THE EFFECTS OF HYPOXIA ON THE BEHAVIOR AND DEVLOPMENT OF LARVAL ESTUARINE FISH

By:

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DEDICATION

To my amazing parents, Paula and Keith, and my brother and sister, Luke, and Lana, for always being there for me no matter what life threw at me and for encouraging me to never give up.

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ABSTRACT

Hypoxia has been proven to severely impair fish survivorship, cause alterations to certain behaviors, and induce embryonic deformities. Despite this, the effects of hypoxia on many areas of larval behavior, such as anxiety-related behaviors and light/dark preference, are not well known. Therefore, I studied its impacts on larval spotted seatrout (*Cynoscion nebulosus*), red drum (*Sciaenops ocellatus*), and striped bass (*Morone saxatilis*) behavior by exposing them to either normoxic or hypoxic conditions. I received four separate shipments of both sciaenid species and two shipments of striped bass. Each shipment corresponded to a separate trial that included three replicates each of normoxia and hypoxia. All fish were acclimated and maintained in six 19-liter aquariums for 24 hours. After acclimation, I utilized ten fish from each aquarium in scototaxis (light/dark preference) testing and ten from each aquarium in novel object testing for a sample size of 240 (120 for striped bass) fish for each behavioral test across all four trials. Scototaxis and novel object protocols were used to examine behavioral changes. Both behavioral tests lasted 180 seconds since it was found larvae "froze" in movement past this time. Once behavioral tests concluded, all tested fish were euthanized and observed under a microscope. ImageJ software was used to check for possible eye deformities that may have led to alterations in visual acuity that could affect behavior. Standard lengths were also taken to determine hypoxia caused impairments to growth rate. In the scototaxis test, seatrout preference was for the light side in normoxia ($p = 0.00104$) and no preference between light and dark under hypoxic

conditions (p = 0.22628). Red drum showed no preferences to light or dark in either normoxia (p = 0.44139) or hypoxia (p = 0.17702). Striped bass showed a strong light preference ($p <$ 0.00001) that did not change after hypoxia exposure (p = 0.00022). In the novel object test, hypoxia-exposed seatrout spent significantly more time in the inner (p = 0.00318) and middle rings (p = 0.00031) compared to normoxia-exposed individuals. Red drum (p = 0.11876) and striped bass (p = 0.92828) showed no significant change in behavior in hypoxia compared to normoxia. None of the three species showed any changes in eye development in terms of eye width and area. There was no significant difference between the left and right eyes either. In terms of standard lengths, the only species that showed a significant difference between control and treatment was red drum (p = 0.02167); hypoxia-exposed individuals were statistically smaller than normoxia exposed ones. Based on my statistics analysis, I can conclude that spotted seatrout are behaviorally influenced by hypoxia exposure whereas red drum are developmentally affected. Striped bass showed impairments to neither behavior or development, but many confounding factors in the testing of this species calls into question the validity of these results. Spotted seatrout behavioral changes likely did not stem from eye deformities, but rather changes to mechanorecptor sensory development. Future testing should look into hypoxia-caused alterations of mechanoreceptor systems within coastal fish species. Further behavioral testing should utilize both camera tracking software along with physical observation.

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CHAPTER I

INTRODUCTION

1.1. Background

Dissolved oxygen concentrations in an estuarine ecosystem dictate which species can live there. Long periods of normoxia, or normal dissolved oxygen conditions of approximately 5-7 mg/L O_2, is what is needed to support diverse and healthy estuarine ecosystems (Landry *et al.* 2007). Due to natural cycles of nutrient and freshwater input, respiration, and temperature, hypoxic conditions (< 3 mg/L O_2) or anoxic conditions (< 0.2 mg/L O_2) occasionally occur (Landry *et* al. 2007, Moore *et al.* 2008, Ludsin *et al.* 2009). These low dissolved oxygen periods are generally short lived and not a severe threat to estuarine organisms. However, in recent years, human alteration to natural nutrient cycles, point and nonpoint source pollution, and climate change have exacerbated these hypoxic periods in both length and intensity (Moore *et al.* 2008, Ludsin *et al.* 2009,).

Hypoxia can have profound negative effects on estuarine fish populations (Wannamaker and Rice 2000). In very severe cases, high temperatures combined with hypoxic or anoxic conditions, have been responsible for countless fish kills in estuaries and coastal waters throughout the United States (Wannamaker and Rice 2000, Cooper *et al.* 2002, Rabalais *et al.* 2002,). At less lethal levels of hypoxia, fish are subjected to an array of metabolic and physiological impairments. (Kramer 1987). According to Pan *et al*. (2016), once dissolved oxygen concentrations drop below the point where aerobic metabolic function becomes impossible, which varies by fish species, they must rely on anaerobic respiration. Due to the reduced ATP yield from this form of respiration, fish are forced to reduce non-vital functions,

such as unnecessary movement, to conserve energy for vital metabolic processes (Pan *et al.* 2016). This reduced ability to function properly make fish in hypoxic conditions less able to avoid predation from hypoxia resistant predators such as gelatinous zooplankton, marine mammals, and seabirds (Kramer 1987, Breitburg *et al.* 1994, Shoji *et al.* 2005). Deformities in larval and embryonic individuals exposed to hypoxia have been reported in many species (Levin *et al*. 2009, Elshout *et al.* 2013, Bardon-Albaret and Saillant 2016, Borgström *et al*. 2017).

Estuarine and coastal hypoxia episodes throughout the world continue to increase due to a combination of direct anthropogenic alterations and climate change (Peperzak 2003, Meier 2006, Bindoff *et al.* 2007, IPCC 2007, Vaquer-Sunyer and Duarte 2008, Levin *et al.* 2009, O'Neil *et al.* 2012, Rabalais *et al.* 2014). Despite this, the acute effects and tolerance of hypoxia on larval marine and estuarine fish have not been well documented (Bardon-Albaret and Saillant 2016). Even less known are the effects of hypoxia on larval estuarine fish behaviors that may allow them to perceive potential threats, perform risk assessment, and retreat if necessary (Breitburg *et al.* 1994, Ohl *et al.* 2008). Alteration of behavior through hypoxia could result in reduced survival of commercially and ecologically important estuarine fish. Reduced recruitment could be potentially devastating to both Mid-Atlantic and Gulf coast estuarine ecosystems as well as fishermen (recreational and commercial) who depend on these species. Therefore, it is critical to understand if larval fish experience abnormal behavior in hypoxia. Further analysis of larval fish morphometrics following hypoxia exposure can give better insight into whether specific deformities are responsible for causing estuarine fish larvae to become more vulnerable to predation or other stimuli.

1.2. Project Goals and Objectives

The first goal of this study was to determine how 1-day post hatch (DPH) larval estuarine fish react to a stimulus under normoxic (control) and hypoxic (treatment) conditions. I tested this using two different behavioral tests: a scototaxis protocol and a novel object test. In both tests, I first needed to determine a threshold for what is considered "normal behavior," which would be behavior exhibited for control fish. The next step would be to test treatment fish by exposing them to hypoxia and determine if their behavioral preferences were different than control fish

The second goal of this study was to determine how hypoxia affected the development of the larvae over the course of the experiment. I specifically wanted to see how hypoxia affected eye growth and development in this timespan. Previous studies by Ingalls and Philbrook (1958) found hypoxia could cause eye deformities in larval fish exposed to hypoxia as embryos. Knowing that much of eye development occurred in the embryonic period, I also wanted to determine if any hypoxia related impairments would occur after the larvae hatched.

Considering teleost ocular tissue requires one of the highest amounts of oxygen to function properly, hypoxia exposure during a period where these organs are in a state of rapid growth could lead to potential developmental issues due to a lack of oxygen required for proper development (Buckley 1984, Osse and Van Den Boogaart 1995, Waser and Heisler 2005). With larval teleost fish exhibit a very fast growth rate upon hatching, I felt it was pertinent to test this knowing that hypoxia causes growth and developmental impairments (Buckley 1984, Osse and Van Den Boogaart 1995, Levin *et al*. 2009, Elshout *et al.* 2013, Bardon-Albaret and Saillant 2016, Borgström *et al*. 2017). If any developmental changes did occur, I could see if a correlation potentially existed with any behavioral alterations seen.

CHAPTER II

LITERATURE REVIEW

2.1. Estuarine Hypoxia

Estuaries are very important and productive ecosystems that boast a high array and abundance of organisms (Beck *et al.* 2001, Vasconcelos 2010). High productivity makes estuaries prime nursery habitat for many fish species (Nixon *et al.* 1986, Boehlert and Munday 1988, Beck *et al.* 2001, Vasconcelos 2007, Vasconcelos 2010). In terms of fish, a nursery habitat refers to a sheltered coastal area where larval individuals metamorphose and grow before returning to the ocean as adults (Beck *et al.* 2001). Estuaries of the Atlantic and Gulf coasts provide this ecological function due to their shallow depth and submerged aquatic vegetation, such as eelgrass (*Zostera marina*) and turtle grass (*Thalassia testudinum*) (Rooker *et al.* 1998). This structural complexity provided by sub aquatic vegetation encourages larval fish recruitment and settling as it gives them refuge from larger predators (Rooker *et al.* 1998). Many commercially, recreationally, and ecologically valuable species, such as weakfish (*Cynoscion regalis*), spotted seatrout (*Cynoscion nebulosus*), black sea bass (*Centropristis striata*), red drum (*Sciaenops ocellatus*), Atlantic menhaden (*Brevoorita tyrannus*), and winter flounder (*Pseudopleuronectes americanus*) utilize estuaries as nursery habitats (Lankford and Targett 1994, Roman *et al.* 2000, Wannamaker and Rice 2000, Ludsin *et al.* 2009).

Despite their importance, estuaries are one of the most threatened ecosystems in the world (Blaber *et al.* 2000, Vasconcelos *et al.* 2007). This threat is mainly from human encroachment and development on estuarine habitat and adjacent areas due to their aesthetic appeal driving tourism (Wannamaker and Rice 2000, Ludsin *et al.* 2009, Jiang *et al.* 2014). Developed residential land on the edge of estuaries has caused higher inputs of nutrients such as

nitrogen and phosphorus due to nonpoint source pollution in the form of runoff from lawn and garden fertilizers and domestic animal waste leading to eutrophication (Figure 2.1) (Cooper *et al.* 2002, Ludsin *et al.* 2009, Jiang *et al.* 2014).

Excess nutrients cause algal blooms that quickly die off after the surge in nutrients is fully utilized (Cooper *et al.* 2002, Ludsin *et al.* 2009, Jiang *et al.* 2014). Populations of herbivorous zooplankton, such as copepods, also spike with this surplus of food and produce copious amounts of waste (Downing *et al.* 1999, Cooper *et al.* 2002, Ludsin *et al*. 2009, Jiang *et al.* 2014) Eventually, zooplankton populations either exhaust this food supply and decrease or crash with declining algae growth (Downing *et al.* 1999, Cooper *et al.* 2002, Ludsin *et al.* 2009, Jiang *et al.* 2014). The result is a buildup of organic matter that heterotrophic bacteria decompose. Respiration and remineralization by these bacteria increase the biological oxygen demand (BOD) of the system. The result is often hypoxic (< 3 mg/L O_2) or anoxic (< 0.2 mg/L $O_{2)}$ waters that produce harmful, if not lethal, effects to many estuarine organisms (Moore *et al.* 2008, Ludsin *et al.* 2009).

Today, as many as 35-60% of estuaries in the United States experience frequent summer hypoxia events (Bricker *et al.* 1999, Scavia *et al.* 2002, Vaquer-Sunyer and Duarte 2008). Hypoxic marine dead zones spurred by similar stimuli, such as the Northern Gulf of Mexico dead zone, are also a common occurrence (Rabalais *et al.* 2002, Rabalais *et al.* 2014, Thrash *et al.* 2017). While the severity and size of these events are expected to increase due to further increases of coastal development, climate change due to excess greenhouse gas emissions will also have a major role in increasing hypoxia events (Peperzak 2003, Meier 2006, Bindoff *et al.* 2007, IPCC 2007, Vaquer-Sunyer and Duarte 2008, Levin *et al.* 2009, O'Neil *et al.* 2012, Thrash *et al*. 2017). Sea surface temperature increases of 0.2°C per decade in the past 40 years are

contributing to increases in coastal hypoxia by decreasing available dissolved oxygen in the water column, establishing a longer lasting thermocline leading to longer periods of vertical stratification, and providing better conditions for eutrophication causing algal blooms (Hansen *et al.* 2006, Thomas *et al.* 2007). Predicted sea level rise of 20-60 cm by the end of the century will further influence hypoxia by increasing salinity and affecting vertical stratification of the water column via a longer lasting halocline (IPCC 2007, Thomas *et al.* 2007, Meier *et al.* 2016). As with a thermocline, a long-lasting halocline would reduce circulation and lead to lower parts of the water column and sediment becoming hypoxic or anoxic (Peperzak 2003, Meier 2006, Bindoff *et al.* 2007, IPCC 2007, Vaquer-Sunyer and Duarte 2008, Levin *et al.* 2009, O'Neil *et al.* 2012, Thrash *et al*. 2017).

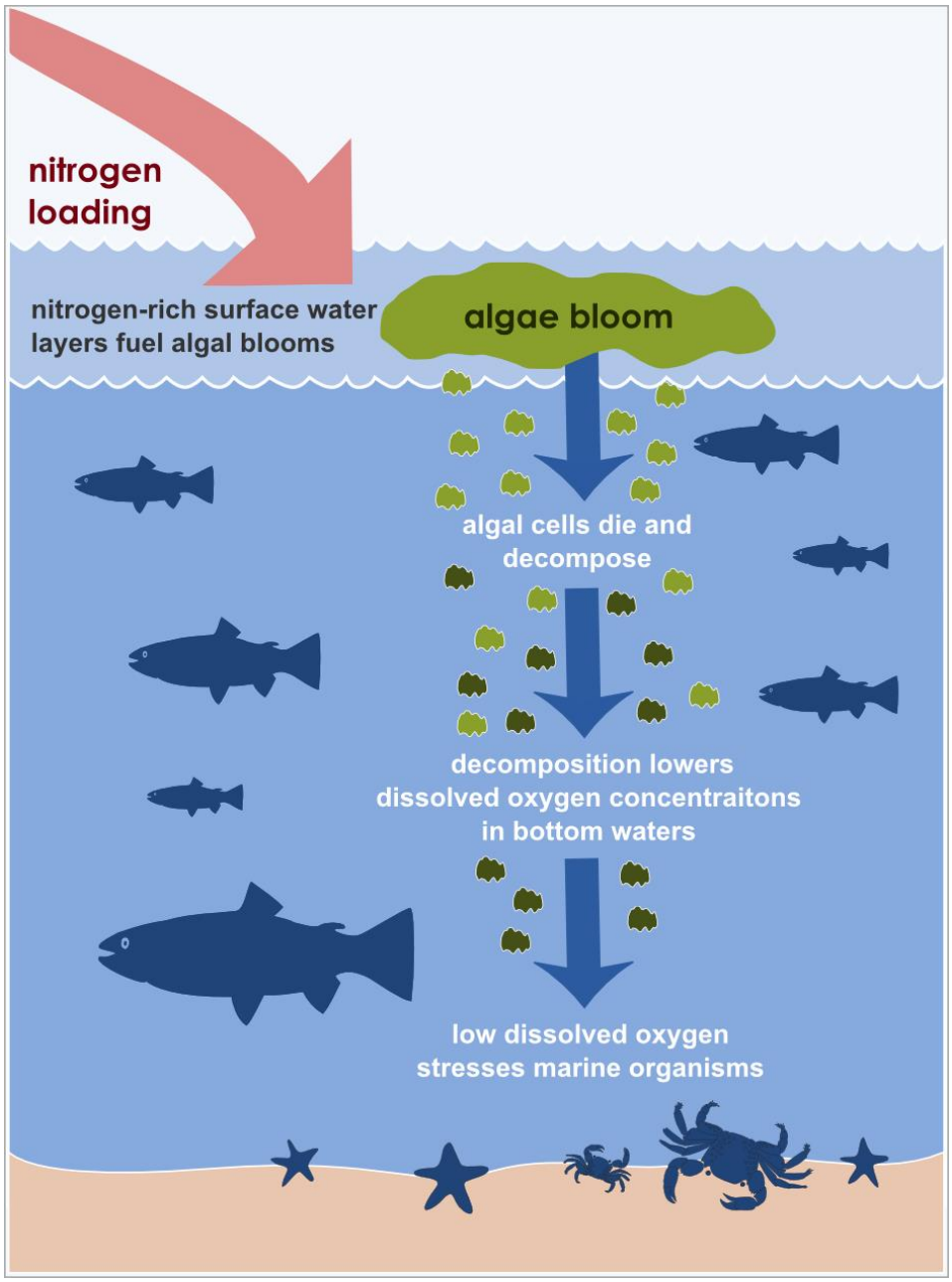


Figure 2.1. The process of eutrophication as illustrated by the State of Washington Department of Ecology (2017). Nutrients from both natural and anthropogenic sources flush into estuaries where algae quickly make use of them. Zooplankton also spike in numbers with this surplus food. When the nutrient load is exhausted, algae die off in large quantities along with the zooplankton. Aerobic bacterial breakdown of this dead organic matter further increases the already heavy biological oxygen demand, which leads to hypoxia.

Similar hypoxia events also occur in freshwater habitats. The process of eutrophication is also present in these ecosystems due to anthropogenic introduction of excess nutrients in the form of runoff and point source pollution, especially as waterfront development continues to increase in these areas (Smith *et al.* 1999, Jenny *et al.* 2015). Increasing global surface temperatures are expected to raise the temperatures of freshwater bodies while also promoting thermal stratification (Jenny *et al.* 2015). As with estuarine and marine hypoxia, freshwater hypoxia events have also increased dramatically over the past few decades (Smith *et al.* 1999, Jenny *et al.* 2015). Jenny *et al.* (2015) determined that freshwater hypoxia has been anthropogenically enhanced for nearly 70 years prior to that in estuarine and marine systems. This was done by using the presence and amount of laminated sediment on the bottom of 365 freshwater sites throughout the globe, which are used as an indicator of mass die outs associated with long increments of hypoxia (Jenny *et al.* 2015).

As in estuaries, hypoxia is detrimental to freshwater biota and can cause mass die offs of finfish and invertebrates as well as alteration in behavior either to cope with or escape hypoxia (Urbina *et al.* 2011, Jenny *et al.* 2015). Species affected may include those with adult forms that live in higher salinity areas. These include anadromous species such as striped bass (*Morone saxatilis*), sturgeon (*Acipenser* spp.), and river herring (*Alosa* spp.) (Burdick and Hightower 2006). These species migrate from the ocean or estuaries into freshwater rivers to spawn (Burdick and Hightower 2006). Larvae develop in freshwater and migrate downstream into higher salinity areas as they mature (Burdick and Hightower 2006). Most of these species use estuaries as nursery habitat in their juvenile stage (Burdick and Hightower 2006). If survival of larvae is compromised, it could greatly impact the adult populations and their trophic relationships.

2.2. Physical Impairments to Fish in Hypoxia

Hypoxia can have profound negative effects on estuarine fish populations (Wannamaker and Rice 2000). In very severe cases, high temperatures combined with hypoxic or anoxic conditions, have been responsible for countless fish kills in estuaries throughout the United States (Wannamaker and Rice 2000, Cooper *et al.* 2002, Rabalais *et al.* 2002). Many fish species that can sense hypoxia will attempt to avoid it if possible. For example, studies on adult summer flounder (*Paralichthys dentatus*) found they increase their swimming speed by as much as 248% when faced with hypoxia to escape to more oxygen rich areas (Brady and Targett 2010, Hanke and Smith 2011). Regardless, many species (or life stages) do not have the means to rapidly escape hypoxic zones and must cope with these conditions (Hanke and Smith 2011).

At less lethal levels of hypoxia, fish are subjected to an array of metabolic and physiological impairments that may reduce survival, but this is dependent on the species (Nilsson and Ostlund-Nilsson 2008, Pan *et al.* 2016). Depending on the species, larval fish may represent a life stage where they may be either more resistant or vulnerable to hypoxia (Levin *et al.* 2009, Hanke and Smith 2011, Elshout *et al.* 2013, Nelson and Lipkey 2015, Bardon-Albaret and Saillant 2016, Pan *et al.* 2016). For example, some species may be more vulnerable to hypoxia as larvae due to a higher dependence on cutaneous respiration and restricted gas exchange (Levin *et al.* 2009, Elshout *et al.* 2013, Bardon-Albaret and Saillant 2016); others, such as red drum, have been found to be more tolerant of hypoxia as larvae than adults due to physiological mechanisms that allow their metabolism to function aerobically at lower dissolved oxygen concentrations (Nilsson and Ostlund-Nilsson 2008, Pan *et al.* 2016).

Early studies performed on larval zebrafish (*Danio rerio*) found that individuals exposed to hypoxia in an embryonic stage suffered from microphthalmia or anophthalmia, curved spines, reduced or absent pectoral fins, and other malformations that inhibited swimming and sensory function (Figure 2.2) (Ingalls and Philbrook 1958). Hassell *et al.* (2008) found that black bream (*Acanthopagrus butcheri*) embryos exposed to hypoxia exhibited reduced hatch rates, deformities, and smaller size. Studies done on larval red snapper (*Lutjanus campechanus*) by Bardon-Albaret and Saillant (2016) found reduced egg viability and larval survival capabilities in hypoxia levels of 3 mg/L O_2. The physical deformities acquired by larvae because of hypoxia exposure can greatly reduce their survival. Eye defects in microphthalmia can cause heavily reduced vision or blindness in one or both eyes while anophthalmia always results in blindness (Ragge *et al.* 2007). Reduced fin development and curvature of the spine can greatly reduce swimming efficiency.

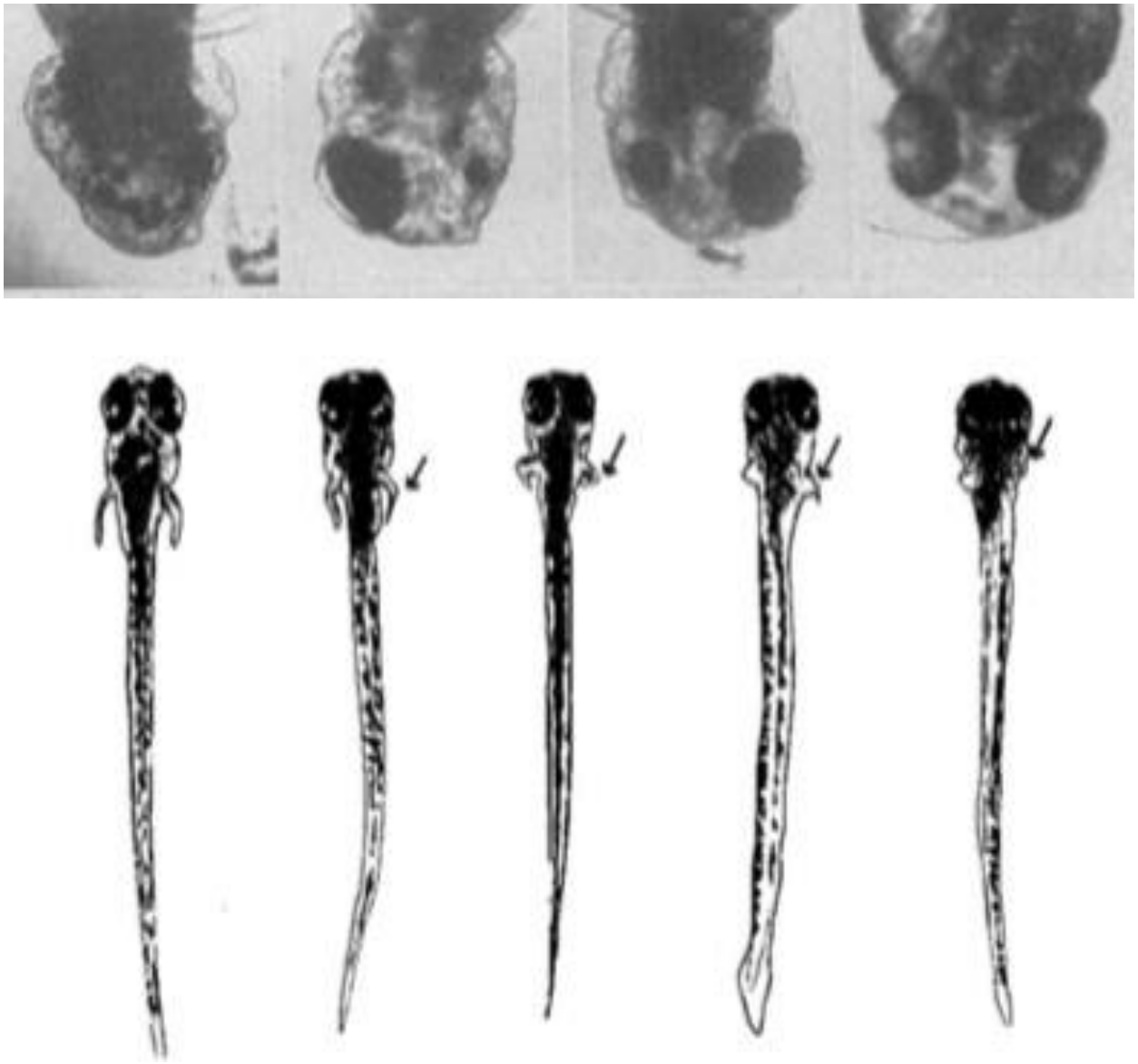


Figure 2.2. Photos by Ingalls and Philbrook (1958) show different levels of deformity in zebrafish larvae exposed to hypoxia as embryos. In the top set of images, the furthest right photo depicts a larva with normal eye development, the two middle depict microphthalmia in the left and right eyes, and the farthest left depicts anophthalmia. The bottom set of photos show reduced levels of pectoral fin development in zebrafish exposed to hypoxia**.**

2.3. Behavioral Impairments to Fish in Hypoxia

Developmental impairments caused by hypoxic conditions can alter how larvae respond to certain environmental stimuli (Pihl *et al.* 1991, Wannamaker and Rice 2000, Vaquer-Sunyer and Duarte 2008). In lower dissolved oxygen situations, fish behavior is prioritized with escaping hypoxic zones or conserving oxygen (Kramer 1987, Brady and Targett 2010, Hanke and Smith 2011). Due to this, these fish may be less likely to avoid predation (Kramer 1987). Some species may resort to a vertical migration in the water column to inhale atmospheric air at the surface, which also makes them more vulnerable to predation by gelatinous zooplankton or seabirds (Purcell 1985, Kramer 1987). Complex antipredatory maneuvers, such as synchronized schooling, may become less efficient or disrupted in hypoxic conditions (Domenici *et al.* 2007). Studies by Breitburg *et al.* (1994) showed that naked goby larvae (*Gobiosoma bosc*) were more vulnerable to predation by hypoxia-resistant predators such as Atlantic sea nettles (*Chrysaora quinquecirrha*) compared to other fish predators. Similar studies by Shoji *et al.* (2005) found that larval red sea bream (*Pagrus major*) predation by moon jellyfish (*Aurelia aurita*) increased under hypoxic conditions while that by Japanese Spanish mackerel (*Scomberomorus niphonius*) decreased. In both studies, the fish's limited mobility due to hypoxia caused the gelatinous animals to capture them more efficiently compared to fish predators (Breitburg *et al.* 1994, Shoji *et al.* 2005).

Both Breitberg *et al.* (1994) and Shoji *et al.* (2005) determined that physiological impairment was likely one of the main causes of the increased predation of fish larvae by hypoxia-resistant predators. However, these studies never determined if hypoxia related impairments might have also altered how fish perceive predators. It is possible that these impairments may weaken a fish's ability to perceive predatory threats leading to reduced

avoidance. These behaviors would fall under the category of anxiety, or, in this case, anxiety-related behavior as to not tie emotional qualities seen in more advanced animals (Ohl *et al.* 2008).

According to Catherall (2003), anxiety-related behaviors are distinguished as an animal's reaction to a possibly dangerous stimulus in its immediate proximity. The animal increases its focus on the potentially dangerous stimulus while in this anxious state and assesses the risks of approaching it (Blanchard and Blanchard 1989, Lang *et al.* 2000, McNaughton and Corr 2004, Ohl *et al.* 2008). If the animal concludes the risks of approaching the stimulus outweigh the possible benefits of investigation, the animal retreats and avoids the stimulus (Ohl *et al.* 2008). This is what would be considered a fear response as the animal perceives the stimulus as dangerous or life threatening (Ohl *et al.* 2008).

Fear responses are a form of anxiety like behavior that has evolved in many types of vertebrate animals as a method of adapting to stressful changes in their environment (Ohl *et al.* 2008). Anxiety-related behaviors are partially the result of activation of the gamma-aminobutyric acid type A ($GABA_A$) receptors within the brain, which, when activated, heighten awareness (Jutfelt *et al.* 2013, Hamilton *et al.* 2014, Thompson *et al.* 2016, Hossein-Javaheri *et al*. 2017, Tsz Kwan *et al.* 2017). The activation of these receptors is known to be altered due to changes in membrane potential caused by alteration of extracellular ion concentrations (Jutfelt *et al.* 2013, Hamilton *et al.* 2014, Thompson *et al.* 2016, Hossein-Javaheri *et al*. 2017, Tsz Kwan *et al.* 2017). This has been shown to occur in high pCO_2 conditions associated with ocean acidification (Jutfelt *et al.* 2013, Hamilton *et al.* 2014). Recently, Hossein-Javaheri *et al*. (2017) also observed changes to GABA receptor activation in goldfish (*Carassius auratus*) kept in anoxia, indicating that dissolved oxygen can play a role in the activation of these receptors. The information from

these cellular reactions is then processed in the frontal cortex of the brain in vertebrates (Catherall 2003, Ohl *et al.* 2008). Previous studies on rats have shown that intermittent hypoxia can permanently damage the frontal cortex resulting in long-term behavioral impairments that affect movement, vigilance, memory, and associative learning (Row *et al.* 2003, Row 2007). Similar symptoms were a result of hypoxia induced brain damage in zebrafish (Yu and Li 2011).

The resulting behavioral alterations may be even more severe if the species in question uses visual recognition of threatening stimuli and cannot properly process an imminent threat through sight. Hypoxia could greatly impair fish vision due to the retina of teleost fish requiring the highest oxygen supply compared to other tissues (Waser and Heisler 2005). Besides the zebrafish study performed by Ingalls and Philbrook (1958), Robinson *et al.* (2013) found visual impairment in adult Australasian snapper (*Pagrus auratus*) in oxygen saturation levels of <25%.

Alteration of anxiety-related behaviors in fish is commonly looked at in many freshwater species, such as zebrafish, in pharmacology and neurology research (Bencan *et al.* 2009, Maximino *et al.* 2010). Scototaxis and novel object tests are commonly used to determine the influence of drugs on the fish's anxiety related behavior. Scototaxis protocols assess how experimental variables affect the anxiety response of an animal by observing its preference for dark or light areas of an arena and the amount of time spent in each (Maximino *et al.* 2010). The theory behind this test is that fish may perceive the light side as a danger zone, and will be more likely to retreat to the dark side of the arena to hide from predators (Maximino *et al.* 2010). Despite this, marine and estuarine fish tend to stray from this theory and may choose a side that most resembles the lighting in their natural environment (Hamilton *et al.* 2014, Thompson *et al.* 2016).

The novel object test determines the extent of an animal's exploratory behavior towards an object it has never seen before (Sneddon *et al.* 2003). The amount of time a fish investigates the object, and the animal's proximity to it, is a method used to measure the level of anxiety experienced by the animal (Sneddon *et al.* 2003, Hamilton *et al.* 2014). In this test, fish may behave vigilantly when presented with a novel object. This would indicate the fish in question perceives the object as threatening and has assessed that the risks outweigh the benefits of investigating the object (Sneddon *et al.* 2003, Ohl *et al.* 2008). However, it is also possible that they naturally will not perceive the object as a threat and investigate it intensely. The natural level of curiosity and likelihood of exploration appears to be based on species preference (Jutfelt *et al.* 2013, Hamilton *et al.* 2014). Hamilton *et al.* (2014) depicted juvenile California splitnose rockfish (*Sebastes diploproa*) in a control setting as being wary when investigating the novel object. This was the opposite for control three-spine sticklebacks (*Gasterosteus aculeatus*), which had a natural preference to explore the novel object (Jutfelt *et al.* 2013).

Recently, such tests have been performed on juvenile California splitnose rockfish, three-spine stickleback, and blacksmith damselfish (*Chromis punctipinnis*) under high pCO_2 conditions to determine the effects of ocean acidification on these behaviors (Jutfelt *et al.* 2013, Hamilton *et al.* 2014, Thompson *et al.* 2016, Tsz Kwan et al. 2017). Even more recently, use of scototaxis testing on red drum (*Sciaenops ocellatus)* under such conditions has also been completed (Lonthair *et al.* 2017).

2.4. Species of Interest: Spotted Seatrout and Red Drum

The estuarine species that will be of most interest in determining how hypoxia affects their larval form will be those that hold high economic or ecological value and are at risk of hypoxia related impairments due to their biology and life history. Two of the species used in this

experiment were spotted seatrout (*Cynoscion nebulosus*), and red drum (*Sciaenops ocellatus*), (Figure 2.3). Both spotted seatrout and red drum are in the family Sciaenidae, a cosmopolitan family of 270 species that includes the croakers and drums (Ramcharitar *et al.* 2006).

Even though both spotted seatrout and red drum rely heavily on estuaries throughout their life history, it has been found that both species are poorly adapted to hypoxia in early life stages compared to other sciaenid species within their ecosystem (Hanke and Smith 2011). Studies by Goodman and Campbell (2007) found that the 24-hour LC^{50} (lethal concentration 50%), or the concentration of dissolved oxygen that killed 50% of the test population, of juvenile red drum was 1.45 mg/L O_2 while for juvenile spotted seatrout it was 1.89 mg/L O_2, which were the highest for all tested sciaenids. Both spotted sea trout and red drum larvae were used in anti-predatory experiments by Poling and Fuiman (1999). They found that spotted seatrout depend mostly on mechanoreception for environmental perception whereas red drum used a combination of mechanoreception, vision, and hearing (Poling and Fuiman 1999). Poling and Fuiman (1999) used larvae of both species at different ages, including one-DPH larvae that measured approximately 2 mm. This indicates that even at this size both spotted sea trout and red drum sensory abilities are developed enough for analysis of their behavior.

More recently, scototaxis tests performed by Lonthair *et al.* (2017) found that control red drum larvae spent approximately 20-40% of their time in the dark zone, which meant that they were phototactic, or preferred lighter surroundings. This also indicates that these larvae are appropriate to use in scototaxis testing. Besides this experiment, there is not much known on larval spotted seatrout or red drum behavior, especially after exposure to hypoxia.

Figure 2.3. Illustrations of adult spotted seatrout (top) and red drum (bottom) by the South Carolina Department of Natural Resources (2015). Both sciaenid fish have similar morphology, life history, and habitat requirements. They have also been the subject of prior larval behavior studies and have been shown to be poorly adapted to hypoxia. These attributes make them adequate test subjects.

2.4.1. Spotted Seatrout Life History

Spotted seatrout, or speckled seatrout, are a popular food fish that are harvested both recreationally and commercially (Atlantic States Marine Fisheries Commission 2016). They range from Massachusetts to southern Florida and west to the Gulf Coast primarily in shallow waters of around 26°C (Atlantic States Marine Fisheries Commission 2016). They are tolerant of a wide range of salinities, but prefer salinity close to full strength seawater (~30 ppt) (Atlantic States Marine Fisheries Commission 2016). Spawning occurs between the months of April and October around each fish's natal estuarine habitat (Saucier and Baltz 1993, Kucera *et al.* 2002, Atlantic States Marine Fisheries Commission 2016). Preferred water parameters for spawning are approximately 16.6 ppt salinity, 29.7 °C, and 7.9 mg/L O_2 (Saucier and Baltz 1993). Fertilized eggs float to the surface where that are transported into estuaries by tidal flow and wind while unfertilized and unviable eggs sink at spawning sites (Powell 2003, Saucier and Baltz 1993, Atlantic States Marine Fisheries Commission 2016). Larvae of approximately 1.5 mm in length hatch from the eggs 16 to 20 hours post fertilization (Saucier and Baltz 1993). In this stage, larval spotted seatrout feed on zooplankton such as copepods and larval bivalves (Holt and Holt 2000, Atlantic States Marine Fisheries Commission 2016).

Overall stability of populations is challenging to predict due to the overall lack of data regarding sensitivity of this species to specific water quality parameters (temperature, pH, etc.) throughout their life history from egg to adult (Atlantic States Marine Fisheries Commission 2016). Hypoxia is already known to pose a significant threat to larval individuals, but effects on adults are not well known (Hank and Smith 2011, Atlantic States Marine Fisheries Commission 2016). Increases in severity of "dead zones" within the Gulf of Mexico will certainly have a negative effect on this species (Rabalais *et al.* 2002, Rabalais *et al*. 2014, Thrash *et al.* 2017).

Anthropogenic changes to these water quality conditions directly or indirectly through nonpoint source pollution and climate change could dramatically reduce survivability throughout at all life stages (Atlantic States Marine Fisheries Commission 2016, Alloy *et al*. 2017).

2.4.2. Red Drum Life History

The red drum is a large sciaenid species that ranges from Long Island, New York south to Florida and west to the Gulf of Mexico, but in recent years are not often found farther north than the Chesapeake Bay (Matlock 1987, Atlantic States Marine Fisheries Commission 2016). They can grow up to 150 cm in length (Atlantic States Marine Fisheries Commission 2016). Red drum prefer temperatures in the mid 20°C range and salinity close to that of seawater (Holt *et al.* 1981). However, this species is tolerant of a wide range of both parameters (Holt *et al.* 1981). Spawning takes place in the late summer around August and ends in November in coastal waters (Peters and McMicheal 1987, Atlantic States Marine Fisheries Commission 2016). Recent studies have found that red drum will utilize high salinity areas of the estuaries for spawning (Atlantic States Marine Fisheries Commission 2016). High salinities of 25 ppt or above are required to keep the buoyant eggs afloat before hatching (Atlantic States Marine Fisheries Commission 2016). Larvae prefer higher salinity areas in estuaries like adults and move from pelagic to demersal habitat within the first few weeks (Atlantic States Marine Fisheries Commission 2016). Red drum larvae, like the adults, are opportunistic feeders and will feed on various species of copepods, mysids, and polychaetes (Atlantic States Marine Fisheries Commission 2016).

Coastal development has threatened red drum populations by altering habitat. (Atlantic States Marine Fisheries Commission 2016). Pollution is another issue, especially that coming from ports where hazardous material has an increased chance of contaminating the water either

through boat waste or accidental spills (Atlantic States Marine Fisheries Commission 2016, Alloy *et al.* 2017, Johansen and Esbauggh 2017). Runoff and other nonpoint source pollution from these developed areas can also reduce water quality through eutrophication (Atlantic States Marine Fisheries Commission 2016). Climate change is expected to increase water temperatures and sea level while decreasing dissolved oxygen and exacerbating coastal dead zones, such as the one in the Gulf of Mexico (Rabalais *et al.* 2002, Rabalais *et al*. 2014, Atlantic States Marine Fisheries Commission 2016, Thrash *et al.* 2017). Besides the physiological effects associated with this, red drum may be more susceptible to pathogens in these conditions reducing survivability throughout all life stages (Atlantic States Marine Fisheries Commission 2016).

2.5. Species of Interest: Striped Bass

The striped bass (*Morone saxitilis*) (Figure 2.4) is a large bass of the family Moronidae, which includes six species of temperate predatory fish found in fresh, brackish, or saltwater (Jobling *et al.* 2010). This species was chosen for this experiment due to its high economic and ecological value. Striped bass harvest represents one of the most important and lucrative fisheries of the Atlantic states and has been for centuries (Atlantic States Marine Fisheries Commission 2016). As an adult, this species is also an important predator that controls populations of estuarine and marine forage species (Jobling *et al*. 2010).

Striped bass adults are known to require a high dissolved oxygen concentration (5-6 mg/L O_2) to maintain their highly active predatory lifestyle (Downing *et al.* 1999). Anything below 1 mg/L O_2 is known to be fatal (Fry 1971). Similarly, Chittenden (1971) found that striped bass abandoned freshwater spawning grounds in the Delaware River due to dissolved oxygen levels less than 3mg/L O_2 caused by urban pollution. Turner and Farley (1971) reported that striped bass egg hatch rate was greatly reduced in dissolved oxygen levels of 4 mg/L O_2. They

also found that survival of hatched larvae was greatly reduced compared to those in normoxia (Turner and Farley 1971). A study by Brandt *et al.* (2009) found that juvenile striped bass decreased in body condition, growth rate, and consumption of food when kept in 4mg/L O_2 or less. Brandt *et al.* (2009) reported adult striped bass are known to be able to sense and avoid hypoxia. However, how their behavior is affected by hypoxia regarding threat perception is not known.

Figure 2.4. The striped bass as illustrated by the California Department of Fish and Wildlife (2017). This large, popular food fish is anadromous and spawns in freshwater. Increasing freshwater hypoxia could be problematic for this species as adults, juveniles, larvae, and eggs have been shown to be sensitive to hypoxia.

2.5.1. Striped Bass Life History

Striped bass are a wide-ranging species found as north as Canada in the St. Lawrence River and Gulf of St. Lawrence down south to Florida's St. John's River as well as the Gulf of Mexico (Merriman 1941, Atlantic States Marine Fisheries Commission 2016). As a naturally anadromous species, striped bass are tolerant of a wide range of salinity levels but prefer temperatures between 15 and 20°C (Atlantic States Marine Fisheries Commission 2016). Due to this, they have been introduced to the Pacific Ocean around California as well as many freshwater lakes and manmade bodies of water for fishing purposes (NOAA Fisheries 2017).

Adults will remain in estuaries or coastal ocean zones around their select spawning locations year-round (Atlantic States Marine Fisheries Commission 2016). In the wild, non-spawning adult striped bass are found in a wide range of coastal habitat types at varying levels of salinity (Atlantic States Marine Fisheries Commission 2016). Spawning season occurs in the spring through summer and is initiated by increases in water temperatures (Setzler-Hamilton *et al.* 1980). During this time, oceanic adult striped bass migrate inland from marine and brackish water to freshwater far up river to spawn (Atlantic States Marine Fisheries Commission 2016). Once fertilized, the buoyant eggs hatch between 1 and 4 days (Coutant 1985). Larvae and juveniles of this species will remain in fresh or brackish water until they are between 2 and 3 years old (Merriman 1941). Larvae and age 0 juveniles feed on zooplankton such as copepods, polychaetes, and mysid shrimp (Hartman and Brandt 1995).

Threats to striped bass are mainly anthropogenic. Non-point source pollution and runoff that trigger eutrophication or temperature in tidal freshwater and estuaries are of greatest concern. Striped bass have proven to be intolerant of hypoxia as seen in studies by Chittenden (1971), Fry (1971), and Turner and Farley (1971). Pollution in the form of pesticides,

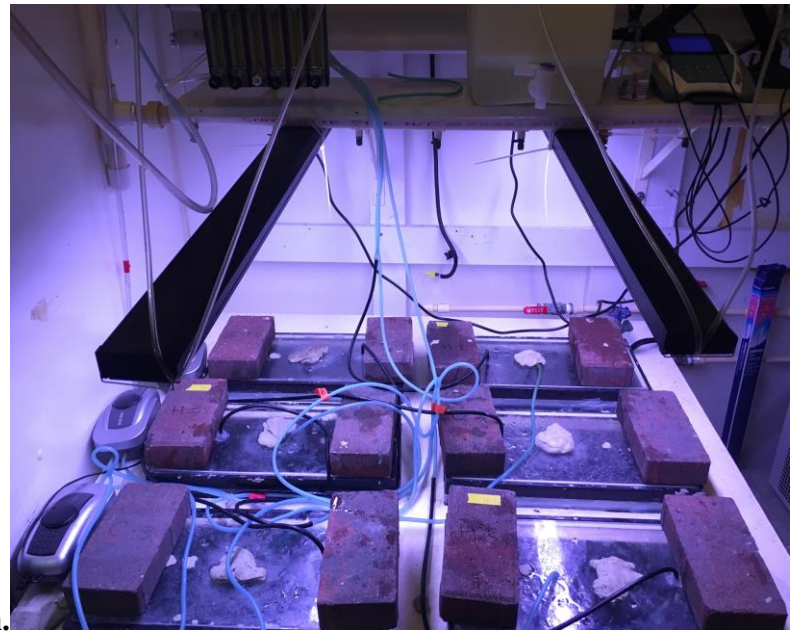
pharmaceuticals, and metals could detrimentally affect hatching success and larval survival (Atlantic States Marine Fisheries Commission 2016). As predators, adult striped bass are also vulnerable to bioaccumulation of toxins such as mercury that could affect reproduction and overall health (Piraino and Taylor 2009). Bacterial infection from mycobacteriosis has also become a source of natural mortality in striped bass that has been increasing since the 1990's in areas such as the Chesapeake Bay (Latour *et al.* 2012). As with many other species, stressors associated with climate change may exacerbate infections from mycobacteriosis and other pathogens (Atlantic States Marine Fisheries Commission 2016). Climate change is expected to exacerbate the issues of hypoxia and warming temperatures throughout the striped bass' range (Atlantic States Marine Fisheries Commission 2016).

CHAPTER III

METHODS AND MATERIALS

3.1. Hypoxia Setup

The system used to house fish in this experiment was originally built by former Delaware State University graduate student Andrea Stoneman, and is located at the Delaware State University Aquaculture Research and Demonstration Facility in Dover, Delaware (Stoneman 2016). The system is comprised of six 19-liter glass aquaria each measuring 20x40x25 cm. Each aquaria was fitted with an acrylic lid to keep temperature and dissolved oxygen as constant as possible with holes (~7 cm diameter) drilled into each lid to allow easy access for water quality testing. Holes were sealed with window sealant putty when not in use to prevent atmospheric disturbance. A pair of bricks was placed on either end of each aquarium to keep the lids in place. Fluorescent aquarium lights over the aquariums provided light for 12 hours during each trial to keep the fish under a normal diel cycle. All six aquaria sat in a common water bath to maintain a constant temperature (Figure 3.1a) (Stoneman 2016). Temperature regulation is accomplished by using an Isotemp® 4100 R20 (Fisher Scientific, Pittsburgh, PA) recirculating water pump (Figure 3.1b) to circulate water through 30 m of copper tubing coiled within the water bath.

a. 

b. 

Figure 3.1. The system used to hold the fish larvae (a.). All six 19-L aquariums sit in a common water bath. Three of the flow meters in the top left were used to regulate the flow of nitrogen into the three treatment aquaria. On the far left are the air pumps used to deliver atmospheric air into all six aquaria. The water bath shared by all aquaria was heated to a specific temperature by the Isotemp® 4100 R20 recirculating water pump (b.), which fed heated water through 30 meters of copper tubing in the bath.

Hypoxic conditions in the experiment were achieved by bubbling pure nitrogen gas (N_2) into the designated aquaria. A single canister supplied nitrogen to the three aquaria assigned to hypoxia within the system. The 1.25 cm diameter airline tubing originating from the nitrogen canister was split into three separate lines, one for each hypoxia assigned aquarium. Each of these airline tubes ran into a flow meter, which regulated the nitrogen flow to 100 mL/min. Airline tubing 0.47 cm in diameter delivered nitrogen from each flowmeter to its assigned aquarium, where it was diffused through a 5 cm air stone in each aquarium. Normoxic conditions were created by bubbling atmospheric air into the designated aquariums at a rate of 100 mL/min using separate aquarium air pump. Airline tubing 0.47 cm in diameter delivered atmospheric air into the three normoxia assigned aquaria, where it was diffused through 5 cm air stones.

Originally, this was all that was done for tanks simulating hypoxic conditions. The first few trials using this method with spotted seatrout, and later red drum, caused significant amounts of mortality. The data from these trials was discarded. Increasing the flow rate of nitrogen gas increased water circulation, but quickly reduced dissolved oxygen to intolerable levels for the larval fish. To amend this issue, I bubbled atmospheric air into each aquarium at a rate of 100 mL/min using an aquarium air pump to keep the larval fish aloft and circulate the water.

To produce hypoxic conditions of approximately 2.5 mg/L O_2, the airflow was regulated even further after the flowmeters with using aquarium valves located on each airline. These valves proved to be relatively unstable in controlling airflow and had to be monitored on a regular basis. This was coupled with water quality testing with a YSI 556 probe (YSI Inc., Yellow Springs, OH) every two hours for the course of the entire trial to assure that each aquaria was being maintained in hypoxia. Normoxic conditions created through the aquarium air pumps were maintained at approximately 5.3-6.3 mg/L O_2.

3.2. Other Water Quality Parameters

Besides dissolved oxygen, water parameter targets were based on those from the facilities in which each respective species was spawned (Table 3.1). These conditions mirrored approximate conditions experienced by wild adult members of their species during spawning as well as by one-DPH larvae reared in these facilities. Due to the sensitive nature of the larvae, using water quality parameters like the spawning source reduced the time needed for acclimation procedures, and decreased the stress level of the fish upon introduction to the aquarium.

Aquarium water was produced in the lab by first filling up a 208-L rain barrel with well water. Instant Ocean© (Spectrum Brands, Blacksburg, VA) sea salt mix was added to achieve the desired salinity, which was verified with a YSI 556 probe (YSI Inc., Yellow Springs, OH). Striped bass water utilized only well water with no added salt mix due to these larvae being found in freshwater at this early life stage. The original pH of the well water was high (~8.4) and was brought down to a value of 8 using of 20 mL of 0.1 M hydrochloric acid (HCl) per 208-L barrel of mixed water. Water was well mixed to ensure homogenization before being equally distributed into each of the six aquaria.

Total ammonia readings were taken in triplicate from each of the 6 experimental tanks approximately 1.5 hr before the end of each trials. I measured total ammonia nitrogen using salicylate reagents and a spectrophotometer (Hach DR 3900). The total ammonia concentrations for each of the three vials for each aquarium were averaged together to give a reading for the aquarium.

Table 3.1. Target water quality parameters for the three tested species. Dissolved oxygen wasn't included due to it being approximately the same for the three species between control and treatment. These parameters mirror each specie's spawning conditions in captivity and what the one-DPH larvae would be reared in there.

Parameter	Spotted Seatrout	Red Drum	Striped Bass
Temperature	26 °C	26 °C	20°C
Salinity	16.50 ppt	30 ppt	0 pp
pH	8.0	8.0	8.0

3.3. Hypoxia and Normoxia Trials

Four trials were planned for each of the three fish species, which would coincide with an individual shipment of larvae from their respective spawning source (Table 3.2). Each trial consisted of three replicates of the control (normoxia) and the treatment (hypoxia) for a sample size (n) of 12 (Table 3.3). The assignment of each aquarium to either normoxia or hypoxia was randomized after each successive trial to prevent biases associated with tank placement within the system.

Table 3.2. The schedule of larval shipments for each specie along with their spawning facility.

Species	Spawning Facility	Trial 1	Trial 2	Trial 3	Trial 4
Spotted Seatrout	Texas Parks and Wildlife Aquaculture	6/23/2016	7/6/2016	7/14/2016	8/25/2016
Red Drum	Texas Parks and Wildlife Aquaculture	8/2/2016	5/2/2017	6/1/2017	6/29/2017
Striped Bass	North Carolina State University	5/17/2017	5/23/2017	X	X

The approximately 2400 eggs of all species were packed and sent one-day post fertilization and hatched in transit. Upon arrival, fish from the single shipping bag were sub-divided into 6 smaller bags each containing 1 L of shipping water. Fish larvae were too small to accurately count when it came to equally split them between the six aquaria. To solve this, the bag of shipping water was gently mixed to ensure larvae were as equally distributed by water

volume. The homogenized shipping water was then poured into each of the six bags, which equated to approximately 400 larvae per aquarium. Larvae were then temperature acclimated for two hours via floating in plastic bags. To reduce stress related to water acclimation, 50 mL of tank water was added to each shipping bag every half hour. Once the acclimation period ended, larvae were gently sieved through 100 µm mesh preventing shipping water from entering the tanks. Larvae were collected on the mesh and directly introduced into their respective aquariums.

Table 3.3. The experimental setup for the behavioral trials. Note fish for both behavioral tests will come from the same aquaria.

Experiment 1: Scototaxis Protocol			
Species	**Treatments (3 Replicates per treatment)**	**Number of Trials**	**n**
Spotted Seatrout	Control, Hypoxia	4	12
Red Drum	Control, Hypoxia	4	12
Striped Bass	Control, Hypoxia	2	6
Experiment 2: Novel Object			
Species	**Treatments (3 Replicates per treatment)**	**Number of Trials**	**n**
Spotted Seatrout	Control, Hypoxia	4	12
Red Drum	Control, Hypoxia,	4	12
Striped Bass	Control, Hypoxia	2	6

The recirculating water pump was turned on for two days prior to the initiation of each trial allowing water temperatures in the experimental tanks stabilize. The nitrogen gas and atmospheric air pumps were turned on in both the hypoxia and normoxia tanks one day prior to the experiment to ensure that dissolved oxygen levels were on target when larval fish were added.

During the trial, temperature, pH, and dissolved oxygen in each tank were recorded every three hours and checked every half hour using YSI 556 multiprobe and Orion pH probe (Thermo Fisher Scientific, Waltham, MA). After the 24-hour exposure period ended, behavioral testing began immediately afterwards. The water bath and pump as well as the nitrogen and air were kept on during sampling to prevent biases between fish sampled earlier compared to those sampled later.

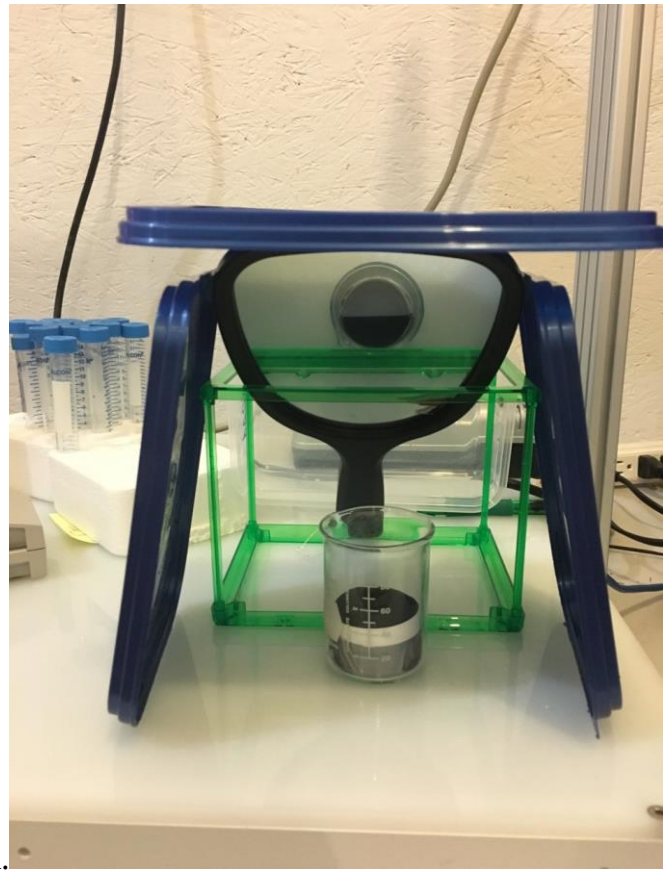
3.4. Behavioral Tests

For each species, 5 fish were tested for each behavioral test per aquaria, which equated to 15 tested from the normoxia replicates and 15 were tested from the hypoxia replicates for each behavioral test. Overall, 60 fish per trial were used for behavioral testing, with 30 assigned to scototaxis and 30 assigned to novel object testing. Overall, 240 fish (N=240) were behaviorally tested per species. This ended up being half this amount for striped bass (N=120) due to availability.

For both the scototaxis and novel object tests, a 100-mL beaker was used as the test arena. This arena type was chosen due to its size in comparison to the size of the larvae. It is small enough to observe the 2 mm sciaenid larvae, but large enough for the fish to maneuver and swim throughout it in a timely manner. Due to the striped bass larvae being larger than the other two species at approximately 3.5 mm, a larger arena was constructed for both tests using a 150-mL beaker instead. This volume arena was calculated as being proportionate to the striped bass larvae as the 100-mL beaker was to for the sciaenid larvae. Fish were observed through an angled mirror from about half a meter away to prevent the observer from influencing the fish's behavior. Plastic blinds were placed around the arena to keep the lighting within the arena uniform and prevent glare (Figure 3.2a). Each fish was tested individually, and no fish was tested twice as done in similar studies.

In the scototaxis test, the beaker was filled with 20 mL of tank water (that was changed out after each fish tested). The bottom and sides of the beaker were covered by waterproof, non-reflective paper that was half black and half white (Figure 3.2b). Fish were not allowed to acclimate to the arena and tested singularly A single fish was placed at the black/white demarcation line using a 1 mL pipette. As soon as this occurred, the timer was started. Each time

the fish moved from the light to the dark side or vice versa of the arena, the time was recorded. After 180 seconds, the fish was removed, and the amount of time spent in each side of the area was totaled. Prior to the next fish being placed in the arena, the arena was rotated 180 degrees ensured that individual left or right preferences of the fish did not influence the results.

a. 

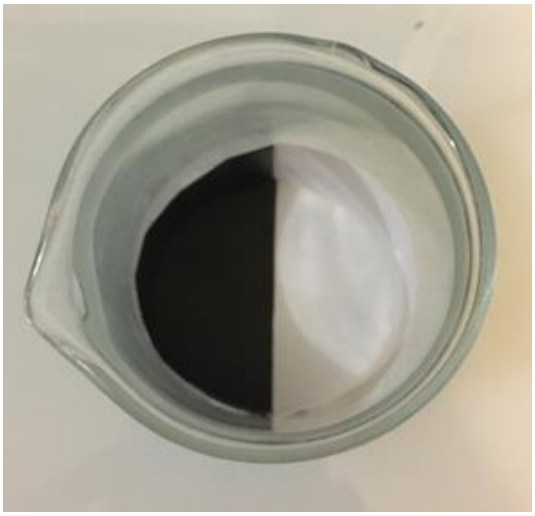
b. 

Figure 3.2. Setup for the scototaxis protocol. (a) The arena was placed underneath an angled mirror and set approximately a third of a meter away from the observer. Plastic lids were used as blinds to keep lighting in the arena uniform. (b) The arena itself was contructed from a 100 mL beaker and nonreflective paper on the bottom and sides that was half white and half black.

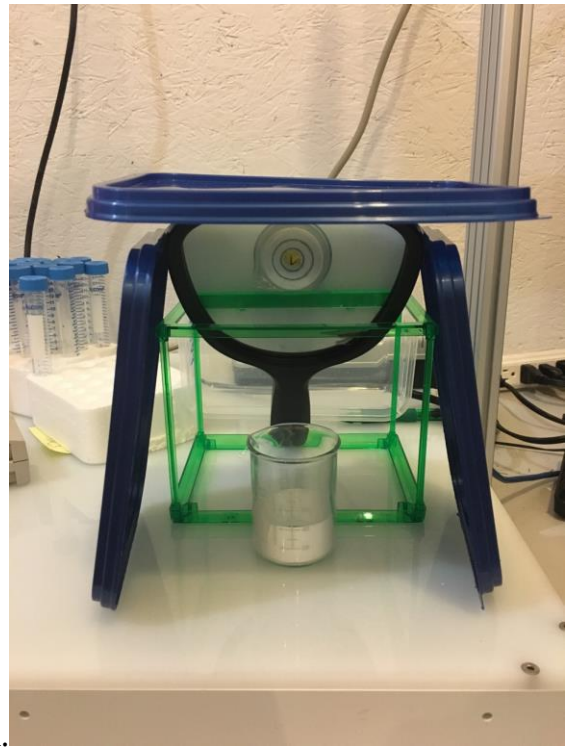
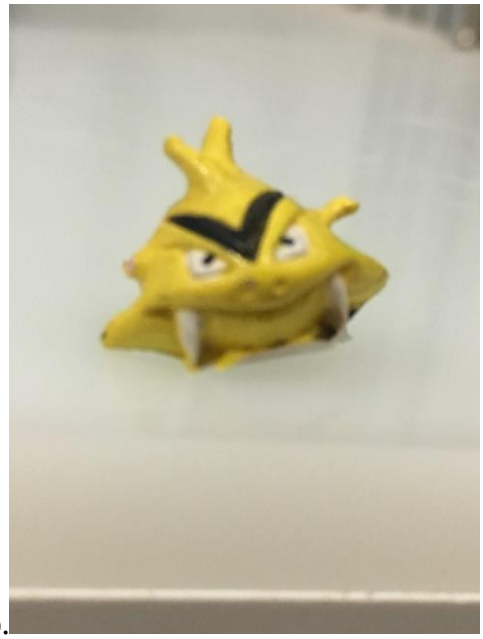
Fish were observed through an angled mirror from about a third of a meter away to prevent the observer from influencing the fish's behavior. Plastic blinds were placed around the arena to keep the lighting within the arena uniform and preventing glare (Figure 3.3a). The arena was filled with 20 mL of tank water and was changed after each tested fish. The arena sides and bottom were covered by only white non-reflective paper. On the bottom of the arena, three rings emanating from the center of the beaker approximately 7 mm apart were drawn. These rings allowed the observer to determine where the fish spent most of its time in relation to the object as seen in Figure 3.3b. These rings were labeled outer, middle, and inner moving from the edge of the arena inward. The novel object chosen for this test was the head of a small monster action figure with a width of 10 mm across (Figure 3.3c). This object was chosen due to its small size and exaggerated predatory appearance (large mouth, big teeth). The object was primarily black and yellow, but was colored in using green, red, orange, purple, and blue pencils. Multiple colors were added to prevent the fish from reacting solely due to innate color preferences (Hamilton *et al*. 2014).

To begin the novel object test, a single fish was placed in the center of the arena without the novel object using a 1 mL pipette. The fish was acclimated to the arena for 120 seconds for it to familiarize itself with the environment briefly before the addition of the object. The novel object was carefully placed in the center of the arena (the inner ring) using a pair of forceps as to not harm the fish. Care was taken to insure the object was placed in the same position each time (bottom up) to prevent differed perception of the object between individuals.

Once the object was placed, the timer was started for the 180-second trial. The observer recorded the time the fish moved from one ring to another. Observations on how the fish

interacted with the object were also taken, but not quantified. At the end of the experiment, the fish was removed and the time the fish spent in each ring of the arena was totaled.

It was soon brought to my attention that I may have been creating a bias in the novel object test by only testing fish on a white background. I made an assumption that the fish would behave the same on a white background compared to a black background (or any other colored background) in the presence of a novel object. To rectify this, I made a separate novel object arena identical to the one normally used, but with a black background. I tested a small sample size of fish 30 normoxia and 30 hypoxia fish from each species and compared the results with the normoxia and hypoxia novel object tests on the white background.

a.  b. 

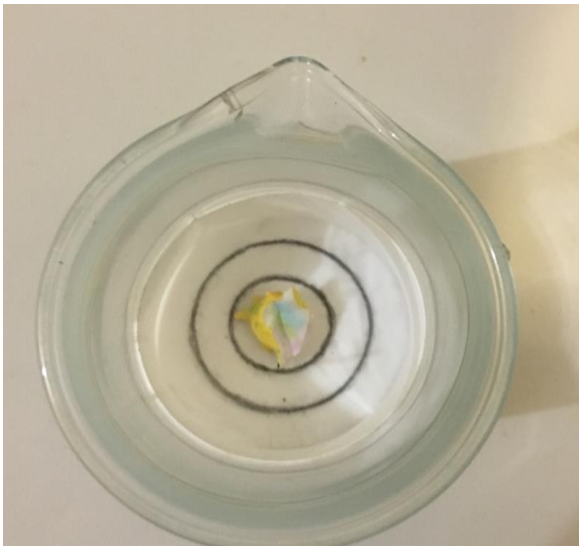
c. 

Figure 3.3. Setup for the novel object test. (a) The arena was placed underneath an angled mirror and set approximately 0.3 meters away from the observer. Plastic lids were used as blinds to keep lighting in the arena uniform. (b) The object used in this experiment was the head of a small action figure. The bottom and sizes were colored various colors to prevent bias. (c) The arena itself was contructed from a 100 mL beaker and white nonreflective paper on the bottom and sides. Three zones each 7mm in width were drawn to gauge the fish's distance from the object.

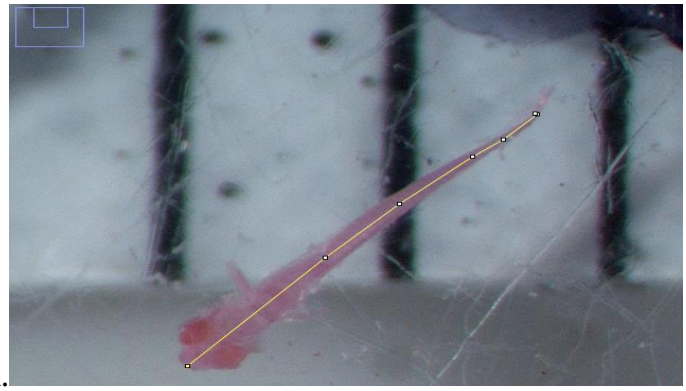
Once all tests concluded, larval fish were euthanized with a lethal dose of tricaine methanesulfonate (MS-222) of 4 mg/19L. Individuals used in the behavioral trials were preserved in 95% ethanol and used for the eye and length measurement portion of the study.

3.5. Eye and Length Measurements

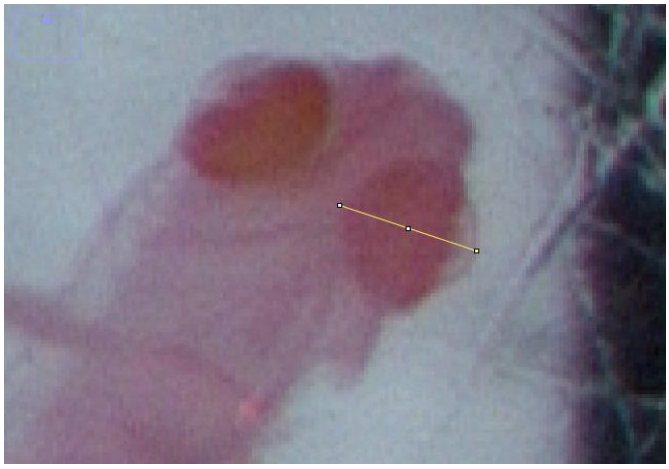
The preserved fish from the previous experiments were taken to the main campus of Delaware State University for measurements. A dissecting scope with a mounted Nikon Digital Sight DS-Fi2 camera connected to a laptop was used as the means of observing these preserved fish. A ruler was first taped down to the base of the microscope so that the 0 to 5 mm lines were visible. A gridded petri dish was taped down over the ruler to hold fish. Before fish were viewed, the ethanol they were preserved in was dyed with Alizarin-Red staining solution, imparting a pink color that made minute details easier to view. Fish were pipetted singly on to the petri dish. Using a dissecting pick, fish were gently positioned so that their dorsal plane faced upwards and that the horizontal plane of their eyes (from lens to retina) was visible. A photograph was then taken. This was done for 60 randomly selected fish of each species that were previously used in behavioral testing, 30 of which were control and 30 treatments. These fish were pulled from euthanized groups used in behavioral testing.

Photographs were analyzed using ImageJ (National Institute of Health, Ver. 1.5) software to accurately measure the standard lengths of each fish as well as eye measurements (Figure 3.4 a,b, and c). Standard lengths were used over total lengths to avoid error due to damage in the caudal fin of some measured individuals. Using the ruler as a scale in all photos, the program measured the standard length, eye area, and eye width (from lens to retina) of all fish in millimeters. Measurements were taken of both eyes in each fish as a method of determining if micropthalmia occurred in one eye or both.

a.



b.



c.

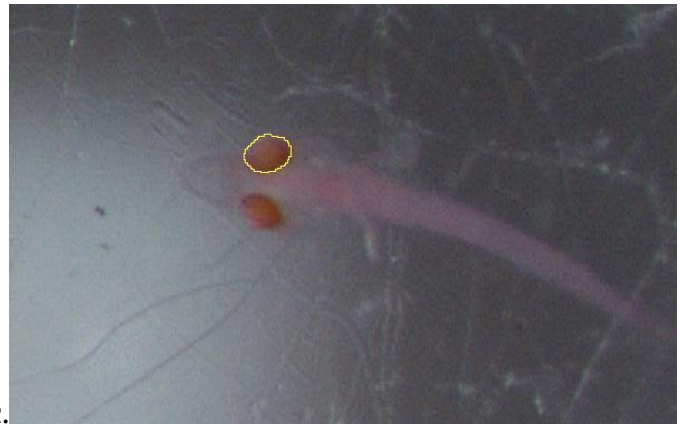


Figure 3.4. Photos taken of larval spotted seatrout under the microscope. Using ImageJ, (a) the standard length (b) eye width from lens to retina, and (c) eye area were analyzed. All eye measurements were done for both eyes.

3.6. Statistical Analysis

Prior to statistical analysis, all behavioral data sets were run through a Shapiro-Wilk normality test to determine if they were parametric. If data was found to be parametric, a *t*-test was run with a 95% confidence interval. If data was non-parametric, a Wilcoxon rank-sum test with a 95% confidence interval was used. For the scototaxis tests, I ran four separate analyses where I compared normoxia light vs. dark, hypoxia light vs. dark, hypoxia light vs normoxia light, and hypoxia dark vs. normoxia dark. For novel object tests, I ran three analyses that compared time spent by fish in each ring of the arena between control and treatment.

I compared the averages of all water quality data between control and treatment using *t*-tests with a 95% confidence interval. For standard length data, *t*-tests with a 95% confidence interval were also used. I used the same test to compare eye widths and areas between left and right eyes within the control and treatment to check for micropthalmia. To compare all measured eyes across both control and treatment, a one-way Analysis of Variance (ANOVA) with a 95% confidence interval was used.

CHAPTER IV

RESULTS

4.1. Spotted Seatrout Results

4.1.1. Water Quality

The averages and standard deviations of each measured water quality parameter can be found in Table 4.1. With the exception of dissolved oxygen, water quality parameters tested (Temperate, salinity, pH and total ammonia), were found to not vary between the normoxic and hypoxic treatment aquaria.

Table 4.1. The average values and standard deviations for each parameter in both control and treatment conditions for spotted seatrout trials along with associated *p*-values. Asterisks denote significant values.

	Average ± Std. Dev		
Parameter	**Normoxia**	**Hypoxia**	***p*-value**
DO	5.47±0.238 mg/L	2.45±0.243 mg/L	< 0.00001*
Temperature	26.55±0.141 °C	26.54±0.070 °C	0.300439
Salinity	16.6±0.065 mg/L	16.62±0.064 mg/L	0.055892
pH	7.97±0.221	7.97±0.229	0.472238
Total Ammonia	0.0400±0.027mg/L	0.0400±0.029 mg/L	0.256642

4.1.2. Behavioral Results

This data was found to be non-parametric and was analyzed using Wilcoxon rank-sum tests. The resulting *p*-values for all the analyses run from the scototaxis tests and novel object tests data can be found in Table 4.2. Figures 4.1 and 4.2 illustrate the results of the scototaxis tests and novel object tests.

In the scototaxis testing, spotted seatrout demonstrated a significant preference to remaining on the light side of the arena (115 seconds on average) over the dark side normoxia (65 seconds on average). In the hypoxia group, seatrout had no preference to the dark or light

side and stayed within each condition approximately 50% of the time (90 seconds on average). This shift from a light preference to a preference to neither indicates a change in behavior.

In novel object test, spotted seatrout also demonstrated a significant change in behavior. While hypoxia exoposed fish still spent the majority of their time in the outer ring on average, it was significantly less than that of the control. This was due to more time being spent in the middle and inner rings.

A second set of Wilcoxon-rank sum tests was completed to determine significance between the use of a dark versus light background during the novel object tests. For the normoxia inner, middle, and outer rings, the *p*-values were 0.500, 0.46654, and 0.154075, respectively. For the hypoxia inner, middle, and outer rings, the *p*-values were 0.413957, 0.250816, and 0.169831, respectively. Overall, not significance occurred between using a light colored and dark colored background.

Table 4.2. The *p*-values from the *t*-tests run on each of the four scototaxis analyses as well as each ring in the novel object test. Asterisks denote a significant value.

Scototaxis Parameters	***p*-value**
Normoxia Light vs. Normoxia Dark	0.00104*
Hypoxia Light vs. Hypoxia Dark	0.22628
Normoxia Dark vs. Hypoxia Dark	0.00386*
Normoxia Light vs.Hypoxia Light	0.00466*
Novel Object Parameters	
Inner (Normoxia vs. Hypoxia)	0.00318*
Middle (Normoxia vs. Hypoxia)	0.00031*
Outer (Normoxia vs. Hypoxia)	>0.00001*

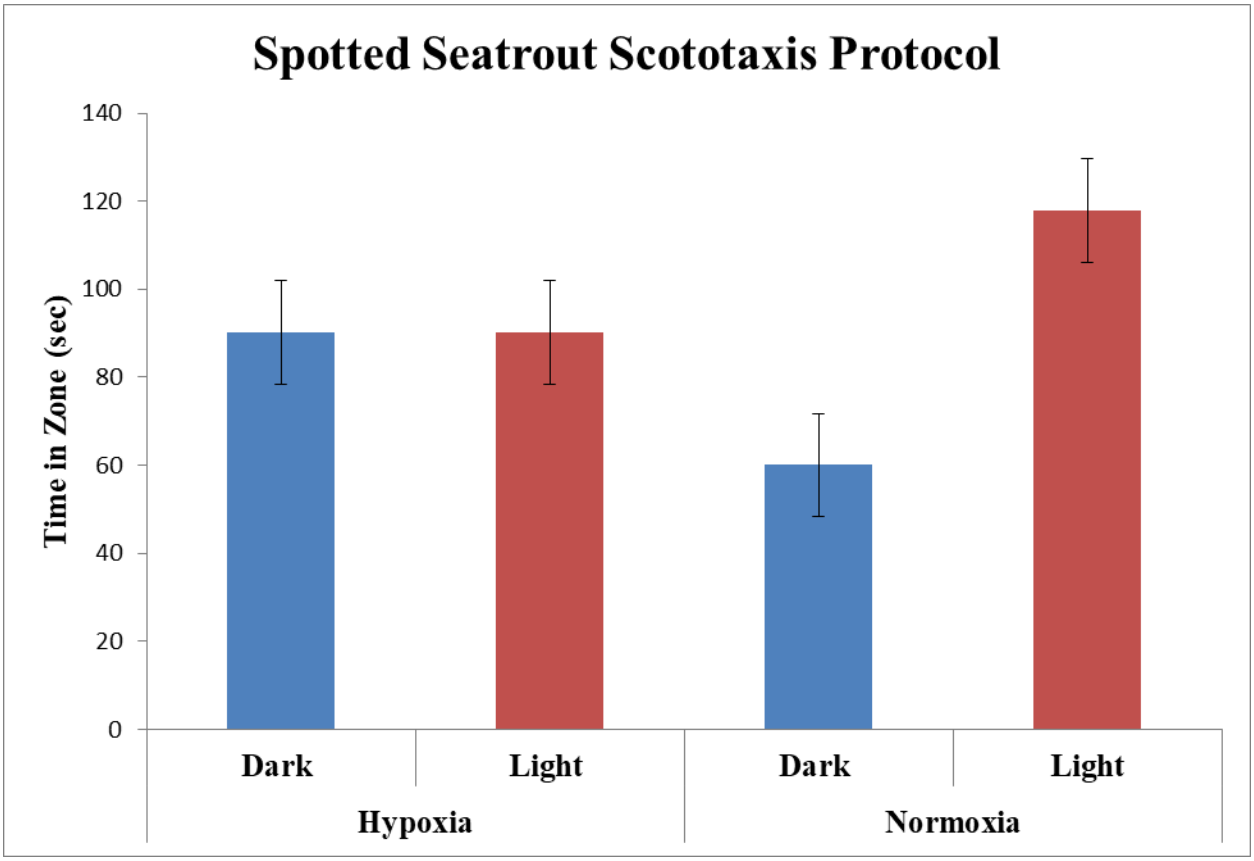


Figure 4.1. The average time spent by fish in zones in the scototaxis protocol. Error bars represent standard error. Fish in hypoxia spent the same amount of time on average in both the light and dark zones (p = 0.22628) where normoxia fish spent significantly more time on the light side (p = 0.00104).

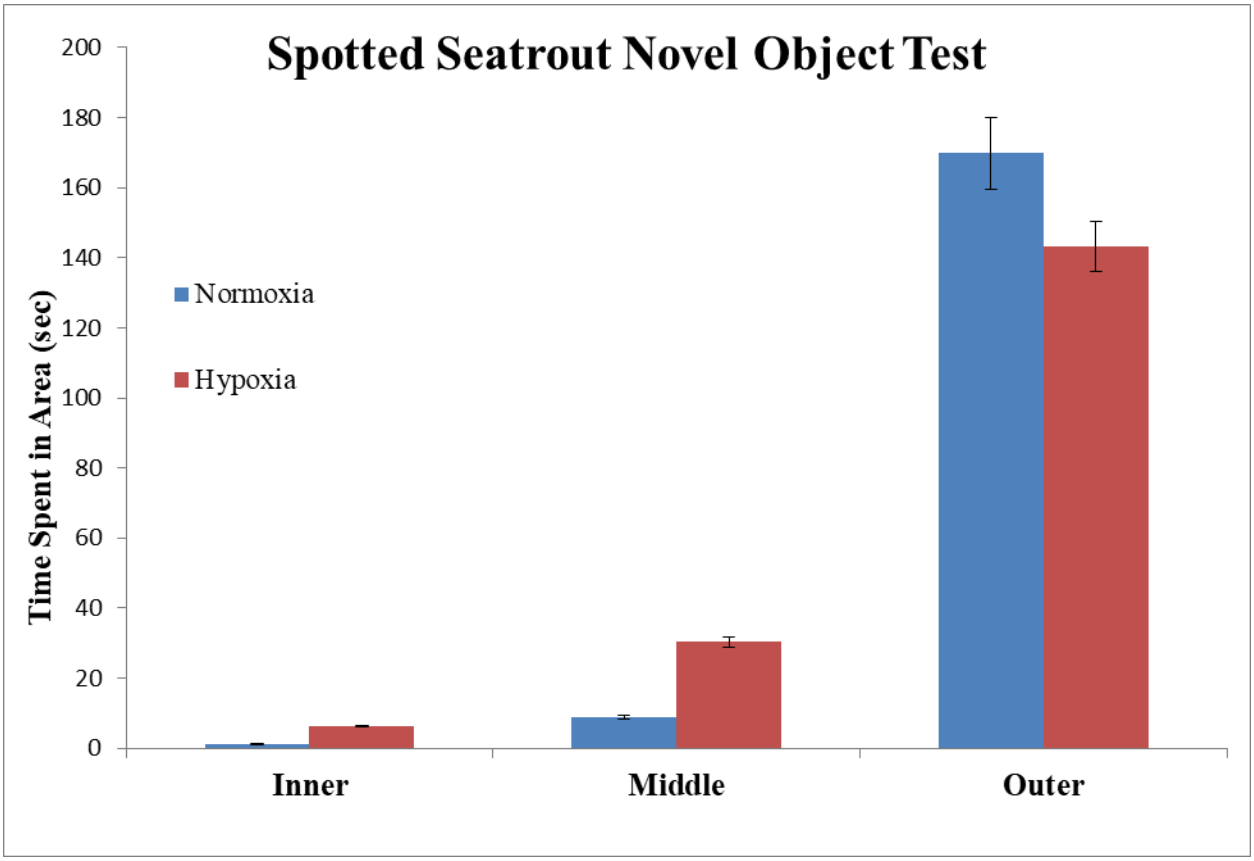


Figure 4.2. Average time spent in each ring of the novel object test by both control and treatmnet fish. Error bars represent standard error. For the outer ring, control fish spent significantly more time here than treatment fish (p = 0.00), but this was reverse for the middle (p = 0.000315) and inner rings (p = 0.00318).

4.1.3. Measurement Data

The averages and standard deviations of each physical measurement can be found in Table 4.3. There were no significant changes in development to spotted seatrout exposed to hypoxia (Table 4.4). Standard lengths were statistically the same. No differences in left and right eye widths or areas were found in within hypoxia or normoxia respectivelyor when comparing dimensions between these two groups.

Table 4.3. The averages and standard deviation of all spotted seatrout measurements.

Parameter	**Normoxia**	**Hypoxia**
Standard Length (mm)	2.25±0.279	2.17±0.374
Right Eye Width (mm)	0.143±0.014	0.143±0.022
Left Eye Width (mm)	0.145±0.016	0.144±0.025
Right Eye Area (mm^2)	0.022±0.005	0.021±0.006
Left Eye Area (mm^2)	0.022±0.006	0.022±0.007

Table 4.4. The *p*-values for standard lengths, eye width, and eye area and *p*-values from ANOVAs for eye width and area between both normoxia and hypoxia. Asterisks denote significant values.

Parameter	***p*-value**
Standard Length	0.50000
Normoxia Right vs. Left Width	0.32768
Hypoxia Right vs. Left Width	0.45339
All Eye Widths	0.98581
Normoxia Right vs. Left Area	0.40714
Hypoxia Right vs. Left Area	0.49177
All Eye Areas	0.99517

4.2. Red Drum Results

4.2.1. Water Quality

The averages and standard deviations of each measured water quality parameter can be found in Table 4.5. With the exception of dissolved oxygen, water quality parameters tested (Temperate, salinity, pH and total ammonia), were found to not vary between the normoxic and hypoxic treatment tanks.

Table 4.5. The average values and standard deviations for each parameter in both control and treatment conditions for red drum along with associated *p*-values. Asterisks denote significant values.

	Average ± Std. Dev		
Parameter	**Normoxia**	**Hypoxia**	***p*-value**
DO	5.89±0.315 mg/L	2.54±0.276 mg/L	< 0.00001*
Temperature	26.30±0.208 °C	26.30±0.209 °C	0.47750
Salinity	24.78±0.696 mg/L	24.80±0.724 mg/L	0.401471
pH	7.97±0.062	7.97±0.044	0.33867
Total Ammonia	0.0446±0.045 mg/L	0.0546±0.031 mg/L	0.24304

4.2.2. Behavioral Results

In the scototaxis testing, red drum showed no preference to either the dark or light side in either normoxia or hypoxia (Figure 4.3). Though there was a slightly higher preference to the dark side on average in normoxia (~95 seconds on average) and a dark side preference in hypoxia (~110 seconds on average), neither were statistically significant compared to the preference of the other respective side. In the novel object test, hypoxia exposed red drum demonstrated no significant difference from the normoxic group in the amount of time spent in the outer, middle, and inner rings (Figure 4.4). The *p*-values for both tests can be found in Table 4.6.

A second set of Wilcoxon-rank sum tests was completed to determine significance between the uses of a dark versus a light background during the novel object tests. For the normoxia inner, middle, and outer rings, the *p*-values were 0.500, 0.299642, and 0.30111, respectively. For the hypoxia, inner, middle, and outer rings, the *p*-values were 0.500, 0.37274, and 0.386151, respectively. Overall, no significance occurred between using a light colored and dark colored background.

Table 4.6. The *p*-values from the *t*-tests run on each of the four scototaxis analyses and three novel object analyses for red drum. Asterisks denote significant values.

Scototaxis Parameters	***p*-value**
Normoxia Light vs. Normoxia Dark	0.44130
Hypoxia Light vs. Hypoxia Dark	0.17702
Normoxia Dark vs. Hypoxia Dark	0.15272
Normoxia Light vs.Hypoxia Light	0.10310
Novel Object Parameters	
Inner (Normoxia vs. Hypoxia)	0.72786
Middle (Normoxia vs. Hypoxia)	0.08914
Outer (Normoxia vs. Hypoxia)	0.11876

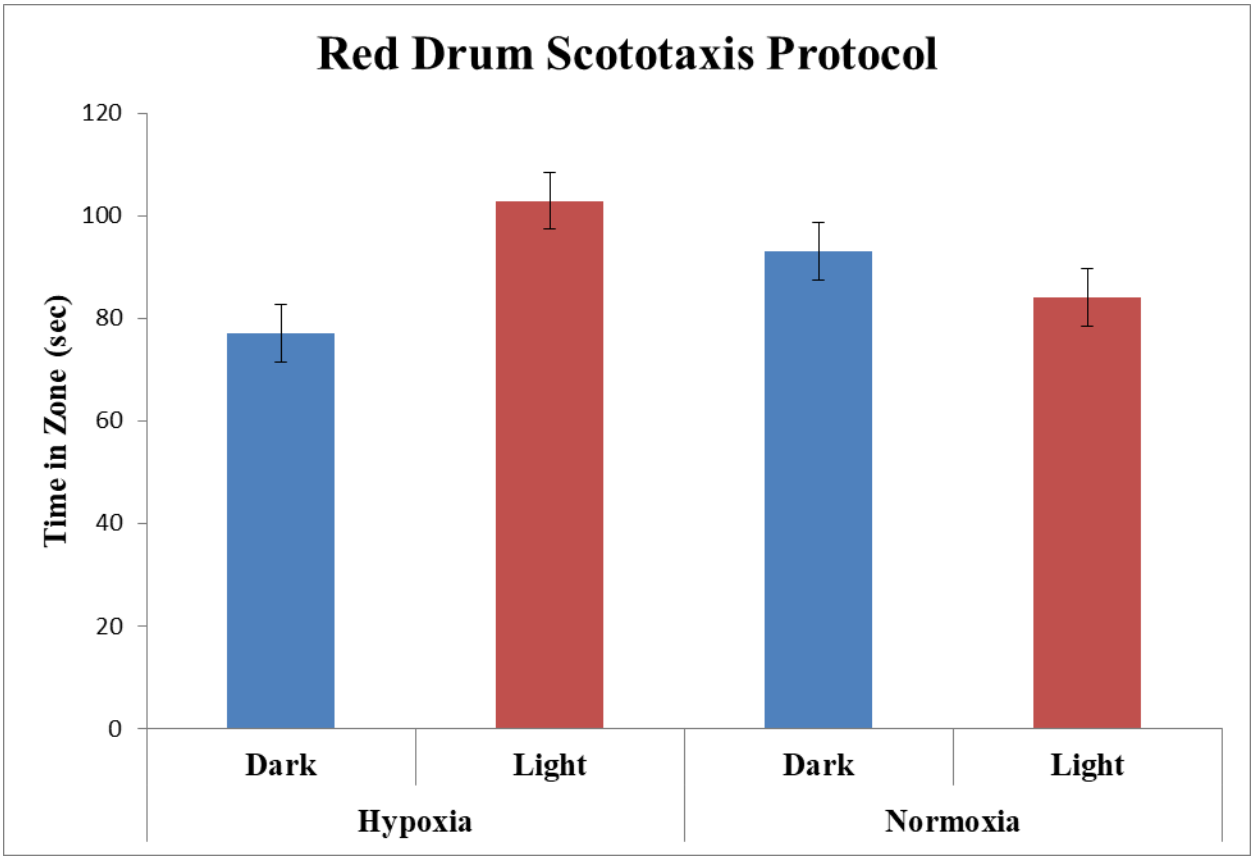


Figure 4.3. The average time spent by fish in both zones in both treatments of the scototaxis protocol. Error bars represent standard error. Overall fish spent a similar amount of time in both the light (p = 0.1031) and dark areas (p = 0.15272) between treatments.

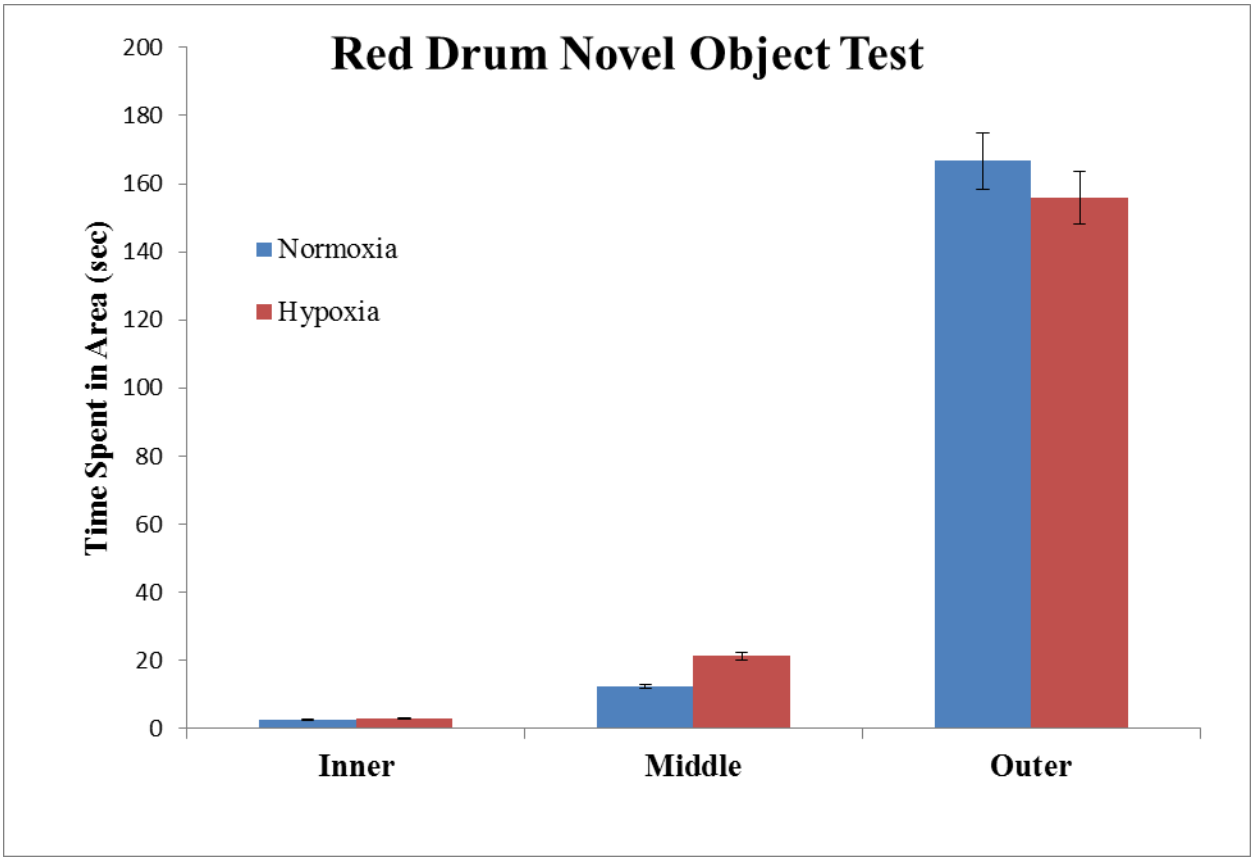


Figure 4.4. Average time spent in each ring of the novel object test by both control and treatment fish. Error bars represent standard error. Between control and treatment, time spent in the outer (p = 0.11876), middle (p = 0.08914), and inner rings (p = 0.72786), was relatively similar.

4.2.3. Measurement Data

The averages and standard deviations of each physical measurement can be found in Table 4.7. In terms of standard length, red drum showed a significant difference in size between normoxia and hypoxia, where hypoxia exposed fish were significantly smaller than normoxia exposed fish (Table 4.8). No differences in left and right eye widths or areas were found in within hypoxia or normoxia respectivelyor when comparing dimensions between these two groups.

Table 4.7. The averages and standard deviation of all red drum measurement

Parameter	**Normoxia**	**Hypoxia**
Standard Length (mm)	2.78±0.383	2.58±0.408
Right Eye Width (mm)	0.176±0.028	0.184±0.034
Left Eye Width (mm)	0.175±0.028	0.185±0.034
Right Eye Area (mm^2)	0.033±0.006	0.035±0.010
Left Eye Area (mm^2)	0.034±0.007	0.022±0.007

Table 4.8. The *p*-values for standard lengths, eye width, and eye area and *p*-values from ANOVAs for eye width and area between both normoxia and hypoxia. Asterisks denote significant values.

Parameter	***p*-value**
Standard Length	0.02616*
Normoxia Right vs. Left Width	0.46583
Hypoxia Right vs. Left Width	0.44766
All Eye Widths	0.48816
Normoxia Right vs. Left Area	0.41468
Hypoxia Right vs. Left Area	0.41871
All Eye Areas	0.82779

4.3. Striped Bass Results

4.3.1. Water Quality

The averages and standard deviations of each measured water quality parameter can be found in Table 4.9. With the exception of dissolved oxygen and total ammonia, water quality parameters tested (Temperate, salinity, pH), were found to not vary between the normoxic and hypoxic treatment tanks. Total ammonia was significantly higher in the hypoxia aquaria than the normoxia aquaria.

Table 4.9. The average values and standard deviations for each parameter in both control and treatment conditions for striped bass along with associated *p*-values. Asterisks denote significant values.

	Average ± Std. Dev		
Parameter	**Normoxia**	**Hypoxia**	***p*-value**
DO	7.43±0.400 mg/L	3.29±0.257 mg/L	< 0.00001*
Temperature	22.28±0.085 °C	22.36±0.659 °C	0.210619
Salinity	0.126±0.008 mg/L	0.123±0.004 mg/L	0.072537
pH	8.03±0.023	8.02±0.024	0.053686
Total Ammonia	0.2411±0.038 mg/L	0.3777±0.084 mg/L	0.000252*

4.3.2. Behavioral Results

For the behavioral trials, I was only able to test half the fish I planned to due to a lack of availability. In the scototaxis protocol, striped bass showed an overwhelming preference for the light side in both normoxic (~150 seconds on average) and hypoxic (~160 seconds on average) conditions, which indicated that no behavioral changes occurred.

In the novel object test, hypoxia exposed striped bass demonstrated no significant difference in amount of time spent in the outer, middle, and inner rings compared to their normoxia counter parts. A second set of Wilcoxon-rank sum tests was completed to determine

significance between the use of a dark versus a light background during the novel object tests. For the normoxia inner, middle, and outer rings, the *p*-values were 0.204011, 0.148499, and 0.327191. For the hypoxia inner, middle, and outer rings, the *p*-values were 0.348409, 0.398743, and 0.341955, respectively. Overall, no significance occurred between using a light colored and dark colored background.

Table 4.10. The *p*-values from the *t*-tests run on each of the four scototaxis analyses and three novel object analyses for striped bass. Asterisks denote significant values.

Scototaxis Parameters	***p*-value**
Normoxia Light vs. Normoxia Dark	0.00022*
Hypoxia Light vs. Hypoxia Dark	0.00000*
Normoxia Dark vs. Hypoxia Dark	0.31732
Normoxia Light vs.Hypoxia Light	0.16152
Novel Object Parameters	
Inner (Normoxia vs. Hypoxia)	0.89656
Middle (Normoxia vs. Hypoxia)	0.77182
Outer (Normoxia vs. Hypoxia)	0.92828

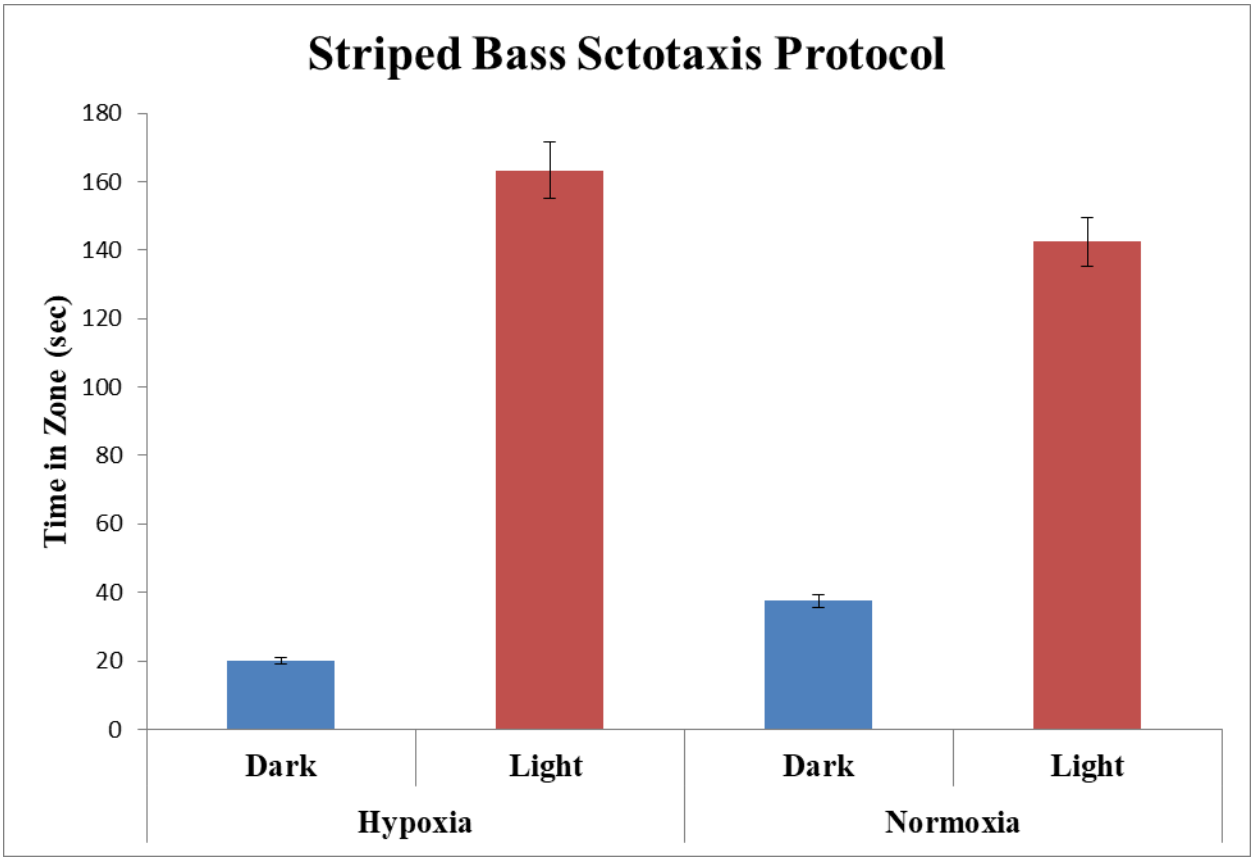


Figure 4.5. The average time spent by fish in both zones in both treatments of the scototaxis protocol. Error bars represent standard error. Overall fish preferred the light area in both the control (p = 0.00022) and treatment (p =0.00).

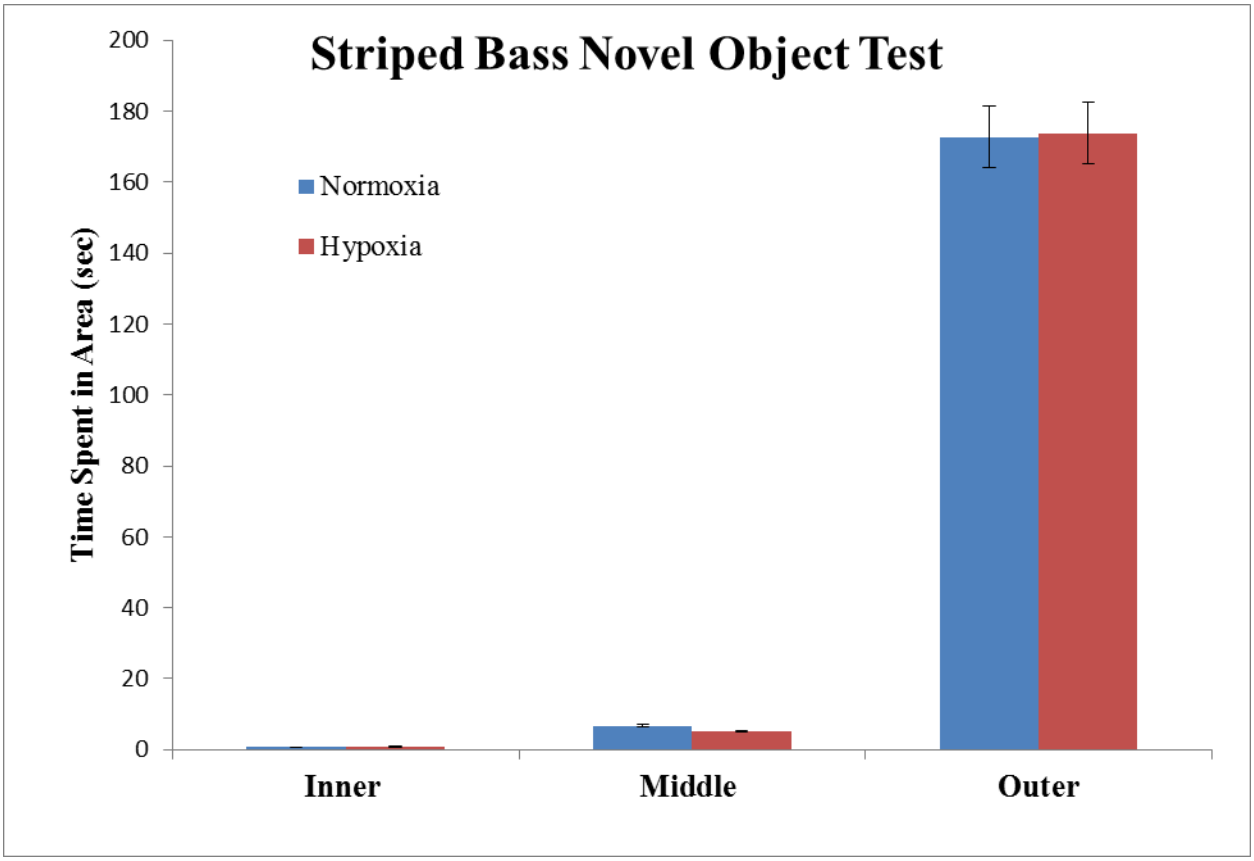


Figure 4.6. Average time spent in each ring of the novel object test by both control and treatmnet fish. Error bars represent standard error. Between control and treatment, time spent in the outer (p = 0.928286), middle (p = 0.77182), and inner rings (p = 0.89656), was relatively similar.

4.3.3. Measurement Data

The averages and standard deviations of each physical measurement can be found in Table 4.11. There were no significant changes in development to striped bass exposed to hypoxia (Table 4.12). Standard lengths were statistically the same. No differences in left and right eye widths or areas were found in within hypoxia or normoxia respectivelyor when comparing dimensions between these two groups.

Table 4.11. The averages and standard deviation of all spotted seatrout measurement data as well as *p*-values for *t*-tests for standard lengths, eye width, and eye area and *p*-values from ANOVAs for eye width and area between both normoxia and hypoxia.

Parameter	**Normoxia**	**Hypoxia**
Standard Length (mm)	5.79±0.464	6.04±0.658
Right Eye Width (mm)	0.254±0.038	0.269±0.035
Left Eye Width (mm)	0.254±0.038	0.267±0.036
Right Eye Area (mm^2)	0.072±0.019	0.080±0.020
Left Eye Area (mm^2)	0.072±0.019	0.080±0.017

Table 4.12. The *p*-values for standard lengths, eye width, and eye area and *p*-values from ANOVAs for eye width and area between both normoxia and hypoxia.

Parameter	***p*-value**
Standard Length	0.02616
Normoxia Right vs. Left Width	0.49375
Hypoxia Right vs. Left Width	0.42527
All Eye Widths	0.35428
Normoxia Right vs. Left Area	0.48133
Hypoxia Right vs. Left Area	0.48441
All Eye Areas	0.35423

CHAPTER V

DISCUSSION

5.1. General Discussion

Maintaining water quality within the sciaenid species' experiments proved to be more successful than striped bass. While both spotted seatrout and red drum are known to be sensitive to hypoxia, the dissolved oxygen concentrations the hypoxia fish were exposed to were high enough to prevent the high mortality that would cause total ammonia levels to rise (Goodman and Campbell 2007). Therefore, they were more or less similar to total ammonia levels in the normoxia aquaria. Striped bass are known to be intolerant of hypoxia in early life stages as well, but I underestimated how much mortality would occur from it during this experiment (Chittenden 1971, Fry 1971, Turner and Farley 1971). I believe the initial high death toll of striped bass in the aquaria set off a positive feedback loop where dead fish caused total ammonia to rise, which then led to more fish dying off, which in turn further exacerbated the continuing mortality caused by hypoxia. It was because of this high mortality that I needed to raise the dissolved oxygen in the striped bass hypoxia aquaria to approximately 3.3 mg/L O_2. Temperature, though consistent between normoxia and hypoxia, could have also been a potential issue. Studies by Secor and Houde (1995) found that larval striped bass cohorts kept in temperatrures over 20°C had increased mortality over those kept between 20°C and 15°C. Regardless, I do not think this had much of an effect since 20°C is the temperature striped bass are spawened and reared at the North Carolina State University facility the fish came from.

The preference of light colored environments favored by spotted seatrout and striped bass indicates that these species feel more secure in better-lit conditions. I hypothesize that this is due to the transparent nature of the larvae that makes them less visible on a light background. Stewart

et al. (2011) and Chen *et al.* (2015) reported a similar preference to light backgrounds, also known as a phototactic preference, in larval zebrafish. Downing and Litvak (2000) found that larval haddock (*Melanogrammus aeglefinus*) survivorship was higher in tanks with lighter backgrounds as it gave the fish better perception to prey. Considering all fish tested were just growing out of their yolk sacs, it is possible that an innate light preference could be the result of the larvae being drawn to areas where possible prey items could be visible and easily captured. On the other hand, red drum showed no significant preference for either side in control conditions ($p = 0.44130$). Recent scototaxis tests performed by Lonthair *et al.* (2017) found that control red drum larvae spent approximately 20-40% of their time in the dark zone, which was slightly lower than the approximately 50% time spent in the dark zone for control red drum in this experiment. Considering red drum larvae do not have an innate preference for either side, it could be that these larvae are more naturally adapted for living in either lighting condition. It is possible that they see the benefit of occasionally entering the light area to look for prey more successfully, but enter the darker and sheltered areas after a while to prevent falling prey themselves.

In all three species, fish in normoxia novel object tests spent most of their time in the outer ring, farthest from the object. In this ring, fish, for the most part, did not swim directly up against the wall in what would be descirbed as a thigmotaxis response (Maximino *et al*. 2010). Since they could not react to their own reflcetion, fish seemed to be keeping what they deemed a safe distance from the novel object while still being able to observe it. In all three species, control fish entered the middle ring and the inner ring, but time spent here was significantly lower than time spent in the outer ring. This was generally due to a few tested fish making quick,

single darts in and out of the center areas. Therefore, it can be said that anxiety-like behaviors in all three species kept them from more through investigation of a potentially dangerous stimulus.

Seatrout showed the most significant change in this behavior. When this occurred, seatrout would continually make quick darts into the center rings, but do so at higher rates. It was noted that occassionally, fish would observe the object from the middle ring briefly, make a quick 1-2 second dart into the inner ring, and quickly return to the outer ring. This occurred at higher frequency the more time passed for some fish tested, which gave the impression that the fish was becoming less anxious about investigating the object over time. This was a similar case with treatment red drum tested; however, none of the changes to their behavior were significantly different from the control. Despite this, the change in behavior of entering the middle ring (p = 0.08914) neared significance. Overall, time spent in each ring between normoxia and hypoxia spotted seatrout (Figure 4.2) and red drum (Figure 4.4) followed a similar pattern, regardless of the results being not significant for red drums. This may indicate that although both species are vulnerable to hypoxia in terms of survival, red drum may be more adaptable to dissolved oxygen conditions when it comes to behavior. I believe that this may due to red drum larvae have been shown to utilize multiple sensory functions (vision, mechanoreception, and hearing) to a higher degree than seatrout, which are more inclined to use mechanoreception (Poling and Fuiman 1999).

Novel object testing on a dark background rather than a light showed no significant differences compared to a light background in all three species. This better supports the idea that the main stimulus in this experiment was the novel object and not the uniform background behind them. If the novel object test were combined with a scototaxis test, as seen in experiments by Jutfelt *et al*. (2013), it is possible that this would not be the case, however.

All three species showed no significant difference in eye width or eye area between control and treatment. There were no significant signs of anopthalmia or micropthalmia between left and right eyes indicating that hypoxia has little effect on eye growth within this short window for larval fish life. Considering seatrout was the only species that illustrated significant behavioral changes in both scototaxis and novel object tests, it can be concluded that these changes were likly not due to visual impairment. If anything, it further supports findings by Poling and Fuiman (1999) that larval seatrout are mechanoreception specialists when sensing the world around them. If this is the case, it is likely that hypoxia somehow altered sensory via mechanorecptors in such areas as the lateral line. Red drum, on the other hand, which are sensory genralists, could avoid hypoxia alteration of mechanoreceptors and instead utilize their unimpaired vision and hearing to better sense the environment around them (Poling and Fuiman 1999, Lonthair *et al*. 2017). This may be a similar scenario for striped bass, but I can not make this conclusion due to their exposure to high ammonia.

In terms of standard length, the only species that showed a significant difference between control and treatment was the red drum ($p = 0.026167$) where hypoxia exposed fish were significantly smaller than normoxia exposed fish. These findings indicate that red drum may be more vulnerable to hypoxia impairments in early periods of growth and development. Recent findings by Pan *et al.* (2016) suggest that larval red drum can aerobially support their metabolism at lower dissolved oxygen levels than their adult counterparts. However, this appears to be limited for baseline functions needed for surival and not nessecarily for proper growth and devlopment (Pan *et al.* 2016). Spotted seatrout larvae were slightly larger after hypoxia exposure, although this wasn't statistically significant and may simply be due to natural variation within the species.

An interesting note from this experiment was that in both sciaenid species, occasional deformed larvae did appear that still had a yolk sac. This was found in both control and treatment groups indicating it can naturally occur even if environmental conditions are optimal. Though these deformed larvae were noted, they were not quantified. Therefore, it is suggested that determining the frequency of this developmental deformity in hypoxia compared to normoxia should be considered. Striped bass standard lengths were similar between control and treatment, but as for all other tests, this does not necessarily mean that they are adapted for growth in hypoxic conditions. This is especially true considering findings by Turner and Farley (1971) and Brandt et al. (2009) that striped bass spawning as well as larval development and condition decreased significantly in any waters less than 4mg/L O_2.

Overall, a lack of significant results in the measurement data may be attributed to not only the length of exposure time, but also the life stage. Hypoxia related deformities found in the studies by Ingalls and Philbrook (1958), Hassell *et al.* (2008), and Bardon-Albaret and Saillant (2016) were all due to exposure during the embryonic stage of the fish, rather than post hatch. This indicates that hypoxia has a higher chance of causing developmental impairments in fish eggs than it does once the larvae are free swimming. Thus, more studies can be focused on hypoxia related developmental impairments in this life stage.

From the conducted experiments and resulting data, I can conclude that spotted seatrout are behaviorally influenced by hypoxia exposure. With no imapairments to eyes, it is unlikley that vision is to blame, which leaves mechanoreception as the possible culprit. As mechanoreception specalists, spotted seatrout larvae sense the world around them utilizing organs such as their lateral line and olfactory (Poling and Fuiman 1999). Thus, it is likley that hypoxia causes devlopmental damage to these systems in seatrout leading to behavioral changes.

Again, red drum likley utilized a wide range of different sensory abilities, such as vision, which seems to be not damaged by hypoxia because no significant changes in eye dimensions were noticed. Thus, behaviorally, red drum showed higher resilience to hypoxia compared to their close relative, the spotted seatrout. This could be the same for striped bass, but these trials should be considered a invalid due to the high total ammonia levels as well as the smaller sample size. With striped bass trials inconclusive, I feel that they should be redone utilizing larger aquaria (considering their larger size compared to the sciaenid larvae). Regardless, all of these tests show that these fish species are all negatively affected by hypoxia in terms of behavior and devlopment to some degree.

5.2. Conclusion and Future Recommendations

I feel this study appropriately shows how both hypoxia can negavtively affect certain fish species and how hypoxia tolerance is related to species of life stage specific adaptations (Engstrom-Ost and Isaksson 2006, Levin *et al.* 2009, Hanke and Smith 2011, Elshout *et al.* 2013, Nelson and Lipkey 2015, Bardon-Albaret and Saillant 2016, Pan *et al.* 2016). While some fish larvae may have low tolerances to hypoxia due to sole dependence on cutaneous respiration, others have high tolerances due to physiological mechanisms that allow their metabolism to function aerobically at lower dissolved oxygen concentrations (Nilsson and Ostlund-Nilsson 2008, Pan *et al.* 2016). This study illustrates that species, such as spotted seatrout, have reduced hypoxia tolerance in their larval stage compared to red drum, regardless of their close taxonomic relationship and habitat.

I feel to further understand behavioral implications associated with larval fish behavior in hypoxia, tests should be completed utilizing tracking software using other important estuarine speices (and striped bass). Besides physically observing the fish in behavioral tests, other

behavioral testing by Hamilton *et al.* (2014), Jutfelt *et al.* (2013), and Tsz Kwan *et al.* (2017) also utilized camera tracking equipment and software to track fish movement more accurately. While this was available for use in this experiment in the form of a Zebracube system, it did not contain the appropriate specifications to be used for fish larvae. Thus, I recommend future studies that use this tracking software along with physical observation. I also believe looking further into how mechanoreception is specifically affected by hypoxia could determine why behavioral changes occur. Studies by Munday *et al.* (2009) using larval clownfish *(Amphiprion ocellaris*) have shown that behavioral changes occur when high pCO_2 disrupt devlopment in olfactory systems. Thus, it is possible that similar effects occur in larval spotted seatrout in hypoxia.

With estuarine and coastal marine water quality being heavily impacted by anthropogenic activity, it is imperative that humans better understand how fish are affected. While hypoxia is known to be a detriment to most fish species, we are just beginning to understand how it changes their behavior. This new insight can allow humans to create better management for effected species and perhaps mitigate some of the alterations we are creating in our coastal systems.

REFERENCES

Alloy, M., Garner, T. R., Bridges, K., Mansfield, C., Carney, M., Forth, H., and Bonnot, S. 2017. Co exposure to sunlight enhances the toxicity of naturally weathered Deepwater Horizon oil to early lifestage red drum (*Sciaenops ocellatus*) and speckled seatrout (*Cynoscion nebulosus*). *Environmental toxicology and chemistry*, *36*(3), 780-785.

Atlantic States Marine Fisheries Commission. 2016. "Spotted Sea Trout." {Accessed 2016 January 10}. http://www.asmfc.org/species/spotted-seatrout

Atlantic States Marine Fisheries Commission. 2016. "Red Drum." {Accessed 2016 July 8}. http://www.asmfc.org/species/red-drum

Atlantic States Marine Fisheries Commission. 2016. "Striped Bass." {Accessed 2016 July 8}. http://www.asmfc.org/species/atlantic-striped-bass

Bardon-Albaret, A. and E. A. Saillant. 2016. Effects of hypoxia and elevated ammonia concentration on the viability of red snapper embryos and early larvae. *Aquaculture*, 459: 148-155.

Beck, M.W., K.L. Heck Jr., K.W. Able, D.L. Childers, D. B. Eggleston, B. M. Gillanders, B. Halpern, C.G. Hays, K. Hoshino, T.J. Minello, R.J. Orth, P.F. Sheridan, and M.P. Weinstein. 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *BioScience*, 51(8): 633-641.

Bencan, Z., D. Sledge, and E.D. Levin ED. 2009. Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety. *Pharmacol. Biochem. Behav*. 94:75-80.

Bindoff N.L., Willebrand J, Artale V, Cazenave A, Gregory J, Gulev S, Hanawa K, Le Quéré C, Levitus S, Nojiri Y, Shum CK, Talley LD, Unnikrishnan A: Observations: Oceanic Climate Change and Sea Level**.** In *Climate Change 2007: The Physical Science Basis Contri- bution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* Edited by: *Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press*; 2007:385-432.

Blaber, S.J.M., D.P. Cyrus, J.J. Albaret, C.V. Ching, J.W. Day, M. Elliot, M.S. Fonseca, D.E. Hoss, J. Orensanz, I.C. Potter, and W. Silvert. 2000. Effects of fishing on the structure and functioning of estuarine and nearshore ecosystems. *ICES Journal of Marine Science*, 57:590-602.

Blanchard, R. J., & D. Blanchard. 1989. Antipredator defensive behaviors in a visible burrow system. *J Comp Psychol*, *103*(1), 70-82.

Boehlert, G.W., and B. C. Mundy.1988. Roles of behavioral and physical factors in larval and juvenile fish recruitment to estuarine nursery areas. *American Fisheries Society Symposium*. 3(5): 51-67.

Borgström, P., J. Strengbom, L. Marini, M. Viketoft, & R. Bommarco, R. 2017. Above-and belowground insect herbivory modifies the response of a grassland plant community to nitrogen eutrophication. *Ecology*, *98*(2), 545-554.

Brady, D. C., & T. Targett. 2010. Characterizing the escape response of juvenile summer flounder Paralichthys dentatus to diel-cycling hypoxia. *Journal of fish biology*, *77*(1), 137-152.

Brandt, S. B., M. Gerken, K. Hartman, & E. Demers, E. 2009. Effects of hypoxia on food consumption and growth of juvenile striped bass (Morone saxatilis). *Journal of Experimental Marine Biology and Ecology*, *381*, S143-S149.

Breitburg, D.L., N. Steinberg, S. DuBeau, C. Cooksey, and E.D. Houde. 1994. Effects of low dissolved oxygen on predation on estuarine fish larvae. *Marine Ecology Progress Series*, 104: 235-246.

Bricker, S. B., C. G. Clement, D. E. Pirhall, S. P. Orlando, and D. R. G. Farrow. 1999. National estuarine eutrophication assessment: A summary of conditions, historical trends, and future outlook. National Oceanic and Atmospheric Administration, Silver Spring, Maryland.

Buckley, L. J. 1984. RNA-DNA ratio: an index of larval fish growth in the sea. *Marine Biology*, *80*(3), 291-298.

Burdick, S. M., & J. Hightower, 2006. Distribution of spawning activity by anadromous fishes in an Atlantic slope drainage after removal of a low-head dam. *Transactions of the American Fisheries Society*, *135*(5), 1290-1300.

Catherall, D. 2003. How fear differs from anxiety. *Traumatology*, 9(2): 76-92.

California Department of Fish and Wildlife. 2017. "Fishing for Striped Bass." {Accessed 5 April 2017}. https://www.wildlife.ca.gov/Fishing/Inland/Striped-Bass.

Chen, F., Chen, S., Liu, S., Zhang, C., & Peng, G. 2015. Effects of lorazepam and WAY-200070 in larval zebrafish light/dark choice test. *Neuropharmacology*, *95*, 226-233.

Chittenden Jr, M. E. 1971. Effects of handling and salinity on oxygen requirements of the striped bass, Morone saxatilis. *Journal of the Fisheries Board of Canada*, *28*(12), 1823-1830.

Cooper, R.U., L.M. Clough, M.A. Farwell, and T.L. West. 2002. Hypoxia-induced metabolic and antioxidant enzymatic activities in the estuarine fish *Leiostomus xanthurus*. *Journal of Experimental Marine Biology and Ecology*, 279: 1-20.

Coutant, C. C. 1985. Striped bass, temperature, and dissolved oxygen: a speculative hypothesis for environmental risk. *Transactions of the American Fisheries Society, 114*(1), 31-61.

Domenici, P., C. Lefrancois, and A. Shingles. 2007. Hypoxia and the antipredator behavior of fishes. *Philosophical Transactions of the Royal Society*, 362: 2105-2121.

Downing, J., N. N. Rabalais, R. J. Diaz, R. J. Zimmerman, J. L. Baker, and T. Pato. 1999. Gulf of Mexico hypoxia: Land and sea interactions. Task Force Report. Council for Agricultural Science and Technology.

Downing, G., and M. Litvak. 2000. The effect of photoperiod, tank colour and light intensity on growth of larval haddock. *Aquaculture International*, *7*(6), 369-382.

Elshout, P.M., D. M. Dionisio Pires, R.S. Leuven, S.E. Wendelaar Bonga, A.J. and Hendriks. 2013. Low oxygen tolerance of different life stages of temperate freshwater fish species. Journal of Fish Biology, 83(1): 190-206.

Engström-Öst, J., and I. Isaksson. 2006. Effects of macroalgal exudates and oxygen deficiency on survival and behaviour of fish larvae. *Journal of experimental marine biology and ecology*, *335*(2): 227-234.

Fry, F. E. J. 1971. The effect of environmental factors on the physiology of fish. *Fish Physiology*, 6: 1-98.

Goodman, L. R., & J. Campbell. 2007. Lethal levels of hypoxia for gulf coast estuarine animals. *Marine Biology*, *152*(1), 37-42.

Hamilton, J., A. Holcombe, and M. Tresguerres. 2014. CO_2 induced ocean acidification increases anxiety in rockfish via alteration of $GABA_A$ receptor functioning. *Proceedings of the Royal Society B*, 281: 1-7.

Hanke, M. H., & K.J. Smith 2011. Tolerance and response of silver perch (Bairdiella chrysoura) to hypoxia. *Aquatic Biology*, *14*(1), 77-86.

Hansen, J., M. Sato, R. Ruedy, K. Lo, D. Lea, & M. Medina-Elizade, 2006. Global temperature change. *Proceedings of the National Academy of Sciences*, *103*(39), 14288-14293.

Hartman, K. J., & S. Brandt. 1995. Trophic resource partitioning, diets, and growth of sympatric estuarine predators. *Transactions of the American Fisheries Society, 124*(4), 520-537.

Hassell, K.L., P.C. Coutin, and D. Nugegoda. 2008. Hypoxia impairs embryo development and survival in black bream (*Acanthopagrus butcheri*). *Marine Pollution Bulletin*, 57: 302-306.

Holt, J., R. Godbout, & C. R. Arnold. 1981. *Effects of temperature and salinity on egg hatching and larval survival of red drum, Sciaenops ocellata*. Texas A & M University, Sea Grant College Program.

Holt, G., & S. Holt. 2000. Vertical distribution and the role of physical processes in the feeding dynamics of two larval sciaenids *Sciaenops ocellatus* and *Cynoscion nebulosus*. *Marine Ecology Progress Series*, 181-190.

Hossein-Javaheri, N., M. Wilkie, W. Lado, & L. Buck. 2017. Stellate and pyramidal neurons in goldfish telencephalon respond differently to anoxia and GABA receptor inhibition. *Journal of Experimental Biology, 220*(4), 695-704.

IPCC Intergovernmental Panel on Climate Change. 2007. Climate change 2007: the physical science basis—contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. In: Solomon S, Qin D, Manning M et al (eds). Cambridge University Press, Cambridge, United Kingdom, p 996

Ingalls, T.H., and F. R. Philbrook. 1958. Monstrosities induced by hypoxia. The New England Journal of Medecine, 259 (12): 558-563

Jenny, J., P. Francus, A. Normandeau, F. Lapointe, M. Perga, A. Ojala, A. Schimmelmann, and B. Zolitschka. 2015. Global spread of hypoxia in freshwater ecosystems during the last t hree centuries is caused by local rising human pressure. *Global Change Biology*, 22(4): 1481-1489.

Jiang, M. H. Chen, Q. Chen, and H. Wu. 2014. Study of landscape patterns of variation and optimization based on non-point source pollution control in an estuary. *Marine Pollution Bulletin*, 87: 88-97.

Jobling, M., S. Peruzzi, & C. Woods. 2010. The temperate basses (Family: Moronidae). In *Finfish aquaculture diversification*: 337-360. CABI, Oxfordshire, UK.

Johansen, J. L., & Esbaugh, A. J. 2017. Sustained impairment of respiratory function and swim performance following acute oil exposure in a coastal marine fish. *Aquatic Toxicology, 187*, 82-89.

Jutfelt, F. K. Bresolin de Souza, A. Vuylsteke, and J. Sturve. 2013. Behaviroal disturbances in a temperate fish exposed to sustained high CO_2 Levels. *PLOS ONE,* 8(6): 1-6.

Khemis, B., C. Audet, R. Fournier, and J. De La Noue. 2003. Early weaning of winter flounder (*Pseudopleuronectes americanus* Walbaum) larvae on a commercial microencapsulated diet. *Aquaculture Research*, 34(6): 445-452.

Kramer, D.L. 1987. Dissolved oxygen and fish behavior. *Environmental Biology of Fishes*, 18(2): 81-92.

Kucera, C. J., C. Faulk, & G. Holt, 2002. The effect of spawning salinity on eggs of spotted seatrout *(Cynoscion nebulosus,* Cuvier) from two bays with historically different salinity regimes. *Journal of experimental marine biology and ecology*, *272*(2), 147-158.

Landry, C. A., S. Steele, S.Manning, S., & A. Cheek, 2007. Long term hypoxia suppresses reproductive capacity in the estuarine fish, Fundulus grandis. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *148*(2), 317-323.

Lang, P. J., M. Davis, & A. Öhman. 2000. Fear and anxiety: animal models and human cognitive psychophysiology. *Journal of affective disorders*, *61*(3), 137-159.

Latour, R., D. Gauthier, J. Gartland, C. Bonzek, K. McNamee, and W. Vogelbein. 2012. Impacts of mycobacteriosis on the growth of striped bass (Morone saxatilis) in Chesapeake Bay. *Canadian Journal of Fisheries and Aquatic Sciences*, 69(2): 247-258.

Lankford, T.E., and T.E. Targett. 1994. Stability of estuarine nursery zones for juvenile weakfish (Cynoscion regalis): effects of temperature and salinity on feeding, growth, and survival. *Marine Biology*, 119: 611-620.

Levin, L.A., W. Ekau, A.J. Gooday, F. Jorissen, J.J. Middlelburg, S.W. A. Naqvi, C. Neira, N.N. Rabalais, and J. Zhang. 2009. Effects of natural and human induced-hypoxia on coastal benthos. *Biogeosciences*, 6, 2063-2098.

Long, Y., J. Yan, G. Song, X Li, X. Li, Q. Li, & Z. Cui. 2015. Transcriptional events co-regulated by hypoxia and cold stresses in Zebrafish larvae. *BMC genomics*, *16*(1), 385.

Lonthair, J., R. Ern, A. Esbaugh, & H. Browman. 2017. The early life stages of an estuarine fish, the red drum (Sciaenops ocellatus), are tolerant to high pCO2. *ICES Journal of Marine Science*, *74*(4), 1042-1050.

Ludsin, S.A. X. Zhang, S.B. Brandt, M.R. Roman, W.C. Boicourt, D.M. Mason, and M. Costantini. 2009. Hypoxia-avoidance by planktivorous fish in Chesapeake Bay: I mplications for food web interactions and fish recruitment. *Journal of Experimental Marine Biology and Ecology*, 381: S121-131.

Matlock, G. 1987. The life history of red drum. *Manual of Red Drum Aquaculture, edited by GW Chamberlain, RJ Miget & MG Haby. Texas Agricultural Extension Service and Sea Grant College Program, Texas A&M University, College Station, Texas*, 1-47.

Maximino, C., T. Marques de Brito, C.A. Dias, A. Gouveia, and S. Morato. 2010. Scototaxis as anxiety-like behavior in fish. *Natural Protocol*. 5(2): 209-216.

McNaughton, N., & P. Corr, P. J. 2004. A two-dimensional neuropsychology of defense: fear/anxiety and defensive distance. *Neuroscience & Biobehavioral Reviews*, *28*(3), 285-305.

Meier, M. 2006. Baltic Sea climate in the late twenty-first century: a dynamical downscaling approach using two global models and two emission scenarios. *Climate Dynamics*, 27(1): 39-68.

Merriman, D. 1941. Studies on the striped bass (Roccus saxatailis) of the Atlantic Coast. U.S. Fish and Wildlife Service Fishery Bulletin 50:1-77.

Moore, S.K. V.L. Trainer, N.J. Mantua, M. S. Parker, E.A. Laws. L.C. Backer, and L.E. Fleming. 2008. Impacts of climate variability and future climate change on harmful algal blooms and human health. *Environmental Health*, 7(Suppl 2):S4.

Munday, P. L., D. Dixson, J. Donelson, G., Jones, M. Pratchett, G. Devitsina, & K. Døving. 2009. Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proceedings of the National Academy of Sciences*, 106(6):1848-1852.

Nelson, J., and G. Lipkey. 2015. Hypoxia tolerance variance between swimming and resting striped bass (*Morone saxatilis*). *Journal of Fish Biology*, 87:510-518.

Nilsson, G., and S. Ostlund-Nilsson, 2008. Does size matter for hypoxia tolerance in fish? *Biology Review*, 83:173-189.

Nixon, S.W., C. A, Oviatt, J. Frithsen, and B. Sullivan. 1986. Nutrients and the productivity of estuarine and coastal marine ecosystems. *Journal of the Limnological Society of Southern Africa*, 12(1-2): 43-71.

NOAA Fisheries. 2017. "Atlantic Striped Bass" {Accessed 2017 June 24]. https://www.fisheries.noaa.gov/species/atlantic-striped-bass.

Ohl, F., Arndt, S. S., & F. van der Staay. 2008. Pathological anxiety in animals. *The veterinary journal*, *175*(1), 18-26.

O'Neil, J.M., T.W. Davis, M.A. Burford, and C.J. Gobler. 2012. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. Harmful Algae, 14: 313-334.

Osse, J. W. M., and J. Van den Boogaart.1995. Fish larvae, development, allometric growth, and the aquatic environment. *ICES Marine Science Symposia*, 201(0): 21-34

Pan, Y, E., Rasmus & A., Esbaugh. 2016. Hypoxia tolerance decreases with body size in red drum *Sciaenops ocellatus*. *Journal of fish biology*. 89.

Peperzak, L. 2003. Climate change and harmful algal blooms in the North Sea. *Acta Oecologica*, 24: S139-S144.

Peters, K., R. McMicheal. 1987. Early life history of the red drum, Scianeops ocellatus (Pisces: Sciaenidae), in Tampa Bay, Florida. *Estuaries*, 10(2): 92-107.

Pichavant, K., J. Persin-Le-Ruyet, N. Le Bayon, A. Severe, A. Le Roux, L. Quemener, V. Maxime, G. Nonnotte, and G. Boeuf. 2000. Effects of hypoxia on growth and metabolism of juvenile turbot. *Aquaculture*, 188: 103-114.

Pihl, L., S. P. Baden, and R. J. Diaz. 1991. Effects of periodic hypoxia on distribution of demersal fish and crustaceans. *Marine Biology*, 108(3): 349-360.

Piraino, M. N., & D. Taylor. 2009. Bioaccumulation and trophic transfer of mercury in striped bass (Morone saxatilis) and tautog (Tautoga onitis) from the Narragansett Bay (Rhode Island, USA). *Marine environmental research*, *67*(3), 117-128.

Poling, K.R., and L.A. Fuiman. 1999. Behavioral specialization in developing scianenids and its relationship to morphology and habitat. *Environmental Biology of Fishes*, 54: 119-133.

Powell, A. B. 2003. Larval abundance, distribution, and spawning habits of spotted seatrout (Cynoscion nebulosus) in Florida Bay, Everglades National Park, Florida. *Fishery Bulletin*, *101*(3), 704-711.

Purcell, J. E. 1985. Predation on fish eggs and larvae by pelagic cnidarians and ctenophores. *Bulletin of Marine Science*, *37*(2), 739-755.

Rabalais, N.N., R.E. Turner, and W.J. Wiseman Jr. 2002. Gulf of Mexico hypoxia, A.K.A. "The Dead Zone". *Annual Reviews of Ecology and Systematics*, 33: 235-263.

Rabalais, N.N., W. Cai, J. Carstensen, D. Conley. B. Fry, X Hu, Z. Quinones-Rivera, R. Rosenberg, C. Slomp, E. Turner, M. Voss, B. Wossel, and J. Zhang. 2014. Eutrophication-driven deoxygenation in the coastal ocean. *Oceanography*, 27(1): 172-183.

Ragge, N. K., I. Subak-Sharpe, & J. Collin 2007. A practical guide to the management of anophthalmia and microphthalmia. *Eye*, *21*(10), 1290-1300.

Ramcharitar, J., D. Gannon, & A. Popper. 2006. Bioacoustics of fishes of the family Sciaenidae (croakers and drums). *Transactions of the American Fisheries Society*, *135*(5), 1409-1431.

Robinson, E., A. Jerrett, S. Black, and W. Davison. 2013. Hypoxia impairs visual acuity in snapper (*Pagrus auratus*). *Journal of Comparative Physiology A*, 199: 611-617.

Roman, C.T., N. Jaworski, F.T. Short. S. Findlay, and R. S. Warren. 2000. Estuaries of the Northeastern United States: Habitat and Land Use Signatures. *Estuaries*, 23(6): 743-764.

Rooker, J. R., S. A. Holt, M. Soto, & G. J. Holt. 1998. Postsettlement patterns of habitat use by sciaenid fishes in subtropical seagrass meadows. *Estuaries*, *21*(2), 318-327.

Row, B. W. 2007. Intermittent hypoxia and cognitive function: implications from chronic animal models. In *Hypoxia and tHe circulation* (pp. 51-67). Springer, Boston, MA.

Row, B. W., R. Liu, W. Xu, L. Kheirandish, & D. Gozal. 2003. Intermittent hypoxia is associated with oxidative stress and spatial learning deficits in the rat. *American journal of respiratory and critical care medicine*, *167*(11), 1548-1553.

Saucier, M., and D. Baltz. 1993. Spawning site selection by spotted seatrout, *Cynoscion nebulosus*, and black drum, *Pogonias cromis*, in Louisiana. *Environmental Biology of Fishes*, 36: 257-272.

Scavia, D., J. Field, D. F. Boesch, R.W. Buddemeier, V. Burkett, D. R. Cayan, & D. J. Reed. 2002. Climate change impacts on US coastal and marine ecosystems. *Estuaries*, *25*(2), 149-164.

Secor, D. H., & E. Houde. 1995. Temperature effects on the timing of striped bass egg production, larval viability, and recruitment potential in the Patuxent River (Chesapeake Bay). *Estuaries and Coasts*, *18*(3), 527-544.

Setzler-Hamilton, E. M., J. Mihursky, W. Boynton, K. Wood, G. Drewry, & T. Polgar. 1980. Striped bass spawning and egg and larval stages. In *Proceedings of the Fifth Annual Marine Fisheries Symposium, Sports Fishing Institute, Washington, DC* (pp. 89-100).

Shoji, J., R. Masuda, Y. Yamashita, and M. Tanaka. 2005. Effect of low dissolved oxygen concentrations on behavior and predation rates on red sea bream *Pagrus major* larvae by the jellyfish *Aurelia aurita* and by juvenile Spanish mackerel, *Scomberomorus niphonius*. *Marine Biology*, 147: 863-868.

Smith, V. H., G. Tilman, and J. Nekola. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental pollution*, 100(1): 179-196.

Sneddon, L.U., V.A. Braithwaite, and M.J. Gentle. 2003. Novel object test: examining nociception and fear in the rainbow trout. *The Journal of Pain*, 4(8): 431-440.

South Carolina Department of Natural Resources. 2015. "SC Marine Stocking Research Program." {Accessed 13 September 2016}. http://www.dnr.sc.gov/marine/stocking/species.html

State of Washington Department of Ecology. 2017. "Effects of Nitrogen." {Accessed 2017 July 10}. http://www.ecy.wa.gov/programs/eap/Nitrogen/Effects.html

Stewart, A., Maximino, C., Marques de Brito, T., Herculano, A. M., Gouveia, A., Morato, S., ... & Kalueff, A. V. (2011). Neurophenotyping of adult zebrafish using the light/dark box paradigm. *Zebrafish neurobehavioral protocols*, 157-167.

Stoneman, A. 2016. The effects of ocean acidification on the otoliths of larval estuarine and marine fish (Master's Thesis). Delaware State University, Dover, Delaware.

Thomas, P., M. Rahman, I. A. Ian, & J. Kummer. 2007. Widespread endocrine disruption and reproductive impairment in an estuarine fish population exposed to seasonal hypoxia. *Proceedings of the Royal Society of London B: Biological Sciences*, *274*(1626), 2693-2702.

Thompson, R.R.J., E.S. Paul, A.N. Radford, J. Purser, and M. Mendl. 2016. Routine handling methods affect behaviour of three-spined sticklebacks in a novel test of anxiety. *Behavioral Brain Research*, 306: 26-35.

Thrash, C., K. Seitz, J. B. Baker, B. Thompson, L. Gillies, N. Rabalais, B. Henrissat, and O. Mason. 2017. Metabolic roles of uncultivated bacterioplankton lineages in the northern Gulf of Mexico" Dead Zone". *American Society of Microbiology*, 8(5):1-17

Tsz Kwan, G. T. Hamilton, and M. Tresguerres. 2017. CO_2-induced ocean cacidification does not affect individual or group bejavior in a temperate damselfish. *Royal Society Open Science*, 4: 170283.

Turner, J. L., & T. Farley. 1971. Effects of temperature, salinity, and dissolved oxygen on the survival of striped bass eggs and larvae. *California Fish and Game*, *57*(4), 268-273.

Urbina, M., M. Forster, and C., Glover. 2011. Leap of faith: Voluntary emersion behaviour and physiological adaptations to aerial exposure in a non-aestivating freshwater fish in response to aquatic hypoxia. *Physiology and Behavior*, 103(2): 240-247.

Vasconcelos, R.P., P. Reis –Santos, A. Maia, V. Fonseca, S. Franca, N. Wouters, M.J. Costa, and H.N. Cabral. 2010. Nursery use patterns of commercially important marine fish species in estuarine systems along the Portuguese coast. *Estuarine, Coastal and Shelf Science*, 86: 613-624.

Vasconcelos, R.P., P. Reis –Santos, V. Fonseca, A. Maia, M. Ruano, S. Franca, C. Vinagre, M.J. Costa, and H. Cabral. 2007. Assessing anthropogenic pressures on estuarine fish nurseries along the Portuguese coast: a multi-metric index and conceptual approach. *Science of the Total Environment*, 374 (2-3): 199-215.

Vaquer-Sunyer, R., and C.M. Duarte. 2008. Thresholds of hypoxia for marine biodiversity. *PNAS*, 105(40): 15452-15457.

Wannamaker, C.M., and J.A. Rice. 2000. Effects of hypoxia on movements and behavior of selected estuarine organisms from the southeastern United States. *Journal of Experimental Marine Biology and Ecology*, 249 (2): 145-163.

Waser, W., and N. Heisler. 2005. Oxygen delivery to the fish eye: root effect as crucial factor for elevated retinal PO_2. *Journal of Experimental Biology*, 208: 20

Yu, X., & Y. Li, 2011. Zebrafish as an alternative model for hypoxic-ischemic brain damage. *International journal of physiology, pathophysiology and pharmacology*, *3*(2), 88.