SOCIAL LEARNING AND ITS EFFECT ON CONSERVATION EFFORTS IN THE BARRENS

TOPMINNOW: AN EVALUATION OF CONDITIONING AND SOCIAL LEARNING

AS A VIABLE LONGTERM SOLUTION TO EVOLUTIONARY TRAPS

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ABSTRACT

The invasive Western Mosquitofish (WMF), *Gambusia affinis*, has facilitated the

extinction and endangerment of multiple freshwater fish species, including the Barrens

Topminnow (BTM), *Fundulus julisia*. In my study, I investigated if BTMs are capable of socially

learning conditioned predator recognition, and I hypothesized that BTMs are capable of social

learning and that such learning might improve survival of BTMs. To explore the role of

conditioning and subsequent learning in the conservation of BTMs, I conducted a series of

experiments in which I 1) attempted to condition BTMs to exhibit antipredator behavior

when faced with WMF, and 2) created a situation in which naive BTMs could potentially learn

from conditioned individuals. I found no evidence of conditioning or of social learning, and

there were no significant differences in behavior, body condition, or survival among my

treatment groups.

ACKNOWLEDGEMENTS

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CHAPTER I

INTRODUCTION

Throughout an animal's life, many behaviors and decisions influence the fitness (i.e., the relative lifetime reproductive success) of an individual. In some cases, an individual's behaviors and decisions are influenced by the observations of other individuals, particularly conspecifics in similar ecological situations. This adjustment of one's own behavior through observation of other individuals, known as social learning, has been observed in a range of vertebrates (Griffin, 2004; Galef and Laland, 2005; Reader and Biro, 2010). Social learning can provide a way for individuals to learn quickly and behaviorally respond to their environment, and it can be associated with a number of fitness benefits. Indeed, social learning has been found to increase individual survival in a range of taxonomic groups (Griffin, 2004; Galef and Laland, 2005; Reader and Biro, 2010).

Social Learning Can Increase Survival

Numerous studies have shown that individuals in populations learn from conspecifics and that this social learning can cause individuals to adjust their own behavior in response to a novel ecological challenge (i.e., an ecological challenge that individuals have observed other individuals face and respond to; Griffin et al., 2004; Galef and Laland, 2005; Bool et al., 2011; Manassa et al., 2013; Reader and Biro, 2010). From an evolutionary viewpoint, social learning is expected to evolve and be maintained in a population if it increases individual fitness.

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Survival has large effects on fitness, and as such, one key fitness benefit of social learning is likely to be increased survival. A major factor that affects survival in many species is predation, and in many animal populations, social learning likely increases survival by allowing individuals to learn to avoid predation (Griffin, 2004; Ferrari et al., 2005; Manassa et al., 2013). Specifically, a change in one's behavior through social learning can potentially allow individuals to exhibit plasticity in response to novel predators in a given environment and therefore increase survival and ultimately an individual's fitness.

In natural ecosystems, the introduction of a novel predator into a naïve prey population has been shown to rapidly lead to the spread of predator recognition among individuals in the population, allowing the individuals to avoid the predators (Chivers and Smith, 1995; Griffin, 2004; Ferrari et al., 2005; Cornell et al., 2012; Manassa et al., 2013). For example, naïve Fathead Minnows (*Pimephales promelas*) introduced into a pond simultaneously with Northern Pike (*Esox lucius*) quickly acquired recognition of the predator's odor and exhibited significantly more dashing and cover use than control individuals (Chivers & Smith, 1995). However, there are limits to the effect that learning has on long-term predator recognition. In Iberian Green Frog tadpoles (*Pelophylax perezi*) individuals acquired predator recognition, but this predator recognition was only retained without reinforcement for nine days, after which there was a lack of response without reinforcement (Gonzalo et al., 2009).

Some studies have found that learning is more likely to lead to anti-predator behavior if individuals observe cues from conspecifics that are in similar life stages. For example, in juvenile Spiny Chromis (*Acanthochromis polyacanthus*), individuals conditioned using juvenile

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chemical cues from a predator displayed antipredator responses, whereas those conditioned

using adult chemical cues did not (Mitchell & McCormick, 2013). Some studies have also

suggested that individuals can even learn to recognize predators by observing experienced

heterospecifics. In one experiment, Ward's Damselfish (*Pomacentrus wardii*) were found to

transmit predator recognition to a closely related fish, Lemon Damselfish (*Pomacentrus*

moluccentris)*,* and to a distant relative, Threespot Cardinalfish (*Apogon trimaculatus*),

through observation (Manassa et al., 2013).

Social Learning as a Way to Escape Evolutionary History?

Environmental change is prevalent on both short and long time scales, and in many

cases environmental change results in organisms experiencing environments that they are

not well adapted to. When organisms are in a novel environment and face novel cues, but

because of their evolutionary history they continue to display responses to those cues that

are not currently adaptive, they are in an evolutionary trap (Schlaepfer, 2002; Robertson,

2013). In other words, evolutionary traps are evolutionary engrained responses to ecological

cues that were once adaptive but are no longer are associated with relatively high fitness.

Behavioral modification has previously been highlighted as a way to rescue organisms

from evolutionary traps (Griffin et al., 2000). For example, reintroduction programs are often

used when a native species faces decimation from an introduced predator, and conditioning

reintroduced individuals to acquire predator recognition is one way to address and mediate

the lack predator experience that individuals in reintroduction programs often have (McLean

et al., 1999; Griffin et al., 2000). However, it is currently unclear how effective conditioning is

as a long- term solution to rescuing organisms from evolutionary traps. To be an effective long-term conservation method that improves overall population persistence, any learned behavior must spread through the population. The spread of learned behavior through a population may occur naturally through social learning.

Relatively few studies have examined the role that social learning can play in conservation efforts; as such, more work is needed to fully understand whether social learning can rescue organisms from novel ecological challenges. In particular, it will be critical to determine whether conditioning followed by social learning can free imperiled populations from evolutionary traps. In one of the few studies on the topic, Ferrari et al. (2005) found that Fathead Minnow can learn by observation, allowing predator recognition to spread to naïve individuals and also suggested that the intensity of the social learning behavior may be associated with the perceived risk of predation (Helfman, 1989).

I hypothesize that conditioning and social learning together can rescue organisms from evolutionary traps. Utilizing the endangered Barrens Topminnow (BTM), *Fundulus julisia*, as a model species, I evaluated this hypothesis using both an experimental lab and field approach. BTMs are ideal for such a study as they are currently victim to an evolutionary trap that was caused by the introduction of the Western Mosquitofish (WMF), *Gambusia affinis*. BTMs have no evolutionary history with WMF, and as such, they exhibit a lack of antipredator behavior. As a result, BTM populations have been decimated (see Chapter 3 for additional discussion of the evolutionary trap that BTMs are in). Previous work has shown that BTMs are capable of being conditioned to alter their behavior in response to a novel predator (Farnsley, 2014); given this, if conditioning followed by social learning can free a population from an

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evolutionary trap, we would expect it to occur in a system such as the BTM. Below, I provide a description of my research goals and approach.

Overview of Research Goals and Approach

To understand if social learning of conditioned antipredator behavior can occur in BTM, there are several questions that will need to be answered:

- **Do BTMs socially learn?**

- **How strong is the conditioned behavior in individual BTMs who learned via social learning (i.e., what is the magnitude of the effect of social learning on behavior)?**

- **Does the conditioned and/or socially learned predator recognition in BTMs lead to increased body condition (e.g., due to better foraging success in a competitive environment) and ultimately increased survival in the wild (i.e., in an environment with predators and inter- and intra-specific competition)?**

To address the above questions, I conducted a series of experiments in which I aimed to:

1) **Condition BTMs to exhibit antipredator behavior when presented with Western Mosquitofish.** During this phase, individual BTMs were trained to recognize and adjust their behavior in response to a novel predator, the WFM, using chondroitin sulfate, an aversive chemical cue previously shown to elicit an altered behavioral response in the BTM. Such conditioning has previously been found to alter BTM behavior in response to presentation of WMF and improve short-term survival in the wild following release of BTMs (Farnsley, 2014).

2) **Determine whether naïve BTMs can learn anti-predator behavior by observing a**

conditioned BTM. During this phase, naïve BTMs were placed with those BTMs conditioned in Phase I and the novel predator, WMF. This phase exposed naïve BTMs to both predators and experienced individuals, thereby providing an opportunity of social learning. I hypothesized that these naïve individuals might socially learn from conditioned individuals to alter their behavior in the presence of WMF.

3) **Assess the effect of conditioning and social learning on BTMs' body condition and survival in a lab setting.** After allowing naïve and conditioned BTMs to interact in the presence of WMF, I explored the effect of conditioning and social learning on BTM body condition and survival in a semi-natural setting in which BTMs and WMF interacted for a two-week period. I predicted that naïve individuals exposed to conditioned individuals would alter their behavior when exposed to WMF and that such social learning would improve the body condition and survival of BTMs.

4) **Assess the effect of conditioning and social learning on BTMs' survival in the wild in a release- and-recapture study**. While the previous phases allowed me to explore the results of conditioning and social learning of BTMs under lab conditions, this phase allowed me to determine how my different experimental groups fare in nature. I expected that conditioned BTMs and BTMs that had the opportunity to socially learn from conditioned fish would have improved survival in the wild relative to a control group of BTMs that did not have the opportunity to learn socially from conditioned fish.

In the following chapter (Chapter 2), I review the literature on the role of learning in conservation. I then describe the experimental work outlined above (Chapter 3) and provide

a brief summary of the conservation implications of my research and avenues of future

research.

CHAPTER II

LEARNING AS A SOLUTION IN
CONSERVATION

What is Learning?

Defining Learning

Learning is defined as "an enduring change in the mechanisms of behavior involving

specific stimuli that results from prior experience with the same or different stimuli and

responses" (Domjan, 2015, p.14). In other words, learning occurs when individuals alter their

behavior in response to either previously experienced or novel stimuli. By altering behavior in

response to novel situations and stimuli, individuals can potentially acclimate quickly to a

changing environment, therefore allowing for greater opportunities for survival, resource

acquisition, and reproduction (Hughes et al., 1992; Snell-Rood, 2013; Liu et al., 2016).

Because learning requires changing behavior, and because behavior is linked to evolutionary

history, the capacity to learn is expected to be highly dependent on the behavioral plasticity

of an organism (i.e., the change in behavior that an individual can exhibit in response to a

change in one or more environmental conditions) (Hughes et al., 1992; Snell-Rood, 2013; Liu

et al., 2016). Specifically, plasticity defines the boundaries of behaviors that are possible, and

thus, determines how individuals can alter their behavior in response to novel environmental

cues. As seen with phenotypic plasticity, having less behavioral plasticity means that a rapidly

changing environment is more likely to create a situation that cannot be acclimated to, as

individuals who are not plastic will be unable to alter their behavior in response to novel environmental cues (Snell-Rood, 2013; Liu et al., 2016). On the other hand, greater phenotypic plasticity is expected to be related to higher potential for acclimation in a changing environment.

Why is learning present in populations?

Plasticity allows individuals to exhibit a range of behaviors, and as such, greater plasticity can allow individuals to acclimate to a diverse set of environments (Snell-Rood, 2013; Liu et al., 2016). This flexibility can provide a way by which one may increase survivorship, resource acquisition, and mate acquisition even when the environment varies (Hughes et al., 1992; Tebbich et al., 2001; Snell-Rood, 2013; Muth et al., 2016). Environmental variation that occurs within an individual's lifespan is expected to select for behavioral plasticity because such plasticity allows the individual to acclimate to its varying environment and have greater lifetime reproductive success (Tebbich et al., 2001; Snell-Rood, 2013; Muth et al., 2016). For example, in Muth et al. (2016), the Common Eastern Bumble Bee (*Bombus impatiens*) was found to learn many different characteristics of flowers to associate with a pollen reward. By learning to associate several different features of flowers to pollen rewards, the bees were able to remember newly discovered food sources and recognize them under varying conditions, which is hypothesized to increase their lifetime inclusive fitness and hence be selected for.

Individual vs. Social Learning

There are two main methods by which individuals may learn: individual learning and social learning (Whalen et al., 2015). Individual learning is the use of personal experience in order to generate behaviors in response to new settings or environmental conditions (Whalen et al., 2015). This contrasts with social learning, which is learning through the experience of another individual in order to behave in a manner appropriate to the environment (Truskanov & Lotem, 2015; Whalen et al., 2015). While these two methods differ, it is important to note that they are not mutually exclusive (Cornell et al., 2012). A behavior that was learned by an individual within the population can be spread throughout the population via social learning, and a single individual often has the potential to learn through individual experience and by observing others.

Costs and Benefits of Individual and Social Learning

To evaluate how and why each of these forms of learning takes place, it is important to understand the relative costs and benefits of individual and social learning, as I would only expect learning that results in a net gain in fitness to be selected for and maintained evolutionarily in a population. Individual learning is expected to be beneficial in the sense that it allows the individual to quickly acquire a behavior (Whalen et al., 2015). Additionally, the information acquired via individual learning is more likely to be accurate and relevant to the individual, as the individual itself has experience with the stimuli (Laland, 2004). However, while individual learning may be advantageous in the sense that it can allow an individual to quickly adjust their behavior, this method of learning can be associated with relatively high

risk. By experiencing a novel situation firsthand, an individual may be harmed by the novel stimulus, and this could increase the potential for mortality and ultimately reduce lifetime reproductive success.

With social learning, individuals need not take the risk of being initially exposed to the stimulus itself in order to exhibit an appropriate response, and this decrease in risk is one potential benefit of social learning that might decrease mortality and increase overall fitness (Whalen et al., 2015). For example, both individual learning and social learning in Ward's Damselfish (*Pomacentrus wardi*) led to similar survivorships, suggesting that social learning, while not direct, can still provide a means by which individuals can acquire valuable information that increases survival (Manassa & McCormick, 2013). While there are benefits to social learning, there is also the risk of unreliable information (Galef & Laland, 2005). If the behavior which is received from another individual is mismatched to the current environment or if copying errors occur, social learning can prove to be detrimental to the individual (Webster & Laland, 2008). This unreliability creates a trade-off with the risk involved with personal experience.

Social learning can be affected by a range of factors, including group size (Lachlan et al., 1998; Griffin et al., 2000; Tebbich et al., 2001). For example, in Lachlan et al. (1998), guppies (*Poecilia reticulata*) were more likely to learn from a group of shoaling individuals than from a single demonstrator. Social learning can also be affected by individual characteristics. For instance, in guppies, smaller individuals preferred small demonstrators in the context of social learning (Lachlan et al., 1998). In addition to reducing risks from novel encounters, social learning can allow a behavior to quickly spread throughout the population at a faster rate

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than if each individual had to experience the novel situation (Chivers & Smith, 1995; Griffin et al., 2000; Galef & Laland, 2005), and this social spread of learned behavior among individuals can alter population dynamics. Individuals can also vary in their propensity to learn socially, and some researchers have suggested that some individuals are more likely to be 'shy' whereas others are more likely to be 'bold' in regard to their behavioral tactics. For instance, in Brown et al. (2013), juvenile Rainbow Trout (*Oncorhynchus mykiss*) who were identified as being either shy or bold were conditioned to respond to Pumpkinseed (*Lepomis gibbosus*) odor. Eight days after conditioning it was found that shy individuals still maintained the conditioned behavior while the bold individuals no longer exhibited the behavior, suggesting that shy and bold tactics influence the behavior exhibited in response to novel stimuli (Brown et al., 2013).

As these learning methods carry with them associated costs and benefits, it may be beneficial for individuals to use both forms of learning in response to different situations. When the cost associated with learning through personal experience is greater than the cost associated with loss of accuracy of social learning, social learning is expected to be favored (Laland, 2004). However, many species use a combination of individual and social learning. For instance, Cornell et al. (2012) conditioned American Crows (*Corvus brachyrhynchos*) to associate certain masks with negative encounters and found that not only did the crows demonstrate individual learning by being successfully conditioned, but individuals who never previously encountered the masks also avoided them or presented antipredator tactics such as the mobbing of the masks. By having individuals in the group who were experienced and others inexperienced, the naïve individuals

learned that the masks were a negative stimulus and remembered in future encounters. This is consistent with social transference of the behavior (Cornell et al., 2012).

While many of the studies discussed above have focused on social learning within a species, social learning among individuals of different species can also occur. In Manassa et al. (2013), individuals were found to learn from heterospecifics in addition to conspecifics. In this study, Ward's Damselfish (*Pomacentrus wardii*) were introduced to a novel predator, Brown Dottyback (*Pseudochromis fuscus*). The Ward's Damselfish were able to socially transmit recognition of the novel predator to both another species of Lemon Damselfish (*Pomacentrus moluccensis*) and also the distantly related Three-spot Cardinalfish (*Apogon trimaculatus*). This suggests that within complex ecosystems, such as those seen in coral reefs, interspecies transmission may act as a way to allow for rapid acquisition of an appropriate response to novel circumstances. (Manassa et al., 2013)

Learning and Conservation

How is learning used in conservation?

Due to the rapid speed at which learning can take place to allow an individual or group to acclimate to new environments, learning can have implications in the field of conservation. One way conservation biologists may use learning is through training of naïve individuals (Griffin et al., 2000). For example, training can allow for individuals who have not been reared in a natural setting to survive upon release by improving success in resource acquisition and/or survival (Griffin et al., 2000). Rodriguez et al. (1995) demonstrated that a captive

raised Iberian Lynx (*Lynx pardinus*) could be trained to improve foraging, and human interaction was avoided in order to prepare the lynx for release. By training prerelease, the lynx was able to overcome a lack of acclimation to the natural environment (Rodriguez et al., 1995). Conditioning, which occurs when a cue is paired with a novel stimulus to bring about a desired effect (Griffin et al., 2000), is also frequently used in a conservation context. For example, Berejikian et al. (1999) conditioned juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) to recognize predators. By pairing an injured fish stimulus with the odor of a Cutthroat Trout (*Oncorhynchus clarki*), which is a predator of Chinook Salmon, the authors hoped to create a negative association with the predator. While earlier attempts at training had little success, there was a significant increase in survival post-release of conditioned individuals in this study relative to unconditioned individuals (Berejikian et al., 1999). Through conditioning, a released organism can be taught to avoid certain objects or organisms such as predators. In an earlier study, New Zealand Robins (*Pertoica* australis) were conditioned using model predators. After release, the birds showed more caution toward the predators than to a control object, showing that the birds could be conditioned to avoid the predators (McLean et al., 1999). It is important to note that conditioning may not be a grand solution that works for all organisms over a long time period. While Iberian Green Frog tadpoles (*Pelophylax perezi*) can be conditioned to recognize novel predators using chemical cues, they fail to respond after nine days of no interaction with the predators (Gonzalo et al., 2009). This suggests that conditioned responses can be easily lost in some cases. Additional research is needed to better understand the role of learning and conditioning in conservation, and in particular, it will be important to consider in future studies the effect of conditioning on the

long-term survival in natural settings.

Why use learning in conservation?

Given that there are costs and benefits to individuals of learning, it is important to consider the question of why learning should be used for conservation. As previously stated, learning allows individuals to quickly acclimate to a rapidly changing environment (Liu et al., 2016). Learning therefore potentially allows individuals to succeed in the face of many of the conservation problems present in today's global environment (McLean et al., 1999). One major cause of rapid environmental change is the introduction of an invasive species. By rapidly invading native populations, invasive species can reduce native populations by replacing niches, predating on native species, and through competition. If the native species are not able to quickly acclimate, populations may be reduced, or even go extinct, particularly since there will very often not be sufficient time or genetic variation for evolutionary adaptation to occur after the introduction of a novel invasive predator. Learning, however, may provide a path by which a species acclimates.

Farnsley (2014) conditioned Barrens Topminnows (BTMs), *Fundulus julisia,* to recognize the Western Mosquitofish (WMF), *Gambusia affinis,* as a predator. WMF are an invasive species known to feed on the young and harass adult of BTMs and have contributed to the decline of several fish and amphibian species (Galat & Robertson, 1992; Goodsell & Kats, 1999; Pyke, 2008). Conditioning the BTMs to recognize the WMF as a predator may create an avenue by which the BTM populations could persist in the presence of the WMF. In Farnsley's (2014) study, BTMs were successfully conditioned and a later release-and-recapture study showed a significant difference in the survival of conditioned and naïve individuals, such that

conditioned individuals were more likely to survive and be recaptured than unconditioned

individuals. As seen in Farnsley's (2014) study, conditioning for predator recognition can

potentially provide an escape to the evolutionary trap caused by the introduction of a novel

predator. However, it will be important in the future to explore the persistence of

conditioned behavior across different time scales.

Synthesis

Can learning be used as a long-term conservation solution?

Learning and conditioning have been shown to increase survival by improving foraging

success and predator recognition. For example, training can allow managers to overcome one

of the common problems of releasing captive-bred individuals, i.e., the problem that captive

settings do not match the wild habitat, leading to inappropriate behaviors (Rodriguez et al.,

1995). Also, socially learning predator recognition has been shown to improve success in the

wild. In Shier and Owings (2007), for instance, Black-tailed Prairie Dogs (*Cynomys*

ludocivianus) were trained via social learning. Captive raised individuals were taught using an

adult individual as a demonstrator for predator recognition. By training in this fashion,

released individuals did as well as wild-raised individuals and better than those who were

trained without a demonstrator (Shier & Owings, 2007). From these studies, it would be easy

to assume that learning is a simple solution to problems found in conservation; however,

there are several issues that potentially limit the effectiveness of learning in the context of

conservation.

First, it is not certain that all learning strategies work for all species. If a species does

not have the capability of learning in a particular manner, individuals of that species may not

be able to gain benefits from training or conditioning. For example, it is not currently known

if Hellbenders (*Cryptobranchus alleganiensis*), an imperiled species that tends to be solitary,

exhibit horizontal transference of information (Crane & Mathis, 2011). This may limit the

methods by which the individuals can acquire conditioned behavior. The ability to learn is

dependent on factors such as what behavior is being learned, the cognitive ability of the

species, and the type of environment the species inhabits (Liu et al., 2016). For instance, it

was demonstrated recently that Poison Dart Frogs (Family Dendrobatidae), which live in a

highly dynamic environment are capable of rule-based learning and of utilizing visual cues

(Liu et al., 2016). These traits may not be general to the Order Anura, as Leopard Frogs (*Rana*

pipiens) are potentially incapable of utilizing spatial orientation cues (Liu et al., 2016).

Second, it is not guaranteed that behavior acquired through conditioning and learning

will be enough to overcome problems associated with environmental change. For instance, if

there is an increase in the density of an invasive predator, learning new behavioral strategies

simply might not be able to overcome novel increases in competition or predation.

Interactions from a novel predator not only affect the ecosystem in a top-down manner, but

also a bottom- up manner (Grosholz et al., 2000; Kagata & Ohgushi, 2006).

Third, many conservation methods utilizing learning, such as those discussed above,

involve conditioning, which relies on individual learning. While individual learning provides

benefit to the individual, that alone does not allow a behavior to persist in a population. In

order to persist, the conditioned behavior needs be transmitted. Without transmission,

conditioning behaviors may only be a temporary aid to the population, failing to help wild

individuals in current or future generations within the released population.

What is missing?

To move forward with understanding how learning can be best used in conservation, it will be key to enhance our understanding of the long-term success of conditioning on a population over a long time scale. For conditioning specifically, this means determining if the behavior is maintained in the population across generations. For instance, it is generally unclear if conditioning followed by social learning can allow learned adaptive behaviors to spread in a population. One problem contributing to this lack of knowledge is that, in nature, learning is difficult to study. For example, it can be difficult to determine the cause of death for individuals (Brown & Day, 2002). The uncontrolled environment can produce deaths due to reasons unaccounted for in the study system.

In summary, additional research is needed to understand the overall effectiveness of conditioning and training as tools for conservation. Long term success of conditioning across generations has yet to be looked at and maintenance of learned behavior in a population is not greatly understood. While there is currently evidence for the importance of learning in conservation on relatively short time scales, it will be critical to explore the long- term benefits of learning in relation to long-term conservation issues.

CHAPTER III

CONDITIONING AND SOCIAL LEARNING IN THE BARRENS TOPMINNOW, *FUNDULUS JULISIA*, IN THE PRESENCE OF A NOVEL PREDATOR, *GAMBUSIA AFFINIS.*

Introduction

Through many human interactions with the environment, ecosystems have been greatly impacted by activities that transform the ecosystem and contribute to the extinction of species. Causes such as habitat loss, extinction cascades, overexploitation, and introduction of species, known under the term the "evil quartet", have contributed to the loss of biodiversity in the ecosystems in which they occur (Diamond, 1989; Brook et al., 2008). Through habitat loss and fragmentation, human activity such as deforestation has created a massive loss of species (Skole & Tucker, 1993). Another way humans have influenced ecosystems is through the introduction of invasive species. In some cases, such as the Zebra Mussel (*Dreissena polymorpha)*, the introduction is incidental; it is believed to have been introduced across the world via infested ballast water (McMahon, 1996). Though it was not intentional, the Zebra Mussel has since caused millions of dollars in damage by clogging water pipes (Pimentel et al., 2004). Additionally, Zebra Mussels utilize their high fecundity to crowd out native fauna (Pimentel et al., 2004). In other cases, the introduction is done with purpose. For example, Mosquitofish (*Gambusia holbrooki* and *G. affinis.*), were introduced to control mosquito populations. However, mosquitofish are now known to harm native species, such as the Barrens Topminnow (BTM), *Fundulus julisia* (Laha & Mattingly, 2007). The introduction of a

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novel predator can have a ripple effect due to trophic cascading, which is when predator-prey effects that change with abundance of predators or prey ripple across multiple trophic levels (i.e., lower predator population sizes can lead to a growing prey population, leading to a shrinking producer population) (Pace et al., 1999). When a novel predator is introduced into a community, naïve prey populations can display inappropriate (i.e., sub-optimal with respect to fitness) antipredator behavior, leading to reduced population numbers, or in some cases, prey species can be outcompeted for resources. Through these mechanisms (i.e., predation and competition with introduced species), many native species can become endangered or extinct, lowering biodiversity (Brooke et al., 2008).

To battle the reduction of biodiversity of an ecosystem, reintroduction projects have shown promise by providing an environment in which an imperiled species can be raised and bred in a safe, predator-free environment and then released into the wild after reaching a size or stage in which predation is likely to be reduced (Rahbek, 1993; Chapter 2). These projects can bolster dwindling populations by preventing harm to individuals during more vulnerable life stages (Witzenberger & Hochkirch, 2011). However, reintroduction projects of threatened or endangered species can fail if there has been a previous introduction of a novel predator that the imperiled species has no evolutionary history with, in that the threatened or endangered species when released may be subject to an evolutionary trap in which they will not recognize the predator as a danger and will not exhibit an appropriate behavioral response (Snyder et al., 1996). One method utilized to combat evolutionary traps is the conditioning of predator recognition, in which a stimulus already associated with a predator response is paired with the presence of a novel predator (Griffin et al., 2000). Conditioning of

predator recognition can allow individuals to learn through experience and exhibit appropriate responses to different dangers found in the wild (Buchholz, 2007). For instance, captive raised New Zealand Robins (*Petroica australis*) have been successfully conditioned to recognize novel predators and, once released into the wild, were significantly more cautious around a predator model versus control individuals (McLean et al., 1999). Previous work has also suggested using conditioning for Rainbow Trout (*Oncorhynchus mykiss*) recognition in the release of Hellbender conservation programs, although the success of such conditioning in this species is currently unknown (Crane & Mathis, 2011).

While conditioning can be a powerful tool in reintroduction efforts, it is important to explore two questions to determine fully the effectiveness of conditioning. Firstly, will the individuals experiencing conditioning effectively learn the behavior in question? And, secondly, is there a mechanism in which the learned behavior is spread and/or maintained within the wild population? While conditioning of predator recognition is a relatively accepted and utilized method for increasing a population's chance at post-release survival, relatively few studies have examined the maintenance of conditioned behavior in natural populations (Griffin et al., 2000; Banks et al., 2002; Vilhunen, 2006; Chapter 2). In cases in which populations successfully maintain conditioned behaviors, the individuals within populations appear to be utilizing social learning (Griffin et al., 2000). Social learning (i.e., learning via observation of another's actions; Chapter 2) has been shown to occur in some fish species, occurring not only in conspecifics, but also heterospecifics (Manassa et al., 2013). For example, it was found that Ward's Damsel (*Pomacentrus wardii*) were able to spread information about a predator not only to the closely related Lemon Damsel (*Pomacentrus moluccensis*), but also

the distantly related Three-spot Cardinalfish (*Apogon trimaculatus*) (Manassa et al., 2013; Chapter 1)*.* By having the capability to learn from other individuals in the community, an individual can rapidly adjust their behavior to novel encounters in the environment. When information is spread in such a manner, it also allows for the behavior to persist as it spreads through the population. For example, when looking at the difference between chemical cues and visual cues in predator recognition, social learning was likely a factor in the relatively fast spread of predator recognition in a population of 78,000 Fathead Minnow (*Pimephales promelas*) (Brown et al., 1997). Additionally, when used with training, social learning can enhance the responses demonstrated by individuals (Shier & Owings, 2007). When juvenile Black-tailed Prairie Dogs (*Cynomys ludovicianus*) were trained to recognize predators, those trained in the presence of an experienced adult were found to have greater survival one year after reintroduction than those trained without a demonstrator (Shier & Owings, 2007). This suggests that social learning is an important mechanism for establishing predator responses in populations where conditioning is used to increase survival following release into the wild (see also related discussion in Chapter 2).

Despite some previous research on the role of conditioning and social learning in conservation (described above and in Chapter 2), it is still unclear how broadly conditioning can be used to increase individual survival and population persistence of threatened and/or endangered species. As such, additional research is needed to understand fully whether conditioning and subsequent social learning can be an effective conservation tool in a range of species. One species that is ideal for examining the effects of conditioning and the maintenance of learned behavior is the BTM. The BTM is listed as an endangered species by

the State of Tennessee and is being considered for federal protections under the Endangered

Species Act (USFWS, 2011; Bettoli, 2015). BTMs are part of a larger group U.S. species in the

United States that have become endangered due to the introduction of the Western

Mosquitofish (WMF), *Gambusia affinis* (Laha & Mattingly, 2007; Westhoff et al., 2013).

Because the BTM had no previous experience or evolutionary history with the WMF, the BTM

has no evolutionarily adaptive antipredator response when they encounter the predator and,

therefore, young are predated upon and adults are harassed by the WMF (Westhoff et al.,

2013; Farnsley, 2014). Recent preliminary work has suggested that behavioral conditioning

can allow BTMs to recognize WMF as a predator and alter their behavior (Farnsley, 2014). By

pairing chondroitin sulfate, a known fish alarm cue, with the presence of WMF, Farnsley

(2014) found that BTMs can be classically conditioned, which involves pairing a conditioned

stimulus to an unconditioned stimulus, to potentially recognize the WMF as a predator

(Farnsley, 2014). Such conditioning is thought to be a possible way of increasing the survival

of BTMs upon reintroduction, providing an answer to the first of my questions above.

However, there remains a question of whether conditioning that elicits antipredator behavior

can act as a long- term conservation solution. For example, Iberian Green Frog (*Pelophylax*

perezi) tadpoles were found to be capable of losing a learned behavior days after exposure

(Goldsworthy & Bettoli, 2006). However, through reinforcement (repeated exposure to the

stimulus), the behavior was found to persist.

　　　To begin to answer the questions of whether conditioning can act as a long-term

solution for BTMs and whether social learning will allow predator recognition to persist in the

population, I explored both the effects on conditioning on 1) the behavior and survival of

conditioned individuals and 2) the behavior and survival of unconditioned, naive individuals who observed conditioned individuals (i.e., those individuals that had the potential to learn socially). Specifically, I used classical conditioning to attempt to condition BTMs to recognize and alter their behavior in response to a novel predator. These focal, conditioned fish were then placed with naïve BTMs in the presence of WMF to determine if the naïve individuals could socially learn from the conditioned individuals. Because previous work has shown that BTM can be conditioned to recognize a predator, and other fish species have been shown to be capable of social learning, both within and across species (discussed above and in Chapter 2), I hypothesized that BTMs can be conditioned to exhibit antipredator behavior in the presence of WMF and that unconditioned BTMs can learn antipredator behavior through social learning. If the BTM proves to be capable of social learning, conditioning fish before reintroduction could have implications toward furthering the continual conservation of BTMs and other related species.

Methods

Acquisition of study organism

A total of 115 Juvenile BTMs were obtained from Conservation Fisheries, Inc from Knoxville, TN. They were an average of 3.60 cm long, ranging from 2.70 cm to 4.70 cm, and 0.33 g, ranging from 0.14 g to 0.71 g. While not in use, fish were housed in 75.7 L aquaria within the University of Tennessee at Chattanooga and fed a mixture of brine shrimp and blood worms *ad libitum*. WMF were obtained from a freshwater spring near Hickory Creek in Vervilla, Warren County TN (35.5875 N, -85.8575 W). This research was performed

associated with the approved IACUC protocol # 15-07.

Phase I: Conditioning of the Barrens Topminnow to a novel predator, the Western

Mosquitofish

To condition the BTM, I utilized classical conditioning, in which BTM were exposed to one of three treatments: 1) chondroitin and three WMF (conditioning treatment), 2) distilled water and three WMF (control in which BTM would have experience with WMF but no conditioning), and 3) distilled water (control in which BTM would have no experience with WMF and no conditioning). Chondroitin was prepared by mixing 0.07 mg into 5 mL of distilled water. Chondroitin was used at this concentration as it was previously determined in another study to be effective when conditioning predator recognition in the BTM (Farnsley, 2014).

Each trial began by placing two random BTMs into a 37.85 L aquarium (i.e., the experimental tank) that was covered by a sheet from all sides except for above. Additionally, a porous and transparent plastic divider was placed halfway across the tank to create two compartments; visual and chemical, but not physical, interaction was possible between fishes in the two compartments of the tank. The two BTM individuals were placed in one compartment of each tank. Fish were allowed to acclimate to the tank for one hour. After acclimation, treatment conditions (described below) were introduced to the tank. In the conditioning treatment (treatment 1) 5 mL of chondroitin was added via syringe and 3 WMF were added for five minutes to the side opposite the BTM individuals immediately after chondroitin was introduced. In the experience treatment (treatment 2), 5 mL of distilled water was added via syringe and 3 WMF were added for five minutes to the side opposite the BTM

individuals immediately after water was introduced. In the no conditioning or experience treatment (treatment 3), 5 mL of distilled water was added via syringe to the tank. After a given trial, the BTMs were temporarily placed in a holding tank and the experimental tanks were thoroughly cleaned using water, soap, and rubbing alcohol. After cleaning and again setting up the experimental tanks, as described above, the BTMs were returned to their original experimental tank and allowed to acclimate overnight. The day following treatment, BTMs in each tank were filmed for five minutes before and after the introduction of 3 new WMF to the side opposite the BTM individuals. This allowed me to record time active (i.e., time spent in motion) and quantify the change in the proportion of time spent active before and after presentation with WMF only, which allowed me to determine whether my conditioning was effective (i.e., whether is caused BTMs to alter their behavioral reaction to WMF).

There were twelve replicates for each treatment, divided into six blocks (i.e., two replicates per block) that took place from December 12, 2015-January 6, 2016. After all replicates and trials for a given block were complete, the BTM individuals from that block were placed in 75.7 L aquarium to await Phase II of the study, and fish from different treatments were housed in separate aquaria.

To determine if there were differences in the change in the proportion of time spent active (i.e., in motion) among treatments (i.e., to determine whether conditioning was effective), Univariate Analysis of Variance (ANOVA) were run using IBM SPSS Statistics 24. Specifically, I focused on the change in the proportion of time spent active before and after presentation of WMF because I hypothesized that conditioned fish would have learned to perceive WMF as a threat and alter their motion relative to fish in the other two treatments.

That is, if conditioning was effective, I expected conditioned fish to alter activity relative to experienced or unconditioned control fish, and specifically, based on previous research (Farnsely, 2014), I expected conditioned fish to increase their activity when presented with only WMF relative to the experienced or unconditioned fish. In the ANOVA, the change in the proportion of time spent active was treated as my response variable, treatment was a fixed factor, and block was a random factor. I also examined whether there was significant interaction between treatment and block. Normality was evaluated using a Shapiro-Wilk test, and the distribution of the change in the proportion of time spent active was not significantly different from normal (p = 0.63).

Phase II: Evaluating the effectiveness of conditioning and social learning on Barrens Topminnow body condition and survival in the lab

To explore differences in the effect of predation recognition conditioning on BTM body condition and on social learning of predator recognition among treatments, a total of 36 naïve BTMs (i.e., those not used in Phase I of the study) were placed in 113.5 L aquaria along with a total of 36 BTMs that had been used in Phase I of the study. Tanks contained cover (i.e., artificial, plastic plants) that was spread out evenly within the tank. This phase was divided into six blocks. In blocks 1 through 5, two replicates of each treatment per block were performed. The sixth block contained four replicates of the chondroitin and WMF treatment, two replicates of the water and WMF treatment, and no replicates of the water only treatment. Specifically, in each replicate, a single naïve BTM was placed into a tank and assigned to one of three treatments: they were placed in a tank with 1) one BTM exposed to

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water only during Phase I, 2) one BTM exposed to water and WMF during Phase I, or 3) one BTM exposed to chondroitin and WMF during Phase I. Additionally, two WMF were added to each tank. I chose to use two WMF in this portion of the study to avoid re-using WMF that had previously been exposed to BTMs in Phase I. Each day one bloodworm per individual was added to the tanks. Each BTM was weighed, measured (i.e., total length was recorded), and marked using elastomer paint before entry into the tank, which allowed me to track individual BTMs throughout the course of Phase II. After being marked, BTMs were immediately placed in the tank and used in the experiment. After a two-week period, all BTMs were removed and weighed and measured before being placed in 37.85 L aquaria; fish from each block were placed in separate aquaria based upon treatment after this portion of the study.

Body condition was calculated using Fulton's body condition factor, where *K* is a standardized measure of body condition, *W* is weight in grams, and *L* is length in centimeters, as seen in the following:

$$K = 100 * \left(\frac{W}{L^3}\right)$$

Eq. 1

This measure of body condition is a well-established method of quantifying body condition in fishes, as it accounts for the scaling relationship between weight and length that is typically observed in fishes (Booth & Hixon, 1999; Sutton et al., 2000; Robinson et al., 2008). In my study, I quantified body condition (*K*) before and after the two-week treatment period of Phase II for all BTM.

To evaluate whether there was an effect of treatment on body condition, Univariate

ANOVAs were run in IBM SPSS Statistics 24. Change in body condition across the two-week

period was my response variable, treatment was a fixed factor, and block was a random

factor. I additionally considered the interaction between block and treatment. Normality was

evaluated using a Shapiro-Wilk test, and the distribution of the change in body condition was

not significantly different from normal for either BTM used in Phase I and II (p = 0.82) or naïve

BTM used in Phase II (p = 0.29). Additionally, two chi-square analyses were run to determine

if there were differences in death among treatment for BTM used in Phase I and II and the

naïve BTM used in Phase II. Given that conditioning is thought to alter activity and improve

survival in this species (Farnsley, 2014), I hypothesized that fish that were conditioned would

have better body condition and be more likely to survive than fish in my other two

treatments. Additionally, if social learning occurred, such that naive BTM individuals learn to

adjust their behavior in the presence of WMF by observing conditioned fish, then I would also

expect naive fish that were paired with conditioned fish to have higher survival and body

condition than fish in my other two treatments.

Phase III: Evaluating the effectiveness of social learning on activity-level of naive fish

To evaluate whether social learning of predator recognition potentially occurred in

the BTM and caused naïve BTM to alter their activity, each naïve individual from Phase II was

individually placed into a 37.85 L aquarium which was covered by a sheet from all directions

except for above. Additionally, a porous, transparent divider was placed halfway across the

tank to separate the halves of the tank, as in Phase I of the study. BTM were then allowed to

acclimate for one hour. After acclimation, time active (i.e., time spent moving) was recorded

for five minutes before and after the introduction of three WMF to the compartment of the tank opposite the BTM. I then quantified the change in the proportion of time individuals were active before and after WMF were introduced to the tank, which allowed me to determine if the naïve BTM from my Phase II potentially learned from the conditioned BTM from Phase I to alter their behavior in response to WMF.

To evaluate differences in the change in proportion of time active among treatments (i.e., the treatment that individuals were exposed to in Phase II), Univariate ANOVAs were run in IBM SPSS Statistics 24. The change in the proportion of time active was my response variable, treatment was a fixed factor and block was a random factor. I additionally tested for an interaction between treatment and block. Normality was evaluated using a Shapiro-Wilk test, and the distribution of the change in proportion of time active was significantly different from normal (p = 0.013). Because the data differed significantly from a normal distribution, I also ran a non-parametric Kruskal-Wallis test, which allowed me to evaluate the effect of treatment and block on the change in the proportion of time spent active; however, Kruskal-Wallis tests are generally less powerful than ANOVA and do not allow one to assess interactions between predictor variables. Given this, and because the results of the Kruskal-Wallis test were quantitatively the same as those of the ANOVA, I present only the results of the parametric ANOVA below. If social learning occurred, I would expect naive individuals who were placed with conditioned individuals to have a change in activity that is different from naive individuals who were placed with fish that were not conditioned during Phase I of the study.

Phase IV: The effect of learned predator recognition on survival in the wild in the Barrens Topminnow

As mentioned above, all BTM individuals were tagged using elastomer paint prior to use in Phase II of the study. All markings had been in place at least 2 weeks before this phase and were checked before release. Specifically, prior to Phase II, I tagged fish from the different treatments differently in order to track the survival of fish from my different treatments following release into the wild. BTM were marked with the colors blue, red, or yellow to show whether the fish was used in Phase I, was a naïve individual from Phase II, or was a nonexperimental BTM, respectively. Additionally, the marking was placed either on the left behind the gills, the right behind the gills, or toward the tail on the right to show whether the individual experienced the chondroitin and WMF treatment, the water only treatment, or the water and WMF treatment, respectively. To determine if learned predator recognition affected survival of the BTM in the wild, I released BTM from my experiment into a spring near Hickory Creek in Warren County, TN (35.5875 N, -85.8575 W) on July 3, 2016. The release site was dammed off with concrete, helping to limit migration in and out of the sample site. The release site was composed of four interconnected pools. Specifically, I released 49 individual BTM, 41 of which were included in my earlier study, 5 of which were non-experimental (i.e, completely naïve), and 3 which were unknown due to loss of marking, into the spring. Included in the release were 23 BTM used in both Phase I and Phase II (from the treatments: water only = 6, water and WMF = 7, and chondroitin and WMF = 10) and 18 BTM from Phase II which had been naïve prior to Phase II (from the treatments: water only = 7, water and WMF= 4, and chondroitin and WMF = 7). All fish were released into the middle

pool of the spring habitat in shallow water (< 0.5 m) near the shoreline. I then sampled two

days later, and I resampled another three times on July 5, August 6, and September 1, 2016.

During my re-sampling, I went over each of the pools and connecting portions with a 3.05 x

1.83 m delta mesh 3.175 mm seine, passing over the entirety of each pool at least once and

making two passes over the shallow connecting areas. Each sampling session lasted

approximately 3-4 hours. During seining sessions, recaptured BTM were kept in a bucket of

water until the end of session as to prevent re-sampling the same individual repeatedly on a

given sampling day. However, after all sampling was completed on a given day, all recaptured

BTM were re-released into the spring, and as such, I cannot rule out the possibility that a

single individual might have been captured on more than one sampling day. For each

recaptured fish, I recorded which treatment the individual had experienced and whether the

individual was used in Phase I and Phase II or was a naïve individual used only in Phase II.

To examine if 1) treatment in Phase I had an effect on recapture and 2) if social learning

opportunity in Phase II had an effect on recapture, I performed two chi-square analyses in

which I compared recapture rates across treatments among BTM used in Phase I and II and

among BTM used only in Phase II. If conditioning was effective in Phase I and improved

survival following release into the wild, I would expect more conditioned fish to be

recaptured relative to the fish in the other two treatments. If social learning occurred during

Phase II and improved survival, I would expect to recapture more naive fish that were paired

with a conditioned fish relative to naive fish that were paired with fish that were either

unconditioned or exposed only to WMF during Phase I.

Results

Phase I: Can the Barrens Topminnow be conditioned using a chemical alarm cue?

Treatment had no effect on the change in proportion of time spent active ($F_{2,10}$= 1.140, P=0.358). Specifically, BTM individuals that were exposed to water, water and WMF, and chondroitin and WMF did not differ significantly in activity (Fig. 1). These results suggest that conditioning did not significantly alter activity associated with the presentation of WMF in this study. However, there was a significant effect of block on the change in the proportion of time spent active ($F_{5,10}$= 7.751, P=0.003), suggesting that on average there were differences among blocks in how individuals altered their activity in response to WMF. There was no significant interaction between block and treatment ($F_{10,18}$= 0.507, P=0.863), though, suggesting that there were no differences among treatments in the way that block affected the change in the proportion of time spent active.
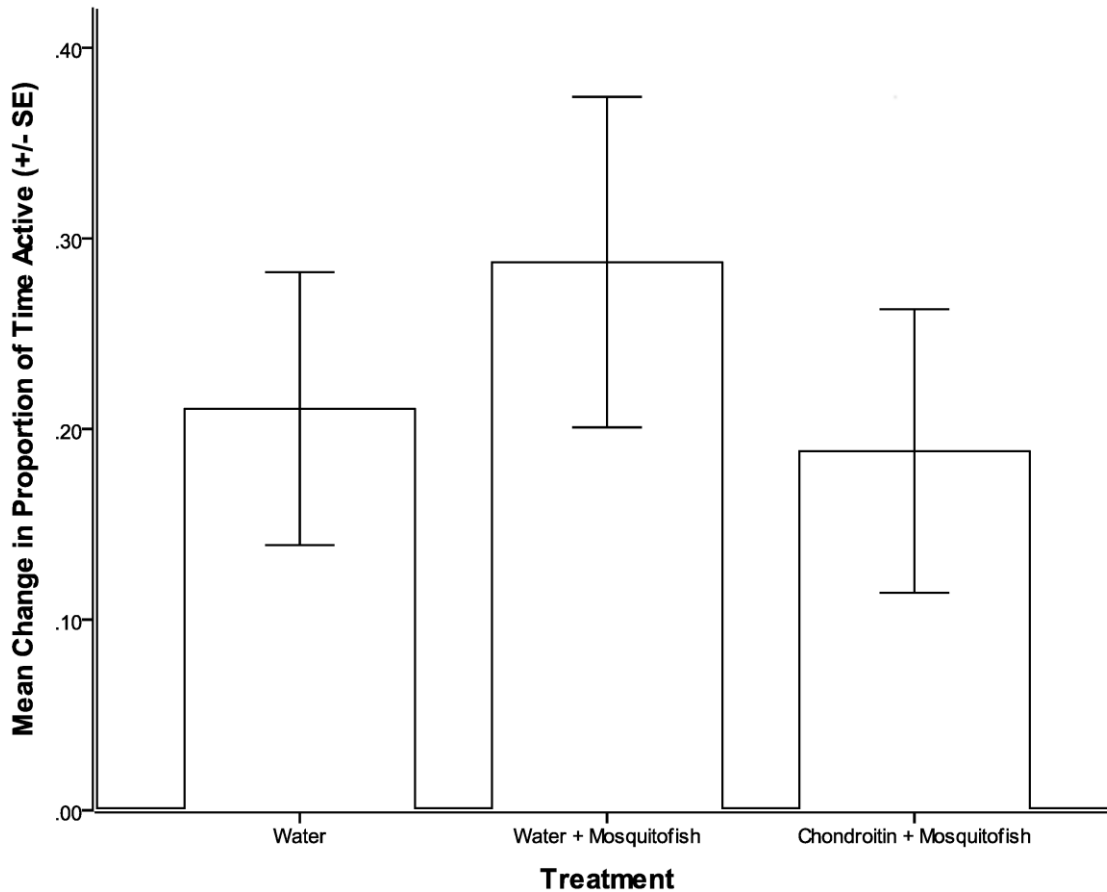
Fig. 1 Change in the proportion of time active before and after presentation of Western Mosquitofish (WMF) (*Gambusia affinis*) among the treatments in which Barrens Topminnows (*Fundulus julisia*) were exposed to water only, water and WMF, and chondroitin and WMF

Phase II: Does conditioning treatment affect change in body condition in the Barrens Topminnow?

Treatment did not affect the change in body condition for either conditioned or naïve individuals (conditioned individuals: $F_{2,9.58}$= 0.105, P=0.902, Fig. 2; naïve individuals: $F_{2,10.15}$= 0.002, P=0.998, Fig. 3). There was no significant effect of block or the interaction between block and treatment on body condition (conditioned individuals (block): $F_{5,8.61}$= 0.378,

P=0.852; naïve individuals (block): $F_{5,8.74}$= 1.613, P=0.253; conditioned individuals (block-treatment): $F_{9,18}$= 0.894, P=0.550; naïve individuals (block-treatment): $F_{9,15}$= 0.774, P=0.643). The average weight of the BTMs changed during my treatments from 0.35 g to 0.33 g for Phase I individuals and 0.31 g to 0.30 g for naïve individuals. The average lengths of the BTMs Phase I individuals changed from 3.65 cm to 3.60 cm for Phase I individuals and 3.55 cm to 3.47 cm for naïve individuals. Dead individuals were not included in the post-treatment means but were included in the pre-treatment means. A total of five BTM died during the course of Phase II. Specifically, one water only fish from Phase I, one water only naïve fish from Phase II, two water and WMF naïve fish from Phase II, and one chondroitin and WMF naïve individual from Phase II. There were no significant differences among treatments in the number of conditioned individuals (χ^2_2 = 2.674, P = 0.263) or naïve individuals (χ^2_2 = 0.611, P = 0.737) that died during Phase II of the study.
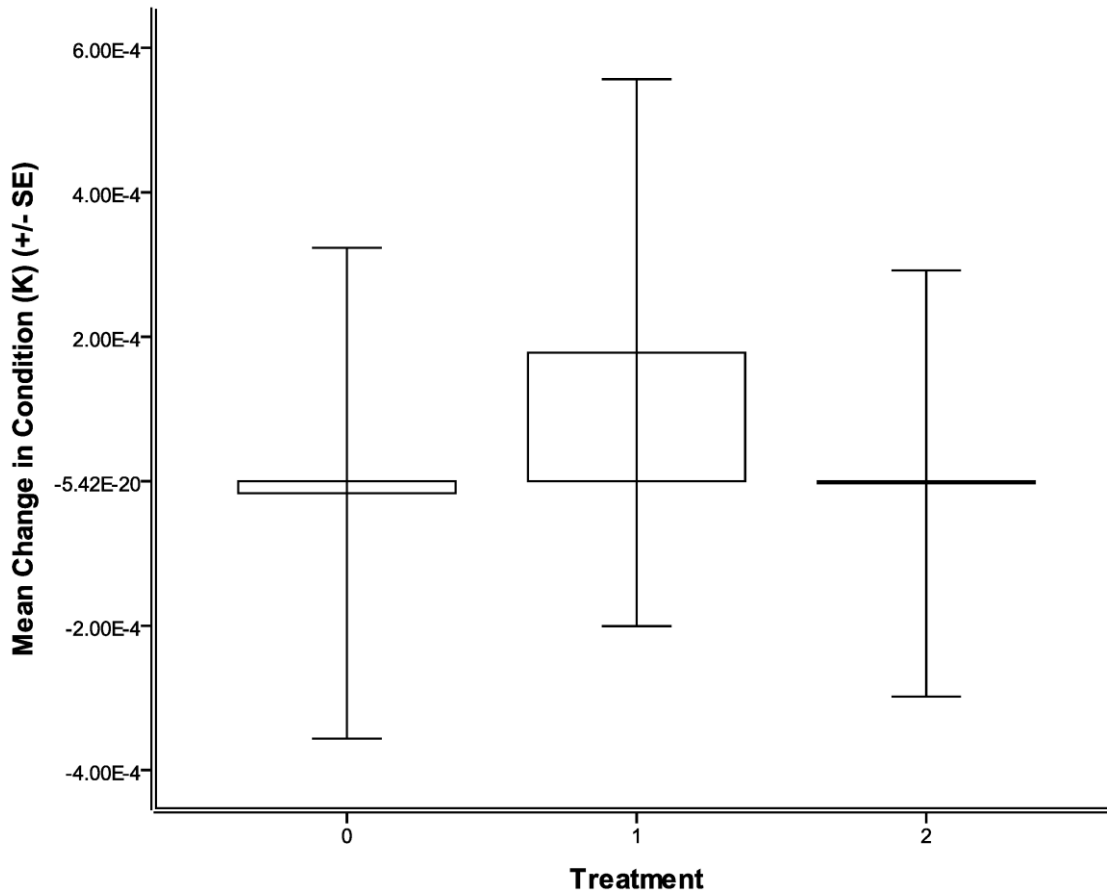
Fig. 2 Change in body condition (*K*) in the Barrens Topminnows (*Fundulus julisia*) used in

both Phase I and Phase II among the treatments in which the BTMs were exposed to water

only, water and Western Mosquitofish (WMF) (*Gambusia affinis*), and chondroitin and WMF
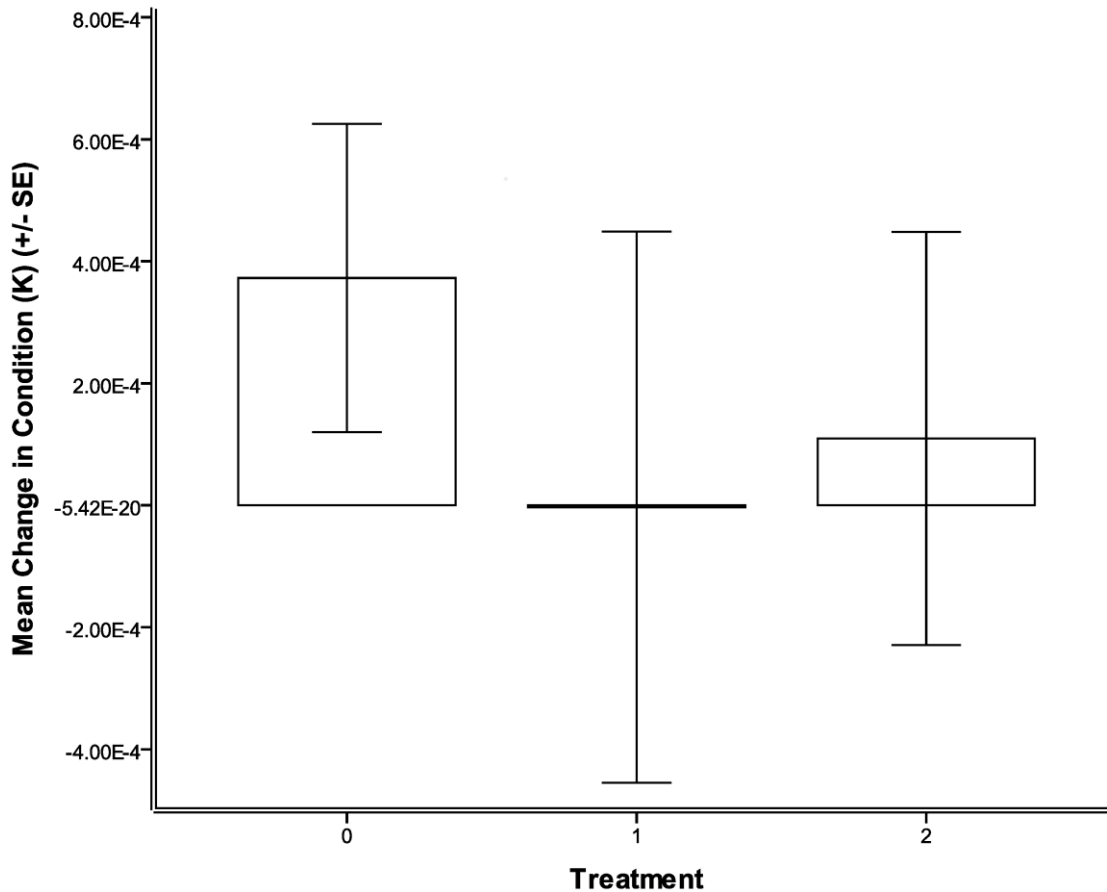
Fig. 3 Change in body condition (*K*) in naïve Barrens Topminnows (*Fundulus julisia*) from Phase II among the treatments in which BTMs were paired with individuals exposed to water only, water and Western Mosquitofish (WMF) (*Gambusia affinis*), and chondroitin and WMF

Phase III: Can the Barrens Topminnow socially learn conditioned predator recognition?

When I focused on the behavior of naive BTMs that spent two weeks with BTMs from Phase I and WMF, there were no significant differences in the change in proportion of time active among treatments after these previously naive individuals were placed with WMF ($F_{2,3.11}$= 0.342, P=0.734). This suggests that the naive fish that were paired with conditioned and unconditioned individuals did not differ in their response to

WMF. I also found no significant effect of block on change in the proportion of time spent active ($F_{3,2.27}= 1.938$, P=0.339), and there was no significant interaction between block and treatment ($F_{3,11}= 0.657$, P=0.595).
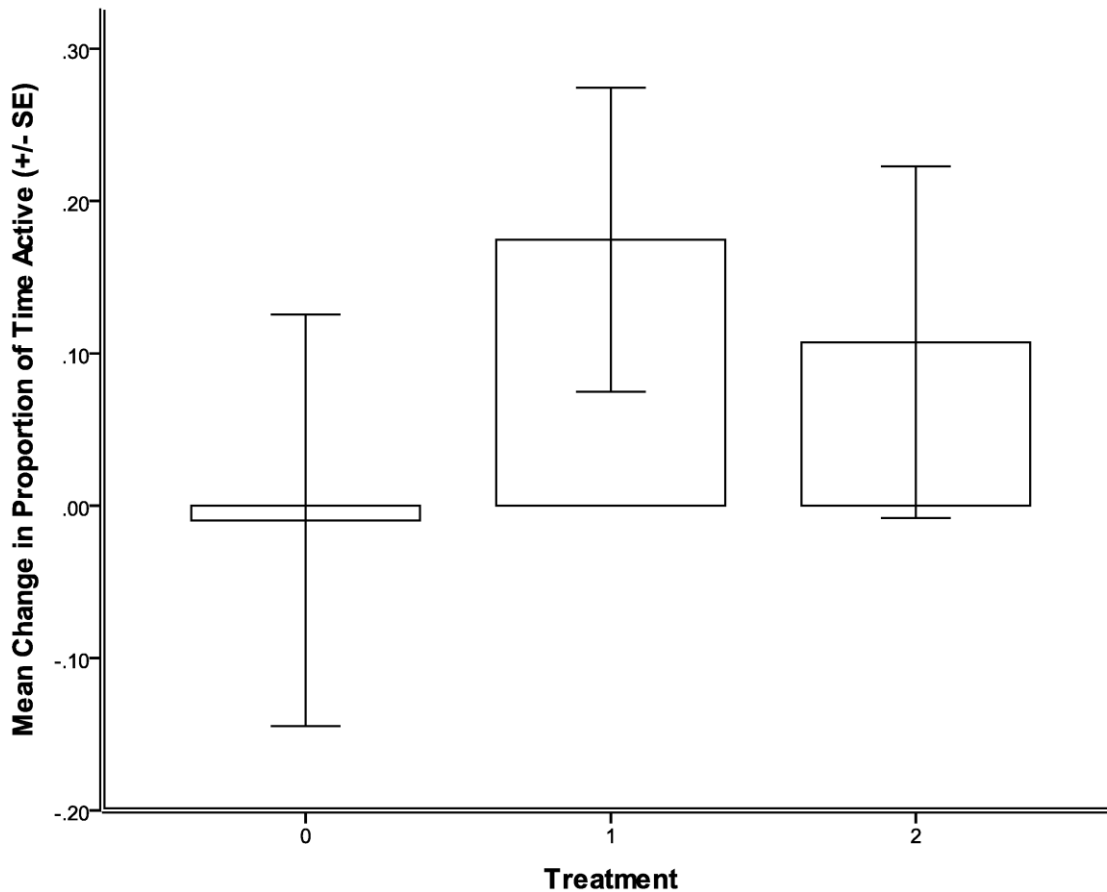
Fig. 4 Change in the proportion of time active in naive phase II Barrens Topminnows (*Fundulus julisia*) before and during exposure to Western Mosquitofish (WMF) (*Gambusia affinis*)h among the treatments of water, water and WMF, and chondroitin and WMF

Phase IV: The effect of learned predator recognition on survival in the wild

  Three fish were captured during my initial sampling, two were captured during my second sampling, and one captured during my third sampling. No individuals were captured

on my final day of sampling. There was no effect of treatment on the recapture rate of either

BTM used in both Phase I and Phase II (χ^2_2 = 0.97, P = 0.953) (Fig. 5) or BTMs used as naïve

individuals in Phase I (χ^2_2 = 3.536, P = 0.171) (Fig. 6). These results suggest that fish from all

treatments were equally likely to be recaptured following release into the wild.
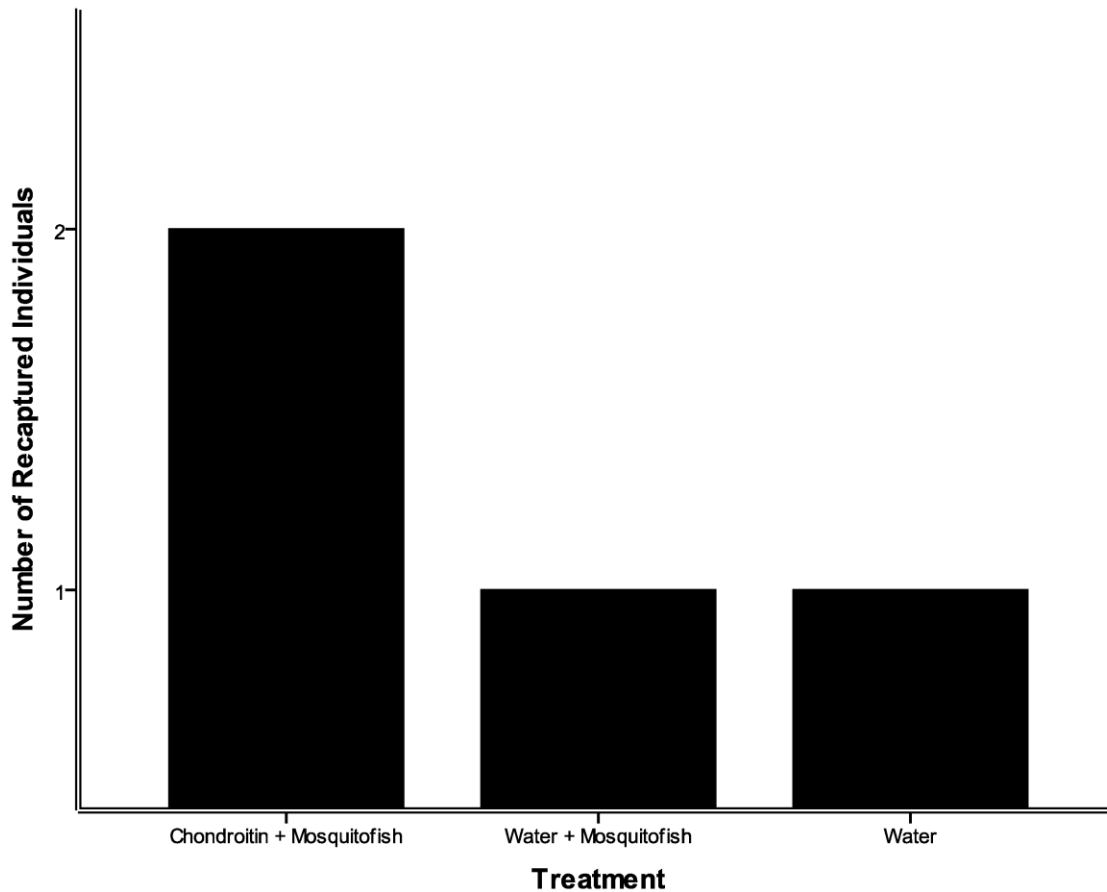
Fig. 5 The number of individual conditioned Barrens Topminnows (*Fundulus julisia*) used in

both Phase I and Phase II that were recaptured among treatments across the four
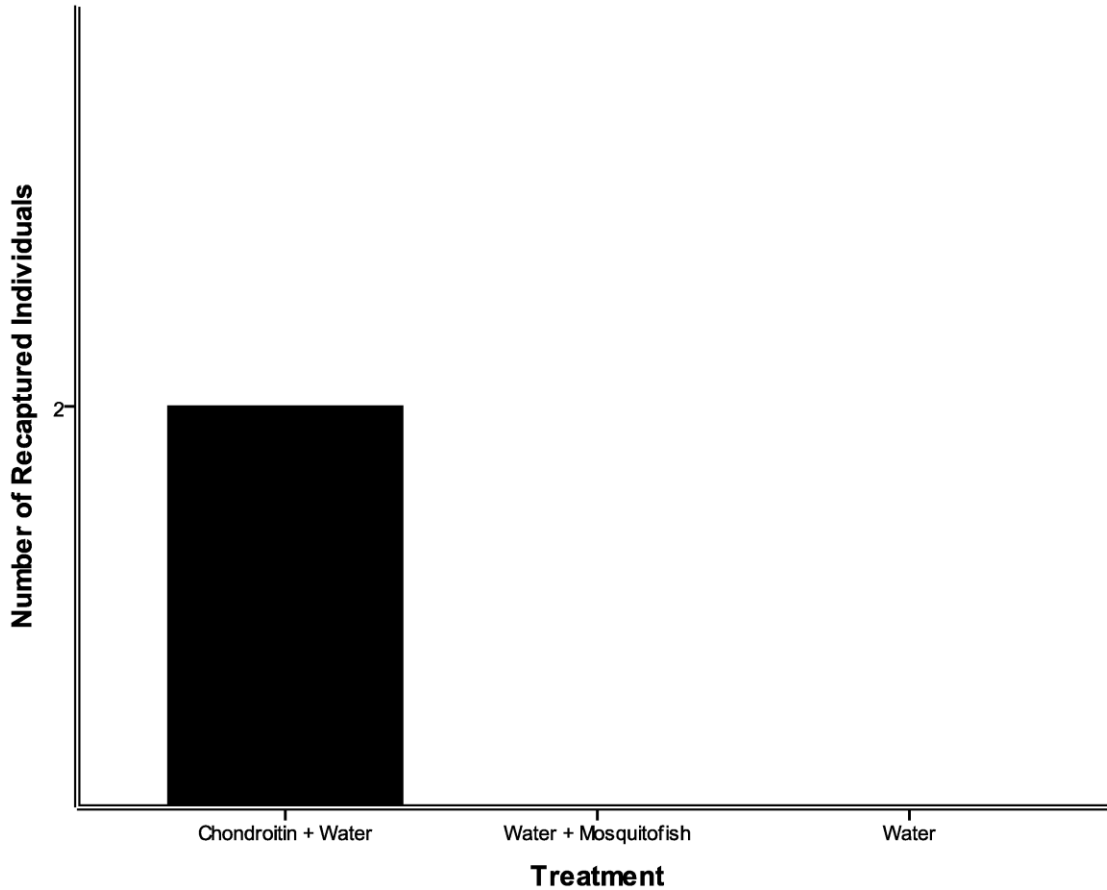
resampling dates

Fig. 6 The number of individual Barrens Topminnows (*Fundulus julisia*) which acted as naïve individuals in Phase II that were recaptured among treatments across the four resampling dates

Discussion

Overall, I found no significant differences among my control groups and the experimental group in conditioning and social learning of predator recognition, and as such, I have no evidence at this time that conditioning and social learning are effective under the conditions of my study. Specifically, these findings indicate that conditioning and subsequent social learning likely did not take place in the BTMs in this study. In the following sections, I

discuss how my results compare to similar works and discuss some alternative explanations of my findings.

Phase I: Can the Barrens Topminnow be conditioned to recognize a novel predator?

There were no significant effects of conditioning on BTM behavior, and thus no evidence that BTM learned predator recognition though conditioning in our study. While this may indicate that my focal fish failed to acquire the predator recognition, previous work has shown that the BTM can learn to alter their behavior through conditioning and potentially acquire predator recognition via the same method utilized for this study (Farnsley, 2014). Given this previous work, and other work suggesting that a range of animals can learn effectively (Chivers & Smith, 1995; Griffin et al., 2000; Bool et al., 2011), it is possible that the individuals used in my study differed in either learning capability or their reaction to the alarm cue from those previously used in Farnsely's (2014) study, and may be less apt to learn and exhibit conditioned behavior. Other work has found evidence of variation in the ability to learn within a given species. For example, in a study looking at spatial learning, Three-Spined Sticklebacks (*Gasterosteus aculeatus*) exhibited population differences in their learning capability; however, it is believed that this is due to the different local environments as different landmarks were utilized within a maze in that study (Girvan & Braithwaite, 1998). Additionally, antipredator behavioral responses may differ among species and individuals. For instance, while previous work has shown the capability of BTM to be conditioned and increase activity in the presence of predators, the closely related species Northern Studfish (*Fundulus catenatus*) was found to decrease activity in response to such conditioning (Farnsley, 2014). Thus, it is

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possible that the lack of conditioning observed in the present study is due to individual variation in antipredator behavior.

It is also possible that the present study differed in some unintended way from that of Farnsley (2014). For example, while every attempt was made to replicate the conditions of Farnsley (2014), there might have been differences in the water chemistry or the chondroitin used in the present study. For instance, it is possible that the chondroitin sulfate used in my experiment was ineffective due to the chemical cue's aging or incompatibility with my specific population of BTM; however, I think that the difference in results in my study relative to those of Farnsley (2014) is less likely due to population differences as my population was received from the same captive breeding program as those used in Farnsley (2014). Additionally, due to my method of measuring change in behavior, exhibiting high activity before presentation of the predator can potentially hide positive changes in activity, as I did not measure intensity of movement; it is therefore possible that my measurement approach was too coarse to detect effects of conditioning.

Despite not finding evidence of predator recognition in my conditioned fish, previous studies have shown that a variety of fish species can be conditioned. For example, the Arctic Charr (*Salvelinus alpinus*) can be conditioned using the odor of Arctic Charr-fed Pikeperch (*Sander lucioperca*). By pairing the odor of Pikeperch with Pikeperch presence, naïve individuals were conditioned to avoid Pikeperch through a single exposure event, showing some species of fish to be readily able to acquire predator recognition through conditioning (Vilhunen, 2006). Fathead Minnow (*Pimephales promelas*) have also shown to learn predator recognition through conditioning by pairing Brook Charr (*Salvelinus fontinalis*) odors, used to

simulate predator presence, and chemical alarm cues to allow the Fathead Minnow to recognize the Brook Charr as a predator (Ferrari et al., 2005). It will be interesting in the future to look at how alarm cue reactions differ among populations and how shy/bold dispositions affect learning in the Barrens Topminnow and other topminnows.

Because there was a chance that my conditioning was effective in Phase I but my measurement approach was too coarse to capture effects of conditioning on behavior, I analyzed the effects of conditioning on 1) body condition in the presence of WMF and 2) the effects of being paired with conditioned fish on the body condition and behavior of naive fish (discussed below and in Methods above).

Phase II: Is Barrens Topminnow body condition affected by the presence of Western Mosquitofish?

I found no significant differences in body condition among treatments for both my conditioned and naïve individuals during the portion of my study in which I exposed the BTM to WMF for two weeks. In general, body condition actually increased in the BTMs during the 2 weeks in Phase II; this increase in body condition was minor, but the lack of a decrease in body condition on average might suggest that there was minimal food competition in Phase II of my study. These results were contrary to my predictions but are consistent with some previous work. According to Laha and Mattingly (2007), WMF have an impact upon the BTM through harassment and physical harm and by consuming small juveniles. Specifically, Laha and Mattingly (2007) allowed for adult BTM and adult WMF to live together over 60 days and observed no significant negative impacts upon the BTM, suggesting that adult BTM are

able to survive with adult WMF. This may suggest that it is the adult WMF's interaction with juvenile individuals which allows the WMF to have a strong negative impact on BTM populations (Laha & Mattingly, 2007). Understanding how an invasive species is negatively impacting a species of interest can provide an apt platform for finding solutions to conservation problems, and my results and those of Laha and Mattingly (2007) suggest that the WMF primarily negatively impacts the BTM through predation and harassment, rather than through competition for food that reduces body condition.

Phase III: Can the Barrens Topminnow socially learn to recognize the Western Mosquitofish as a predator?

I found no significant effects of treatment on predator recognition among my control and experimental groups, suggesting that social learning did not take place or was simply not possible due to lack of initial conditioning and learning in my study. This finding that no social learning occurred is likely related to my earlier finding that behavior did not differ among treatments during Phase I. Specifically, given that I found no evidence that BTM were conditioned to alter their behavior in the presence of WMF, the naive BTM likely had no potential to learn socially from conspecifics. There is also the possibility that the BTM does not socially learn predator recognition at the juvenile stage. However, further work would be needed to make any conclusions about social learning in this species. Additionally, by placing my observer individuals in long contact with the desired predators, the BTM may have become habituated to the presence of the WMF. For example, Brachetta et al. (2016) showed that repeated exposure to a predator odor caused loss of defensive behaviors in the Talas

Tuco-tuco (*Ctenomys talarum*), a subterranean rodent, due to habituation to the presence of the odor.

Overall, and despite a lack of evidence for conditioning or social learning in the present study, I do think that it is likely that the BTM has the potential to learn through experience and socially given work in other systems. For example, Mathis et al. (1996) demonstrated that naïve Fathead Minnow exhibit fright responses in the presence of the Northern Pike (*Esox lucius*) when paired with experienced conspecifics and will retain the predator recognition. Additionally, Brook Stickleback (*Culaea inconstans*) can also learn and retain predator recognition from experienced Fathead Minnows (Mathis et al., 1996). In another study, Suboski et al. (1990) found that Zebra Danio (*Brachydanio rerio*) can transmit conditioned predator recognition to naïve observers. One way that my experiment differs from the examples given was the method by which the observers were allowed to observe. In my experiment, observers were allowed prolonged contact with the predator during Phase II, perhaps giving time for habituation of the predator presence; while there were five BTM deaths and two WMF deaths, I never informally observed any significant harassment being done to the fish that might have affected body condition and survival. It is also important to note that while I had only one demonstrator for each observer, other work demonstrated that guppies, which are of the Order Cyprinodontiformes like the BTM, socially learn more effectively while shoaling as a group (Lachlan et al., 1998). Additionally, the fact that I observed one individual BTM during Phase III and two BTMs during Phase I might have created differences in behavior between the two phases. For example, there might have been interactive effects in Phase I such that the behavior of one individual BTM affected the

behavior of the other BTM in Phase I. Such interactive effects might have created variation in behavior that either prevented conditioning or made it impossible to detect the effect of conditioning behaviorally. In the future, it will be key to examine the ability of BTM to learn socially across different scenarios that vary in predator exposure and under conditions in which they have the potential to learn socially from conspecifics that exhibit anti-predator behavior.

Phase IV: The effect of learned predator recognition on survival in the wild in the Barrens Topminnow

There was no significant effect of treatment on recapture rate of either conditioned BTM or BTM given the opportunity to learn socially during Phase II. This likely indicates that there was no effect of treatment on survival in the wild in my study. Due to the finding that conditioning and social learning did not appear to take place in my study, it is difficult to determine if learned predator recognition can act as a conservation tool in the BTM. It is also important to note that with the low sample size of my recapture study, my statistical power was very low, making it difficult to detect effects of treatment on recapture rate if such effects existed. In general, my findings differ from those of Farnsley (2014) and Berejikian et al., (1999). In both Farnsley (2014), which focused on the BTM and Berejikian et al., (1999), which focused on the Chinook Salmon (*Oncorynchus tshawytscha*), conditioning using a predator odor increased post release survival. This difference is likely related to my findings that the BTM showed no differences among treatments in activity in relation to conditioning or social learning.

Conclusions:

Predator recognition is an important tool for survival as it allows individuals to assess risk and exhibit an appropriate behavioral response. However, when exposed to a novel predator, a species may find itself within an evolutionary trap, presenting behaviors that are inappropriate to the actual risk. This misappropriated behavior can cause a decline in the population and may lead to extinction as the species is predated and harassed through time. To help battle against the loss of populations and species, captive breeding and reintroduction programs have begun to incorporate behavioral approaches into their methods, including training, conditioning, and social learning. However, there is still some question as to the full effectiveness of these methods. In the case of the BTM, my results indicate that there was no effect of treatment on conditioning or social learning, suggesting that conditioning might be ineffective under some conditions. These results have implications in the conservation of the BTM, as there remains uncertainty to the persistence of conditioned predator recognition upon release in this species. As with any conservation project, the ultimate goal is to have a stable population that can persist without further input from conservation managers. Without knowing if learned predator recognition will persist in the population, and without knowing under what precise settings conditioning is likely to be effective, conditioning may prove to only be a temporary aid that is only effective under certain conditions. While such results may appear bleak, it is important to note that this study only looked at one method of maintaining behavior in a population. There is much still to be understood in the subject of learning and behavior and there is still much to be learned about utilizing behavioral and conservation sciences together.

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