THE PENNSYLVANIA STATE UNIVERSITY
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# THE EFFECT OF ENVIRONMENTAL ENRICHMENT ON AGILITY, EXPLORATION AND ANXIETY LEVELS OF HATCHERY-REARED FISH

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# ABSTRACT

In an attempt to battle the negative effects of habitat degradation and over-exploitation on wild fish populations, researchers have begun to look to hatcheries as a component of ex-situ conservation. Hatcheries are utilized as breeding grounds for fish, which are ultimately released as a way to bolster and help maintain wild populations Unfortunately, released fish often display behavioral deficits and are unable to function as effectively as their wild counterparts, with large proportions of released fish lost to mortality. It is hypothesized that poor post-release survival arises because of adaptation to captivity, relaxed natural selection in the captive breeding environments, and the experience of being maintained in a safe, unchanging captive environment. This thesis project examined the potential benefits of environmental enrichment in a captive breeding environment on the behavior and swimming performance of rainbow trout (*Oncorhynchus mykiss*). Trout aged 11 months, were exposed to environmental enrichment by adding and exposing the fish to different kinds of novel objects, such as plastic models of aquatic weeds, PVC pipes, and floating plants that provided the fish with areas to explore, hide and interact. After two months of enrichment, the fish were tested in an Open Field Novel Object Test to assess their explorative nature, levels of anxiety and their overall swimming agility. These assays revealed that compared to fish reared in standard hatchery conditions, the trout reared in enriched conditions showed a lower level of anxiety and a higher level of activity. The enriched fish were also more explorative, but there were no clear effects on swimming performance and the fish were not significantly more agile in terms of the number of collisions the fish made within the test tank walls.

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# Chapter 1

## Introduction

Like many wild species, several fish populations have come under threat of endangerment or extirpation in recent decades (Burkhead, 2012). The influence of a growing human population has altered the size and survival of fish species worldwide, and researchers do not estimate a reversal in the trend. While most focus, in terms of fish population declines, has been on marine species, freshwater species have been facing similar declines. As a whole, freshwater biodiversity has declined faster than terrestrial or marine biodiversity in recent decades (Xenopoulos et al, 2005). In the twentieth century, freshwater fishes have had the highest extinction rate among vertebrates (Burkhead, 2012). Furthermore, loss scenarios indicate that up to 75% of local fish biodiversity in freshwater systems may be lost by 2070 (Xenopoulos et al, 2005).

Fish therefore face endangerment and extinction in water systems worldwide. In North America, freshwater fish have seen a 25% increase in extinctions since 1989, and at least 57 species and subspecies have been lost since 1900 (Burkhead, 2012). In the Chinese Yangtze River Basin, an area populated by both freshwater and non-freshwater species, 25 native fish were considered to be endangered in 2003 (Fu et al, 2003). Worldwide, 158 species of fish have been rendered extinct since 1900, and studies indicate that these numbers may be larger, as only 38% of total fish species have been evaluated (Ceballos et al, 2015).

Several major causes of fish decline have now been identified. Habitat fragmentation, modification of water flow, and the introduction of alien species are large contributors, especially in freshwater fish populations (Dudgeon et al, 2006; Burkhead, 2010). In marine species, over-exploitation and water pollution have been shown to contribute to population decline. Over-exploitation may account for up to 55% of marine extinctions (Dulvy, Sadovy and Reynolds, 2003), while water pollution in combination with elevated water temperatures has been shown to yield increased susceptibility to disease in Pacific salmon (Dietrich et al, 2014). It seems that these multiple stressors have an additive effect and together they contribute to the global decrease in fish populations.

One way to mitigate the effects of population decline is to manage fish numbers through captive rearing programs in which fish are bred and reared in hatcheries and subsequently released into natural water bodies. Fish hatcheries have been in existence since the 19th century and were specifically developed to address aspects of population decline. The first species targeted by hatcheries was cod, but today, hatchery-raised fish bolster many wild populations (Kurlansky, 1999). Initially, the principle aims of these programs were to overcome anthropogenic environmental disturbance, compensate for overfishing, and to maintain the highest possible level of productivity in fisheries (Brown and Laland, 2001). However, these captive breeding programs are now widely utilized as a tool to help restore and supplement declining populations in the wild (Araki et al, 2008).

At the turn of the 21st century, researchers began to investigate the success rates of fish bred in hatcheries and released into the wild to supplement populations (Brown and Laland, 2001). While large amounts of fish were released from hatcheries each year, the chance of the fish surviving to adulthood was low. As hatcheries grew, practices were adopted to improve total production, but methods to improve post-release survival remained inadequate. One study indicated that of five billion hatchery-reared salmon released, less than five percent survived to adulthood (McNeil, 1991). The massive loss of fish post-release rendered the conservation-based bolstering of wild populations ineffective.

It became clear that hatchery-reared fish possess behavioral deficits compared to their wild counterparts (Olla, Davis and Ryer, 1998). The behavioral differences between wild and hatchery-reared fish are likely the result of their development in very different environments (Braithwaite and Salvanes, 2006). These deficiencies, which develop early in the life of the fish, subsequently affect the success of the fish when released into the wild (Heenan et al, 2009).

Standard hatchery conditions are very different from conditions experienced in the wild. Hatchery rearing at higher than natural densities may contribute to poor anti-predator responses as well as delayed time finding and capturing prey (Brockmark, Adriaenssens and Johnsson, 2010). Anti-predator responses may also be hindered by the lack of predation experienced in hatchery environments (Olla, Davis and Ryer, 1998; Vilhunen, Hirvonen and Laakkonen, 2005). Furthermore, hatchery-reared fish are fed a rich, nutritious diet with excess food, resulting in well-fed fish that possess high body condition. Both the low levels of predation and the good nutrition ensure successful development in the hatchery environment, but interestingly, these probably both contribute significantly to poor post-release survival statistics (Ahlbeck, Salvanes and Braithwaite, 2015). Fish reared in hatchery conditions have been shown in behavior trials to fail at avoiding predators, with hatchery fish often moving closer to a predator than their wild counterparts (Braithwaite and Salvanes, 2006). The hatchery fish are also less adept at capturing live prey, and in some instances will even feed on small stones and pebbles that resemble food pellets typically used in hatchery rearing (Braithwaite and Salvanes, 2006; Strand et al, 2010).

To address the impact these behavioral deficiencies have on post-release mortality, researchers have attempted to identify the core differences in the development between hatchery-reared and wild fish. To date, the most prominent effects appear to stem from the artificial breeding program and an enrichment-lacking rearing environment that limits the amount of learning by fish throughout the first few months of life (Brown and Laland, 2001; Braithwaite and Salvanes, 2006).

Growing evidence indicates that artificial breeding of hatchery populations has resulted in changes to the behavioral phenotypes of fish in addition to their genetic divergence from wild populations (Huntingford, 2004). Genotypic divergences can occur in only a few generations of hatchery-reared fish (Araki et al, 2008), and may contribute to maladaptive behaviors such as increased aggression and risk-taking (Sundström, Lõhmus and Johnsson, 2003). Further risk associated with divergence in the genotype of wild and hatchery-reared fish comes when fish are released. Released fish that survive to reproductive maturity can interbreed with native species, but if the genotypic differences between wild and hatchery-reared fish are too great, it is highly likely that long-term reproductive success of the population will be negatively affected (Braithwaite and Salvanes, 2006).

The negative effects of artificial breeding are only worsened by alterations of natural and sexual selection pressures that occur within the hatchery environment (Brown and Laland, 2001). Studies indicate that hatchery rearing tends to yield fish that are more aggressive than their wild counterparts (Huntingford, 2004). This could be due, in part, to the unnaturally high densities of confinement in hatchery conditions (Brockmark, Adriaenssens and Johnsson, 2010). Feeding procedures for these tanks may introduce a competitive pressure that indirectly selects for aggressive fish that are successful in acquiring food by fighting other fish in the tank (Huntingford, 2004). While the aggression may lead to success in the hatcheries, this behavior does not translate to success in the wild. Atlantic salmon capable of dominating a localized food source through aggressive behavior have been shown to do poorly in natural environments in which food is spatially unpredictable (Olla et al, 1994; Huntingford, 2004).

Hatchery environments, unlike the changing natural world, provide very little experience of variation. Rearing animals in monotonous, unvarying conditions has long been known to under-stimulate the brain and sensory systems and generally leads to a number of behavioral deficits in adulthood (Strand et al, 2010). A long history of research with mammals has explored the effects of different kinds of enrichment in the captive environment. In the 1940's, Donald Hebb was the first to anecdotally propose the theory that an enriched environment could lead to neural benefits in laboratory rodents when he compared the behaviors of his pet rats with those he worked with in the laboratory (Van Praag, Kempermann and Gage, 2000). Several years later, Cross and Harlow expanded the discussion when they proved that monkeys bred in full or partial social isolation in bare wire cages failed to develop appropriate social responses (Suomi, Harlow and Kimball, 1971).

To quantify the potential benefits of enrichment, researchers first established experiments with rodents. These experiments exposed rodents to assorted types of enrichment, including access to voluntary exercise, environmental complexity, and frequent variation in the environment. In early experiments, Rosenzweig et al. (1996) proved that differential experience in rodents resulted in neurochemical changes in the brain and that enriched experiences improved learning (Rosenzweig and Bennett, 1996). The researchers also showed positive effects of environmental stimulation on brain function (Rosenzweig, 1979; Renner and Rosenzweig, 1987). Other experiments showed how environmental enrichment could elicit neural plasticity in the forms of gliogenesis, neurogenesis, or altered biochemical parameters such as growth factors and the neurotransmitter acetylcholine (Van Praag, Kempermann and Gage, 2000).

Similar benefits on neural plasticity were observed in experiments conducted with other species, including gerbils, ground squirrels, cats and monkeys (Rosenzweig and Bennett, 1996). Consequently, researchers began to consider utilizing enrichment in fish hatchery environments to determine if it would alter the development of behavior in hatchery-reared fish (Ebbesson and Braithwaite, 2012; Salvanes et al, 2013).

Although the methods used to introduce enrichment vary, studies have shown that in general, enrichment can be very beneficial to the success of an individual upon release from the hatchery environment (Braithwaite and Salvanes, 2005; Brown and Laland, 2001; Heenan et al, 2009). Enrichment allows the opportunity for individuals to develop survival skills such as predation avoidance, foraging, social interaction and mating, and habitat selection (Reading, Miller and Shepherdson, 2013). Furthermore, enrichment may improve physical conditions of target species while improving animal welfare (Reading, Miller and Shepherdson, 2013).

Since studies indicate most post-release mortality occurs immediately or soon after release, a key target of enrichment endeavors is the behavioral flexibility of hatchery-reared fish (DePasquale et al, 2016). The transition from life in the hatchery, where conditions are manipulated to yield many fish, to the wild, where conditions favor the best-adapted fish, often prove fatal. Behavioral flexibility allows the fish to adapt to its new environment while acquiring the skills required for success post-release (Ebbesson and Braithwaite, 2012).

In a study of the benefit of altering landscapes in hatchery environments, juvenile cod were exposed to a variable environment (Braithwaite and Salvanes, 2005). Cod reared in enriched tanks, which included varying and changing environments, developed more flexible behavioral traits compared to those reared in standard hatchery conditions (Braithwaite and Salvanes, 2005). These traits included faster exploration of an area with a stimulus fish, faster recovery from a stressful experience, and earlier consumption of live prey (Braithwaite and Salvanes, 2005). This flexible behavior was even found to result in fish with enhanced social behavior skills that resulted in decreased levels of aggression (Braithwate and Salvanes, 2006).

Enrichment has also been shown to generate fish more capable of social learning. In a study conducted with juvenile cod, fish that were exposed to enrichment were better able to socially learn how to forage on novel prey items by observing tutor fish demonstrating the necessary skills. Both enriched fish and those reared in standard conditions were exposed to tutor fish foraging for prey, but only fish that had been reared in an enriched environment had improved consumption of prey at the conclusion of the study, indicating that experience with enrichment helps fish to acquire behaviors that they visualize around them (Strand et al, 2010). Social learning may also help improve predator avoidance and antipredator behavior in naïve fish (Olla, Davis and Reyer, 1998).

While many experiments show that enrichment can be beneficial to hatchery-reared fish, there are instances in which enrichment fails to alter post-release survival (Archer and Crowl, 2014; Ersbak and Haase, 1983). Why some aspects of enrichment have positive effects, while others have little or no effect remains unknown (Braithwaite and Salvanes, 2005).

Much of the research completed to date has focused training programs that work to develop cognitive aspects of behavior. These aspects may include the ability to learn to feed on prey, detect and understand predation threats, or generally acquire new skills (Braithwaite and Salvanes, 2005; Suboski and Templeton, 1989; D’Anna et al, 2012; Roberts et al, 2014). Enrichment presumably also shapes other aspects of behavior such as general temperament; for example, propensity to explore, activity levels, relative boldness versus timidity. The experiment described in this these was therefore designed to study these aspects in fish reared with or without enrichment.

The effects that enrichment may have on physical fitness, rather than cognitive abilities, of fish are also unknown. The introduction of environmental enrichment into a tank, as opposed to training programs that aim to influence cognitive development, may allow the development of physical benefits to the fish. Environmental variability may provide the opportunity for fish to maneuver and swim around obstacles or dart away and hide from aggressive tank mates. These traits may provide fish with a higher chance of post-release survival. Therefore, the current study was also designed to assess whether enrichment inside a rearing tank influences the physical fitness and agility of the fish.

In this study, hatchery reared trout (11 months of age) were either exposed to environmental enrichment or reared in standard conditions as a control for two months. Enrichment included introduction and rotation of novel objects, such as imitation plants, PVC pipes, and floating plants. Following the enrichment period, the fish were tested in an Open Field Novel Object Test.

Open Field tests are regularly used as a means to measure behavioral responses of animals in a new environment (De Passillé, Rushen, and Martin, 1995; Warren and Callaghan, 1975; Levin and Cerutti, 2009). This new environment provides the test subjects with exposure to unfamiliar stimuli, which can elicit a variety of responses. One of the most prevalent measurements in an Open Field test is that of “freeze,” the portion of time the subject spends in a nonmoving state. The “freeze” state is adaptively significant because a nonmoving animal may be more hidden from a predator, and therefore allows a quantification of the level of emotion and fear an animal may be experiencing (Denenberg, 1969). The Novel Object test was utilized to measure stress response and approach behavior in the fish. The test is commonly used because it relies on the subject’s innate exploratory behavior in an environment with unknown stimuli (Antunes and Biala, 2012).

Behavior changes as a result of enrichment were measured through a combination of the aforementioned tests. Agility and exploration were also investigated to determine if either of these swimming performance measures was altered by exposure to enrichment.

## Chapter 2

## Methods

A total of 81 rainbow trout were divided among four standard hatchery tanks (opaque, green PVC tanks measuring 60x60x95 cm). All tanks were set up at the Pennsylvania State University Rock Springs aquaria facility. The tanks were supplied with spring water in a continuous flow through system without any other objects inside the tanks. All fish were kept on a 12 hour light and 12 hour dark schedule with the lights set to fade in slowly as they turned on and off to simulate sunrise and sunset. The fish were fed to satiation on a daily basis with commercially produced fish pellets.

At 11 months of age, the fish were divided into six groups of either 13 or 14 trout. Three of the groups were transferred to tanks (60x60x95 cm) that contained environmental enrichment while the remaining three groups of fish were housed in the same standard hatchery tanks lacking enrichment (60x60x95 cm). Enriched tanks contained one plastic plant imitation with long fronds (50-60 cm long) that floated vertically in the water column and provided areas for fish to swim through. Enriched tanks also contained a single PVC pipe and one PVC pipe pyramid to provide fish with areas of shelter. Fish could swim freely through these structures and utilize the areas as refuge to avoid interaction with other fish. Finally, three plastic floating plants were present on the water's surface to provide an area of cover. The enrichment items were repositioned weekly in conjunction with tank cleaning. All tanks, both standard and enriched, were cleaned once a week. Additionally, some degree of novelty and environmental variability were provided in the enriched tank through weekly rotations of different novel objects. Novel objects included floating plants of various size and color and floating Ping-Pong balls. These items were selected due to their novelty to the fish and because of the ease with which they could be cleaned and sterilized for use at a later date. The fish were housed in their respective tanks for two months before their behavior was screened in a Novel Object test. All fish husbandry, handling and behavioral testing was approved by the Pennsylvania State University IACUC committee under protocol number 45265.

### ***Novel object assessment***

In order to test individual fish, single trout were gently captured via netting and were then transferred from their home tank into an Open Field trial tank measuring 45x45 cm with a 10 cm water depth. The tank held four objects, one in each corner of the tank, and each item was novel to the fish (Figure 1). The novel objects were: a stone, a soda can, a tube and a pot. The four objects created a shadowed edge effect, and lines were drawn between novel objects to distinguish the center and outer edge of the tank. This created two zones that the fish were free to move between, but also allowed us to determine where the fish were spending their time.

At the start of each trial, a fish was placed in a start cylinder (17 cm diameter) in the center of the tank and allowed to settle. If the fish did not escape from the cylinder on its own within 10 seconds, the experimenter gently lifted the cylinder, allowing the fish to move throughout the tank. An overhead camera recorded the fish movements for three minutes. After three minutes, the fish was removed from the tank using a small dip-net. The fish were returned to a new holding tank and kept separate from the untested fish to ensure no fish was retested.

The videos were then viewed and analyses of various parameters associated with movement and exploration were performed as the fish swam around the tank and interacted with the different novel objects. Latencies to first cross the boundary marking the edge area and to reenter the middle of the tank were recorded in addition to the total crossings between the middle and the edge of the tank and the proportion of time spent in the middle and edge of the tank. The time spent active, still, and frozen (i.e. stationary) were also recorded. Additionally, the numbers of contacts with novel objects and with the tank walls were logged. These measures were used to quantify activity, explorative nature and levels of anxiety.

Animals naturally express a range of temperament traits that are sometimes described as personality (Réale et al 2007). Individuals that are nervous or timid tend to express more thigmotactic behaviors, staying close to the walls of an arena, and take longer to move into the open central area of the arena. These individuals also typically freeze more often, move less and take longer to move towards and explore novel objects in their environment. Bolder, less anxious and more exploratory individuals tend to exhibit opposite behavior (Burns 2008).

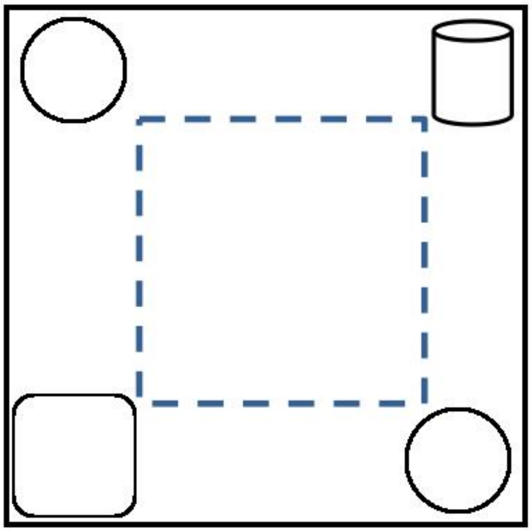

**Figure 1.** Novel object test tank with four novel objects in corners and drawn lines to distinguish outer edge from middle of tank.

### *Statistical analysis*

The data were checked for equality of variance and log or inverse square-root transformations were used where necessary to ensure that the data conformed to the assumptions of analysis of variance (ANOVA) tests. The data were analyzed using one-way ANOVAs. An alpha level of 0.05 is used throughout.

# Chapter 3

## Results

### *Activity Levels*

Fish reared in enriched tanks exhibited significantly higher activity levels than their non-enriched counterparts. Enriched fish spent more time moving ($F_{1,78}$=19.667, p<0.001; Figure 2) and less time stationary ($F_{1,78}$=4.671, p=0.034; Figure 3) than non-enriched fish.

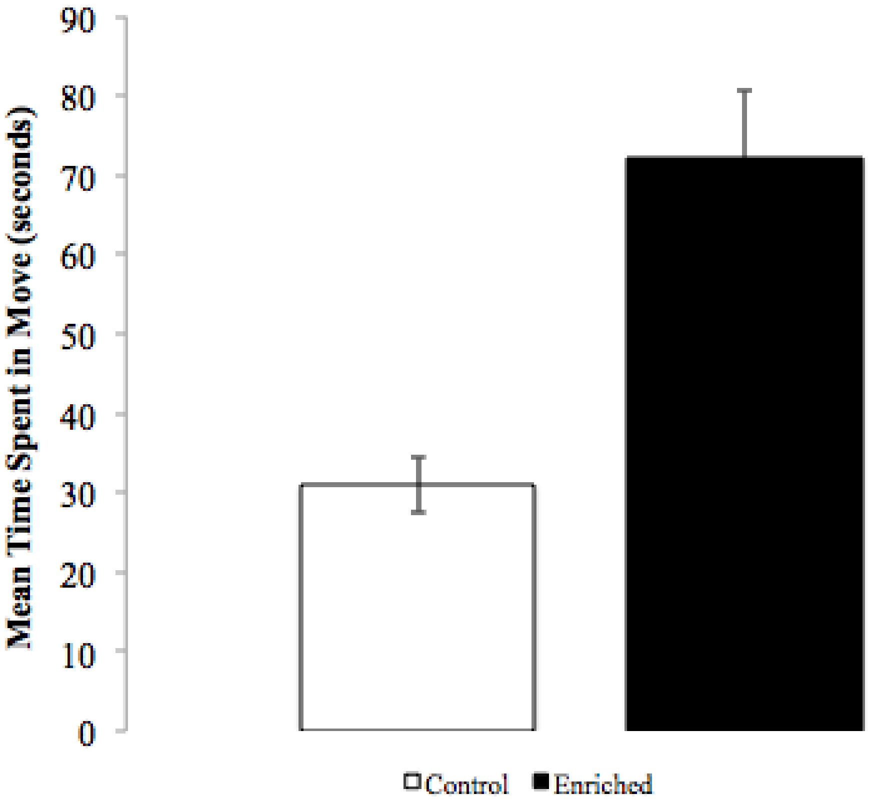

**Figure 2.** The mean time trout spent actively moving during the three-minute novel object test. Trout reared in environmentally enriched hatchery conditions spent significantly more time actively moving throughout the tank (72.200 ± 8.570 seconds) than their non-enriched counterparts (31.073 ± 3.563 seconds).

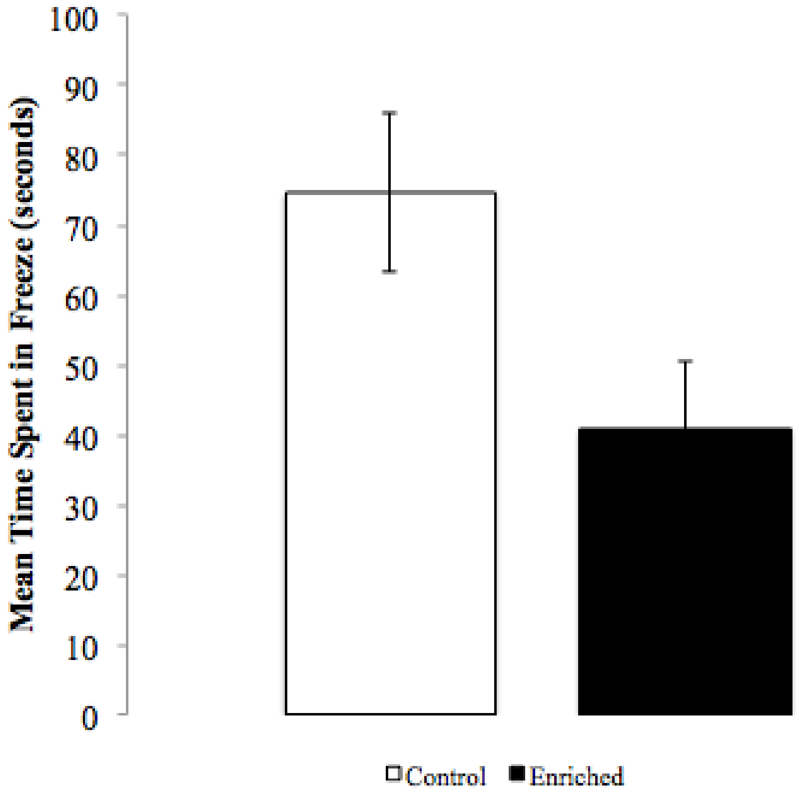

**Figure 3.** The mean time trout spent frozen (characterized as any period of inactivity or immobility) during the three-minute novel object test. Fish reared in standard hatchery conditions spent significantly more time in a frozen state (74.683 ± 11.362 seconds) than those reared in environmentally enriched habitats (40.925 ± 9.711 seconds).

### *Exploration*

Enriched fish interacted significantly more with the four novel objects (a stone, a soda can, a tube, and a pot) than non-enriched fish ($F_{1,72}$=5.973, p=0.017; Figure 4), indicating that fish reared in enriched environments were more explorative than non-enriched counterparts.

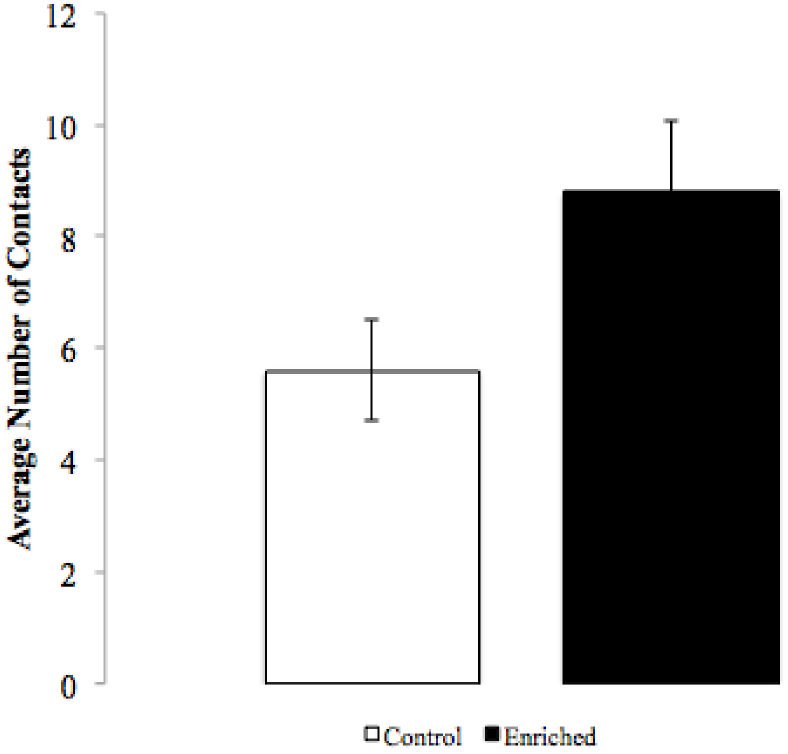

**Figure 4.** The mean number of contacts fish made with novel objects throughout the three-minute novel object test. Any period of time spent within half of the body length of the fish to any of the four novel objects was characterized as contact. Trout reared in environmentally enriched habitats interacted significantly more with novel objects (8.800 ± 1.266 contacts) than their non-enriched counterparts (5.610 ± 0.898 contacts).

*Anxiety*

Fish reared in an enriched environment appeared to exhibit less anxiety-related behavior, spending more time moving between the middle and outer edges of the tank ($F_{1,73}$=14.182, $p<0.001$; Figure 5).

However, measures of time spent in the middle of the tank compared to the outer edges ($F_{1,79}$=0.161, p=0.689), time to reach the outer edge of the tank from the middle ($F_{1,78}$=0.295, p=0.589), and time to re-enter the middle of the tank from the outer edge ($F_{1,73}$=0, p=0.985) showed no differences between the control and enriched fish.

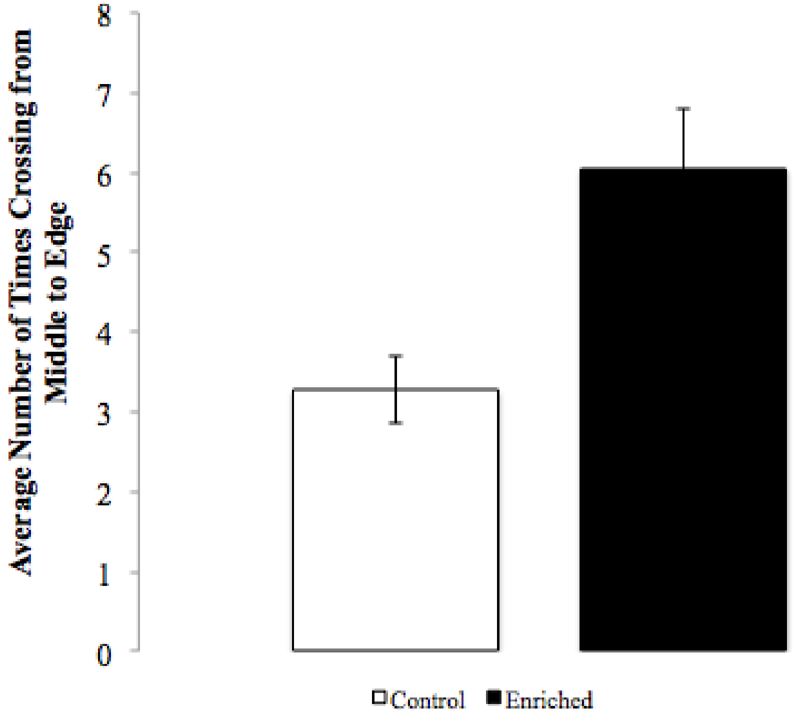

**Figure 5.** The mean number of times fish crossed from the middle to the outer edges of the tank during the three-minute novel object test. The shading in the outer edges of the tank provided a shelter for fish, and a lack of movement between the inner and outer regions of the tank was therefore indicative of anxiety-induced behavior. Environmentally enriched trout crossed between shaded and non-shaded regions of the tank significantly more (6.050 ± 0.748 crossings) than their non-enriched counterparts (3.268 ± 0.420 crossings).

### *Agility*

Agility was quantified as the number of collisions made with the walls of the tank during the three-minute novel object test. There was not a significant difference in the number of wall collisions made by fish reared in enriched environments compared to their non-enriched counterparts ($F_{1.78}$ = 2.635, p = 0.150).

# Chapter 4

## Discussion

*Activity*

Activity levels were measured by the periods of time spent moving versus frozen or stationary throughout the duration of the novel object test. Our hypothesis predicted that fish reared in environmentally enriched habitats would have higher levels of activity than their non-enriched counterparts, due in part to the utilization of a “freeze” state by non-enriched fish as an anti-predator survival strategy (Denenberg, 1969). Our hypothesis was supported by the results; environmentally enriched fish spent more time moving throughout the tank, and less time in a frozen or stationary position, than their non-enriched counterparts. Being active and mobile is important in a real-world, natural context because the fish need to balance hiding from predators in shelter with being out in the open foraging. While it is important to seek shelter at times of real threat, spending too much time frozen or hidden will decrease the amount of time the fish have to forage (Lima & Bednekov 1999).

*Exploratory Behavior*

Exploratory behavior was quantified by the number of interactions between individual fish and the four novel objects, a stone, soda can, tube and pot, throughout the Novel Object test. We hypothesized that fish reared in environmentally enriched conditions would interact more frequently with novel objects than their non-enriched counterparts. The results of our experiment supported this hypothesis, because fish reared in environmentally enriched habitats interacted more frequently with novel objects than their non-enriched counterparts.

Although these results did support our hypothesis, it should be noted that the results from previous experiments with other species indicate the affect of enrichment on exploratory behavior remains unclear. While some studies support the claim that enrichment increases exploration of novel environments (Simpson and Kelly, 2011), other studies find the opposite to be true. In one study of rats, for example, enrichment-reared subjects actually exhibited less explorative behavior than their counterparts reared in isolation (Varty et al, 2000). In another study, exploration was linked to periods of deprivation in chickens (Nicol and Guilford, 1991).

Therefore, while the results of our experiment provide support for the benefits of enrichment on exploratory behavior in hatchery-reared fish, more research is needed to understand how enrichment alters the behavioral phenotype of the fish. Differences in species, aspects of the experimental design or the stage of development of subjects (i.e. juveniles versus adults) may have contributed to reports that indicate no benefit to exploratory behavior following periods of environmental enrichment. To be sure of the role of enrichment on exploratory behavior in hatchery-reared fish, further work is necessary.

### *Anxiety*

Anxiety was quantified by observing the interactions trout made with the middle and edges of the open field tank throughout the novel object test. Edges were created in the tank through the walls of the arena and the placement of the four novel objects in the tank corners (Figure 1). Measurements of anxiety included the number of times a fish moved between the edge and middle of the tank and the total time spent in each area of the tank.

These kinds of parameter were chosen because they have previously been widely applied in other studies investigating anxiety. For example, such variables are commonly used in assessments of anxiety and other fear-based behaviors in both rodent and other fish models (Maximino et al, 2010). Edge effects created by novel object placement introduce the principles of scototaxis and thigmotaxis, which have both been utilized as measures of fear-avoidance behaviors.

Scototaxis, or the movement of an animal with a preference for either dark or light conditions, has been widely utilized as an indicator of anxiety behavior, with animals that express higher levels of anxiety and fear-avoidance behaviors spending more time in the dark portion of the environment than the light (Maximino et al, 2010). “Shaded” areas existed in our experiment between novel objects. Thigmotaxis, or the movement of an animal towards or away from a mechanical stimulus, has also been utilized as a measure of anxiety in rodents and other species of fish. Animals that express higher than normal anxiety or fear-avoidance behaviors spend more time in contact with walls and other objects within an experimental model (Maximino et al, 2010). Therefore, increased time spent within the edges of the open field tank could be indicative of anxiety and fear-avoidance behavior due to the thigmotactic effects of proximate tank walls and novel objects.

Additional measures of anxiety used in this experiment were the time to reach the outer edge of the tank from the centrally located starting cylinder and the time to initially re-enter the center of the tank from the edge. These variables were selected to quantify the readiness to exit the open portion of the tank and willingness to re-enter after experiencing the scototactic and thigmotactic effects of the tank edge.

As we hypothesized that environmentally-enriched trout would exhibit less anxiety and fear-avoidance behavior than their non-enriched counterparts, we suggested that enriched trout would spend comparably more time in the middle of the tank, have more crossings between the middle and edges of the tank, take more time to enter the edge from the centrally-located starting cylinder and re-enter the middle of the tank quicker than non-enriched counterparts. Of these measurements, however, only the number of times a fish moved from the edge to the center of the tank was significantly different between enriched and non-enriched fish. While these results may reflect a genuine lack of difference between the control and enriched fish, there are several other confounding factors that may have influenced the other measures. For example, eager exploration of novel objects, which would be expected in environmentally enriched fish, would likely increase the amount of time fish spend in the edges of the tank. Alternatively, an environmentally enriched fish may have higher activity and therefore may quickly enter the edge of the tank irrespective of a scototactic or thigmotactic influence. These alternative explanations could be further investigated by including an open field assay among the tests. Here the fish are placed into an empty arena with no novel objects, and the proportion of time spent at the edges or in the center, swimming or active can be measured (Archard & Braithwaite 2011).

As environmentally enriched fish passed through the edges and center of the tank significantly more than their non-enriched counterparts, this experiment does suggest that elements of anxiety were consistent with our hypothesis that anxiety behavior would be lower in environmentally enriched fish. An abundance of similar results from experiments with other species indicates that this result is a real reflection of decreased anxiety in enriched fish, supporting the anti-anxiety benefits of environmental enrichment. However, further research is needed to determine why only some of the anxiety behavior measures showed consistent differences between the control and enriched fish.

### *Agility*

A goal of this study was to address the potential benefits of environmental enrichment on physical capabilities of captive-reared fish, an aspect of hatchery-implemented enrichment that has been minimally researched. Improvements to these capabilities could be important because of their potential enhancement of post-release survival. Agility and more adept swimming may allow hatchery-reared fish to avoid predators or dangers or enable them to be more adept at foraging in a novel and wild environment.

In this study, agility was measured as the number of collisions fish made with the wall of the tank throughout the three-minute novel object test. We wanted to determine whether previous experience of swimming in and out of enrichment objects would help the enriched fish to avoid running into the walls of the test tank. However, the results showed that there was no difference in agility between enriched and non-enriched fish.

While these results do not support our hypothesis, it is possible that the measurement of interactions with the wall was not an appropriate quantification for agility. As in the parameters used to quantify anxiety behavior, potential confounding variables exist that could have affected the number of collisions made with the walls of the tank. Both a desire to remain close to walls, as would be expected for non-enriched fish, or a desire to interact with novel objects placed near tank walls, a predicted behavior of enriched fish, could have altered the number of collisions made with a wall irrespective of a subject's agility. The inadequacy of the selected parameters and assay to measure agility may have been a leading factor in the lack of correlation between environmental enrichment and increased agility.

A proven connection between environmental enrichment and agility was recently made in a sister experiment conducted just prior to the one described in this thesis. In that experiment, individual trout were made to swim the length of a long tank that contained a series of obstacles resulting in fish making a sinusoidal track down the tank in order to reach a reward at the far end. In this study, there was a significant difference in the number of wall collisions made by enriched and non-enriched fish. Enriched fish made fewer wall contacts and appeared to have an overall superior agility compared to control fish (Ahlbeck et al. in prep.).

Given the importance of swimming behavior and agility for everyday existence in the wild, it is surprising that so little work has been done to address potential ways to improve agility in hatchery-reared fish. High levels of agility are beneficial for many aspects of life, including rapidly locating and reaching areas of shelter, avoiding predation, and speed and accuracy of acquiring prey during foraging. The adoption of enrichment procedures in fish hatcheries that may increase agility and swimming strength may be critical for improving post-release survival of captive-reared fish. Therefore, more research should be conducted to determine the benefits environmental enrichment may have on the agility of captive-reared fish.

### *Summary*

Implementation of environmental enrichment in fish hatcheries has been proven beneficial in many instances to the post-release survival of fish. In this experiment, we investigated the effects of environmental enrichment on activity, exploration, anxiety and agility in rainbow trout (*Oncorhynchus mykiss*). Environmentally enriched fish exhibited higher levels of activity and exploration, as measured by the time spent moving and the frequency of interactions with novel objects, respectively. Enriched fish also had lower levels of anxiety, as measured by the amount of time spent within the middle and edges of the tank. There was no observed difference in agility between enriched and non-enriched fish, although this may have been reflective of a poorly chosen assay.

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### Activities

The Braithwaite Lab Undergraduate Research Member, 2014-2016
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Penn State Homecoming University Relations Director, 2013-2014
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