OVERCOMING EVOLUTIONARY HISTORY: CONDITIONING THE ENDANGERED BARRENS TOPMINNOW

TO AVOID PREDATION BY THE INVASIVE WESTERN MOSQUITOFISH

By

Sarah Farnsley

Hope Klug
Assistant Professor
Biological and Environmental Sciences
(Chair)

David Aborn
Associate Professor
Biological and Environmental Sciences
(Committee Member)

Bernard Kuhajda
Tennessee Aquarium Conservation Institute
(Committee Member)

OVERCOMING EVOLUTIONARY HISTORY: CONDITIONING THE ENDANGERED BARRENS TOPMINNOW

TO AVOID PREDATION BY THE INVASIVE WESTERN MOSQUITOFISH

By

Sarah Farnsley

A Thesis Submitted to the Faculty of the University of Tennessee at Chattanooga
in Partial Fulfillment of the Requirements of the Degree of
the Master of Science: Environmental Science

The University of Tennessee at Chattanooga
Chattanooga, Tennessee
May 2014

ABSTRACT

Organisms use cues to assess their environment; however, environmental changes can create a mismatch between cues and the conditions with which they were historically associated. An evolutionary trap is when past selection pressures shaped cue-response systems that were once adaptive for an organism but no longer are. Invasive species are one cause of evolutionary traps, and the Barrens Topminnow (BTM), *Fundulus julisia* serves as an example of an imperiled species trapped by the introduction of an invasive predator: the Western Mosquitofish (WMF), *Gambusia affinis*. This study used conditioning to help the BTM escape its evolutionary trap, and data showed that conditioned fish responded significantly different to the presentation of *WMF* compared to control fish, and sampling following release of BTMs resulted recapturing exclusively conditioned individuals. These results suggest that conditioning may encourage BTM recognition of *WMF* as a predator and increase long-term survival of the BTM.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

The introduction of non-native species is one of the greatest current threats to biodiversity (Wilcove et al., 1998). In addition to their potentially negative impact on the economy, human health, and ecosystem services, non-native species can threaten the survival of native species (Pejchar and Mooney, 2009). For example, the zebra mussel, *Dreissena polymorpha*, arrived in the Great Lakes through the ballast water of transatlantic ships and now causes $1 billion annually in damages and control costs (Pimentel et al., 2005). These invasive mussels reduce food availability and oxygen levels for native mussels, clams, and snails, and physically cover native mollusks, compromising their ability to survive (Pimentel et al., 2005).

Another way in which non-native species severely threaten natives is through predation. A native species has no evolutionary history with an introduced, non-native predator, and hence, often completely lacks adaptations that would allow it to avoid predation. This has been referred to as an evolutionary trap; individuals become "trapped" by their evolutionarily engrained responses to ecological cues, and these responses are often no longer adaptive following the introduction of a novel predator species (Schlaepfer et al., 2005). Without any historical experience with which to base an appropriate response to an introduced predator, native prey may display ineffective, weak, or non-existent antipredator behavior and consequently experience reduced survival (Schlaepfer et al., 2005; Sih et al., 2010). For example, upon its introduction into islands of the West Indies as a mechanism of rat control, the Indian mongoose, *Herpestes auropunctatus*, preyed upon native ground-nesting birds and herpetofauna. As these native species evolved in an environment free of the Indian mongoose, they failed to demonstrate appropriate defensive behaviors against this exotic predator, and the islands consequently suffered at least seven native amphibian and reptile extinctions (Pimentel et al., 2005).

Compared with terrestrial and marine systems, freshwater systems are thought to be even more strongly impacted by predator introductions, owing to the patchy, heterogeneous distribution of predators and predator-free refuge in freshwater environments (Cox and Lima, 2006). This spatial variability allows for prolonged isolation from certain predator archetypes and promotes prey naiveté (Cox and Lima, 2006). Freshwater prey may therefore display enhanced sensitivity and vulnerability to an introduced predator.

The Barrens topminnow (BTM), *Fundulus julisia*, serves as a local example of a native freshwater species detrimentally impacted by the introduction of a non-native predator. BTMs are imperiled due in part to predation by the invasive Western Mosquitofish, *Gambusia affinis*. The BTM, endemic to the Barrens Plateau in Middle Tennessee (Williams and Etnier, 1982), is confined to the headwaters of three watershed: the Duck River, Elk River, and Caney Fork River (Westhoff et al., 2013). It is currently listed as state-endangered in Tennessee (Withers, 2009) and is considered endangered by the American Fisheries Society's Endangered Species Committee (Jelks et al., 2008) but has no protection under the Endangered Species Act. This species was proposed for federal listing in the late 1970s but the proposal was never finalized and BTMs were subsequently classified as a Category 2 Candidate for Federal listing until that category was discontinued in 1996. The BTM has recently been petitioned to be federally listed by the Center for Biological Diversity and other conservation organizations (USFWS, 2011).

Although native to southern parts of the United States, *WMF* populations on the Barrens Plateau are present as a result of human introduction for the purpose of mosquito control (Westhoff et al., 2013). Mosquitofish species have been implicated in the eradication or decline of native fish species such as the Least Chub, *Iotichthys phlegethontis*, the threatened Railroad Valley Springfish, *Crenichthys nevadae*, and the endangered Gila Topminnow, *Poeciliopsis o. occidentalis*, as well amphibians like the California Newt *Taricha torosa* (Gamradt,. and Katz, 1996; Galat and Robertson, 1992; Fuller et al., 1999; Millis et al., 2004). This is generally accomplished through aggressive behavior and competitive

interactions, especially concerning the early life-stages of other species (Westhoff et al., 2013). The WMF possesses several characteristics that allow for successful invasion at the expense of native species, including short generation times, broad physiological tolerances, and the ability of females to colonize novel habitats (Goldsworth and Bettoli, 2006).

As *WMF* is an introduced species, the BTM has no evolutionary history or experience with this predator and therefore a greater probability it will not be able to avoid predation by *WMF*. Despite extensive efforts to preserve BTM populations through a re-introduction program and other conservation activities, the BTM remains imperiled and its populations continue to decline (Goldsworth and Bettoli, 2006).

The lack of BTM adaptation to avoid predation by *WMF* may be leading to high BTM mortality. As a result, conservation efforts focused on BTMs must attempt to reduce *WMF* predation on BTMs. Previous research has shown that conditioning individuals to avoid a predator has increased survival in species such as Chinook Salmon (*Oncorhynchus tshawytscha*), Rainbow Trout (*Oncorhynchus mykiss*), and Brook Trout (*Salvelinus fontinalis*) (Berejikian et al., 2009; Brown et al., 1998; Mirza and Shivers, 2000). As such, I hypothesize that conditioning can be used to rescue the BTM from its evolutionary trap. Specifically, I predict that the BTM can learn to recognize *WMF* as a predator and avoid predation, which will ultimately be expected to increase longterm survival of the BTM. In order to demonstrate this, my research will aim to condition, (i.e. train), BTMs to avoid *WMF*. Specifically, I will condition BTMs to associate *WMF* with an aversive stimulus, a fish alarm pheromone that has been shown to ellicit a fear response in a range of fish species (Mathuru et al., 2012), and avoid contact with *WMF*, with the ultimate goal of increasing long-term survival of the BTM in its natural habitat.

Overview of Thesis Goals:

To assess whether BTM can escape its evoltuionary trap and be conditioned to avoid predation by *WMF*, I will conduct a three-phase study. My specific goals are to:

1. **Synthesize the literature on evolutionary traps:** As mentioned above, the BTM's lack of evolutionary history with *WMF* has caused it to fall into an evolutionary trap in which BTM individuals are highly susceptible to *WMF* predation. To begin to understand how conditioning can be used to rescue BTM from its evoltuionary trap, I will first review and synthesize the literature on evolutionary traps. In particular, I will focus on 1) the causes of evolutionary traps, 2) mechanisms by which evolutionary traps can be escaped, and 3) the likely outcome of a species or population that is rescued from an evolution trap. This review will help me identify whether conditioning is likely to rescue the BTM from its evolutionary trap **(Chapter 2)**.

2. **Determine whether the Northern Studfish, (*Fundulus catenatus*), a close relative of the BTM, responds to potential conditioning agents:** To minimize use of an imperiled species, I will first determine if the common, unprotected Northern Studfish shows any behavioral response to chondroitin sulfate, which is a standard fish alarm cue and has been found to elicit a fear response in a number of fish species. The Northern Studfish is a close relative of the BTM. As such, the Northern Studfish is expected to behave similarly to the BTM. If the Northern Studfish responds to chondroitin, it is likely that chondroitin will be an effective aversive stimulus that could be used to condition the BTM to avoid *WMF* **(Chapter 3).**

3. **Condition the BTM to escape their evolutionary trap**: If the Northern Studfish responds to chondroitin, I will determine whether the BTM also responds to this alarm cue. If the BTM does respond to chondroitin, I will condition the BTM to associate *WMF* with danger by pairing an alarm cue with the visual presentation of *WMF*. I will evaluate the conditioned and non-conditioned BTM response to *WMF* to determine if BTM can be conditioned to avoid *WMF*. To assess whether

conditioning BTMs can lead to increased survival of BTM, I will reintroduce conditioned and non-conditioned BTM into their natural habitat and estimate survival of each group in the wild **(Chapter 4)**.

4. Discuss the long-term effectiveness of conditioning in the BTM's natural habitat: In my final chapter, I will discuss the potential long-term conservation implications of conditioning the BTM to avoid predation by *WMF*. I will additionally discuss the role that conditioning has in more generally helping imperiled species escape evolutionary traps **(Chapter 5)**.

CHAPTER 2

EVOLUTIONARY TRAPS AND POTENTIAL EFFECTS OF ESCAPE

What's in a trap?

Organisms use cues to assess their environment; however, changes to the environment can

cause a mismatch between current cues and the conditions with which they were historically associated

(Schlaepfer et al., 2002; Schlaepher et al., 2005; Robertson et al., 2013). Individuals can consequently

become "trapped" by their evolutionarily engrained responses to novel environmental cues and may

experience reduced fitness when a behavioral response to a cue is no longer adaptive or beneficial

(Schlaepfer et al., 2002). A disconnect between a current environmental cue and the effectiveness of an

organism's evolutionary response to that cue can hinder the organism's ability to make appropriate life-

history decisions involving reproduction, migration, foraging, mating, or, in the case of ecological traps,

habitat (Schlaepfer et al., 2002). An evolutionary trap can therefore be broadly characterized as an

instance in which past selection pressures shaped cue-response systems that were once adaptive for an

organism but no longer are (Schlaepfer et al., 2002; Robertson et al., 2013).

More specific definitions exist in the literature, some considering human-induced environmental

change (Robertson et al., 2013), rapid change, (Robertson et al., 2013; Schlaepfer et al., 2002), or the

loss of fitness incurred from change (Robertson et al., 2013; Schlaepfer et al., 2002) as necessary and

defining features of an evolutionary trap. While it is useful to identify factors that influence or

characterize evolutionary traps (e.g. rapid or human-induced change), applying any one of these explicit

definitions could perhaps fail to recognize the existence of an evolutionary trap that lacks the particular

factors required. Below, I discuss limitations of these more specific definitions.

First, must traps always result from human-induced change? Any of various natural disturbances

would seem to have the potential to initiate an evolutionary trap; a species essential to the trophic

structure of an island ecosystem could be eliminated following a hurricane, suitable habitat may be

destroyed as a result of fire, or a specialized food source might become scarce following a drought.

These examples involve natural occurrences interfering with the reliability of past environmental cues

and could consequently result in inappropriate responses by affected organisms, leading to evolutionary

traps.

In regards to definitions requiring "rapid" change, rather than consider the amount of time it

takes for an evolutionary trap to develop, it seems more appropriate to recognize that change occurs

faster than populations can adapt. Additionally, certain organisms or populations have been

hypothesized to be capable of more readily adapting to environmental change than others, (i.e. larger

populations) (Robertson et al., 2013; Schlaepfer et al., 2002) and other factors besides time are thought

to influence susceptibility to traps (Robertson et al., 2013). This would therefore make time a relative

component in the characterization of evolutionary traps but not necessarily a defining feature.

Finally, must there always be documented negative effects associated with an evolutionary

trap? Some would argue that the American Pronghorn, *Antilocapra americana*, appears to be trapped by

anti-predator behavior that was evolutionarily engrained more than 10,000 years ago (Byers, 1998).

Speeds of 60 miles per hour are no longer an appropriate response to predation, as no animal in North

America could catch this prey, but the behavior seems to occur with no apparent major negative

impacts to its survival (Byers, 1998).

For the operational purposes of this paper, an evolutionary trap will be therefore defined as a

phenomenon in which an organism displays an inappropriate response to a novel environmental cue

based on a mismatch between the current cue and the evolutionary conditions with which it was

associated. This definition recognizes that environmental change can cause the disruption of cue-

response systems upon which organisms have historically relied, but it does not include specific,

obligatory conditions under how and when this change must occur.

Two main questions concerning evolutionary traps have received a large amount of attention in the literature: 1) What causes evolutionary traps? (reviewed in Schlaepfer et al., 2002, Schlaepfer et al., 2005) and 2) How can evolutionary traps be escaped? (Schlaepfer et al., 2002; Keeler and Chew, 2008). Research into these questions, especially regarding manipulative experiments, is still needed, as populations continue to be devastated by traps and new instances of traps are continually being recognized (reviewed by Schlaepfer et al., 2002). However, there is a third, largely unexplored question: what happens once a species escapes from an evolutionary trap? For example, is an organism that falls into a trap once relatively likely to fall into another trap in the future? Will an organism that is freed from a trap have similar or different ecological impacts than it did prior to falling into the trap? It has been broadly determined that traps are caused by change and escaped through change (Schlaepfer et al., 2002; Robertson et al., 2013) but we must also consider what changes could result from a species being freed from an evolutionary trap.

Questions that work on evolutionary traps aim to understand

What causes evolutionary traps?

Evolutionary traps are caused when environmental cues are altered and initiate an inappropriate response because of the past conditions with which that cue was associated, or when novel conditions reduce the fitness incurred from choosing a specific resource, or, in the case of the most severe traps, when both situations occur (Robertson et al., 2013). Novel environmental circumstances can result from anthropogenic activities including agricultural practices, ecotourism, and the introduction of exotic species (Robertson et al., 2013). For instance, without any historical experience with which to base an appropriate response to a novel predator, native prey may display ineffective, weak, or non-existent anti-predator behavior and consequently experience reduced survival (Schlaepfer et al., 2005; Sih et al., 2010). As an example, upon its introduction into islands of the West

Indies as a mechanism of rat control, the Indian Mongoose, *Herpestes auropunctatus*, preyed upon

native ground-nesting birds and herpetofauna (Pimentel et al., 2005). As these native species evolved in

an environment free of the Indian Mongoose, they failed to demonstrate appropriate defensive

behaviors against this exotic predator, and the islands consequently suffered at least seven native

amphibian and reptile extinctions (Pimentel et al., 2005).

Additionally, various ecological factors can trigger a trap, such as reduced prey availability,

increased resource competition, or the presence of inappropriate mates (Robertson et al., 2013). An

invasive frog species, the American Bullfrog, *Rana catesbeiana,* may have initiated an evolutionary trap

for native California Red-legged Frogs, *Rana draytonii*, by acting as an unsuitable mate (D'Amore et al.,

2009). The presence of *R*. *catesbeiana* in the native range of *R*. *draytonii* potentially interfered with the

previously adaptive sexual selection mechanisms of the native species, suggested by observed attempts

at interspecific mating (D'Amore et al., 2009). Regardless of the mechanism or cause behind an

evolutionary trap, if the extent of environmental change is greater than the range that has been

historically experienced, an organism may not be equipped to survive (Schlaepfer et al., 2002).

There is believed to be variation in ability to respond to novel circumstances, and thus variation

in susceptibility to evolutionary traps (Robertson et al., 2013). These differences are thought to have an

evolutionary basis and can be largely explained by cue-response systems, response thresholds, and cost-

benefit analyses (Robertson et al., 2013). For example, organisms are more likely to get trapped if a

novel cue representing a poor option strongly mimics a cue that historically represented a good option

(Robertson et al., 2013). This especially holds true if the past cue indicating a good option was reliable

(Robertson et al., 2013). Furthermore, ifin its evolutionary history, an organism incurred a high cost

when rejecting a good option, but did not necessarily suffer a high cost when mistakenly choosing a

poor option, the organism is more likely to be susceptible to evolutionary traps (Robertson et al., 2013).

Individual variation among members of the same species has also been suggested, in that bolder

individuals are more likely to ignore a novel predation threat and exploit novel resources versus fearful individuals, making daring individuals more likely to be trapped as a result (Robertson et al., 2013).

How can a species escape a trap?

The literature identifies two major means by which a population of trapped individuals can escape an evolutionary trap: phenotypic plasticity and natural selection (Barrett and Hendry, 2012; Schlaephfer et al., 2002). Organisms have been shown to make non-genetic, plastic modifications in response to an evolutionary trap, such as adjusting behavior via experience-based learning (Schlaepfer et al., 2002). Behavioral phenotypes can therefore be altered developmentally and may mitigate the effects of evolutionary traps (Barrett and Hendry, 2012). For example, a study focused on the behavioral response of the House Finch, *Carpodacus mexicanus,* to sick conspecifics and suggested that the observed plastic quality of disease defense behavior could be especially valuable when novel pathogens emerge (Zylberberg et al., 2012).

By means of conditioning and associative learning, it seems possible to take advantage of an organism's behavioral plasticity by encouraging a desired response through the use of external cues (Sih et al., 2011). Prior to reintroduction into the wild, juvenile Black-tailed Prairie Dogs, *Cynomys ludovicianus,* were conditioned by pairing visual exposure to a predator with alarm vocalizations, and results showed that this associative learning promoted post-release survival (Shier and Owings, 2006). Following conditioning experiments such as this, prey are no longer naïve to a predator and are better equipped to assess risk of predation and exhibit effective defensive behaviors (Sih et al., 2010). Conditioning efforts may even benefit the entire population if the conditioned behavior can be horizontally transmitted, (Sih et al., 2011) which can be especially useful in long-lived species (Schlaepfer et al., 2002).

Although these plastic responses could cause shifts in the frequencies of phenotypes of polyphonic traits, this may not result in any significant evolutionary change in the population (Strauss et al., 2006). Furthermore, although phenotypic plasticity can serve as a first defense against evolutionary traps, these adjustments may have associated costs and can even be maladaptive in some instances (Barrett and Hendry, 2012).

It therefore appears that in many cases a population must ultimately overcome evolutionary traps by responding with evolutionary change (Barrett and Hendry, 2012). The opportunity for adaptation by natural selection will not only depend on a population's size and underlying genetic variation but also on the strength of the effect that the novel environmental condition has on individuals and ultimately the population (Schlaepfer et al., 2002). If the environmental change strongly impacts individual fitness and the population is large enough that it contains sufficient genetic variation so that genotypes and associated phenotypes are affected differentially, then an adaptive evolutionary response would be expected (Schlaepfer et al., 2002).

If a trapped population can either adjust to environmental novelty by means of phenotypic plasticity or adapt to change via natural selection acting on existing genetic variation, then a population may have the capacity to escape from its evolutionary trap (Schlaepfer et al., 2002; Barrett and Hendry, 2012). One study suggests that a native butterfly may have freed itself from its evolutionary trap by adapting to an invasive plant host (Keeler and Chew, 2008). In areas where it was not as prominent, invasive Garlic Mustard, *Alliaria petiolata,* was shown to serve as a population sink and evolutionary trap for the native Mustard White butterfly, *Pieris oleracea*, because the butterfly larvae do not develop well on the exotic plant (Keeler and Chew, 2008). However, in areas where Garlic Mustard was well-established, the native butterfly seemed to have adapted to the invasive plant and even preferred it for oviposition (Keeler and Chew, 2008).

What happens once a species escapes from a trap?

In the same way that environmental changes can initiate changes in an organism and within a population, it can be assumed that once an organism escapes a trap via adaptation, there will be subsequent changes to the organism, the population, the surrounding ecosystem, or perhaps to all. There are published examples of changes in morphological, physiological, and life history traits that are thought to have evolved as a result of selection associated with novel environments (Strauss et al., 2006). As an example, character displacement can occur in fish as a result of the introduction of novel competitors. Fish adapt by exhibiting specialized polymorphic feeding types (Bourke et al., 1999) or altering life history traits (Crowder, 1986), and while such adaptations often prove to be beneficial, it is also possible that resultant changes can constrain evolutionary diversification. For example, behavioral changes in foraging from terrestrial to arboreal in pigeons and doves lead to associated changes in morphology and specialized functionality. The cost of arboreal foraging limited subsequent diversification of arboreal-dwelling pigeons and doves and inhibited reversals to terrestrial foraging in these lineages, which may have led to an evolutionary dead end (Lapiedra et al., 2013).

Escape from an evolutionary trap could result in energy reallocation, perhaps by sacrificing on other essential energy expenditures. One evolutionary trap example may be increased seawater temperatures as a result of anthropogenic activity. This change has created a novel environmental condition, and coral species react to this cue with a stress response, which causes bleaching and disruption of the symbiosis between coral hosts and dinoflagellates. In simulated bleaching events, it was found that bleaching can have sub-lethal effects by causing coral to reduce reproductive output as a result of the energetic costs associated with the loss of their photosynthetic partners. In an attempt to adapt and escape this evolutionary trap, it seems that the coral species are sacrificing energy that would otherwise be put into reproductive effort. In fact, response to increased sea water temperatures is so

12

energetically costly that it is was shown to affect reproductive output for two years (Michalek-Wagner and Willis, 2001).

Following escape from a trap, the adaptations of the population may affect other interacting species and the ecosystem as a whole, perhaps by initiating trophic cascades. One study examining a food web dynamic involving predatory spiders, herbivorous grasshoppers, and a herbaceous grass. Faced with the risk of predation by spiders, grasshoppers exhibited defensive behavior by reducing the amount of grass that they consumed and decreasing feeding time, which in turn lowered the impact of this herbivore on grass biomass (Schmitz et al., 1997). This scenario suggests that a behavioral response is enough to affect other ecosystem components (Schmitz et al., 1997) and supports the idea that if a population attempts to escape an evolutionary trap by means of a behavioral adjustment, other trophic levels may subsequently be affected.

An escaped population may experience dramatic increases in abundance as a result of no longer being constrained by a trap, which could create or increase competition, deplete resources, or shift ecological niches (Levin, 2003). If the escaped population is forced to specialize on a new resource, this could push another less competitively equipped population out of their established niche, thus threatening the persistence of a newly affected population (Levin, 2003).

Escape of an evolutionary trap by one species could simply shift the negative effects to another species, initiating another evolutionary trap. Consider the previously mentioned example of the native Mustard White butterfly escaping from its evolutionary trap involving the invasive plant host (Keeler and Chew, 2008): in the highly specialized insect-plant relationship, the butterfly's escape from a trap via adaption to a novel, invasive host plant could detrimentally affect native plants.

Finally, the permanence of overcoming an evolutionary trap must be examined. As previously stated, some organisms are especially susceptible to traps (Robertson et al., 2013; Schlaepfer et al., 2002) and could therefore be more prone to being trapped by a completely new cue following escape,

or even fall right back into the same trap. The latter scenario may be especially likely if escape from a trap simply initiates or maintains an evolutionary arms race. For instance, ocean acidification as a result of increased anthropogenic CO_2 emissions represents a novel environmental condition for marine organisms. Bivalves, such as the Blue Mussel, *Mytilus edulis*, normally employ immune defenses, such as production of cuticle, mucous, enzymes and peptides, against invasive microorganisms; however, when experimentally exposed to levels of acidification relevant to global ocean acidification, the Blue mussel was forced to adapt to this novel environmental condition by devoting energy away from immune defenses to shell formation and growth, leaving the bivalve more susceptible the bacterial pathogen, *Vibrio tubiashii*. In response, the pathogen is simultaneously adapting to changes in the bivalves by displaying increased rates of mutation, shorter generation times, and horizontal gene-transfer, thus engaging the bivalves in an evolutionary arms race,in which the pathogens appear to currently be winning (Asplund et al., 2013).

Discussion

Environmental change can lead to evolutionary traps, which may force populations of organisms to change or face possible extinction (Schlaepfer et al., 2002). Before encouraging these populations to escape their traps through implementation of conservation efforts, it is necessary to first try and anticipate what changes may result from escape (Table 1). However, just as some of the causes behind traps and the mechanisms of escape remain unpredictable (Robertson et al., 2013), such may be the changes that occur once a population is freed from a trap. Furthermore, each case regarding escape from an evolutionary trap should be addressed on a case-by-case basis, as each layer of change involved in the steps of an evolutionary trap adds complexity to the specific system.

Table 2.1 Questions to address prior to implementing conservation efforts to free a species from an
evolutionary trap

Does the apparently maladaptive response to the trap possibly represent a long-term adaptive strategy?
What morphological, physiological, behavioral, and/or life-history traits are likely to be affected following escape?
What effects will the proposed action have on the overall ecosystem? Is it likely to initiate a trophic cascade?
Is it likely that proposed action will initiate another trap?
Will escape from the trap be accomplished via exploitation of a population's plasticity, or will the population be encouraged to evolve?
- If plasticity will be utilized via conditioning efforts, what is the likelihood of horizontal transfer within the population?
- If conservation efforts will utilize selective pressures to encourage the population to evolve, does the population contain sufficient genetic variation for natural selection to act on?
Will proposed action induce an evolutionary arms race?

Nevertheless, some issues would seem to broadly apply to the general phenomenon of populations escaping from an evolutionary trap. Some expected questions may be: Do populations even stand a chance against a backdrop of a rapidly changing environment? Will escaped populations just be trapped by the next anthropogenically-induced novel environmental cue? Can rates of adaptation keep up with global change? While issues linking climate change, phenotypic plasticity, biodiversity, and microevolution have been explored in depth, there is still more information needed (Visser, 2008).

In attempting to free a population from an evolutionary trap, deciding on a plan of conservation action, including the option of "no action," requires that we have a sense of what happens once a population escapes. There may be instances in which no conservation action should be taken, for example, if the apparent maladaptive response to the trap may possibly represent a long-term adaptive

strategy (Robertson et al., 2013). By integrating predictive models of environmental change with

knowledge of a population's behavioral ecology and life-history traits it may be possible to better

predict the changes that will result from a population escaping its evolutionary trap (Visser, 2008;

Robertson et al., 2013).

CHAPTER 3

NORTHERN STUDFISH (*FUNDULUS CATENATUS*) RESPONSE TO THE ALARM CUE CHONDROITIN SULFATE

Introduction

Organisms assess and respond to their environment based on detection of visual, auditory, and/or olfactory cues, with specific ecological conditions often dictating the most effective mode of signal transmission (Meuthen et al., 2012). In aquatic environments, chemical signals mediate essential behavioral interactions between individuals when visual cues are limited, as in instances of high turbidity or in areas of dense vegetation (Mirza and Chivers, 2000). The olfactory reception of chemical cues in aquatic habitats can increase individual fitness by allowing individuals to identify a conspecific mate (Rafferty and Boughman, 2006), locate spawning sites (Sorensen et al., 2005), and establish social structure (Moore and Bergman, 2005). Chemical signals can also act to decrease the likelihood of a fatal encounter, as various groups of aquatic organisms interpret certain chemical cues as a threat of predation and consequently respond with antipredator behavior (Ferrari et al., 2010). The odor of a nearby predator (Tollrian and Harvell, 1999), the urinary ammonia discharged from startled prey (Kiesecker et al., 1999), and/or substances released following injury inflicted by a predator have all been shown to serve as alarm signals that initiate defense responses in prey (Wisenden, 2003).

For example, in many prey species of fishes, such as minnows and other members of the superorder Ostariophysi, predatory damage to an individual's epidermal cells will release a substance that functions as a chemical alarm cue to neighboring conspecifics (Pfeiffer, 1977; Ferrari et al., 2010). Originally known as *Schreckstoff,* this alarm substance was first discovered to elicit a fright response in minnows (Von Frisch, 1941) and has been the focus of several evolutionary, physiological, and chemical studies (Chivers et al., 2007; Pfeiffer et al., 1985; Mathuru et al., 2012). Evidence of response to an alarm cue has now been found in fish species outside the superorder Ostariophysi, including Sculpins

(Cottidae; Bryer et al., 2000), Trouts (Salmonidae; Mirza and Chivers, 2000), and Sunfishes

(Centrarchidae; Marcus and Brown, 2003) (see also Ferrari et al., 2010 for a review).

Chemical alarm cue recognition is hypothesized to lead to increased survival of neighboring

individuals by eliciting an ecologically appropriate defense behavior (Smith, 1992; Lonnstedt et al., 2012;

Mirza and Chivers, 2000), which is expected to differ in different prey species. While it is likely most

effective for some fishes to respond to alarm cue detection with area avoidance (e.g. Pumpkinseeds,

Lepomis gibbous; Golub et al., 2005) others increase shoaling and shelter use (e.g. Fathead Minnows,

Pimephales promelas; Mathis and Smith, 1993), alter feeding activity, (e.g. Brook Trout, *Salvelinus*

fontinalis; Mirza and Chivers, 2001), and/or cease motion all together (e.g. Blackspotted Topminnow,

Fundulus olivaceus; Reed, 1969). The evolutionary significance of alarm cue production continues to be

debated, as many scientists question how this substance can be maintained through natural selection if

the only apparent benefit is to the receiver of the alarm signal and none to the sender (Meuthen et al.,

2012; Ferrari et al., 2010). It has been suggested that the epidermal cells containing the alarm substance

serve as a layer of protection from UV radiation (Manek et al., 2012; Chivers et al., 2007), parasites,

and/or disease in fish (Pollock et al., 2012; Chivers et al., 2007), suggesting their role as an alarm system

to be merely a secondary one.

Recent research has focused on the chemical makeup of the cells responsible for producing the

alarm signal. Mathuru et al. (2012) utilized biochemical fractionation to show that chondroitin

fragments are a key component of the alarm substance that elicits an anti-predator response in

Zebrafish (*Danio rerio)*.

Despite a large amount of research on alarm behavior in fishes (Smith, 1992; Chivers and Smith,

1998; Ferrari et al., 2010), it remains unclear in most species whether chondroitin acts as an alarm cue,

and if so, what response it elicits. The goal of this study was to investigate the ability of chondroitin to

elicit an alarm response in the Northern Studfish (*Fundulus catenatus*), a stream fish widely distributed in the Ohio and Mississippi River drainages (Page and Burr, 2011).

Alarm responses have been well-documented in stream fishes, for example in Brook Trout, (Mirza and Chivers, 2000; Mirza and Chivers, 2001), the Slimy Sculpin (*Cottus cognatus*; Bryer et al., 2001) the Iowa Dater (*Etheostoma exile*; Smith, 1979), and the congeneric Blackspotted Topminnow (Reed, 1969). As such, if chondroitin functions generally as an alarm cue in fishes, I would expect chondroitin to elicit a behavioral response in the Northern Studfish. Topwater prey fishes that live near aquatic or overhanging terrestrial vegetation have been shown to cease movement in the presence of predators (Reed, 1969), and I therefore hypothesized that freezing would be a likely response of the Northern Studfish to this alarm substance. To assess this, behavior was observed before and after addition of chondroitin, and changes in behavior were compared to those following the addition of a control substance. I discuss my findings in relation to potential uses of the alarm cue chondroitin in the conservation of stream fishes.

Methods

Fish acquisition and maintenance

Adult and juvenile Northern Studfish were collected using seines from the Collins River in McMinnville, Tennessee on May 23, 2013. When not in use in experimental trials, fish were housed in groups of 8 in 75.7 L aquaria at the University of Tennessee Chattanooga. Fish were fed frozen bloodworms daily and maintained on a 14:10 h light:dark cycle. Water temperature was maintained at approximately 18°C.

Experimental trials

Experiments took place during June and July of 2013. To evaluate the effect of the alarm substance chondroitin on Northern Studfish behavior, I conducted an experiment that consisted of two treatments: 1) exposure to chondroitin and 2) exposure to a control substance (distilled water). Just prior to use in an experimental trial, I measured the total length of each fish and categorized it as either a male, female, or juvenile. Each replicate began by randomly selecting one fish and placing it in a 75.7 L observation tank for experimentation. The observation tank was isolated from all other tanks (i.e. fish in the observation tank did not have any contact with other fish) and from the behavioral observer. Specifically, observations were performed from behind an opaque plastic barrier through eye slits and with the aid of mirrors positioned above the experimental tanks. This set-up allowed us to minimize exposure to any cues other than the substance added to the tank. Fish were allowed to acclimate for approximately one hour. For 10 minutes following acclimation and prior to substance addition, fish behavior was observed. During this time, movement (i.e. whether the fish was motionless or in motion) and vertical distribution (top, middle, or bottom third) in the tank were recorded every 30 seconds via scan sampling. At the completion of 10 minutes, the substance (either chondroitin or distilled water) was added through tubing (approximately 5 mm in diameter) that had previously been run into the tank. Preliminary studies in which dye was added to the substance confirmed that this method led to the substance being dispersed throughout the tank within seconds. The control substance was 5 mL of distilled water. The chondroitin treatment consisted of 0.07mg of commercially available chondroitin (Sigma C4384) dissolved in 5 mL of distilled water. This concentration of chondroitin is consistent with levels used in the Mathuru et al. (2012) study that elicited an alarm response in Zebrafish. Another 10 minute observational period followed substance addition, with the same behaviors being assessed. Each fish was only used once and a total of 18 trials per treatment were completed.

Statistical Analysis

I calculated the percentage of time spent motionless prior to and after substance addition. This allowed me to then calculate the change in activity for each individual fish following the addition of the substance (chondroitin or distilled water). The percentage of time spent on the bottom third of the tank was calculated before and after substance addition for each trial, and I calculated the change in use of this lower third of the tank for all individuals.

I used stepwise ANCOVA (remove if $P > 0.15$) to examine the effect of chondroitin on the change in 1) activity (i.e. the change in the proportion of the time spent inactive) and 2) the proportion of time spent in the bottom third of the aquarium. Treatment (chondroitin or distilled water) was treated as a fixed factor. Because I hypothesized that size and/or group (male, female, or juvenile) might also influence behavior, I included these variables in the statistical analyses. Group was treated as a random factor and total length was treated as a covariate. I additionally examined the interaction between group and treatment.

Results

A total of 36 Northern Studfish were used in the study, including 5 juveniles, 13 males, and 18 females. Individuals ranged in total length from 42 mm to 128 mm. Chondroitin significantly decreased activity in the Northern Studfish (treatment effect: $F_{1, 31} = 9.371$, $P = 0.004$; Fig. 3.1).
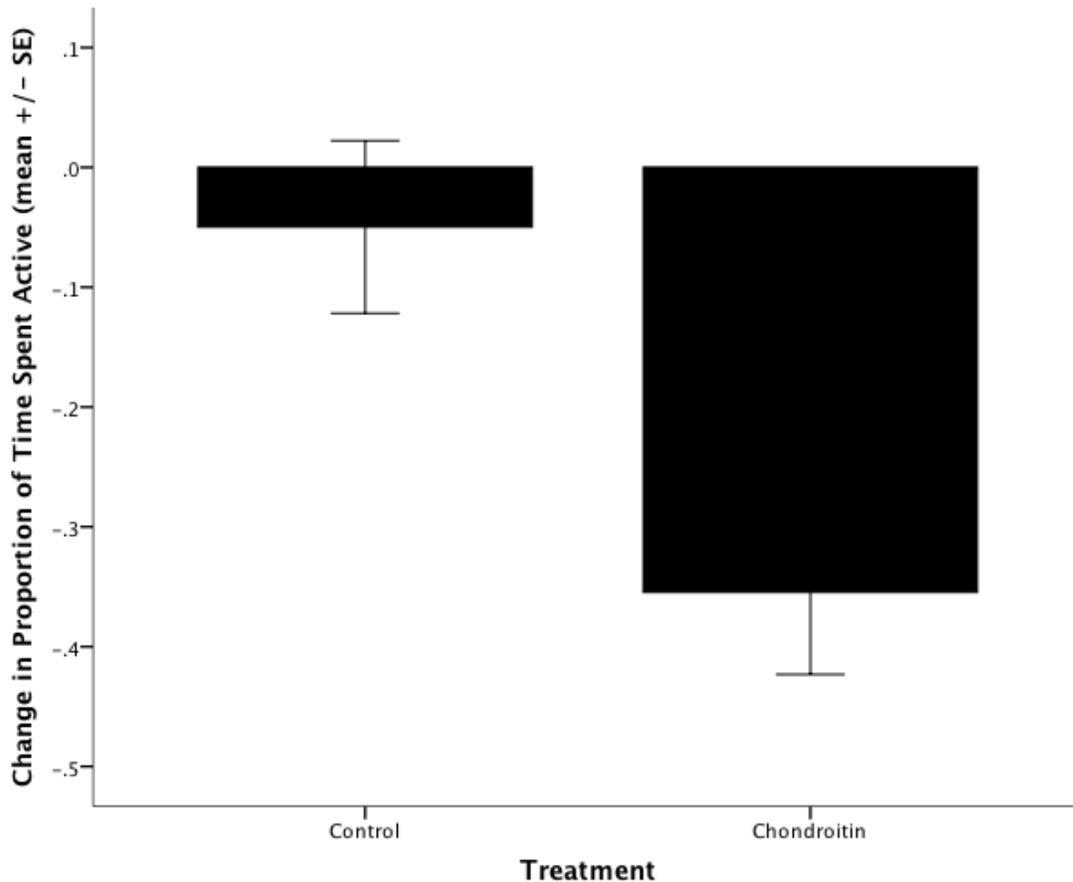
Fig. 3.1. Change in the proportion of time spent in motion by specimens of the Northern Studfish (*Fundulus catenatus*) before and after substance addition for control (distilled water) and chondroitin treatments (whiskers represent ± standard error).

Following substance addition, fish that were exposed to chondroitin tended to reduce their activity by sevenfold relative to the control group, with change in proportion of time spent in motion as -0.354 versus -0.050. However, there was no relationship between either length or the group of the fish and the decrease in activity (total length: $F_{1, 31} = 0.088$, $P = 0.769$; group: $F_{2, 31} = 0.794$, $P = 0.183$). Additionally, there was no significant interaction between treatment and group (treatment x group: $F_{2, 29} = 0.998$, $P = 0.154$).

Fish exposed to chondroitin did not significantly differ from the control group in regards to a change in the use of the bottom third of the tank following substance addition, with change in

proportion of time spent on the bottom third as -0.185 for the chondroitin treatment versus -0.026 for the control group (treatment effect: $F_{1, 31}$ = 0.488, P = 0.232). Likewise, there was no relationship between either length or group and the change in the use of the bottom third of the tank (total length: $F_{1, 31}$ = 0.29, P = 0.866; group: $F_{2, 31}$ = 0.144, P = 0.866).

Discussion

Chondroitin significantly reduced activity in the Northern Studfish, suggesting that it serves as an alarm cue in this species. As this species lives in or near aquatic or overhanging terrestrial vegetation, freezing or decreasing motion is likely an adaptive response to a predation threat (Reed, 1969). This is the first study to demonstrate Northern Studfish response to an alarm cue, and the first time that chondroitin sulfate has been shown to elicit alarm behavior in a stream fish.

The finding that chondroitin functions as an alarm response is consistent with work by Mathuru et al. (2012), who found that chondroitin fragments trigger a fear response in Zebrafish. Likewise, the finding that the Northern Studfish exhibits anti-predator behavior in response to an alarm cue is consistent with work in other stream fishes. For example, Bryer et al. (2001) found that Slimy Sculpins increased shelter use in response to damage-released cues from conspecifics and that the presence of these cues intensified alarm response to predator odor. Brook Trout have been shown to respond to alarm signals released upon injury to conspecifics by decreasing activity and reducing foraging (Mirza and Chivers, 2001).

The finding that chondroitin functions as an alarm cue in the Northern Studfish has implications for the conservation of imperiled fishes, many of which are stream-dwelling species. We hypothesize that by pairing a predatory cue with an alarm substance, fish can be conditioned to associate a predator with an anti-predator response. Once released back into their natural habitat, fish may then experience increased survival as a result of this associative learning. Mirza and Chivers (2000) showed increased

survival among juvenile Brook Trout conditioned to associate a damage-released alarm cue with

predator odor. These conditioned fish retained predator recognition in response to predator odor for 10

days following conditioning (Mirza and Chivers, 2000). Other staged predator encounters leading to

increased survival based on past experience with predator cues include juvenile Rainbow Trout

(*Oncorhynchus mykiss*; Mirza and Chivers, 2003) and Fathead Minnows (Gazdewich and Chivers, 2002*)*.

Predator recognition conditioning is particularly useful for conservation when predators are an

unfamiliar, introduced species and represent a considerable threat to prey species survival. Following

conditioning, prey are no longer naïve to a predator and are better equipped to assess risk of predation

and exhibit effective defensive behaviors. More research is needed in order to illustrate whether

freezing would be an effective defensive behavior to condition in response to a predation threat.

This work has further implications for the preservation of imperiled fish species for research

purposes; as chondroitin can induce an alarm response, sacrificing individuals for the purpose of

harvesting alarm substance material may not be necessary. However, chondroitin is known to be only

one component of the alarm substance, and anti-predator response to chondroitin could be relatively

weaker than epidermal club cell material. Future studies should focus on identifying the capability of

imperiled fishes to respond to chondroitin as an alarm substance and investigate the use of this

chemical cue in conditioning prey fishes to associate it with a predation threat.

CHAPTER 4

TEACHING AN IMPERILED SPECIES TO ESCAPE AN EVOLUTIONARY TRAP

Introduction

One of the most pressing conservation concerns is the increasing emergence of evolutionary

traps (Hobbs et al. ,2009). An evolutionary trap occurs when past selection pressures shaped cue-

response systems that were once adaptive for an organism but no longer are (Schlaepfer et al., 2002;

Robertson et al., 2013). Once in an evolutionary trap, an organism displays an inappropriate (i.e. sub-

optimal with respect to fitness) response to novel ecological conditions due to the lack of evolutionary

history with those conditions. Evolutionary traps are increasing in prevalence due to climate change, the

introduction of invasive species, and anthropogenic activities such as agriculture, harvesting, and

ecotourism (reviewed in Visser, 2008, Schlaepfer et al., 2005, Robertson et al., 2003). For example,

climate change can shift both phenology (i.e. timing of development, reproduction, and migration)

(Menzel and Fabian, 1999, BeeBee, 1995, Huppop and Huppopp, 2003) and species' range (Parmesan et

al., 1999, Thomas and Lenoon,, 1999, Austin and Rehfisch, 2005). Additionally, worldwide examples

have demonstrated that the introduction of exotic species dramatically alters communities and

ecosystems by displacing, and even extirpating, native species (Gurevitch and Padilla, 2004).

We now have a basic understanding of the general factors that are likely to lead to an

evolutionary trap and the factors that make certain populations more or less able to naturally recover

from a trap (reviewed in Robertson et al., 2013, Schlaepfer et al., 2002, Visser, 2008). For example, if an

organism experiences environmental change that occurs to a greater extent than has been historically

experienced, the organism is likely to become trapped by its evolutionary past (Schlaepfer et al., 2002),

and organisms are even more likely to fall into, and less likely to recover from, a trap if a novel cue

representing a poor option strongly mimics a cue that historically represented a good option (Robertson

25

et al., 2013). Surprisingly, though, we know relatively little about how to effectively rescue organisms from evolutionary traps (Robertson et al., 2013).

Various conservation efforts have been suggested to mitigate the effects of evolutionary traps. For example, some authors have proposed artificially creating selective pressures on native species in order to initiate the evolution of adaptive behaviors (Robertson et al., 2013). However, this may only be effective for species with ranges small enough to allow for all populations to experience the selective pressure and have the opportunity to adapt (Robertson et al., 2013). Additionally, an adaptive response to artificial selection might take too long to rescue a population. In instances when a trap is created as a result of changes to the ecosystem, habitat restoration is an obvious solution, but it may prove difficult to adequately restore a habitat. For instance, birds drawn to habitat along the forest edge created by logging may consequently suffer as mammals inhabiting these same attractive edge areas prey on bird eggs and young (Weldon and Haddad, 2005). However, removal of this cue may be difficult, since trap habitat is continually created by timber-cutting patterns. Also, if novel circumstances have been created due to the introduction of an exotic species, complete and thorough removal is improbable once these invasive populations have become largely established (Mooney and Hobbs, 2000).

In addition to more traditional conservation approaches, rescuing a populationor even an entire species from potential extinction due to an evolutionary trap may require an integrative approach that incorporates knowledge from behavioral ecology, learning and cognition, evolution, and ecology. In particular, there is evidence suggesting that, in some cases, we can teach individuals to alter their behavior in response to a novel ecological challenge (Shier and Owings, 2006; Manassa and McCormick, 2012; Mirza and Chivers, 2000; Mirza and Chivers, 2003, Gazdewich and Chivers, 2002). Below, I outline the role that learning, and in particular conditioning, can play in freeing organisms from evolutionary traps, and then describe an empirical study in which conditioning is used to potentially free an imperiled fish from an evolutionary trap.

Organisms have been shown to make plastic modifications in response to an evolutionary trap, such as adjusting behavior via experience-based learning (Schlaepfer et al., 2002). Behavioral phenotypes can therefore be altered developmentally and may mitigate the effects of evolutionary traps (Barrett and Hendry, 2012). As such, conditioning has the potential to rescue organisms from evolutionary traps, and previous work conducted in other contexts (i.e. in relation to captive breeding programs and fisheries research) suggests that conditioning can aid in conservation efforts. Prior to reintroduction into the wild as part of a captive breeding program, juvenile Black-tailed Prairie Dogs, *Cynomys ludovicianus,* were conditioned by pairing visual exposure to a predator with alarm vocalizations, and results showed that this associative learning promoted post-release survival (Shier and Owings, 2006). Following conditioning experiments such as this, prey are no longer naïve to a predator and are better equipped to assess risk of predation and exhibit effective defensive behaviors (Sih et al., 2010). Fisheries research has shown that conditioning has increased survival in species such as juvenile Brook Trout (*Salvelinus fontinalis*; Mirza and Chivers, 2000), juvenile Rainbow Trout (*Oncorhynchus mykiss*; Mirza and Chivers 2003) and Fathead Minnows (*Pimephales promelas*; Gazdewich and Chivers 2002*)* when presented with a non-native predator to which these individuals would have otherwise have been naïve to. Conditioning efforts may even benefit the entire population if the conditioned behavior can be horizontally transmitted (Sih et al., 2011). Naïve damselfish, *Acanthochromis polyacanthus*, have been shown to respond to a novel predator after observing the behavior of experienced members of the same species who were previously conditioned with chemical alarm cues to recognize a predator (Manassa and McCormick, 2012). It is therefore likely that conditioning can be used to modify a sub-optimal response to a novel ecological cue, and that such behavior modification can free organisms, and potentially entire populations, from an evolutionary trap.

To begin to understand if conditioning can be used to free individuals from an evolutionary trap and increase survival, a study was conducted in which I attempted to free an imperiled fish species from

its evolutionary trap by conditioning individuals to avoid an introduced predator. I focused on the

Barrens Topminnow (BTM), *Fundulus julisia*, a small-bodied freshwater fish that is state endangered in

Tennessee. It is currently listed as state-endangered in Tennessee (Withers, 2009) and is considered

endangered by the American Fisheries Society's Endangered Species Committee (Jelks et al., 2008) but

has no protection under the Endangered Species Act. The BTM is ideal for such a study because it is in an

evolutionary trap initiated by the introduction of the invasive Western Mosquitofish (WMF), *Gambusia*

affinis. The WMF is thought to prey on and harass larval and juvenile BTMs, which leads to high BTM

mortality (Goldsworth and Bettoli, 2006). As *WMF* is an introduced species, the BTM has no evolutionary

history or experience with this predator and therefore no evolutionarily engrained antipredator

response to *WMF*. Previous conservation efforts for the BTM, including a large reintroduction program

and extensive habitat restoration of particular springs, have had limited success, and recently, there has

been discussion about whether effort should continue to be invested in saving this imperiled fish given

the lack of success of previous conservation work (personal communication, October 12th, 2012). Given

that fish learn through conditioning (Mirza and Chivers, 2000; Manassa and McCormick, 2012;

Gazdewich and Chivers, 2002), I hypothesized that BTM could be trained (i.e. conditioned) to recognize

WMF as a predator and respond with ecologically-appropriate antipredator behavior, which could in

turn improve BTM survival. To my knowledge, this is the first study to use conditioning with the specific

aim of freeing individuals from an evolutionary trap.

Specifically, I used an alarm cue found in fish epidermal cells, chondroitin sulfate, that elicits a

fear response in other fish species (Mathuru et al., 2012; Farnsley et al. in review) to condition BTM to

avoid predation and/or harassment by *WMF*. In the first phase of the study, I examined if the BTM

responds to chondroitin sulfate to verify that this substance can be used as an aversive stimulus in

conditioning. BTMs did respond to the alarm cue (described below), and in the second phase of the

study, I conditioned BTM individuals to associate *WMF* with potential danger by pairing this aversive

alarm cue with the visual presentation of *WMF*. I then evaluated the behavioral response of conditioned and non-conditioned BTMs to the presentation of *WMF* to determine if the BTM can be conditioned to avoid *WMF*. Finally, to establish whether such conditioning could potentially free BTM from their evolutionary trap, I assessed the survival effects of conditioning following release of the BTMs into their natural habitat.

Methods

Fish acquisition and maintenance

I obtained juvenile BTMs propagated and reared in captivity from Conservation Fisheries Inc. in Knoxville, TN. *Gambusia affinis* were collected from a spring where BTMs had been reintroduction, the Ramsey Barn site, and where BTMs from this experiment were to be released at a later date. When not in use in experimental trials, fish of each species were housed in groups of 25 in 75.7 L aquaria at the University of Tennessee Chattanooga, fed live brine shrimp daily, and maintained on a 14:10 h light:dark cycle. Water temperature was maintained at approximately 18°C.

Phase I: Testing BTM response to alarm cue

The first experiment took place during November and December of 2013. To evaluate the effect of the alarm substance chondroitin on BTM behavior, I conducted an experiment that consisted of two treatments: 1) exposure to chondroitin and 2) exposure to a control substance (distilled water). Each replicate began by randomly selecting one fish and placing it in a 37.85 L observation tank isolated from all other tanks (i.e. fish in the observation tank did not have any physical, visual, or chemical contact with other fish) and from the behavioral observer. Specifically, observations were performed from behind an opaque plastic barrier through eye slits and with the aid of mirrors positioned above the

29

experimental tanks. This set-up allowed minimal exposure to any cues other than the substance added

to the tank.

Fish were allowed to acclimate for approximately one hour. For 10 minutes following

acclimation and prior to substance addition, fish behavior was observed. During this time, movement

(i.e. whether the fish was motionless or in motion) was recorded. This experiment focused on motion

because change in movement patterns is indicative of anti-predator behavior in other topwater prey

species (Reed, 1969; Farnsley et al., in review). At the completion of 10 minutes, the substance (either

chondroitin or distilled water) was added through tubing (approximately 5 mm in diameter) that had

previously been run into the tank. Preliminary studies in which dye was added to the substance

confirmed that this method led to the substance being dispersed throughout the tank within seconds.

The control substance was 5 mL of distilled water. The chondroitin treatment consisted of 0.07mg of

commercially available chondroitin (Sigma C4384) dissolved in 5 mL of distilled water. This concentration

of chondroitin is consistent with levels used hat elicited an alarm response in Zebrafish (*Danio* rerio) and

Northern Studfish (*Fundulus catenatus*) (Mathuru et al., 2012; Farnsley et al., in review). Another 10

minute observational period followed substance addition, with the same behavior being assessed. Each

fish was only used once and a total of 10 replicates per treatment were completed.

A Univariate Analysis of Variance was used to examine the effect of chondroitin on the change

in activity (i.e. the change in the proportion of the time spent active). Treatment (chondroitin or distilled

water) was treated as a fixed factor with change in activity as the dependent variable.

Phase II a: Conditioning BTM to associate WMF with an aversive stimulus

Conditioning took place during December of 2013. Tanks were isolated and observed in the

same manner as in Phase I, but for Phase II, a transparent plastic divider with 5 mm holes (which

allowed for visual and chemical, but not physical, interaction among fishes) was used to divide each

aquarium in half.

Fig. 4.1 Experimental tank setup

None of the BTMs used in the previous phase of testing for a response to chondroitin were used

for the conditioning trials (i.e. all fish were naïve to chondroitin). The following treatments were used: 1)

control: two BTMs, no *WMF*, distilled water added to aquarium, 2) conditioning: two BTMs,two *WMF*

added to aquarium (separated from BTMs by transparent divider), chondroitin sulfate added.

Specifically, each replicate began by adding two BTM to the tank and allowing the fish to

acclimate for six to eight hours. For the conditioning treatment, chondroitin was added via tubing and

two adult *WMF* were simultaneously added to the tank on the side of the divider opposite the BTMs. For the control treatment, distilled water was added to the tank.

Each fish was only used once and twelve replicates of the control treatment and eleven replicates of the conditioning treatment were completed. Conditioned fish were then tagged with elastomer paint on their left side and control fish with elastomer paint on their right side. Fish from each treatment were housed in separate tanks until they were used in the next phase of the study.

Phase II b: Evaluating effectiveness of conditioning

Aquaria were set up in the same manner as in the conditioning trials, and the same individual fish were used from Phase II a. Fish were tested from both treatments approximately 24 hours after conditioning for a behavioral response to the visual and chemical presentation of *WMF*, with no substances added to the tank. Two BTMs from the same treatment (conditioned or control) were put into each tank and were allowed to acclimate for six to eight hours. During an initial 10 minute observational period, the time that each fish spent in motion was recorded. Two adult *WMF* were then added to the tank on the side of the divider opposite the BTMs, followed by another 10 minute observational period in which each fish's time spent in motion was recorded. Change in motion for each BTM was then calculated. A Univariate Analysis of Variance was used to examine the effect of conditioning on the change in activity (i.e. the change in the proportion of the time spent active). To avoid pseudoreplication, each tank (rather than each fish) served as a replicate. Treatment (conditioning or control) was treated as a fixed factor with change in activity of the fish in each tank as the dependent variable.

Phase III: Release and recapture:

With conditioned and non-conditioned fish marked differently with elastomer paint (described above), and following a negative disease screening, fish from both treatments were released into the wild at the Ramsey Barn site 14 days following conditioning.

Fig. 4.2 Release site

A total of 22 conditioned BTMs and 24 control BTMs were released. Three days later, sampling was performed by seining 20 times for approximately four hours in an attempt to recapture marked fish from this study. As mentioned above, I hypothesized that conditioning BTM to associated *WMF* with an alarm cue would increase BTM survival. As such, I expected to re-capture more conditioned than unconditioned BTMs.

I used a Chi Square test to determine if conditioned fish more likely to be recaptured than unconditioned fish. I anticipated that I would be able to recapture a relatively small number of fish (as recapturing small fish by seining is logistically difficult). Due to the small expected number of recaptured

fish, I used a Likelihood Ratio test, which is the most appropriate Chi-Square analysis when expected

values are relatively low.

Results

BTMs respond to the chondroitin alarm cue

There was a significant difference between the BTM's response to the addition of the control

substance (distilled water) and the addition of chondroitin (treatment effect: $F_{1,17} = 6.723$, $P = 0.020$).

Specifically, chondroitin significantly decreased activity in the BTM with a mean change in proportion of

time active of -0.21 (+/- 0.087) following the addition of chondroitin. Control fish exhibited a mean

change in proportion of time active of 0.00035 (+/- 0.027) following the addition of distilled water.

Conditioning affects BTM response to the novel predator

As previously stated, the same fish used in the conditioning trials were used to test the effects

of conditioning. There was a significant difference between the response of conditioned fish to *WMF*

and the response of the control group to *WMF* (treatment effect: $F_{1,21} = 4.397$, $P = 0.048$; Fig. 2).

Specifically, conditioned fish increased activity in the presence of *WMF* significantly more than
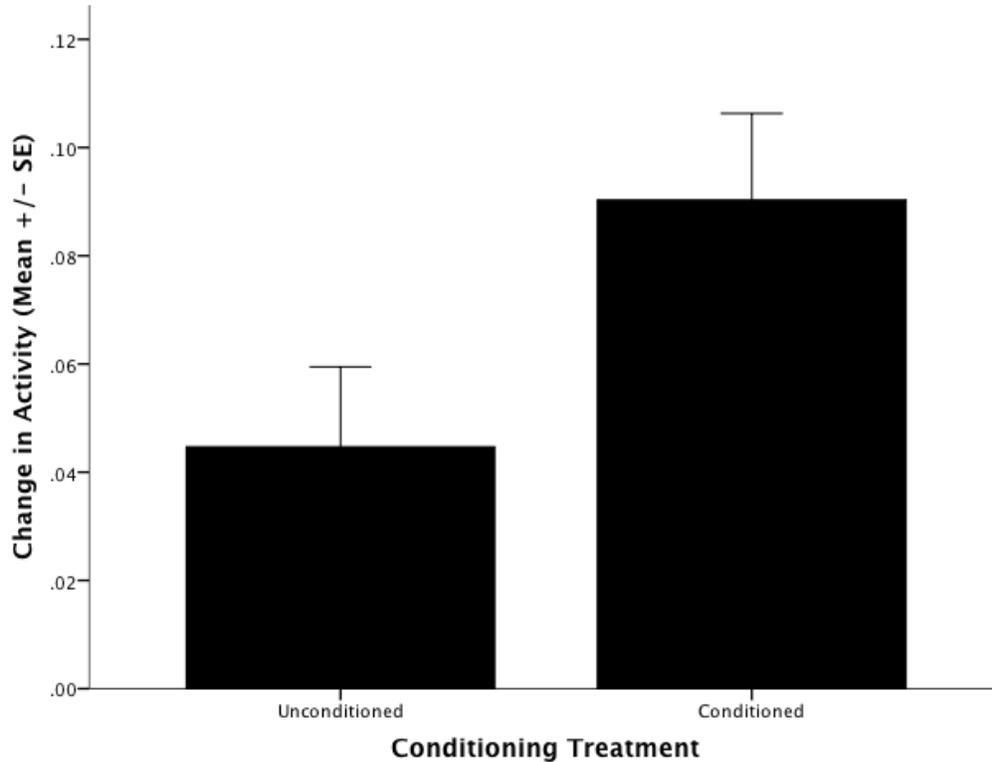
unconditioned fish did (Fig. 4.1).

Fig. 4.3 Change in the proportion of time spent in motion by specimens of Barrens Topminnows (*Fundulus julisia*) before and after presentation of Western Mosquitofish (*Gambusia affinis*) for unconditioned and conditioned treatments (whiskers represent ± standard error).

Conditioning has the potential to free BTM from its evolutionary trap

A total of three marked fish from this study were recaptured, with all three identified as conditioned fish (marked on their left side with elastomer paint). While five resident, unmarked BTMs were captured at this site during the 20 sampling attempts, no control fish from this study were recaptured. This represented a significant difference between expected and observed recapture rates for the two groups ($\chi^2_1 = 4.655$, *P* = 0.031). In other words, conditioned fish were significantly more likely to be re-captured than unconditioned fish, and this is potentially because conditioned fish were more

likely to survive than unconditioned fish. A total of 269 *WMF* were collected during sampling on this date.

Discussion

Evolutionary traps are a pressing conservation concern, yet relatively little is known about how to effectively rescue organisms from evolutionary traps (Schlaepfer et al., 2002). Here, I have shown that learning can be used to potentially rescue an organism from its evolutionary trap. In particular, my work illustrates that conditioning individuals to associate an introduced predator with an aversive stimulus can elicit behavioral changes and make it more likely that those individuals survive when exposed to the predator in the wild. Specifically, BTMs that were conditioned to associate the introduced predator *WMF* with the alarm cue chondroitin altered their behavior relative to unconditioned fish and were more likely to be re-captured after release into the wild than unconditioned fish, suggesting that they had higher survival than unconditioned fish. To my knowledge, my study is the first to directly demonstrate that conditioning can potentially be used to rescue organisms from an evolutionary trap. However, the finding that conditioning can alter the behavioral response to a predator is consistent with work done in other species (e.g. Shier and Owings, 2006). Likewise, the finding that conditioning has the potential to improve survival is consistent with research done in other contexts (Mirza and Chivers, 2000; Gazdewich and Chivers, 2002; Mirza and Chivers, 2003).

Importantly, the sample sizes of my study were small and my assessment of survival (i.e. recapture likelihood) was conducted over a relatively short time frame (three days). The results of this work therefore provide only a first look at how conditioning can be used to rescue organisms from evolutionary traps. However, it is still noteworthy that all recaptured individuals from this project were those that were conditioned to associate *WMF* with an alarm cue, and this is especially relevant considering the high density of *WMF* at the site. In the future, it will be important to explore the role of

conditioning in rescuing individuals from evolutionary traps in studies with larger sample sizes and over

a longer time period. Additionally, in order to escape the evolutionary trap in a meaningful way,

successful BTM reproduction and recruitment would be needed (i.e. the reintroduced

population would have to become self-sustaining). Regardless, the use of conditioning to rescue

populations from evolutionary traps warrants further attention.

In addition to demonstrating a potential role for conditioning in rescuing organisms from

evolutionary traps, my study also revealed that the alarm cue chondroitin affects behavior in the BTM.

Addition of chondroitin alone significantly reduced activity of the BTM, suggesting that this substance

serves as an alarm cue in this species. As a topwater prey species living near vegetation, freezing is likely

one adaptive antipredator behavior (Reed, 1969; Chapter 3; Farnsley in review). The finding that

chondroitin potentially functions as an alarm cue is consistent with work done in other species (Mathuru

et al.,2012; Chapter 3; Farnsley in review).

Conditioning BTMs to associate the predator *WMF* with the alarm cue chondroitin affected their

response to *WMF*. Compared to the control BTMs, the conditioned BTMs had roughly twice an increase

in activity as the control fish when *WMF* was presented. Because my first experiment showed that

addition of chondroitin alone caused a decrease in activity in the BTM, it is interesting that conditioning

that paired the alarm cue and the predator caused increased activity. This difference in response (i.e.

increased versus decreased activity) could be due to the different levels of threat posed by the two

different experiments (Helfman 1989, Chivers et al. 2001). As only one identified component of the

known alarm substance in Zebrafish, chondroitin sulfate may elicit a weaker antipredator behavior in

the BTM as compared to the alarm substance coupled with the visual and chemical presence of two

nearby predators. Also, the BTM's increased activity in the presence of *WMF* could be attributed to

predator-inspection behavior, which has been documented in many fish species and other taxa as well

(Brown et al. 2001). In the future, it will be interesting to examine how different levels of perceived threat influence a conditioned antipredator response.

In summary, my study illustrates that conditioning may allow for BTM recognition of *WMF* as a predator and consequently lead to increased survival of the imperiled BTM in its natural habitat. More generally, it may be possible for a range of organisms to escape from evolutionary traps by means of conditioning. Conditioning will only be feasible in organisms that can be captured and released or bred in captivity and released into the wild. Thus, the use of conditioning to free organisms from evolutionary traps will likely be an additional conservation tool that can complement other existing approaches, including artificial selection, removal of invasive species, and habitat restoration (Schlaepfer et al., 2002).

CHAPTER 5

LONG-TERM EFFECTIVENESS OF CONDITIONING IN THE BARRENS TOPMINNOW'S NATURAL HABITAT
AND FUTURE DIRECTIONS

Rapid changes at global and local levels have created novel ecosystems that are functionally and compositionally different than their historic states, and one of the most concerning ecological changes is the loss of biodiversity (Visser 2008). Rates of species' loss are unprecedented and unlikely to improve, as contributing factors, including the spread of invasive species, do not show signs of slowing or reversing (Mace and Purvis, 2008).

While studies have shown that conservation efforts can limit species' loss (Butchart, 2006), it has become increasingly necessary to approach conservation challenges with novel tools and by integrating knowledge from multiple disciplines (e.g. ecology, evolution, behavioral ecology, psychology). For example, past efforts to reintroduce imperiled species have often been aimed at mitigating population declines and preventing local extinction, but these reintroductions may prove unsuccessful if animals no longer exhibit behavior appropriate to the altered environment in which they are released (Griffin, 2000).

Given this dilemma, conservation biology can benefit from accounting for evolutionary dynamics by considering the environment in which an organism has evolved. As my study demonstrates, learning and conditioning can potentially play a role in helping imperiled species escape evolutionary traps. Additionally, inoculation of conditioned individuals into a naïve population may allow for the horizontal transmission of effective learned behavior, demonstrated by numerous studies (as reviewed in Chapter 4).

The ultimate goal of my thesis project was to increase the long-term survival of the BTM in its natural habitat. In approaching this objective, I first considered the BTM's lack of evolutionary history

with an introduced predator, *Gambusia affinis*, and then determined that the BTM would need to be

conditioned to avoid *WMF*. The subsequent selection, testing, and application of an appropriate alarm

cue allowed me to condition BTMs to associate *WMF* with danger, and measuring the effectiveness of

conditioning demonstrated that conditioned BTMs responded significantly different from control fish to

the visual and chemical presentation of this predator. Furthermore, sampling efforts following

conditioning and release resulted in the capture of only conditioned individuals, suggesting that

conditioned individuals potentially have higher survival than unconditioned individuals over a short time

frame. Results from this study suggest that there are potential long-term conservation implications of

conditioning the BTM to avoid predation by *WMF.*

In the future, it will be critical to expand upon my work. Specifically, future studies should focus

on conditioning and releasing larger sample sizes and examining the long-term trends of conditioning.

Upcoming work should also examine the role that social learning may play in overcoming evolutionary

traps**.** More generally, based on my findings, conditioning should be considered as a potential

conservation tool when dealing with organisms in evolutionary traps, as this work demonstrates that it

may be possible for a species to overcome its evolutionary history.

REFERENCES

Asplund, Maria E., Baden, Susanne P., Russ, Sarah, Ellis, Robert P., Gong, Ningping, and Hernroth, Bodil E. 2013. Ocean acidification and host–pathogen interactions: blue mussels, *Mytilus edulis*, encountering *Vibrio tubiashii. Environmental Microbiology*. 4: 1029-1039.

Austin, G.E. and Rehfisch, M.M. 2005. Shifting nonbreeding distributions of migratory fauna in relation to climatic change. *Global Change Biology*. 11: 31-38.

Barrett, Rowan D. H., and Hendry, Andrew P. 2012. Evolutionary rescue under environmental change. Behavioural Responses to a Changing World. First Edition. Oxford University Press.

BeeBee, T.J.C. 1995. Amphibian breeding and climate. *Nature*. 374: 219-220.

Berejikian, Barry A., Smith, Jan F., Tezak, E. Paul, Schroder, Steven L. and Knudsen, Curtis M. 1999. Chemical alarm signals and complex hatchery rearing habitats affect antipredator behavior and survival of Chinook salmon (Oncorhynchus tshawytscha) juveniles. *Canadian Journal of Fish and Aquatic Science*. 56: 830–838.

Bourke, P., Magnan, P. & Rodriguez, M.A. 1999. Phenotypic responses of lacustrine brook charr in relation to the intensity of interspecific competition. *Evolutionary Ecology*. 13: 19–31.

Brown, Grant E. and Smith, R. Jan F. 1998**.** Acquired predator recognition in juvenile rainbow trout (Oncorhynchus *mykiss):* conditioning hatchery-reared fish to recognize chemical cues of a predator. *Canadian Journal of Fish and Aquatic Science*. 55: 611-617.

Bryer, P., Mirza, R.S., and Chivers, D.P. 2001. Chemosensory Assessment of predation risk by slimy sculpins (*Cottus cognatus*): responses to alarm, disturbance, and predator cues. *Journal of Chemical Ecology*. 27: 533-546.

Butchart, S.H.M., Stattersfield, A.J., and Collar, N.J. 2006. How many bird extinctions have we prevented? *Oryx*. 40: 266-278.

Byers, John A. 1997. American pronghorn: social adaptations and the ghosts of predators past. University of Chicago Press.

Chivers, D.P., and Smith, R.J.F. 1998. Chemical alarm signaling in aquatic predator-prey systems: a review and prospectus. *EcoScience*. 5: 338-352.

Chivers, D.P., Mirza, R.S., Bruer, P.J., and Kiesecker, J.M. 2001. Threat-sensitive predator avoidance by slimy sculpins: understanding the role of visual versus chemical information. *Canadian Journal of Zoology*. 79: 867-873.

Chivers, D.P., Wisenden, B.S., Hindman, C.J., Michalak, T., Kusch, R.C., Kaminskyj, S.W., Jack, K.L. Ferrari, M.C.O., Pollock, R.J., Halbgewachs, C.F., Pollock, M.S., Alemadi, S., James, C.T., Savaloja, R.K., Goater, C.P., Corwin, A., Mirza, R.S., Kiesecker, J.M., Brown, G.E., Adrian, J.C., Jr., Krone, P.H.,

Blaustein, A.R., and Mathis, A. 2007. Epidermal 'alarm' substance cells of fishes maintained by non-alarm functions: possible defence against pathogens, parasites and UVB radiation. *Proceedings of the Royal Society London Biological Sciences*. 274: 2611-2619.

Cox, Jonathan G., and Lima, Steven L. 2006. Naïveté and an aquatic-terrestrial dichotomy in the effects of introduced predators. *Trends in Ecology and Evolution*. 21: 674-680.

Crowder, L.B. 1986. Ecological and morphological shifts in Lake Michigan fishes – glimpses of the ghost of competition past. *Environmental Biology of Fishes*. 16: 147–157.

D'Amore, Antonia, Kirby, Erik, and Hemingway, Valentine. 2009. Reproductive Interference by an invasive species: an evolutionary trap? *Herpetological Conservation and Biology*. 4: 325-330.

Ferrari, M.C.O., Wisenden, B.D., and Chivers, D.P. 2010. Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. *Canadian Journal of Zoology*. 82: 698-724.

Ferriere, R. and Legendre, S. 2013 Eco-evolutionary feedbacks, adaptive dynamics and evolutionary rescue theory. *Philosophical Transactions of the Royal Society Biological Sciences*. 368: 20120081.

Fuller, P.L., L.G. Nico and J.D. Williams. 1999. Nonindigenous Fishes Introduced into Inland Waters of the United States. Special Publication 27. *American Fisheries Society*. 613.

Galat, D. L. and Robertson, B. 1992. Response of endangered *Poeciliopsis occidentalis sonoriensis* in the Rio Yaqui drainage, Arizona, to introduced *Gambusia affinis*. *Environmental Biology of Fishes*. 33: 249-264.

Gamradt, S.C. and Katz, L.B. 1996. The effect of introduced crayfish and mosquitofish on California newts (*Taricha torosa)*. *Conservation Biology*. 10: 1155-1162.

Gazdewich, K.J., and Chivers, D.P. 2002. Acquired predator recognition by fathead minnows: influence of habitat characteristics on survival. *Journal of Chemical Ecology*. 28: 439-445.

Goldsworth, Cory A. and Bettoli, Phillip W. 2006. Growth, body condition, reproduction and survival of stocked Barrens topminnows, *Fundulus julisia* (Fundulidae). *American Midland Naturalist*. 156: 331-343.

Keeler, Margaret S. and Chew, Frances S. 2008. Escaping an evolutionary trap: preference and performance of a native insect on an exotic invasive host. *Oecologia*. 156: 559–568.

Golub, J.L., Vermette, V., and Brown, G.E. 2005. Response to conspecific and heterospecific alarm cues by pumpkinseeds in simple and complex habitats: field verification of an ontogenetic shift. *Journal of Fish Biology*. 66: 1073-1081.

Griffin, A.S., Blumstein D.T., and Evans, C. 2000. Training captive-bred or translocated animals to avoid predators. *Conservation Biology*. 14: 1317-1326.

Gurevitch, Jessica, and Padilla, Dianna K. 2004. Are invasive species a major cause of extinctions? *TRENDS in Ecology and Evolution*. 19: 470-474.

Helfman, G.S. 1989. Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. *Behavioral Ecology and Sociobiology*. 24: 47-58.

Huppop O. and Huppopp K. 2003. North Atlantic oscillation and timing of spring migration in birds. *Proceedings of the Royal Society*. 270: 233-240.

Jelks, H. L., S. J. Walsh, N. M. Burkhead, S. Contreras-Balderas, E. Díaz-Pardo, D. A. Hendrickson, J. Lyons, N. E. Mandrak, F. McCormick, J. S. Nelson, S. P. Platania, B. A. Porter, C. B. Renaud, J. J. Scmitter-Soto, E. B. Taylor, and M. L. Warren, Jr. 2008. Conservation status of imperiled North American freshwater and diadromous fishes. *Fisheries*. 33: 372-407.

Kiesecker, J.M., Chivers, D.P., Marco, A., Quilchano, C., Anderson, M.T., and Blaustein, A.R. 1999. Identification of a disturbance signal in red-legged frogs, *Rana aurora*. *Animal Behavior*. 57: 1295-1300.

Lapiedra, O., Sol, D., Carranza, S.,and Beaulieu, J.M. 2013. Behavioural changes and the adaptive diversification of pigeons and doves. *Proceedings of the Royal Society of Biological Sciences*. 280: 20122893.

Levin, Donald A. 2003. The ecological transition in speciation. *New Phytologist*. 161: 91-96.

Lönnstedt, O.M., McCormick, M.I., Meekan, M.G., Ferrari, M.C.O., and Chivers, D.P. 2012. Learn and live: predator experience and feeding history determines prey behaviour and survival. *Proceedings of the Royal Society Biological Sciences.* 279: 2091-2098.

Mace, G.M. and Purvis, A. 2008. Evolutionary biology and practical conservation: bridging a widening gap. *Molecular Ecology*. 17: 9-19.

Manassa, R.P. and McCormick, M.I. 2012. Social learning and acquired recognition of a predator by a marine fish. *Animal Cognition*. 15: 559-565.

Manek, A.K., Ferrari, M.C.O., Sereda, J.M., Niyogi, S., and Chivers, D.P. 2012. The effects of ultraviolet radiation on a freshwater prey fish: physiological stress response, club cell investment, and alarm cue production. *Biological Journal of the Linnean Society*. 105: 832-841.

Marcus, J.P. and Brown, G.E. 2003. Response of pumpkinseed sunfish to conspecific chemical alarm cues: an interaction between ontogeny and stimulus concentrations. *Canadian Journal of Zoology*. 81: 1671-1677.

Mathis, A., and Smith, R.J.F. 1993. Chemical alarm signals increase the survival time of fathead minnows (*Pimephales promelas*) during encounters with northern pike (*Esox lucius*). *Behavioral Ecology*. 4: 260-265.

Mathuru, Ajay S., Kilbat, Caroline, Cheong, Wei Fun, Shui, Guanghou, Wen, Markus R., Friendrich, Rainer W., and Jesuthasan, Suresh. 2012. Chondroitin fragments are odorants that trigger fear behavior in fish. *Current Biology*. 22: 538-544.

Menzel, A. and Fabian, P. 1999. Growing season extended in Europe. *Nature*. 397: 659.

Meuthen, D., Baldauf, S.A., and Thünken, T. 2012. Evolution of alarm cues: a role for kin selection? *F1000 Research*. 1: 1-27.

Michalek-Wagner, K. and Willis, B.L. 2001. Impacts of bleaching on the soft coral *Lobophytum compactum*. Fecundity, fertilization, and offspring viability. *Coral Reefs*. 19: 231-239.

Millis, M.D., Rader, R.B., and Belk, M.C. 2004**.** Complex interactions between native and invasive fish: the simultaneous effects of multiple negative interactions. *Oecologica*. 141: 713-721.

Mirza, R. and Chivers, D. 2000. Predator-recognition training enhances survival of brook trout: evidence from laboratory and field-enclosure studies. *Canadian Journal of Zoology*. 78: 2198-2208.

Mirza, R.S., and Chivers, D.P. 2001. Are chemical alarm cues conserved within salmonid fishes? *Journal of Chemical Ecology*. 27: 1641-1655.

Mirza, R.S., and Chivers, D.P. 2003. Response of juvenile rainbow trout to varying concentrations of chemical alarm cue: response thresholds and survival during encounters with predators. *Canadian Journal of Zoology*. 81: 88-95.

Moore, P.A., and Bergman, D.A. 2005. The smell of success and failure: the role of intrinsic and extrinsic chemical signals on the social behavior of crayfish. *Integrative and Comparative Biology*. 45: 650-657.

Page, L.M., and Burr, B.M. 2011. Peterson Field Guide to Freshwater Fishes of North America North of Mexico, second ed. Houghton Mifflin Harcourt Publishing, New York, NY.

Parmesan, C. et al. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature*. 399: 579-583.

Pejchar, Liba and Mooney, Harold A. 2009. Invasive species, ecosystem services and human well-being. *Trends in Ecology and Evolution*. 24: 597-504.

Pfeiffer, W. 1977. Distribution of fright reaction and alarm substance cells in fishes. *Copeia*. 4: 653-665.

Pfeiffer, W., Riegelbauer, G., Meier, G., and Scheibler, B. 1985. Effect of hypoxanthine-3(N)-oxide and hypoxanthine-1(N)-oxide on central nervous excitation of the black tetra *Gymnocorymbus ternetzi* (Characidae, Ostariophysi, Pisces) indicated by dorsal light response. *Journal of Chemical Ecology*. 4: 507-523.

Pimentel, David, Zuniga, Rodolfo, and Morrison, Doug. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*. 52: 273-288.

Pollock, R.J., Pollock, M.S., Ferrari, M.C.O., Kaminskyj, S.G.W., and Chivers, D.P. 2012. Do fathead minnows, *Pimephales promelas* Rafinesque, alter their club cell investment in responses to variable rish of infection from *Saprolegnia*? *Journal of Fish Disease*. 35: 249-254.

Rafferty, N.E., and Boughman, J.W. 2006. Olfactory mate recognition in a sympatric species pair of three-spined sticklebacks. *Behavioral Ecology*. 17: 965-970.

Reed, J.R. 1969. Alarm substances and fright reaction in some fishes from the Southeastern United States. *Transactions of the American Fisheries Society*. 4: 664-668.

Robertson, Bruce A., Rehage, Jennifer S., and Sih, Andrew. 2013. Ecological novelty and the emergence of evolutionary traps. *TRENDS in Ecology and Evolution*. 28: 552-560.

Schlaepfer, Martin A. Runge, Michael C., and Sherman, Paul W. 2002. Ecological and evolutionary traps. *TRENDS in Ecology and Evolution*. 17: 474-480.

Schlaepfer, Martin A., Sherman, Paul W., Blossey, Bernd, and Runge, Michael C. 2005. Introduced species as evolutionary traps. *Ecology Letters*. 8: 241-246.

Schmitz, Oswald J., Beckerman, Andrew P., and O'Brien, Kathleen M. 1997. Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. *Ecology*. 78: 1388-1399.

Shier, D. M., and Owings, D. H. 2006. Effects of predator training on behavior and post-release survival of captive prairie dogs (*Cynomys ludovicianus*). *Biological Conservation*. 132: 126-135.

Sih, Andrew, Bolnick, Daniel I., Luttbeg, Barney, Orrock, John L., Peacor, Scott D., Pintor, Lauren M., Preisser, Evan, Rehage, Jennifer S., and Vonesh, James R. 2010. Predator-prey naïveté, anti-predator behavior, and the ecology of predator invasions. *Oikos*. 119: 610-621.

Sih, Andrew, Ferrari, Maud C. O., and Harris, David J. 2011. Evolution and behavioural responses to human-induced rapid environmental change. *Evolutionary Applications*. 4: 367–387

Smith, R.J.F., 1979. Alarm reaction of Iowa and Johnny darters (*Etheostoma*, Percidae, Pisces) to chemicals from injured conspecific. *Canadian Journal of Zoology*. 57: 1278-1282.

Smith, R.J.F. 1992. Alarm signals in fishes. *Reviews in Fish Biology and Fisheries*. 2: 33-63.

Sorensen, P.W., Fine, J.M., Dvornikovs, V., Jeffrey, C.S., Shao, F., Wang, J., Vrieze, L.A., Anderson K.R., and Hoye, T.R. 2005. Mixture of new sulfated steroids functions as a migratory pheromone in the sea lamprey. *Nature Chemical Biology*. 1: 324-328.

Strauss, Sharon Y., Lau, Jennifer A., and Carroll, Scott P. 2006. Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? *Ecology Letters*. 9: 357-374.

Thomas, C.D. and Lenoon, J.J. 1999. Birds extend their ranges northward. *Nature*. 399: 213.

Tollrian R., and Harvell, C.D. 1999. The evolution of inducible defenses. Princeton University Press, Princeton, NJ.

USFWS (U.S. Fish and Wildlife Service). 2011. Endangered and threatened wildlife and plants; partial 90 day finding on a petition to list 404 species in the southeastern United States as endangered or threatened with critical habitat. Federal Register. 76: 59836-59862.

Visser, Marcel E. 2008. Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proceedings of the Royal Society of Biological Sciences*. 275: 649–659.

von Frisch, K. 1941. Uber einen Schreckstoff der Fischhaut und seine biologische Bedeutung. *Zeitchrift fur vergleichende Physiologie*. 29: 46-145.

Wilcove, David S., Rothstein, David, Dubow, Jason, Phillips, Ali, and Losos, Elizabeth. 1998. Quantifying Threats to Imperiled Species in the United States. *BioScience*. 48: 607-615.

Williams, J.D. and Etnier, D.A. 1982. Description of a new species, *Fundulus julisia*, with a redescription of *Fundulus albolineatus* and a diagnosis of the subgenus *Xenisma* (Teleostei: Cyprinodontidae). Occasional Papers of the Museum of Natural History, University of Kansas. 102: 1-20.

Withers, D. I. 2009. A Guide to the Rare Animals of Tennessee. Tennessee Natural Heritage Progam, Nashville, TN.

Westhoff, Jacob T., Watts, Allison V., and Mattingly, Hayden T. 2013. Efficacy of artificial refuge to enhance survival of young Barrens topminnows exposed to western mosquitofish. *Aquatic Conservation: Marine and Freshwater Ecosystems*. 23: 65-76.

Wisenden, B. D. 2003. Chemically mediated strategies to counter predation. *In Sensory processing in aquatic environments*. 236-251. Springer.

Zylberberg M., Klasing, K.C., and Hahn, T.P. 2012. House finches (*Carpodacus mexicanus*) balance investment in behavioural and immunological defences against pathogens. *Biology Letters*. 9.

VITA

Sarah Farnsley was born in Nashville, TN to the parents of Pat and Linda Farnsley. She is the oldest of two brothers, Reed and Kyle. She attended Ooltewah High School in Ooltewah, TN and, after graduation, attended the University of Tennessee, Knoxville and obtained a Bachelors of Communications degree. Sarah then returned to UTK to complete a Bachelors of Science degree in Ecology and Evolutionary Biology, which she completed in May of 2010. Sarah performed field work on Lake Erie, Lake Tai in China, and in the Mojave Desert before accepting a graduate teaching assistantship at the University of Tennessee at Chattanooga in the Environmental Sciences Program. Sarah graduated with a Masters of Science degree in Environmental Science in May 2014.