

Summer 2019

## Thermal and Low Oxygen Tolerance of a Southern Population of Striped Bass (*Morone saxatilis*)

Daniel A. Lleras

Follow this and additional works at: <https://digitalcommons.georgiasouthern.edu/etd>



Part of the [Other Physiology Commons](#)

---

### Recommended Citation

Lleras, Daniel A., "Thermal and Low Oxygen Tolerance of a Southern Population of Striped Bass (*Morone saxatilis*)" (2019). *Electronic Theses and Dissertations*. 1964.  
<https://digitalcommons.georgiasouthern.edu/etd/1964>

This thesis (open access) is brought to you for free and open access by the Graduate Studies, Jack N. Averitt College of at Digital Commons@Georgia Southern. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of Digital Commons@Georgia Southern. For more information, please contact [digitalcommons@georgiasouthern.edu](mailto:digitalcommons@georgiasouthern.edu).

THERMAL AND LOW OXYGEN TOLERANCE OF A SOUTHERN POPULATION OF  
STRIPED BASS (*MORONE SAXATILIS*)

by

DANIEL LLERAS

(Under the Direction of Johanne Lewis)

ABSTRACT

Climate change projections estimate a 2-3°C increase in water temperatures by the end of the century. The amount of habitat with suitable temperature and oxygen concentration for aquatic organisms will also be reduced. Striped bass (*Morone saxatilis*) inhabiting the rivers in Southeastern Georgia make an interesting study system as they do not participate in summer coastal migrations typical of their northern conspecifics. Instead, fish in this southern population remain in freshwater environments that experience warming and associated decreases in dissolved oxygen. The present study aims to determine the thermal and low oxygen tolerance of juvenile striped bass collected from southeast Georgia through the measurement of aerobic metabolic scope (AMS), loss of equilibrium (LOE<sub>crit</sub>), and critical oxygen tension (P<sub>crit</sub>). Fish were acclimated to one of four experimental temperatures (20, 25, 30, and 33°C), representing the range of temperatures typical of the natural environment in the summer as well as the anticipated increase in temperature due to climate change (33°C). Additionally, plasma samples were analyzed for lactate levels to assess the metabolic state of the fish. Results indicate fish acclimated to 30 and 33°C have reduced performance (lower AMS) and low oxygen tolerance (LOE<sub>crit</sub>). The findings of this study determined that southern striped bass are susceptible to projected increases in temperature where an increase of 3°C will push them close to the thermal lethal limit and lower their ability to survive in hypoxic environments.

INDEX WORDS: Metabolism, Thermal tolerance, Hypoxia, Aerobic scope, Striped bass, Climate change, Savannah River

THERMAL AND LOW OXYGEN TOLERANCE OF A SOUTHERN  
POPULATION OF STRIPED BASS (*MORONE SAXATILIS*)

by

DANIEL LLERAS

B.S., Georgia Southern University, 2016

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in

Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

STATESBORO, GEORGIA

© 2019

DANIEL LLERAS

All Rights Reserved

THERMAL AND LOW OXYGEN TOLERANCE OF A SOUTHERN POPULATION OF  
STRIPED BASS (*MORONE SAXATILIS*)

by

DANIEL LLERAS

Major Professor: Johanne Lewis

Committee: Christine Bedore

Jamie Roberts

Electronic Version Approved:

July 2019

## DEDICATION

I dedicate this thesis to my mom for the tremendous love and support throughout my life and educational career;

To my fiancé who has never given up on me and followed me through all of my endeavors;

To my grandmother who I miss dearly and encouraged me throughout all of my life and obstacles.

## ACKNOWLEDGMENTS

I want to thank Dr. Johanne Lewis for the tremendous support, patience, and guidance throughout my entire graduate career along with guidance and assistance throughout the project; Dr. Jamie Roberts and Dr. Christine Bedore for intellectual guidance; Richmond Hill Hatchery for providing the striped bass used for the project; Robbie Deal for providing technical support; Danilo Orellana for assisting with animal care and data collection; Sigma Xi for financial funding towards the project; the Graduate Student Organization for financial funding towards the project and for travel to present my research; the Georgia Southern University department of Biology for funding and support.

## TABLE OF CONTENTS

|  | Page |
|--|------|
| ACKNOWLEDGMENTS.....   | 3    |
| LIST OF TABLES.....  | 5    |
| LIST OF FIGURES.....   | 6    |
| CHAPTER  |      |
| 1 GENERAL INTRODUCTION.....  | 7    |
| Effects of Increased Temperature on Aquatic Poikilotherms.....                                       | 8    |
| Effect of Decreased Oxygen on Aquatic Poikilotherms.....   | 12   |
| Combined Effects of Increased Temperature and Decreased Oxygen on Aquatic<br>Poikilotherms.....      | 16   |
| 2 CLIMATE CHANGE EFFECT ON A SOUTHERN POPULATION OF STRIPED BASS<br>( <i>MORONE SAXATILIS</i> )..... | 18   |
| Materials and Methods.....   | 21   |
| Results.....   | 30   |
| Discussion.....  | 40   |
| REFERENCES.....  | 52   |

LIST OF TABLES

|  | Page |
|--|------|
| Table 2.1: $Q_{10}$ values for RMR between each acclimation temperature..... | 32   |

## LIST OF FIGURES

Page

|   |    |
|---|----|
| Figure 1.1: Temperature effect on AMS as shown by MMR and RMR.....                  | 12 |
| Figure 1.2: Graph of $MO_2$ vs. $PO_2$ used to determine $P_{crit}$ .....           | 15 |
| Figure 2.1: Temperature and dissolved oxygen data from the Savannah River.....      | 21 |
| Figure 2.2: Respirometry experimental set-up.....                                   | 26 |
| Figure 2.3: Rate of oxygen consumption at the different phases of respirometry..... | 26 |
| Figure 2.4: Exercise tank for striped bass.....                                     | 27 |
| Figure 2.5: Results for AMS through RMR and MMR.....                                | 31 |
| Figure 2.6: Results for AMS through RMR and MMR without the 25°C group.....         | 31 |
| Figure 2.7: AMS at the four acclimation temperatures.....                           | 33 |
| Figure 2.8: AMS at the four acclimation temperatures without the 25°C group.....    | 33 |
| Figure 2.9: $LOE_{crit}$ (kPa).....   | 35 |
| Figure 2.10: $LOE_{crit}$ (total time).....   | 35 |
| Figure 2.11: $P_{crit}$ graphs for 12 different individuals.....                    | 37 |
| Figure 2.12: Lactic acid concentrations following $LOE_{crit}$ .....                | 39 |
| Figure 2.13: Lactic acid accumulation rate.....                                     | 39 |

## CHAPTER ONE: GENERAL INTRODUCTION

Climate change projections estimate a 2-3°C increase in aquatic temperatures by the end of the century. The degree of susceptibility to thermal stress depends on the thermal tolerance of an organism such that temperatures above the thermal tolerance range are correlated with reduced performance of various biological activities such as growth and foraging (Storey and Tanino 2012; Rummer et al. 2013). Organisms must occupy temperatures within their thermal range for optimal performance, therefore climate change is expected to affect the distribution of species where those that are less tolerant to changes in temperature are expected to shift northward with increasing temperatures while those with a wider thermal tolerance are expected to expand their range (Woodward et al. 2010; Storey and Tanino 2012). Increases in temperature do not act in isolation and interact with other abiotic factors such as oxygen in complex ways. For example, nutrient run-off from fertilizers used in agriculture results in algal blooms that deplete dissolved oxygen (DO) in freshwater systems (Kernan et al. 2011). DO is depleted even further as oxygen consumption of microbes increases with temperature and results in low oxygen conditions that pose a severe threat to native fauna (Kernan et al. 2011; Storey and Tanino 2012).

Temperature also affects the physical properties of water resulting in reduced oxygen solubility at higher temperatures which can cause thermal stratification of oxygen that further limits the available suitable habitat for freshwater organisms. High temperature and low oxygen can synergistically interact and negatively impact performance of freshwater organisms through a reduction in the ability to tolerate thermal stress as shown by the oxygen and capacity limited thermal tolerance (OCLTT) hypothesis (Pörtner et al. 2017). Lower oxygen availability along with increased oxygen demand at higher temperatures limits the available oxygen for the

performance of biological processes and results in a reduction in performance and survival of freshwater organisms (Pörtner et al. 2017).

### *Effects of Increased Temperature on Aquatic Poikilotherms*

Most freshwater organisms are poikilothermic, meaning that they have no physiological control over their internal temperature, instead their core body temperature matches that of the environment. Similarly, most freshwater organisms are also ectothermic meaning that heat is gained mainly from the environment rather than from internal heat production as observed in homeotherms. Due to this, the rate of most biochemical reactions used to fuel biological processes are directly affected by temperature where reaction rates increase with rising temperatures. Therefore, more oxygen is required to provide energy for basal metabolic reactions required for survival and maintenance of an organism.

Metabolic rate, defined as energy consumption per unit time and accounts for the sum of all the chemical reactions that occur within an organism, represents the energetic cost of sustaining life in an organism and provides valuable information on the effect of temperature on the organism as a whole (Ferreira et al. 2014; Rosewarne et al. 2016). Standard metabolic rate (SMR) is defined as the oxygen consumption ( $MO_2$ ) of a resting, undisturbed, and fasted organism allowed to acclimate to experimental conditions. This rate represents the metabolic oxygen demand required for basal cellular processes such as respiration (Rosewarne et al. 2016; Rummer et al. 2013; Ferreira et al. 2014). Routine metabolic rate (RMR) is also the oxygen consumption of a post-absorptive undisturbed fish but differs from SMR by accounting for minimal activity such as maintaining posture and minimal swimming activity (Rosewarne et al. 2016; Ferreira et al. 2014). Both SMR and RMR are good measures of the basic cost of living in an organism but RMR is more easily measured and likely to be observed in nature than SMR

(Wood 2018). As with most biochemical reactions, SMR and RMR increase by 1.5-2 times per 10°C increases in temperature as shown by the temperature coefficient ( $Q_{10}$ ) of various teleost species signifying a higher cost of life in ectothermic poikilotherms (Figure 1.1.) (McBryan et al. 2013).

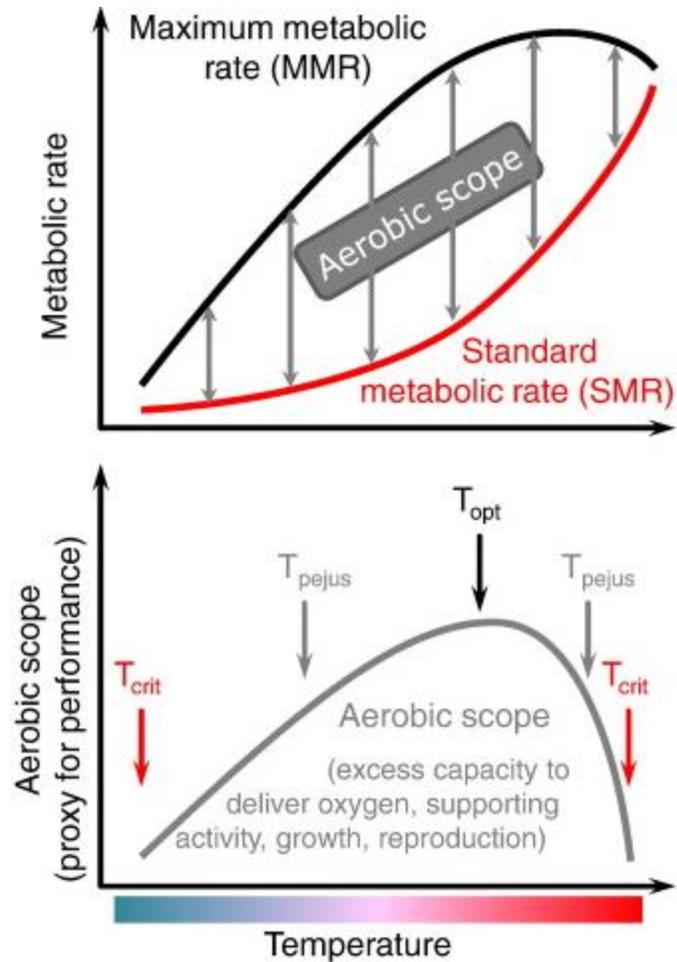
Oxygen consumption must be elevated above SMR in order to obtain energy for non-basal biological processes (i.e. feeding, digestion, activity, growth, etc.) but oxygen consumption is limited by an upper boundary referred to as maximum metabolic rate (MMR) which signifies the maximum aerobic capability of an organism (Rosewarne et al. 2016; Ferreira et al. 2014; McBryan et al. 2013). MMR also increases at  $Q_{10}=1.5-2$  until it is limited by oxygen delivery mechanisms at temperatures above peak MMR, so elevations in SMR with increasing temperature brings oxygen consumption closer to its MMR limit (Figure 1.1.) (Farrell 2015; Rosewarne et al. 2016; Verberk et al. 2016; McBryan et al. 2013). At the temperature where SMR is equal to MMR, aerobic metabolism cannot be maintained, and organisms are forced to switch to anaerobic respiration (lactic acid fermentation) to meet energetic needs. Anaerobic respiration is less efficient than aerobic respiration and survival is time limited due to a limitation on fuel stores and the accumulation of a toxic end product – lactic acid.

The difference between MMR and SMR (or RMR) represents the amount of oxygen available for biological activities beyond the basal oxygen needs and is defined as aerobic metabolic scope (AMS) (Rosewarne et al. 2016; Farrell 2015; Verberk et al. 2016). AMS is used to understand how changes in temperature affect the ability to perform various biological functions essential for survival (McBryan et al. 2013). Therefore, AMS is commonly used to determine how climate change will affect performance in organisms (McBryan et al. 2013; Rosewarne et al. 2016; Farrell 2015; Verberk et al. 2016; Rummer et al. 2014).

Thermal performance curves (Figure 1.1) for AMS provide thermal tolerance values that can be used to determine how temperature change will affect the performance of an organism. At the peak of the curve, aerobic capability for biological processes is maximum. The width of the tolerance curve is representative of the extent of thermal tolerance. Eurythermal organisms, with a wide range of thermal tolerance have wide curves and can occupy a wider variety of habitats. Whereas stenothermal organisms would have a much narrower curve due to their limited thermal tolerance range. As such, stenothermal organisms are more affected by warming events (Storey and Tanino 2012).

The susceptibility of freshwater poikilotherms to changes in temperature also depends on the current temperature they experience in their habitat and the effect that these changes will cause can be observed in several points along a thermal performance curves (Figure 1.1). Temperatures within the thermal optimum ( $T_{opt}$ ) represent the temperature range where AMS is maximized (McBryan et al. 2013; Schulte et al. 2011). If temperatures increase above  $T_{opt}$  into the pejus range ( $T_{pejus}$ ), then significant reductions in AMS occur (McBryan et al. 2013). If temperatures increase above  $T_{pejus}$  to  $T_{crit}$ , AMS reaches zero and the organisms must rely on anaerobic respiration to meet its metabolic demands (Farrell 2015). Temperatures above  $T_{crit}$  are fatal to the organism. Increases of 3°C from climate change may benefit organisms in habitats with temperatures below  $T_{opt}$  by causing a right shift in the temperature performance curve from  $T_{pej}$  (at temperatures below  $T_{opt}$ ) to  $T_{opt}$ . However, organisms already inhabiting temperatures that occur at or above  $T_{opt}$  can shift the organism towards  $T_{pejus}$  or  $T_{crit}$ , respectively, resulting in reduced AMS and an overall reduction in performance (Storey and Tanino; Rummer et al. 2013; Rosewarne et al. 2016; McBryan et al. 2013). These reductions in performance can have significant negative effects on the survival of the population.

Reductions in AMS have shown subsequent reductions in performance of important biological processes including growth, digestion, and activity since more oxygen is required for SMR and less can be attributed to these traits at higher temperatures (Hartman and Brandt 1995; Cox and Coutant 1981; Cook and Bradford 2006). Studies on equatorial fish including blackfin chromis (*Chromis actripectoralis*), lemon damsel (*Pomacentrus moluccensis*), and several others showed significant reductions in AMS and overall performance in fish acclimated to a 3°C increase in summer temperatures resulting from climate change (Rummer et al. 2013). Similarly, African cichlids (*Pseudocrenilabrus multicolor victoridae*) exhibited increased AMS at the highest acclimation with subsequent reductions in critical swimming speed signifying that more energy was attributed to regulating SMR at the cost of reduced activity (McDonnell 2016).



**Figure 1.1.** The effect of temperature on standard metabolic rate and maximum metabolic rate (MMR). The difference between MMR and SMR signifies aerobic metabolic scope (AMS) (Top panel). Thermal performance curve for AMS where the thermal optimum ( $T_{opt}$ ) signifies temperature where AMS is maximized, pejus range ( $T_{pejus}$ ) signifies the temperatures where AMS declines, and critical temperature ( $T_{crit}$ ) signifies the temperatures where AMS=0. Graph obtained from Verberk et al. (2016).

### *Effect of Decreased Oxygen on Aquatic Poikilotherms*

Hypoxia, or oxygen depletion, is an environmental phenomenon where concentrations of dissolved oxygen (DO) in the water decrease to a level that can no longer support aerobic metabolism in organisms (<2-3 mg/L  $O_2$ ). Hypoxic conditions can be caused by low DO solubility from high temperatures, excess nutrient loads resulting in eutrophication, and other

factors which makes it more likely for aquatic poikilotherm to be exposed to fluctuations in environmental oxygen than terrestrial organisms. Prolonged exposure to hypoxia, and subsequently time spent relying on anaerobic means of producing energy, results in tissue damage to organs and is also correlated with reduced performance in organisms.

An organism's ability to withstand hypoxic environments depends on its hypoxia tolerance or ability to extract oxygen from the environment and efficiently supply it to the tissues (Nelson and Lipkey 2015). Fish will typically exhibit various responses to hypoxia. At the behavioral level, fish will exhibit avoidance of hypoxic zones and move towards higher oxygen concentrations (Wannamaker and Rice 2000). At the physiological level, fish may exhibit higher respiration and circulation rates to increase delivery of oxygen to tissues within minutes or hours of exposure to hypoxia (Chen et al. 2015). At the cellular level, fish may exhibit gill remodeling to increase lamellar surface and increase oxygen uptake when exposed to chronic hypoxia (Wu et al. 2017). If the above measures are not sufficient to meet oxygen demand during hypoxia, then the fish will shift from aerobic to anaerobic respiration. Anaerobic respiration sets an important biochemical breakpoint used to commonly estimate the hypoxia tolerance of an organism.

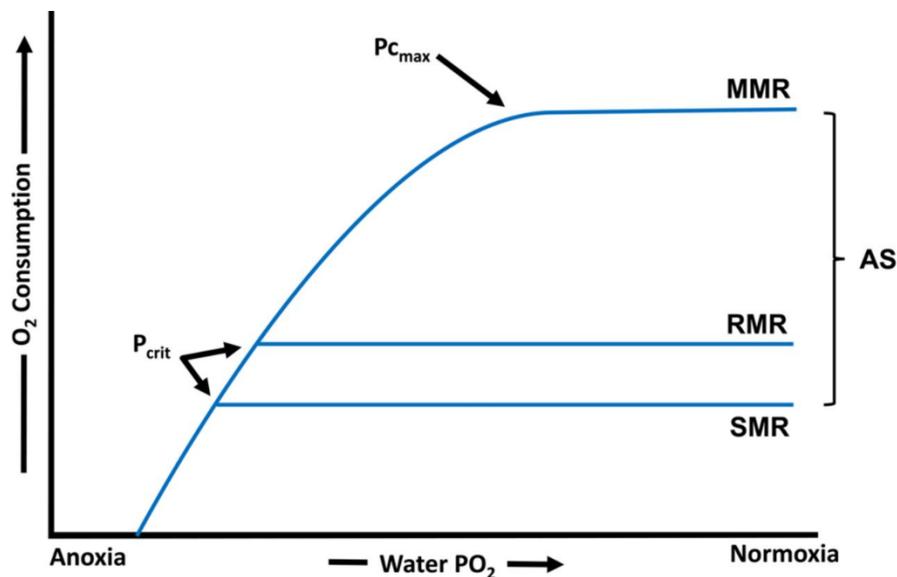
Three common parameters used to measure hypoxia tolerance include determining the point of loss of equilibrium (LOE), critical oxygen tension ( $P_{crit}$ ), and the point at which organisms switch to anaerobic metabolism (most commonly assessed by increased lactate production). LOE is the  $PO_2$  (partial pressure of oxygen which signifies the oxygen concentrations of water) where the fish fails to maintain an upright position in the water column. This loss of equilibrium is due to a shortage of oxygen delivery to the body tissues and if the environment is not rapidly reoxygenated this level of environmental oxygen will be fatal to the organism (He et al. 2015). The most commonly observed measures for LOE in literature are

LOE<sub>crit</sub> and LOE<sub>50</sub> (Wood 2018). Both measurements of LOE involve gradual reductions in DO either through nitrogen gas or closed loop respirometry. LOE<sub>50</sub> measures the PO<sub>2</sub> or total time until 50% of the fish sampled lose equilibrium (Babikian et al. 2017) while LOE<sub>crit</sub> measures the PO<sub>2</sub> or total time until loss of equilibrium occurs in each individual fish (He et al. 2015). Regardless of the methodology, as the tolerance of the organism to hypoxia increases, the value for LOE<sub>crit</sub> or LOE<sub>50</sub> will decrease.

Determination of hypoxia tolerance via estimation of P<sub>crit</sub> relies on estimating the PO<sub>2</sub> at which fish can no longer regulate their oxygen consumption (oxy-regulation) and oxygen consumption decreases with water PO<sub>2</sub> below this point (oxy-conformation) (He et al. 2015; Rogers et al. 2016). To estimate P<sub>crit</sub> the oxygen consumption or metabolic rate of the organism is plotted against the oxygen availability in the environment (Figure 1.2). Higher P<sub>crit</sub> is associated with lower hypoxia tolerance and there are several physiological and ecological points along a MO<sub>2</sub> vs. PO<sub>2</sub> curve used to determine P<sub>crit</sub> that are also relevant to AMS (Figure 1.2.). At P<sub>crit-max</sub>, MMR is limited by availability of ambient DO and begins decreasing proportionally with PO<sub>2</sub> which represents the PO<sub>2</sub> where AMS is initially compromised by hypoxia (Rosewarne et al. 2016; Rogers et al. 2016). At P<sub>crit-RMR</sub>, RMR begins decreasing proportionally with PO<sub>2</sub> and resembles the point where fish switch from oxy-regulation to oxy-conformation (Rogers et al. 2016). At P<sub>crit-SMR</sub>, AMS reaches zero and fish are unable to regulate SMR resulting in a shift to anaerobic respiration (Rogers et al. 2016 and Rosewarne et al. 2016).

Additional methods to estimate hypoxia tolerance use biochemical markers to indicate the level of ambient DO at which the fish is forced to switch to anaerobic means of energy production. Lactate accumulation following hypoxia exposure can be used to determine efficiency in regulating SMR before shifting to anaerobic respiration (Speers-Roesch et al 2013;

Wood 2018). Another common tissue biomarker measured is tissue glycogen. As carbohydrates are the sole fuel source under anaerobic conditions, a decrease in glycogen indicates an increased reliance upon glycolysis coupled with lactic acid fermentation for ATP production. As such, organisms with larger glycogen stores are typically more hypoxia tolerant since higher rates of lactic acid fermentation occur towards ATP production during anaerobic respiration (Speers-Roesch et al. 2013). Typically, two or more of the above methods of analysis are combined to provide a detailed description of the physiological and ecological effects of hypoxia on an organism.



**Figure 1.2.** Plot of oxygen consumption ( $MO_2$ ) vs. water partial pressure of oxygen ( $PO_2$ ) during hypoxia exposure in relation to aerobic scope (AS) determined as the difference between maximum metabolic rate (MMR) and standard metabolic rate (SMR). Maximum critical oxygen tension of oxygen ( $P_{cmax}$ ) represents the  $PO_2$  where AS is initially limited by hypoxia through reductions in MMR. Critical oxygen tension ( $P_{crit}$ ) resembles the  $PO_2$  where routine metabolic rate cannot be maintained and oxy-conformation occurs.  $PO_2$  below  $P_{crit}$  signifies the transition from aerobic to anaerobic respiration. Figure obtained from Rogers et. al (2016).

*Combined Effects of Increased Temperature and Decreased Oxygen on Aquatic Poikilotherms.*

Because DO decreases as temperature increases projected increases in temperature from climate change will inevitably lead to subsequent reductions in DO in aquatic environments. The interaction between low DO solubility and high temperatures poses the risk of a reduction in suitable aquatic habitats where cooler, deeper waters hold less oxygen than the warmer and more oxygenated surface limiting fish to an intermediate area with suitable oxygen and temperature. Therefore, it is proposed that fish are forced in thermal refuges during the summer where they will typically aggregate around cool thermal refuges when exposed to hypoxia, and the limitation in suitable habitat makes the fish more susceptible to disease, predation, and other crowding events (Coutant 1985).

Furthermore, MMR is limited by reductions in DO (Figure 1.2.) and SMR increases with supra-optimal temperature as metabolic reactions increase (Figure 1.1.) so a combination of hypoxia and high thermal stress exhibits a significant reduction in AMS and overall thermal tolerance as described by the oxygen and capacity limited thermal tolerance (OCLTT) hypothesis (McBryan et al. 2013; Rosewarne et al. 2016; Cook et al. 2006; Rogers et al. 2016; Farrell 2015). Similarly, regulating SMR becomes more costly at elevated temperatures leading to earlier onset of anaerobic respiration and lower hypoxia tolerance (Cook et al. 2006). However, some fish that are acclimated to warmer environments may show increased hypoxia tolerance since acclimation will give allow changes at the cellular level to increase oxygen uptake, delivery, and reduce energy demand (McBryan et al. 2016; Raby et al. 2016; Healy and Schulte 2012). The effect of temperature acclimation and hypoxia tolerance typically depends on the species, but less tolerant

species exhibit elevated RMR,  $LOE_{crit}$  and  $P_{crit}$  along with reduced MMR at high thermal and hypoxic conditions (Yang Yang et al. 2015; He et al. 2015; Del-Toro Silva et al. 2008).

Projected increases of 3°C in aquatic environments pose possibilities of high thermal and subsequent hypoxic stress on aquatic poikilotherms (IPCC 2007; Rummer et al. 2013; Rosewarne et al. 2016). Rising temperatures will have many negative impacts on overall performance through reductions in AMS which will be synergistically exacerbated by hypoxia resulting in both reduced thermal and hypoxia tolerance as shown by the OCLTT hypothesis (Rosewarne et al. 2016; Cook et al. 2006; Rogers et al. 2016; Farrell 2015). Fish acclimated to higher temperatures are therefore expected to exhibit lower AMS along with higher  $P_{crit}$  and  $LOE_{crit}$ . Freshwater fish will most likely rely primarily on avoidance behavior which may be limited by a reduction in suitable habitat through a temperature and oxygen squeeze (Coutant 1985). Limitations in habitat further pose a threat of crowding events which can have significantly negative implications on fish populations. Therefore, it is important to understand how climate change will impact the survivability of fish species in future generations to better guide conservation efforts.

## CHAPTER TWO: CLIMATE CHANGE EFFECT ON A SOUTHERN POPULATION OF STRIPED BASS (*MORONE SAXATILIS*)

Striped bass (*Morone saxatilis*) are a species of piscivorous fish whose geographic range extends along the North American Atlantic coast from the St. Lawrence River and the Bay of Fundy in the north to the St. John's River and the Gulf of Mexico in the south (COSEWIC 2012; Coutant 1985). Along this range striped bass are traditionally divided into two populations, northern and southern, where the dividing line occurs in Cape Hatteras, North Carolina (Coutant 1985; Dudley et al. 1977). The northern population includes all the groups present northward of Cape Hatteras to the St. Lawrence River in Canada and are mainly characterized by anadromous behavior and northerly coastal migrations during the summer to avoid high thermal stress in freshwater ecosystems (Coutant 1985; Dudley et al. 1977). The southern population which includes all the populations present from Cape Hatteras to the St. John's River and Gulf of Mexico do not exhibit anadromous behavior but mainly remain in estuaries and freshwater ecosystems. The differences in migratory behavior in southern populations may be attributed to higher ocean temperatures relative to estuary and river temperatures which limit survival of striped bass during the summer (U.S. and Wildlife Service 1983; Bjorgo et al. 2000; Dudley et al. 1977).

Together, temperature and DO play a significant role in habitat selection and survival of striped bass (Coutant 1985 and Kraus et al. 2015). Coutant's temperature-oxygen squeeze hypothesis states that temperature and oxygen synergistically confine striped bass into limited areas of habitat with suitable temperature and DO during the summer (Coutant 1985). As water temperatures increase, surface water temperatures reach supra-optimal temperatures and DO in

the surface (where DO is highest) becomes inaccessible to striped bass. Similarly, DO is lower in deeper waters and may be insufficient for optimal aerobic respiration to occur.

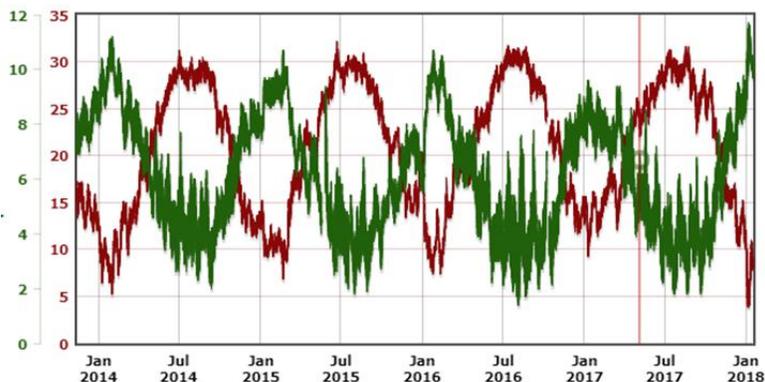
Confinement into smaller thermal refuges makes the striped bass more susceptible to mortality events associated with crowding such as rapid spread of disease, predation, overfishing, etc. (Coutant 1985; Kraus et al. 2015; Lapointe 2014; Bettoli 2013; Hedrick et al. 1987). For example, thermal and hypoxic stress are correlated with higher prevalence of mycobacteriosis in adult striped bass which results in observed reductions in AMS and  $P_{crit}$  (Lapointe 2014). Crowding can further intensify transmission of the infection and put the fish at higher risk of contracting the bacteria (Lapointe et al. 2014; Hedrick et al. 1987). Similarly, anthropogenic effects that damage these thermal refuges pose a severe threat to striped bass restricted to limited refuges as has been observed in past mortality cases where reductions in DO below 2 mg/L along a thermal refuge in Lake Norman caused significant mortalities in the summer (Coutant 1985; Thompson and Rice 2013; Bettoli 2013).

Climate change is projected to influence striped bass habitat and distribution where the effect it will have depends on latitude and whether the population is anadromous or not (Coutant 1990). An overall increase from one to three months of unsuitable conditions during the summer months with supra-optimal temperatures and hypoxic conditions is projected to occur along habitats occupied by non-anadromous southeastern populations (Coutant 1990). Fresh water habitats of these populations are already restricted by high temperatures and low oxygen availability during the summer so rising temperatures and longer summers pose a significant threat to these ecosystems (Coutant 1990). The southeastern populations are therefore the most susceptible to climate change and understanding their abiotic requirements is essential in their future conservation in the inevitable reality of climate change (Coutant 1990).

The current study focuses on a non-anadromous population of striped bass found along the Savannah River in Southeastern Georgia, USA which are stocked from a population naturally found along the Coosa River in Alabama. The Back River, which occurs closest to the mouth of the Savannah River, serves as the main spawning site for adults and is an important nursery site for young of the year and juvenile striped bass (Reinhert 2004; Dudley et al. 1977). During the summer, temperatures in the Back River exhibit daily average minimum and maximum temperatures of 25 and 30°C, respectively, with mean temperatures of 29°C (U.S. GEOL Survey). Concentrations of DO range from 4.3-7 mg/L with an average DO of 5.4 mg/L but can drop as low as 2.0 mg/L during the summer (Figure 2.1) (U.S. GEOL Survey). Research on various populations of striped bass shows that they prefer temperatures  $\leq 25^{\circ}\text{C}$  and DO concentrations  $\geq 3$  mg/L (Coutant 1985; Kraus et al. 2015; Mohan et al. 2014). Maximum summer temperatures in the Back River exceed the optimal range of juveniles (24-28°C) observed in northern populations of striped bass (Duston et al. 2004; Cox and Coutant 1981; U.S. and Wildlife Service 1983) posing the question whether temperature is limiting the success of juveniles along the Savannah River, and whether projected increases of 3°C resulting from climate change will threaten the future success of this population.

The current study aimed to determine the thermal tolerance of juvenile striped bass in relation to current thermal ranges experienced in the Savannah River, how projected increases of 3°C from climate change will affect their thermal tolerance, and the effect of current and projected temperatures on hypoxia tolerance. I hypothesized that thermal and hypoxia tolerance will be negatively affected by acclimation to increased temperatures. I predicted that i)  $T_{\text{opt}}$  occurs at temperatures  $\leq 20^{\circ}\text{C}$ , ii) AMS will be significantly reduced at elevated temperatures (30 and 33°C), and iii) a decrease in hypoxia tolerance which is demonstrated by an increase in

LOEcrit and  $P_{crit}$  at higher acclimation temperatures. These physiological indicators of decreased hypoxia tolerance will be supported by biochemical indicators of a switch to anaerobic metabolism at a higher  $PO_2$  (increased lactate production).



**Figure 2.1.** Data and graph obtained from the U.S. Geological Survey from 2014 to 2018 where temperature is shown in red and DO in green. Temperatures for the summer are significant since they exceed the observed optimal thermal range (24-28°C) of juveniles striped bass (*Morone saxatilis*). Data was collected from the Back River section of the Savannah River in Savannah, Georgia from gauge number 0219897945.

## MATERIALS AND METHODS

### *Animal Collection & Holding Conditions*

Juvenile striped bass ( $n=30$ ) were acquired from the Richmond Hill Hatchery (Richmond Hill, Georgia, USA). The fish were held in aerated, 100-gallon aquariums in the aquatic animal facility at Georgia Southern University and allowed to acclimate to room temperature water (20°C) with a salinity of 6ppt for at least 2 weeks prior to experimentation. Fish were exposed to a 12:12 light/dark photoperiod cycle. They were fed cichlid pellets daily (except 24 hours prior to experimentation) and a water change of 30-40% was conducted twice a week. Mean fish weight was  $176.17 \pm 8.44$ g and ranged from 107.95-252.50g. All experimental protocols were

approved by the Georgia Southern University Animal Care Committee in accordance with policies by the Institutional Animal Care and Use Committee (IACUC protocol #I17011).

### *Experimental Design*

#### *a) Temperature Acclimation*

Overall sample size was determined by availability of subjects where a portion of the fish (26) were separated into four different treatment groups and each group was exposed to one of four different water temperatures (20, 25, 30, or 33°C). The first temperature (20°C, n=6) was the typical room temperature of the aquatic facility and was the temperature at which the holding tank for the striped bass was maintained. The second (25°C, n=6) and third (30°C, n=6) temperatures were selected based on temperature ranges from the U.S. Geological Survey gauge collected on the Back River section of the Savannah River (gauge number 0219897945) over a three-year time span from 2014 to 2016 (US GEOL Survey). The second temperature (25°C) was the lowest mean daily temperature in the selected summer months (June, July, and August), and the third temperature (30°C) was the highest mean daily temperature in the selected summer months. The fourth temperature (33°C, n=8) was selected using anticipated increases in water temperature (3°C) as a result of global warming. During experimentation, there were 1 and 4 mortalities in the 25 and 33°C acclimation temperature groups, respectively. The sample size for the data set was n=5 and n=4 for the 25 and 33°C, respectively. At the onset of each acclimation period, water was heated at a rate of 0.5°C per day to the test temperature. Fish were held at the set temperature for 15-24 days (average 19 days) to allow them to acclimate to the temperature change.

b) *Maximum and Routine Oxygen Consumption*

Intermittent-flow through respirometry was employed to measure dissolved oxygen (mg/L) in 10 second intervals using Pyro Oxygen Logger V3. 213. The respirometry set-up (total of 4.76 Liters in system) followed the design of Svendsen et al. 2015 and consisted of a chamber where the fish were placed, flush and recirculation pumps to circulate water through the system, a FireSting O<sub>2</sub> sensor, and tubing to interconnect the system (Figure 2.2.). The flush pump allowed aerated water to enter and leave the system while the recirculating pump kept water circulating within a closed loop. When both pumps were running, water ran through the closed/recirculating system and the open/flush system. Turning off the flush pump allowed water to circulate within the closed loop without allowing aerated water from the tank to enter the system. This allowed the fish to naturally bring down DO to measure oxygen consumption.

The intermittent-flow through respirometry followed three phases in the following order: flush, wait, and measurement (Figure 2.3.). In the flush phase (4 minutes), both pumps were connected, and oxygen levels balanced out with those from the tank. In the wait phase (2 minutes), oxygen levels dropped slightly. In the measurement phase (4 minutes), oxygen levels linearly declined as they were consumed by the fish. The initial (prior to the fish being placed in the chamber) and final (after fish was removed from chamber following LOE) background respiration rates were measured (10 minutes) to compensate for oxygen consumption from microbial activity that may have accumulated during experimentation. Background respiration rate was determined under the assumption that background oxygen consumption accumulated linearly between the initial and final background measurements (Svendsen et al. 2015).

For each individual experimental trial, one fish was removed from its acclimation tank, moved to another 20-gallon tank (with aerated water at test temperature), and manually chased as

described in Figure 2.4. until exhaustion (~5 minutes) occurred. Fish were considered exhausted when they did not respond to touch or were unable to increase speed to escape touch. To ensure exhaustion, fish were exposed to 1 minute of air while they were weighted using a standard gram scale and were immediately transferred to the respirometry chamber to measure MMR for 4 minutes following the respirometry protocol outlined above. The flush pump was activated and the fish remained undisturbed in the chamber for at least 12 hours following the MMR measurement. The walls of the 40-gallon tank holding the respirometry chamber were covered in black plastic bags and noise levels were kept to a minimum to prevent the fish from being disturbed. All trials were initiated at approximately the same time of day (5:00 pm Eastern Time) to prevent any differences due to diurnal patterns in metabolic rate. After 12 hours, a measurement of RMR was obtained over a 4 minute measurement period following the respirometry protocol outlined above.

Both RMR and MMR were calculated using the following formula:

$$y = \frac{[\beta(K_1 * (V - M)) - (K_2 * V)]}{M}$$

Where  $y$  is  $\text{MO}_2$  (kPa  $\text{O}_2/\text{h}/\text{kg}$ ) corrected for fish mass, background respiration, time between measurements, and volume of the chamber.  $\beta$  is the oxygen solubility coefficient (mg  $\text{O}_2/\text{L}/\text{kPa}$ ),  $K_1$  is the rate of oxygen consumption (kPa/hour) during the 4 minute MMR or RMR measurement,  $V$  is the volume of the chamber (Liters),  $M$  is the mass of the fish (kg), and  $K_2$  is the estimated background consumption rate (kPa/hr).  $K_2$  was calculated as:

$$K_2 = (K_i + K_f) * t$$

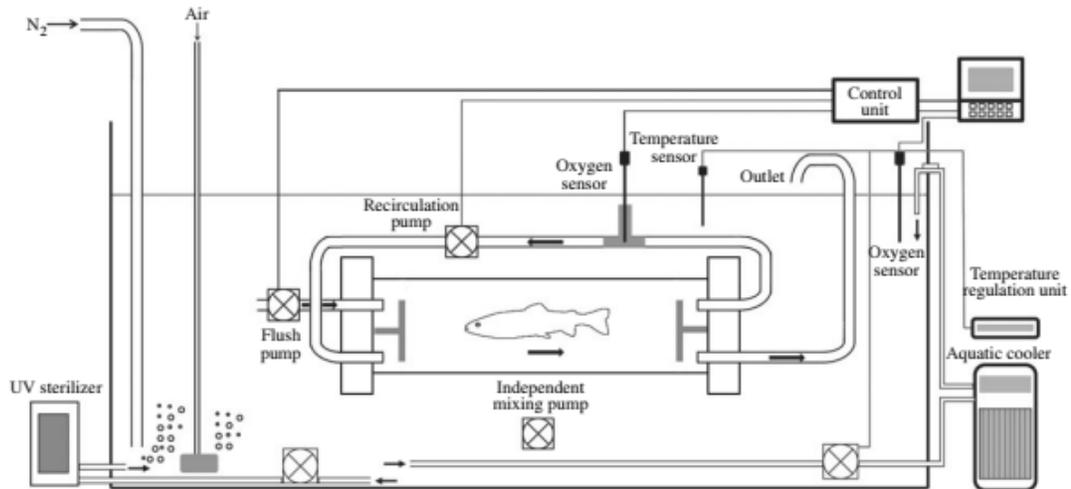
Where  $K_i$  is the initial background respiration,  $K_f$  is the final background respiration and  $t$  is the time of oxygen consumption measurement since the initial background measurement.

Temperature coefficients ( $Q_{10}$ ) were calculated using the following formula:

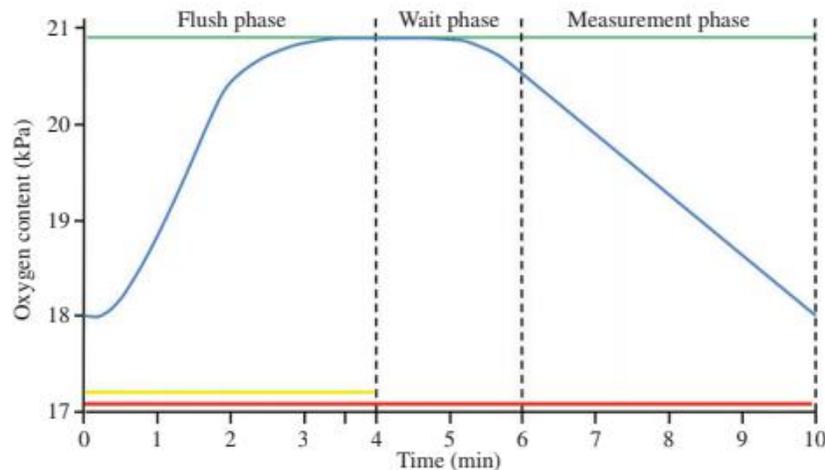
$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{T_2 - T_1}}$$

Where  $R_1$  and  $R_2$  are the mean RMR at the lower and higher temperatures, respectively.  $T_1$  and  $T_2$  correspond to the lower and higher acclimation temperature, respectively. RMR at all temperatures were compared with each other.

Aerobic scope (mg O<sub>2</sub>/h/kg) was calculated as the difference between maximum and routine metabolic rate.



**Figure 2.2.** Set-up of the intermittent-flow through respirometry chamber where arrows describe the movement of water. Water enters the system through the flush pump, leaves through the outlet, or recirculates within the system through the recirculation pump. Dissolved oxygen is measured as water flows through the oxygen sensor. Oxygen consumption is measured when the system is closed (flush “off”) and oxygen reductions are measured as the fish consumes oxygen in the water flowing through the closed system. Figure obtained from Rosewarne et al.(2016).



**Figure 2.3.** Changes in  $O_2$  content at the different phases of intermittent flow respirometry. During the flush phase, water is entering the system from the tank through the flush pump and the respirometry system is open. During the wait phase, the system is closed (flush pump “off”) and oxygen levels are stabilized within the closed system. During the wait phase oxygen levels decrease as they are consumed by the fish within the chamber. Figure obtained from Rosewarne et al. (2016).



**Figure 2.4.** Tank where fish were manually chased to exhaustion. A large obstacle was placed in the middle to allow the fish to swim around in a circle (rather than randomly throughout the tank) while being chased by either a net or the researcher's hand. The caudal fin of fish were lightly pressed between two fingers or the fish was softly touched with the net to stimulate movement in fish away from the stimuli.

c) *Hypoxia Tolerance*

i)  $LOE_{crit}$

After measuring RMR, the flush pump remained off to allow oxygen levels to drop below normoxic conditions. DO was reduced by the fish in the closed loop chamber, continuously recorded at 10 second intervals, and the fish was visually monitored until loss of equilibrium occurred. Loss of equilibrium was confirmed when the fish failed to maintain upright position for 10 seconds and was unable to gain equilibrium when touched within the chamber. If the fish gained equilibrium, it was kept in the chamber. When it did not regain equilibrium, the fish was removed from the chamber, the  $PO_2$  (kPa) where LOE occurred was recorded, and the fish was terminally sampled for blood (procedure described below)..

ii) *Critical Oxygen Tension ( $P_{crit-RMR}$ )*

$MO_2$  was calculated in 4-minute intervals using the formula described above. Mass corrected  $MO_2$  values were plotted against their corresponding initial  $PO_2$  values (Figure 1.2) and analyzed using the rMR-package which utilizes the BASIC program designed by Yeager and Ultsch (1989) to determine the inflection point signifying  $P_{crit}$ .

iii) *Biochemical Analysis*

Blood was drawn from the caudal artery of the fish and centrifuged at 1500 rpm for 10 minutes to separate the blood and plasma (stored at  $-80^{\circ}C$  for biochemical analysis). The fish were then killed with a blunt trauma to the head followed by cervical displacement.

Plasma was deproteinated by adding 5X volume of 6% perchloric acid, vortexed and chilled on ice for five minutes and centrifuged at 1500 rpm for 10 minutes at  $4^{\circ}C$ . Additionally, a serial 6-point, two times serial dilution using 6% PCA as the solvent was performed to create a set of L-(+)-Lactic Acid (Sigma-Aldrich, L1750) standards ranging from 1.65 to 0 mM. Lactate was measured in each sample or standard (in triplicate) by adding 25  $\mu$ l of the resulting sampling supernatant or standard to 200  $\mu$ l assay buffer containing glycine buffer (Sigma-Aldrich, G5418) and 2.5 mM  $NAD^+$  in each well of a 96-well microplate and incubated (while shaking) for 15 minutes at room temperature. The reduction of  $NAD^+$  to  $NADH$  was initiated by adding 10 units/ml of L-Lactic Dehydrogenase from rabbit muscle (Sigma L-2500) to each well. Samples were incubated (while shaking) for an additional 30 minutes at room temperature. Assay buffer without  $NAD^+$  and 25  $\mu$ L of the LDH-assay buffer solution were loaded in each well. The microplate was left on the shaker for an additional 30 minutes and the samples were then assayed on a SpectraMax Plus 384 Spectrophotometer at a wavelength of 340 nm using SoftMax Pro

software. Lactate accumulation rate was calculated as lactate concentration divided by the total time spent in the closed respirometry chamber following the RMR measurement until LOE occurred. Initial lactate concentration was assumed to be negligible for all fish.

### *Statistical Analysis*

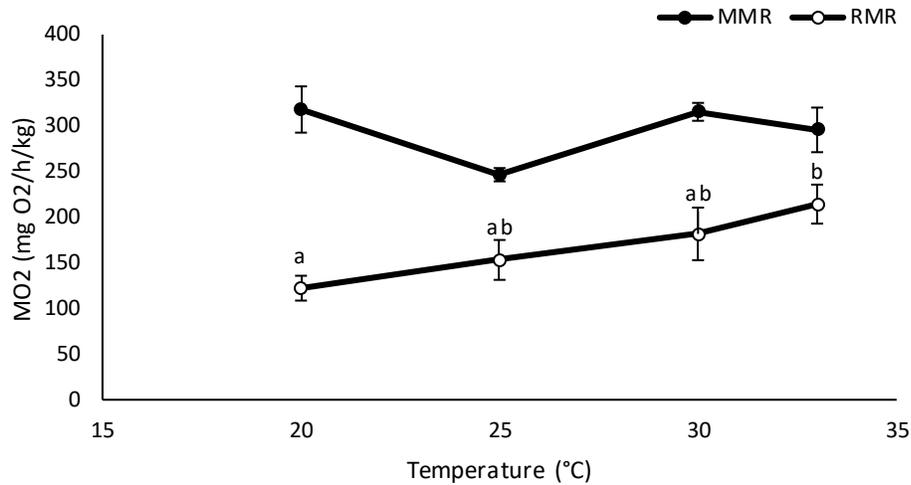
The statistical program JMP PRO13 was used to analyze among group variation in aerobic scope, MMR, RMR, LOE, and lactate accumulation rates across temperature acclimation groups using a one-way ANOVA. RMR, LOE<sub>crit</sub> (PO<sub>2</sub> and total time), and lactate accumulation rates were converted to natural logs to fit the normal distribution and equal variance assumptions of ANOVAs. Lactate concentrations did not fit the equal variance assumption of ANOVAs so a Welch's test was used to determine among group variation across temperature acclimation groups. A Tukey-Kramer test was used to determine within group variation across treatments. The Shapiro-Wilk W Test was used to test for normality and a Levene Test was used to test for equal variances for all analyses. P values below 0.05 were considered statistically significant.

## RESULTS

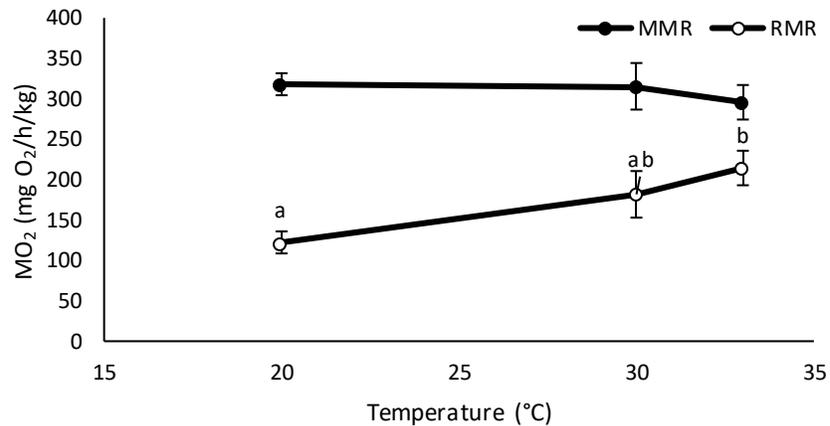
### *Thermal Tolerance:*

#### *a) Routine and Maximum Oxygen Consumption:*

Mean mass corrected RMR was  $122.23 \pm 13.62$ ,  $153.19 \pm 21.85$ ,  $181.69 \pm 28.80$ , and  $214.30 \pm 21.35$  mg O<sub>2</sub>/h/kg for acclimation treatment groups 20, 25, 30, and 33°C, respectively. Acclimation temperature had a significant effect on RMR where RMR significantly increased by 75.32% from 20 to 33°C (Figure 2.5. and Figure 2.6.). Q<sub>10</sub> was highest between 30 and 33°C (1.7) and all Q<sub>10</sub> values were above 1 (Table 2.1.). Mean mass corrected MMR was  $317.94 \pm 25.33$ ,  $246.51 \pm 7.27$ ,  $315.37 \pm 9.80$ , and  $295.60 \pm 24.45$  mg O<sub>2</sub>/h/kg for acclimation temperature groups 20, 25, 30, and 33°C, respectively. Acclimation temperature had a significant effect on MMR but *post hoc tests* were not significant. When the 25°C group was removed, acclimation temperature did not have a significant effect on MMR (Figure 2.6.).



**Figure 2.5.** Mean oxygen consumption (mg O<sub>2</sub>/h/kg) in juvenile *Morone saxatilis* acclimated to 20, 25, 30, or 33°C. Acclimation temperature had a significant effect on RMR (one-way ANOVA,  $F=3.4156$ ,  $P=0.0413$ ) and MMR (one-way ANOVA,  $F=3.2441$ ,  $P=0.0480$ ). RMR values were converted to natural logs to fit the normal distribution assumption of ANOVAs. Different letters indicate a significant difference ( $P<0.05$ ) between temperature groups (Tukey *post hoc* test). All data are means  $\pm$  SEM  $n=6$  for 20°C,  $n=5$  for 25°C,  $n=6$  for 30°C, and  $n=4$  for 33°C.



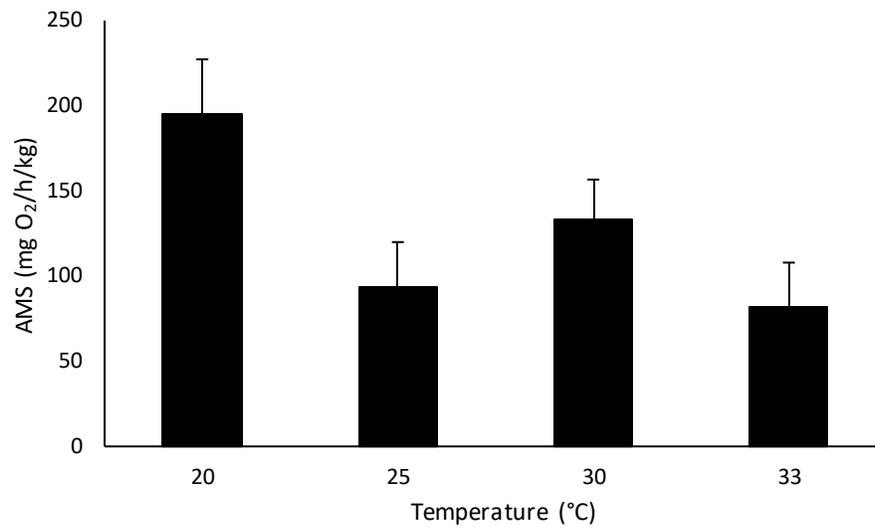
**Figure 2.6.** Mean oxygen consumption (mg O<sub>2</sub>/h/kg) in juvenile *Morone saxatilis* acclimated to 20, 30, or 33°C. Acclimation temperature had a significant effect on RMR (one-way ANOVA,  $F=5.5802$ ,  $P=0.0178$ ). RMR values were converted to natural logs to fit the normal distribution assumption of ANOVAs. Acclimation temperature did not have a significant effect on MMR (one-way ANOVA,  $P>0.05$ ). Different letters indicate a significant difference between temperature groups (Tukey *post hoc* test). All data are means  $\pm$  SEM  $n=6$  for 20°C,  $n=5$  for 25°C,  $n=6$  for 30°C, and  $n=4$  for 33°C.

**Table 2.1.** Temperature coefficients in juvenile *Morone saxatilis* calculated for changes in RMR between acclimation temperatures.

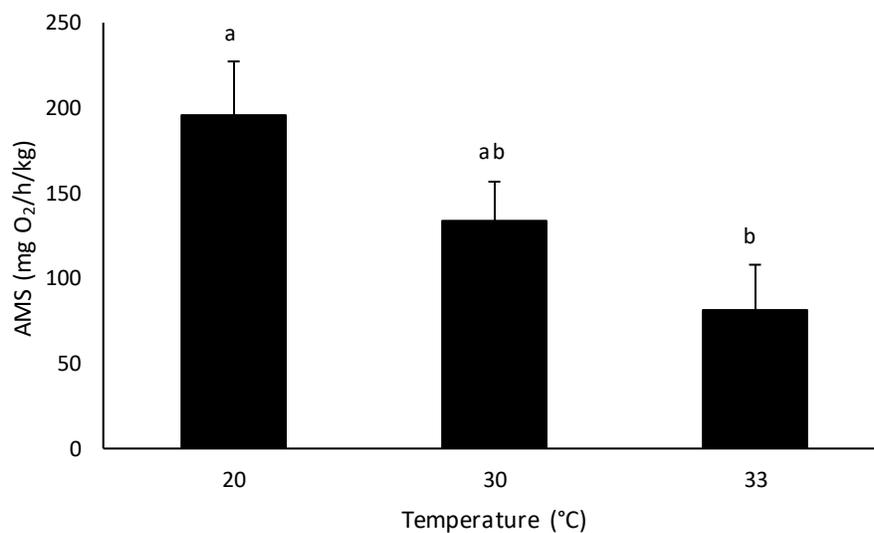
| Temperatures (°C) | Q <sub>10</sub> |
|-------------------|-----------------|
| 20-25             | 1.6             |
| 20-30             | 1.5             |
| 20-33             | 1.5             |
| 25-30             | 1.4             |
| 25-33             | 1.5             |
| 30-33             | 1.7             |

*b) Aerobic Scope*

Mean mass corrected AMS was  $195.70 \pm 31.57$ ,  $93.33 \pm 26.65$ ,  $133.69 \pm 22.97$ , and  $81.30 \pm 26.60$  mg O<sub>2</sub>/h/kg for acclimation treatment groups 20, 25, 30, and 33°C, respectively. Acclimation temperature had a significant effect on AMS but *post hoc* tests were not significant (Figure 2.7.) When the 25°C temperature acclimation group was removed, acclimation temperature had a significant effect on AMS where AMS significantly decreased by 74.4% between the 20 and 33°C acclimation groups (Figure 2.8.).



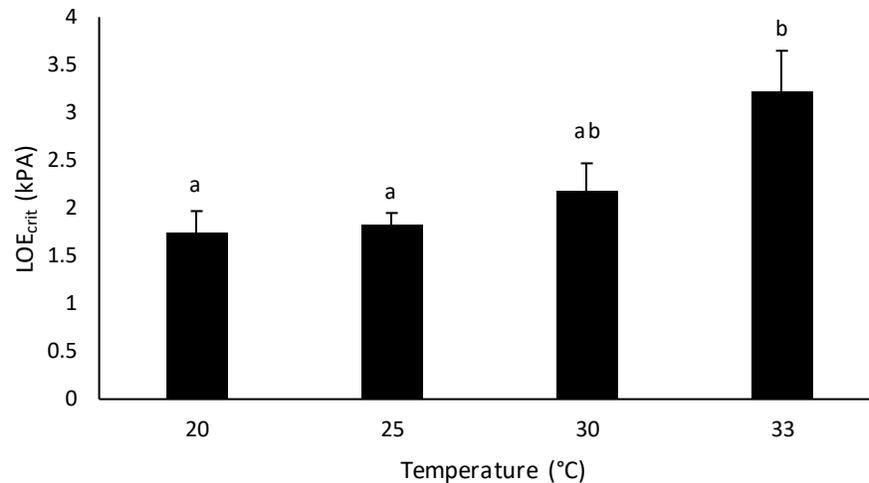
**Figure 2.7.** Mean AMS (mg O<sub>2</sub>/h/kg) in juvenile *Morone saxatilis* acclimated to 20, 25, 30, or 33°C. Acclimation temperature had a significant effect on AMS (one-way ANOVA,  $F=3.4882$ ,  $P=0.338$ ). There was no significant difference in AMS between acclimation temperature groups (Tukey *post hoc* test). All data are means  $\pm$  SEM  $n=6$  for 20°C,  $n=5$  for 25°C,  $n=6$  for 30°C, and  $n=4$  for 33°C.



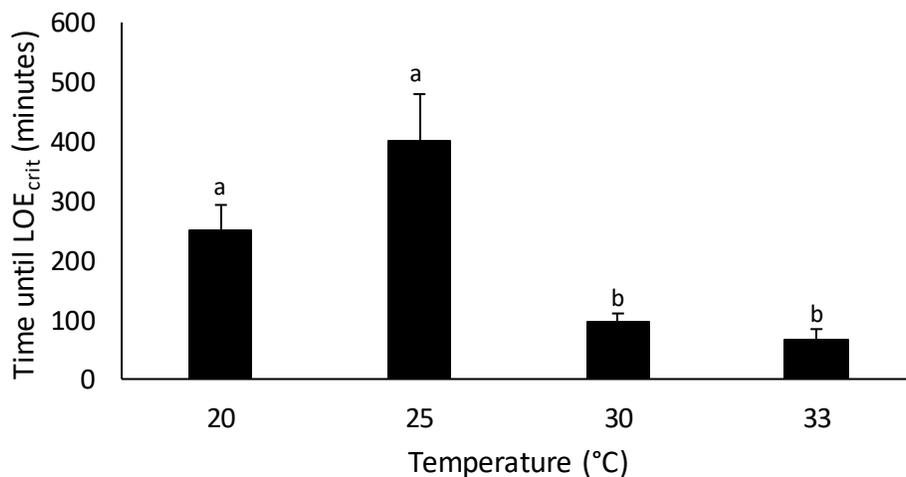
**Figure 2.8.** Mean AMS (mg O<sub>2</sub>/h/kg) in juvenile *Morone saxatilis* acclimated to 20, 30, or 33°C. Acclimation temperature had a significant effect on AMS (one-way ANOVA,  $F=3.8837$ ,  $P=0.0476$ ). Different letters indicate a significant difference between temperature acclimation groups (Tukey *post hoc* test). All data are means  $\pm$  SEM  $n=6$  for 20°C,  $n=5$  for 25°C,  $n=6$  for 30°C, and  $n=4$  for 33°C.

*Hypoxia Tolerance:**a) LOE<sub>crit</sub>*

Mean LOE<sub>crit</sub> was  $1.74 \pm 0.23$ ,  $1.82 \pm 0.13$ ,  $2.18 \pm 0.29$ , and  $3.22 \pm 0.43$  kPa at acclimation temperatures 20, 25, 30, and 33°C, respectively. Acclimation temperature had a significant effect on LOE<sub>crit</sub> where LOE<sub>crit</sub> significantly increased at temperatures above 25°C with a significant effect occurring at 33°C (Figure 2.9.). Mean total time until LOE<sub>crit</sub> was  $250.92 \pm 41.97$ ,  $399.87 \pm 79.52$ ,  $94.72 \pm 15.56$ , and  $66.75 \pm 17.25$  minutes at acclimation temperatures 20, 25, 30, and 33°C, respectively. Acclimation temperature had a significant effect on total time until LOE<sub>crit</sub> occurred where time until LOE decreased significantly at acclimation temperatures above 25°C (Figure 2.10.). Both measures of PO<sub>2</sub> and time where LOE<sub>crit</sub> occurred show the same trend that hypoxia tolerance decreases at temperatures above 25°C with the most significant effect occurring at 33°C.



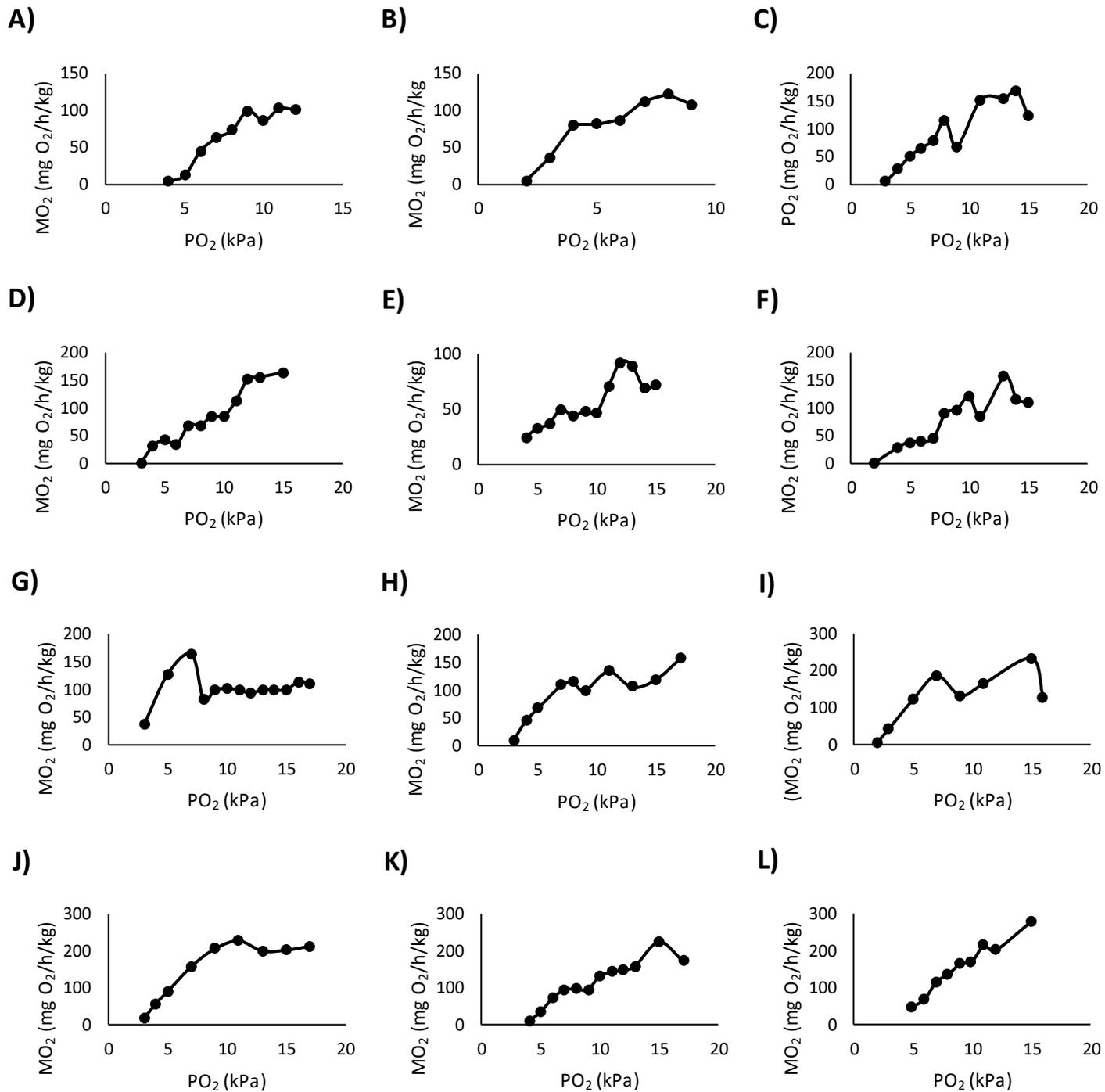
**Figure 2.9.** PO<sub>2</sub> (kPa) where LOE<sub>crit</sub> occurred in juvenile *Morone saxatilis* acclimated to 20, 25, 30, or 33°C. Temperature had a significant effect on LOE<sub>crit</sub> (one-way ANOVA,  $F=4.5523$ ,  $P=0.0185$ ). kPa values were converted to natural logs to fit normal distribution assumptions of ANOVAs. Different letters indicate a significant difference between temperature groups (Tukey *post hoc* test). All data are means  $\pm$  SEM  $n=4$  for 20°C,  $n=5$  for 25°C,  $n=6$  for 30°C, and  $n=4$  for 33°C.



**Figure 2.10.** Mean total time (minutes), following measurement of RMR where chamber remained closed allowing juvenile *Morone saxatilis* to bring down DO until LOE occurred, in juveniles acclimated to 20, 25, 30, or 33°C. Temperature had a significant effect on time until LOE occurred (one-way ANOVA,  $F=14.4944$ ,  $P=0.0001$ ). Time values were converted to natural logs to fit normal distribution assumptions of ANOVAs. Different letters indicate a significant difference between temperature groups (Tukey *post hoc* test). All data are means  $\pm$  SEM  $n=4$  for 20°C,  $n=5$  for 25°C,  $n=6$  for 30°C, and  $n=4$  for 33°C.

b)  $P_{crit}$

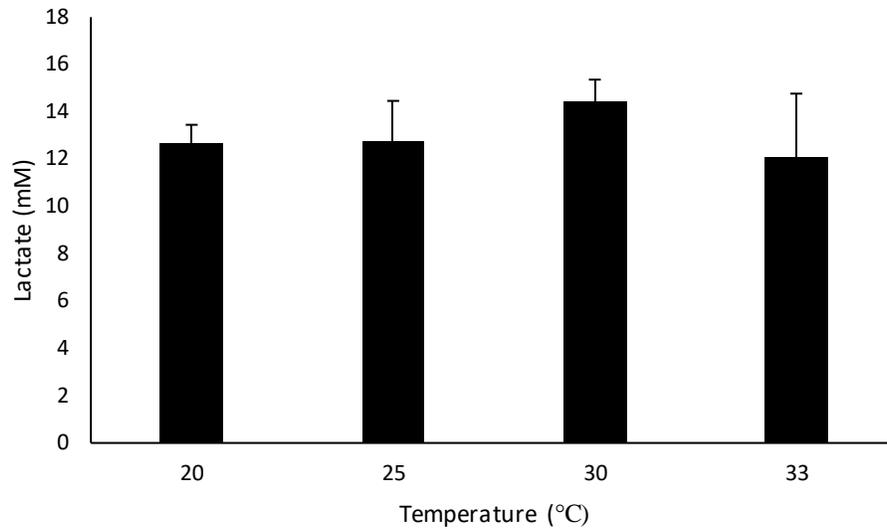
Due to the large amount of interindividual variability we were not able to confidently obtain mean values for  $P_{crit}$  data for each acclimation temperature. Instead, three individuals from each acclimation temperature, whose curves most closely exhibited a theoretical relationship (Figure 1.2) between external  $PO_2$  and RMR were selected to present the trends in the data set (Figure 2.11.) Only fish #17 (Figure 2.11G.) and fish #12 (Figure 2.11J.) acclimated to 30 and 33°C, respectively, exhibited curves that fit the stereotypical plot for  $P_{crit}$  analysis.  $P_{crit}$  was higher (8.51 kPa) in the individual acclimated to 33°C relative to the individual (7.50 kPa) acclimated to 30°C.  $P_{crit}$  was determined at the x-intercept between the two linear regression lines for Fish #12 but  $P_{crit}$  in fish #17 was determined as the mid-point between the two linear regression lines since the continuity assumption of the model was violated (Yeager and Ultsch 1989) and the intercept between the lines was skewed right.



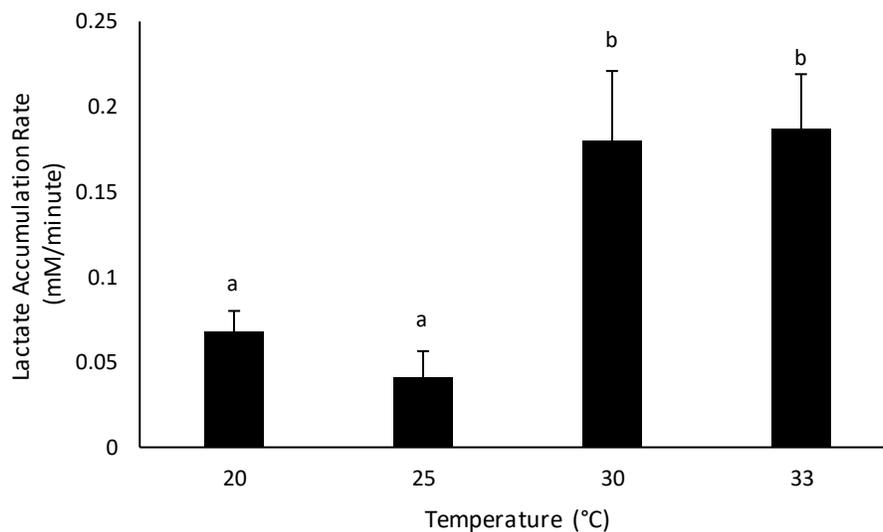
**Figure 2.11.** Oxygen consumption (mg  $O_2$ /h/kg) at specified  $PO_2$  (kPa) in individual juvenile *Morone saxatilis*. Figures A-C, D-F, G-I, and J-L correspond to individuals acclimated to 20, 25, 30, and 33°C, respectively. The three curves that most closely resemble the theoretical  $MO_2$  vs.  $PO_2$  (Figure 2.) relationship were selected for 3 fish from each temperature acclimation group.

c) *Lactate*

Mean plasma lactic acid concentration was  $12.65 \pm 0.80$ ,  $12.75 \pm 1.71$ ,  $14.45 \pm 0.91$ , and  $12.06 \pm 2.71$  mM at acclimation temperatures 20, 25, 30, and 33°C, respectively. Temperature did not have a significant effect on lactic acid concentration (Figure 2.12.). However, acclimation temperature had a significant effect on lactate produced over the time when fish were in the closed respirometry system which increased significantly at acclimation temperatures above 25°C. Lactate accumulation rate was  $0.07 \pm 0.013$ ,  $0.042 \pm 0.015$ ,  $0.18 \pm 0.04$ , and  $0.19 \pm 0.03$  mM/minute at acclimation temperatures 20, 25, 30, and 33°C, respectively (Figure 2.13.). Lactate levels were assumed to be negligible or at baseline levels when fish were in an open respirometry set-up as they had access to fully aerated water and fish were at rest under normoxic conditions for at least 12 hours prior to measurement.



**Figure 2.12.** Mean plasma lactate concentrations (mM) following LOE in juvenile *Morone saxatilis* acclimated to 20, 25, 30, or 33°C. Temperature did not have a significant effect on plasma lactate concentrations (one-way ANOVA,  $P > 0.05$ ). All data are means  $\pm$  SEM N=6 for 20°C, N=5 for 25°C, N=6 for 30°C, and N=4 for 33°C.



**Figure 2.13.** Mean plasma lactate production (mM after  $LOE_{crit}$  / Total time until LOE) following LOE in juvenile *Morone saxatilis* acclimated to 20, 25, 30, or 33°C. Temperature had a significant effect on plasma lactate production (one-way ANOVA,  $F = 12.7804$ ,  $P = 0.0002$ ). Lactate accumulation rate values converted to natural logs to fit normal distribution assumption of ANOVAs. Different letters indicate a significant difference between temperature groups (Tukey *post hoc* test). All data are means  $\pm$  SEM N=6 for 20°C, N=5 for 25°C, N=6 for 30°C, and N=4 for 33°C.

## DISCUSSION

The data obtained from this study demonstrated that chronic exposure to temperatures in the upper summer range result in an overall decrease in performance and low oxygen tolerance in southern juvenile striped bass. The significant reduction in aerobic scope paired with the 50% mortality that occurred in fish acclimated to 3°C above current-day summer high temperatures indicates the southern populations of striped bass may be at risk due to a reduction in energy being available for vital life history processes such as growth, reproduction and predatory-prey interactions during a substantial portion of the year. Furthermore, these supra-optimal temperatures occur at a section of the Savannah River that serves as the main nursery for juveniles during the summer posing a threat to the success of future populations.

### *Thermal Tolerance*

As expected, the routine metabolic rate of striped bass was directly affected by acclimation temperature. RMR steadily increased with acclimation temperature, with the highest rate occurring in the 33°C acclimation group (Figure 2.5.). Most biological reactions, including metabolic rate, exhibit a predictable response to changes in environmental temperature, with rates doubling or tripling for every 10°C increase in temperature ( $Q_{10} = 2-3$ ) (Clarke and Johnson 1999). The  $Q_{10}$  for routine metabolic rate for the striped bass was 1.4 – 1.7 across the acclimation temperatures, with the highest  $Q_{10}$  between 30 and 33°C (Table 2.1.). Although these  $Q_{10}$  values were slightly lower than the standard 2-3 range for metabolic reactions, they are consistent with the typical range ( $Q_{10} = 1.5 - 2$ ) observed for teleost fish (White et al. 2006). Also, it has been shown that a reduction in  $Q_{10}$  can result from partial compensation following thermal acclimation through changes at the cellular level that increase efficiency of cellular respiration (Lurman et al. 2013; Kruger and Brocksen 1978; George and Hochachka 1971).

As RMR increases with temperature, so does the minimum energy required for basic life sustaining processes within the organism and as a result less energy can be used for foraging, digestion, and growth resulting in reduced performance of these traits (Farrell 2015; Rosewarne et al. 2016). Hartman and Brandt (1995) demonstrated this tradeoff in the scope for growth of Northern juvenile striped bass from Chesapeake Bay. Scope for growth measures the amount of energy used for growth relative to basal metabolism under maximum food consumption at variable temperatures and was determined using a bioenergetics model based on Winberg's balanced energy equation where energy for growth is calculated as consumed energy minus the energy utilized for RMR, egestion, and lost during excretion. Juveniles acclimated to 29°C (the average summer temperature of the water in their habitat) exhibited elevated RMR and reduced growth relative to those in other temperatures. Similarly, food consumption decreased with increased RMR at higher temperatures in Chesapeake juveniles signifying reduced foraging activity (Cox and Coutant 1981). Comparison between previous studies and the results from our study suggest that RMR, and therefore the basic costs of sustaining life, may be similar throughout striped bass populations.

The northern populations of striped bass appear to rely upon the coastal migration to keep themselves within their optimal temperature range, but this behavioral response is not observed in the southern populations. Instead, adults migrate upstream towards thermal refuge and the juveniles remain within the estuary where they are chronically exposed to supra-optimal temperatures and to prolonged periods of elevated costs of life (Dudley et. al 1977). Prolonged exposure to current temperatures poses a threat to the performance of the population since less energy can be devoted to essential biological processes, and future projections pose a more significant risk since the basic cost of life accounted for most of the consumed oxygen in fish

acclimated to these temperatures. Therefore, striped bass in the Savannah River could be more vulnerable to climate change since they experience higher temperatures relative to northern populations during the summer that already result in reduced performance.

MMR remained relatively constant with increasing acclimation temperature (Figure 2.5. and Figure 2.6.). Typically, reductions in MMR are associated with DO limitations (Rosewarne et al. 2016; Rogers et al. 2016) so the reduction in MMR at 25°C is unexpected. There were unexpected elevated ammonia levels in the acclimation tank that could have placed fish under additional stress resulting in a slight, but non-significant decrease in MMR at 25°C. Although it did not result in a significant decrease in MMR, it did result in a decrease in AMS at this temperature. Otherwise, MMR exhibited expected results across all temperature treatment groups since MMR exponentially increases with temperature until a peak temperature is reached and then remains relatively constant as observed in the data from our study (McBryan et al. 2013). The results from this study are consistent with observations made in adult striped bass from Chesapeake Bay where MMR did not exhibit significant changes between fish acclimated to 20 and 28°C (Lapointe et al. 2014).

Most notably, projected increases of 3°C had a large negative effect on fish survival. Mortalities were significantly high in the 33°C group where 50% of the the fish died in the respirometry chamber following measurements of MMR signifying that half of the fish were not even able to perform activities requiring spurts of activity above RMR. This poses serious consequences for survival where the fish will be unable to escape predators, forage, or exhibit avoidance behavior towards areas with suitable temperatures for extended periods of time in future summers.

The reduction in AMS is attributed to the increase in RMR with relatively constant MMR as acclimation temperature increases (Figure 2.8.). The increasing cost of life at higher temperatures brings RMR closer to its upper boundary of aerobic metabolism resulting in less oxygen for non-basal biological processes (Rosewarne et al. 2016; Ferreira et al 2015). The observed results suggest that the  $T_{opt}$  for AMS occurs at temperatures  $\leq 20^{\circ}\text{C}$  where available energy for non-basal biological processes is maximized.  $T_{pejus}$  appears to occur at temperatures above  $20^{\circ}\text{C}$  as was observed in a reduction in AMS at  $30^{\circ}\text{C}$  signifying that AMS is already negatively impacted by current maximum summer temperatures. To our knowledge,  $T_{crit}$  for juvenile striped bass exposed to chronic increases in temperature have not been determined, but the high mortality rate of 50% coupled with a significant reduction of 74.4% in AMS between  $20^{\circ}\text{C}$  and  $33^{\circ}\text{C}$  suggests  $T_{crit}$  may be around  $33^{\circ}\text{C}$ . Therefore, projected temperatures of  $33^{\circ}\text{C}$  result in failure to maintain aerobic metabolism along with the basic mechanisms of life so the fish must rely on anaerobic respiration for its energetic demands.

Temperatures in the Savannah River range from  $25\text{-}30^{\circ}\text{C}$  in the summer and are  $\sim 29^{\circ}\text{C}$  on average (US GEOL Survey; Will et al. 2001; Dudley et al. 1977). Based on the performance curves generated from our data, these mean and maximum summer temperatures are already high enough to result in reductions in AMS and overall performance for these juveniles. Temperatures above  $30^{\circ}\text{C}$  severely limit AMS where the cost of life is so high that short spurts of activity are highly costly and can result in fatality from energetic limitations. If current climate change projections persist, populations of southern striped bass face significant challenges from metabolically limited performance and may have to rely on avoidance or migratory behavior to find thermal refuge.

Adult striped bass in the Savannah exhibit upstream migrations towards cooler waters upstream and are even hypothesized to cross towards neighboring rivers like the Ogeechee River allowing them to temporarily escape stressful thermal conditions (Dudley et al. 1977; Roberts personal communication). However, juveniles are less likely to undertake such migrations due to the increased threats of predation, exhaustion, and starvation that are possible during migration (Coutant 1985). Juveniles in the Savannah River may currently rely on behavioral avoidance of supra-optimal temperatures towards cooler refuges such as near dams, shores, or sandbars as has been observed in juveniles along an estuary of the Chesapeake Bay during the summer (Kraus et al. 2015).

The future survival of juveniles in the Back River relies on the preservation of thermal refugia, and studies on spatial variation in juveniles along the Back River in relation to temperature should be conducted to observe the variation in temperature between thermal refuges and calculated parameters (such as range and mean) of the overall system. This information can be used to provide a better understanding of their current thermal tolerance in relation to temperatures in thermal refuges, the impact climate change will have on these thermal refuges, and preservation of these thermal refuges. It would also be important to further study thermal and hypoxia tolerance in larger individuals to establish whether the potential impacts of climate change will be primarily on younger fish or the entire population.

### *Hypoxia Tolerance:*

As predicted, there was a significant reduction in hypoxia tolerance in fish at the highest acclimation temperatures (30 and 33°C) representing the current summer high and projected 3°C above the current summer high. This was exemplified by a 32% increase in PO<sub>2</sub> where LOE<sub>crit</sub> occurred between 30 and 33°C (Figure 2.9.), and a 76% decrease in total time until LOE<sub>crit</sub> occurred between 25 and 30°C (Figure 2.10.). This high temperature related reduction in hypoxia tolerance is likely a result of the fish acclimated to warmer temperatures requiring a higher minimum level of environmental oxygen to sustain elevated rates of oxygen consumption due to a temperature induced increase of up to 43% in RMR.

Hypoxia tolerance in acclimated juvenile striped bass at rest depends on the ability to depress RMR through reduction of oxygen-demanding activities such as digestion, locomotion, and growth (Nelson and Lipkey 2015; Matthews and McMahon 1999). Therefore, elevated RMR with temperature may offset their ability to depress RMR resulting in reduced hypoxia tolerance (Nelson and Lipkey 2015; Kruger and Brocksen 1978; McBryan et al., 2016.) which likely explains why LOE<sub>crit</sub> significantly increased with temperature. Reduction in hypoxia tolerance at higher acclimation temperatures through measures of LOE<sub>crit</sub> were also observed in Chinese crucian carp, cardinal fish, lemon damsel fish, and blunt snout bream (Yang Yang et al. 2015; Nilsson et al. 2010; Wu et al. 2017).

However, PO<sub>2</sub> where LOE<sub>crit</sub> occurs decreases with temperature in killifish when exposed to hypoxic conditions following thermal acclimation (McBryan et al. 2016). Differences between acute increases in temperature and acclimation to temperature affect hypoxia tolerance. Acute increases in temperature directly affect hypoxia tolerance since higher SMR from elevated temperatures is more costly to maintain requiring higher DO in the environment. However, high

thermal acclimation can sometimes result in compensatory mechanisms that make the fish more efficient in obtaining oxygen from the environment, as observed in killifish whose increase gill surface area increased at higher acclimation temperatures, resulting in increased hypoxia tolerance (McBryan et al. 2016). Our data suggests that southern striped bass are likely not capable of making major compensatory changes such as the gill remodeling seen in southern populations of killifish that allow them to become more efficient at obtaining oxygen from the environment at higher temperatures.

The reduction in hypoxia tolerance at higher temps was also supported by evidence at the biochemical level showing higher rates of lactic acid production at acclimation temperatures above 25°C with a significant increase of 77% between 25 and 30°C (Figure 2.13.). This signifies that anaerobic respiration occurred earlier and at higher rates in high temperature acclimation groups which shows that they were less able to regulate RMR at higher temperatures resulting in an earlier shift to anaerobic respiration, and higher temperatures elevated the rate of lactic acid fermentation which can result in lactic acidosis at high concentrations. Similarly, plasma lactate concentrations (Figure 2.12.) are ~7 and 1.5 times higher following hypoxia induced  $LOE_{crit}$  relative to juvenile striped bass at rest and following exhaustive exercise, respectively (Nickinmaa et al. 1984). When mean lactic acid concentrations for all four temperature treatment groups were calculated, they equaled 1169 mg/L which is consistent with lactic acid concentrations (1170 mg/L) obtained from adult striped bass along the Sacramento-San Joaquin river system that were characterized as being in poor condition and subsequently experienced high mortality following tagging protocol (Chadwick 1968).

i)  $P_{crit}$ 

The partial pressure of environmental oxygen at which supply no longer meets the resting demand for oxygen is termed the critical oxygen tension ( $P_{crit}$ ). This vital point has been widely accepted, and used, as an indicator of the hypoxia tolerance level of a fish and it is generally observed that an increase in RMR due to acclimation to higher temperatures is accompanied by an elevation in  $P_{crit}$  (He et. al 2015; Rogers et al. 2016; Rosewarne et al. 2016). Recent literature criticizes the use of  $P_{crit}$  as a measure of hypoxia tolerance stating that it allows researchers to select their data, there are multiple way to determine it (MMR, RMR, and SMR), it is not biologically justifiable, and several other reasons that make it an unsuitable measure of hypoxia relative to other methods (Wood 2018). Wood (2018) suggests using other methods such as LOE and lactate analysis as measures of hypoxia tolerance. Similarly, Speers-Roesch et al. (2013) suggest coupling LOE and biochemical analyses with  $P_{crit}$  studies to receive conclusive results for comparative studies on hypoxia tolerance.

This study took into consideration the criticism of  $P_{crit}$  and several precautions were taken to test the  $P_{crit}$  of juvenile striped bass. When selecting the  $P_{crit}$  data, we measured  $MO_2$  at each value of  $PO_2$  possible in incremental increases or decreases of 1 kPa, and in incremental increases of 2 kPa when there was overlap between initial DO values to calculate  $MO_2$  (Figure 2.11). When selecting the appropriate initial DO value to calculate  $MO_2$  at a specific  $PO_2$ , we converted DO from mg/L to kPa and chose the value that was at least within a hundredth of the selected kPa. Similarly,  $P_{crit}$  was measured along with LOE and plasma lactate production to further strengthen the hypoxia tolerance results. A high degree of interindividual variability, multiple transition points, and inability to establish a oxy-regulator line made determination of

$P_{crit}$  challenging so  $P_{crit}$  results were excluded from the interpretation of hypoxia tolerance (Figure 2.11).

We can conclude from this study that projected increases in temperature reduce hypoxia tolerance in juvenile striped bass through elevations in RMR that exceed compensation mechanisms to depress RMR resulting in earlier onset of anaerobic respiration coupled with elevated lactic acid fermentation. Temperature induced hypoxia conditions will likely increase in aquatic environments like the Savannah River and limit the ability of juvenile striped bass to uptake oxygen from their environment through limitations in MMR. Similarly, higher temperatures will limit the amount of suitable habitat and alternations in DO of thermal refuges will be less tolerated. Availability of areas with suitable temperature and DO may be a key factor in striped bass preservation and survival in the future. Future studies on the biochemical mechanisms involved in hypoxia tolerance in striped bass populations should be pursued to better understand their susceptibility to changes in temperature.

#### *Future Directions and Conclusions:*

Climate change is projected to influence striped bass habitat and distribution (Coutant 1990). Riverine or semi-riverine populations are expected to become fully riverine and make further up-stream migrations during the summer months (Coutant 1990). An increase from one to three months of unsuitable conditions during the summer months characterized by supra-optimal temperatures followed by hypoxic conditions is projected to occur. Similarly, cool spring and water discharge that feed the rivers may diminish in the future due to reduced groundwater recharge and increased human groundwater withdrawals, resulting in higher water temperatures and fewer available cool water refuges with sufficient DO (Coutant 1990).

Thermal and hypoxia tolerance decreases with acclimation temperature in juvenile striped bass from the Savannah River. Daily mean maximum summer temperatures already exhibit reductions in AMS which result in reduced overall performance of non-basal biological processes like growth, activity, and foraging (Ferreira et al. 2014; Rosewarne et al. 2016; Rummer et al 2013). Anticipated increases of 3°C resulting from climate change by the end of the century pose a significant threat to juvenile striped bass in the Savannah River where half of the subjects did not survive experimentation and the remaining exhibited significantly low AMS that severely limits performance and makes them very susceptible to predation, starvation, and entrapment in unsuitable habitats. Extended summer conditions could result in local extirpation of striped bass if the predicted changes in temperature occur.

Hypoxia tolerance is low in current summer conditions and will decrease further with anticipated increases in temperature in these fish. Higher temperatures will further limit their performance indirectly through AMS or directly through limitations in RMR depression resulting in time limited survival through anaerobic respiration. Furthermore, inverse reductions in DO with temperature will further limit the striped bass habitat in the Savannah River and may increase the risk of mortalities due to crowding in thermal refuges along the Savannah River (Coutant 1985).

Striped bass suffered significant declines in population following construction of the Tide Gate along the Back River section of the Savannah River which served as a major nursery for striped bass larvae and juveniles. Removal of the Tide Gate along with stocking efforts by the GA-DNR has resulted in an estimated survival of 35-45% in stocked juveniles exhibiting an overall population growth (Reinert 2004; Will et al. 2001). Although spawning is being observed, it is not sufficient for a self-sustaining population that can occur without active

stocking (Will et al. 2001). High temperatures in the Savannah River during spawning season may be playing a role through a reduction in reproductive performance.

Similarly, growth may be limited in juveniles at high temperatures resulting in a longer time until sexual maturity is reached. Results from this study suggest that temperature may be a limiting factor in striped bass success along the Savannah River. As temperatures rise within the following century, striped bass may have to embark in longer migrations upstream or make migrations to other, and more suitable, bodies of water as has been hypothesized for Savannah River striped bass. The future of these striped bass relies on a more thorough understanding of their life history and how abiotic factors affect it. Results from this study provide evidence that temperature plays a significant effect on striped bass performance and should be taken into account when making conservation decisions. Thermal refuges for striped bass should be investigated in more detail so they can be protected from exploitation and degradation. Conservation efforts should also take into account the possibility of stocking juveniles towards more suitable habitats within the Savannah River.

Future investigation into differences in thermal tolerance between northern and southern populations using AMS at higher and lower temperatures would provide valuable information regarding whether thermal tolerance exhibits counter-gradient latitudinal variation as is seen in growth patterns in striped bass populations (Conover et al. 1985, Brown et al. 1998). Application of AMS to determine thermal tolerance should also be applied to determine differences in tolerance between different life stages of striped bass and see if it plays a part in thermal niche selection differences between juveniles and adults (Coutant 1985). Furthermore, juvenile striped bass from this study exhibited high hypoxia tolerance as was similarly observed by Nelson and Lipkey (2015) in juveniles from Chesapeake Bay. Further studies should attempt to obtain

uniform hypoxia tolerance data for comparison between different populations and determine the physiological and biochemical mechanisms employed by striped bass to tolerate hypoxic conditions.

## REFERENCES

- Aisamie et al. 2017 Speers-Roesch, B., Mandic, M., Groom, D. J. E., & Richards, J. G. (2013). Critical oxygen tensions as predictors of hypoxia tolerance and tissue metabolic responses during hypoxia exposure in fishes. *Journal of Experimental Marine Biology and Ecology*, 449, 239–249. <https://doi-org.libez.lib.georgiasouthern.edu/10.1016/j.jembe.2013.10.006>
- Babikian, J., Nasser, N., Monzer, S., & Saoud, I. P. (2017). Survival and respiration of marbled rabbitfish (*Siganus rivulatus*) fingerlings at various oxygen tensions. *Aquaculture Research*, 48(8), 4219-4227. doi:10.1111/are.13242
- Bettoli, P. W. (2013). Challenges and Opportunities in Studying and Managing Striped Bass and its Hybrids in the 21st Century. *Biology and management of inland striped bass and hybrid striped bass*, 80, 575-588.
- B.W. Green, S.D. Rawles, S.A. Fuller, B.H. Beck, M.E. McEntire (2015). Hypoxia affects performance traits and body composition of juvenile hybrid striped bass (*Morone chrysops* × *M. saxatilis*) *Aquacult. Res.*
- Boag, T. H., Stockey, R. G., Elder, L. E., Hull, P. M., & Sperling, E. A. (2018). Oxygen, temperature and the deep-marine stenothermal cradle of Ediacaran evolution. *Proceedings of the Royal Society B: Biological Sciences*, 285(1893), 20181724. doi:10.1098/rspb.2018.1724
- Brandt, S. B., Gerken, M., Hartman, K. J. & 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.
- Brougher, Daniel S., et al. Comparative Oxygen Consumption and Metabolism of Striped Bass *Morone saxatilis* and its Hybrid *M. chrysops* x *M. saxatilis* . *Journal of the World Aquaculture Society*, vol. 36, no. 4, 2005, pp. 521–529., doi:10.1111/j.1749-7345.2005.tb00400.x.
- Brown, J. J., Ehtisham, A., & Conover, D. O. (1998). Variation in Larval Growth Rate among Striped Bass Stocks from Different Latitudes. *Transactions of the American Fisheries Society*, 127(4), 598-610. doi:10.1577/1548-8659(1998)127<0598:vilgra>2.0.co;2
- Bjorgo, K. A., Isely, J. J., & Thomason, C. S. (2000). Seasonal Movement and Habitat Use by Striped Bass in the Combahee River, South Carolina. *Transactions of the American Fisheries Society*, 129(6), 1281-1287. doi:10.1577/1548-8659(2000)129.0.co;2
- Chadwick, H. K. (1968). Mortality rates in the California striped bass population. *Calif. Fish and Game*, 54(4), 228-246.
- Clarke A, Johnston NM (1999) Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology*, 68, 893–905.

- Cook, A. M., Duston, J., & Bradford, R. G. (2006). Thermal tolerance of a northern population of striped bass *Morone saxatilis*. *Journal of Fish Biology*, 69(5), 1482-1490.
- Conover, D. O., Brown, J. J., & Ehtisham, A. (1997). Countergradient variation in growth of young striped bass (*Morone saxatilis*) from different latitudes 1. *Canadian Journal of Fisheries and Aquatic Sciences*, 54(10), 2401-2409. doi:10.1139/f97-147
- COSEWIC. 2012. COSEWIC assessment and status report on the Striped Bass *Morone saxatilis* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. iv. + 82 pp. (www.registrelep-sararegistry.gc.ca/default\_e.cfm).
- Coutant, C. C.. *Environmental quality for striped bass*. United States: N. p., 1980. Web.
- Coutant, C. C. (1985). Striped Bass, Temperature, and Dissolved Oxygen: A Speculative Hypothesis for Environmental Risk. *Transactions of the American Fisheries Society*, 114, 31-61.
- Coutant, C. C. (1990). Temperature-Oxygen Habitat for Freshwater and Coastal Striped Bass in a Changing Climate. *Transactions of the American Fisheries Society*, 119(2), 240-253. doi:10.1577/1548-8659(1990)119<0240:thffac>2.3.co;2
- Cox, D. K., & Coutant, C. C. (1981). Growth Dynamics of Juvenile Striped Bass as Functions of Temperature and Ration. *Transactions of the American Fisheries Society*, 110(2), 226-238. doi:10.1577/1548-8659(1981)110<226:gdojsb>2.0.co;2
- Del Toro-Silva FM, Miller JM, Taylor JC, Ellis TA. Influence of oxygen and temperature on growth and metabolic performance of *Paralichthys lethostigma* (Pleuronectiformes: Paralichthyidae), *J Exp Mar Biol Ecol* , 2008, vol. 358 (pg. 113-23)
- Dudley, R. G., Mullis, A. W., & Terrell, J. W. (1977). Movements of Adult Striped Bass (*Morone saxatilis*) in the Savannah River, Georgia. *Transactions of the American Fisheries Society*, 106(4), 314-322.
- Duston, J, et al. Effect of Body Size on Growth and Food Conversion of Juvenile Striped Bass Reared at 16–28 °C in Freshwater and Seawater. *Aquaculture*, vol. 234, no. 1-4, 2004, pp. 589–600., doi:10.1016/j.aquaculture.2004.01.005.
- Farrell, A. P. (2015). Pragmatic perspective on aerobic scope: peaking, plummeting, pejus and apportioning. *Journal of Fish Biology*, 88(1), 322-343.
- Ferreira, Elizabeth O., Katja Anttila, and Anthony P. Farrell (2014). Thermal Optima and Tolerance in the Eurythermic Goldfish (*Carassius auratus*): Relationships between Whole-Animal Aerobic Capacity and Maximum Heart Rate. *Physiological and Biochemical Zoology* 87.5: 599-611.
- George N. Somero, Peter W. Hochachka, Biochemical Adaptation to the Environment, *Integrative and Comparative Biology*, Volume 11, Issue 1, February 1971, Pages 159–167, <https://doi.org/10.1093/icb/11.1.159>
- Green, B. W., Rawles, S. D., Fuller, S. A., Beck, B. H. & Mcentire, M. E. (2015). Hypoxia affects performance traits and body composition of juvenile hybrid striped bass (*Morone chrysops* × *M. saxatilis*). *Aquaculture Research*, 47, 2266–2275.

- Hartman, Kyle J., and Stephen B. Brandt. Comparative energetics and the development of bioenergetics models for sympatric estuarine piscivores. *Canadian Journal of Fisheries and Aquatic Sciences* 52, no. 8 (1995): 1647-666. doi:10.1139/f95-759.
- He, W., Cao, Z., & Fu, S. (2015). Effect of temperature on hypoxia tolerance and its underlying biochemical mechanism in two juvenile cyprinids exhibiting distinct hypoxia sensitivities. *Comparative Biochemistry And Physiology, Part A*, 187232-241. doi:10.1016/j.cbpa.2014.05.004
- Healy, T. M., & Schulte, P. M. (2012). Thermal Acclimation Is Not Necessary to Maintain a Wide Thermal Breadth of Aerobic Scope in the Common Killifish (*Fundulus heteroclitus*). *Physiological & Biochemical Zoology*, 85(2), 107-119. doi:10.1086/664584
- Hedrick, R. P., Mcdowell, T., & Groff, J. (1987). Mycobacteriosis In Cultured Striped Bass From California. *Journal of Wildlife Diseases*, 23(3), 391-395. doi:10.7589/0090-3558-23.3.391
- Hundt, M., Schiffer, M., Weiss, M., Schreiber, B., Kreiss, C., Schulz, R., & Gergs, R. (2015). Effect of temperature on growth, survival and respiratory rate of larval allis shad *Alosa alosa*. *Knowledge and Management of Aquatic Ecosystems*, (416), 27. doi:10.1051/kmae/2015023
- IPCC, 2007: <http://www.slvwd.com/agendas/Full/2007/06-07-07/Item%2010b.pdf>
- Kernan, M., Battarbee, R. W., & Moss, B. R. (2011). *Climate Change Impacts on Freshwater Ecosystems*. Somerset: Wiley.
- Kraus, R. T., Secor, D. H. & Wingate, R. L. (2015). Testing the thermal-niche oxygen-squeeze hypothesis for estuarine striped bass. *Environmental Biology of Fishes*, 98, 2083–2092.
- Kruger, Richard L., and Robert W. Brocksen. Respiratory metabolism of striped bass, *Morone saxatilis* (Walbaum), in relation to temperature. *Journal of Experimental Marine Biology and Ecology*, vol. 31, no. 1, 1978, pp. 55–66., doi:10.1016/0022-0981(78)90136-3.
- Lapointe, D., Vogelbein, W., Fabrizio, M., Gauthier, D., & Brill, R. (2014). Temperature, hypoxia, and mycobacteriosis: effects on adult striped bass *Morone saxatilis* metabolic performance. *Diseases of Aquatic Organisms*, 108(2), 113-127.
- Lurman, G., Walter, J., & Hoppeler, H. H. (2013). Seasonal changes in the behaviour and respiration physiology of the freshwater duck mussel, *Anodonta anatina*. *Journal of Experimental Biology*, 217(2), 235-243. doi:10.1242/jeb.093450
- McBryan, T. L., Healy, T. M., Haakons, K. L., & Schulte, P. M. (2016). Warm acclimation improves hypoxia tolerance in *Fundulus heteroclitus*. *The Journal of Experimental Biology*, 219(4), 474-484. doi:10.1242/jeb.133413
- McDonnell, L. H., & Chapman, L. J. (2016). Effects of thermal increase on aerobic capacity and swim performance in a tropical inland fish. *Comparative Biochemistry And Physiology, Part A*, 19962-70. doi:10.1016/j.cbpa.2016.05.018
- Milton A. Matthews, Robert F. McMahon, Effects of temperature and temperature acclimation on survival of zebra mussels (*Dreissena polymorpha*) and Asian clams (*Corbicula*

- fluminea*) under extreme hypoxia, *Journal of Molluscan Studies*, Volume 65, Issue 3, August 1999, Pages 317–325, <https://doi.org/10.1093/mollus/65.3.317>
- Mohan, J. A., Halden, N. M., & Rulifson, R. A. (2014). Habitat use of juvenile striped bass *Morone saxatilis* (Actinopterygii: Moronidae) in rivers spanning a salinity gradient across a shallow wind-driven estuary. *Environmental Biology of Fishes*, 98(4), 1105–1116.
- Nelson, J. A. & Lipkey, G. K. (2015). Hypoxia tolerance variance between swimming and resting striped bass (*Morone saxatilis*). *Journal of Fish Biology*, 87, 510–518.
- Nikinmaa, M., Cech, J. J., & Mckenroe, M. (1984). Blood oxygen transport in stressed striped bass (*Morone saxatilis*): role of beta-adrenergic responses. *Journal of Comparative Physiology B*, 154(4), 365–369. doi:10.1007/bf00684443.
- Nilsson, G. E., Östlund-Nilsson, S., & Munday, P. L. (2010). Effects of elevated temperature on coral reef fishes: Loss of hypoxia tolerance and inability to acclimate. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 156(4), 389–393. doi:10.1016/j.cbpa.2010.03.009
- Patricia M. Schulte, Timothy M. Healy, Nann A. Fangue, Thermal Performance Curves, Phenotypic Plasticity, and the Time Scales of Temperature Exposure, *Integrative and Comparative Biology*, Volume 51, Issue 5, November 2011, Pages 691–702, <https://doi.org/10.1093/icb/icr097>
- Pörtner, H., Bock, C., & Mark, F. C. (2017). Oxygen- and capacity-limited thermal tolerance: Bridging ecology and physiology. *The Journal of Experimental Biology*, 220(15), 2685–2696. doi:10.1242/jeb.134585
- Raby, G. D., Casselman, M. T., Cooke, S. J., Hinch, S. G., Farrell, A. P., & Clark, T. D. (2016). Aerobic scope increases throughout an ecologically relevant temperature range in coho salmon. *The Journal of Experimental Biology*, 219(12), 1922–1931.
- Reinert, T. R. (2004). *Decline and recovery of striped bass in the Savannah River Estuary: synthesis and re-analysis of historical information and evaluation of restoration potential* (Unpublished doctoral dissertation).
- Rogers, N. J., Urbina, M. A., Reardon, E. E., Mckenzie, D. J., & Wilson, R. W. (2016). A new analysis of hypoxia tolerance in fishes using a database of critical oxygen level (Pcrit). *Conservation Physiology*, 4(1). doi:10.1093/conphys/cow012
- Rosewarne, P. J., Wilson, J. M., & Svendsen, J. C. (2016). Measuring maximum and standard metabolic rates using intermittent-flow respirometry: a student laboratory investigation of aerobic metabolic scope and environmental hypoxia in aquatic breathers. *Journal of Fish Biology*, 88(1), 265–283.
- Rummer, J. L., Couturier, C. S., Stecyk, J. A., Gardiner, N. M., Kinch, J. P., Nilsson, G. E., & Munday, P. L. (2013). Life on the edge: thermal optima for aerobic scope of equatorial reef fishes are close to current day temperatures. *Global Change Biology*, 20(4), 1055–1066.

- Shang, E.H.H., and Wu, R.S.S. 2004. Aquatic hypoxia is a teratogen and affects fish embryonic development. *Environ. Sci. Technol.* 38: 4763–4767. doi:10.1021/es0496423. PMID:15487785.
- Shingles, A., Mckenzie, D.J., Taylor, E.W., Moretti, A., Butler, P.J., & Ceradini, S. (2001). Effects of sublethal ammonia exposure on swimming performance in rainbow trout (*Oncorhynchus mykiss*). *The Journal of experimental biology*, 204 Pt 15, 2691-8 .
- Speers-Roesch, B., Mandic, M., Groom, D. J. E., & Richards, J. G. (2013). Critical oxygen tensions as predictors of hypoxia tolerance and tissue metabolic responses during hypoxia exposure in fishes. *Journal of Experimental Marine Biology and Ecology*, 449, 239–249. <https://doi-org.libez.lib.georgiasouthern.edu/10.1016/j.jembe.2013.10.006>
- Storey, K. B., & Tanino, K. K. (2012). *Temperature adaptation in a changing climate nature at risk*. Wallingford: CAB International.
- Survey, U.S. Geological. Water Resources of the United States. Water Resources of the United States: U.S. Geological Survey. N.p., n.d. Web. 13 Apr. 2017.
- Svendsen, M. B. S., P. G. Bushnell, and J. F. Steffensen. Design and setup of intermittent-flow respirometry system for aquatic organisms. *Journal of Fish Biology* 88.1 (2015): 26-50.
- T. L. McBryan, K. Anttila, T. M. Healy, P. M. Schulte, Responses to Temperature and Hypoxia as Interacting Stressors in Fish: Implications for Adaptation to Environmental Change, *Integrative and Comparative Biology*, Volume 53, Issue 4, October 2013, Pages 648–659, <https://doi.org/10.1093/icb/ict066>
- Thompson JS, Rice JA (2013) The relative influence of thermal experience and forage availability on growth of age 1–5 striped bass in two southeastern reservoirs. *Am Fish Soc. Symp* 80:93–120
- U.S. Fish and Wildlife Service. 1983-19\_. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates. U.S. Fish Wildl. Serv. Biol. Rep. 82(11). U.S. Army Corps of Engineers TR EL-82.-4.
- Verberk, W. C., Overgaard, J., Ern, R., Bayley, M., Wang, T., Boardman, L., & Terblanche, J. S. (2016). Does oxygen limit thermal tolerance in arthropods? A critical review of current evidence. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 192, 64-78. doi:10.1016/j.cbpa.2015.10.020
- Wannamaker, C. M., & Rice, J. A. (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.
- White, C. R., Phillips, N. F., & Seymour, R. S. (2006). The scaling and temperature dependence of vertebrate metabolism. *Biology letters*, 2(1), 125–127. doi:10.1098/rsbl.2005.0378
- Will, T. A., Reinert, T. R., & Jennings, C. A. (2001). Assessment of spawning sites and reproductive status of striped bass, *Morone saxatilis*, in the Savannah River Estuary. *Final report for project*, 10-21

- Wood, C. M. (2018). The fallacy of the Pcrit – are there more useful alternatives? *The Journal of Experimental Biology*, 221(22). doi:10.1242/jeb.163717
- Woodward, G., Perkins, D. M., & Brown, L. E. (2010). Climate change and freshwater ecosystems: impacts across multiple levels of organization. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 365(1549), 2093–2106. doi:10.1098/rstb.2010.0055
- Wu, C., Liu, Z., Li, F., Chen, J., Jiang, X., & Zou, S. (2017). Gill remodeling in response to hypoxia and temperature occurs in the hypoxia sensitive blunt snout bream (*Megalobrama amblycephala*). *Aquaculture*, 479479-486. doi:10.1016/j.aquaculture.2017.06.020
- Yang, Yang, et al. Variations in temperature acclimation effects on glycogen storage, hypoxia tolerance and swimming performance with seasonal acclimatization in juvenile Chinese crucian carp. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, vol. 185, 2015, pp. 16–23., doi:10.1016/j.cbpa.2015.03.009.
- Yeager, D. P., & Ultsch, G. R. (1989). Physiological Regulation and Conformation: A BASIC Program for the Determination of Critical Points. *Physiological Zoology*, 62(4), 888-907. doi:10.1086/physzool.62.4.30157935
- Zhongqi Chen, c., Snow, M., Lawrence, C. S., Church, A. R., Narum, S. R., Devlin, R. H., & Farrell, A. P. (2015). Selection for upper thermal tolerance in rainbow trout (*Oncorhynchus mykiss* Walbam). *Journal Of Experimental Biology*, 218(5), 803-812.