Eastern Illinois University

The Keep

Masters Theses

Student Theses & Publications

2018

Impact of Temperature Increase on Freshwater Fish Species: Energetics and Muscle Mechanics of Two Centrarchids

Israt Jahan

Recommended Citation

The Graduate School

Thesis Maintenance and Reproduction Certificate

FOR: Graduate Candidates Completing Theses in Partial Fulfillment of the Degree
Graduate Faculty Advisors Directing the Theses

RE: Preservation, Reproduction, and Distribution of Thesis Research

Preserving, reproducing, and distributing thesis research is an important part of Booth Library's responsibility to provide access to scholarship. In order to further this goal, Booth Library makes all graduate theses completed as part of a degree program at Eastern Illinois University available for personal study, research, and other not-for-profit educational purposes. Under 17 U.S.C. § 108, the library may reproduce and distribute a copy without infringing on copyright; however, professional courtesy dictates that permission be requested from the author before doing so.

Your signatures affirm the following:

• The graduate candidate is the author of this thesis.

• The graduate candidate retains the copyright and intellectual property rights associated with the original research, creative activity, and intellectual or artistic content of the thesis.

• The graduate candidate certifies her/his compliance with federal copyright law (Title 17 of the U. S. Code) and her/his right to authorize reproduction and distribution of all copyrighted materials included in this thesis.

• The graduate candidate in consultation with the faculty advisor grants Booth Library the nonexclusive, perpetual right to make copies of the thesis freely and publicly available without restriction, by means of any current or successive technology, including but not limited to photocopying, microfilm, digitization, or internet.

• The graduate candidate acknowledges that by depositing her/his thesis with Booth Library, her/his work is available for viewing by the public and may be borrowed through the library's circulation and interlibrary loan departments, or accessed electronically. The graduate candidate acknowledges this policy by indicating in the following manner:

_____ Yes, I wish to make accessible this thesis for viewing by the public

___✓___ No, I wish to quarantine the thesis temporarily and have included the *Thesis Withholding Request Form*

• The graduate candidate waives the confidentiality provisions of the Family Educational Rights and Privacy Act (FERPA) (20 U. S. C. § 1232g; 34 CFR Part 99) with respect to the contents of the thesis and with respect to information concerning authorship of the thesis, including name and status as a student at Eastern Illinois University. I have conferred with my graduate faculty advisor. My signature below indicates that I have read and agree with the above statements, and hereby give my permission to allow Booth Library to reproduce and distribute my thesis. My adviser's signature indicates concurrence to reproduce and distribute the thesis.

_____
Graduate Candidate Signature

ISRAT JAHAN
Printed Name

M.S. BIOLOGICAL SCIENCES
Graduate Degree Program

/ Faculty Adviser Signature

ANABELA MARIA REDONDE DA MAIA
Printed Name

12/11/18
Date

Please submit in duplicate.

Impact of Temperature Increase on Freshwater Fish Species:

Energetics and Muscle Mechanics of Two Centrarchids

(TITLE)

BY

Israt Jahan

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

MS in Biological Sciences

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

2018

YEAR

I HEREBY RECOMMEND THAT THIS THESIS BE ACCEPTED AS FULFILLING
THIS PART OF THE GRADUATE DEGREE CITED ABOVE

6/14/18

THESIS COMMITTEE CHAIR   DATE

6/11/18

DEPARTMENT/SCHOOL CHAIR   DATE
OR CHAIR'S DESIGNEE

6/11/18

THESIS COMMITTEE MEMBER   DATE

THESIS COMMITTEE MEMBER   DATE

6/11/18

THESIS COMMITTEE MEMBER   DATE

THESIS COMMITTEE MEMBER   DATE

IMPACT OF TEMPERATURE INCREASE ON FRESHWATER FISH: ENERGETICS

AND MUSCLE MECHANICS OF TWO CENTRARCHIDS

By

Israt Jahan

B.S. (Honors), MS

University of Dhaka

A Thesis

Prepared for the Requirements for the Degree of

Master of Science

Department of Biological Sciences

Eastern Illinois University

November 2018

ABSTRACT

Fishes have evolved physiologically to live within a specific range of environmental variation and existence outside of that range can be stressful or fatal. These ranges can coincide for fishes that evolved in similar habitats. This study investigates physiological processes limiting thermal tolerance, specifically how changes in water temperature affect the swimming energetics and muscle mechanics in largemouth bass (*Micropterus salmoides*) and bluegill (*Lepomis macrochirus*). I focused on the impact of temperature change at the muscle level in these two species and the capacity to adapt to rapid changes in the environment. Fish were housed at 20°C and then tested in a recirculating flow tank 2 body length (BL)/sec at 16°C, 20°C and 22°C while measuring water oxygen consumption as a proxy of metabolism. Fish were fasted for at least 24 hours and acclimatized for 2 hours prior each trial. All temperatures were controlled by chiller and heater. Fish were also implanted with bipolar electrodes to record muscle activity using electromyography (EMG) standard techniques. Mass corrected oxygen consumption was different between fish ran at the three different temperatures. Overall, metabolic rate was higher at higher temperatures. Mass corrected oxygen consumption was influenced not only by temperature but also by both species and species and temperature interactions. Active metabolic rate was higher in largemouth bass than in bluegill at 16°C and 20°C. However, at 22°C bluegill had almost 1.35-fold higher active metabolic rate. The temperature quotient Q_{10} is the measure of temperature sensitivity of a biological system and for current study it was calculated from 16°C to 20°C and 22°C for both species to measure how the physiology is affected by increase in temperature. Calculated Q_{10} at 2 BL/s was 1.43 for largemouth bass and 7.31 for bluegill (16-22°C). At higher temperatures oxygen consumption increases in fish while oxygen content decreases in water due to a lower saturation pressure, making oxygen a clear limiting factor. This is likely to affect health

and growth of individuals, especially when mobility to a lower temperature environment is not possible. While swimming at the same speed (2BL/s), electromyography recordings showed swimming characterized by activation of the musculature. With increased temperature fish start recruiting more red muscles and white muscle by showing greater magnitude and longer duration of muscle activation. Fish are thus capable of changing muscle mechanics to adapt to change in temperature, although bluegill and largemouth bass use distinct strategies. Largemouth bass has less or no activity of axial red muscles at 22°C while bluegill successfully recruit red muscles at all three temperatures. Using more white muscles at higher temperature may lead largemouth bass to an additional demand of oxygen consumption after swimming at higher temperature to maintain functional balance.

ACKNOWLEDGMENTS

Finally, I am thankful to my parents and my in-laws for helping me directly or indirectly during my MS studies. Last but not the least, I offer my sincere thanks to my husband Mohammad Manirul Haque who helped me in every possible way form to accomplish my study.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 1

LIST OF FIGURES

Chapter 1

Chapter 2

INTRODUCTION

Global warming is highly concerning issue as a universal threat to ecological integrity and function, highlighting the urgent need for a better understanding of the impact of heat exposure on the resilience of ecosystems (IPCC, 2014). The effects of climate change are expected to differ in both magnitude and direction among geographic areas (IPCC, 2007). Though projected that sea level rise will cause destruction on islands and coastal places, places like Illinois will not be spared, and Illinois policymakers would be well advised to start considering the impact climate change will have in the state. According to the studies done by the US Climate Change Science Program 2016, the ice-free season along the Great Lakes is also becoming longer. Between 1994 and 2011, reduced ice cover lengthened the shipping season on the lakes by eight days. The Great Lakes are likely to warm another 3° to 7°C in the next 70 years (USCCSP, 2016), which will likely strongly impact fish communities.

Global environmental change has both direct and indirect impacts on fish stocks that are exploited commercially (Lehodey et al., 2006). Direct effects act on physiology and behavior and alter growth, development, reproductive capacity, mortality, and distribution of fish and other aquatic organisms. Indirect effects alter the productivity, structure, and composition of the ecosystems on which aquatic organisms depend for food and shelter (Brander 2007). These will not necessarily operate independently, and the possibility of synergy or interactions between them is probable. It will affect a range of abiotic factors that are tightly linked to the production and distribution of fish populations, and these climate-driven biotic changes will likely differ between open ocean, continental shelf habitats, coastal waters (Walther et al., 2002; Lehodey et al., 2006), as well as a variety of freshwater habitats. Although the importance of the different environmental factors varies regionally, some general principles can be derived. Temperature, because of its pervasive effect on organismal physiology, is likely to affect all regions (Rijnsdorp et al., 2009).

1

Water temperature plays an important role in the lives of fishes and is an ecological resource, like food and habitat which can influence individual fitness (Magnuson *et al.*, 1979). When temperature departs from optimal for an organism, it will act as a stressor and impair physiology and behavior (Fry, 1947; Beyers and Rice, 2002; Donaldson *et al.*, 2008).

Resolving the effects of climate change on fish populations is complicated, mainly since it is characterized by changes in a multitude of environmental factors which affect various processes at different levels of biological organization (Harley et al., 2006; Lehodey et al., 2006; Anker-Nilssen, 2008). For example, even when the effects of changes in an environmental factor such as temperature on the physiology of a given organism is known, it will be difficult to evaluate the outcome of this organism-level physiological response at the population or ecosystem levels (MacKenzie and Koster, 2004). Thermal regimes in river and stream ecosystems are fundamentally important to fish and other aquatic organisms because most of these animals are ectotherms with physiologic processes directly controlled by temperatures of the ambient environment (Neuheimer and Taggart 2007; Buisson et al. 2008; Pörtner and Farrell 2008; Durance and Ormerod 2009). As a result, temperature strongly dictates the distribution and abundance of individual species across many spatial and temporal scales (Brannon et al. 2004; Rieman et al. 2007; Wenger et al. 2011).

The effects of increasing temperature on marine and freshwater ecosystems are already evident, with rapid poleward shifts in distributions of fish and plankton in regions such as the North East Atlantic, where temperature change has been rapid (Turrel 2003, Beaugrand et al 2002, Hughes and Holliday 2006). High levels of loss of warm water fish habitat are also documented in the Midwest due to increases in temperature in streams of this region (Eaton and Scheller, 1996). Moreover, human alterations of river flows have already caused the extinction of at least a dozen

freshwater fish worldwide (Xenopoulos et al 2005). Further changes in distribution and productivity are expected due to continuing warming and evaporation of the Arctic (Drinkwater, 2005).

Fishing and climate change are strongly interrelated pressures on fish production and must be addressed jointly. Loss of biodiversity and reductions in demographic structure due to fishing result in greater sensitivity of fish stocks and marine ecosystems to climate change (Meyer 1999, Magnuson 2001). Recruitment in fish populations has long been known to be a key process that is strongly influenced by climate variability (Cushing 1995). Responses by individual species to climate change may disrupt their interactions with others at the same or adjacent trophic levels (Walther 2002).

We have a limited understanding of how aquatic species might be able to alter their physiology over multiple generations to enable persistence in a warmer environment (Donelson 2012). Studying how fish can cope physiologically with these challenges is of the utmost importance. In my current study, I address how climate change effects, namely increased water temperature, will affect fish physiology and their major activity locomotion. The two main goals of this thesis are to better understand the impact of temperature on energetics (Chapter 1) and to investigate how muscle mechanics of axial musculature change with increased temperature (Chapter 2) in two Centrarchidae species during steady swimming. I will measure oxygen consumption in a closed respirometer during steady swimming at three different temperatures 16ºC, 20ºC and 22ºC in largemouth bass and bluegill. To accomplish the second goal I will record muscle activation patterns from axial and caudal white and red muscle at the same three distinct temperatures in these two warmwater species.

LITERATURE CITED

Anker-Nilssen, T., Borges, F., Brander, K., Duplisea, D., Frederiksen, M., Gallego, A., Gislason, A., Van Hal, R., Heath, M., Hofstedte, R.T. and Holliday, A.P., 2008. The effect of climate change on the distribution and abundance of marine species in the OSPAR Maritime Area. ICES.

Beaugrand, G., Reid, P.C., Ibanez, F., Lindley, J.A. and Edwards, M., 2002. Reorganization of North Atlantic marine copepod biodiversity and climate. *Science*, *296*(5573), pp.1692-1694.

Beyers, D. W. and Rice, J. A. (2002). Evaluating stress in fish using bioenergetics-based stressor-response models. In *Biological Indicators of Aquatic Ecosystem Stress* (Adams, S. M., ed.), pp. 289–320. Bethesda, MD: American Fisheries Society.

Brander, K.M., 2007. Global fish production and climate change. *Proceedings of the National Academy of Sciences*, *104*(50), pp.19709-19714.

Claireaux, G., Couturier, C. and Groison, A.-L. (2006). Effect of temperature on maximum swimming speed and cost of transport in juvenile European sea bass (*Dicentrarchus labrax*). Journal of Experimental Biolology 209, 3420-3428.

Cushing, D.H., 1995. *Population production and regulation in the sea: a fisheries perspective*. Cambridge University Press.

Donaldson, M. R., Cooke, S. J., Patterson, D. A. and MacDonald, J. S. (2008). Cold shock and fish. *Journal of Fish Biology* 73, 1491–1530.

Donelson, J.M., Munday, P.L., McCormick, M.I. and Pitcher, C.R., 2012. Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nature Climate Change*, *2*(1), p.30.

Drinkwater, K.F., 2005. The response of Atlantic cod (Gadus morhua) to future climate change. *ICES Journal of Marine Science*, *62*(7), pp.1327-1337.

Eaton, J. G., & Scheller, R. M. (1996). Effects of climate warming on fish thermal habitat in streams of the United States. Limnology and oceanography, 41(5), 1109-1115.

Fry, F. E. J. (1947). *Effects of the Environment on Animal Activity.* Toronto, ON: University of Toronto Press.

Harley, C.D., Randall Hughes, A., Hultgren, K.M., Miner, B.G., Sorte, C.J., Thornber, C.S., Rodriguez, L.F., Tomanek, L. and Williams, S.L., 2006. The impacts of climate change in coastal marine systems. *Ecology letters*, *9*(2), pp.228-241.

Hofmann, G.E. and Todgham, A.E., 2010. Living in the now: physiological mechanisms to tolerate a rapidly changing environment. *Annual review of physiology*, *72*, pp.127-145.

Hughes, S.L. and Holliday, N.P., 2006. ICES report on ocean climate 2005.

IPCC. 2007. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

IPCC. 2014. Climate Change 2014: Impacts, Adaptation, and Vulnerability. Cambridge University Press, Cambridge, UK.

Lehodey, P., Alheit, J., Barange, M., Baumgartner, T., Beaugrand, G., Drinkwater, K., Fromentin, J.M., Hare, S.R., Ottersen, G., Perry, R.I. and Roy, C., 2006. Climate variability, fish, and fisheries. *Journal of Climate*, *19*(20), pp.5009-5030.

MacKenzie, B.R. and Köster, F.W., 2004. Fish production and climate: sprat in the Baltic Sea. *Ecology*, *85*(3), pp.784-794.

Magnuson, J.J., 2001. A future of adapting to climate change and variability. *Fisheries in a changing climate*, *32*, pp.273-282.

Magnuson, J. J., Crowder, L. B. and Medvick, P. A. (1979). Temperature as an ecological resource. *American Zoologist* **19,** 331–343.

Meyer, J.L., Sale, M.J., Mulholland, P.J. and Poff, N.L., 1999. Impacts of climate change on aquatic ecosystem functioning and health. *JAWRA Journal of the American Water Resources Association*, *35*(6), pp.1373-1386.

Rijnsdorp, A.D., Peck, M.A., Engelhard, G.H., Möllmann, C. and Pinnegar, J.K., 2009. Resolving the effect of climate change on fish populations. *ICES journal of marine science*, *66*(7), pp.1570-1583.

Roessig, J. M., Woodley, C. M., Cech, J. J., and Hansen, L. J. (2004). Effects of global climate change on marine and estuarine fishes and fisheries. Reviews in Fish Biology and Fisheries, 14(2), 251-275.

Skelly, D.K., Joseph, L.N., Possingham, H.P., Freidenburg, L.K., Farrugia, T.J., Kinnison, M.T. and Hendry, A.P., 2007. Evolutionary responses to climate change. *Conservation biology*, *21*(5), pp.1353-1355.

Turrell, W. ed., 2003. Hydrobiological Variability in the ICES Area, 1990-1999: A Symposium Held in Edinburgh, 8-10 August 2001. ICES.

Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J., Fromentin, J.M., Hoegh-Guldberg, O. and Bairlein, F., 2002. Ecological responses to recent climate change. *Nature*, *416*(6879), p.389.

Xenopoulos, M.A., Lodge, D.M., Alcamo, J., Märker, M., Schulze, K. and Van Vuuren, D.P., 2005. Scenarios of freshwater fish extinctions from climate change and water withdrawal. *Global Change Biology*, *11*(10), pp.1557-1564.

IMPACT OF TEMPERATURE INCREASE ON ENERGETICS OF TWO FRESHWATER CENTRARCHIDS

ABSTRACT

Higher water temperatures are expected to increase energetic costs of locomotion, which in turn may impair the capacity of fishes to grow and reproduce. An increase of a few degrees in atmospheric temperature will not only raise the temperature of the waterbody, but it can also cause major hydrologic changes affecting the physical and chemical properties of water. There is already evidence of loss of warm water fish habitat in the Midwest, due to an increase in temperature in local streams. It is thus crucial to assess how fish respond to these changes physiologically to better protect fish populations from climate change through informed management policies. This study investigates how energetics are affected by thermal tolerance in largemouth bass and bluegill. Fish were housed at 20°C and then tested in a closed swim tunnel at 2 body length/s at 20°C, 22°C and 16°C, while recording decreasing concentrations in oxygen due to respiration. Prior to each trial fish were fasted for 24-48 hours and acclimatized for two hours at 0.5 body length/s at the experimental temperature. Mass corrected oxygen consumption was higher in largemouth bass than in bluegill at 16°C and 20°C, whereas bluegill showed significantly higher swimming metabolic rate at 22°C. The temperature quotient Q_{10} is the measure of temperature sensitivity of an enzymatic reaction rate or a physiological process with temperature increase by 10°C. Calculated Q_{10} (16°C-22°C) for bluegill was almost three times higher than that of largemouth bass, indicating that species with higher site fidelity might have more problems seeking optimal habitats as temperature increases. To protect these fish populations as the climate changes, information on bioenergetics needs to be taken into account in terms of management policies.

INTRODUCTION

Temperature is an important environmental variable; it influences other environmental variables and affects the metabolism of all living organisms directly. It has a greater impact on poikilothermic animal than on those who can regulate their body temperature (Bardach and Bjorklund, 1957). There is a vast array of research on how aquatic species will respond to those changes in both their distribution and at the physiological level (Di Santo, 2016). Temperature has been coined the 'ecological master factor' for fish (Brett, 1971), and important physiological functions such as growth, swimming performance and active metabolic rate can have species-specific temperature optima that are near a species-preferred or acclimated temperature (Fry, 1947; Brett, 1971; Dickson and Kramer, 1971; Beamish, 1978; Houston, 1982; Bernatchez and Dodson, 1985; Johnston and Temple, 2002).

The progressive warming of aquatic ecosystems is of critical conservation concern for fish as it has been associated with shifts in phenology, distribution and abundance (e.g. Perry et al., 2005; Pörtner and Knust, 2007; Martins et al., 2011), yet the temperature sensitive mechanisms driving these phenomena remain hypothetical. A leading hypothesis is that these population-level changes result from a decrease in aerobic metabolic performance of fish with increasing temperature, caused by a gradual decline in the capacity of the ventilatory and circulatory systems to deliver oxygen to the respiring tissues, i.e. oxygen- and capacity-limited thermal tolerance (OCLTT) (Pörtner and Knust, 2007; Pörtner and Farrell, 2008; Martins et al., 2011). This decline in aerobic metabolic performance affects critical biological functions, including behavior, growth and reproduction, owing to limited capacity of circulatory and ventilatory systems to match oxygen demands (Portner and Knust, 2007; Portner and Ferrel, 2008). Previous research has shown that increased temperature increases metabolism and oxygen consumption (Pettersson and

Hedenström, 2000). Also, dissolved oxygen is negatively impacted by water temperature, which leads to higher temperatures affecting fish by both lower oxygen availability and higher energy requirements (Claireaux et al., 2006).

Oftentimes when we study responses of fish to temperature change, we mostly talk about lethal temperature or critical thermal maxima (e. g. Gollock et al 2006; van Maaren 2000). However, lethal limits are often more extreme than the temperatures that an animal will experience in its environment (Portner et al, 2017). Thus, when fish are exposed to temperature changes, they can obtain optimal performance by altering either their behavior (preference/avoidance) or their physiology (adaptation and acclimation), when the temperature change is sufficiently long (Portner et al, 2017). One approach to resolving the adaptive value of a precise thermal preference in many fish is to compare optima of various functions *(e.g.,* metabolism, growth, swimming speed) with thermal preferences (Beitinger and Fitzpatrick, 1979).

In aquatic respiratory physiology, two types of respirometry chambers are commonly used to conduct either swimming (Fry and Hart, 1948; Steffensen et al., 1984) or resting respirometry measurements (Hemmingsen and Douglas, 1970; Fry, 1971). Resting respirometry is sometimes also referred to as static respirometry (e.g. Reidy et al., 2000; Barnes et al., 2011). Despite differences in their complexity and ease of use, both methods allow measuring of mass corrected oxygen consumption rates (MO_2) to estimate metabolic rates during or following varying levels of activity (e.g. resting *versus* active swimming) (Reidy et al., 1995; Peake and Farrell, 2006; Killen et al., 2007). In the current study we are calculating the mass corrected oxygen consumption rate of bluegill and largemouth bass at 2BL/s which is expected to be close to their regular swimming speed in a riverine environment. I have chosen bluegill and largemouth bass to study the impact of temperature on fish energetics because both species are well studied both in terms of locomotion

10

and ecological roles. Data is available on the cost of locomotion and their performance in different ecological conditions (Maia et al, 2015; Han et al, 2017).

In this study, my objectives were to determine how temperature influences oxygen consumption rate during routine swimming of two species of freshwater centrarchids, bluegill and largemouth bass. Largemouth bass and bluegill are both warm water fish with a tolerance 15°C-30°C. According to Coutant and Cox (1976), the upper lethal temperature for largemouth bass in Midwest is 35.5°C, while for bluegill it is 36.0°C (Sarig 1966). Largemouth bass finds suitable habitat in latitudes from the mid-south to the northern border of the U.S. (Eaton and Scheller, 1996). The optimum temperature for growth is 23-28°C for largemouth bass (Diez et al, 2007) and 24-27°C for bluegill (Pflieger et al, 1975). Bluegill lives in shallow water with high site fidelity (Hubbs et al, 1958).

My hypothesis is that, at higher temperatures, fish will have increased oxygen consumption rate regardless of species and that has the temperature increases the increase in oxygen consumption will be more noticeable.

MATERIALS AND METHODS

Experimental Animals

Six largemouth bass, *Micropterus salmoides* (Lacépède, 1802) were collected from Embarras River, IL during the Fall 2016 and housed in rectangular 50L tanks at 20±1°C and fed everyday initially on live earthworms and then regularly on soy free fish food pellets. Total length of individuals studied ranged from 150-300mm.

Six bluegill *Lepomis macrochirus* (Rafinesque, 1810) were collected from Embarras River, IL during the Fall 2016 and housed in rectangular 50L tanks at 20±1°C and fed everyday initially on frozen bloodworm and then regularly on soy free fish food pellets. Total length of individuals studied ranged from 120-170mm.

Oxygen Consumption

Before each trial fish were fasted for at least 24 hours and transferred to a 185L Loligo closed respirometry flume (Figure 1.1). To reduce stress levels, fish were acclimated in the 30 cm-long working sections of the flow chamber at low flow (0.5 BL/s) with the lid open for a minimum of two hours prior to testing at the experimental temperature. Oxygen consumption was measured while the fish was swimming steadily at 2BL/s for 2 hours using closed swim tunnel respirometry standard techniques (Maia et al. 2015) at three different temperatures, 16, 20 and 22±0.5°C. Six fish from both species were run for three trials at each temperature. Temperature in the experimental tank was controlled using a chiller and titanium heaters in the water bath. Dissolved oxygen and temperature were recorded using a YSI 6050020 Pro20 ODO optical probe. Linear regressions between oxygen concentration and time were made for each experiment and slopes derived from the regressions were used to calculate MO_2 (mg of O_2 min^{-1} kg^{-1}) after accounting for the volume of the respirometer and the mass of the fish. Only data with r^2 value greater than 0.97 were used (Figure 1.2). The oxygen consumption rate was calculated as a difference in dissolved oxygen concentration over time, considering the weight of the fish and the volume of the chamber, using the following formula:

$$MO_2 = \frac{m * V}{W}$$

where, MO_2 is the mass corrected oxygen consumption rate ($mgO_2min^{-1}\ kg^{-1}$), m is the slope of regression line in $mgO_2/L/min$, V is the volume of respirometer chamber in liters and W is the weight of fish in kilograms and all fish were weighed before the experiment, t2 is the higher temperature and t1 is the lower temperature.

From the oxygen consumption rates, Q_{10} was calculated as:

$$Q_{10} = \left(\frac{MO_2\ at\ t2}{MO_2\ at\ t1}\right)^{\frac{10}{(t2-t1)}}$$

Statistical analyses

Three-way analysis of variance (ANOVA) was used to test for differences in mass corrected oxygen consumption among individuals, species and temperatures. Since individual effects were negligible a two-way ANOVA was subsequently used to test for temperature and species interactions. Tukey's post hoc tests were used to test differences (Zar, 2009). All modeling was completed in R (R Core Team, 2016). Values are means ± S.E.M. and $P<0.001$ was used as the level of statistical significance.

RESULTS

Increase in temperature caused an increase in oxygen consumption in both largemouth bass and bluegill. Fish were run for 2 hours at 2BL/s; this interval was chosen to maximize the resolution needed to assess changes to metabolism between swimming trials. During the 2-hour period fish were actively swimming and kept their position in the flume. In my current study, I found that regardless of species when the temperature increases mass corrected oxygen consumption (MO_2) also increase (Figures 1.2, 1.3). Three-way ANOVA on MO_2 considering

three variables 1) individual, 2) species and 3) temperature, showed the individual effects to be negligible ($p>0.05$). Thus, the statistical analysis was simplified to a 2-way ANOVA with species and temperature and species-temperature interaction as predictors. Both species and temperature had an impact on active metabolism (MO_2). Higher temperatures increased oxygen consumption in both species of I also found interactive effects of species and temperature, driven specifically by an increased active metabolic rate of bluegill at 22°C ($p<0.05$, Figure 1.4). In contrast, there was no statistical significant between largemouth bass at 20 and 22°C. Overall, largemouth bass increased its oxygen consumption by 22% with increasing temperature, while bluegill increased its oxygen consumption by 231%.

Both species have active metabolic rates with increased dependence on temperature, as indicated by Q_{10} values greater than 1. Q_{10} of active metabolic rate from 16 to 20°C at 2 BL/s was 1.28 for bluegill and 1.04 for largemouth bass (Table 1.1). However, Q_{10} of active metabolic rate from 16 to 22°C was almost 5-fold higher in bluegill than the same Q_{10} of largemouth bass. It appears that bluegill is not as affected by changes in water temperature from 16 to 20°C as largemouth bass. However, a temperature of 22°C is enough to substantially increase active metabolism in bluegill.

DISCUSSION

The results of this study partly support my hypothesis is that, at higher temperatures, fish have higher active metabolic rates. However, at higher temperatures the two centrarchid species appear to have different strategies with the bluegill increasing oxygen consumption at a substantially higher rate than largemouth bass. These differences must be taken into account when developing management strategies for different species.

The impact of current and future warming on ectotherms in the wild is somewhat uncertain (Payne, 2016). Biochemical reaction rates, metabolic rates and nearly all other biological rates depend strongly on temperature (Brown et al. 2004), so for ectotherms, environmental temperature has a profound influence on physiology and fitness (Payne, 2016). My recent study was carried out at temperatures within the tolerance limit of largemouth bass and bluegill (15°C-32°C). Mass corrected oxygen consumption during swimming at 2BL/s at 22°C when compared to 16°C has a Q_{10} of 7.31 in bluegill, which means that this behavior is highly temperature dependent ($Q_{10}>5$) (Rome, 2007). An increase in temperature results in an increase in the rates of enzymatic reactions resulting in general in an increase in metabolic rates (Hochacka and Somero, 2001).

These findings suggest that with a higher temperature dependency, bluegill at 22°C are likely incurring in an energy deficit. According to the energy budget theory we know that if an organism need to spend more energy for metabolism their growth would be hindered (Forseth et al, 1994). Consequently, the mass growth of the population could be compromised at higher temperature. Moreover, reproduction of fish population is dependent on available energy, they can grow physically or can reproduce with emaciated body mass (Forseth et al, 1994). While individuals survive at non-lethal thermal constraints, the resulting reductions in available energy will endanger reproduction and thus population survival. Another study showed that at higher temperature bluegills have shorter life spans (Martinez et al, 2016) which may impact the community composition and indicate physiological tradeoffs. On the other hand, largemouth bass did not show as much vulnerability with temperature change. This poses the question how different fish species differ in dealing with challenges of higher temperature. One hypothesis is that muscle recruitment in largemouth bass and bluegill will be different. Largemouth bass and bluegill differ in their body shape and in their locomotor mode. Largemouth bass are more elongated and swim

by powering of axial musculature (Han et al, 2017), while bluegill have higher body depths and swim with the use of both axial musculature and pectoral, dorsal and anal fins (Drucker and Lauder, 2001). Since the dependence on aerobic and anaerobic metabolism of these different components is likely to be distinct, locomotor patterns can also influence cost of transport (Svendsn, 2010). Another possible explanation for the different physiological strategies of bluegill and largemouth bass could be the post exercise oxygen debt—a substitution for anaerobic metabolism in intact fish even during swimming at same speeds at different temperature. This study stands in sharp contrast with the common assumption that fish use slow-twitch red fiber muscles at low speeds (aerobic metabolism) and should not recruit fast glycolytic white muscle (generating anaerobic metabolites) until they approach their maximum sustainable speed (Di Santo et al, 2017; Priede and Holliday, 1980). The detection of anaerobic metabolism at similar speeds is particularly important considering that the most common approach to investigate fish energetics has critical impact at different temperature even though the temperature is within the tolerance limit. Future research should focus on how these two species change their locomotor strategies with increased temperature and how their muscle mechanics respond to that. In addition, studies at a wider range of temperatures and with longer acclimation periods can improve the understanding of the physiology these fish.

While considerable information on the temperature effects on swimming and oxygen consumption (MO_2) exists for Pacific salmon (*Oncorhynchus* spp.) (e.g. Griffiths and Alderdice, 1972; Beamish, 1978), there are very few works done on bluegill and largemouth bass. Lee et al (2003) established that salmon acclimated in different environment have different energetics and metabolic rate at same temperature. So, the temperature optima are not the same for all fish of same species from different habitats. Unlike a previous study on bluegill (Sarig, 1966), this study

does not show the similar temperature optima for bluegill of 17-25°C, since at 22°C locomotion is costly. This may because Illinois fish have a narrower or lower optimal temperature interval than other bluegill populations. Care needs to be taken when extrapolating results to other areas, fish sizes or life stages and acclimation temperatures.

This study clearly demonstrates that even within non-lethal temperature intervals, changes in temperature can affect the physiology and energy budgets of fish drastically and that these effects are temperature and species specific. As oxygen concentration in water also becomes a limiting factor with increased temperature (Banrnes et al, 2011), fish are likely to experience serious physiological stress with increased water temperatures.

LITERATURE CITED

Bardach, J.E. and Bjorklund, R.G., 1957. The temperature sensitivity of some American freshwater fishes. *The American Naturalist*, *91*(859), pp.233-251.

Barnes, R.K., King, H. and Carter, C.G., 2011. Hypoxia tolerance and oxygen regulation in Atlantic salmon, Salmo salar from a Tasmanian population. *Aquaculture*, *318*(3-4), pp.397-401.

Beamish, F.W.H., 1978. Swimming capacity. *Fish physiology*, *7*, pp.101-187.

Beitinger, T.L. and Fitzpatrick, L.C. 1979. Physiological and ecological correlates of preferred temperature in fish. *American Zoologist*, *19*(1), pp.319-329.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. and West, G.B., 2004. Response to forum commentary on" toward a metabolic theory of ecology". *Ecology*, *85*(7), pp.1818-1821.Coutant, C.C. and Cox, D.K., 1976. Growth Rates of Subadult Largemouth Bass at 24 to 35. 5 C. In *Thermal Ecology II, Proceedings of a Symposium held at Augusta Georgia*

April 2-5, 1975. CONF-750425, 1976. p 118-120, 1 fig, 1 tab, 12 ref.

Di Santo, V., 2016. Intraspecific variation in physiological performance of a benthic elasmobranch challenged by ocean acidification and warming. *Journal of Experimental Biology*, *219*(11), pp.1725-1733.

Di Santo, V., Kenaley, C.P. and Lauder, G.V., 2017. High postural costs and anaerobic metabolism during swimming support the hypothesis of a U-shaped metabolism–speed curve in fishes. *Proceedings of the National Academy of Sciences*, p.201715141.

Drucker, E. and Lauder, G., 2001. Wake dynamics and fluid forces of turning maneuvers in sunfish. *Journal of Experimental Biology*, *204*(3), pp.431-442.

Eaton, J. G. and Scheller, R. M. (1996). Effects of climate warming on fish thermal habitat in streams of the United States, *41*(5), 1109–1115.

Forseth, T., Ugedal, O. and Jonsson, B., 1994. The energy budget, niche shift, reproduction and growth in a population of Arctic charr, Salvelinus alpinus. *Journal of Animal Ecology*, pp.116-126.

Fry, F.E.J., 1971. The effect of environmental factors on the physiology of fish. *Fish physiology*.

Fry, F.A. and Hart, J.S., 1948. Cruising speed of goldfish in relation to water temperature. *Journal of the Fisheries Board of Canada*, *7*(4), pp.169-175.

Gollock, M.J., Currie, S., Petersen, L.H. and Gamperl, A.K., 2006. Cardiovascular and haematological responses of Atlantic cod (Gadus morhua) to acute temperature increase. *Journal of Experimental Biology*, *209*(15), pp.2961-2970.

Griffiths, J.S. and Alderdice, D.F., 1972. Effects of acclimation and acute temperature experience on the swimming speed of juvenile coho salmon. *Journal of the Fisheries Board of Canada*, *29*(3), pp.251-264.

Han, A.X., Berlin, C. and Ellerby, D.J., 2017. Field swimming behavior in largemouth bass deviates from predictions based on economy and propulsive efficiency. *Journal of Experimental Biology*, *220*(18), pp.3204-3208.

Hemmingsen, E.A. and Douglas, E.L., 1970. Respiratory characteristics of the hemoglobin-free fish Chaenocephalus aceratus. *Comparative biochemistry and physiology*, *33*(4), pp.733-744.

Hochochka, P.W. and Somero, G.N., 2002. Biochemical adaptation.

Hubbs, C.L., Lagler, K.F. and Smith, G.R., 1958. Fishes of the Great Lakes region (revised). *Cranbrook Inst. Sci. Bull*, *26*, pp.1-213.

Killen, S.S., Costa, I., Brown, J.A. and Gamperl, A.K., 2007. Little left in the tank: metabolic scaling in marine teleosts and its implications for aerobic scope. *Proceedings of the Royal Society of London B: Biological Sciences*, *274*(1608), pp.431-438.

Lee, C.G., Farrell, A.P., Lotto, A., MacNutt, M.J., Hinch, S.G. and Healey, M.C., 2003. The effect of temperature on swimming performance and oxygen consumption in adult sockeye (Oncorhynchus nerka) and coho (O. kisutch) salmon stocks. *Journal of Experimental Biology*, *206*(18), pp.3239-3251.

Maia, A., Sheltzer, A. P., and Tytell, E. D.;2015. Streamwise vortices destabilize swimming bluegill (Lepomis macrochirus). *Journal of Experimental Biology*, *218*(5), 786-792.

Martinez, E., Porreca, A.P., Colombo, R.E. and Menze, M.A., 2016. Tradeoffs of warm adaptation in aquatic ectotherms: Live fast, die young?. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *191*, pp.209-215.

Martins, E.G., Hinch, S.G., Patterson, D.A., Hague, M.J., Cooke, S.J., Miller, K.M., Lapointe, M.F., English, K.K. and Farrell, A.P., 2011. Effects of river temperature and climate warming on stock-specific survival of adult migrating Fraser River sockeye salmon (Oncorhynchus

nerka). *Global Change Biology*, *17*(1), pp.99-114.

Martin, T.L. and Huey, R.B., 2008. Why "suboptimal" is optimal: Jensen's inequality and ectotherm thermal preferences. *The American Naturalist*, *171*(3), pp.E102-E118.

Norin, T. and Clark, T.D. (2016). Measurement and relevance of maximum metabolic rate in fishes. *Journal of Fish Biology*, *88*(1), pp.122-151.

Norin, T. and Malte, H. (2011). Repeatability of standard metabolic rate, active metabolic rate and aerobic scope in young brown trout during a period of moderate food availability. *Journal of Experimental Biology, 214*(10), 1668-1675.

Payne, N.L., Smith, J.A., Meulen, D.E., Taylor, M.D., Watanabe, Y.Y., Takahashi, A., Marzullo, T.A., Gray, C.A., Cadiou, G. and Suthers, I.M., 2016. Temperature dependence of fish performance in the wild: links with species biogeography and physiological thermal tolerance. *Functional Ecology, 30*(6), pp.903-912.

Perry, A.L., Low, P.J., Ellis, J.R. and Reynolds, J.D., 2005. Climate change and distribution shifts in marine fishes. science, 308(5730), pp.1912-1915.

Pflieger, W.L., Sullivan, M. and Taylor, L., 1975. *The fishes of Missouri* (Vol. 343). Jefferson City: Missouri Department of Conservation.

Pörtner, H.O., Bock, C. and Mark, F.C., 2017. Oxygen-and capacity-limited thermal tolerance: bridging ecology and physiology. *Journal of Experimental Biology, 220(15)*, pp.2685-2696.

Pörtner, H.O. and Farrell, A.P., 2008. Physiology and climate change. *Science*, pp.690-692.

Pörtner, H.O. and Knust, R., 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science, 315(5808)*, pp.95-97.

Priede, I.G. and Holliday, F.G.T., 1980. The use of a new tilting tunnel respirometer to investigate some aspects of metabolism and swimming activity of the plaice (Pleuronectes platessa L.). Journal of Experimental Biology, 85(1), pp.295-309.

van Maaren, C.C., Kita, J. and Daniels, H.V., 2000. Temperature tolerance and oxygen consumption rates for juvenile southern flounder Paralichthys lethostigma acclimated for five different temperature. UJNR Technical Report, 28, pp.135-140.

Reidy, S.P., Kerr, S.R. and Nelson, J.A., 2000. Aerobic and anaerobic swimming performance of individual Atlantic cod. Journal of Experimental Biology, 203(2), pp.347-357.

Reidy, S.P., Nelson, J.A., Tang, Y. and Kerr, S.R., 1995. Post-exercise metabolic rate in Atlantic cod and its dependence upon the method of exhaustion. Journal of Fish Biology, 47(3), pp.377-386.

Roche, D.G., Binning, S.A., Bosiger, Y., Johansen, J.L. and Rummer, J.L., 2013. Finding the best estimates of metabolic rates in a coral reef fish. Journal of Experimental Biology, 216(11), pp.2103-2110.

Sarig, S., 1966. A review of diseases and parasites of fishes in warm water ponds in the Near East and Africa. In FAO World Symposium on Warm Water Pond Fish Culture (pp. 278-89).

Steffensen, J.F., Johansen, K. and Bushnell, P.G., 1984. An automated swimming respirometer. Comparative Biochemistry and Physiology--Part A: Physiology, 79(3), pp.437-440.

Svendsen, J.C., Tudorache, C., Jordan, A.D., Steffensen, J.F., Aarestrup, K. and Domenici, P., 2010. Partition of aerobic and anaerobic swimming costs related to gait transitions in a labriform swimmer. Journal of Experimental Biology, 213(13), pp.2177-2183.

Zar, J.H. (2009) Biostatistical Analysis. 5th Edition. Prentice Hall, New Jersey.

TABLES

Table 1.1: Temperature quotient Q_{10} of largemouth bass and bluegill at 20°C and 22°C comparing

to 16°C

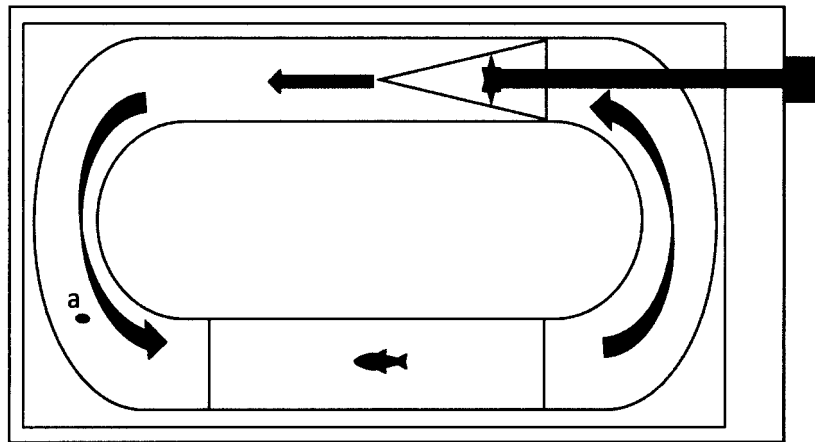| Fish species	20°C	22°C
Largemouth bass	1.28	1.40
Bluegill	1.04	7.31

FIGURES

Figure1.1: Closed flume tunnel setup used for respirometry experiments. Water flows counter clockwise, oxygen probe placed at position (a) and fish swim at compartment (b), this compartment was sealed using a lid throughout the two hours experimental period

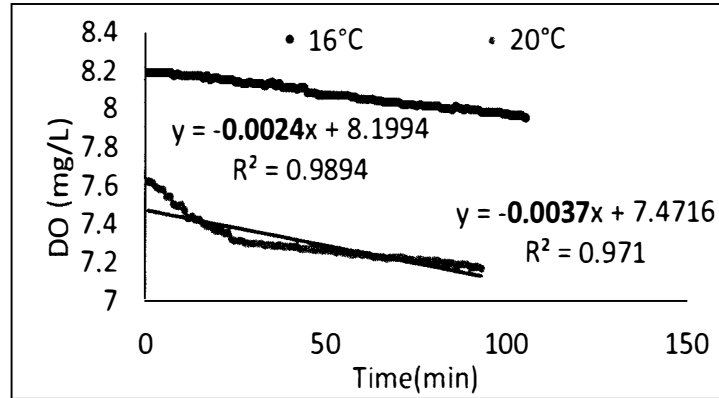Figure 1.2: Oxygen depletion at different temperatures for one largemouth bass individual.
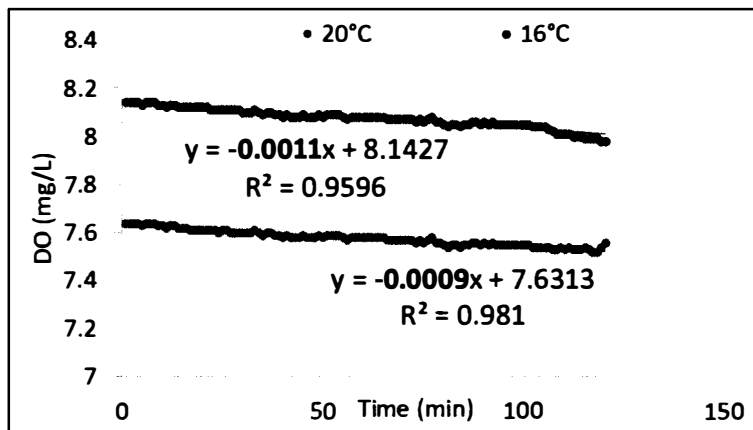

Figure 1.3: Oxygen depletion at different temperatures for one bluegill individual
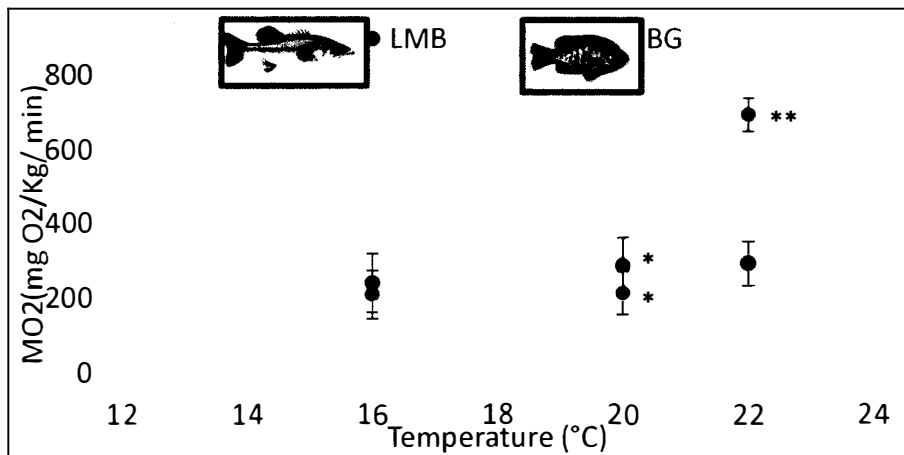

Figure 1.4: Mass corrected oxygen consumption in largemouth bass and bluegill at different temperatures. LMB, n=6; BG, n=4. Each fish was run for three trials. The asterisks denote differences at different temperatures for both species.

23

IMPACT OF TEMPERATURE INCREASE ON MUSCLE MECHANICS OF TWO FRESHWATER CENTRARCHIDS

ABSTRACT

Temperature strongly regulates the distribution and fitness of ectotherms, and many studies have measured temperature dependent effects on physiological performance. Recent years have shown a rise in mean global temperatures and a shift in the geographical distribution of ectothermic animals. Fish have evolved physiologically to live within a specific range of environmental variation and existence outside of that range can be stressful or fatal. This study investigates physiological processes limiting thermal tolerance, specifically how changes in water temperature affect the swimming muscle mechanics in largemouth bass (*Micropterus salmoides*) and bluegill (*Lepomis macrochirus*). I focused on the impact of temperature change at the muscle level in these two species and the capacity to adapt to rapid changes in the environment. Fish were housed at 20°C and then tested in a recirculating flow tank 2 body length/sec at three different temperature at 16°C, 20°C and 22°C. To perform intramuscular electromyography (EMG), bipolar electrodes were inserted through the skin into epaxial white muscle at 0.5 and axial red muscle at 0.5BL and 0.75BL to record contraction parameters using electromyography standard techniques and allowed to recover for 1h before being tested during steady swimming at 2body length /s. Electromyographic recordings were analyzed for magnitude, frequency, onset, offset, duration and duty cycle. According to my data there are significant trends of increased recruitment of white muscle and increases in duty cycle with increase temperature in both species. At higher temperature fish have greater magnitude in all muscles, except in axial red muscles at 22°C which are not recruited. Also, the duration of muscle activation is higher at 22°C for the muscles that are

active. Freshwater fish species that live in greatly static environment over the course of different seasons may fall into more difficulties with increased temperature. This study reveals that to a certain extent fish change their muscle mechanics to attempt to cope with increases in temperature due to climate change, which could in turn have serious metabolic impacts.

INTRODUCTION

In most fishes a change in aquatic temperature results in a change in body temperature since they lack the capacity to raise their temperature above ambient (Cossins and Crawford, 2005). Fish growth can be influenced not only by the genetic makeup but also by environmental factors (Lee et al, 2003). Swimming is the most common behavior and a demanding metabolic activity for fish (Eaton et al., 1996) and thus species have developed different strategies to reduce energy expenditure (Pettersson and Hedenström, 2000). Environmental temperature changes profoundly affect the locomotor performance of ectotherms (e.g. Rome and Alexander, 1970; Rome, 1982; Rome, 2007). Unlike a simple chemical reaction, where a decrease in temperature merely slows its rate, locomotion is a complex integrative process with muscular, neural, biomechanical and metabolic components (Rome, 2007).

Locomotion is powered by musculoskeletal movement and tension generation at the level of the muscle fibers (Altringham and Ellerby, 1999). The muscles of fish are layered rather than bundled as in the other vertebrates. Each section, or sheet, of muscles is called a myomere or myotome and is separated from its neighbor by a sheet of connective tissue (Altringham and Ellerby, 1999). Other sheets of connective tissue, called septa occur along the vertical midline of the body separating the muscles of the left and right sides of the body, and horizontally separating the muscles of the upper and lower halves of the body (Altringham and Ellerby, 1999). The

muscles of the upper half of the body are the epaxials and those of the lower half are called hypaxials. Fish muscles can be clasified into two principal types, fast twitch or white muscle which relies on anaerobic pathways, and slow-twitch or red muscle which relies on aerobic energy sources (Altringham and Ellerby, 1999). Fast muscle makes up the bulk of the fish, typically 80–100 % of the fish cross section at a given point (Altringham and Ellerby, 1999). The proportion of red muscle is 10% - 30% depending upon the ecosystem of that fish and can be entirely absent in some fish like stickleback (Videler, 1993; Sanger and Stoiber, 2001; Syme, 2005). The decreased muscle mass towards the tail places the power source for fast swimming far anterior to the tail, and a power-transmitting role for posterior fast muscle seems likely (Altringham and Ellerby, 1999). The slow muscle is usually confined to a zone beneath the lateral line, making up an increasing proportion of the body cross-section towards the tail (Videler, 1993). The proportion of slow muscle is related to the ecology of the fish, with constantly swimming pelagic species having a higher percentage of slow-twitch muscle than benthic, less active species (Boddeke et al., 1959; Videler, 1993).

   With their slow twitch properties, red fibers are not capable of producing the rapid oscillations in force required to produce rapid tail beats for fast swimming but are well suited to power slow body undulations for regular swimming (Syme, 2005). It has been shown that slow fibers are activated during steady, cruise swimming while white fibers power burst swimming activity such as kick-and-glide and the startle response (Rome et al., 1992a; Sisson and Sidell, 1987; Jayne and Lauder, 1994; Coughlin and Rome, 1999; Ellerby et al., 2000; Ellerby and Altringham, 2001; Sanger and Stoiber, 2001). Whereas white fibers are the main source of power during high-speed swimming, continued recruitment of red fibers at high speeds may aid power production (Johnson et al., 1994) or force transmission (Altringham and Ellerby,1999). Muscle

level measurements of activity, strain and force production have revealed the mechanical roles of many specific muscles in locomotion (Ellerby et al, 2000). A wave of contraction propagates down the length of the fish body during swimming (Syme 2006). Peake and Farrell (2004) noted a progressive switch from aerobic to aerobic/anaerobic to exclusively anaerobic metabolism with increased swim speed in smallmouth bass, suggesting a red to red/white to white sequence of muscle recruitment.

In swimming fish, muscle mechanical power is transferred to the water to generate lift and thrust (Rome et al., 1993; Altringham and Ellerby, 1999; Drucker and Lauder, 1999; Coughlin, 2002). As stated above fish use lateral muscles during steady swimming, showing that red and white muscle can be recruited at different speeds and behaviors to generate thrust (Altringham and Ellerby, 1999, Westneat et al, 1998). To more precisely describe muscle mechanics, *in vitro* experiments have investigated muscle power output under simulated swimming conditions such as repeated cycles in several fish species (e.g. Altringham et al.,1993; Rome et al., 1993; Johnson et al., 1994; Hammond et al., 1998). Some studies found that at 20°C scup use their red muscles in a way that produces 87–98% of maximum power. But at 10°C, the result is dramatically different, with the muscles producing only about 20% of the power they can produce and producing maximum power at a cycle frequency about half of the tail-beat frequency observed in fishes at this temperature (Rome and Swank, 1992; Swank and Rome, 2000; Rome et al., 2000).

This study investigates if fish are capable of changing muscle mechanics to cope with changes in water temperature. My hypothesis for the study is that at higher temperatures fish will maximize force production in both red and white muscles by increasing parameters such as magnitude, frequency and duration of muscle contraction as well as duty cycle.

MATERIALS AND METHOD

Experimental Fishes

Six largemouth bass, *Micropterus salmoides* (Lacépède, 1802) were collected from Embarras river IL during Fall 2016 and housed in rectangular 50L tank at 20°C ((±1ºC) and fed everyday initially on live earthworm and then regular soy free fish food pellets. Total length of individual fish studied were ranged from 150-300mm.

Six bluegill *Lepomis macrochirus* (Rafinesque, 1810) were collected from Embarras river IL during Fall 2016 and housed in rectangular 50L tank at 20°C ((±2ºC) and fed everyday initially on frozen bloodworm and then regular fish food pellets. Total length of individual studied ranged from 120-170mm.

Gross Anatomy

Freshly frozen fish of both species were collected and transferred into two separate jars with Formaldehyde Acetic Acid Ethanol (FAA). They were kept in fixative for four days and preserved in 70% ethanol. Scales and the delicate layer of skin removed using fine surgical forceps (Figure 2.2) to study the muscle arrangement in both species.

Electromyography

Bipolar EMG electrodes, constructed from 0.0434 mm diameter insulated stainless-steel wire (California Fine Wire Co., Grover Beach, CA, USA), were implanted through the skin in the white into the epaxial white muscle at 0.5BL and midline red muscle at 0.5BL and 0.75BL to record muscle contraction using standard techniques (Gerry and Ellerby, 2014; Ellerby and

Altringham, 2001). Insulation was removed from a 2 mm section of each wire to form an electrode tip with two bare wire sections facing oppositely. Electrodes were threaded through a 25-gauge needle for implantation into muscles. Before performing intramuscular electromyography (EMG) recordings, fish were sedated by tricaine methane sulfonate (MS222, Argent Chemical Laboratories) at 0.075g/L and fish was immersed in the anesthetics for 10 minutes. After electrode implantation, fish could recover for 1h before being tested in a Loligo 295L flow tank while steadily swimming at 2BL/s at three different temperatures 16, 20 and 22±0.5°C (Figure 2.1 A). EMGs were recorded and digitized using 8-Channel Recorder iWorx device with BIO4 iWire,4 channel biopotential amplifier and sampling frequency was 100kHz. Recorded data were analyzed (Figure 2.1 B) for magnitude of muscle contraction, onset, offset and duration of muscle activation and duty cycle associated with epaxial white and axial white and red muscles (Ellerby and Altringham, 2001).

Statistical analyses

Two-way ANOVAs were used to test for individual effects along with one of each of the following variables: 1) temperature (16°C, 20°C and 22°C), 2) muscle (epaxial white muscle at 0.5BL, red muscle at 0.5BL and red muscle at 0.75BL) and 3) species.

RESULTS

Muscle Arrangement

Largemouth bass axial musculature presents red muscle that is more superficial and runs along the horizontal septum with 5mm width and 2mm depth in most of the areas (Figure 2.2 A),

with the width of red muscle increasing towards the tail. Fiber typing of dorsal and anal fin musculature appears to indicate presence of only red musculature attaching to the lepidotrichia (Figure 2.2 A).

In contrast, white muscle occupies most the axial musculature in bluegill; red muscle occupies along the lateral line in a very thin layer and gradually become wider but still shallow along caudal fin region (Figure 2.2 B).

Despite the close phylogenetic relationship of these two species, muscle arrangement differs substantially. Largemouth bass has more prominent and deeper red muscle than bluegill. Moreover, red muscles in bluegill are wider near the caudal region than in largemouth bass (Figure 2.2).

Muscle Activity

Largemouth bass

Largemouth bass swimming at 2BL/s show muscle activity in caudal red muscle and epaxial white muscle at all temperatures. However, muscle recruitment and muscle mechanics differed with temperature. At 22°C axial red muscles are not active thus muscle mechanics cannot be determined.

At 22°C largemouth bass shows an increase in recruitment of white muscle fibers when compared to lower temperatures (Figure 2.2). Muscle mechanics are also affected, especially in magnitude and duration, which indicate an increase in overall strength of contraction ($p < 0.05$, Figure 2.3). It is also apparent that at the higher temperature (22 °C) muscle relaxation takes longer ($p < 0.05$). Axial red muscle shows little or no activity at 22°C in contrast to cooler temperatures (16°C and 20°C).

Greater magnitude of white muscles ($p<0.05$) with increasing temperature indicates additional recruitment of these fibers (Figure 2.4). Duration of contraction and concurrently duty factor of white muscles were longer at 22°C when compared to 16°C (Figures 2.5, 2.6). Interestingly, frequency which is considered an intrinsic muscle property varied with temperature but only in the axial white musculature, where it was lower at 20°C, higher at 22°C and the highest at 16°C (Figure 2.7).

When analyzing the relative timing of activation of the three muscles studied, it is clear to see that overall muscles have higher duty cycles at higher temperatures (Figure 2.8). The pattern of activation of axial red and axial white is also distinct, with a clear phase shift at lower temperatures and a higher overlap at higher temperatures.

Bluegill

Bluegill swimming at 2BL/s show activity in all muscles at all temperatures tested (Figure 2.9). However, the axial white muscles do not show as high muscle recruitment and muscle mechanics at 22°C as at lower temperatures (Figure 2.9). Axial red muscle shows significantly greater magnitude at 22°C ($p<0.05$, Figure 2.10) and both axial and caudal red muscles were activated for longer period at higher temperature ($p<0.05$, Figure 2.11). Axial white muscle does not show any difference in muscle recruitment and duration of muscle activation between 16°C and 22°C.

DISCUSSION

The results of this study support my hypothesis that at higher temperatures fish maximize force production. However, axial red muscle is sometimes no longer active. As predicted, fish are

capable of changing muscle mechanics at different temperatures through a combination of differential recruitment and changes in each muscle parameters.

Bluegill and largemouth bass are two Centrarchidae species that differ in their ecology and behavior (Fish and Savitz, 1983). While largemouth bass have a more fusiform body shape and relies mainly on axial muscle contraction for locomotion, bluegill have a deeper body and are known to be more maneuverable (Fish and Savitz, 1983). In terms of their muscle anatomy, these two species also differ substantially. Largemouth bass has more prominent and deeper red muscle than bluegill. Moreover, red muscles in bluegill are wider near the caudal region than in largemouth bass. It is thus not surprising that these two species have distinct muscle mechanics.

This study was particularly interested in the capacity of these two species to change muscle mechanics to cope with changes in environmental temperature while performing the same behavior, swimming at 2BL/s. I was interested in the role of the axial muscles especially since the arrangement is also distinct in these two species. In general, these two species coped with temperature by increasing muscle recruitment and force production through increasing contraction magnitude and duration of muscle activation. However, while bluegill increased these parameters for both the caudal white and red muscle, largemouth bass only changed the muscle mechanics of white caudal muscle.

Coughlin (2003) noted that the relaxation and contraction times of red muscles from rainbow trout are faster than for scup when compared at the same temperature, and Ellerby et al. (2001) noted the relatively short relaxation times and high operating frequencies in red muscle of scup versus the long relaxation times and slow operating frequencies in red muscle of eels. The electromyographic data of largemouth bass has shown that at higher temperatures fish start to recruit more white muscle. Even though white muscle uses anaerobic energy sources, it is well

documented that the use of this type of muscle has a higher cost (Altringham, 2000). The elevated mass corrected oxygen consumption following exhaustive exercise, termed 'excess post-exercise oxygen consumption' (EPOC) (Gaesser and Brooks, 1984) can be used to assess both recovery time and the non-aerobic oxygen cost of exercise. EPOC, which replaces the term 'oxygen debt' (Hill et al., 1924) to avoid causal implications, reflects the increased quantity of oxygen required to restore tissue and cellular stores of oxygen, biochemical imbalances in metabolites such as lactate and glycogen, and other functions such as ionic and osmotic balance (Lee et al, 2003). This could explain the fact that largemouth bass did not increase oxygen consumption with temperature as much as bluegill while exercising (this thesis, Chapter 1).

My current research work also elucidates that fish with lesser proportion of red muscles does not necessarily mean they are more dependent on anaerobic white muscle. Bluegill are able to change muscle mechanics to produce comparable swimming speeds at higher temperature. However, as seen in Chapter 1 this comes at a higher metabolic cost than in largemouth bass.

Fish are capable of changing muscle recruitment and muscle mechanics in the presence of temperature stressors, however, these behaviors are likely to still be costly over time. Largemouth bass would need more oxygen after the swimming at higher temperature to get rid of the lactic acid produced and accumulated in white muscles. Moreover, to produce the same amount of energy anaerobically white muscles will incur into higher ATP usage than that of aerobic red muscles. Thus, the whole locomotion process at raised temperature would affect the energy budget of fish. Consequently, the physical growth and overall population growth would be challenged. My study on how the physiological processes are affected by temperature holds potential applications to management and stronger community wide conservation efforts of these recreational species.

LITERATURE CITED

Altringham, J.D., Wardle, C.S. and Smith, C.I., 1993. Myotomal muscle function at different locations in the body of a swimming fish. *Journal of Experimental Biology*, *182*(1), pp.191-206.

Altringham, J.D. and Ellerby, D.J., 1999. Fish swimming: patterns in muscle function. *Journal of Experimental Biology*, *202*(23), pp.3397-3403.

Askew, G.N. and Ellerby, D.J., 2007. The mechanical power requirements of avian flight. *Biology Letters*, *3*(4), pp.445-448.

Bennett, A.F., 1985. Temperature and muscle. *Journal of Experimental Biology*, *115*(1), pp.333-344.

Cossins, A. R., and Crawford, D. L. (2005). Fish as models for environmental genomics. Nature Reviews Genetics, 6(4), 324-333.

Coughlin, D.J., 2003. *Steady swimming by fishes: kinetic properties and power production by the aerobic musculature* (pp. 55-72). Science Publishers Inc., Enfield.

Ellerby, D. J., and Altringham, J. D. 2001. Spatial variation in fast muscle function of the rainbow trout Oncorhynchus mykiss during fast-starts and sprinting. *Journal of Experimental Biology, 204*(13), 2239-2250.

Ellerby, D.J., Spierts, I.L. and Altringham, J.D., 2001. Slow muscle power output of yellow-and silver-phase European eels (Anguilla anguilla L.): changes in muscle performance prior to migration. *Journal of Experimental Biology, 204*(7), pp.1369-1379.

Fish, P.A. and Savitz, J., 1983. Variations in home ranges of largemouth bass, yellow perch, bluegills, and pumpkinseeds in an Illinois lake. *Transactions of the American Fisheries Society*, *112*(2A), pp.147-153.

Gaesser, G.A. and Brooks, C.A., 1984. Metabolic bases of excess post-exercise oxygen. *Medicine and science in sports and exercise*, *16*(1), pp.29-43.

Gerry, S.P. and Ellerby, D.J., 2014. Resolving shifting patterns of muscle energy use in swimming fish. *PloS one*, *9*(8), p.e106030.

Hill, A.V., Long, C.N.H. and Lupton, H., 1924. Muscular exercise, lactic acid, and the supply and utilisation of oxygen. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character*, *97*(681), pp.84-138.

Lee, C.G., Devlin, R.H. and Farrell, A.P., 2003. Swimming performance, oxygen consumption and excess post-exercise oxygen consumption in adult transgenic and ocean-ranched coho salmon. *Journal of Fish Biology*, *62*(4), pp.753-766.

Lee, C.G., Farrell, A.P., Lotto, A., Hinch, S.G. and Healey, M.C., 2003. Excess post-exercise oxygen consumption in adult sockeye (Oncorhynchus nerka) and coho (O. kisutch) salmon following critical speed swimming. *Journal of Experimental Biology*, *206*(18), pp.3253-3260.

Rome, L.C., 1982. Energetic cost of running with different muscle temperatures in Savannah Monitor lizards. *Journal of Experimental Biology*, *99*(1), pp.269-277.

Rome, L.C., 2007. The effect of temperature and thermal acclimation on the sustainable performance of swimming scup. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *362*(1487), pp.1995-2016.

Rome, L.C. and Alexander, R.M., 1990. The influence of temperature on muscle velocity and sustained performance in swimming carp. *Journal of Experimental Biology*, *154*(1), pp.163-178.

Sanger, A.M. and Stoiber, W., 2001. Muscle fiber diversity and plasticity. *Fish physiology*, *18*, pp.187-250.

Syme, D.A., 2005. Functional properties of skeletal muscle. *Fish Physiology*, *23*, pp.179-240.

Videler, J.J., 1993. *Fish swimming* (Vol. 10). Springer Science & Business Media.

Videler, J.J. and Wardle, C.S., 1991. Fish swimming stride by stride: speed limits and endurance. *Reviews in Fish Biology and Fisheries*, *1*(1), pp.23-40.

Westneat, M.W., Hale, M.E., McHenry, M.J. and Long, J.H., 1998. Mechanics of the fast-start: muscle function and the role of intramuscular pressure in the escape behavior of Amia calva and Polypterus palmas. *Journal of Experimental Biology*, *201*(22), pp.3041-3055.

FIGURES
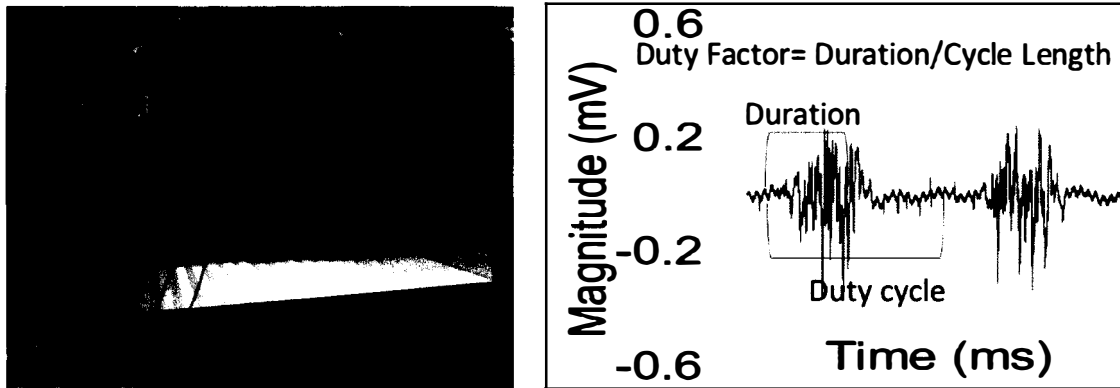
Figure 2.1: Experimental setup of electromyography trial of largemouth bass in (a) loligo 295L flow tank, (b) EMG recordings from the mid red muscle (0.5BL) at 20°C in largemouth bass.
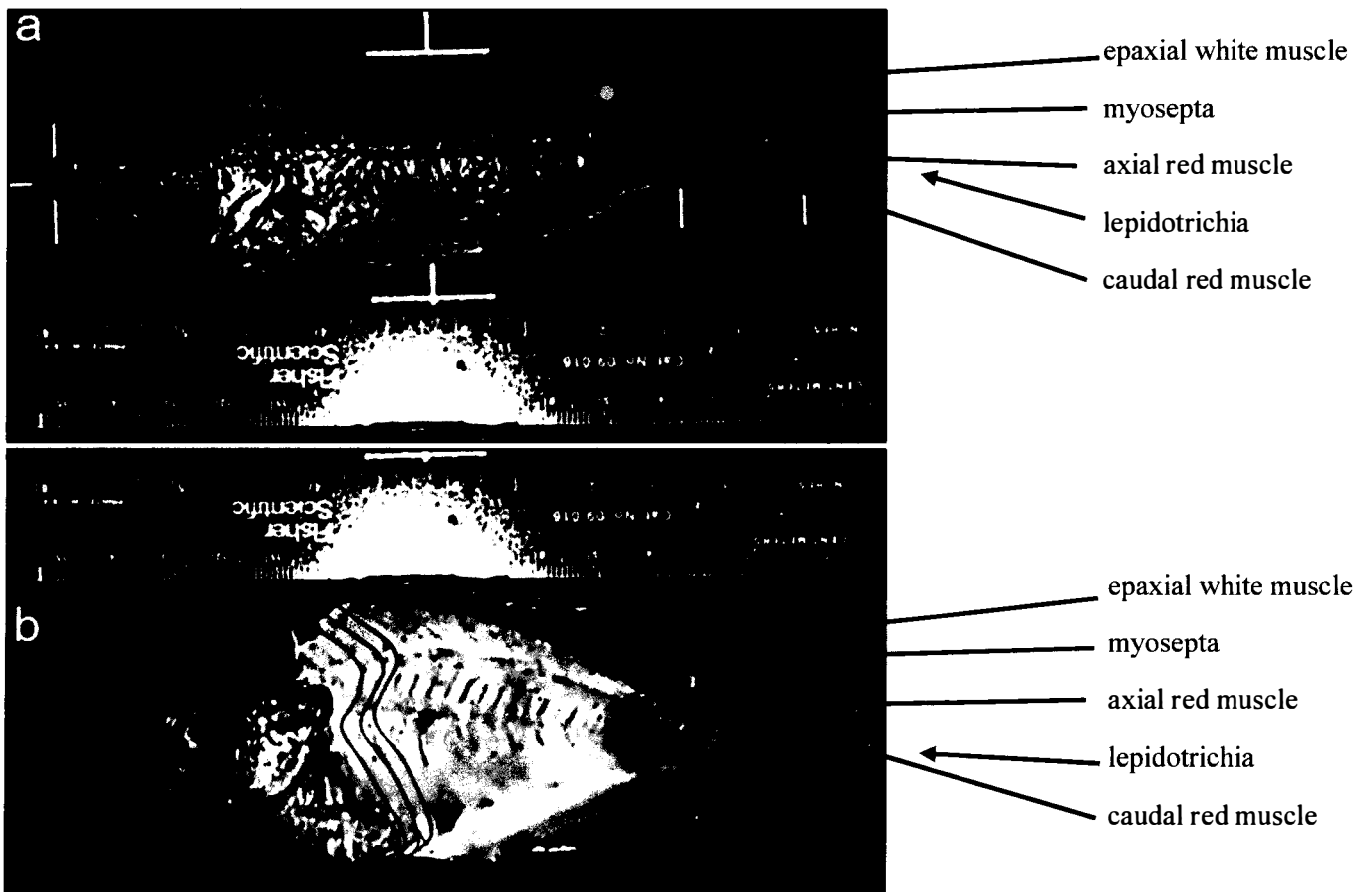
Figure 2.2: Fish muscle arrangement in (a) largemouth bass (*Micropterus salmoides*) and (b) bluegill (*Lepomis macrochirus*).
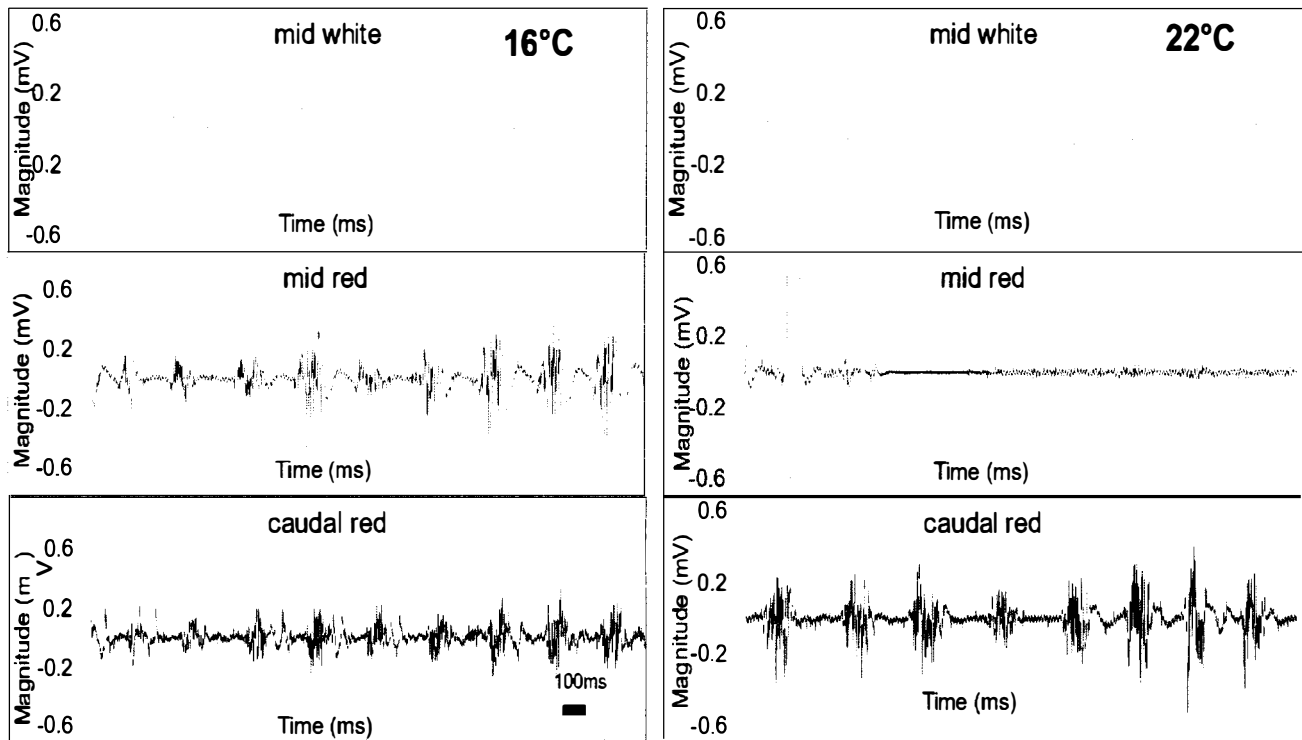
Figure 2.3: Electromyography signals from different muscles of largemouth bass at 16°C and 22°C
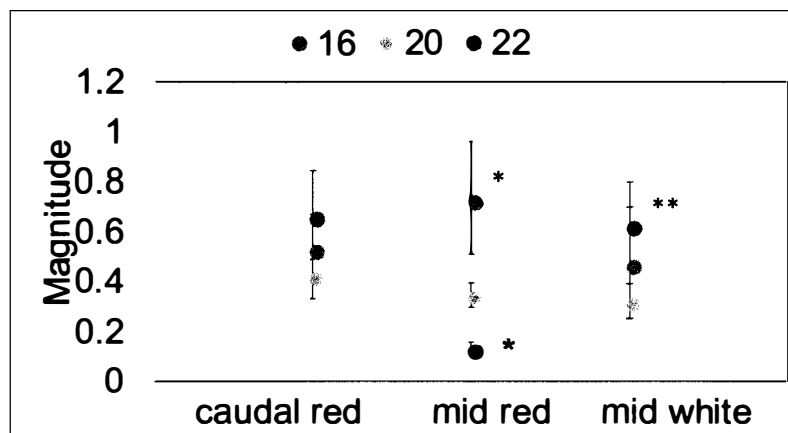

Figure 2.4: Magnitude of different muscles of largemouth bass at 16°C, 20°C and 22°C. Asterisks
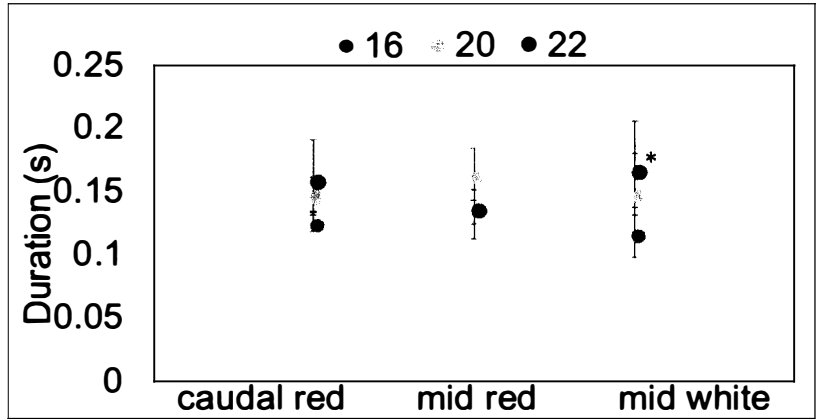
denote significance at p<0.05.

Figure 2.5: Duration of activation of different muscles of largemouth bass at 16°C, 20°C and 22°C. Asterisks denote significance at p<0.05.

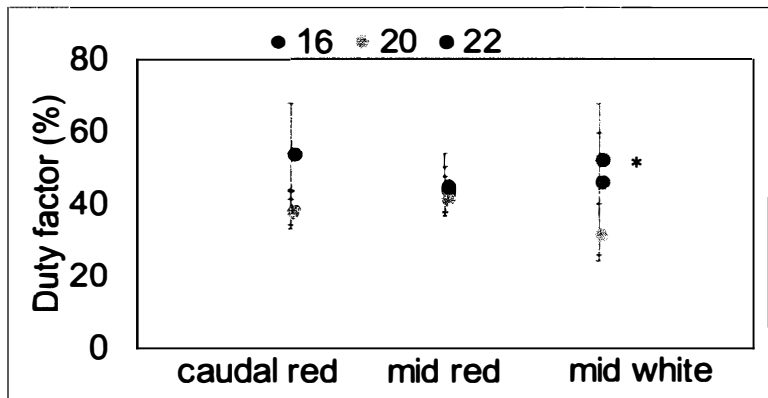Figure 2.6: Duty factors of different muscles of largemouth bass at 16°C, 20°C and 22°C. Asterisks denote significance at p<0.05.
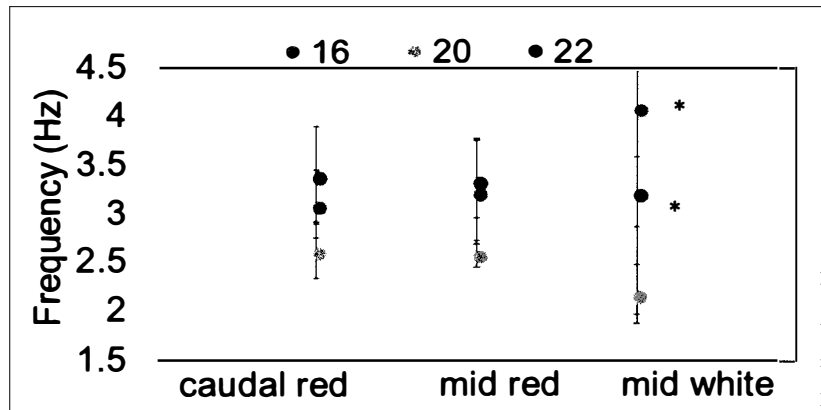

Figure 2.7: Frequency of different muscles of largemouth bass at 16°C, 20°C and 22°C. Asterisks denote significance at p<0.05.
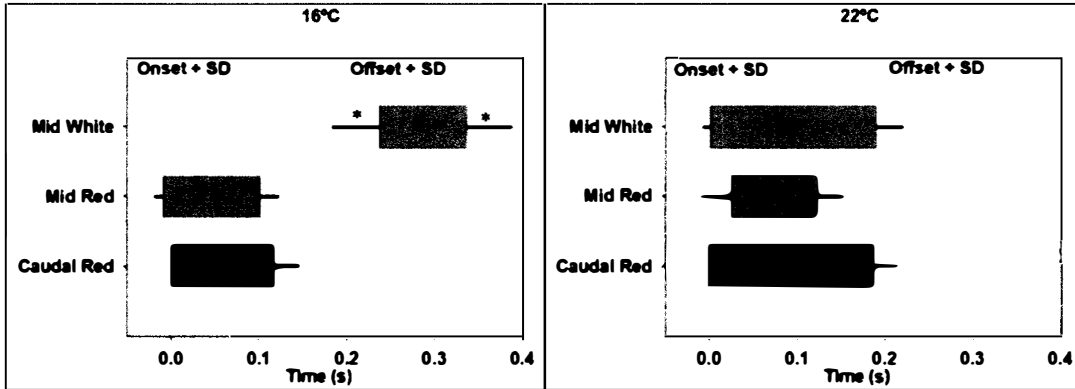
Figure 2.8: Onset and offset of activation of different muscles in largemouth bass at 16°C and 22°C. Letters and asterisks denote significant differences. Caudal red is the reference muscle.
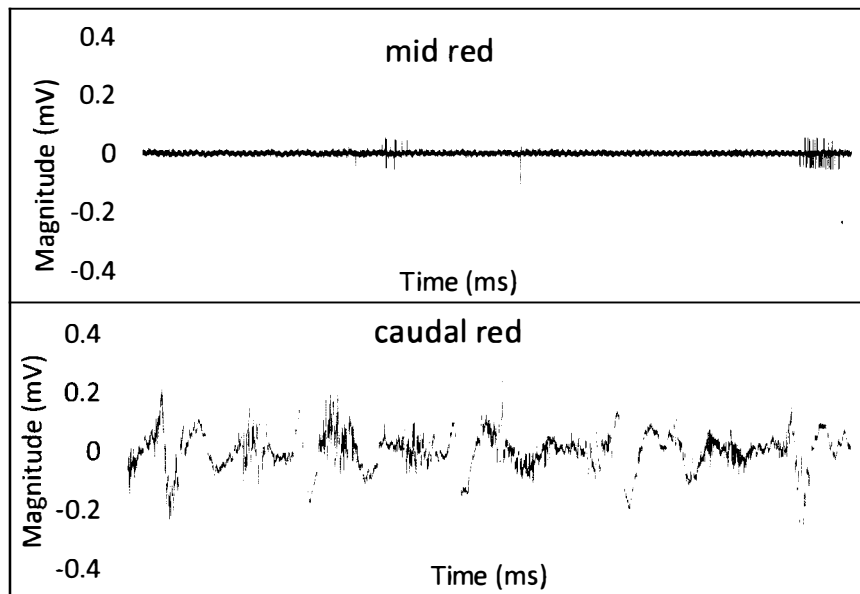

Figure 2.9: Representative electromyography traces from different muscles of bluegill at 22°C
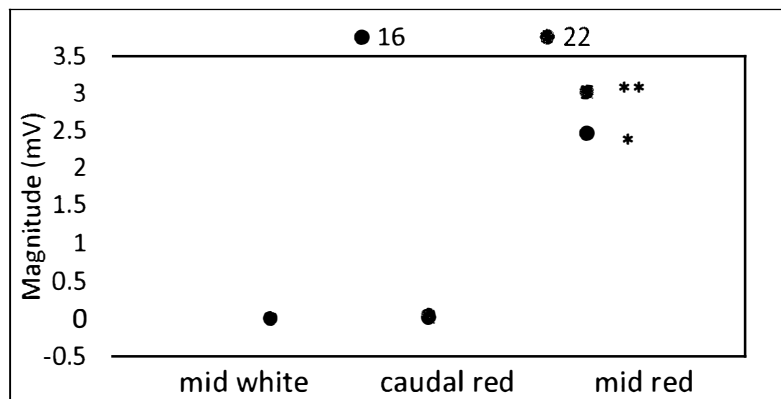
Figure 2.10: Magnitude of different muscles of bluegill at 16°C and 22°C. Asterisks denote significance at p<0.05.
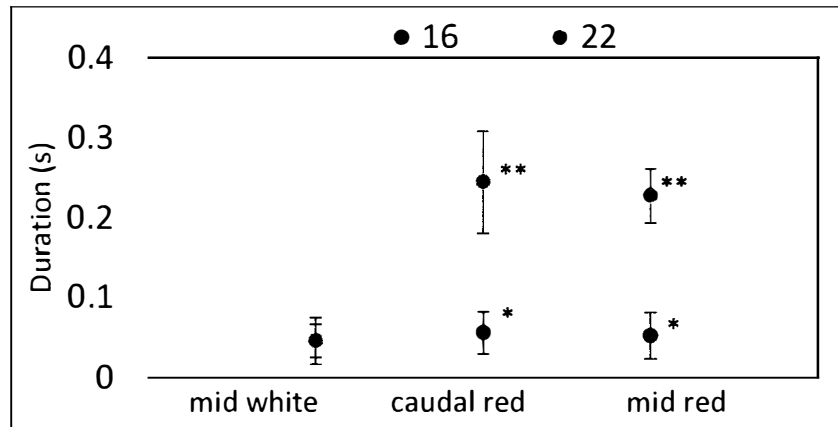

Figure 2.11: Duration of activation of different muscles of bluegill at 16°C and 22°C. Asterisks denote significance at p<0.05.

CONCLUSIONS, FUTURE RESEARCH DIRECTIONS, AND RECOMMENDATIONS

Changes in temperature have profound effects on ectothermic fish since environmental temperature will directly affect the rate of physiological processes (Herbing, 2002). The findings from my study provide important guidelines for understanding the adverse effects of temperature exposure in a native fish species, bluegill and largemouth bass, which had not been previously investigated. This information is crucial not only for Illinois but throughout the Midwest as there is already evidence of loss of warm water fish habitat in the Midwest, due to an increase in temperature in local streams. Moreover, most of the tributaries of this region are covered with dams, which stop fish from moving to their suitable environment. To protect fish populations from environmental stressors like temperature we need more information on how temperature affects physiological processes to improve management policies. This study shows that different fish species are negatively impacted by increase in temperature, although the mechanisms are species specific. Active metabolic cost or cost of transport was shown to be highly affected by temperatures within the non-lethal limits in both bluegill and largemouth bass. However, the negative impact of increased temperature in bluegill was substantially higher than in largemouth bass. This suggests that populations of bluegill are more likely to be affected by climate change than those of largemouth bass. As cost of transport increases, there is likely less resources available to the fish that can be allocate for growth and reproduction which can have a strong negative impact on population dynamics.

This study also shows that temperature has a significant effect not only on cost of transport but also on muscle mechanics of swimming in centrarchid fish. Bluegill is more affected energetically as their locomotion at higher temperature leads them to a greater increase in cost of transport. On the other hand, largemouth bass can limit their extra metabolic rate by altering muscle utilization

for extra energy, recruiting more white muscle and potentially increasing the oxygen debt after stressful swimming event at higher temperature to dispose of accumulated lactic acid. The temperatures tested are well below the lethal temperatures for this species (Coutant and Cox,1976; Sarig 1966), but by changing the energetics throughout a longer part of the year, cumulative effects can easily lead to stunt growth and lower fecundities. In addition, increased temperatures lead to lower oxygen solubility (Claireaux et al., 2006) and oxygen extraction can also be more energetically demanding. These fish populations could easily fall into a negative energy budgets which can lead to migration when possible or to local extinctions. These findings should be further integrated in a However, there is not so much study has done on native fish species from the local rivers and tributaries yet.

Based on my research I recommend that careful consideration is taken for implementation of new dams and that stream restoration efforts are strengthened. While dams are sometimes necessary they need to be considered in the light of the current climate change scenarios as they hinder the ability of the species to find suitable habitat in terms of temperature and can accelerate local extinctions. In addition, habitat restoration efforts that recover faster and turbulent flows along with increased local depths can serve as a buffer for increase water temperatures and oxygen depletions and provide environmental refugia to temperature sensitive species. Another important factor to consider is bank vegetation which can not only provide shade and thus ameliorate temperature effects but also contribute to higher oxygen concentrations. Expanding on how fish are affected by current and future climate change events should also be the focus of additional research. Finally, I believe it is high time to focus on climate change issues and dedicate more efforts to understand how every native fish will be affected and how can they have protected them from extinction and better managed with sustainability as an end.

LITERATURE CITED

Claireaux, G., Couturier, C. and Groison, A.-L. (2006). Effect of temperature on maximum swimming speed and cost of transport in juvenile European sea bass (*Dicentrarchus labrax*). Journal of Experimental Biolology 209, 3420-3428.

Coutant, C.C. and Cox, D.K., 1976. Growth Rates of Subadult Largemouth Bass at 24 to 35. 5 C. In *Thermal Ecology II, Proceedings of a Symposium* held at Augusta Georgia April 2-5, 1975. CONF-750425, 1976. p 118-120, 1 fig, 1 tab, 12 ref.

Herbing, I.H.V., 2002. Effects of temperature on larval fish swimming performance: the importance of physics to physiology. *Journal of Fish Biology*, *61*(4), pp.865-876.

Sarig, S., 1966. A review of diseases and parasites of fishes in warm water ponds in the Near East and Africa. In FAO World Symposium on Warm Water Pond Fish Culture (pp. 278-89).