A Dissertation
Entitled

The Physiological and Behavioral Responses of Yellow Perch to Hypoxia

by

Betsy L. Bodamer Scarbro

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the
Doctor of Philosophy Degree in Biology (Ecology)

_________________________________________
Dr. Thomas B. Bridgeman, Committee Chair

_________________________________________
Dr. Jessica Head, Committee Member

_________________________________________
Dr. Christine M. Mayer, Committee Member

_________________________________________
Dr. Randall J. Ruch, Committee Member

_________________________________________
Dr. W. Von Sigler, Committee Member

_________________________________________
Dr. Patricia R. Komuniecki, Dean
College of Graduate Studies

The University of Toledo

May 2014
An Abstract of

The Physiological and Behavioral Responses of Yellow Perch to Hypoxia

by

Betsy L. Bodamer Scarbro

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Doctor of Philosophy Degree in Biology (Ecology)

The University of Toledo

May 2014

Yellow Perch within Lake Erie’s Central Basin must contend with the development of hypolimnetic hypoxia, which generally occurs August - October and reaches thicknesses of up to 8 meters off the lake bottom. Since Yellow Perch are primarily demersal benthivores, large portions of their primary habitat becomes unsuitable during hypoxic events. Field studies have shown that while Yellow Perch largely avoid hypoxia, they continue to forage for benthic prey despite hypoxic conditions. Little is known about the fine-scale behavioral changes of Yellow Perch during hypoxia, or the physiological consequences of hypoxic foraging. In controlled laboratory experiments, I analyzed the behavioral changes of Yellow Perch under simulated hypolimnetic hypoxia, and determined the physiological response of Yellow Perch to hypoxic exposure by measuring the response of a hypoxia-responsive protein, Hypoxic Inducible Factor -1α.

Yellow Perch were subjected to normoxic (~8 mg O₂/L), moderate hypoxic (~4 mg O₂/L) or severe hypoxic (<2 mg O₂/L) dissolved oxygen concentrations for durations...
of up to 8 hours, followed by a 40-hour normoxic recovery period. Baseline HIF-1α levels were detected in Yellow Perch liver tissues under normoxic conditions, and increased significantly after two hours of hypoxic exposure. HIF-1α peaked at 2 and 4 hours of hypoxic exposure under severe and moderate hypoxic conditions, respectively, but returned to levels similar to normoxic treatments by 8 hours of exposure. These results suggest Yellow Perch are well adapted to hypoxic conditions and that a direct negative feedback mechanism may aide survival under prolonged hypoxia.

In order to observe the behavioral changes of Yellow Perch in stratified hypoxic conditions, I designed and constructed two experimental tank systems that simulated hypoxic conditions characteristic of temperate freshwater lakes. Using these systems, two behavioral experiments were conducted examining changes in behavior and consumption of Yellow Perch subjected to various thicknesses of hypolimnetic hypoxia. While the number of hypolimnetic forays did not differ between hypoxic and normoxic treatments, dive duration decreased significantly during hypoxia, resulting in less time total time in the hypolimnion. Consumption did not significantly decrease until hypoxic thickness reached 4.0 meters. These findings suggest that the ability of Yellow Perch to forage benthically is not greatly affected by hypoxia \( \leq 2.6 \) meters in thickness; however, increasing hypoxic thickness likely decreases the energetic gain of benthic foraging, driving horizontal shifts in Yellow Perch populations to areas where hypoxia is thinner \((\leq 2.6 \) m). Increases in the duration or spatial extent of hypoxia resulting from forecasted global climate conditions are likely to lead to further changes in community distributions, increased competition, and altered trophic interactions.
To Mom and Dad
for instilling upon me the love of nature and water from the very start

&

Rocky
for giving me another reason to stay
Acknowledgements

This dissertation has been a long, but worthwhile journey, one that would not have been possible without all the support I received from advisors, colleagues, friends, and family. First and foremost, I would like to thank my advisor, Dr. Tom Bridgeman, for helping me to grow as a scientist, and encouraging me to keep going when things didn’t work out as planned. I would also like to thank the other members of my graduate committee, Drs. Christine Mayer, Randall Ruch, Von Sigler, and Jessica Head for all their input and guidance throughout this project. A special thanks to Drs. Patrick Kocovsky and Richard Kraus for their support, understanding and advice during this final stretch. To Pat, Meredith, and Rachel - thank you for all your administrative assistance, moral support, and for always taking care of us. And to Amanda, Kristen, Rachel – thank you for being my support group and sounding board, and making this a journey of friendship as well as scientific growth. I could not have made it this far without the love and support from my parents and my family – who have ever only believed in me and encouraged me to follow my dreams. To my husband, thank you for being my “Rock” (yeah, I did), my coach, and my biggest fan throughout this journey, and for always helping me keep life in perspective. And last but not least, to my Scarbro family and to all my other dear friends for all your love and moral support, and for not taking it personally when I forget to phone back. To all of you – thank you.
Table of Contents

Abstract ........................................................................................................................................ iii
Acknowledgements ......................................................................................................................... vi
Table of Contents ........................................................................................................................... vii
List of Tables ................................................................................................................................... xi
List of Figures ................................................................................................................................. xii
Preface .............................................................................................................................................. xiii
1 Introduction ................................................................................................................................. 1
2 Assessment of Hypoxia Inducible Factor in Yellow Perch *Perca flavescens*:
  Can HIF be used as an indicator of hypoxic exposure? ....................................................... 5
    2.1 Abstract ................................................................................................................................. 5
    2.2 Introduction .......................................................................................................................... 6
    2.3 Methods .............................................................................................................................. 10
      2.3.1 Experimentation ........................................................................................................... 10
      2.3.2 Protein reparation and analysis ................................................................................... 11
      2.3.3 Data analyses ............................................................................................................... 13
    2.4 Results ................................................................................................................................. 13
    2.5 Discussion ............................................................................................................................ 15
      2.5.1 Conclusions and Future recommendations. ............................................................... 18
2.6 Acknowledgements.................................................................19

3 Experimental dead zones: two designs for creating oxygen gradients in aquatic ecological studies..........................................................23

3.1 Abstract....................................................................................23

3.2 Introduction..............................................................................24

3.3 Methods..................................................................................28

3.3.1 Hypoxitron 1: Water column stratification with hypolimnetic hypoxia..............................................................................28

3.3.1.1 Thermal stratification.........................................................28

3.3.1.2 Creating hypolimnetic hypoxia.........................................29

3.3.1.3 Adding fish.........................................................................30

3.3.2 Hypoxitron 2: Hypoxic channels .........................................32

3.3.2.1 Creating hypoxia...............................................................33

3.3.2.2 Adding fish.........................................................................34

3.4 Assessment..............................................................................34

3.4.1 Hypoxitron 1 – Hypoxic water column..............................34

3.4.2 Hypoxitron 2 – Hypoxic channel.........................................35

3.5 Discussion and Recommendations.........................................37

3.6 Acknowledgements................................................................39

4 Bobbing for benthos: Yellow Perch (Perca flavescens) foraging behavior in simulated hypolimnetic hypoxia ..............................................51

4.1 Abstract....................................................................................51

4.2 Introduction..............................................................................52
4.3 Methods ............................................................................................................. 55

4.3.1 Experiment 1: Changes in diving behavior and consumption ........ 55

4.3.1.1 Tank system ................................................................................................. 56

4.3.1.2 Animals ........................................................................................................ 56

4.3.1.3 Fish acclimation and experimentation ............................................... 57

4.3.1.4 Data collection ............................................................................................ 57

4.3.1.5 Statistical Analysis ..................................................................................... 58

4.3.2 Experiment 2: Changes in consumption with increasing hypoxic thickness .............................................................................................................. 58

4.3.2.1 Tank system ................................................................................................. 58

4.3.2.2 Animals ........................................................................................................ 59

4.3.2.3 Fish acclimation and experimentation ............................................... 60

4.3.2.4 Statistical Analysis ..................................................................................... 61

4.4 Results .................................................................................................................. 62

4.4.1 Experiment 1: Changes in diving behavior and consumption ....... 62

4.4.2 Experiment 2: Changes in consumption with increasing hypoxic thickness .............................................................................................................. 63

4.5 Discussion ............................................................................................................ 64

4.6 Acknowledgements ............................................................................................. 68

5 Discussion .............................................................................................................. 78

5.1 General conclusions ........................................................................................... 78

5.1.1 Short-term hypoxic exposure has little effect on Yellow Perch physiology or consumption potential ........................................... 79
5.1.2 Yellow Perch continue to forage during hypoxic conditions, however benthic foraging is limited by hypoxic thickness ..........80

5.1.3 Possible consequences for trophic interactions and bioenergetics. ..80

5.1.4 Implications of hypoxic depth thresholds and Lake Erie hypoxia, and possible consequences of global warming..............................82

5.2 Future Research .........................................................................................................................................................83

5.2.1 Further exploration into the HIF pathway in Yellow Perch ...........83

5.2.2 Examining hypoxic tolerance of Yellow Perch over genetically or geographically different populations .....................84

5.2.3 Stratified hypoxic behavioral studies with live prey.................85

References.........................................................................................................................................................................86
List of Tables

3.1 Materials and sources for major components of Hypoxitron systems ..................41
3.2 Summarized pros and cons of Hypoxitron 1 and Hypoxitron 2 .........................42
4.1 Average DO and temperatures of normoxic and hypoxic experimental zones .....69
List of Figures

2-1 Image of HIF protein bands .................................................................21
2-2 HIF response to hypoxic exposure in Yellow Perch...........................................22
3-1 Diagram of Hypoxitron 1 ........................................................................44
3-2 Diagram of acclimation cage ......................................................................45
3-3 Diagram of Hypoxitron 2 ........................................................................46
3-4 Detail of experimental chamber section ......................................................47
3-5 DO and temperature profiles from Hypoxitron 1 ........................................48
3-6 Hypolimnetic DO in Hypoxitron 1 ..............................................................49
3-7 Average DO for each section of Hypoxitron 2 ........................................50
4-1 Diagram of Hypoxitron 1 system used in experiment 1 .............................72
4-2 Diagram of Hypoxitron 2 system used in Experiment 2 ............................73
4-3 Diagram of Benthic Foraging Apparatus ....................................................74
4-4 Number of dives, dive duration, and total time spent in hypolimnion ..........75
4-5 Average consumption of Yellow perch in normoxic and hypoxic hypolimnias....76
4-6 Consumption of Yellow Perch across varying thickness of hypoxia ............77
Preface

The chapters of this dissertation are organized in order of their scope for understanding the physiological and behavioral responses of Yellow Perch to hypoxic environments. Each chapter has been written as a manuscript to be submitted for publication to a peer reviewed journal. Chapter 3 is largely identical to the submitted manuscript version, with only slight re-wordings.

Chapter 2 will be submitted for potential publication to the Journal of Fish Biology. Chapter 3 is has received a conditional accept for potential publication as:


and is largely identical to the submitted manuscript version with only slight re-wordings.

Chapter 4 will be submitted for potential publication to *Freshwater Biology*.

This is publication #2014-05 from the University of Toledo Lake Erie Center. This work was funded by a University Research Award to T. Bridgeman et al. from U. Toledo and the Ohio Board of Regents. Funding for BBS was supplied by University of Toledo teaching and research assistantships, and a Robert Brundage Memorial Scholarship. Additionally, NSF GK-12 DGE#0742395 provided two years of stipend, tuition, and fees.
support, as part of "Graduate Fellows in High School STEM Education: An Environmental Science Learning Community at the Land-Lake Ecosystem Interface."

All fish handling and experimentation described in this paper was carried out in accordance with the University of Toledo Institutional Animal Care and Use Committee (IACUC protocol # 106201).
Chapter 1

Introduction

The Central Basin of Lake Erie (LECB) has been historically prone to oxygen depletion and the formation of hypoxia due to the lake’s morphology and high productivity (Delorme 1982). The size, location, and duration of the central basin’s hypoxic zone varies year to year depending on meteorological processes that influence stratification, total phosphorous loadings, hypolimnetic dissolved oxygen depletion rates, and dissolved oxygen concentrations at the time of stratification (Burns et al., 2005; Rao et al., 2008). Dissolved oxygen concentrations within the hypolimnion generally reach hypoxic levels as early as July, and continue to decline, often forming areas of anoxia (<1mg/L) before the fall turnover in September or October (USGS Lake Erie Biological Station, unpublished data; Arend et al. 2011). Hypoxia develops upward from the benthic layer, a result of biological processes depleting available oxygen via decomposition and respiration. Depending on hypolimnetic depth and the persistence of stratification, the LECB hypoxic layer can reach depths ranging from 1 to 8 meters (avg. = 6 m at deepest point; Arend et al. 2011), leading to changes in species distributions and trophic interactions (Roberts et al., 2009; Vanderploeg et al. 2009a; Vanderploeg et al. 2009b).
The Yellow Perch (*Perca flavescens*) is one of the most important fisheries species in Lake Erie, providing essential linkages between benthic and pelagic communities (Knight et al. 1984; Cobb & Watzin 1998), and contributing a large percentage of the Lake Erie fishing industry with 5393 tons of Yellow Perch commercially harvested from Lake Erie in 2012 (YPTG 2013). Since Yellow Perch are primarily demersal benthivores (Knight et al., 1984), a large portion of their primary habitat becomes unsuitable during periods of hypoxia. Field studies have reported shifts in Yellow Perch distributions during periods of hypolimnetic hypoxia within the LECB, migrating both vertically to normoxic (normal oxygen concentrations) water above the thermocline, and horizontally to areas where the hypoxic layer is thin or nonexistent (Roberts et al. 2009). Changes in species distributions are common responses to hypoxic conditions, as species undergo behavioral responses to avoid hypoxic conditions and migrate to areas with normal oxygen concentrations (Wannamaker & Rice 2000; Baldwin et al. 2002; Roberts et al. 2009; Vanderploeg et al. 2009b; Zhang et al. 2009). These shifts in species distributions result in lower community diversity and/or changes in community structures not only within the hypoxic zone, but within neighboring oxygenated areas as well (Britt 1955; Magnuson et al. 1985; Rahel & Kolar 1990; Breitburg 1992; Wu 2002; Bridgeman et al. 2006; Ludsin et al. 2009). The migration of species to areas with more suitable oxic conditions may reduce the direct negative consequences of hypoxia; however migration subjects organisms to new environmental conditions, including: changes in thermal, optic, and chemical properties; habitat substrates; population densities; novel predator and prey species (Kersten et al. 1991;
Domenici et al. 2000; Domenici et al. 2007); and an overall increase in predation risk (Rahel & Kolar 1990; Vanderploeg et al. 2009a). Consequently, long durations of hypoxia can have negative impacts on the physiology of organisms, leading to reduced growth and reproductive potential (Marcus et al. 2004; Thomas et al. 2007).

The formation of hypolimnetic hypoxia may create a potential barrier between Yellow Perch and their primary food source, benthic invertebrates (Phil et al. 1992; Aku & Tonn 1999; Zhang et al. 2009), as hypoxic areas have been shown to provide refuge for hypoxia-tolerant prey from their larger, less tolerant predators (Magnuson et al. 1985; Chapman et al. 1995; Robb & Abrahams 2002;). However, predators have also been shown to make forays into hypoxic water to forage for preferred prey species (Rahel & Nutzman 1994; Robb & Abrahams 2002). Diet analyses of LECB Yellow Perch have shown shifts in foraging patterns from benthic invertebrates to pelagic zooplankton during extensive hypoxic events (Roberts et al. 2009). However, bottom water trawls within the LECB hypoxic zone captured Yellow Perch that had recently consumed benthic prey items. These findings suggest Yellow Perch continued to forage benthically despite severe hypoxia (Roberts et al. 2009).

Although there is evidence that Yellow Perch subject themselves to hypoxia in order to forage for benthic invertebrates, little is known about the hypoxia-induced behavioral changes undergone by Yellow Perch, the full extent of hypoxic exposure, or the physiological consequences that result. The central objectives of this dissertation research are to analyze the behavioral changes of Yellow Perch in stratified, hypoxic hypolimnetic conditions, and to determine their physiological response via the production
of hypoxia-responsive proteins. This study utilizes controlled laboratory experiments to examine small-scale behavioral and physiological processes that drive larger shifts in Yellow Perch distribution and prey selection. The specific objectives of this study are:

1) To identify a hypoxia-responsive protein (specifically Hypoxia-Inducible Factor, HIF) in Yellow Perch that may be used to indicate the hypoxic exposure of wild caught fish, and to quantify the physiological response in Yellow Perch over various degrees of hypoxic exposure (Chapter 2);

2) To develop an experimental tank system that simulates hypolimnetic hypoxic conditions, allowing for the study Yellow Perch under environmentally relevant oxygen gradients (Chapter 3); and

3) To use the experimental system in objective 2 to examine and quantify the changes in Yellow Perch behavior and benthic foraging when confronted with hypolimnetic hypoxia (Chapter 4).
Chapter 2

Assessment of Hypoxia Inducible Factor in Yellow Perch *Perca flavescens*: Can HIF be used as an indicator of hypoxic exposure?

2.1 Abstract

The Yellow Perch *Perca flavescens* is one of the most important Great Lakes fish species in terms of economic and ecological value. During the summer months, Yellow Perch within Lake Erie’s Central Basin must contend with a hypolimnetic hypoxia. Although these low oxygen conditions are thought to create a barrier between Yellow Perch and their primary food source – benthic invertebrates – studies have shown that Yellow Perch make forays into hypoxic water in order to forage. However, the extent of hypoxic exposure experienced by Yellow Perch and the potential physiological consequences that result are unknown. In this study, I quantified the response of Hypoxia-Inducible Factor-1-alpha (HIF-1α) in Yellow Perch tissues to examine the potential of HIF-1α as a biomarker of hypoxic exposure in wild caught fish. I conducted a controlled laboratory experiment in which Yellow Perch were subjected to normoxic (~8 mg O₂/L), moderate hypoxic (~4 mg O₂/L) or severe hypoxic (<2 mg O₂/L) dissolved oxygen concentrations for durations of up to 8 hours, followed by a 40 hour normoxic
recovery period. Baseline HIF-1α levels were detected in Yellow Perch liver tissues under normoxic conditions, and increased significantly after two hours of hypoxic exposure. HIF-1α peaked at 2 and 4 hours of hypoxic exposure under severe hypoxic and moderate hypoxic conditions, respectively, but returned to levels similar to normoxic treatments by 8 hours of exposure, and continued to decline to levels approximately half of that of control levels. While the mechanisms regulating HIF levels in Yellow Perch are still uncertain, these results suggest a direct negative feedback mechanism that prevents cell death and increases the probability of survival under prolonged hypoxia. High individual variability within treatments and the increase/decrease response indicate that HIF-1α may not be the most useful indicator of hypoxic exposure in wild-caught fish. Regardless, this study serves as an important first step in characterizing the molecular response of Yellow Perch to hypoxia.

2.2 Introduction

The Yellow Perch *Perca flavescens* is one of the most economically and ecologically important Great Lakes fish species. Prized as a food and sport fish, the Yellow Perch ranks as one of the top three recreational and commercial fishery species, and over 5000 tons of Yellow Perch were harvested from Lake Erie in 2012 (YPTG 2013). Ecologically, Yellow Perch provide essential linkages between benthic and pelagic communities by benthic foraging (Cobb & Watzin 1998) and seasonal migrations between nearshore and offshore regions (Brown et al. 2009).
During the summer months, Yellow Perch within Lake Erie’s Central Basin (LECB, avg. depth = 18.3 m) must contend with a thermally stratified water column in which the hypolimnion steadily becomes depleted in dissolved oxygen (DO) until hypoxic (≤2mg O₂/L) or even anoxic (<1 mgO₂/L) conditions result. The breadth and duration of hypolimnetic hypoxia varies year to year depending on seasonal factors, however dissolved oxygen levels within the hypolimnion generally fall to hypoxic levels by early August. Dissolved oxygen concentrations continue to decrease, often forming areas of anoxia before the fall turnover in September or October. The late-summer hypoxic layer creates a potential barrier between Yellow Perch and their primary food source, benthic (bottom dwelling) invertebrates. Yellow perch select for pelagic prey during periods of hypoxia; yet, bottom trawl samples within the LECB hypoxic zone produced Yellow Perch that had recently consumed benthic prey items (Roberts et al. 2009). These findings suggest that Yellow Perch continue to forage benthically despite severe hypoxic conditions. The physiological consequences of foraging in a hypoxic environment are unknown but may result in physiological stress and decreased fitness (Roberts et al. 2011).

Studies examining the hypoxic tolerance of Yellow Perch offer a range of results. While some studies have found Yellow Perch to be extremely sensitive to hypoxia, experiencing mortality within 24 hours exposure to only moderately low oxygen conditions (3.5 mg/L at 19°C; Moore 1942), other studies have found Yellow Perch to be extremely hypoxic tolerant, surviving in oxic conditions as low as 0.25 mg/L (at winter temperatures) and 2 mg/L (at room temperatures) for 5 and 6 days, respectively (Petrosky & Magnuson 1976; Head et al. unpublished data). Hypoxic tolerance has been shown to
differ greatly not only among species, but also within species, where different age and size classes exhibited different thresholds to hypoxia (Tinson & Laybourn-Parry 1985; Casselman & Harvey 1975). These studies have demonstrated that hypoxic tolerance among fish is dependent on various factors, including fish size or age class, environmental temperature, the rate of oxygen decrease and/or acclimation to hypoxic conditions, and possibly even scientific interpretation and the designated indicator of hypoxic stress (i.e. Rees et al. 2001; Moore 1952; Robb & Abrahams 2003; Petrosky & Manguson 1976; Head et al. *unpublished data*).

Recently, the study of physiological responses of fish to hypoxia has included the classification and examination of Hypoxia Inducible Factor (HIF) and its pathways. Hypoxic Inducible Factor is a heterodimer consisting of α and β subunits that are basic-helix-loop-helix PAS proteins. HIF-1α serves as the master switch for the induction of hypoxia related genes that regulate anaerobic energy production and cell survival under hypoxic conditions (reviewed by Benizri et al. 2008; Heise et al. 2007; Bracken et al. 2003). The HIF alpha subunit (HIFα) is continuously produced under normal oxygen conditions; however it is exceptionally short-lived (half-life < 5 min) due to rapid ubiquitination and proteasomal degradation (Huang et al. 1998). Under hypoxic conditions, the oxygen-dependent degradation process is terminated and HIFα accumulates and dimerizes with the constitutively expressed HIFβ (ARNT), translocates into the nucleus, and binds with coactivators p300/CBP and Src-1 (Bruick & McKnight, 2001). The HIF/coactivator complex binds to hypoxia-responsive element (HRE) promotor regions and activates the transcription of a number of target genes that regulate oxygen transport and cell adaption through decreased locomotion, muscle contraction,
protein translation, increased amino acid metabolism, the up-regulation of ATP metabolism and glycolysis, and anti-growth (Wu, 2002; Semenza, 1999).

HIFα has been documented in insect, mammal, and fish species (e.g. Arquier et al., 2006; Bernaudin et al., 2002; Gorr et al., 2006; Gracey et al., 2001), suggesting that HIFα is present in all complex animals. Most of the available data on HIFα and its target genes have been obtained through human and other mammalian studies. Fish, mammals, and insects share many of the same biochemical and physiological responses to hypoxia (Soitamo et al., 2001; Nikinmaa & Rees, 2005; Law et al., 2006; Rahman & Thomas, 2007; Gracey et al., 2001); however, fish are exposed to a broader range of environmental oxygen concentrations and hence may exhibit different behavioral and molecular adaptations to hypoxia.

Four HIFα homologs (HIF-1α/-2α/-3α/-4α) have been documented in rainbow trout *Oncorhynchus mykiss*, killifish *Fundulus heteroclitus*, zebra fish *Danio rerio*, and grass carp *Ctenopharyngodon idella*, respectively (Nikinmaa & Rees 2005; Law et al. 2006; Rahman & Thomas 2007; Soitamo et al. 2001, Powel & Hahn 2002). However, the molecular functions and pathways of HIFα in fish are still poorly understood. HIF-1α accumulation and DNA binding activity increase during hypoxia in several fish species, including rainbow trout (Soitamo et al. 2000; van Heerden et al. 2004; Nikinmaa et al. 2004), grass carp and crucian carp *Carassius carassius* (Sollied et al. 2006; Law et al. 2006; Rissanen et al. 2006), and atlantic croaker *Micropogonias undulatus* (Rahman and Thomas, 2007). However, individual and species responses can be highly variable, and the significance of this to fish physiology and hypoxic tolerance is still unclear.
Characterizing the molecular response of Yellow Perch to hypoxia is an important first step in understanding the physiological (and perhaps behavioral) responses of perch exposed to hypoxic conditions. In this study, I measured HIFα protein in gill and liver tissue of Yellow Perch exposed to environmentally relevant dissolved oxygen concentrations. I hypothesized that the concentration of HIFα in Yellow Perch would increase with decreasing dissolved oxygen concentration and increased length of exposure to low oxygen. Characterizing the HIF response in fish exposed to naturally relevant oxygen conditions is a first step in the development of HIF as a potential bio-indicator of hypoxic stress in wild-caught fish, and will increase our knowledge of the role of HIF in fish physiology and hypoxic tolerance.

2.3 Methods

2.3.1 Experimentation

I conducted a controlled laboratory experiment in which Yellow Perch were subjected to several levels of stable dissolved oxygen concentrations for durations of up to 8 hours. Fingerling Yellow Perch (80-130 mm total length) were obtained from a local fish hatchery and housed in holding tanks until experimentation began. Three treatment tanks (20 gallon aquaria) were assigned to each dissolved oxygen treatment level: normal (~8 mg O₂/L), moderately hypoxic (~4 mg O₂/L) or hypoxic (<2 mg O₂/L), a range relevant to the LECB hypolimnion. Dissolved oxygen concentrations were manipulated by injecting combinations of nitrogen and atmospheric gas through air stones until the
desired oxygen concentrations were achieved. Temperature was maintained at room temperature for the duration of the experiment (~20°C).

At the beginning of the experiment (T=0), six Yellow Perch were added to each of the nine treatment tanks (N= 54). At the end of each pre-determined time duration (T+1, 2, 4, and 8 hours), one fish from each tank was harvested (N=3 for each T x DO treatment). In addition, one fish was harvested from the holding tank at each time treatment to serve as a control group. Hypoxic conditions were maintained in moderate and severe hypoxic treatment tanks for the first eight hours, after which nitrogen was turned off and compressed air was bubbled into hypoxic tanks, returning oxygen concentrations to normoxic levels. After 24 and 48 hours, the fifth and sixth fish were harvested from each tank, respectively. These last two treatments allowed for the examination of possible recovery from hypoxic-induced stress.

At time of harvest, fish were immediately euthanized by a swift blow to the head followed by clipping of the spinal column directly behind the skull (Van Heerden et al., 2004; Sollid et al., 2006). Handling and euthanization time were minimized (<30 sec) to reduce error and prevent non-treatment level responses. After euthanization, liver and gill tissues were removed and frozen in liquid nitrogen. Samples were stored at -80°C until time of analysis.

2.3.2 Protein Preparation and Analysis

To prepare sample extracts for analysis, fish tissues were homogenized using a Dounce All-Glass Tissue Grinder (Kontes) in ice-cold RIPA lysis buffer with protease inhibitor PMSF (1 mg:10 μg ratio) and sonicated (Branson Sonifier 250, output 3 at 20%
for 20 pulses) on ice to disrupt cell membranes and release cell contents. Extracts were aliquoted into microcentrifuge tubes (~200 µl/tube) and centrifuged at 13000 rpm for 10 minutes. The supernatants were saved and their total protein concentrations were determined with the DC protein assay (BioRad). The supernatants were frozen at -20 C until time of analysis.

HIF-1α content in gill and liver tissues was analyzed by immunoblotting. Supernatants (total proteins = 40 µg/well) were separated on 10% Bis-Tris SDS-PAGE gels (NuPage) along with a common standard (High DO/48hr extract from 1 fish) and a molecular weight standard (Gibco BRL BenchMark Prestained Protein Ladder), and transferred to Immobilon-P membranes (0.45 µm, PVDF, Millipore). Membranes were prestained reversibly (Novex Reversible protein stain kit), and a prominent 120 kDa band was measured by density to verify uniform protein loading (loading standard).

Membranes were blocked in a 5% non-fat dry milk in TBS-Tween-20 blocking solution, incubated with Donkey Normal Serum (1:100 dilution, Jackson ImmunoResearch) for 30 minutes, rinsed, and incubated with anti-HIF antibody (1:500 dilution, see below) for 4 hours. After primary incubation, membranes were rinsed and incubated with the secondary antibody (anti-rabbit IgG-Biotin, 1:500 dilution, GE Healthcare) for 1 hour. Streptavidin-alkaline-phosphatase (Amersham Pharmacia Biotech UK Limited) was used to detect biotin, and membranes were washed in a BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate disodium/nitro blue tetrazolium chloride) color development solutions until bands developed. The resulting bands were scanned (Canon 8800F Color Photo Scanner) and densities determined (quantification with ImageJ software, v1.40g; National Institute of Health, USA)
In preliminary studies, four HIF antibodies were tested against Yellow Perch gill and liver tissues (anti-rabbit HIF-1α and -HIF-4α; anti-gcHIF-1α and -HIF-4α, gc = grass carp *Ctenopharyngodon idellus*, obtained from Dr. Richard Y C Kong, City University of Hong Kong). The anti-gcHIF-1α best detected HIF-1α proteins in Yellow Perch liver tissue. HIFα was not detected in gill tissue using any of these antibodies. Thus, the anti-gcHIF-1α antibody was used to measure HIF-1α in liver tissue in this study.

2.3.3 Data Analysis

HIF-1α band densities were normalized by standardizing raw values against the loading (~120 kDa prestain band) and common standards (1 common sample ran on all gels). The resulting values were normalized against the mean HIF-1α value of the control (T=0) group, setting control values to one and measuring HIF in terms of fold increase from control levels. Statistical significances between combined oxygen and time treatment groups were determined with a repeated measures analysis of variance (ANOVA) followed by a Tukey HSD post hoc test using SAS 9.2 Enterprise software (SAS Institute, Cary NC, USA). Correlation analysis between water temperature and total length and HIF content of high and control treatment levels were analyzed in RStudio 0.97.551 (R Core Team, 2012) using the Hmisc package (Harrell, 2014) rcorr() function. All alphas were set to 0.05.

2.4 Results
Grass carp HIF-1α antibody detected HIFα at ~80 kDa (Fig 1). In other fish studies, HIF-1α proteins range from approximately 82 to 94 kDa (Soitamo et al., Law et al. 2006; Sollid et al. 2005). Two hours of exposure to severe hypoxia (≤2 mg/L) resulted in a significant increase in HIFα in Yellow Perch liver tissue, increasing 4.5 fold above control levels ($p=0.0141$, Fig 2). HIFα levels decreased and returned to levels not different than that of fish held in normoxic conditions after 4, 8, 12, and 24 hours of exposure. HIFα levels also increased significantly after 2 hours of moderate hypoxia treatment and peaked at 5.3-fold above control at 4 hours treatment ($p=0.0029$). By the 8 hour treatment, HIFα levels declined to control level. A marked decrease in HIFα levels was observed in control and treatment groups after the 8 hour treatment and 16 hours of recovery (24 hour group), dropping to levels approximately half of control levels, and remained stable for the remainder of the recovery period. A single mortality occurred during the experiment within a hypoxic treatment tank, shortly after 2 hours of exposure (@T=2:07). This fish was not included in the analysis, and had a HIFα level of only 0.224x control levels.

There was high individual variation in HIFα protein content in both normoxic and hypoxic fish. Although smaller fish have been found to accumulate HIFα more quickly (Rissanen et al. 2006), there was no correlation between total fish length and HIFα levels in normoxic treatments to account for this variation ($R^2 = -0.01835, p=0.4338$). Temperature can influence HIFα levels in fish liver tissues, however water temperature of experimental tanks fluctuated by only 2.3°C over the 48 hour period (mean temp = 20.9°C) and showed no correlation with HIF levels ($R^2 = 0.03809, p=0.6118$).
2.5 Discussion

This study shows that HIF-1α content increases dramatically in Yellow Perch liver within 2 hours of exposure to moderate and severe hypoxia. Interestingly, HIF accumulated at similar rates in both hypoxic treatments during the first 2 hours of low oxygen exposure (Fig 2). However, under hypoxia, the ability of Yellow Perch to maintain elevated HIF levels decreased so that by 4 hours of exposure, HIF-1α levels were similar to control. Under moderate hypoxia, HIF-1α levels peaked at 4 hours then declined to control at 8 hours. Although this pattern only partially supports my original hypothesis, similar patterns have been found in other fish studies, citing both DO and time dependent HIFα thresholds. HIF-1α accumulated within in vitro tissues of the hypoxic sensitive rainbow trout after 4 hours exposure to 5% oxygen, but tended to decrease at lower oxygen levels (Soitamo et al. 2001). In crucian carp liver tissues, HIF levels peaked at 6 hours, and thereafter declined despite continued hypoxia (Rissanen et al. 2006). On the other hand, in grass carp, a hypoxic tolerant species, HIF-1α and -4α content increased steadily (1.5 and 2.5 fold, and 2.5 and 3 fold) after 4 and 24 hours of hypoxic exposure (Law et al. 2006).

These results suggest that there is an oxygen or time threshold in which maximal HIF accumulation occurs, and conditions exceeding these limits result in feedback regulation that reduces HIF content. Similar decreases in HIF-1α have been observed within human and mouse tissues under prolonged hypoxia (Marxsen et al. 2004; Uchida et al. 2004; Ginouves et al. 2008). Uchida et al. (2004) suggested that initial increases in HIF-1α simultaneously increase natural antisense HIF-1α (αHIF) which bind to and
destabilize HIF-1α mRNA, leading to decreased HIF-1α protein synthesis. Despite decreases in HIF-1α, HIF-2α remained stable, which suggested that the initial accumulation of HIF-1α may be critical to an organism’s survival under hypoxic conditions, and that different HIFα analogs likely have separate roles in hypoxia-induced responses (Uchida et al. 2004). Alternatively, decreases in HIF-1α during chronic hypoxia were found to result from the overexpression of prolyl-hydroxylase (PHD), which hydroxylates HIF-1α and marks it for degradation (Ginouves et al. 2008; Marxsen et al. 2004). Under normoxic conditions, PHD maintains HIF-1α at low levels, but under hypoxic conditions, PHD activity decreases and HIF-1α levels increase. However, it was found that the intracellular O₂ availability is increased due to the inhibition of mitochondrial respiration by HIF (Ginouves et al. 2008). With oxygen once again available, PHD activity increased and reinitiated the HIF-1α degradation pathway, leading to decreased HIF-1α levels (a.k.a. desensitization).

Furthermore, HIFα levels declined to normoxic levels before the termination of hypoxic treatment, however levels continued to decline after re-oxygenation to approximately half of control levels and remained steady for the duration of the recovery period. Though this response has not been previously described in fish, Manxsen et al. (2004) reported PHD levels increased strongly in human cells in response to prolonged hypoxia. After re-oxygenation, PHD levels declined but remained elevated even after 24 hours of normoxic conditions. The result was a more rapid HIF-1α degradation, shortening the half-life of HIF-1α and decreasing HIF-1α content (Manxsen et al. 2004). The similarity between the HIF response in Yellow Perch observed in this study and the
PHD regulated negative feedback of HIF in human cells suggests that PHD may play a role in the regulations of HIF-1α in Yellow Perch, limiting the accumulation of HIF-1α under chronic hypoxia.

The high survival rate of Yellow Perch in hypoxic treatments adds further support to the decline in HIFα after initial elevation during hypoxia being due to HIF desensitization and physiological adaptation, and not due to general metabolic failure. Based on the results of this study, the extremely low level of HIF-1α in the non-surviving fish (death at T=2:07, HIF=0.224x control levels) suggests either 1) the HIF mechanism failed and HIF did not accumulate at all, or 2) HIF peaked and declined within the first 2 hours of hypoxic exposure. Though there was no visible ailment, other physiological stressors or illness may have affected this individual’s ability to respond to hypoxia. Regardless, this single Yellow Perch further demonstrates the necessity of HIF for fish survival under hypoxic conditions.

Yellow perch appear to maintain baseline levels of HIF-1α, a characteristic that has been emerging with the increased knowledge of the role of HIF in fish physiology. HIF has been detected at significant levels under normal oxygen conditions within rainbow trout, grass carp, and crucian carp, suggesting that HIF has important roles in fish metabolism even under normoxic oxygen conditions (Soitamo et al. 2001; Law et al. 2006; Rissanen et al. 2006). Variation in the HIF response has been shown to be influenced both by temperature and body size, with lower temperatures and smaller mass leading to increased or faster HIF accumulation (Rissanen et al. 2006), but no correlation between HIF accumulation and body size or temperature was observed in this study. The cause for the variability in HIF-1α content in control groups over 48 hours is uncertain,
but could result from changes in environment (tank transfer & acclimation), tank disturbance at harvest times, or capture efficiency.

2.5.1 Conclusions and Future recommendations

This study further indicates that Yellow Perch are very tolerant of hypoxia and can survive under low oxygen conditions for several hours (present study, Petrosky & Magnuson 1976; Head et al. unpublished data). This study was an important first step in the understanding of the HIF response in Yellow Perch and demonstrates varying HIF levels across oxygen gradients and over time. The results suggest, however, that HIF-1α content alone will not be a useful indicator of hypoxic exposure in wild-caught fish. Although the HIF response is sufficiently rapid (1-2 hrs) to potentially identify fish exposure to hypoxic waters, it does not appear to correlate solely with duration of exposure or level of hypoxia. Stress and other factors (such as capture/handling stress, temperature changes, and hypoxic exposure >2 hour) may affect HIF-1α content. Additionally, it is unknown how repeated short hypoxic exposures (<1 hour) alternated with normoxic recovery periods affect HIF accumulation. Identifying and characterizing additional Yellow Perch HIF analogs would provide a more complete picture of HIF response and function, and the development of Yellow Perch-specific HIF antibodies would increase sensitivity of HIF detection, possibly detecting finer scale changes. Furthermore, studies addressing alternating short term hypoxic and normoxic recovery exposures under the associated low and high temperatures of stratification may provide more environmentally relevant data compatible with the benthic foraging behavior of Yellow Perch within stratified hypoxic waters (Roberts et al, 2009; see also Chapter 4).
Studies examining the genetic targets or regulators of HIF, such as VEGF or PHD, would help to better understand the role HIF plays in hypoxic adaptation and the mechanisms driving the negative feedback of HIF under prolonged hypoxia. Furthermore, HIF target genes or regulators may exhibit responses more useful for determining hypoxic exposure in wild caught fish.

2.6 Acknowledgements

I would like to thank Dr. Richard Kong of the City University of Hong Kong Department of Biology and Chemistry for the supply of the grass carp HIF antibody. The experimental portion of this project was conducted in partnership with T. Höök, J. Roberts, and with assistance from D. Warner. V. Sigler, J. Head, and C. Mayer provided scientific advice and input throughout this project. This research was supported by a University Research Award from U. Toledo and the Ohio Board of Regents to T. Bridgeman, V. Sigler, R. Ruch, and J. Turner. Support for BLB was proved by a NSF GK-12 DGE#0742395 fellowship and a Robert Brundage Memorial Scholarship.
**Figure 2-1** Scanned image of immunoblot membrane demonstrating HIF protein accumulation in Yellow Perch liver tissues after 2 hours of normoxic (8 mg/L), moderate hypoxic (4 mg/L), and severe hypoxic (2 mg/L) exposure.

**Figure 2-2** Average HIF-1α content (± 1 SE, n=3) in Yellow Perch liver tissue relative to control group (control = 0 hr treatment). Yellow perch were exposed to normoxic (8 mg/L), moderate hypoxic (4 mg/L), or severe hypoxic (2 mg/L) conditions for up to 8 hours. 24 and 48 hour treatments represent the 16 and 40 hour recovery time treatments, respectively. Significant differences from normoxic levels within time treatments are dictated by * at $p < 0.05$. Significant differences among time treatments not shown.
Figure 2-1

At T=2 hrs
Figure 2-2
Chapter 3

Experimental dead zones: two designs for creating oxygen gradients in aquatic ecological studies

3.1 Abstract

Hypoxia is commonly defined as dissolved oxygen (DO) concentrations below 2 mg L$^{-1}$. The occurrence of hypoxia within freshwater and marine ecosystems is stressful for respiring organisms across all trophic levels, leading to changes in community diversity, altered trophic interactions, and physiological fitness of organisms. While many field studies have identified changes in diets and species distribution due to hypoxia, the individual behavioral changes that drive these patterns is poorly understood. Additionally, many laboratory studies that subject organisms to a fixed DO concentration may not be entirely applicable to natural environments because many organisms are capable of sensing and avoiding hypoxic areas. Herein I describe two experimental tank systems developed to study the effects of oxygen gradients on fish behavior. These systems are novel in that 1) fish and potentially other aquatic organisms can freely move between hypoxic and well oxygenated areas, 2) the thermocline or oxycline is easily adjusted for multiple treatment levels, and 3) they are constructed with affordable, readily-available materials and are easily maintained. In both systems, I was able to
conduct fish behavior studies under stable thermal and oxic stratification comparable to conditions found in temperate freshwater lakes.

3.2 Introduction

Hypoxia (dissolved oxygen concentrations of 2 mg L\(^{-1}\) or less) is a growing environmental problem affecting marine, estuarine, and freshwater systems globally. Since the 1960s, the number of low oxygen “dead zones” has increased exponentially, with over 470 coastal hypoxic areas reported in 2007 (Diaz and Rosenberg 2008). Although hypoxia is a naturally occurring process (particularly in stratified eutrophic lakes), both the frequency and duration of hypoxic events have increased with the expansion of urban and agricultural land use and the consequential eutrophication of aquatic ecosystems (Diaz and Rosenberg 2001). Efforts to reduce nutrient loadings to lake and river systems have resulted in improved conditions and reduced hypoxia in some instances (i.e. Elbe Estuary, Germany; Barrow Estuary, Ireland; Sarasota Bay, Florida, US; Lake Macquarie, Australia; see Diaz et al. 2011), while similar efforts in other watersheds have remained unsuccessful (i.e. Lake Erie, US/Canada, Zhou et al. 2012; also see Diaz et al. 2011).

Effects of global climate change may result in further expansion of hypoxia as well as more extensive hypoxic events. Hydrodynamic and water quality models examining the potential effects of global climate change on aquatic systems predict increases in both the occurrence and spatial extent of hypoxic events as a result of increasing water temperatures. (Magnuson et al. 1997, Kling et al. 2003, Blumberg and
Di Toro 2011). Rises in water temperatures can trigger a cascade of biological and chemical changes, including decreases in oxygen solubility, longer duration of summer stratification, and increases in biological oxygen demands (Kling et al. 2003). These complex biological and chemical changes can affect the intensity and duration of hypolimnetic hypoxia.

The occurrence or intensification of hypoxia is stressful for aquatic organisms across all trophic levels, leading to disruptions in important ecosystem functions (Magnuson et al. 1985; Aku and Tonn 1999, Diaz and Rosenberg 2008). Hypoxia can lead to increased mortality in both sessile and mobile organisms (Wu 2002, Diaz 2001, Diaz and Rosenberg 2008, Hernandez-Miranda et al. 2010), or to the extirpation or migration of oxygen sensitive species (Britt 1955, Wu 2002, Bridgeman et al. 2006). These changes lead to decreases in community diversity and changes in community structure, not only within the hypoxic zone but neighboring oxygenated areas as well (Magnuson 1985, Rahel and Kolar 1990, Breitburg 1992, Ludsin et al. 2009, Vanderploeg et al. 2009a). The migration of organisms to areas with higher dissolved oxygen concentrations may reduce the direct negative consequences of hypoxia, but may subject the organisms to new environmental stresses, including: changes in thermal, optic, and chemical properties; habitat substrates; population densities; increased competition for resources (Kersten et al. 1991, Domenici et al. 2000); and an overall increase in predation risk (Wolf and Framer 1987, Rahel and Kolar 1990, Vanderploeg et al. 2009a). Shifts in species placement not only alter trophic interactions (Pihl et al. 1992, Breitburg et al. 1997, Ludsin et al. 2009), but over the long term can have negative impacts on the physiology of organisms, leading to reduced growth and reproductive
potential (Marcus et al. 2004, Thomas et al. 2007). The specific food web and physiological effects of hypoxia are highly case-specific and depend on a variety of factors including physiological tolerances to hypoxia and the effects of low oxygen concentrations on escape behavior, feeding behavior, and swimming speed of the species involved (Breitburg et al. 1997).

Understanding individual changes in behavior and physiology is necessary to fully explain the ecological consequences of hypoxia. Field methods such as hydroacoustics and depth stratified trawling have been utilized in studies examining the responses of aquatic organisms – primarily fishes and zooplankton – to hypoxic conditions. These studies documented diet shifts and changes in distributions (i.e. Wanink et al. 2001, Pothoven et al. 2009, Roberts et al. 2009, Vanderploeg et al. 2009b, Roberts et al. 2012), however provided only limited insight on the fine-scale mechanisms and behaviors that drive these patterns. Laboratory studies have documented physiological and behavioral changes of aquatic organisms under hypoxic conditions, however these studies have generally subjected organisms to a fixed dissolved oxygen concentration for a predetermined duration (i.e., McIntyre and McCollum 2000, Brandt et al. 2009), or to gradually altered dissolved oxygen concentrations of the entire experimental environment (i.e. Chapman et al. 1995, Tiffany et al. 2010). While such studies help to explain physiological changes resulting from hypoxic exposure, the behavioral aspect may not be entirely applicable to natural environments since many organisms are capable of sensing and avoiding hypoxic areas (Kramer 1987, Wannamaker and Rice 2000, Pollock et al. 2007). While a few studies have created artificially stratified small-scale environments in order to examine the behaviors of
zooplankton, macroinvertebrates, and larval fishes under hypoxic conditions (i.e. Tinson and Laybourn-Parry 1985, Rahel and Kolar 1990, Breitburg 1994, Stalder and Marcus 1997, Weltzien et al. 1999), only two of these have been conducted using larger fish species. A study conducted by Claireaux et al. (1995a) examined the oxygen consumption and heart rates of Atlantic cod within a thermally stratified 10.5 m high x 4 m diameter tower tank in which fish were free to move between temperatures, and in a later study (Claireaux et al. 1995b), varying oxygen concentrations. However, constructing or gaining access to a tank of this scale is likely unfeasible for most research facilities.

My objective was to design and create an experimental tank system in which: 1) fish could freely move between hypoxic and well oxygenated areas; 2) the thermocline and/or depth of hypoxia could be easily adjusted for multiple treatment levels; and 3) could be constructed from readily available and affordable materials totaling ≤$5000. This experimental system would be valuable for studying behavioral responses to hypoxia due to the fact that it would be more comparable to real-world hypoxic environments, and hence would produce results more applicable to natural populations. Such a system could be used to better examine small-scale hypoxia-induced changes in behavior, physiology, and species/trophic interactions for various aquatic and marine taxa, hence furthering our ability to understand and predict the full ecological consequences of hypoxia.

The design and construction of my experimental oxygen gradient systems stemmed from my desire to further understand the effects of Lake Erie’s summer “dead zone” on the foraging behavior of Yellow Perch (*Perca flavescens*). As my research
progressed, my experimental system underwent several modifications in order to better mimic natural Lake Erie condition and to provide increased control of experimental variables. Herein I describe two resulting designs.

3.3 Materials and Procedures

3.3.1 Hypoxitron 1: Water column stratification with hypolimnetic hypoxia

3.3.1.1 Thermal Stratification

My goal in this initial design was to closely emulate the natural process of freshwater stratification by controlling the temperature, and hence the density, of specific water column strata. This system consisted of two treatment tanks (1.5-m-high x 0.45-m-diameter, 1-mm translucent polymer fiberglass tank), allowing for a control and a hypoxic treatment, partially immersed within a larger basin of cold water (0.8-m-high x 1.2-m-diameter fiberglass tank, Fig. 1, Table 1). Tanks were filled with de-chlorinated tap water rather than lake water in an effort to minimize biological activity that could potentially alter set dissolved oxygen concentrations. Water within the outer tank was cooled using a water chiller unit (Frigid Units, Inc.), and circulated with a pond pump (~250 gph) to ensure constant and uniform cooling. The rapid equilibration of water temperature between the external tank and the immersed portion of the treatment tanks allowed us to manipulate the depth and temperature of the treatment tank hypolimnia by controlling external tank water level and temperature. Epilimnetic temperature was regulated by ambient air temperature and/or by the use of immersed aquaria heaters. To
help monitor stratification, four thermometers were mounted within each treatment tank at depths corresponding to mid-hypolimnion, mid-epilimnion, and ~ 0.25m above and below the predicted thermocline. Target temperatures for the hypolimnion and epilimnion were 7°C and 20°C, respectively, similar to that experienced by temperate zone lakes during summer stratification (Wetzel 2001).

3.3.1.2. Creating hypolimnetic hypoxia

Because the bubbling of gas creates water column mixing and disrupts stratification, it was necessary to achieve the desired hypolimnetic oxygen concentration before the initiation of thermal stratification. Hypolimnetic dissolved oxygen concentrations (control ≥ 8 mg L⁻¹; hypoxic ≤1 mg L⁻¹) were obtained by bubbling either nitrogen gas (hypoxic treatment) or compressed air (control treatment) throughout the entire water column. Chillers and external circulation pumps were turned on prior to this aeration period to minimize changes in hypolimnetic temperature and dissolved gas saturation potential. Once the desired oxygen concentrations were achieved, I ceased aeration and allowed the tanks to stratify thermally. Stratification was considered to be stable once epilimnetic and hypolimnetic temperatures reached >20°C and <10°C, respectively. Once stable stratification was achieved, epilimnetic oxygen concentrations could be manipulated and returned to normal saturations (≥8 mg L⁻¹) by delicately bubbling compressed air at a mid-epilimnnetic level. Water circulation from this created normoxic conditions without disrupting thermocline. In order to minimize the absorption of oxygen from the air back into the water during de-oxygenation, I covered the hypoxic treatment tank with a vented lid until thermal stratification was complete.
3.3.1.3. Adding Fish

I tested the functionality of my system using hatchery-obtained Yellow Perch (115-220 mm), reared in 2-m-diameter tanks and habituated to eat sinking food pellets. The addition of fish created additional challenges in the maintenance of water stratification and oxygen gradients. The swimming motion of fish creates water movement, which can potentially disrupt thermal stratification. Since fish activity increased immediately following introduction into a new environment, it was necessary to restrict fish movement during acclimation periods by adding a three-stage acclimation process to my experimental protocol. For the first stage of acclimation, fish were transferred to an acclimation chamber in each of the two treatment tanks. Acclimation chambers consisted of a wire mesh cage (0.25 m dia. x 0.36 m) with a weighted door that could be opened to release the fish with minimal human interference (Fig. 2). This initial stage of acclimation restrained the fish to a small area within the epilimnion, minimizing frantic behavior and water column mixing. Once fish appeared calm and unstressed (~ 4 hours), the weighted door was opened and secured, allowing the fish to exit the acclimation chamber at will.

The initial response of fish after being released from the acclimation chambers was to swim to the bottom, regardless of oxygen conditions. This behavioral response to a new environment may not only prove lethal to fish, but may also skew experimental results. In nature, hypoxia forms gradually over time giving fish and other organisms time to recognize and adapt to changing environmental conditions. To better simulate an organism’s exposure to natural hypoxia formation, I placed adjustable barriers in each
experimental tank prior to stratification. These barriers were positioned slightly above the thermocline, giving fish free range of the epilimnion yet preventing passage into the hypolimnion. This second stage of acclimation lasted ~18 hours or until fish ceased hiding behavior and began to swim freely. Approximately 2 hours before the start of an experiment, the barriers were gradually lowered, allowing fish to slowly acclimate to the entire water column and the associated oxygen conditions (third stage of acclimation). Not only did this gently introduce fish to hypoxia, but also reduced cross-thermocline movement and helped prolong stratification. Barriers were left to rest flush on the bottom and were left on bottom for duration of the experiment to minimize disturbance.

Once fish were fully acclimatized, I placed food pellets at the bottom of each treatment tank to encourage forays into the hypolimnion. I continued to aerate the epilimnion throughout the experiment to ensure the epilimnion remained normoxic regardless of any mixing. During the experiment, tanks were left undisturbed with the exception of hourly monitoring of thermal stratification and hypolimnetic dissolved oxygen concentrations.

3.3.2 Hypoxitron 2: Hypoxic Channels

While Hypoxitron 1 proved useful for examining the behavioral responses of fish to hypoxic hypolimnnetic environments, it became apparent that constructing a stratified hypoxic system at a larger scale could pose logistic challenges and potential hazards. For instance, hypolimnetic hypoxia within the Central Basin of Lake Erie can reach thicknesses of 4 meters or more – meaning that studies examining specific behavioral
changes or trophic interactions, particularly those involving benthic organisms and/or benthic foragers, would require tanks with total depths of 6 meters or more. Since this was both financially and physically unfeasible, I redesigned my initial hypoxic system to function horizontally rather than vertically. In this manner, hypoxic “depth” is limited only by the space and/or materials available.

Because my research interests addressed Lake Erie’s central basin hypoxia, I designed this new system with the intent of developing corridors in which fish would be separated from their food by up to 4 meters of hypoxic water (with 4 treatment levels - 0, 1.3, 2.6, and 4 m hypoxia). This design consisted of one large holding tank (2 m x 5 m x 1 m) containing four separated experimental units (Fig. 3). Each experimental unit comprised an introduction tank (0.3 m x 0.3 m x 1 m) conjoined with a four meter experimental chamber (30-cm-diameter, clear 0.5-mm PVC ducting). End caps constructed of 0.3-m-diameter Plexiglas windows were installed at the end of each chamber to contain fish and prevent water exchange between the outer basin and the experimental chamber. Additionally, 10 x 7-cm access doors were cut and hinged to the end caps to allow for the easy access of food placement and/or retrieval.

3.3.2.1 Creating Hypoxia

In order to create normoxic and hypoxic areas within each unit, I stretched three separate lengths of diffuser hose (1.3 m, or increment of treatment levels) end-to-end along the bottom of each experimental chamber (Fig. 4). Each section of diffuser hose was connected to either a nitrogen or compressed air gas source, depending on the designated DO level of that section. Gas vents (3 per chamber, modified 12-mm-male
PVC coupling plus ~20-cm length of 12-mm inside diameter vinyl tubing) were added to prevent the accumulation of gas and potential floatation of the chambers. Setting a low water level (equal throughout experimental chambers and the outer basin) to allow for a headspace of ~25 mm within the experimental chambers was necessary to prevent water loss during gas escape, causing potential influxes of oxygenated water (See Fig. 4). Additionally, plastic baffles were added between each 1.3-m section to prevent lateral air movement within the headspace, minimizing the absorption of unassigned gases and further stabilizing the desired oxic status of each section.

To allow for the easy monitoring of dissolved oxygen concentrations along the entire length of each experimental chamber, access ports were installed near the center of each 1.3-m section. Each port was constructed using a threaded, male/female 50-mm PVC coupling and capped with a 38-mm rubber stopper. These dimensions allowed me to monitor DO concentrations throughout each experimental chamber using a YSI ProODO hand-held dissolved oxygen meter. Stoppers prevented air exchange but were easily removed at time of DO measurement. Once construction was complete and water filled to the appropriate level, diffuser hoses were connected to the appropriate gas sources and bubbled until the desired oxygen concentration was reached.

3.3.2.2 Adding Fish

The horizontal nature of this design allowed for the introduction and acclimation of fish prior to the formation of hypoxia. Three fish were added to each experimental unit and allowed to acclimate for 24 hours before de-oxygenation was initiated. Diffusers were attached to air sources so that one of each treatment level (0, 1.3, 2.6, or 4 m) was
assigned to an experimental unit. After desired oxygen concentrations were reached, DO concentrations throughout each experimental chamber were monitored for 24 hours. Fish were allowed free range of their assigned experimental unit for all stages. As before, food was placed at the far end of each experimental chamber to encourage fish movement.

3.4 Assessment

3.4.1 Hypoxitron 1 – Hypoxic water column

I successfully developed and maintained hypolimnetic hypoxia using the vertically stratified tank system. Oxygen depletion, stratification, and re-oxygenation of the epilimnion was usually accomplished within 60 hours, and resulted in ideal thermal and oxic profiles with a distinct epilimnion, thermocline, and hypolimnion (Fig. 5). Hypolimnetic and epilimnetic temperatures differed by approximately 15°C, similar to temperatures of natural stratified freshwater lakes, creating a density gradient sufficient to maintain stable stratification (Wetzel 2001). Oxygen concentrations within the control tank were maintained at normoxic levels (~8 mg L\(^{-1}\)) throughout, and were often higher in the hypolimnion due to the greater saturation of oxygen in colder water. Dissolved oxygen within the hypoxic tank was easily decreased to hypoxic (≤2 mg L\(^{-1}\)) or even anoxic (≤1 mg L\(^{-1}\)) concentrations. Before fish introduction, tank stratification and hypoxia were extremely stable, standing for several days with no noticeable change in dissolved oxygen concentrations or hypolimnion temperatures.

Controlling the initial frantic swimming of introduced fish greatly improved my ability to minimize water mixing and maintain hypolimnetic hypoxia. Even with the
continuous normal swimming behavior of experimental fish throughout each trial, dissolved oxygen concentrations within the bottom layer of water remained at hypoxic levels for a minimum of 6 hours – long enough to run short behavioral experiments (Fig 6).

Over the duration of trial experiments (6 hours), overall temperature and dissolved oxygen concentration within the top water layers and the normoxic hypolimnion did not change. Dissolved oxygen concentrations within the designated hypoxic hypolimnion increased slowly at an average rate of 0.135 mg L\(^{-1}\) hr\(^{-1}\) as a result of fish movement and water mixing. Over the course of 6-hour trials, the average total increase in dissolved oxygen concentrations was 0.92 mg L\(^{-1}\) with a maximum total increase of 1.47 mg L\(^{-1}\) (Fig. 6).

3.4.2 Hypoxitron 2 – Hypoxic Channel

Although this second design excluded thermal stratification, I was able to develop and maintain oxygen gradients with varying lengths of hypoxia (Fig.7). Average dissolved oxygen concentrations of the oxygenated and hypoxic zones were 6.76 and 1.81, respectively. Because of the horizontal design of this system, bubbling could continue throughout the entire experiment or as needed without disrupting oxygen gradients. As a result, areas could be easily deoxygenated or re-oxygenated with fish present, allowing fish to be introduced and acclimated to the experimental chamber prior to hypoxic formation. In this system, de-oxygenation of designated hypoxic areas to hypoxic concentrations (to <2.0 mg L\(^{-1}\)) required significantly less time (~ 4-5 hours, 4 psi) but varied greatly depending on regulator settings.
Due to the horizontal nature and lack of thermal stratification, water mixing between designated hypoxic and oxygenated sections was more difficult to control despite the help of the added baffles. Fortunately, this effect could be largely offset by the ability to continuously bubble nitrogen or oxygen to specified areas. Adjacent normoxic and hypoxic areas experienced greater variability in oxygen concentrations and were more difficult to maintain. Consequently, DO concentrations decreased with increased size of hypoxic zones – i.e. hypoxic zones with the 1.3-m hypoxia treatment level had DO concentrations averaging 2.59 mg L\(^{-1}\), compared to 1.45 mg L\(^{-1}\) in the 4-m hypoxic treatment. Regardless, I was able to achieve the desired range of oxygen gradients and maintain these gradients for a minimum of 48 hours (Fig. 7). Oxygen gradients were often disrupted due to removing the fish at the end of the 24-hour trial experiments; however I believe that these oxygen gradients could be maintained for as long as desired with proper monitoring.

3.5 Discussion and Recommendations

The ability to examine individual behavioral changes is essential in understanding the full effect of hypoxia on aquatic organisms, populations, and communities. In this project, I have developed two separate systems in which organisms can be observed in oxygen gradients similar to those that occur in nature. Compared to previous laboratory
hypoxia studies, organisms have free range of these systems and can therefore choose when they enter hypoxic conditions and for how long, enabling studies that are more ecologically relevant to naturally occurring hypoxia. These free-range systems present new opportunities to examine hypoxia-induced behavioral changes and how they influence physiology, trophic interactions, and other community dynamics.

Hypoxitron 1 performed well using small to medium sized (≤25 cm) fishes, and could easily be adapted for larger fishes given availability of materials and the ability to maintain a low surface area to height ratio (≤ 1:3). Due to the lack of water circulation or pump hazards, this system would also be advantageous for hypoxia-related studies regarding zooplankton, macroinvertebrates, or a combination of organisms for trophic or community level studies. This design incorporates a natural temperature gradient, minimizing the likelihood of results being skewed by the influence of temperature on behavior and physiological processes of study organisms. Consequently, experiments comparing behaviors in normoxic and hypoxic thermally stratified conditions are more easily and accurately extrapolated to natural ecosystems.

Due to the potential of water mixing with the movements of larger mobile organisms, this stratification system is likely best for short-term studies. It may be possible to extend the duration of hypoxia by increasing the total height of both the internal tank and external tanks, thereby creating a larger metalimnion zone, helping to reduce mixing of further spaced epilimnetic and hypolimnetic waters. Hypoxia might also be “refreshed” periodically by the careful addition of cold, de-oxygenated water through a tube extending from the water surface to the bottom. This method could
potentially decrease oxygen concentrations within the epilimnion, however this may have minimal impact on experimentation due to the ability to re-oxygenate epilimnetic waters.

Depending on the desired depths of hypoxia, use of the Hypoxitron 1 design may be limited to available materials, physical stability, and accessibility. In studies in which deeper or multiple hypoxic depths are desired, the Hypoxitron 2 design may prove more practical (Table 2). Using this horizontally-designed system, I was able to achieve and maintain oxygen gradients with up to 4 meters of hypoxia. Despite the lack of thermal stratification, the oxygen gradients proved to be more stable and more easily maintained than in Hypoxitron 1.

The ability to continuously bubble gasses within the channels makes this design extremely versatile. Firstly, hypoxic areas can likely be maintained indefinitely with careful monitoring, or re-oxygenated and de-oxygenated at will, allowing for great experimental flexibility. Additionally, because organisms can be added prior to de-oxygenation, responses to hypoxic formation can be observed and quantified as well. This presence of study organisms during hypoxic formation is likely more comparable to real world conditions since in natural environments, organisms are gradually acclimated to the changing oxic conditions.

Even with continuous bubbling of nitrogen and oxygen, I experienced issues with water mixing between neighboring hypoxic and normoxic sections. This was not surprising due to the lack of thermal stratification. While I was still able to achieve the desired oxygen gradients, this cross mixing of water between neighboring sections may also be mediated by allowing for longer mixing zones – adding distance between designated hypoxic and normoxic areas. Still, examining the effects of shallow, marginal
hypoxia may be beneficial as such conditions are comparable to early stages of hypoxic formation, areas at the perimeter of hypoxic zones, and eutrophic systems that experience episodic stratification.

These experimental systems create new opportunities for the study and observation of aquatic organisms under various oxygen gradients. The ability to quantify hypoxic-induced changes in behavior, physiology, and trophic interactions among aquatic organisms in a controlled laboratory setting will create a more comprehensive understanding of how hypoxia alters ecosystem functions and community structure. By creating an experimental environment in which organisms can react to hypoxia as they would in nature, we are able to discern hypoxia-induced behavioral changes and physiological tolerances. My hope is that these designs will be used in future studies examining the effects of hypoxia on individual species, as well as species interactions.

3.6 Acknowledgements

I would like to thank C. Nagel and J. Scarbro for assistance with material acquisition and construction. I would also like to thank R. Ruch, V. Sigler, C. Mayer, and J. Head for scientific advice throughout this project. This research was supported by a University Research Award from U. Toledo and the Ohio Board of Regents. Water chillers were provided by the USGS Lake Erie Biological Station. Support for BLB was provided by a NSF GK-12 DGE#0742395 fellowship and a Robert Brundage Memorial Scholarship.
Table 3.1 Materials and sources for major components of Hypoxitrons 1 and 2

<table>
<thead>
<tr>
<th>Hypoxiton</th>
<th>Component</th>
<th>Material</th>
<th>Supplier</th>
<th>Part No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Treatment tanks</td>
<td>Translucent Tank, Flat Bottom</td>
<td>Pentair Aquatic Ecosystems</td>
<td>T185</td>
</tr>
<tr>
<td>1</td>
<td>Outer basin</td>
<td>Fiberglass Tanks, Circular Flat Bottom</td>
<td>Pentair Aquatic Ecosystems</td>
<td>TR280</td>
</tr>
<tr>
<td>1</td>
<td>Aquaria heaters</td>
<td>Hydor® Theo Submersible Heater 100 watt</td>
<td>Pentair Aquatic Ecosystems</td>
<td>T11202</td>
</tr>
<tr>
<td>1</td>
<td>Thermometers</td>
<td>Lifegard® Digital Thermometer with Light</td>
<td>Pentair Aquatic Ecosystems</td>
<td>TH22A</td>
</tr>
<tr>
<td>1</td>
<td>Acclimation cage</td>
<td>Wire mesh waste paper basket</td>
<td>local office supply store</td>
<td>na</td>
</tr>
<tr>
<td>1</td>
<td>Acclimation cage</td>
<td>1 oz. rubber grip lead sinker</td>
<td>Bass Pro Shops</td>
<td>BPRG1</td>
</tr>
<tr>
<td>1 and 2</td>
<td>Chiller unit</td>
<td>i.e. Model D1-33 or D1-100, <em>(Model used discontinued)</em></td>
<td>Frigid Units, Inc.</td>
<td>na</td>
</tr>
<tr>
<td>1 and 2</td>
<td>Circulation pumps</td>
<td>Mag Drive Pump 250 gph</td>
<td>Pentair Aquatic Ecosystems</td>
<td>MD22</td>
</tr>
<tr>
<td>2</td>
<td>Outer basin</td>
<td>Livestock gates, wire filled</td>
<td>Tractor Supply Company</td>
<td>na</td>
</tr>
<tr>
<td>2</td>
<td>Outer basin</td>
<td>JobSmart® 1.2 cm polypropylene diamond braided rope</td>
<td>Tractor Supply Company</td>
<td>na</td>
</tr>
<tr>
<td>2</td>
<td>Outer basin</td>
<td>Firestone® EPDM pond liner</td>
<td>Pentair Aquatic Ecosystems</td>
<td>SZ22</td>
</tr>
<tr>
<td>2</td>
<td>Experimental chambers</td>
<td>PVC Flexduct Light-Duty Clear Duct Hose 300 mm</td>
<td>Ducting.com</td>
<td>na</td>
</tr>
<tr>
<td>2</td>
<td>Introduction tanks</td>
<td>Rectangular Polyethylene Tank, no longer available</td>
<td>Pentair Aquatic Ecosystems</td>
<td>na</td>
</tr>
<tr>
<td>2</td>
<td>Diffuser hose</td>
<td>i.e. Perfaerated® diffuser tubing or Air Curtains</td>
<td>Pentair Aquatic Ecosystems</td>
<td>WBT2, AC90</td>
</tr>
<tr>
<td>2</td>
<td>Air vents</td>
<td>Vinyl clear tubing</td>
<td>Pentair Aquatic Ecosystems</td>
<td>TV60</td>
</tr>
<tr>
<td>2</td>
<td>Baffles</td>
<td>Flexible plastic cutting boards– cut to fit</td>
<td>local home wares store</td>
<td>na</td>
</tr>
<tr>
<td>2</td>
<td>Access ports</td>
<td>Rubber airlock stoppers</td>
<td>home-brewing supplies catalog</td>
<td>na</td>
</tr>
</tbody>
</table>
Table 3.2 Summarized pros and cons of Hypoxitron 1 and Hypoxitron 2.

<table>
<thead>
<tr>
<th></th>
<th><strong>Pros</strong></th>
<th><strong>Cons</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypoxitron 1</strong></td>
<td>1. Good for fish up to sizes of ≤ 25 cm (using specified dimensions)</td>
<td>1. Cannot bubble gas in hypolimnion after stratification or start of experiment.</td>
</tr>
<tr>
<td></td>
<td>2. Uses an environmentally relevant thermal gradient</td>
<td>2. Likely limited to short-term trials (~6 hours, but dependent on organism size and movement)</td>
</tr>
<tr>
<td></td>
<td>3. Easily adaptable for zooplankton, macroinvertebrates, or combination</td>
<td>3. Hypoxic depth limited</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. Only two treatment levels (hypoxic and control) can be tested simultaneously.</td>
</tr>
<tr>
<td><strong>Hypoxitron 2</strong></td>
<td>1. Good for fishes of 10-25 cm (using specified dimensions)</td>
<td>1. Isothermal/No thermal stratification</td>
</tr>
<tr>
<td></td>
<td>2. Greater range of hypoxic treatment levels</td>
<td>2. Potential of water mixing in adjacent hypoxic and normoxic sections.</td>
</tr>
<tr>
<td></td>
<td>3. Can test various hypoxic treatment levels simultaneously.</td>
<td>3. Does not reproduce physiological changes that organisms may require to adjust to vertical pressure changes.</td>
</tr>
<tr>
<td></td>
<td>4. Ability to bubble gasses throughout trials.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Ability to run longer trials</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6. Ability to create or alter oxygen gradients with organisms present</td>
<td></td>
</tr>
</tbody>
</table>
**Figure 3-1.** Diagram of Hypoxitron 1.

**Figure 3-2.** Diagram of acclimation cage used in the first stage of fish introduction to Hypoxitron 1. Fish were placed inside the basket to minimize frenzied swimming which can lead to water column mixing. Once fish appeared calm, fish were allowed to exit through a hinged door (A). The door was held closed with a weight (B), but could be opened with minimal human interference using an attached pull cord (C) pull cord.

**Figure 3-3.** Diagram of Hypoxitron 2 with side view (A) and top view (B).

**Figure 3-4.** Detail of experimental chamber section: water level, air vent, DO probe port, baffle, and diffuser hose.

**Figure 3-5.** Average dissolved oxygen and temperature profiles of Hypoxitron 1 control and experimental tanks over the duration of 5 trials. Error bars represent the full range of observed values (i.e. min to max).

**Figure 3-6.** Dissolved oxygen within the hypolimnion of the hypoxic treatment tank of Hypoxitron 1, tracked over 4 6-hour fish foraging trials.

**Figure 3-7.** Average dissolved oxygen (± 1 SE) for each 1.3 m section of the 4 experimental chambers in Hypoxitron 2 over the course of four 24-hour trials. Blue and red bars designate sections intended to be normoxic or hypoxic, respectively.
Figure 3-1
Figure 3-2
Figure 3-3

A) Introduction Tanks

1 m

NORMAL OXYGEN

HYPOXIA

Experimental Chambers

Food

B) 0 m

1.3 m

2.6 m

4 m

2 m

5 m
Figure 3-5
Figure 3-6
Figure 3-7

Intended DO concentration
- normoxic
- hypoxic

DO mg/L

Treatment Levels (meters of hypoxia)
Chapter 4

Bobbing for benthos: Yellow Perch (*Perca flavescens*) foraging behavior in simulated hypolimnetic hypoxia

4.1 Abstract

Fish are generally believed to avoid hypoxic habitats via daily or seasonal migration to more desirable habitats. In the Central Basin of Lake Erie, late summer hypoxia within the hypolimnion has been shown to result in changes in fish distribution. While data suggest Yellow Perch *Perca flavescens* largely avoid hypolimnetic hypoxia by vertical or horizontal migration to normoxic waters, there are also data that suggest that Yellow Perch within the central basin of Lake Erie continue to forage benthically despite hypoxic conditions. I conducted two laboratory behavioral studies examining changes in behavior and consumption of Yellow Perch subjected to various degrees of hypolimnetic hypoxia. First, I examined changes in dive frequency and duration, total time spent in hypolimnion, and consumption of Yellow Perch exposed to thermally stratified conditions with normoxic and hypoxic hypolimnia that simulated Lake Erie conditions. Secondly, I examined changes in consumption with varying thickness of hypolimnetic hypoxia (0, 1.3, 2.6, 4.0, and 6.3 m), that encompass the range of summer hypoxia within the central basin. I found that while the number of forays made by
Yellow Perch into the hypolimnion did not decrease in hypoxic conditions, the average duration of each dive decreased significantly, resulting in less time spent in the hypolimnion overall. Despite reduced foraging time, consumption did not significantly decrease until hypoxic thickness reached 4.0 meters. These findings suggest that the ability of Yellow Perch to forage benthically is not greatly affected by hypoxic conditions when the hypoxic layer is 2.6 meters or less, however hypoxic events exceeding 2.6 meters in thickness may result in the horizontal movement or altered diet of affected Yellow Perch populations.

### 4.2 Introduction

Hypoxia is a growing concern in aquatic, estuarine, and marine systems worldwide (Diaz & Rosenberg 2008). Although oxygen depletion is a naturally occurring process in many freshwater and coastal systems, human-intensified nutrient loadings and subsequent eutrophication has been shown to directly correlate with the formation, extent, and duration of hypoxic zones (Diaz & Rosenberg 2010). The Lake Erie Central Basin (LECB) is naturally prone to hypoxia due to the lake’s morphology and high productivity (Hawley et al. 2006; Delorme, 1982); however, additional nutrient enrichment from point and nonpoint sources has led to larger and more severe hypoxic events (Burns et al. 2005; Edwards et al. 2005). With the onset of thermal stratification generally occurring in May (Arend et al, 2011), dissolved oxygen concentrations within the hypolimnion often decline to hypoxic levels by August. Large areas within the LECB hypolimnion can remain hypoxic for up to three months, or until the fall turnover in
September or October (Arend et al, 2011). While nutrient loading has direct correlations to the extent of oxygen depletion (Bertram 1993), the reach and duration of the central basin’s hypoxic zone is also dependent on various meteorological and hydrological processes that influence stratification and water currents (Burns et al., 2005; Rao et al., 2008; Zhou et al. 2013). During stratified conditions, hypoxia develops upward from the benthic layer, a result of biological processes depleting available oxygen via decomposition and respiration. Depending on hypolimnetic depth and the duration of stratification, the LECB hypoxic layer can reach depths ranging from 1 to 8 meters (Arend et al. 2011) leading to changes in species distributions and trophic interactions (Robert et al. 2009; Vanderploeg et al. 2009a, Vanderploeg et al. 2009b).

Due to differences in hypoxic tolerances, changes in distributions and behavior can be highly variable among taxa, species, sex, and size or age classes (Farwell & Fox 2007; Rees et al. 2001; Robb & Abrahams 2003). Though hypoxic tolerance differs among fishes, low oxygen conditions generally have negative effects as oxygen is required for all basic physiological processes (Pollock et al. 2007). Consequently, fish tend to avoid hypoxia in both freshwater (Aku et al. 1997; Baldwin et al. 2002; Wannamaker & Rice 2000) and marine (Wannamaker & Rice 2000; Stierhoff et al. 2009; Breitburg 1994) systems. However there are many reports of fish entering or residing in hypoxic areas in order to either avoid predation (Chapman et al. 1995; Ludsin et al. 2009; Parker-Stetter & Horne 2009) or capture prey (Rahel & Nutzman 1994). Bottom hypoxia can create a barrier or refuge between hypoxia tolerant benthic invertebrates and prey fish and their larger, less tolerant predators (Wanink et al. 2001; Aku & Tonn 1999; Zhang et al. 2009). However, some predator species have been found to hover near the
thermocline and make short forays into the hypoxic environment to capture prey or forage for benthos (Rahel & Nutzmann 1994; Vanderploeg et al. 2009b). This hypoxic foraging behavior may be driven by the predators’ high energy demands but may result in predators enduring stressful conditions in order to capture energetically rich macroinvertebrates and prey fish (Graeb et al. 2006; Schaeffer et al. 1999).

Fish within the LECB have been shown to avoid hypoxic areas via vertical or horizontal migration (Pothoven 2012; Vanderploeg et al. 2009a, Roberts et al. 2009). This change in distribution has been observed in Yellow Perch, one of Lake Erie’s most important fisheries species in terms of both economic and ecological value. Primarily a benthivorous demersal species, Yellow Perch within the LECB are confronted with hypolimnetic hypoxia on an annual basis. During periods of hypoxia, Yellow Perch have been shown to migrate both vertically to metalimnetic layers, and horizontally to the edge of hypoxic zones where the hypoxic layer is thin (Roberts et al. 2009). Stomach content analyses have also shown shifts in Yellow Perch prey selection during hypoxic periods to incorporate more pelagic prey items, primarily mesozooplankton (zooplankton >200μm; Roberts et al. 2009). Although these findings suggest hypoxic avoidance by Yellow Perch, there is evidence that Yellow Perch continue to forage benthically despite hypoxic conditions. Yellow Perch collected via bottom trawls within hypoxic zones were found to have recently consumed benthic invertebrates, though this was more common at sites where the hypolimnion was thinner (~ 2 m; Roberts et al. 2009). Roberts et al. (2009) detected Yellow Perch entering or exiting the hypolimnion during stationary hydroacoustic surveys over LECB hypoxic zones, though only partial fish tracks were captured, leaving the time spent in hypoxic conditions unknown.
Hypoxia-induced behavioral changes on larger mobile organisms are largely understudied in contrast to benthic invertebrates, zooplankton, and larval fishes (Tinson & Laybourn-Parry 1985, Rahel & Kolar 1990, Breitburg 1994, Stalder & Marcus 1997, Weltzien et al. 1999). Understanding the fine scale behavioral changes that lead to broader shifts in species distributions and prey selection undergone by Yellow Perch and other freshwater fish species is crucial to determining the full impact of hypoxia on fish and trophic interactions in Lake Erie and other freshwater systems. In this study, I examined changes in dive behavior and benthic consumption of Yellow Perch subjected to hypolimnetic hypoxia. I conducted two controlled behavioral experiments in which I exposed Yellow Perch to various hypoxic conditions using two oxygen graduated tank systems (detailed in Chapter 3). These systems permitted Yellow Perch to range freely between normoxic and hypoxic areas, allowing for the observation and quantification of behavioral changes and consumption under environmentally relevant hypoxic conditions. It was hypothesized that while Yellow Perch continue to make foraging forays through hypoxia in order to obtain food, the number of forays will decrease under hypoxic conditions, resulting in less overall consumption with increasing hypoxic depths. Consequently, I expected a threshold of hypoxic depth beyond which Yellow Perch cease to forage benthically. Herein, I describe the hypoxic-driven behavioral changes of Yellow Perch and the impacts on benthic consumption.

4.3 Methods

4.3.1 Experiment 1: Changes in diving behavior and consumption
4.3.1.1 Experimental tank system

For this controlled behavioral experiment, I utilized the vertical stratified hypoxic system described in Chapter 3 (Fig. 1) in order to observe Yellow Perch foraging behavior within a thermally stratified environment with normoxic and hypoxic hypolimnetic conditions. This system consisted of two vertical tanks (1.5-m-high x 0.45-m-diameter) placed within a larger outer basin, allowing for simultaneous observation and quantification of Yellow Perch foraging under stratified normoxic and hypoxic hypolimnetic conditions. For each of the five trials conducted, tanks were cleaned and filled with de-chlorinated tap water. Hypolimnetic dissolved oxygen concentrations (Control ~9 mg/L control; Hypoxic ~1 mg/L) were manipulated with the appropriate bubbling of nitrogen or compressed atmospheric air. For these experiments, hypolimnetic and epilimnetic temperatures were set to approximately 7°C and 20°C, respectively. This temperature difference not only created a density difference sufficient to maintain stable stratification, but also resulted in a temperature gradient similar to that experienced in the central basin during late-summer stratification (Boegman 2006).

4.2.1.2 Animals

Yellow Perch (150-220 mm) were obtained from Freshwater Farms of Ohio (Urbana, OH). Fish were housed in large holding tanks and fed sinking pellet food *ad libitum*, but not within 48 hours prior the start of a trial in order to standardize fish for hunger. The use of sinking pellet food ensured that fish were preconditioned to forage benthically.
4.3.1.3 Fish Acclimation and Experimentation

For each trial, three fish were introduced and acclimated to each of the control and hypoxic treatment tanks as described in Chapter 3. The three-stage acclimation process – cage, epilimnion and slow access to hypolimnion – minimized water movement and helped prolong stratification while allowing fish to slowly acclimate to the entire tank. At the beginning of each trial, 30 sinking food pellets were sent to the bottom of each tank using a clear tube, ensuring fish could see, but not consume food until reaching the bottom. Fish behavior was video recorded for 6 hours after food introduction, and human presence was minimized in an effort to avoid disturbance. I made efforts to maintain external environmental factors at a constant level during foraging periods in order to minimize disturbances that may have resulted in altered fish behavior (i.e. lights remained off, noise minimized, etc.) Five six-hour trials were conducted. At the end of each trial, fish were euthanized via a swift blow to head and brain stem severance, and stomach contents were collected.

4.3.1.4 Data Collection

Video surveillance of the trials was analyzed to track fish movement in and out of the hypolimnion. A dive was defined as the amount of time from when the operculum of the fish descended below the thermocline entering the hypolimnion to the time when the operculum of the fish ascended above the thermocline. Due to the difficulty in distinguishing individual fish, the group of three fish was considered as one experimental subject. I was able to track individual fish over the course of a dive, allowing for the calculation of dive duration. For each tank, the total number of dives, duration of each
dive, and the total amount of time spent within the hypolimnion was determined. Because the amount of food eaten could not be obtained from the video, consumption was analyzed using the dry weights of stomach contents.

4.3.1.5 Statistical Analysis

Due to the inability to distinguish individual fish in behavioral trials, number of dives and total time in hypolimnion observation was the sum of the three fish. Paired t-tests were used to compare number of dives, percent time in hypolimnion, and consumption. Dive duration was compared using all observed dives across treatments in a two-tailed t-test. Percent values were ArcSin square-root transformed prior to t-test. Paired t-tests helped to account for variations between trials that may have resulted from differences in lighting, noise, or other external factors. Consumption was analyzed using both individual and group data, however results were similar in paired t-tests. A nested ANOVA was run on consumption data to compare differences in variance. Additionally, correlation analysis using the rcorr() function of the Hmisc package (Harrel 2014) was used to examine any relationship between time spent in hypolimnion and total consumption. All statistical analyses were conducted in RStudio 0.97.551 (R Core Team 2012).

4.3.2 Experiment 2: Changes in consumption with increasing hypoxic thickness

4.3.2.1 Experimental tank system

Vertical hypoxia simulators become less practical as hypoxic thicknesses
increase, therefore I used the horizontal hypoxic channel system described in Chapter 3 (Fig. 2) to determine changes in consumption of Yellow Perch over varying thickness of the hypoxic layer. This system consisted of 4 experimental chambers (5 m-length x 0.3 m-diameter) with dissolved oxygen concentrations being manipulatable in 1.3-meter sections, allowing us to test consumption from forays through 0, 1.3, 2.6, and 4.0 meters of hypoxic water. Prior to experiments, tanks were filled with tap water that was treated with TetraPond Aqua Safe and aerated for several days to ensure chlorine removal. I ensured dissolved oxygen levels were at normoxic levels (~8 mg DO/L) throughout all chambers prior to fish introduction.

4.3.2.2 Animals

Yellow Perch (115-165 mm) were obtained from Freshwater Farms of Ohio (Urbana, OH). Fish were housed in holding tanks and fed frozen krill. One week prior to the start of a behavioral trial, twelve fish were chosen at random and transferred to a training arena, where fish learned to associate the end of an experimental chamber with food presence. The training arena was a single introduction tank/experimental chamber pairing, consisting of a 4-foot section of 0.3m-tubing situated within a FrigidUnits Living stream rectangular tank. Fish could freely move between an open “introduction” area, and the inside of the tube, Fish were fed frozen krill daily from a benthic foraging apparatus (BFA, Fig. 3) at the end furthest from the introduction area. The benthic foraging apparatus consisted of a rubber-bristled brush that held the krill in place sufficiently to prevent floating yet allow the krill to be easily removed by fish, creating a more realistic simulation of natural benthic foraging than free floating food items. Food
security within the BFA was tested by placing two krill-loaded BFAs between three rapidly bubbling diffusers (to alternate diffuser and BFA) for the duration of 24 hours. This verified that the force of aeration or water currents would not dislodge food items from the BFA, permitting us to conclude that any food missing from BFA at the end of a trial would have most likely been removed and consumed by fish.

4.3.2.3 Fish Acclimation and Experimentation

At the start of each trial, three Yellow Perch were added to each introduction tank using the container method for 20-30 minutes or until water temperatures were equal. Fish were allowed free range of their designated experimental chamber for approximately 48 hours. After the acclimation period, food was placed at the end of each lane to get a standard consumption for each treatment. In preliminary consumption trials, six Yellow Perch in individual containers were given a surplus of food for 24 hours, and krill consumption averaged 9.38 (SD=1.91) krill per fish. Thus, each treatment was issued thirty whole (with eyes removed) medium-sized krill that were weighed and placed within a benthic foraging apparatus. Food was left in place for 24 hours to allow fish to forage. I made efforts to maintain external environmental factors at a constant level during foraging periods in order to minimize disturbances that may have resulted in altered fish behavior (i.e. lights remained off, noise minimized, etc.) At the end of the 24-hour foraging period, the BFA was removed and any remaining krill was removed, counted and weighed.

After completion of the normoxic consumption trial, DO levels within the specified hypoxic areas were decreased to the desired levels (<2 mg DO/L) by bubbling
nitrogen gas to the corresponding sections. Once oxygen levels were set, fish were left to acclimate to the new conditions for a minimum of 24 hours. In order to maintain comparable hunger levels, I did not initiate hypoxic foraging until 48 hours after the end of the normoxic foraging period, at which time food was placed at the end of each lane in the same manner as before. Fish were allowed to forage for 24 hours with minimal disturbance before the BFA was removed and the remaining krill were counted and weighed. Four trials were conducted, each testing the normoxic and hypoxic consumption of each group. Each trial tested one of each hypoxic thickness level: 0, 1.3, 2.6, and 4 meters of hypoxia, measured from the end of each experimental lane. Each trial used a new cohort of fish.

In a post hoc experiment, I modified the experimental system to contain two 8-meter experimental corridors, and conducted two trials (4 replicates) measuring consumption resulting from forays through 6.3 meters of hypoxic water using the same methods described above.

4.3.2.4 Statistical Analysis

Consumption data was calculated as percent of available food consumed by weight, and was analyzed by Analysis of Variance (ANOVA), followed by a post-hoc Tukey multiple comparisons test. A correlation analysis (rcorr(), Hmisc package, Harrel 2014) and a linear regression were run to examine the relationship between consumption by weight and the depth of hypoxic treatment. All statistical analyses were done using RStudio 0.97.551 (R Core Team 2012).
4.4 Results

4.4.1 Experiment 1: Changes in diving behavior and consumption

Epilimnetic and hypolimnetic temperatures averaged 19.7 and 8.97°C, respectively, helping to maintain stable stratification and desired oxygen concentrations (Table 1). Oxygen levels within the control tank averaged 9.25 mg/L throughout. Oxygen levels within the hypolimnion of the hypoxic treatment tank averaged 1.2 mg/L, while epilimnetic waters remained normoxic at approximately 7.43 mg/L.

Yellow Perch spent significantly less time within the hypolimnion when conditions were hypoxic ($p=0.034$, Fig. 4). Under normal oxygen conditions, Yellow Perch spent approximately 57.9% of their total time within the hypolimnion, reducing this time by nearly half (26.8%) under hypoxic conditions. This reduction in total time was largely driven by a significant decrease in the average dive duration ($p=0.021$), from 124 sec/dive in normoxic conditions, to 45 seconds/dive in hypoxic treatments.

The reduction in time did not result in a difference in consumption. The number of forays into the hypolimnion did not significantly differ between normoxic and hypoxic treatments (Fig. 4), however more dives were observed in hypoxic treatments overall with a total of 1956 and 1463 dives in hypoxic and control tanks, respectively. Additionally, in hypoxic treatments, a thermocline “turn around” behavior was observed in which fish swimming downward towards the hypolimnion would change direction at the thermocline, remaining within in the epilimnetic layer. Despite spending less total time in the hypolimnion, there was no significant decrease in consumption among hypoxic treatment groups (Fig. 5). There was, however, a significant difference in
variance between the two groups ($p<0.0001$) as a result of a much greater range of consumption within the control group (0 - 0.83g) compared to hypoxic group (0 - 0.195g). Additionally, more empty stomachs were observed among hypoxic fish (9 of 15), than normoxic fish (6 of 15). There was correlation between total time spent in hypolimnion and total consumption among either hypoxic or control group ($p=0.862$ and $p=0.294$, respectively); however, consumption was highly variable between individuals and individual dive data was not available.

4.4.2 Experiment 2: Changes in consumption with increasing hypoxic thickness

Dissolved oxygen concentrations averaged 6.76 and 1.81 mg DO/L in normoxic and hypoxic areas, respectively. Oxygen levels within adjacent normoxic and hypoxia areas experienced greater variability in oxygen levels due to water mixing. This resulted in slightly higher DO levels in shorter hypoxic sections (Table 1). Dissolved oxygen within the 1.3 m and 4 m treatments averaged 2.63 and 1.84 mg/L, respectively. Regardless, oxygen levels remained within the desired range for the duration of hypoxic treatments. Temperature was homogenous throughout all four treatment tanks at any one time, and ranged between 19.7 and 23.2°C over the duration of the experiment.

Fish ate 97% and 100% of available food in all normoxic consumption trials and 0 meter hypoxic treatment groups, respectively. During hypoxia, consumption remained high in 0-, 1.3- and 2.6-m treatment groups with fish consuming >90% of available krill by weight (Fig. 6). A significant decrease in Yellow Perch consumption was observed in 4- and 6.3-meter hypoxic treatments ($p<0.0004$), with fish consumption averaging 60.0% and 50.5% of available krill by weight, respectively.
4.5 Discussion

Yellow Perch continued to forage benthically despite hypoxic conditions. Contrary to my hypothesis, hypolimnetic hypoxia did not result in a decreased number of forays into hypoxic waters, but instead, significantly decreased the duration of each dive. Although the number of dives into the hypolimnion was not significantly different between treatments, Yellow Perch in hypoxic treatments appeared to be slightly more active, making more forays into the hypolimnion than control groups. The decrease in dive duration resulted in Yellow Perch spending significantly less time within the hypolimnion when oxygen conditions were hypoxic, however consumption of benthic food sources did not significantly differ with 1 meter of hypolimnetic hypoxia.

Significant decreases in consumption were not observed until hypoxic thickness reached 4 meters, where consumption decreased by 40.0 and 49.5% in 4-m and 6.3-m treatments, respectively. The significant decrease in consumption at 4 meters may signify a critical threshold in which Yellow Perch switch to more accessible food sources, i.e. mesozooplankton. However, hypoxic foraging continued through hypoxic depths of 6.3 meters, suggesting that Weight of food consumed was highly correlated with hypoxic thickness, and a linear trend line extended to the x-axis indicates that Yellow Perch may continue to forage at hypoxic thicknesses of as severe as 10 meters (Fig 7). Although, the weight of food consumed was highly correlated with hypoxic thickness, the significant difference between 0-2.6 meter treatments and 4.0-6.3 treatments indicates that the response to hypoxia is not linear but instead a function of behavioral and physiological thresholds. It is therefore likely that a second threshold exists after 6.3 meters in which
hypoxic foraging does cease. Regardless, the continuance of benthic foraging through hypoxic thickness of up to 6.3 meters indicates that hypoxia of these extents do not entirely isolate Yellow Perch from benthic prey.

Stomach content analysis of Yellow Perch captured from hypoxic areas show shifts in prey selection from benthic chironomid larvae and pupae to mesozooplanktonic species (Roberts et al. 2009). This shift was the most prevalent in sites with thicker hypolimnetic depths (~6 m), however chironomid larvae were present in the stomach contents of Yellow Perch captured at sites with hypolimnetic depths of 5.8 meters. Hypolimnetic hypoxia has been shown to condense hypoxic-intolerant zooplankton species (i.e. Daphnia mendotae, Bythotrephes longimanus) in narrow bands at the metalimnion in the LECB (Vanderploeg et al. 2009a). Likewise, Yellow Perch also tend to congregate above the thermocline during hypoxic conditions (Roberts et al. 2009). The resulting increase in density and overlap of both predator and prey species is likely to cause increased predation on zooplankton by Yellow Perch. While Yellow Perch tend to hover above the thermocline, horizontal migration to areas with thinner hypolimnetic or hypoxic depth has also been documented in Yellow Perch (Roberts et al. 2009; Vanderploeg et al. 2009a). As my study demonstrated minimal changes in benthic consumption when hypoxic thickness was 2.6 meters or less, the congregation of Yellow Perch in areas in which 1) hypolimnetic hypoxia is thin and 2) zooplankton are condensed may provide the most energetically efficient scenario during stratified hypoxia, minimizing the effort needed to forage for both benthic invertebrates and zooplankton. The continuation of benthic foraging under shallow hypoxic conditions is likely energetically beneficial to Yellow Perch since chironomid larvae offer a higher
energy density then most zooplankton (3,138 J/g and 2,510, J/g, respectively; Schaeffer et al. 1999).

Field studies found decreased Yellow Perch stomach biomass during and after LECB hypoxia (Roberts et al., 2009), yet the reasons for this are still unclear. Warmer epilimnetic temperatures that are characteristic of the epilimnion in later summer may result in increased metabolic rates (Hettler, 1976; Clarke & Johnston 1999), increasing the rate of digestion and decreasing residence time of stomach contents (Brett & Higgs 1970; Jobling 1980). Additionally, hypoxia may increase Yellow Perch energy expenditure due to migration, or to changes in foraging activity (i.e. zooplankton predation, hypoxic diving). While dive activity was not significantly different in this study, my results suggested that Yellow Perch activity may increase as a result of hypoxia, possibly increasing the energy expended vs energy gained ratio for benthic forays. Hypoxia has been documented to increase fish swimming speed (Herbert & Steffensen 2005; Domenici et al. 2000), as well as decrease consumption (Brandt et al. 2009; Roberts et al. 2011). Roberts et al. (2011 & 2012) found Yellow Perch consumption and growth to decrease under static hypoxic conditions in laboratory experiments, but not under short-term hypoxic exposures. Likewise, there was no evidence of decreased growth potential in Yellow Perch collected from stratified hypoxic zones in LECB. This study further demonstrates that Yellow Perch consumption is unlikely to be affected by intermittent hypoxic exposures, suggesting that it is instead limited by hypoxic depth and the maximum amount of time Yellow Perch are able to spend under hypoxic conditions before experiencing physiological stress or discomfort.
Specifically, assuming the maximum exposure time is constant, the amount of time spent foraging decreases as hypoxic thickness and swimming time increase.

Evidence suggests Yellow Perch are physiologically well adapted to hypoxia, surviving in hypoxic conditions for up to 5 days in laboratory experiments (Petrosky & Magnuson 1976; Head et al. *unpublished data*), and experience minimal physiological stress in exposures lasting 1 hour or less (Chapter 2). This study demonstrates that benthic foraging is unlikely to be affected in hypoxic conditions in which hypoxia thickness is 2.6 m or less, and consumption of zooplankton prey may be more out of convenience than necessity. However, more research is needed examining the effects of hypoxia on Yellow Perch behavior and prey selection. Similar studies using live prey items would provide a better understanding on how hypoxia affects prey behavior, food web interactions, prey selection of Yellow Perch, and bioenergetics of hypoxic foraging.

Incorporating the behavioral changes of prey items under hypoxic conditions would be extremely beneficial in understanding changes in trophic interactions that result from hypolimnnetic hypoxia. Hypoxia has been found to alter behavior in benthic invertebrates, increasing their risk of predation as they abandon benthic refugia for areas with higher dissolved oxygen concentrations (Kolar & Rahel, 1993). While chironomids are highly tolerant of hypoxic conditions, they have been observed to change burrowing behavior under oxic stress, switching from U-burrows to chimney-like structures (Stief et al. 2005). This change in behavior may potentially increase predation risk by hypoxic tolerant benthic foragers. Further, the presence or absence of Dreissenid mussels (*Dreissena bugensis* and *Dreissena polymorpha*) may also affect macroinvertebrate behavior and foraging efficiency of Yellow Perch during hypoxic periods as dressenids
have been found to increase the abundance of benthic invertebrates (Ricciardi et al. 2011; Stewart & Haynes 1994; Horvath et al. 1999) while simultaneously making them less accessible to predatory fish (DeVanna et al. 2011).

Forecasts of global climate change predict increased temperatures in many parts of the world. A rise in average air temperature carries the potential of increased surface temperatures and lengthened stratification (Blumberg and Di Toro 1990; Mortsch & Quinn 1996, Conley et al. 2009), leading to expanded hypoxia in both freshwater and marine ecosystems (Conley et al. 2009). Understanding the effects of hypoxia on species behaviors and trophic interactions will allow us to make better predictions of the consequences of expanding hypoxia, and provide information needed to make effective conservation and management plans.

4.6 Acknowledgements

This research was supported by a University Research Award from U. Toledo and the Ohio Board of Regents I acknowledge the technical assistance of C. Wade, C. Nagel, J. Scarbro, A. Haponski and R. Sullivan. C. Mayer, R. Ruch, J. Head, and V. Sigler offered scientific expertise. Water chillers were provided by the USGS Lake Erie Biological Station. Support for BLB was provided by a NSF GK-12 DGE#0742395 fellowship and a Robert Brundage Memorial Scholarship.
Table 4.1 Average (±SD) of dissolved oxygen concentrations (mg/L) and temperatures (°C) of normoxic and hypoxic zones for each treatment group in Experiment 1 and Experiment 2.

<table>
<thead>
<tr>
<th></th>
<th>Normoxic</th>
<th>Hypoxic</th>
<th></th>
<th>Normoxic</th>
<th>Hypoxic</th>
<th>Normoxic</th>
<th>Normoxic</th>
<th>Hypoxic</th>
<th>Normoxic</th>
<th>Normoxic</th>
<th>Hypoxic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temp</td>
<td>DO</td>
<td>Temp</td>
<td>DO</td>
<td>Temp</td>
<td>DO</td>
<td>Temp</td>
<td>DO</td>
<td>Temp</td>
<td>DO</td>
<td>Temp</td>
</tr>
<tr>
<td>Control</td>
<td>18.92</td>
<td>8.62</td>
<td>19.82</td>
<td>7.43</td>
<td>6.09</td>
<td>6.47</td>
<td>5.95</td>
<td>7.22</td>
<td>6.3</td>
<td>4</td>
<td>2.65</td>
</tr>
<tr>
<td>Hypoxic</td>
<td>(2.15)</td>
<td>(0.04)</td>
<td>(2.31)</td>
<td>(0.50)</td>
<td>21.3</td>
<td>(2.63)</td>
<td>21.57</td>
<td>(0.89)</td>
<td>21.57</td>
<td>(0.41)</td>
<td>21.35</td>
</tr>
<tr>
<td></td>
<td>9.4</td>
<td>9.89</td>
<td>8.53</td>
<td>1.2</td>
<td>(0.50)</td>
<td>(0.45)</td>
<td>(0.60)</td>
<td>(0.61)</td>
<td>(0.62)</td>
<td>(0.62)</td>
<td>(0.89)</td>
</tr>
<tr>
<td></td>
<td>(0.40)</td>
<td>(0.66)</td>
<td>(0.05)</td>
<td>(0.80)</td>
<td>(0.32)</td>
<td>(0.78)</td>
<td>(0.82)</td>
<td>(0.82)</td>
<td>(0.76)</td>
<td>(0.76)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4-1. Diagram of experimental tank system used in experiment 1 (detailed in Chapter 3) used to observe behavior and benthic foraging of Yellow Perch under hypoxic conditions. Two water towers (1.5-m-high x 0.45-m-diameter) were thermally stratified in order to create normoxic and hypoxic hypolimnetic environments. Before stratification, the desired hypolimnetic oxygen concentrations in the normoxic and hypoxic tanks were obtained by the bubbling of compressed air or nitrogen gas through the entire water column of the respective treatment tank. Water towers were thermally stratified using a cold water basin, ambient air temperature and aquaria heaters. The epilimnion of the hypoxic tank was then re-oxygenated by the gentle diffusion of compressed air before introducing fish.

Figure 4-2. Diagram of experimental tank system used in experiment 2 (detailed in Chapter 3). Side (A) and top views (B) show four 5-meter long x 0.3 m diameter experimental chambers used to test the consumption of Yellow Perch via forays through various degrees of hypoxic thickness. Oxygen levels were controlled through the continuous bubbling of either compressed air or nitrogen gas to create hypoxic zones ranging from 0 to 4 meters of hypoxia, measured from the end of the chamber where food was placed. A post-hoc modification was made to enable the testing of 6.3 meters of hypoxic thicknesses.

Figure 4-3. Diagram of a benthic foraging apparatus (BFA). Rubber bristled brushes were used to hold krill in place during consumption trials. The BFA held the krill securely enough to prevent dislodging by aeration or water currents but allowed for
removal by fish.

**Figure 4-4.** Average number of dives, dive duration, and total time spent (± 1 SE) in hypolimnion of Yellow Perch in stratified tanks with normoxic (Control) and hypoxic hypolimnions (Experiment 1). Significant differences are indicated with a * and reported $p$ value.

**Figure 4-5.** Average Consumption (± 1 SE) of Yellow Perch when faced with 0.75 m normoxic (control) and hypoxic hypolimnions (Experiment 1).

**Figure 4-6.** Average consumption (% available by weight) across varying thicknesses of hypoxia (Experiment 2). Error bars report one standard error. A-grouped treatments are significantly different from B-grouped treatments at $p < 0.0004$. 
Figure 4-1

- **Normoxic**
- **Hypoxic**

**Control**
- Thermal Stratification

**Experimental**
- Thermal & Oxygen Stratification

**Cameras**

**Warm Water**
- Ambient air temp or heated

**Outer Water Level**
- Thermo/oxy-cline

**Cold Water**
- Circulating from chiller

- \( N_2 \)
- \( \text{Air} \)
Figure 4-2
Figure 4-3

Side View

Top View
Figure 4-4

- **Number of Dives**
  - CONTROL: 300
  - HYPOXIC: 500
  - *P=0.021*

- **Dive Duration (sec)**
  - CONTROL: 120
  - HYPOXIC: 40

- **Percent Time in Hypoxia**
  - CONTROL: 50%
  - HYPOXIC: 30%
  - *P=0.034*
Figure 4-5

Dry weight gut analysis (g)

CONTROL  HYPOXIC
Figure 4-6

![Bar chart showing consumption (% available by %) with hypoxic thickness in meters (0, 1.3, 2.6, 4.0, 6.3). The chart indicates a significant decrease in consumption as hypoxic thickness increases, with p < 0.0004.]
Chapter 5

Discussion

5.1 General conclusions

This Ph.D. dissertation investigates the physiological and behavioral responses of Yellow Perch to hypoxia and provides insights as to how hypoxia-driven changes in behavior may lead to community wide changes in distributions. Chapter 2 evaluates the physiological responses of Yellow Perch by measuring HIF-1α at various degrees and durations of hypoxia, serving as one of the first examinations of the HIF response in this species. Chapter 3 details the design and function of two experimental tank systems that enable the observation of behavioral changes of benthic-linked species during hypolimnetic hypoxia. Not only do these systems add new possibilities to hypoxia research, but they can also be constructed from readily available materials for minimal costs. Lastly, Chapter 4 describes the changes in Yellow Perch behavior and benthic consumption that result from the formation of hypolimnetic hypoxia. Overall, these three chapters demonstrate the fine-scale effects of hypoxia on Yellow Perch, suggesting that Yellow Perch may be well adapted to hypoxic environments, however extensive hypoxic events result in behavioral changes that likely drive shifts in distributions and prey...
selection, which in turn alter trophic interactions, intraspecific competition, bioenergetics, and species fitness.

5.1.1 *Short-term hypoxic exposure has little effect on Yellow Perch physiology or consumption potential.*

Yellow Perch demonstrated high survivorship when exposed to hypoxic conditions for up to 8 hours. Furthermore, HIF levels did not significantly increase above normoxic levels after 1 hour of hypoxic exposure (Chapter 2). These results suggest that Yellow Perch are fairly tolerant of hypoxic conditions and experience minimal physiological stress under short-term hypoxic exposures. Additionally, repeated short-term hypoxic exposure resulting from forays through shallow hypoxia did not result in decreased consumption (avg. dive duration =29 sec, Chapter 4, experiment 1; see also Roberts et al. 2012). During shallow hypoxic conditions (~0.75 m hypoxia) 98% of Yellow Perch benthic forays were ≤1 minute in duration, and 99.6% of dives within the hypoxic treatments lasted less than 1 hour (Chapter 4, Experiment 1). Considering the rate of HIF accumulation compared with the range of dive durations, it seems unlikely that voluntary hypoxic exposure by Yellow Perch would exceed thresholds in which Yellow Perch begin to experience physiological stress. Hence, under natural hypoxic conditions, hypoxic exposure alone is unlikely to cause decreased fitness in Yellow Perch. Instead, it is possible that any decreased fitness experienced by Yellow Perch during extensive hypoxic events (as suggested by Roberts et al. 2009) results from changes in foraging behavior that may lead to changes in bioenergetics and growth rates.
5.1.2 Yellow Perch continue to forage during hypoxic conditions, however benthic foraging is limited by hypoxia thickness.

   Yellow Perch continued to forage benthically in all hypoxic thicknesses tested in laboratory experiments (up to 6.3 m). Significant decreases in amount consumed occurred at 4.0 m of hypoxic thickness, suggesting a critical threshold exists between 2.6 and 4 meters in which benthic foraging becomes stressful and/or less energetically profitable (Chapter 4). In general, benthic invertebrates have a higher energy density than zooplankton (chironomids and zooplankton, 3,138 and 2,510 J/g wet weight, respectively; Schaeffer et al. 1999) and offer a much higher energetic gain (Graeb et al. 2011). Hypoxia, however, likely increases the energy expenditure needed to forage for benthic organisms due to the necessity to make more frequent forays of shorter duration in order to capture similar amounts of prey (Chapter 4). Assuming the duration of voluntary hypoxic exposure remains constant, increases in hypoxic thickness also increase the amount of swimming time per foray, and foraging time decreases. A threshold most likely exists at the hypoxic thickness in which benthic foraging is no longer energetically profitable, and Yellow Perch switch to more available food sources.

5.1.3 Possible consequences for trophic interactions and bioenergetics.

   Many factors can influence diet and prey selectivity in predators including prey density, encounter rate, capture efficiency, foraging costs, prey size or energy density, and the total energetic gain of the prey item. In a laboratory experiment, Graeb et al. (2011) found Yellow Perch prey selectivity to be determined primarily by a combination of energetic gain and foraging costs, and not by relative prey abundance. However, it is
unknown how prey selectivity may be affected by changes in foraging costs for a particular prey item. Although benthic invertebrates and prey fish offer the most energetic gain under normal oxygen conditions, hypoxia (particularly hypoxia > 2.6 m in thickness) may offset energetic gains due to increased foraging costs. Additionally, hypoxia may also alter prey behavior and distribution. In particular, hypoxia has been shown to condense Yellow Perch, prey fish species and zooplankton within the metalimnion during hypoxic events (Vanderploeg et al. 2009b). This condensation of both predator and prey species likely increases both prey density and encounter rate. Hence, shifts in prey selection by Yellow Perch during hypoxic events may not be driven solely by the direct effects of hypoxia on Yellow Perch, rather by the indirect effects of community-wide changes in behavioral and distributions.

Laboratory experiments examining the ontogentic diet shifts of Yellow Perch found Yellow Perch between 40-60 mm to positively select solely for benthic invertebrates. Smaller Yellow Perch (≤20 mm selected for zooplankton), and those ≥80 mm selected equally for both benthic invertebrates and prey fish (Graeb et al. 2011). This suggests that Yellow Perch in the 40-60 mm size class may be the most affected by hypoxia. Although Yellow Perch ~ 80 mm negatively selected for zooplankton, zooplankton offered the highest capture efficiency and the lowest handling time, and consumption of zooplankton was still evident (Graeb et al. 2011). While benthic invertebrates are the main contributors to Yellow Perch diet in the spring (74.6%, fish=14.2%, zooplankton=10.0%), diet analyses of LE western basin Yellow Perch (age 2+) have shown increases in the occurrence of prey fish in the fall, accounting for 62.9% of diet composition (dry weight, 2013 USGS LEBS data). Furthermore, the frequency of
prey fish in fall Yellow Perch diets has increased over recent years relative to historical data (USGS LECB unpublished data). These finding suggest that even in the absence of hypoxia, predation on fish increases from spring to fall as young-of-the-year prey fish grow. Coincidently, hypoxia forms in mid to late-summer and expands into the fall, while Yellow Perch diets are shifting to include more prey fish in their diet. Hence, shifts in Yellow Perch prey selection during periods of hypoxia are likely to be the result of multiple factors, and not only hypoxia avoidance.

5.1.4 Implications of hypoxic depth thresholds and Lake Erie hypoxia, and possible consequences of global warming.

The hypoxic thickness threshold for benthic foraging of 2.6 meters has significant implications for LECB Yellow Perch populations. Minor hypoxic events that fall below this threshold are likely to have little effect on Yellow Perch. However, increasing hypoxic thicknesses likely drive shifts in distributions of Yellow Perch populations, condensing them vertically to normoxic metalimnetic layers and horizontally to areas where hypoxic thickness is ~2.6 meters or less. Currently, hypoxia within the LECB can reach thicknesses of up to 8 meters at the deepest point (Arend et al. 2011), and has been shown to result in shifts in Yellow Perch distribution to metalimnetic layers or outer edges of the hypoxic zone (Roberts et al 2012; Vanderploeg et al. 2009b). Forecasts of global climate change predict larger and more severe hypoxic events due to warmer air temperatures and increased stratification (Magnuson et al. 1997; Kling et al. 2003; Blumberg & Di Toro 2011). Under these scenarios, Yellow Perch populations are likely to experience greater condensation during the summer months, and as a result may
experience greater interspecific competition for food resources. However, Yellow Perch have been shown to select for the most energetically profitable food resource and, as a result, select for pelagic prey items when foraging for benthic invertebrates is no longer energetically profitably (>2.6 m of hypoxic thickness, Chapter 4; Roberts et al 2009). Under worsening hypoxic conditions in which hypoxic thicknesses of >2.6 m persist for sustained periods, Yellow Perch may be driven to adapt by increasing piscivory in order to meet energetic needs.

5.2 Future research

The present study examines fine scale changes in the benthic foraging patterns of Yellow Perch faced with hypolimnetic hypoxia and the potential physiological consequences that result from hypoxic exposure, demonstrating important implications of worsening hypoxia that may occur with changes in environmental or climatic conditions. The results discerned here raise several additional questions and suggestions for further study.

5.2.1 Further exploration into the HIF pathway in Yellow Perch

Although HIF may not be a useful indicator of hypoxic exposure in fish due to its high individual variability, slow response time, and negative feedback response (Chapter 2), this study demonstrated some aspects of the HIF pathway that are still largely not understood in fish. Understanding the role of additional factors such as temperature, individual variations (i.e. sex and age class), and acclimation time would broaden our understanding of the HIF pathway and its role in fish metabolism and hypoxic tolerance.
among species. Additionally, the examination of HIF target and/or regulator proteins would not only further our understanding of the role of HIF in fish, but may potentially serve as better indicators of hypoxic exposure.

The rate of acclimation to hypoxia has been shown to affect HIF response in zebrafish *Danio rerio* (Rees et al. 2011). This may have implication to Lake Erie Yellow Perch as wind-driven upwellings of hypoxic water result in rapid changes in nearshore oxygen conditions in the LECB (Rao et al. 2014). Consequently, determining how quickly Yellow Perch can adapt to changes in oxygen conditions would broaden our understanding of the effects of hypoxia of LECB Yellow Perch.

5.2.2 Examining hypoxic tolerance of Yellow Perch over genetically or geographically different populations

Studies examining the genetics of Yellow Perch have found high variation across its range, indicating that unique genetic sources have adapted to warmer environments and that Yellow Perch have persisted in and adapted to a wide range of environmental conditions (Sepulveda-Villet & Stepien 2012). Genetic variations have also been found among different spawning groups within the LECB (Sepulveda-Villet & Stepien 2011), and between near-shore and off-shore populations in Lake Michigan (Miller 2003). This evidence of fine-scale genetic divergence raises questions regarding possible differences in hypoxic tolerance between Yellow Perch populations that are regularly exposed to hypoxia (i.e. those within the LECB), and those that are not. Comparing HIF responses and pathways (as well as perhaps behaviors) between genetically divergent Yellow Perch populations or populations exposed to varying ranges of environmental conditions could
offer important insight to the potentially evolutionary consequences of changing environmental conditions. For instance, worsening hypoxic conditions in the LECB might lead to further divergence between Lake Erie Yellow Perch stocks. Conversely, the expansion of hypoxia within the Lake Erie western basin, or other Yellow Perch habitats, as a result of climate change could potentially lead to selection for hypoxic tolerance.

5.2.3 Stratified hypoxic behavioral studies with live prey

Conducting behavioral experiments with Yellow Perch and live prey would offer great insight into how hypolimnetic hypoxia effects trophic interactions in the LECB. As stated previously, changes in prey selectively may be a result of various factors that co-occur during hypoxia. Determining changes in prey behavior during hypoxia in the presence of Yellow Perch may demonstrate the driving forces behind shifts in trophic interactions. More specifically, does Yellow Perch diet change as a result of 1) hypoxic avoidance, 2) changes in prey behavior, density of availability or 3) a combination of these factors?

Furthermore, such behavioral experiments could additionally be used to examine changes in Yellow Perch bioenergetics that result from changes in foraging conditions and prey selection. For example: 1) how does the total energetic gain of prey taxa change during hypoxia?, and 2) what is the full energetic cost of hypoxia on Yellow Perch?

Additionally, environmental conditions simulating forecasted climate change scenarios could predict the consequences of worsening hypoxic conditions on LECB Yellow Perch.
References


Southwick Associates (2012). Sportfishing in America: An Economic Force for Conservation. *Produced for the American Sportfishing Association (ASA) under a U.S. Fish and Wildlife Service (USFWS) Sport Fish Restoration grant (F12AP00137, VA M-26-R) awarded by the Association of Fish and Wildlife Agencies (AFWA).*


van Heerden, D., A. Vosloo, and M. Nikinmaa. 2004. Effects of short-term copper exposure on gill structure, metallothionein and hypoxic-inducible factor-1α (HIF-1α) levels in rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology.* 69(271-280).


Wannamaker, C.M., & J.A. Rice (2000). Effects of hypoxia on movements and behavior of selected estuarine organisms from the southeastern United States. *Journal of*


