelter-seeking behavior), hellbender larvae have evolved an innate ability to distinguish between chemical stimuli from predatory and nonpredatory species. In contrast, the potential for salmonid predation was absent in habitats occupied by Missouri hellbenders. The limited evolutionary history between these species is likely responsible for hellbenders relatively weak fright behavior in response to trout stimuli. As a result, hellbenders may be more vulnerable to predation from nonnative trout than from native fish predators.

Although there is evidence from my study that both rainbow and brown trout are a threat to hellbenders, rainbow trout may be of a less immediate concern than brown trout. Larval hellbenders exhibited only weak fright responses toward rainbow trout stimuli, suggesting some vulnerability (Chapter 4). However, rainbow trout were not stimulated

to approach or forage on prey in the presence of a hellbender secretion (Chapter 2, note also that rainbow trout were not repelled by the secretion). In addition, rainbow trout expelled more secretion-soaked food pellets than control pellets (Chapter 3), and in the wild, a secretion-producing hellbender that was inadvertently eaten might also be expelled from the trout's mouth. This latter result should be taken with two caveats, however: (1) some secretion-soaked pellets were consumed by rainbow trout, so any unpalatability defense is likely not always successful for this predator; and (2) small larvae that do not produce the secretion or that produce only small amounts of the secretion would still be in danger of consumption. Unpalatability of amphibian larvae has been implicated in the survival of some amphibian species in the face of trout introductions. Although populations of three palatable amphibian species were negatively correlated with the presence of introduced trout in sub-alpine lakes in the western United States, the distribution of *Bufo boreas*, an unpalatable species, was positively correlated with trout presence; survival was most likely due to unpalatability and population growth likely resulted from the competitive release of available resources (Welsh et al. 2006).

Several results of my study indicate that brown trout are a cause for immediate concern. First, larval hellbenders also showed only weak fright responses when exposed to brown trout (Chapter 4); note that this response could be mitigated by diet effects in wild-caught trout. The foraging behavior of brown trout in the presence of hellbender secretion was markedly different from that of the rainbow trout. Brown trout were stimulated to approach prey in the presence of the hellbender secretion and consumed more food pellets when they were soaked in the hellbender secretion (Chapter 2, 3). I

hypothesize that the combination of (1) an absence of antipredator behavior exhibited by hellbenders, (2) increased activity and foraging by brown trout in response to hellbender stimuli and (3) the palatability of hellbenders to brown trout has resulted in greater predation pressure on hellbenders by introduced brown trout.

Conservation Recommendations

Because trout have the potential to negatively impact hellbender populations, hellbender conservation efforts should focus on strategies that minimize the potential interactions of hellbenders and nonnative trout. Suitable hellbender habitat is relatively heterogeneous throughout many river systems (personal observation), and a large proportion of the hellbender population in many rivers can be found in relatively few areas (Jeff Briggler, personal communication). Hellbender predation by trout could be minimized if fisheries management biologists reduced trout stocking and immigration to these "quality" hellbender habitats. Restriction of trout introductions to areas that are not occupied by hellbenders could still provide angling opportunities in other areas of the river.

Other conservation tasks should attempt to reduce the probability of predation on hellbender eggs, larvae and juveniles. For example, collecting hellbender eggs from the wild and hatching collected eggs in captivity eliminates wild predation on at least some young hellbenders. Because larval and juvenile hellbenders are more vulnerable than adults to predation (Nickerson and Mays 1973), captive-rearing and head-start programs with the ultimate goal of re-release should increase the survivorship of early hellbender life stages. A. Mathis and A. Crane (personal communications) are developing protocols

for training captive-reared hellbenders to recognize trout as predators. Wide-spread

implementation of this protocol for all head-starting programs could potentially increase

survival of released hellbenders.

Global amphibian populations have been declining for several decades, already

resulting in the extinction of some species (Schloegel et al. 2006). If Missouri's

hellbender populations are to avoid extinction, efforts must be established to ensure the

survival of these unique creatures. These conservation efforts, along with additional

research on the potential causes of hellbender population declines, will likely have a

positive and long-term effect on hellbender populations in Missouri.

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APPENDIX 1

Species of fishes tested in Chapter 2, treatment, sample size, mean fish length (± SE), 2-sample t-test test statistic (t) and corresponding P-value.

Species	Treatment	N	Mean ± SE	Test Stat	P-value
Redhorse spp.	Control	9	33.78 ± 1.50	-1.64	0.135
Moxostoma spp.	Hellbender	7	36.43 ± 0.53		
Rainbow trout (hatchery)	Control	17	24.39 ± 0.61	1.26	0.219
Oncorhynchus mykiss	Hellbender	17	23.48 ± 0.40		
Rainbow trout	Control	13	29.42 ± 0.92	-1.34	0.194
Oncorhynchus mykiss	Hellbender	16	30.91 ± 0.63		
Brown trout (hatchery)	Control	13	24.38 ± 0.56	-0.58	0.565
Salmo trutta	Hellbender	14	24.83 ± 0.53		
Brown trout	Control	17	33.11 ± 0.88	-0.67	0.510
Salmo trutta	Hellbender	17	33.94 ± 0.89		
Smallmouth bass	Control	13	30.28 ± 0.99	1.08	0.291
Micropterus dolomieu	Hellbender	15	28.63 ± 1.20		
Ozark bass	Control	14	20.31 ± 0.47	0.72	0.480
Ambloplites constellatus	Hellbender	15	19.79 ± 0.55		
Spotted bass	Control	14	27.61 ± 0.83	-0.20	0.843
Micropterus punctulatus	Hellbender	13	27.96 ± 1.60		
Walleye	Control	12	21.17 ± 0.32	0.89	0.390
Stizostedion vitreum	Hellbender	10	20.56 ± 0.60		
Banded sculpin	Control	13	11.72 ± 0.90	0.55	0.589
Cottus carolinae	Hellbender	12	11.05 ± 0.82		

APPENDIX 2

Species of fishes tested in Chapter 2, including response variables (No. = number; Lat = latency; App = approach), treatment, sample size, mean (± SE), median, Mann-Whitney test statistic (W) and corresponding P-value.

Species	Response Variable	Treatment	N	Mean ± SE	Median	Test Stat	P-value
Redhorse spp. *Moxostoma* spp.	No. Pellets Eaten	Control	9	13.1 ± 3.28	19	65	0.157
		Hellbender	7	17.1 ± 2.86	20		
Rainbow trout (hatchery) *Oncorhynchus mykiss*	Lat to App	Control	17	162 ± 60	65	319	0.480
		Hellbender	17	95 ± 27	62		
	Lat to Strike	Control	17	265 ± 84	120	299	0.973
		Hellbender	17	348 ± 102	130		
	No. App	Control	17	5.94 ± 0.77	6.00	287	0.729
		Hellbender	17	6.59 ± 0.73	7.00		
	No. Strikes	Control	17	5.18 ± 0.85	5.00	299	0.972
		Hellbender	17	5.47 ± 1.09	5.00		
Rainbow trout *Oncorhynchus mykiss*	Lat to App	Control	13	735 ± 147	1028	180	0.500
		Hellbender	16	870 ± 102	1051		
	Lat to Strike	Control	13	1022 ± 98	1200	182	0.405
		Hellbender	16	1158 ± 35	1200		
	No. App	Control	13	2.46 ± 0.93	1.00	210	0.513
		Hellbender	16	1.25 ± 0.40	0.50		
	No. Strikes	Control	13	1.77 ± 1.12	0.00	209	0.386
		Hellbender	16	0.19 ± 0.14	0.00		
Brown trout (hatchery) *Salmo trutta*	Lat to App	Control	13	929 ± 119	1200	236	0.007**
		Hellbender	14	482 ± 146	85		
	Lat to Strike	Control	13	1140 ± 60	1200	182	1.000
		Hellbender	14	1115 ± 85	1200		
	No. App	Control	13	0.77 ± 0.38	0.00	145	0.061*
		Hellbender	14	1.50 ± 0.36	1.00		
	No. Strikes	Control	13	0.23 ± 0.23	0.00	182	1.000
		Hellbender	14	0.50 ± 0.50	0.00		
Brown trout *Salmo trutta*	Lat to App	Control	17	579 ± 135	262	319	0.480
		Hellbender	17	327 ± 118	99		
	Lat to Strike	Control	17	1028 ± 87	1200	299	0.973
		Hellbender	17	1083 ± 62	1200		
	No. App	Control	17	1.35 ± 0.35	1.00	287	0.729
		Hellbender	17	1.82 ± 0.34	2.00		
	No. Strikes	Control	17	1.59 ± 0.83	0.00	299	0.972
		Hellbender	17	1.06 ± 0.67	0.00		

Smallmouth bass *Micropterus dolomieu*	Lat to App	Control	13	430 ± 120	209	168	0.356
		Hellbender	15	586 ± 122	307		
	Lat to Strike	Control	13	982 ± 107	1200	205	0.403
		Hellbender	15	836 ± 123	1200		
	No. App	Control	13	2.46 ± 0.47	3.00	174	0.500
		Hellbender	15	3.27 ± 0.73	4.00		
	No. Strikes	Control	13	0.62 ± 0.29	0.00	175	0.495
		Hellbender	15	1.2 ± 0.56	0.00		
Ozark bass *Ambloplites constellatus*	Lat to App	Control	14	571 ± 130	381	228	0.443
		Hellbender	15	455 ± 104	224		
	Lat to Strike	Control	14	706 ± 122	652	226	0.508
		Hellbender	15	586 ± 103	534		
	No. App	Control	14	1.86 ±0.64	1.00	191	0.393
		Hellbender	15	1.80 ± 0.31	2.00		
	No. Strikes	Control	14	1.50 ± 0.40	1.50	207	0.910
		Hellbender	15	1.53 ± 0.36	1.00		
Spotted bass *Micropterus punctulatus*	Lat to App	Control	14	353 ± 130	110	186	0.626
		Hellbender	13	449 ± 148	99		
	Lat to Strike	Control	14	520 ± 146	240	174	0.280
		Hellbender	13	721 ± 143	915		
	No. App	Control	14	5.36 ± 1.23	5.00	201	0.826
		Hellbender	13	4.85 ± 1.22	5.00		
	No. Strikes	Control	14	2.79 ± 0.85	2.00	211	0.481
		Hellbender	13	2.15 ± 0.86	1.00		
Walleye *Sander vitreus*	Lat to App	Control	12	1008 ± 102	1200	177	0.009**
		Hellbender	10	481 ± 128	374		
	Lat to Strike	Control	12	1169 ± 21	1200	149	0.346
		Hellbender	10	984 ± 116	1200		
	No. App	Control	12	0.67 ± 0.31	0.00	102	0.014**
		Hellbender	10	2.10 ± 0.43	2.00		
	No. Strikes	Control	12	0.17 ± 0.11	0.00	128	0.392
		Hellbender	10	0.50 ± 0.27	0.00		
Banded sculpin (small) *Cottus carolinae*	Lat to App	Control	7	516 ± 202	484	42	0.184
		Hellbender	7	859 ± 181	1200		
	Lat to Strike	Control	7	518 ± 202	499	41	0.133
		Hellbender	7	917 ± 183	1200		
	No. App	Control	7	0.86 ± 0.26	1.00	58	0.487
		Hellbender	7	1.00 ± 0.69	0.00		
	No. Strikes	Control	7	1.71 ± 0.64	1.00	66	0.072*
		Hellbender	7	0.29 ± 0.18	0.00		
Banded sculpin (large) *Cottus carolinae*	Lat to App	Control	6	736 ± 150	818	45	0.144
		Hellbender	5	329 ± 230	26		
	Lat to Strike	Control	6	781 ± 159	777	44	0.167
		Hellbender	5	378 ± 218	248		
	No. App	Control	6	2.17 ± 0.79	1.50	33	0.580
		Hellbender	5	3.00 ± 1.00	3.00		
	No. Strikes	Control	6	1.33 ± 0.62	1.00	32	0.456
		Hellbender	5	2.20 ± 0.86	2.00		

APPENDIX 3

Eastern hellbender larvae treatments, 3 individual fish stimuli, sample size, fish length (cm), mean (± SE) and median latency to move of larval hellbenders exposed to individual stimuli from 11 different treatments, and Kruskel-Wallis test statistic (H) and corresponding P-value comparing individual fish stimuli within each treatment. For all other response variables (latency to cross line and number of lines crossed) responses to individual fish stimuli were not significantly different in any treatment (P > 0.05).

Treatment	Fish No.	N	Fish Length	Mean ± SE	Median	Test Stat	P-value
Control	1	13	n/a	17.3 ± 7.17	10		
	2	11	n/a	17.2 ± 3.20	15	2.51	0.285
	3	14	n/a	21.8 ± 5.04	15		
Redhorse	1	10	25.5	169.2 ± 75.1	34		
	2	8	24.7	83.8 ± 39.3	40	2.83	0.243
	3	10	25.8	33.0 ± 13.5	12		
Rainbow trout (H)	1	7	30.8	181.1 ± 87.0	56		
	2	6	25.4	308.5 ± 95.4	230	4.68	0.096
	3	7	26.3	122.4 ± 80.9	6		
Rainbow trout	1	18	24.7	181.8 ± 54.6	77		
	2	18	28.3	282.8 ± 63.2	132	0.78	0.678
	3	20	26.5	206.6 ± 52.2	63		
Brown trout (H)	1	7	23.5	323.3 ± 91.7	377		
	2	6	26.0	232.3 ± 9.5	198	5.05	0.080
	3	7	26.5	89.9 ± 30.0	66		
Brown trout	1	18	27.0	226.2 ± 59.0	69		
	2	18	23.2	260.6 ± 55.8	186	1.65	0.439
	3	20	25.0	307.3 ± 54.2	289		
Largemouth bass	1	10	31.1	328.1 ± 74.8	190		
	2	9	28.2	451.8 ± 67.7	600	1.04	0.594
	3	10	26.5	382.5 ± 77.7	490		
Smallmouth bass	1	12	21.3	442.7 ± 68.1	600		
	2	10	26.0	358.5 ± 84.1	444	0.62	0.735
	3	14	30.3	354.4 ± 69.6	452		
Rock bass	1	10	23.0	380.7 ± 70.4	420		
	2	8	20.6	420.8 ± 87.8	600	0.91	0.635
	3	9	20.4	477.7 ± 58.8	600		
Walleye	1	13	23.3	403.5 ± 68.7	600		
	2	12	22.2	463.2 ± 68.1	600	1.01	0.604
	3	14	22.5	362.1 ± 63.0	370		
Banded sculpin	1	10	14.0	600.0 ± 0.00	600		
	2	8	12.8	540.5 ± 29.5	591	10.11	0.006
	3	9	16.2	305.1 ± 92.0	194		