Measuring the influence of winter conditions on largemouth bass behaviour using both biotelemetry and laboratory studies

by

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Dedication

To all of my grandparents, especially Jack, who once, and only once, let me go fishing with him.

Abstract

Studying the winter ecology of freshwater fishes has been a focus of much past and present research. Because of obvious constraints with studying fish *in situ* during the winter, few studies have made links between laboratory findings and observations made in the natural environment. Recently, new developments in biotelemetry have provided a way of assessing the winter ecology of fish in a natural setting. At present, however, there are few, if any, studies that attempt to make direct links between field and laboratory results.

This research focuses on the reaction of largemouth bass (*Micropterus salmoides*) to winter conditions using both biotelemetry and laboratory components. In the first part of the study, biotelemetry-derived swimming activity is assessed across a range of temperatures (4.0-25°C). As well, swim tunnel-derived U_{crit} swimming speeds and burst swimming ability across similar temperatures were evaluated. In both cases, swimming activity and speeds decreased as temperatures decreased. In biotelemetry-derived measurements, swimming activities increase late in winter, possibly suggesting acclimatization.

In the second part of the study the effect of hypoxia on winter habitat selection was investigated. Biotelemetry-obtained locations of largemouth bass were compared to the distribution of dissolved oxygen throughout the lake at numerous times throughout the winter. In addition, experiments were conducted in a laboratory setting on winter juvenile fish to determine the behavioural and physiological impacts of hypoxia. The results of these experiments revealed a behavioural response in the lab and habitat

avoidance in the field at an environmental oxygen level of approximately 2 mg/L of dissolved oxygen.

The combination of biotelemetry with laboratory data has demonstrated that more informative results about the winter ecology of freshwater fishes can be derived. In the future, usefulness of this combined approach in assessing the impacts of climate change on fish populations will be invaluable.

Co-Authorship

Chapter 2: The effect of temperature on activity and swimming performances of adult largemouth bass

Prepared for *Physiological and Biochemical Zoology*

Authors: Caleb T. Hasler, Cory D. Suski, Kyle C. Hanson, Steven J. Cooke, and Bruce L. Tufts.

Comments: This project was conceived by C. Hasler, Dr. C. Suski, and Dr. B. Tufts. Telemetry field work and surgeries were performed by Dr. C. Suski and Dr. S. Cooke and assisted by K. Hanson and C. Hasler. Field work was performed by C. Hasler and Dr. B. Tufts. Data analysis and processing for telemetry data and swimming data were performed by C. Hasler. Swimming protocol was designed by Dr. S. Cooke assisted by C. Hasler. Data were interpreted by C. Hasler, Dr. C. Suski, and Dr. B. Tufts. C. Hasler was responsible for all statistical analyses, figure creation, and manuscript writing.

Chapter 3: The influence of dissolved oxygen on winter habitat selection by largemouth bass: an integration of biotelemetry and laboratory experiments

Prepared for *Oecologia*

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Comments: This project was conceived by C. Hasler, Dr. C. Suski, and Dr. B. Tufts. Telemetry field work was performed by C. Hasler and K. Hanson and surgeries were performed by Dr. C. Suski and Dr. S. Cooke and assisted by C. Hasler and K. Hanson. Telemetry data processing and analyses were performed by C. Hasler. Laboratory

experiment apparatus conceived by Dr. C. Suski and designed and built by C. Hasler. All laboratory work was performed by C. Hasler. C. Hasler was responsible for all sample analyses, statistical analyses, figure creation, and writing. Data were interpreted by C. Hasler, Dr. C. Suski, K. Hanson, Dr. S. Cooke, and Dr. B. Tufts.

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Chapter 1. General Introduction

The responses of fish to environmental variables have been studied extensively (reviewed by Fry 1971). However, when the entire suite of interacting variables in a fish's habitat is considered, more studies are needed to understand the effect of environmental variables. Currently, understanding the winter biology of fishes is becoming more important because of climate change and anthropogenic forces altering thermal regimes and other environmental variables of many North American lakes. These changing thermal regimes may decrease the length of winter conditions, or in some locals may increase the length and severity of winter (Eaton & Scheller 1996). Thus, it is important for fish scientists to consider winter conditions and their influence on fish behaviour and physiology because, if solutions to changing lake environments are to be made, baseline knowledge of fish winter biology is essential. There are two main environmental changes during the winter in most northern lakes: variable ambient temperature and dissolved oxygen (Greenbank 1945). Cold temperature and hypoxia have both been shown to alter fish behaviour and physiology; however, few studies have addressed how the environment affects physiology and behaviour by linking laboratory and field studies.

Environmental Variation

All environmental resources vary across landscapes. For example, at any particular time of day, the surface temperature of a freshwater lake differs regionally (Wetzel 2001). Similarly, the zooplankton abundance may vary with depth (Wetzel 2001). Within lakes, environmental resources vary at spatial scales ranging from entire ecosystems to the distinct components of ecosystems. Environmental resources also vary temporally. Dissolved oxygen in most water bodies increases during the day due to

photosynthesis (Wetzel 2001) and varies seasonally due to ice cover (Mathias and Barica 1980). Thus, environmental resources vary spatially both spatially and temporally.

Variations in environmental resources (e.g., temperature, dissolved oxygen, salinity) are likely to elicit a response from an animal if resource extends beyond that individual's preferenda. These limits are specific to species (De Staso & Rahel 1994), location (i.e., latitudinal gradients, elevation gradients) (Addo-Bediako et al. 2000), and life stage (i.e., temperature requirements of younger fish are different than temperature requirements of adult fish) (Otto et al. 1976). When resource levels exceed an individual's tolerable limits, a stress response (referred to as "Fight or Flight") can occur (Cannon 1929). In fish, changes that arise as part of the stress responses include increased oxygen uptake and transfer, breakdown and mobilization of energy stores in the liver, allocation of energy away from growth and reproduction, and suppression of immune function (see Wendelaar Bonga 1997). To alleviate the stress response, fish can either acclimate/acclimatize to the stressful resource level(s), thereby altering their tolerance, or they can move to areas that satisfy their tolerance limits.

Movement

Movement is a short-term response and is defined as an animal changing location by either the animal's own means (i.e., the beat of a fish tail propelling the fish forward), or by the means of its environment (i.e., the wave action of a lake causing phytoplankton to change location). For fish, movement is a requirement for survival. If a fish inhabits a connected stream/river network, migration from a seasonally unfavourable habitat to a seasonally favourable habitat can occur (Swingland & Greenwood 1983). Spawning behaviour of many salmonid species (e.g., Coho salmon *Oncorhynchus kisutch*) also

requires migration, as spawning in freshwater natal streams is required for successful reproduction (Healey 1983). However, for most freshwater species, especially those that inhabit less connected river systems and lakes, movements within the habitat are the only option. Many fish species have been shown to move to areas based on environmental variables such as dissolved oxygen and temperature (e.g., Whitmore et al. 1960; Magnuson et al. 1985; Suthers & Gee 1986; Coutant 1987; Kramer 1987; Gent et al.1995; Burleson et al. 2001; Albanese et al. 2004; Schreer & Cooke 2004; Balleby et al. 2006; Bauer & Schlott 2006; Gillette et al. 2006; Lefrançois & Domenici 2006). Other reasons for fish to move within their habitat are to avoid predators, to seek out prey, and to reproduce (McIvor & Odum 1988; Eklöv 1997). In general, fish will be motivated to move for a variety of ecological and behavioural reasons. Movement studies are often focused on the reproductive period (i.e., migrating salmonids), and on the effect of environmental variables on movement. Few studies have investigated "everyday" movements of fish and how movements change seasonally.

Fish exploit three types of swimming behaviour when they are motivated to move (Beamish 1978). The first type is sustained swimming, and it utilizes red muscle and aerobic respiration. This type of swimming is generally considered to last indefinitely and does not lead to fatigue. Fish will use sustained swimming during migration and when simply moving throughout their environment. The second type is prolonged swimming, and it uses both red and white muscle, as well as both aerobic and anaerobic respiration. This type of swimming begins aerobically and uses red muscle; once energy stores are depleted and oxygen consumption decreases, white muscle and anaerobic respiration take over as the primary muscle and respiration. Prolonged swimming can last for multiple hours and will result in the fish being physiologically exhausted. Fish will

rarely swim in this manner, but measurements of prolonged swimming allow for the quantification of muscle capacity and energy stores. The last type of swimming is burst swimming and it uses white muscle. Because burst swimming utilizes white muscle, anaerobic respiration supplies the muscle with energy. Thus, burst swimming only lasts for brief periods of time, and, typically, fish exploit this type of swimming to pursue prey, escape predators, or to move quickly through fast-flowing areas. Data for prolonged swimming speed and burst swimming speed exist for many fishes; however, sustained swimming speeds are not as well understood.

The act of movement, so vital for survival, is greatly influenced by the ability of an organism to respond to a changing environment. A fish's ability to respond to changes in their external environment relies on how much or how little they can alter their cellular and muscular activity (Hochachka & Somero 2002). If a fish can perform the same way when displaced from a warm temperature environment to a cold temperature environment, it is known as a eurythermal species; fish that can not perform as well are known as a stenothermal species (Hochachka & Somero 2002). All fish species are considered to fall along this spectrum. Most often, adjustments are required because of a change in temperature or climate. For fish, this typically occurs during seasonal changes, when temperatures may increase or decrease to levels near their thermal tolerance limits. Many species have demonstrated cellular changes in terms of increased density of mitochondria and increased/decreased enzyme activity, which leads to muscular changes that allow fish to respond to the changed environment (Rome et al. 1985; Johnston & Dunn 1987; Guderley & Blier 1988; Egginton & Sidell 1989; Guderley 1990; Battersby & Moyes 1998). Few studies have examined the effect of acclimatization on wild fishes or have shown that acclimatization has any long-term effects.

Largemouth bass are a freshwater species that has a natural range extending from the southern United States to southern Canada. Because of their widespread range, populations of largemouth bass can be exposed to year-long 'summer-like' conditions in the southern portion of their range, conditions that are annually quite variable (e.g., lakes in Eastern Ontario will range from as high as 30+°C in the summer to as low as 4°C in the winter). Largemouth bass are thought to be the least able to acclimate to cold temperatures of all tested centrarchids (Tschantz et al. 2002) and are considered to be dormant during the winter because of this in ability to react to cold temperatures. Also, largemouth bass swimming behaviour has been sparsely studied, so it is unclear as to what ability they have to swim at cold temperatures. Kolok (1992) and Farlinger and Beamish (1977) measured the swimming performance of juvenile largemouth bass in swim tunnels and found summer acclimatized fish swam less than 40 cm/s. However, this is the extent of current knowledge about the species' swimming performance. Thus, there is a need to measure the ability of adult largemouth bass have to swim at all temperatures. Hypoxia may also have an effect on swimming ability (Hughes 1973) – especially during the winter when northern lakes may experience prolonged periods of low amounts of dissolved oxygen.

Hypoxia

One of the main factors that influences fish behaviour is environmental hypoxia, an environment with less than normal amounts of ambient oxygen (normoxia). Numerous studies that have measured the effect of environmental hypoxia have shown that it influences the behaviour, physiology, and biochemistry of many fishes (e.g.,

Furimsky et al. 2003). However, few studies have examined the response to environmental hypoxia in free-ranging fish.

For fish, hypoxia can occur at all parts of the respiratory chain, from the external environment to the gill to the cell. The different types of hypoxia include: environmental hypoxia (reduced PO_2 in ambient water); hypoxia resulting from a loss of hemoglobin in the blood; overuse of oxygen by the cells; poor circulation, resulting in less oxygenated blood reaching the cells; thickening of the gill surface (e.g., from pollutants) interfering with diffusion exchange; and others (Hughes 1973). For this thesis, environmental hypoxia was the only type of hypoxia to have been measured, and all responses to hypoxia have been attributed to the decrease in ambient amounts of oxygen.

In order for a fish to respond (i.e., behaviourally, physiologically, or biochemically) to hypoxia, it first must sense it. The principal site for oxygen sensing in fish is the gill, where branchial oxygen chemoreceptors respond to increases and decreases in ambient oxygen (Holeton & Randall 1967). There are two categories of oxygen receptors in the fish gill: external (detect oxygen in the ambient water) and internal (detect dissolved oxygen in the internal environment). Once the external receptors recognize hypoxia in the environment, fish will alter their behaviour. If the behavioural responses fail to alleviate the effect of hypoxia, the fish's internal environment will begin to become hypoxic, and physiological responses will takeover (Perry & Gilmour 2002). In cases when both behavioural and physiological responses fail to alleviate the negative effects of hypoxia, biochemical responses will be initiated. Thus, a fish sensing hypoxia will induce numerous behavioural, physiological, and biochemical responses whose purpose is to either change the external environment or to prevent cellular damage to its internal environment.

Fish exhibit several categories of behavioural response to hypoxia, including changes in activity, increased use of air breathing, increased use of aquatic surface respiration, and vertical or horizontal habitat changes (Kramer 1987). The foremost response is a change in activity because of the coupling between oxygen and energy budgets. Most often fish will increase ventilation rate and amplitude as oxygen decreases (Holeton 1980). Feeding is also altered, as hypoxic conditions limit the energy available to search, digest, and assimilate food (reviewed in Doudoroff & Shumway 1970). Other activities that can be affected by hypoxic conditions are growth, reproduction, predator avoidance, and spontaneous locomotor activity (Cichocki 1977; Casselman 1978; Whoriskey et al. 1985). Another important behavioural response to hypoxia is air breathing. This process involves fish moving to the surface and breathing in oxygen that is available in the atmosphere. Because air breathing requires a specialized swim bladder, not all fish are capable of air breathing. For fish unable to air breathe, an alternative is aquatic surface respiration, a process that involves fish moving to the surface or near ice to exploit oxygen in bubbles. In some cases, aquatic surface respiration can lead to increased levels of activity (Weber & Kramer 1983). For fish inhabiting a spatially variable habitat, selection of a higher-oxygen habitat can alleviate hypoxic stress. This response involves the fish recognizing hypoxia and actively moving to an area that is higher in dissolved oxygen. However, habitat selection is a costly option because food availability could be less and the risk of predation could be higher in the new habitat. These four behavioural responses (change in activity, air breathing, aquatic surface respiration, and habitat selection) are undertaken to minimize the negative effects of environmental hypoxia. However, they come at a cost, as energy is expended in most cases.

Physiological (or respiratory/metabolic) responses to environmental hypoxia seek to minimize the energetic cost of hypoxia. Fishes will demonstrate a range of responses to hypoxia with two extremes: oxygen conformation or oxygen regulation. Oxygen conformers reduce metabolic demand so that oxygen consumption is coupled with ambient oxygen levels (Loudon 1988; Boutilier 2001), whereas oxygen regulators maintain a constant level of oxygen consumption independent of ambient oxygen until the amount of oxygen needed for aerobic processes becomes limiting (Herreid 1980; Hochachka 1988). Oxygen regulators typically increase the ventilation of the gills and increase gill stroke volume to enhance the amount of oxygen presented to the gill surface (reviewed by Hughes 1973). Because of the rise in stroke volume and decrease in heart rate, blood pressure during hypoxia increases; this will result in more gill lamellar being recruited and make the lamellar more rigid (Randall 1982). Hypoxia in all fish alters the blood's oxygen-carrying capacity. During chronic hypoxia, Wood and Johansen (1972) found the properties of eel hemoglobin change so that more oxygen molecules can be carried, and there is a higher affinity for oxygen. This change is associated with a reduction in the amount of ATP in red blood cells. Physiological responses to hypoxia in fish will either attempt to lower the need for oxygen (conformers) or will attempt to move greater amounts of oxygen through the circulatory system (regulators).

If hypoxic conditions persist, and behavioural and physiological responses have failed to meet the energetic demand for oxygen, biochemical responses to hypoxia take over. The most likely biochemical response is a switch from aerobic respiration to anaerobic respiration. In the presence of oxygen, oxidative phosphorylation will be the primary pathway for producing ATP. However, in hypoxic conditions, when oxygen is limiting, there are few oxygen molecules to act as final electron acceptors in the electron

transport chain. In this instance, ATP synthase will reverse and actively pump protons from the mitochondrial matrix to try to maintain the mitochondrial membrane's potential. Failing a restoration to normoxic conditions, metabolism will switch to anaerobic respiration. This process will metabolize the end product of glycolysis (i.e., pyruvate) and produce a small amount of ATP relative to the aerobic pathway. However, there is a cost to respiring in this manner, as lactate is produced (an accumulation of lactate will limit locomotion; Beamish 1978) and cellular pH rises; both of these end products of anaerobic respiration need to be expelled upon arrival into normal conditions. In summary, if oxygen requirements are not restored by means of behavioural and physiological responses, biochemical responses will attempt to meet the oxygen demand by altering metabolism so that energy can be produced in the absence of oxygen.

Though behavioural, physiological, and biochemical responses to hypoxia counter the negative impacts of hypoxic conditions, many environments are chronically hypoxic, and if fish (and other taxa) are to survive in these environments, they must adapt by becoming hypoxia tolerant. Because largemouth bass have not evolved to be hypoxia tolerant, a discussion of different adaptations to hypoxia is not needed. (However, it should be noted that numerous fish species (e.g., central mud minnow [*Umbra libra*]) have so evolved.) Typical responses by largemouth bass to hypoxia include increases in ventilation rate and decreases in cardiac output (Furimsky et al. 2003). It has also been suggested that largemouth bass gill receptors are more responsive to changes in oxygen levels in the internal environment and not the external environment (Furimsky et al. 2003). Behavioural responses to hypoxia have been shown: Whitmore et al. (1960) demonstrated that largemouth bass in a laboratory gradient avoided water with less than 1.5 mg/L of dissolved oxygen, and largemouth bass have been found to over-winter in

sites that had greater than 2 mg/L of dissolved oxygen (Raibley et al. 1997). However, still unknown is the effect temperature has on largemouth bass response to hypoxia and in largemouth bass's general over-winter habitat preference.

Monitoring Fish

Monitoring and assessing fish movement has been difficult in the past because of the innate difficulties associated with observing life in an aqueous environment. Most studies using laboratory techniques involve removing fish from their natural habitat and housing them in a laboratory tank. This allows for easy manipulation of environmental variables and for a high degree of control. Also, housing of fish in a laboratory setting enables easy observation and measurement of fish behaviour and provides the capability of using mechanisms to assess fish physiology (i.e., swim tunnels). Overall, bringing fish into a laboratory to assess their behaviour and physiology is beneficial for understanding many aspects of their biology. However, it provides little insight into how fish react to their natural environment because, in a laboratory setting, they are exposed to multiple stressors and biases (e.g., crowding, chlorinated water, handling, the presence of observers). Because of the need to remove stress and bias in studies, so that valid conclusions can be made, researchers are using biotelemetry to observe fish behaviour in the wild.

Biotelemetry is the use of radio or acoustic transmitters attached to individuals to find their spatial and temporal location. The first studies using telemetry to study fish in freshwater environments were completed in the 1950s (Trefethen 1956), and a plethora of studies have followed (see Cooke et al 2004 for a review on the topic). Benefits to using biotelemetry include: nearly unbiased data collection (if proper transmitters and tagging

procedures are followed); continuous tracking of multiple fish at small time intervals; and the ability to observe fish *in situ*. However, there are some weaknesses that need to be considered when using biotelemetry: uncertainties when positioning fish in space; statistical issues associated with pseudoreplication; biases linked to the correct temporal interval to use when tracking the animal; and costs associated with starting long-term extensive studies (Rodgers & White in press). Through recent developments in biotelemetry technology and more sophisticated laboratory study designs, scientists are able to quantify many aspects of fish behaviour and physiology. However, one component of fish behaviour and physiology that remains to be sparsely understood is the effect winter has on fish.

Winter

In lakes at northern latitudes, winter conditions can cause resource levels to be near the tolerance limits of most fish. Winter is characterized as a period of cold water temperatures, shortened days, and often ice cover on more northern lakes (Wetzel 2001; Suski & Ridgway In review). Water temperatures in Canadian lakes typically range from sub-zero to 6°C. Fish, being poikliotherms, respond by decreasing activity and movement (Crawshaw 1984; Lemons & Crawshaw 1985; Tschantz et al. 2002; Hanson et al. 2007) and selecting the warmest environments available (Gent et al. 1995; Bauer & Schlott 2004). The ice cover in many northern lakes results in low light intensity, low dissolved oxygen, and confinement (Greenbank 1945). Low amounts of dissolved oxygen are the most detrimental condition for a fish population because extremely low levels of dissolved oxygen during the winter period cause them to suffocate (Greenbank 1945; Cooper & Washburn 1949). In winter, dissolved oxygen decreases for several

reasons: reduced light and temperature, decomposition of macrophytes, oxygen consumption by aquatic organisms (e.g., fish), and the inability of atmospheric air to circulate into water because of ice cover (Greenbank 1945; Cooper & Washburn 1949). Most fish show similar responses to winter hypoxia, but the onset of negative effects occurs at different levels of dissolved oxygen for each species.

Largemouth bass respond to winter in a manner typical of most centrarchids (the sunfish family of fish). They select habitat based on water temperature, water velocity, and dissolved oxygen (Whitmore et al. 1960; Beamish 1970; Rice et al. 1983; Kolok 1992; Gent et al. 1995; Raibley et al. 1997; Cooke et al. 2003). Numerous studies have shown that the thermal tolerance of largemouth bass ranges from 2-32°C (Venables et al. 1978; Guest 1985). Furthermore, telemetry studies have demonstrated that largemouth typically stay in water above 4°C in the wild (Raibley et al. 1997). According to a laboratory study by Kolok (1992), largemouth bass can swim 20 cm/s in 5°C water. Notably, however, Raibley et al. (1997) never found wild fish in water with a velocity over 2 cm/s during the winter. Laboratory studies have also found that largemouth bass will avoid water below 1.5 mg/L of dissolved oxygen (Whitmore et al. 1960). Largemouth bass have been found to demonstrate few changes in the enzyme activities that would maintain their normal range of activity during the winter (Tschantz et al. 2002). However, several recent telemetry studies have challenged pre-existing dogmas of winter fish activity and behaviour (Hanson et al. 2007; Hasler et al. 2007). It can be argued that accurate descriptions of the winter behaviour and physiology of most fishes, including largemouth bass, are still largely unknown– mainly because of the difficulty of studying fish *in situ* during the winter period (Suski & Ridgway in review).

To alleviate the discomforts and costs of studying fish winter ecology, many studies have been completed in a laboratory setting. In particular, laboratory settings allow for controlled microcosms, which can be manipulated and observed with relative ease, and offer a scientifically accepted alternative to field studies. For example, Breder & Nigrelli (1935) describe the winter aggregations of the sunfish (*Lepomis auritus*) in the New York Aquarium; Beamish (1970) describes the effects of water temperature on oxygen consumption in laboratory housed largemouth bass; Farlinger & Beamish (1977) measure the critical swimming speeds of largemouth bass using a swimming tunnel; Petrosky & Magnuson (1983) explain the behaviour and mortality associated with winter hypoxia on bluegill (*L. macrochirus*), northern pike (*Esox lucius*), and yellow perch (*Perca flavescens*) by simulating winter in a microcosm; and Furimsky et al. (2003) document physiological changes to hypoxia-exposed smallmouth bass (*M. dolomieu*) and largemouth bass housed in a laboratory. All of these studies, although completed in controlled laboratory settings, offered valuable information to the knowledge of Centrarchid winter ecology. However, no direct links to natural settings were documented, and until the development of biotelemetry, these links were nearly impossible to make (Lucas & Baras 2000; Cooke et al. 2004).

Recent developments in biotelemetry have allowed for *in situ* studies on winter behaviour of fish (Lucas & Baras 2000; Hanson et al. 2007; Hasler et al. 2007). Fully submerged hydrophones systems, underwater cameras, and sonar, have facilitated the study of fish *in situ* during the winter period (Lucas & Baras 2000). Perhaps the most intriguing development is that of underwater hydrophone systems. Coupled with new developments in tag technology, such as acoustic tags equipped with code division multiple access (CDMA) technology, these systems are now capable of tracking multiple

individual fish at sub-meter accuracy and every few seconds (Niezgoda et al. 2002; Cooke et al. 2005). These are spatial and temporal scales never before studied in fish (Cooke et al. 2005). Also, these new biotelemetry innovations are enabling scientists to confirm laboratory behavioural and physiological experiments using *in situ* data. In this thesis, it will be assumed that movement observed using the telemetry system will indicate a measure of activity (not swim speed).

Hypotheses

It is the purpose of this thesis is to couple studies using the most recent innovations in biotelemetry technology to laboratory findings. I hypothesize that by comparing observations made in the field and laboratory (instead of choosing to use only one type of study) a better understanding of overwintering fish behaviour and physiology will result. Chapter 2 will present a study investigating the *in situ* activity patterns of nine telemetered largemouth bass across a range of ambient temperatures. Also, swimming speeds of largemouth bass captured at a similar range of ambient temperatures will be calculated using a swim tunnel. It is hypothesized that as water temperatures decrease, activity and swimming performance in both studies will decrease because of the dependence of activity and swimming performance on enzymatic Q_{10} values. The winter behaviour of these fish should be better understood if all types of winter activity and swimming are analyzed. This study is unique as it is the first time activity will be assessed in the wild and is the first time the swimming performance of adult largemouth bass will be assessed using swim tunnels. Chapter 3 will present a study analyzing the effects of hypoxia on the winter habitat selection of nine adult largemouth bass. Additionally, the behavioural, physiological, and biochemical responses to winter

hypoxia by juvenile largemouth bass will be measured in a laboratory setting. Tolerance thresholds to hypoxia during the winter period will be measured in both the telemetry and the laboratory studies. It is hypothesized that fish will demonstrate an avoidance of water with low amounts of dissolved oxygen (likely near the limits found by Whitmore (1960) and Raibley (1997). Also, it is expected that the amount of dissolved oxygen that triggers avoidance will be mirrored in all responses (behavioural, physiological, and biochemical). Chapter 4 will explore future research and the usefulness of comparing telemetry studies to laboratory results. Overall, this thesis will attempt to broaden the knowledge of the winter ecology of centrarchids.

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Chapter 2. The effect of temperature on activity and swimming performance of adult largemouth bass

Abstract

Temperature is a crucial environmental factor for all ectotherms, including fish. In this study, biotelemetry and swim tunnel assessments of swimming activity and performances of individual largemouth bass (biotelemetry study, n = 9; swim tunnel, n = 15 at 25.0°C, 7 at 14.0°C, 10 at 7.5°C) were measured across all seasons. In the biotelemetry portion of the study, largemouth bass were tracked throughout the year using a submerged, fixed station telemetry array. Mean daily activity, mean directional swimming activity, and maximum swimming speed decreased with lower water temperature and were lowest during early January (mean daily activity = 0.62 cm/s; mean directional swimming activity = 1.7 cm/s; maximum swimming speed = 6.5 cm/s). Both mean daily activity and maximum swimming speed increased during the late March (to 0.83 cm/s and 9.3 cm/s respectively). In the swim tunnel portion of the study, fish acclimated to 25.0°C, 14.0°C, and 7.5°C decreased swimming performance with water temperature (25.0°C (61.1 cm/s); 14.0°C (46.8 cm/s); 7.5°C (32.9 cm/s). The ability fish had to swim at 3.0 body length/s also decreased significantly when temperature dropped (19.7 to 2.7 s). Results of these assessments indicate that all types of swimming are reduced by cooler temperatures, and winter acclimatization may be a possible explanation for increases in activity late in the winter.

Introduction

Fish are poikilothermic and, as such, their body temperature varies with the surrounding environment. Some fish species, for example, centrarchids, have the ability to withstand a broad range of temperatures, while other fish, such as Antarctic fishes (suborder Notothenioidei) can only survive a narrow range of temperatures. Temperature is a controlling factor in all physiological processes (Fry 1971) and is an important ecological resource that has profound impact on niche selection because an adequate range of body temperature is necessary for proper metabolism, growth, and reproduction (Magnuson et al. 1979; Tracy and Christian 1986). During the winter, fish at northern latitudes experience temperatures that are often near lower critical temperature limits. One way to gain insight into how cold temperatures affect fish is to study swimming activity and performance (Beamish 1978).

Swimming activity and performance can be measured in a variety of ways ranging from cellular enzymatic activities (relating to the ability of a fish to move) to measuring the distance and time it takes a telemetered fish to move between two points (Brett 1964; Guderley 1990; Lucas and Baras 2000; Cooke et al. 2004a; Cooke et al. 2004b; Bauer and Schlott 2004; Hanson et al. 2007). One additional method many fish scientists have employed to quantify swimming performance is the use of swim tunnels (Brett 1964; Farrell et al. 1998). These tunnels involve presenting a changing flow of water to an individual fish and forcing it to swim against the current. Once the fish fails to swim, its prolonged swimming speed (U_{crit}) can be equated to the speed of the flow of water (Brett 1964). Many laboratory studies have found that as temperatures change, the swimming performance of fishes also changes. For example, juvenile European sea bass

(*Dicentrarchus labrax*) increased U_{crit} swimming speed two-fold as the temperature rose from 7°C - 30°C (Claireaux et al. 2006), and changes to smallmouth buffalo (*Ictiobus bubalus*) swimming performance demonstrated significant seasonal effects (Adams & Parsons 1998). However, little is known about the swimming activity of fish in their natural setting.

Seasonal acclimatization and thermal acclimation are important processes to consider when assessing swimming activity and performance, as swimming speeds may change depending on the length of time fish are exposed to an altered environment (Rome et al. 1985; Heap and Goldspink 1986; Guderley and Blier 1988; Adams and Parsons 1998). Acclimation and acclimatization are phenotypic changes in response to environmental change (Hochachka and Somero 2002). Acclimation is typically a laboratory response to one environmental variable such as temperature, whereas acclimatization is normally considered to be an *in situ* response to changes in multiple environmental variables (Hochachka and Somero 2002). Essentially, acclimation can only be assessed in a laboratory setting as it requires a stable controlled environment. However, acclimatization can be assessed by observing fish in the wild through biotelemetry or by capturing fish throughout the season and quickly testing them in the laboratory (Kolok 1992; Adams and Parsons 1998). Although there is a plethora of research investigating the effect of cold temperature and acclimation to cold temperature on fish (e.g. Rome et al. 1985; Heap and Goldspink 1986; Guderley and Blier 1988), only a few studies have measured the effect of seasonal acclimatization on fish mobility and cellular processes (Johnson and Johnston 1991; Kolok 1992; Guderley et al. 1997; Adam and Parsons 1998). As well, to date, there have been no biotelemetry studies that have attempted to evaluate the effect of acclimatization on the swimming behaviour of fishes.

Biotelemetry is the use of manual- or automatic-telemetry systems to track transmitters attached to individuals (Lucas and Baras 2000). In the past, this method has been used to study swimming performance and activity levels of fish in their natural setting (Cooke et al. 2004a; 2004b). The majority of studies measuring swimming performance cover large spatial and temporal scales, often utilizing hydrophone or antenna arrays distributed along the length of a river (Hinch and Bratty 2000). For many lake telemetry studies, the distance between subsequent points and the time interval between the points are used to assess activity (Rogers and White in press). Often, the time intervals can be multiple days and the points can be hundreds of metres apart (meaning only coarse assessments of activity can be made) (Bauer and Schlott 2006). Likewise, telemetry has been unable to accurately assess seasonal acclimatization because fine-scale measurements of swimming activity are difficult. However, new innovations in telemetry, which include near-real time positioning of multiple individual fish using submerged fixed acoustic hydrophone systems and code division multiple access- (CDMA-) enabled transmitters, have provided the ability to assess fine-scale activity and possibly acclimatization (Niezgoda et al. 2002; Cooke et al. 2005).

Largemouth bass (*Micropterus salmoides*) have been the focal species of a CDMA-based acoustic-telemetry system situated in Eastern Ontario. Because of the geographical location of the acoustic-telemetry array, these fish are exposed to temperatures ranging from 4°C in the winter months (November - April) to temperatures greater than 25°C in the summer months (July-August). The purpose of this study is to measure the effect of low temperature on swimming activity of these telemetered largemouth bass at fine temporal (minute and daily) scales and to assess whether seasonal acclimatization is affecting movement during the winter. As well, in order to better

understand largemouth bass winter activity patterns observed in the telemetry portion of this study, we assess swimming performance of fish tested in a swim tunnel.

Materials and Methods

Biotelemetry

Warner Lake (8.3 ha), located entirely within the property of the Queen's University Biological Station, is a freshwater lake. The lake is mainly littoral and is typical of the lakes in Eastern Ontario. The main body of the lake comprises two basins: a deep (6 m) and a shallow (3 m) basin. Warner Lake is equipped with a fixed-station, submerged acoustic-telemetry array consisting of a CDMA-based telemetry system (Lotek MAP 600, Lotek Wireless Inc., Newmarket, Canada) (Cooke et al. 2005). For this study, temperature thermochrons (DS1921Z, iButton, Maxim Integrated Products and Dallas Semiconductor, Sunnyvale CA, United States of America) were deployed at numerous depths (0 - 5 m) and at multiple locations throughout the lake from November 2005 - November 2006 to record ambient temperature at four-hour intervals. A fixed-station acoustic telemetry array consisting of two multi-port MAP 600 (Lotek Wireless Inc., Newmarket ON, Canada) receivers (one for each basin), and 13 hydrophones (8 in the deep basin; 5 in the shallow basin) moored approximately 2 m below the water surface were installed in 2003. Each hydrophone has a cable extending to a central location on shore where it connects to its receiver. The receivers are connected to a desktop computer and are controlled by MAP 600 PC HOST (V3.09, Lotek Wireless Inc., Newmarket ON, Canada). Data are stored on flash cards and are later transferred to personal laptops for interpretation and positioning in BioMAP (v. 2.1.12.1, Lotek

Wireless Inc., Newmarket ON, Canada). Filters within BioMAP process the data, removing erroneous positions using a wavelet analysis (Hess-Nielsen and Wickerhauser 1996; Akay and Mello 1997) and provide daily estimates of both mean and maximum swimming speeds for each fish (Niezgoda et al. 2002). For additional information on Warner Lake ecology, see Suski (2000), and for additional information on the telemetry system arrangement, performance, and accuracy, see Cooke et al. (2005).

In October of 2005, nine largemouth bass (Table 2.1) were angled and implanted with CDMA temperature-pressure sensing acoustic transmitters (Lotek CTPM11-18, 11mm x 60mm, repetition rate 59.5 seconds, life expectancy of 3 years, Newmarket ON, Canada), using the surgical approaches outlined in Cooke et al. (2003). The total lengths of the telemetered fish were uniformly distributed (Shapiro-Wilk W Test; $W = 0.89$, $P = 0.20$). Each fish was released following the surgery, and tracking of the animal by the telemetry system began immediately (via the system described above). Tracking of all the fish continued into the following summer, with the exception of one fish (transmitter number 30700) that was no longer being tracked after April 2006 (for the realized sampling rates, please see Table 2.1). Using the filter, outputs from BioMAP activity per day for each fish were assessed during five different time periods: 10 days @ water temperature 11.5°C (October 16-25, 2006); 10 days @ 4.5°C (January 01-10, 2006); 10 days @ 5°C (March 10-19, 2006); 10 days @ 7.5°C (March 27-April 05); and 10 days @ 25°C (July 30-August 8, 2006) (Figure 2.1). This method utilizes all of the filtered data points and calculates the activity of each fish. Because this method includes all the positions of the fish, periods when fish are 'resting' and showing no signs of directional tracks are included; thus the estimates of mean daily activity underestimate mean directional swimming activity. To assess fish moving in a directional manner, the

distance between each successive position was measured, and the distance between the two positions was calculated using the Pythagorean Theorem. The time difference between each pair of positions was calculated, and the instantaneous swimming activity of the fish determined. To eliminate periods when the fish are 'resting', only instantaneous swimming activities that were above the 75 % quartile (specifically measured for each fish) were used to calculate the mean swimming activity. As well, the maximum swimming speed was measured by recording the daily maximum swimming activity value of each fish.

Swim Tunnel Experiments

A 54 litre Blazka-type swim tunnel (Blazka et al. 1960; Beamish 1978) was used to measure the U_{crit} and burst swimming ability of multiple largemouth bass at three different seasonal temperatures throughout the year:15 fish @ 25.0°C (July-August); 7 fish @ 14.0°C (October); and 10 fish @ 8.5°C (early April) (Brett 1964) (It was not possible to obtain sufficient numbers of fish during the winter, so experiments were conducted immediately after ice out). The tunnel was 120 cm in length and 24 cm in diameter, with a dark screen on the upstream section motivating the fish to occupy this section. Nearby Lake Opinicon water was used to fill the swim tunnel, and the water in the tunnel was held at ambient water temperature by allowing a small flow of water into and out of the swim tunnel throughout the swimming trial. For the U_{crit} swimming speed protocol (Brett 1964; Beamish 1970; Kolok 1992), each fish was accustomed to the swim tunnel at 0.5 body length per second (BL/s) for 1 hour, after which the speed was increased in a step-wise fashion by 0.5 BL/s every half hour until exhaustion. The fish was considered exhausted once it rested on the grate with no reaction to disturbance from

the observer, or when it was overwhelmed by the current and forced against the grate. At that point, the motor speed was decreased to stimulate the fish to begin swimming again and quickly brought back to the exhaustion speed. The time measurement ended when the fish rested against or was forced against the grate for a second time. Fish were allowed 20 minutes to recover before the step-wise test was repeated (Farrell et al. 1998). The results of the second swimming test were used for analysis.

A measure of burst swimming ability (Gregory and Wood 1998) was calculated using the same fish from the U_{crit} swimming test. Burst swimming ability was measured because largemouth bass rarely encounter prolonged flow rates that would require them to swim at U_{crit} swimming speeds, but are more likely to use burst speeds in the wild (Raibley et al. 1997; Hanson et al. 2007). For the burst swimming ability protocol, the fish was accustomed to the swim tunnel for five minutes at 0.5 BL/s. In a step-wise fashion, the speed was increased by 0.5 BL/s every 30 seconds until 3.0 BL/s was reached. Once a speed of 3.0 BL/s was reached, a stop watch was started; it was stopped once the fish rested its caudal fin against the grate.

Data Analysis

Biotelemetry-derived activity were compared across temperatures using repeated-measures one-way analysis of variance and a Tukey HSD post-hoc test. Laboratory measurements of U_{crit} swimming performance were compared across temperatures using one-way analysis of variance and a Tukey HSD post-hoc test. Burst swimming versus temperature was compared using an unpaired student t-test. Total lengths of individual fish in each treatment were linearly regressed against biotelemetry-derived directional swimming and laboratory-derived U_{crit} swimming speeds. All tests were performed using

JMP 6.0.2 software (SAS Institute Inc., Cary, NC), with the level of significance (α) for all tests being 0.05.

Results

Biotelemetry

The mean daily activity of telemetered largemouth bass decreased significantly at cooler water temperatures (repeated-measures ANOVA; $F_{4,35.2} = 18.5$, $P < 0.0.001$) (Figure 2.2a). The mean daily activity of fish during the summer (25°C) was 2.5 cm/s (± 0.3, n = 7), and there was a 50 % reduction in mean daily activity during the fall and early spring when water temperature was 11.5°C and 7.5°C respectively. A further 50 % reduction, relative to the fall, in mean daily activity was found during the early winter when water temperature was 4.5°C. After three months of water temperature ranging between 4.0°C and 5.5°C, mean activity increased 33 % relative to the early winter sample period and was no longer statistically different from the fall and early spring sample period (least means differences Tukey's HSD test; $P > 0.05$) (Figure 2.2a).

When periods of resting were eliminated, largemouth bass mean directional swimming activity decreased significantly as water temperature decreased (repeated-measures ANOVA; $F_{4,34.14} = 20.9$, $P < 0.0001$) (Figure 2.2b). The mean directional swimming activity of fish during the summer (25°C) was 6.6 cm/s (± 0.3, n = 7), and there was ~ 60% reduction in mean directional swimming activity when water temperature was 11.5°C (fall), relative to the summer. There was a further 40% reduction, relative to the fall, in mean directional swimming activity when water temperature was 4.5°C (early winter). No change in mean directional swimming activity

was found in the late winter, but mean directional swimming activity in the early spring was 9 % greater than during the late winter. No statistical difference was found between the late winter and the early spring period (least means differences Tukey's HSD test; $P > 0.05$) (Figure 2.2b). Mean directional swimming activity of telemetered fish was not significantly influenced by total length of fish (Table 2.2).

Maximum swimming speed of telemetered largemouth bass decreased significantly at cooler water temperatures (repeated-measures ANOVA; $F_{4,26.2} = 11.4$, $P < 0.0001$) (Figure 2.3). The maximum swimming speed at 25°C was 18.0 cm/s (± 2.0, n = 7), and there was approximately a 67 % reduction in maximum swimming speed during the 4.5°C sample period (6.5 ± 1.3, n = 9). Maximum swimming speeds during the 25°C and the 11.5°C sample periods were not significantly different however, maximum swimming speed were significantly different during the 4.5°C and the late winter sample periods (least means differences Tukey's HSD test; $P > 0.05$). Likewise, there was no significant difference between the 7.5°C, 4.5°C, and the late winter sample periods (least means differences Tukey's HSD test; $P > 0.05$) (Figure 2.3).

Swim Tunnel Experiments

Seasonal changes in largemouth bass swimming speed (U_{crit}) were measured, and it was found that as ambient water temperatures decreased, so did swimming speed (one-way ANOVA; $F_{2,29} = 26.3$, $P < 0.001$) (Figure 2.4). Fish that were tested in the fall were 25 % slower than fish tested in the summer, and fish tested in the early spring were 54 % slower than fish tested in the summer (least means differences Tukey's HSD test; $P < 0.05$). No significant relationship between total length of fish and swimming ability was found (Table 2.2). The ability of fish to endure burst swimming (3.0 body length/s) was

also assessed, and fish had decreased ability to burst swim as temperature decreased (unpaired student t-test; t ratio$_{12.93}$= -3.1; $P < 0.008$). Fish could burst swim for 19.7 s at 25.0°C (± 4.7, n=9) but only 2.7 s (± 2.7, n=10) at 8.5°C (Figure 2.5).

Discussion

Results from both the biotelemetry and swim tunnel studies show that both largemouth bass activity and swimming performance decrease as water temperature decreases. In the biotelemetry study, the mean activity of nine largemouth bass decreased 50 % during the fall (water temperature = 11.5°C) as compared to the summer (25.0°C); further 50 % reduction was measured during the winter (4.0°C). Likewise, swimming speeds (U_{crit}) of largemouth bass tested in the swim tunnel decreased 25 % and 54 % in the fall (14.0°C) and early spring (7.5°C), respectively, in comparison to summer (25.0°C) swimming speed. Locomotor capacity in ectotherms is directly linked with ambient temperature, as body temperature influences many physiological processes because of the dependence of enzyme activity on temperature coefficients (Q_{10} values) (Hochachka and Somero 1984; Bennett 1990). The optimal temperature range for largemouth bass performance is 27.0-32.0°C (Venables et al. 1978). Swimming speeds measured outside of this preferenda should fall on a 'bell curve', with the maximum being within the preferred range of temperatures (Brett 1971; MacNutt et al. 2004). In this study, activity and swimming speeds in both the biotelemetry and swim tunnel portions decreased in this manner. There are undoubtedly many reasons for the decrease in performance outside of the preferred range. These may include reductions in cardiac performance (Brett 1971), reductions in oxygen-carrying capacity of the blood (Fry and

Hart 1948; Randall and Brauner 1991; Farrell et al. 1996), and hindrance of muscle contractile kinetics (Bennett 1984). The activities and speeds measured in both the biotelemetry study and the swim tunnel study demonstrated progressively lowered movement and performance as the ambient temperature fell below 25°C. Thus, it is likely that a reduction in a number of physiological processes was occurring as temperature decreased.

Previous studies assessing the effect of temperature on the activity and swimming performance of adult largemouth bass have been inconclusive regarding winter behaviour (e.g., Lemons & Crawshaw 1985); as a result, largemouth bass winter behaviour is not well understood. To address winter behaviour, we examined the three types of swimming performance that exist in teleost fish: sustained, prolonged, and burst swimming (Beamish 1978; Webb 1994). Mean directional swimming activity from the biotelemetry study assumes sustained swimming; prolonged swimming was measured in the U_{crit} test; burst swimming was assessed in the swim tunnel study (Bone et al. 1978; Johnston and Moon 1980; Burgetz et al. 1998). In all cases, a significant seasonal decrease was found. Specifically, during the winter, sustained swimming demonstrated a 75 % reduction from the summer, prolonged swimming a 54 % reduction, and burst swimming an 88 % reduction. The general reduction in all types of swimming indicates that a physiological change in swimming muscles, along with other physiological processes, occurs at colder temperatures (Randall and Brauner 1991; Battersby and Moyes 1998; Tschantz et al. 2002). In addition, depleted energy stores during cold periods (Lemons and Crawshaw 1991) may have an effect on swimming performance, as sufficient fuel is needed to supply the swimming muscles with energy (Beardall and Johnston 1983; Lowery and Somero 1990; Randall and Brauner 1991). Another key point is the substantial difference

between prolonged swimming and sustained swimming. Though each type of swimming is non-comparable and measured separately, it appears that largemouth bass in a natural setting during the winter have the muscle capacity and energy stores to swim faster than they actually do. This study has demonstrated that each swimming type is greatly reduced by seasonal decreases in temperature and, for the first time, indicates that *in situ* movement of largemouth bass is considerably lower than the swimming performance of exercised fish.

Seasonal swimming performance in fish has also been linked to length of acclimation and/or seasonal acclimatization time prior to an assessment (Rome et al. 1985; Heap and Goldspink 1986; Guderley and Blier 1988; Adams & Parsons 1999). When the mean activity of the telemetered fish was compared to the early winter, it had statistically increased 33 %, despite the fact that temperature was relatively stable throughout this period. This increase in largemouth bass activity suggests that some degree of acclimatization to winter has occurred. Multiple cold acclimation studies have demonstrated that water temperature is a major contributor to seasonal variation in swimming performance (Heap et al. 1985, Kolok 1991; Taylor et al. 1996; Battersby and Moyes 1998; Tschantz et al. 2002). These changes may be beneficial, as they may increase the ability of fish to withstand winter hypoxia (Guderley 1990). Few laboratory studies have directly assessed acclimatization and swimming performance in fish (Kolok 1992; Adams and Parsons 1998). In this study, the 33 % increase in biotelemetry-derived activities during the winter demonstrates the importance of timing when assessing fish performance in the wild. Also this study, for the first time, shows possible seasonal acclimatization by wild fish assessed in a natural setting.

Conclusion

Activity and swimming speed studies are most often completed using either biotelemetry or swim tunnels. In the present study, both of these methods were used to obtain further insights about the impact of winter temperatures on largemouth bass. Temperature was shown to have a profound impact on largemouth bass in both the biotelemetry and swim tunnel studies across seasons. When the winter activity and swimming values were assessed, along with the relative reductions from summer activity and swimming values, reductions in all swimming types were found, suggesting possible effects from multiple physiological processes associated with swimming performance. The biotelemetry portion of the study revealed an increase in mean daily movement of fish as winter persisted, indicating acclimatization may be occurring. Clearly, winter has an impact on naturally occurring activity and on laboratory-tested swimming performance.

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Tables and Figures

Table 2.1. Transmitter number, total length (mm), and detection rates (detections/min) during each biotelemetry sampling period (summer, fall, early [E.] winter, late [L.] winter and early spring). Mean and standard error of the total length of fish sampled is given.

Transmitter number	Total Length	Summer Detection Rate	Fall Detection Rate	E. Winter Detection Rate	L. Winter Detection Rate	E. Spring Detection Rate
31400	400	0.24(0.02)	0.33(0.04)	0.67(0.02)	0.66(0.01)	0.07(0.02)
31200	378	0.05(0.01)	0.42(0.05)	0.72(0.02)	0.63(0.02)	0.18(0.02)
31100	390	0.19(0.02)	0.55(0.06)	0.67(0.03)	0.61(0.02)	0.12(0.02)
30700	426	-	0.45(0.03)	0.76(0.04)	0.35(0.07)	-
29900	378	0.17(0.2)	0.42(0.03)	0.68(0.02)	0.65(0.02)	0.17(0.02)
30300	424	-	0.29(0.04)	0.73(0.02)	0.61(0.03)	0.13(0.02)
30000	396	0.12(0.01)	0.48(0.02)	0.68(0.02)	0.48(0.03)	0.09(0.02)
29700	405	0.18(0.01)	0.30(0.02)	0.68(0.03)	0.53(0.03)	0.09(0.01)
30400	377	0.03(0.01)	0.10(0.10)	0.74(0.01)	0.64(0.02)	0.13(0.01)
Mean (S.E.)	**397(6)**	**0.14(0.03)**	**0.37(0.04)**	**0.70(0.01)**	**0.57(0.03)**	**0.12(0.01)**

Table 2.2 Treatment (Study: ST = Swim Tunnel; BT = Biotelemetry), sample size (n), total length (cm) (±S.E.), linear fit of total length regressed against swimming speed (SS; U_{crit} for swim tunnel study; Mean swimming activity for biotelemetry study), and p value of linear fit for fish swum in swim tunnel at Lake Opinicon.

Treatment(Study)	n	TL±S.E.	Linear Fit (TL vs. SS)	P value
Summer (ST)	15	29.2(1.4)	65.985818 - 0.1680221 TL	0.690
Fall (ST)	7	27.8(1.6)	31.361564 + 0.53799 TL	0.636
Early Spring (ST)	10	28.1(1.5)	2.7087788 + 1.0752748 TL	0.157
Fall (BT)	8	39.9(0.65)	0.0532505 - 0.0000991 TL	0.463
Early Winter (BT)	9	39.7(0.63)	-0.005962 + 0.0000030 TL	0.533
Late Winter (BT)	9	39.7(0.63)	0.0186112 - 0.0000025 TL	0.653
Early Spring (BT)	8	39.4(0.58)	0.0151695 - 9.6748e-6 TL	0.782
Summer (BT)	7	38.9(0.44)	0.0177515 + 0.0000195 TL	0.957

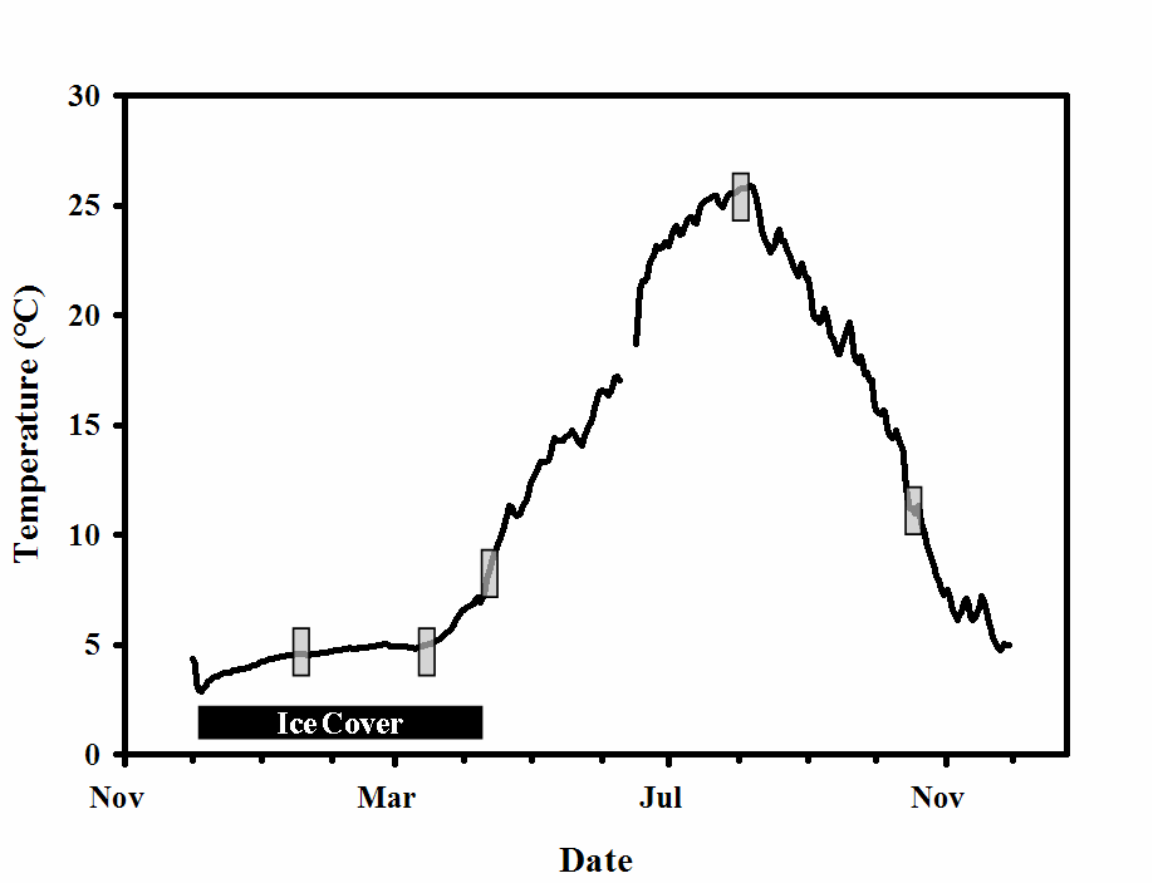

Figure 2.1. Warner Lake water temperature (°C) sampled from December 1, 2005 - November 29, 2006. The solid black line represents temperature (note the break in the line due to retrieval and redeployment of the temperature sensors). The ice cover period is represented by the horizontal black bar, and the periods when the fish speeds were assessed are represented by vertical grey bars.

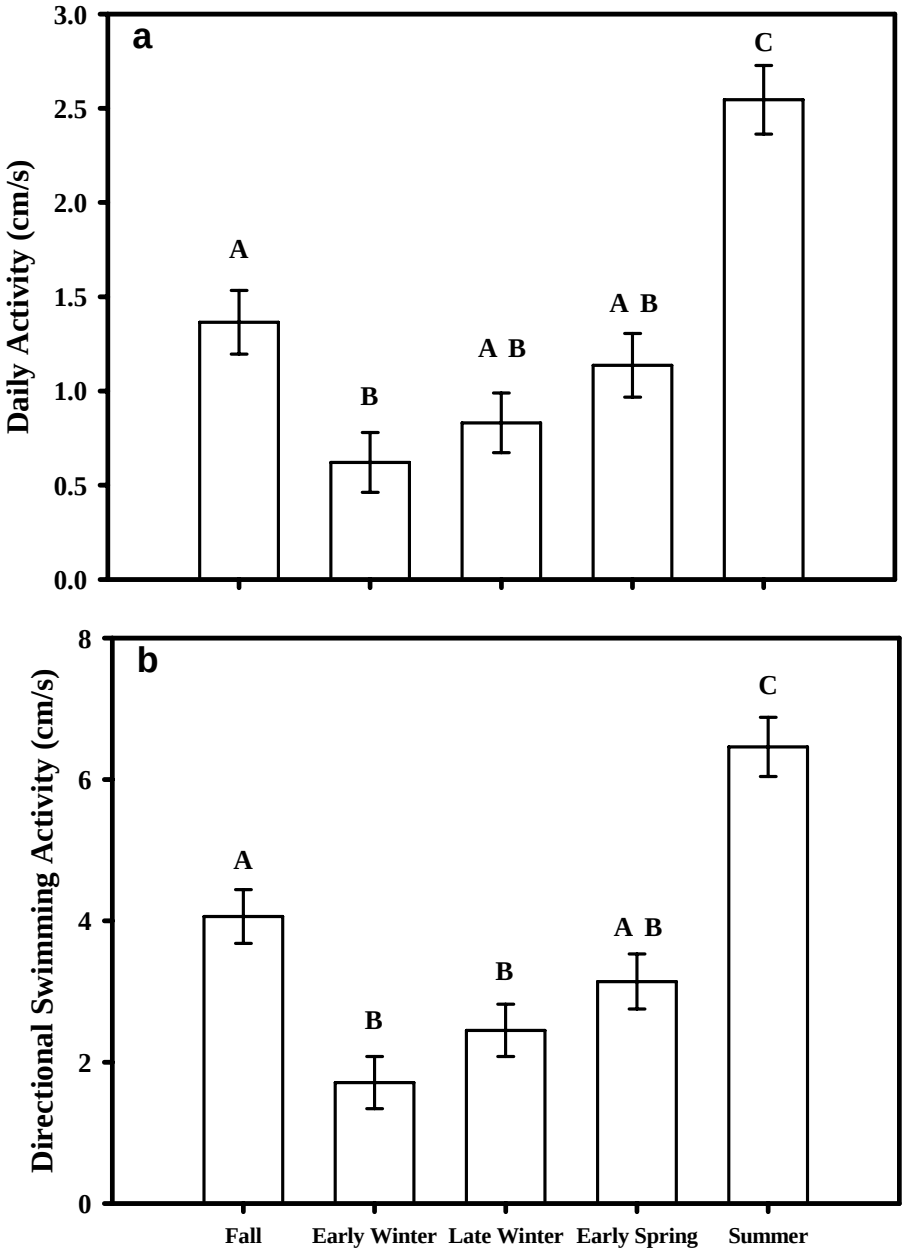

Figure 2.2. Mean (± SE) daily activity (**a**) and mean (± SE) direction swimming speed (**b**) of telemetered largemouth bass during fall (11.5°C), early winter (4.5°C), late winter (5.0°C), early spring (7.5°C), and summer (25°C). Bars not sharing the same letter are statistically different (repeated-measures ANOVA; Tukey's HSD test; $P < 0.05$).

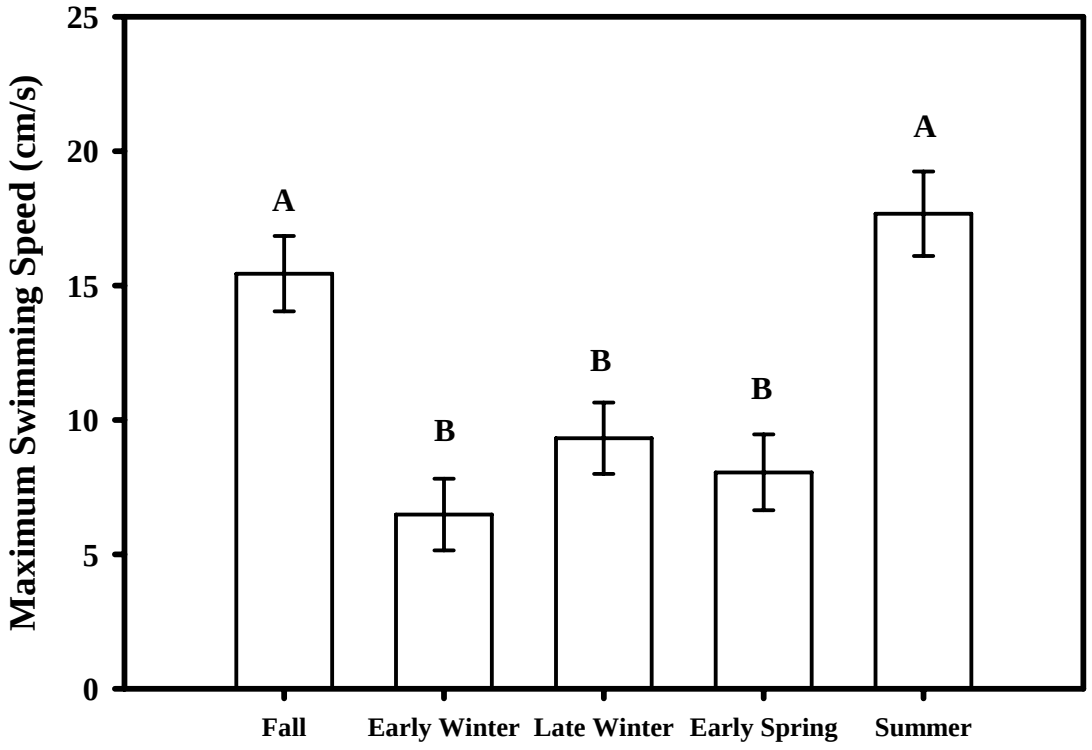

Figure 2.3. Mean maximum (± SE) swimming speed of telemetered largemouth bass during fall (11.5°C), early winter (4.5°C), late winter (5.0°C), early spring (7.5°C), and summer (25°C). Bars not sharing the same letter are statistically different (repeated-measures ANOVA; Tukey's HSD test; $P < 0.05$).

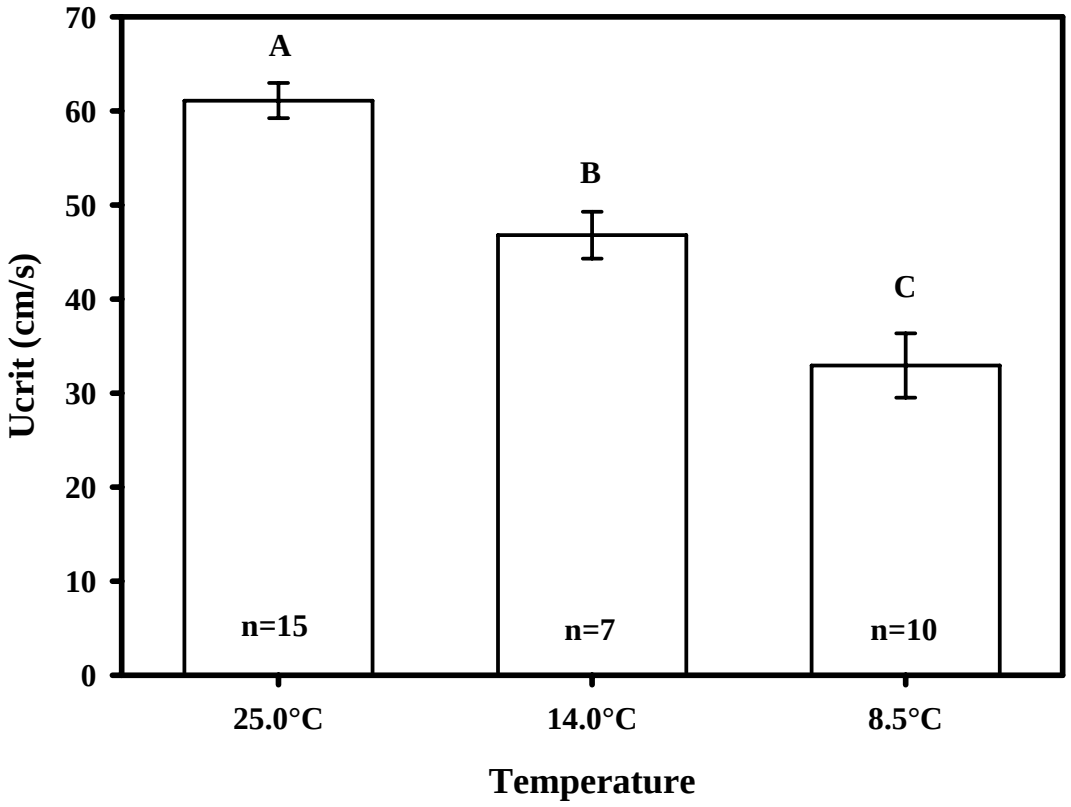

Figure 2.4. Critical swimming speeds (U_{crit}) (± SE) of wild largemouth bass captured from Lake Opinicon when ambient temperatures were 25.0°C, 14.0°C, and 8.5°C. Sample sizes for each group are shown in bars. Bars not sharing the same letter are statistically different (one-way ANOVA; Tukey's HSD test; $P < 0.05$).

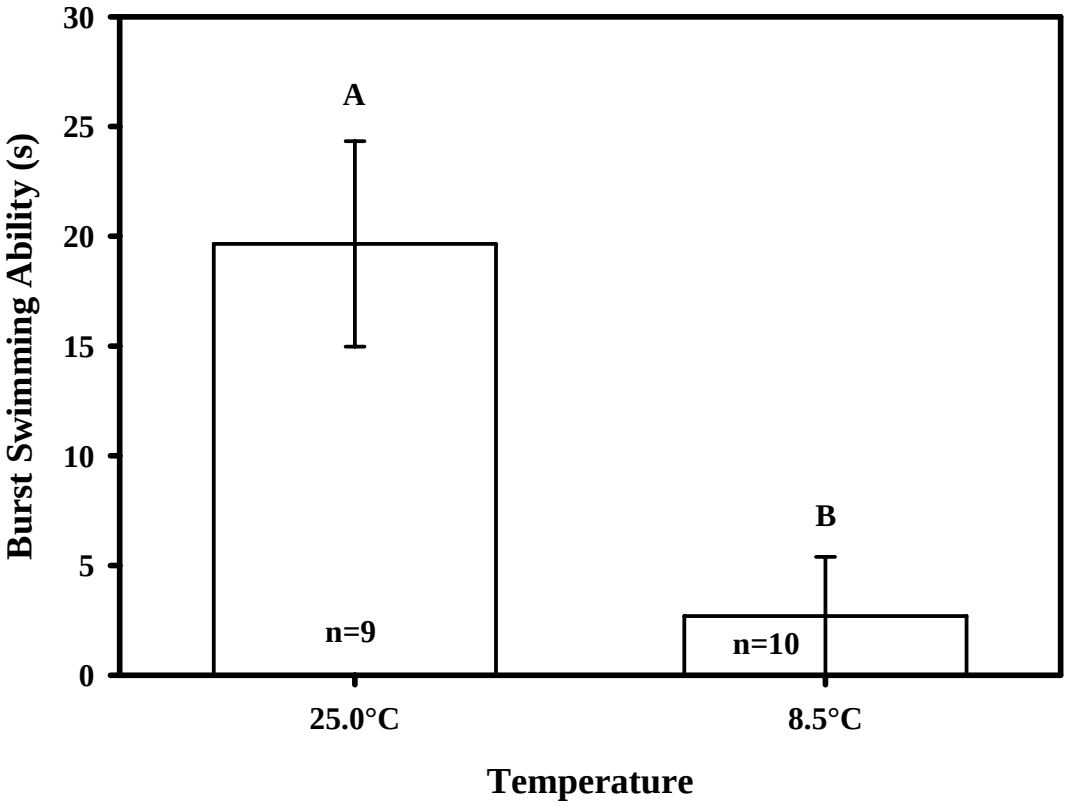

Figure 2.5. Time spent burst swimming (3.0 body length/s)(± SE) of wild largemouth bass captured from Lake Opinicon when ambient temperatures were 25.0°C and 8.5°C. Sample sizes for each group are shown in bars. Bars not sharing the same letter are statistically different (student t-test; $P < 0.05$).

Chapter 3. The influence of dissolved oxygen on winter habitat selection by largemouth bass: an integration of biotelemetry and laboratory experiments

Abstract

In this study, biotelemetry and laboratory approaches were undertaken to understand the relationship of behavioural and physiological responses in fish to winter hypoxia, as well as to link between behavioural patterns observed in the laboratory and behavioural patterns observed in the field. The biotelemetry study compared dissolved oxygen amounts measured throughout the winter period to continually tracked locations of nine adult largemouth bass obtained from a whole-lake submerged telemetry array. Fish habitat usage was compared to habitat availability to assess whether fish were selecting for dissolved oxygen. The laboratory study examined behavioural and physiological responses to progressive hypoxia in juvenile largemouth bass acclimated to winter temperatures. Results from the dissolved oxygen measurements made during the biotelemetry study showed high variance in under-ice dissolved oxygen levels. Avoidance of water with dissolved oxygen < 2.0 mg/L by telemetered fish was demonstrated, but significant use of water with intermediate dissolved oxygen levels was also found. Results from the lab experiments showed marked changes in behaviour (i.e., yawning and vertical movement) < 2.0 mg/L, but no change to tissue lactate. Combined results of the biotelemetry and laboratory studies demonstrate that a dissolved oxygen content of 2.0 mg/L may be a critical threshold that induces behavioural responses by largemouth bass during the winter. In addition, the use by fish of areas with intermediate levels of dissolved oxygen suggests that there are multiple environmental factors influencing winter behaviour.

Introduction

Aquatic systems are spatially heterogeneous for a number of variables across a range of scales (Wiens 1976, 1989; Thompson et al. 2001). Environmental heterogeneity exists because of spatial and temporal variations of different abiotic (e.g., temperature, dissolved oxygen, wave action, sunlight, and salinity) and biotic (e.g., prey abundance, vegetation cover, and conspecific location) factors that results in patches of optimal habitat mixed with patches of sub-optimal and intermediate habitat. For example, lakes have some patches that are warmer than others, and these temperatures change daily and seasonally for a variety of reasons. Thus, aquatic organisms must continually seek out and compete for habitats that optimize their requirement for a suite of environmental resources based on a lake's abiotic and biotic factors (Hutchinson 1957; Hutchinson 1965; Fretwell and Lucas 1970; Wiens 1976).

One important environmental resource that influences fish habitat selection and many physiological processes is dissolved oxygen (Petrosky and Magnuson 1973; Raibley et al. 1997; Furimsky et al. 2003). Oxygen is the final electron acceptor in the major energy-producing pathways and a necessary requirement for aerobic metabolism for most organisms, including fish. Fish respond to reduced levels of dissolved oxygen in a variety of ways. Typically, a behavioural response occurs first and may include: change in activity, use of air breathing, increased use of aquatic surface respiration, and habitat shifts (Kramer 1987). Biotelemetry and laboratory studies have shown that fish will avoid areas of hypoxic water and choose to inhabit areas with greater dissolved oxygen concentrations, with the assumption that this minimizes the physiological burden (Magnuson et al. 1985; Kramer 1987; Gent et al. 1995; Raibley et al. 1997; Burleson et

al. 2001). Secondly, a physiological response to reduced levels of dissolved oxygen occurs: as fish increase ventilation rate and decrease cardiac output (Furmisky et al. 2003). Lastly, if no other response is adequate, fish will alter biochemical pathways, such as increasing anaerobic metabolism. Anaerobic metabolism produces far less ATP per glucose molecule, and the negative consequences include production of lactic acid and a decrease in blood pH; both must be actively cleared upon return to an oxygenated environment (Bennett 1978; Wendelaar Bonga 1997; Furimsky et al. 2003; Martinez et al. 2006). The behavioural physiological and biochemical responses to winter hypoxia are not fully understood, as cold temperature may be an important co-variable.

During winter, in lakes located at high latitudes, dissolved oxygen is often less abundant than in summer and therefore winter can have a strong influence on fish behaviour and habitat selection (Suski and Ridgway in press). Many northern temperate lakes can experience hypoxia (or even anoxia) during winter, as ice cover, low light intensity, reduction in photosynthetic biomass, benthic decomposition, and crowding of fish can combine to reduce dissolved oxygen concentrations in localized areas. In severe cases, winter hypoxia can lead to winterkill (Greenbank 1945; Cooper and Washburn 1949). Winter hypoxia has already been shown to influence centrarchid movement, activity, species richness, species ranges, and population structure (Shuter and Post 1990; Fox and Keast 1991; Gent et al. 1995; Nürnberg 1995; Raibley et al. 1997; Tonn and Magnuson 1982; Farwell et al. 2007). However, no study has measured the impact of winter hypoxia on seasonal fish habitat selection or has linked field- and laboratory-based responses to winter hypoxia.

To study the impacts of hypoxia on winter fish responses and populations, many past studies have used controlled laboratory settings to reproduce conditions that can

occur in nature (e.g., Petrosky and Magnuson 1973; Furimsky et al. 2003). Results obtained from these studies are valuable, as they allow for the effect of individual environmental variables on habitat selection to be determined in a controlled setting; however, they do not consider the suite of factors that can influence habitat selection for free-swimming fish. A technique that has been recently used to quantify habitat selection and environmental variables of free-swimming fishes is biotelemetry. This approach provides clues about why fish choose particular habitats over others and allows for assessment of environmental variables utilized at the individual level (Lucus and Baras 2000; Cooke et al. 2004).

In the current study, a combined approach involving both biotelemetry- and laboratory-based experiments was used to better understand the influence of low dissolved oxygen levels in winter on the behaviour, physiology, and ecology of temperate fishes. Largemouth bass (*Micropterus salmoides*) was chosen as the study species because it is abundant in northern lakes that frequently experience winter hypoxia and winterkill (Scott and Crossman 1973). For the biotelemetry portion of the study, a whole-lake acoustic telemetry array was used to compare the locations of fish in the winter with the dissolved oxygen concentrations. For the laboratory study, the behavioural and physiological responses of winter fish to progressive hypoxia were quantified. The combined results from these two studies will improve the understanding of how environmental variables influence habitat selection in fishes, and will also allow a direct coupling between field- and laboratory-based observations.

Materials and Methods

Biotelemetry Study

Study lake and sample sites

The biotelemetry study was carried out in Warner Lake, eastern Ontario, Canada. Warner Lake, located entirely within the property of the Queen's University Biological Station (QUBS) (44°31'N, 76°22'W) (Figure 3.1a), is a freshwater lake with a naturally self-sustaining population of largemouth bass. The lake has a surface area of 8.3 hectares, comprising two basins: a smaller shallow basin defines the north section of the lake (maximum depth of 3 m) and a large deeper basin (maximum depth of 7 m) defines the southern section of the lake. There is little flow of water into and out of the lake, resulting in long resident times for water. In the current study, dissolved oxygen and temperature were sampled at 21 sites throughout Warner Lake on seven different occasions from November 02, 2005 to April 12, 2006 (Figure 3.1a). At each of the 22 sites, dissolved oxygen was measured at 1 m increments starting at the surface, and the number of measurements made at each site varied because depth ranged from 1-5 m; dissolved oxygen concentrations for all depths were combined to generate a single mean oxygen concentration for each site. Sites were chosen using a bathymetric map to provide full coverage of the littoral and pelagic zones of the lake, as well as to sample areas of the lake known to be frequented by largemouth bass (Hasler, unpublished data). Sites were marked with a global positioning system (GPS), and dissolved oxygen and temperature were measured using a submersible dissolved oxygen and temperature probe (Model 55, YSI Inc., Yellow Springs, Ohio). Dissolved oxygen sampling dates were chosen to ensure the lake was sampled during the pre-ice period (November 02, 2005;

December 01, 2005), during the period of time when ice was present across the entire surface of the lake (January 22, 2006; February 14, 2006; February 24, 2006; March 21, 2006), and during the post-ice period (April 12, 2006). For dates when ice was covering the lake, an 18 cm auger was used to drill through the ice to access the water.

To quantify movements of largemouth bass in Warner Lake, a stationary acoustic telemetry array consisting of a code division multiple access- (CDMA-) based telemetry system (Lotek MAP_600, Lotek Wireless Inc., Newmarket, Ontario, Canada) was used. The system and its accuracy are summarized in Cooke et al. (2005) and Hanson et al. (2007). Briefly, the array consisted of 13 hydrophones, 5 located in the shallow basin and 8 located in the deep basin; the hydrophones were distributed to allow for sub-metre position solutions to be calculated during periods when tagged fish were within the footprint of the array (Niezgoda et al. 2002). Position solutions were recorded on flash cards that were then collected and downloaded to personal laptops for processing and filtering in BioMAP (V2.1; Lotek Wireless Inc., Newmarket, Ontario, Canada), which uses a wavelet analysis to remove uncorrelated noise from a time series (Hess-Nielsen and Wickerhauser 1996; Akay and Mello 1997).

Study Animals

For the field study, nine Warner Lake resident largemouth bass were surgically implanted with CDMA-enabled acoustic tags that emitted a position signal every 60 sec (Lotek CTPM11-18, 11mm x 60mm, repetition rate 59.5 seconds, life expectancy of 3 years, Newmarket ON, Canada). To obtain fish for surgery, largemouth bass were captured using standard hook-and-line angling on October 05 and 06, 2005, held in net pens until all fish had been collected (~ 2 hr), and then removed from the net pens and

taken onshore for surgical implantation of transmitters. Details of the surgery are given in Cooke et al. (2003). Briefly, fish were anaesthetized until unresponsive using a 60 ppm clove oil bath for 6 min and placed on a foamed surgical platform; their gills were then irrigated with a flowing bath of 30 ppm clove oil. A small incision was made on the abdomen and the tag was inserted into the body cavity. The incision was then sutured using polypropylene non-absorbable suture (prolene, sterile, Ethicon Inc., New Jersey), and the fish were placed in a cooler of fresh lake water to recover (< 1 hr) prior to release and the initiation of the tracking study.

Data analysis

In order to consider which time periods would have enough variation to allow habitat choice to be assessed, a Levene's test was performed across sampling periods to test for unequal variances in dissolved oxygen concentrations. This was followed by a non-parametric Kruskal-Wallis and a non-parametric post-hoc to test for differences in mean dissolved oxygen concentrations across sampling periods (Zar 1984).

When analyzing data from the biotelemetry study, we were interested in comparing the ambient dissolved oxygen with fish location and understanding whether the preference was biased because of dissolved oxygen availability. For this, 500 randomly chosen X and Y positions for each fish (n = 9) during each of the five days centered around the dissolved oxygen sampling day (January 20-24; February 12-16, February 22-26; and March 19-23) were first separately plotted in ArcGIS (V9.1, ESRI Inc., Redlands, California) (De Solla et al. 1999). Thiessen Polygons using the dissolved oxygen sample sites as center points were then created using ArcGIS (V9.1, ESRI Inc., Redlands, California; Figure 3.1b). When Thiessen Polygons are used, all areas of the

lake can be assigned to the nearest sample site (center point; and therefore a dissolved oxygen concentration) (Aurenhammer 1991; see appendix 3.1). Next, the numbers of fish positions (per fish, per day) in each Thiessen Polygon were counted, and direct comparison to dissolved oxygen concentration were made. However, with respect to the Thiessen Polygon method, if the sampling sites are not uniformly distributed, the variation in the dataset can be effected. In this case, the distribution of sampling sites was not uniform. Most importantly, there were no sampling sites in the middle of the lake and any dissolved oxygen comparisons with fish positions were grouped with dissolved oxygen readings taken from nearer shore. Because dissolved oxygen is likely to be lower near shore than in the center of the lake (Greenbank 1945), fish positions measured in the center of the lake were attributed with lower amounts of dissolved oxygen than they likely should have been.

In order to assess whether fish locations were related to the availability of dissolved oxygen, chi-square goodness-of-fit tests were used to compare the number of fish locations across the dissolved oxygen concentrations found in the lake (observed) compared with the theoretical number of fish locations expected if fish were uniformly distributed across the different dissolved oxygen concentrations (Zar 1984). Significantly greater or fewer numbers of fish observed for each dissolved oxygen concentration would mean that the fish are occupying areas of the lake based on dissolved oxygen, but independent of availability. However, as a result of the distribution of the dissolved oxygen sampling sites, specifically the lack of sample sites in the central portion of the lake, the likelihood of finding significant results was reduced (Figure 3.1b). Actual variations in dissolved oxygen were decreased by the construction of the Thiessen polygons. Sites in the center of the lake (likely to be high in dissolved oxygen;

Greenbank (1945) were assigned to near shore sites (sites typically lower in dissolved oxygen) in the Warner Lake polygons. This is seen clearly in Figure 3.1.

All tests were performed using JMP 6.0.2 software (SAS Institute Inc., Cary, North Carolina). The level of significance (α) for all tests was 0.05, and all means are reported ± 1 standard error (SE) where appropriate.

Laboratory Study

The laboratory component of this study was designed to quantify the behavioural and physiological responses of largemouth bass to progressive hypoxia. For this, 42 juvenile largemouth bass (Size range: 77mm-110mm[total length]) were obtained from Pure Springs Trout Farm (Shannonville Ontario, Canada) and held in a tank of dechlorinated 5°C tap water for two weeks prior to the experiments. The 42 fish were divided into seven groups of six fish (1 group for behavioural observation and 6 groups for lethal sampling). Fish did not feed during this acclimation period.

To assess behavioural changes caused by exposure to progressive reductions in dissolved oxygen concentration, we placed 6 largemouth bass [size range = 77 mm – 99 mm total length; weight range = 5.5 g – 11.5 g) in separate Erlenmeyer flasks (see appendix 3.2). Graded hypoxia was achieved by first gassing water in a central basin with compressed nitrogen gas (99.95% pure); this deoxygenated water was then pumped from the central basin into individual Erlenmeyer flasks using a submersible pump. Water that overflowed from the flasks was collected and returned to the central basin to create a closed circuit. Dissolved oxygen concentrations in this circuit were monitored with a dissolved oxygen meter (Model 55, YSI Inc., Yellow Springs, Ohio). The

experimental treatments were as follows: 10 + mg/L (11.44 mg/L ± 0.04 mg/L, n = 20); 8 mg/L (7.93 ± 0.08 mg/L, n = 20); 6 mg/L (6.01 ± 0.05 mg/L, n = 20); 4 mg/L (3.92 ± 0.02 mg/L, n = 20); 2 mg/L (1.99 ± 0.02 mg/L, n = 20); and 1.5 mg/L (1.42 ± 0.03 mg/L, n = 20) (see appendix 3.3).

For the experiment, six largemouth bass in Erlenmeyer flasks were first exposed to 10 mg/L dissolved oxygen (approximately 100 % saturation) for 1 hr. After 1 hr of exposure, each individual fish was observed for 2 min, during which time the amount of time spent yawning (wide opening of the operculum and subsequent closing in an irregular pattern, also known as gill flaring) and the amount of time spent moving towards the top of the flask was quantified using a stopwatch. Following this observation period, the amount of nitrogen gas delivered to the central basin was increased resulting in a concomitant reduction in dissolved oxygen within the circuit. Once the new dissolved oxygen concentration had been established and stabilized (~ 30 s), fish were left at this concentration for 1 hr, before being observed for 2 min for the same variables described above. This series of reductions was repeated until the fish were exposed to each of the six dissolved oxygen concentrations.

To quantify the production of lactate in white muscle during progressive hypoxia, 6 juvenile largemouth bass per group (6) [size range = 78 mm – 110 mm total length; 6.7 g – 15.3 g] were exposed for one hour to one dissolved oxygen concentration (either 10.0 mg/L [mean ± standard error; 10.83 ± 0.04, water samples; n = 20]; 8.0 mg/L [7.94 ± 0.06 , n = 20]; 6.0 mg/L [5.96 ± 0.07, n = 20]; 4.0 mg/L [4.11 ± 0.06, n = 20]; 2.0 mg/L [2.00 ± 0.06, n = 20]; or 1.5 mg/L [1.49 ± 0.04, n = 20] of dissolved oxygen) (see appendix 3.4). After the 1-hr exposure to each dissolved oxygen concentration, fish were anaesthetized using a buffered mixture of anesthetic (tricaine methane sulfonate [250

mg/L] and sodium bicarbonate [$NaHCO_3$] [500mg/L]) by quickly pouring the fish from the Erlenmeyer flask into a container filled with anaesthetic (Summerfelt and Smith 1990). Once fish had completely lost equilibrium, most epaxial white muscle above the lateral line was excised with a razor blade, freeze clamped in pre-cooled aluminium tongs, and immediately frozen in liquid nitrogen. Tissue was stored at -80°C until processing.

Metabolite extraction and analysis of tissue lactate were performed following methods outlined in Suski et al. (2003). Briefly, approximately 1 g of frozen tissue was ground under liquid nitrogen using a mortar and pestle. The grounded tissue was transferred to a tarred vial, and a solution of 8 % perchloric acid and 1mM EDTA was added. The mixture was allowed to incubate for 10 min and then centrifuged for 5 min. The supernatant was divided into microcentrifuge tubes, and a neutralizing solution (containing 2 M KOH, 0.4 M KCL and 0.3 M Imidazole) equal to 55 % of the weight of the supernatant was added. The solution was again centrifuged, and the supernatant was transferred to 1.5 ml microcentrifuge tubes and immediately frozen until the analysis of tissue lactate occurred.

Quantification of lactate in the prepared muscle samples followed the enzymatic methods of Lowry and Passonneau (1972) using a 96-well plate spectrophotometer (Spectra MAX Plus; Molecular Devices Corp., Sunnyvale, CA). Briefly, frozen prepared muscle samples were thawed and centrifuged for 3 min. Pre-prepared lactate standards and each sample were injected in duplicate into separate wells of a plate well (7.89 µL of each). Approximately 150 µL of a hydrazine solution (consisting of 200 mM of hydrazine sulfate and 0.016585 g of nicotinamide adenine dinucleotide [NAD]) was then added to each well. The plate was then briefly vortexed, and the absorbance at 340 nm

was read and recorded. Following this, 1 μL of lactate dehydrogenase was added to each well and the well was again vortexed briefly; final absorbance at 340 nm was then read and recorded. Lactate concentration was calculated using a series of functions based on the two absorbance readings, mass of tissue sample, mass of supernatant, and the volume of solutions added (Lowry and Passonneau 1972).

Statistical analysis

Differences in behavioural responses across dissolved oxygen concentrations were assessed using a one-way repeated-measures analysis of variance, followed by a Tukey's HSD test to determine differences between treatments (Zar 1984). Differences in concentrations of white muscle lactate across the different treatments were assessed using a one-way analysis of variance followed by a Tukey's HSD test (Zar 1984). All tests were performed using JMP 6.0.2 software (SAS Institute Inc., Cary, North Carolina). The level of significance (α) for all tests was 0.05, and all means are reported as ± 1 SE where appropriate.

Results

Biotelemetry Study

The mean concentration of dissolved oxygen in Warner Lake decreased from 10.9 mg/L on December 01, 2005 to 4.8 mg/L on January 21, 2006 and was lowest on February 14, 2006 at 2.7 mg/L (Kruskal-Wallis test; $X^2_6 = 119.98$; $P<0.0001$; Figure 3.2). During the sample dates prior to ice formation on the lake (sample dates in November and December), all sample sites during had > 10 mg/L of dissolved oxygen, and all sites were within 1 mg/L of the mean dissolved oxygen of the lake (Figure 3.2). Once complete ice

cover occurred (sample dates from January-March), there was high variance between the sample sites as dissolved oxygen concentrations ranged from < 2 to > 8 mg/L (Levene; $F_{6, 144}$ ratio= 28.15; P<0.001; Figure 3.2). During the period of ice cover, most of the lake (57 - 86 % of sample sites) also had < 5 mg/L of dissolved oxygen (Figure 3.2). Sample sites that did have dissolved oxygen concentrations above 5 mg/L (14 - 43 %) were located in the large deep basin, but the majority of sample sites in the lake were not above 5 mg/L. The portion of sites < 2 mg/L throughout the lake was greatest on the February 14, 2007 sample date. At this time, 41% of the sample sites had dissolved oxygen concentrations below 2 mg/L. Interestingly, the majority of sites in the small shallow basin and all the near-shore sites were < 2 mg/L (Figure 3.1a; data not shown) during the period of ice cover. Once the ice cover melted in April, dissolved oxygen concentrations increased to pre-ice cover values (Figure 3.2).

Telemetered fish were consistently found in intermediate dissolved oxygen polygons (Figure 3.3). From January 20 - 24, fish were primarily located in water with dissolved oxygen > 4 but < 9 mg/L of dissolved oxygen (Figure 3.3), with the bulk of fish locations (49 %) counted in areas with dissolved oxygen between 6 and 7 mg/L. From February 12 - 16, 48 % of fish locations were found in areas of the lake with dissolved oxygen concentrations between 4 and 5 mg/L, while the remaining fish locations were counted in areas of the lake ranging from 2 - 7 mg/L (Figure 3.3). Between February 22 - 26, 77 % of fish locations were found in areas of the lake with dissolved oxygen greater than 3 mg/L but less than 4 mg/L; however, fish did inhabit areas with as much as 7 mg/L and as little as 2 mg/L of dissolved oxygen (Figure 3.3). From March 19 - 23, fish locations were more dispersed across a wider range of dissolved oxygen, as fish locations were counted in areas of the lake with no detectable dissolved oxygen and in areas

measured to have up to 7 mg/L of dissolved oxygen (Figure 3.3). During this period, 81 % of fish locations were counted in areas with 3 - 6 mg/L of dissolved oxygen.

During each sampling period, usage of habitat by fish was independent of dissolved oxygen availability, as observed distributions of fish across the sample sites were significantly more dispersed than the expected theoretical distribution (if fish were uniformly distributed) across the sample sites (Figure 3.4). The significance of these results are encouraging, as the loss of variation in dissolved oxygen levels inherent in the Thiessen construction may mask an even stronger relationship than that suggested here. During each of the sampling periods, over 20 % of the sites had ambient dissolved oxygen below 2 mg/L, but during the three sampling periods in January and February, no fish were found to be located in these areas. Likewise, areas with more than 6 mg/L of dissolved oxygen were present (26 % during January 20-24; 10 % during February 12-16; 3 % during February 22-26; and 12 % during March 19-23), but during the two February sampling periods and the March sampling period, only 19 %, 5 %, and 3 % (respectively) of fish locations were counted within these areas. Overall, fish locations were consistently found in water with ambient dissolved oxygen > 2 mg/L and < 6 mg/L.

Laboratory Study

Juvenile largemouth bass exhibited pronounced behavioural responses when exposed to increasingly anoxic water. After exposure to either 10.0, 8.0, 6.0 or 4.0 mg/L dissolved oxygen, there were no observations of yawning activity in any of the fish (Figure 3.5). However, when the same bass were exposed to water at 2.0 mg/L dissolved oxygen, fish yawned for the majority of the 2-min monitoring period (repeated-measures ANOVA; $F = 50.41$; $df = 5$; $P < 0.0001$; Figure 3.5a); following exposure to water at 1.5

mg/L dissolved oxygen, there was a further increase in yawning rate. A similar pattern was seen in the amount of time spent moving to the surface of the flasks. Vertical movements were not observed until dissolved oxygen concentrations fell to 2.0 mg/L, at which time the fish were observed moving to the surface for a significant amount of time (repeated-measures ANOVA; $F = 32.98$; $df = 5$; $P<0.0001$; Tukey's HSD test; $P > 0.05$; Figure 3.5b). Following 1-hr exposure to dissolved oxygen concentrations of 1.5 mg/L, there was a further increase in the rate of vertical movement (Tukey's HSD test; $P < 0.05$).

The juvenile largemouth bass did not demonstrate significant changes in white muscle lactate concentrations when exposed to increasingly hypoxic water (ANOVA; $P > 0.05$; Figure 3.6). Tissue lactate ranged from 2.02 (± 0.51, n = 6) to 3.87 (± 1.12, n = 6) μmol/g of wet weight of tissue, but no significant variation was found across treatments.

Discussion

Eastern Ontario's Warner Lake experiences considerable variation in concentrations of dissolved oxygen available to largemouth bass over the winter. During this study's sampling dates when ice was not present on the lake, the mean dissolved oxygen concentrations ranged from 10.2 - 11.3 mg/L, the 25^{th} and 75^{th} quartiles on these dates ranging from 10.1 - 11.4 mg/L. Hence, fish were only exposed to water with dissolved oxygen concentrations above 10 mg/L, which is well within the range of adequate dissolved oxygen for largemouth bass. In contrast, during the ice-cover sample dates, mean dissolved oxygen concentrations across all of Warner Lake ranged from 2.7 - 3.8 mg/L, with the 25^{th} and 75^{th} quartiles on these dates ranging from 0.6 - 6.5 mg/L.

Consequently, fish experienced water with a greater range of dissolved oxygen available at these times, but the mean dissolved oxygen concentration was lower than during ice-free periods. In addition, there was significant variation in dissolved oxygen concentration laterally across different sampling sites in the lake, providing a patchwork of available oxygen concentrations for largemouth bass to inhabit. The variance across sample dates in dissolved oxygen, as well as the overall reduction in dissolved oxygen concentration during ice-cover periods, is a result of a variety of inherent characteristics of temperate lakes at high latitudes (Greenbank 1945; Mathias & Barica 1980; Meding & Jackson 2003). During ice cover, dissolved oxygen was generally both lower and more variable, providing largemouth bass with a variety of dissolved oxygen concentrations to choose from during ice-cover periods, in contrast to ice-free periods when the lake was oxygenated.

The combined results of both the biotelemetry and laboratory studies demonstrate that largemouth bass show behavioural changes to water with < 2 mg/L dissolved oxygen during winter. Sites in Warner Lake that had dissolved oxygen concentrations < 2 mg/L contained significantly fewer largemouth bass than would be statistically expected (if fish were uniformly distributed with respect to oxygen concentration) while significantly more largemouth bass than statistically expected were found in areas > 2 mg/L and < 6 mg/L of dissolved oxygen. In addition, during the laboratory study, largemouth bass significantly increased yawning and vertical movements when exposed to water with dissolved oxygen concentrations of 2 mg/L or less. Previous laboratory studies with yellow perch (*Perca flavescens*) and bluegill (*Lepomis macrochirus*) have shown that activity levels increase when fish are exposed to hypoxic conditions, presumably as they attempt to seek out more oxygenated habitat (Scherer 1971; Petrosky and Magnuson 1973). In our lab study,

yawning activity (or gill flaring) increased significantly at 2 mg/L for largemouth bass, probably to increase water flow across gill lamellae in an effort to increase the amount of dissolved oxygen entering the blood stream (Randall 1982; Perry and Gilmour 2002). In winter field studies, Raibley et al. (1997) measured the dissolved oxygen (among other environmental variables) at specific locations of multiple telemetered fish, and found the fish to be consistently in water with dissolved oxygen concentrations > 2 mg/L; however, they did not consistently record the dissolved oxygen at fish locations, and they did not quantify dissolved oxygen throughout the river system. Avoidance of anoxic water would be advantageous for largemouth bass because prolonged exposure to hypoxic water could lead to costly, inefficient anaerobic metabolism, suffocation, and even death (Greenbank 1945; Cooper & Washburn 1949). The combined results of our biotelemetry and laboratory studies suggest that a minimal level of dissolved oxygen near 2 mg/L is a threshold where behavioural changes in over-wintering largemouth bass are induced.

Interestingly, even though telemetered largemouth bass in our study showed an aversion to water that contained less than 2 mg/L dissolved oxygen, they did not choose to inhabit the most oxygenated water available. Specifically, during the entire ice-cover period, those fish were found to inhabit sites with intermediate levels of dissolved oxygen (concentrations between 7 and 2 mg/L), even though water with 8 and 9 mg/L of dissolved oxygen was present. Their selection of intermediate levels of dissolved oxygen when higher levels of dissolved oxygen is available can be explained by the fundamental niche concept, which explains that a niche is an *n*-dimensional hypervolume, with *n* representing a number of continuous variables that permit survival and reproduction of a species (Hutchinson 1957; 1965). Further studies have documented an animal's niche to be a combination of physical and biotic interactions, with the two types of interaction not

always acting independently in space and time (Chapman 1966; Tracey and Christian 1986). Fry (1971) suggested that there are at least seven factors in a fish's fundamental niche: temperature, dissolved oxygen, toxicity, metabolites, food, salinity, and carbon dioxide. More recent work suggests other physical and biotic characteristics are equally important as amount of cover, water velocity, depth, establishments of home ranges and territory, and aggregations with conspecifics also alter fish habitat selection (Stoot and Cross 1973; Suthers and Gee 1986; Kramer 1987; Spoor 1990; Heggenes et al. 1999; Hasler et al. 2007). If fish in Warner Lake are selecting habitat based on some or all of these parameters, fish using intermediate oxygen patches must have benefits that outweigh the negative effects associated with low dissolved oxygen. Warner Lake is fairly consistent thermally and has no significant water velocity, so, in addition to oxygen, largemouth bass may be selecting habitat based partially on proximity to conspecifics (Breder and Nigrelli 1935; Hasler et al. 2007), cover, and prey abundance. If fish were selecting habitat based exclusively on oxygen, they would likely go to the most oxygenated areas. However, during periods when fish are avoiding water with < 2 mg/L of dissolved oxygen, they are consistently found in water with intermediate not high concentrations of dissolved oxygen.

Although there was a significant general avoidance of areas with dissolved oxygen < 2 mg/L, a small number of fish spent some time in such water: two of the nine telemetered fish inhabited areas with < 2 mg/L dissolved oxygen during the March sampling period, and all telemetered fish were occasionally found in areas < 3 mg/L throughout each study period. Moreover, during the laboratory study, fish did not exhibit increased lactate concentrations in white muscle despite 1 hr exposure to dissolved oxygen concentrations < 2 mg/L, indicating that they were still respiring aerobically,

despite this low oxygen concentration. It is important to consider, however, that oxygen requirements in winter will be low, as two separate studies have concluded that the metabolic rate of largemouth bass during the winter months is greatly reduced, and that they are essentially dormant (Beamish 1970; Crawshaw 1984; Lemons and Crawshaw 1985). In this study, largemouth bass use of intermediate areas is not unexpected, as previous studies have shown that hypoxia is not an absolute barrier to fish movements, and that fish will use hypoxic zones for opportunistic feeding (Pihl et al. 1992; Rahel and Nutzman 1994). One possible reason is that slightly higher temperatures in hypoxic zones would allow for increased metabolism and activity (Fry 1971; Gee et al. 1978; Burleson et al. 2001). In the wintertime, it may be beneficial for fish to tolerate lower dissolved oxygen concentrations that may be lethal during warmer periods when the oxygen requirements are higher (Fry 1971). However, there are trade-offs, as temperature negatively influences both the amount of oxygen in water and a variety of physiological parameters such as hemoglobin oxygen affinity and the rate of enzymatic activity (Fry 1971; Cech et al. 1979; Huey 1991; Somero 1995). However, our laboratory study did not find a physiological change when fish were exposed to hypoxia: tissue lactate, an indicator of anaerobic respiration, did not change. Tissue lactate would be expected to increase once environmental oxygen levels fall below a specific threshold, and the environment becomes more hypoxic (Jenson et al. 1993). It is evident from the current biotelemetry and laboratory studies that largemouth bass tolerate low levels of dissolved oxygen during winter, potentially to benefit from slight metabolic gains from increased temperature. As well, short-term exposure to hypoxia did not facilitate a physiological change, suggesting the physiological consequence of winter habitat selection is minimal.

Conclusion

Biotelemetry and laboratory studies conducted to determine the factors affecting behaviour and physiology are most often performed independently. In the present study, however, these two approaches were used to examine the effect of winter hypoxia on largemouth bass. Dissolved oxygen was found to influence not only their individual laboratory behavioural responses, but also their habitat selection in the wild. In particular, telemetered largemouth bass tended to avoid areas with dissolved oxygen < 2.0 mg/L and laboratory-tested largemouth bass exhibited behavioural responses, such as yawning and vertical movement, when exposed to water with dissolved oxygen levels near 2 mg/L. A small number of largemouth bass, however, were found to occasionally inhabit areas of the lake with below 2mg/ L of dissolved oxygen. Also, no physiological change in white muscle was detected when laboratory-tested fish were exposed to water with less than 2 mg/L of dissolved oxygen. Overwintering largemouth bass appear to avoid water with less than 2 mg/L of dissolved oxygen, but further research is needed to understand the extent to which prolonged exposure to low amounts of dissolved oxygen affects physiological processes.

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Figures

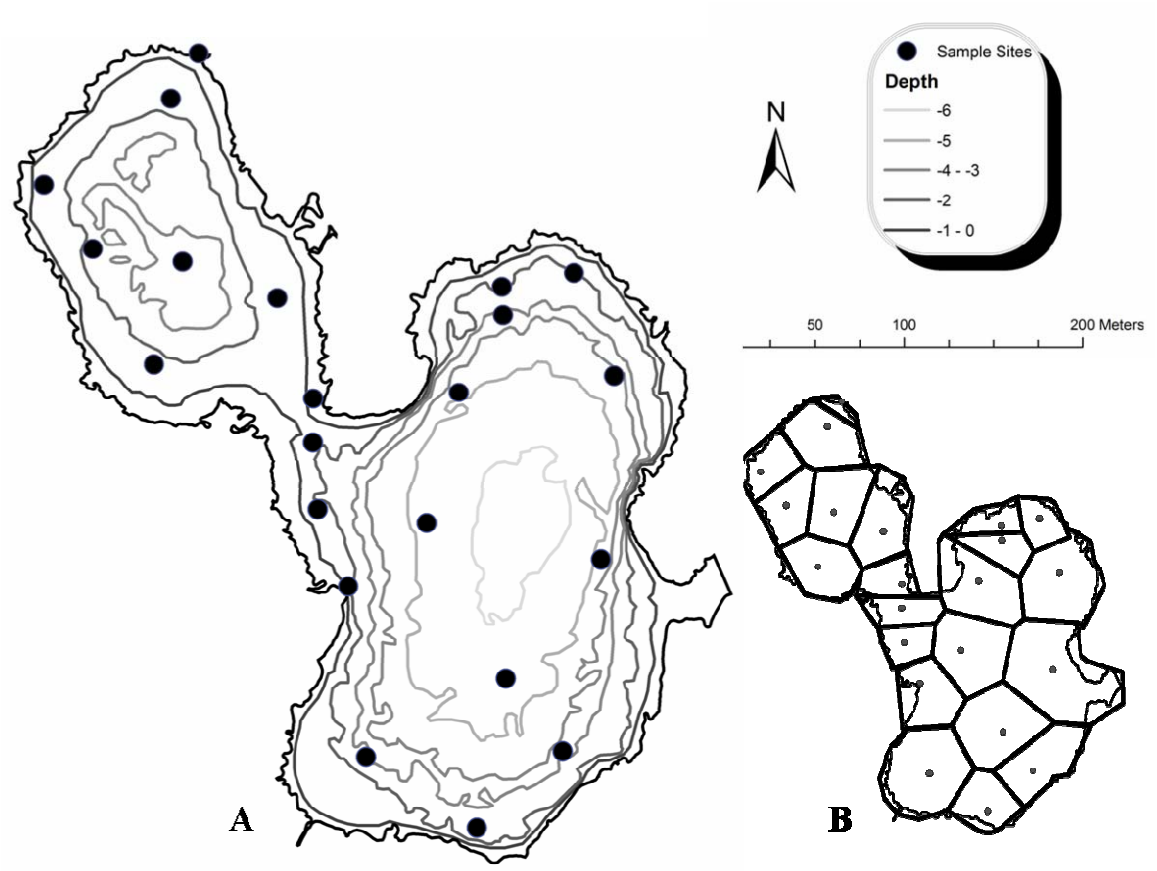


Figure 3.1. Map of Warner Lake depth contours (m) (grey lines) and dissolved oxygen sampling sites (solid black dots) (A). Thiessen polygons used (thick black lines) to assess dissolved oxygen and fish positions (B).

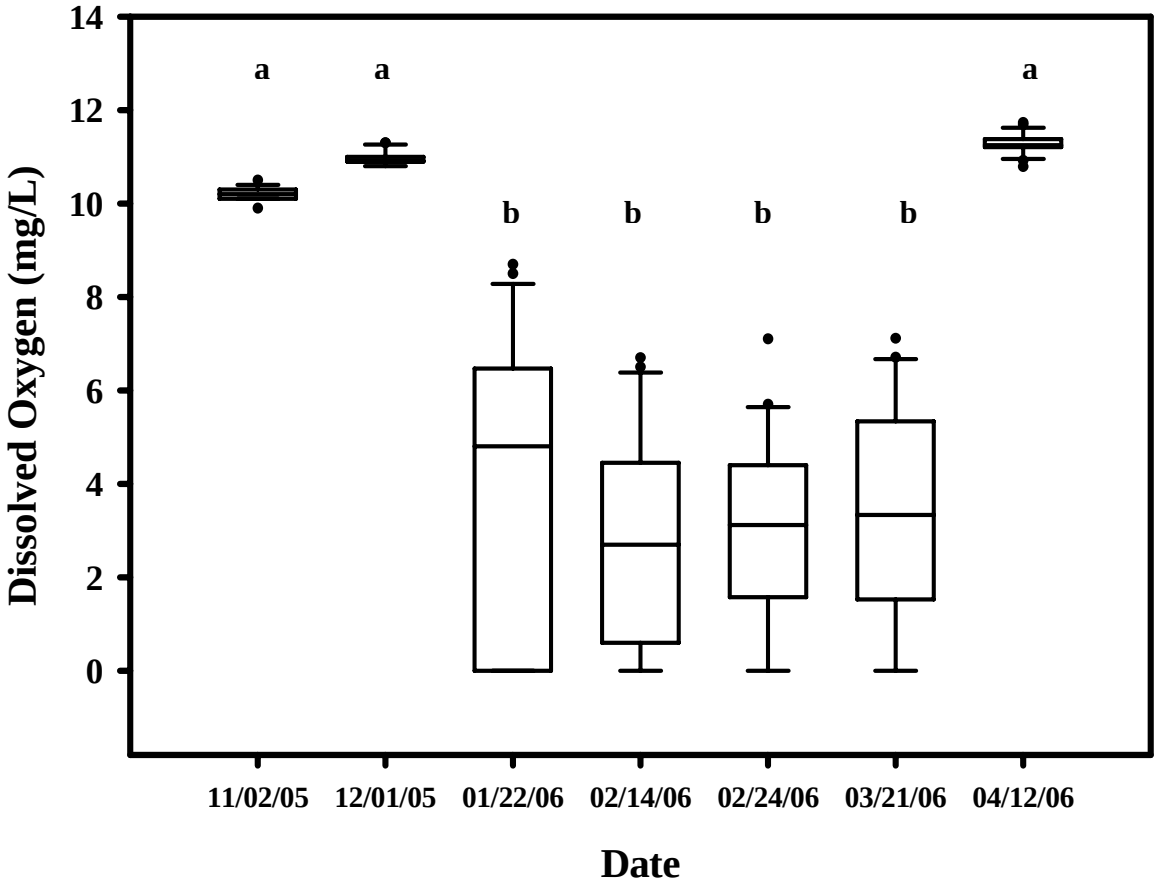


Figure 3.2. Concentrations of dissolved oxygen (mg/L) sampled at Warner Lake on dates given. Horizontal bars in box plots indicate the 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentiles and black dots represent outliers. Sites not connected with similar letters are statistically different (Kruskal-Wallis test; $X^2_6 = 119.98$; $P < 0.0001$; Nonparametric post-hoc $P < 0.05$).

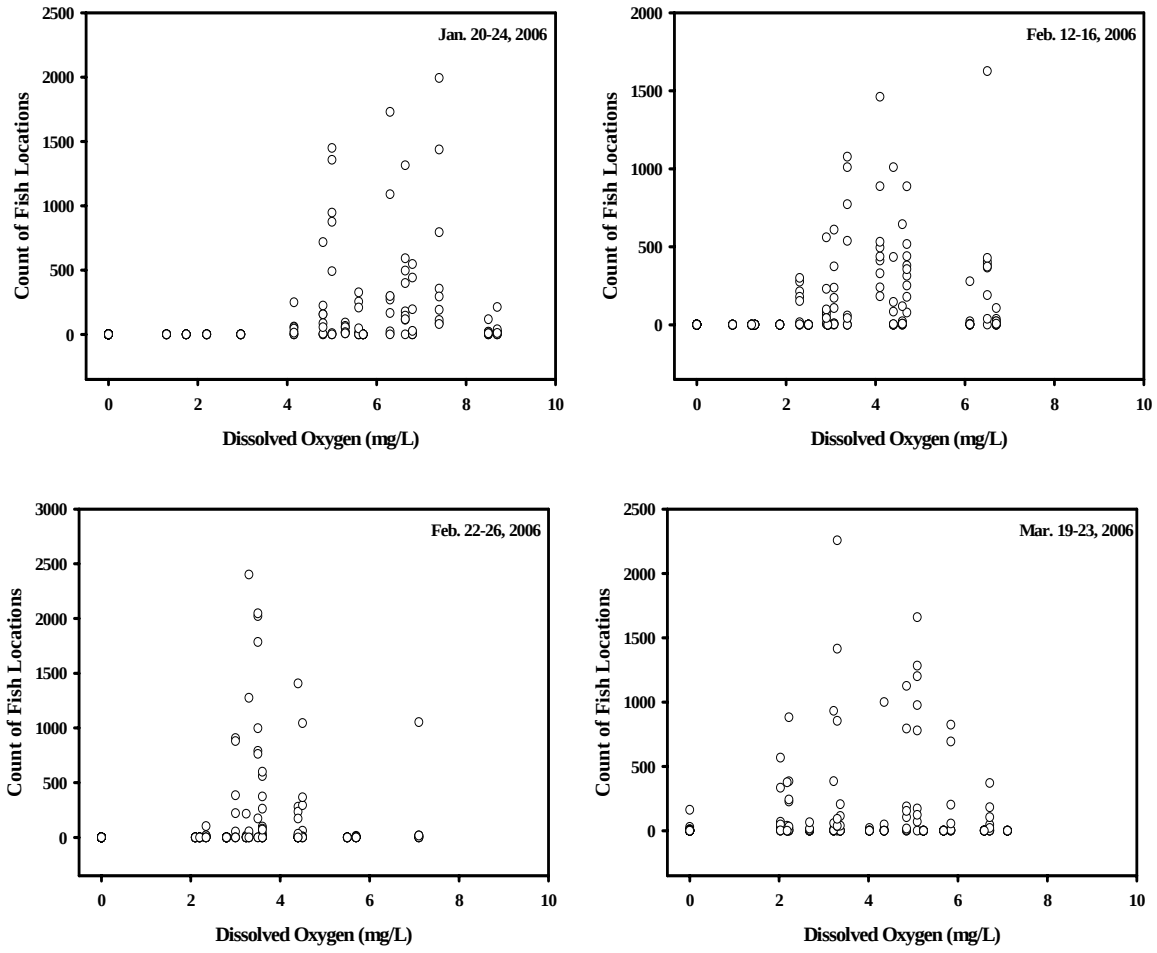


Figure 3.3. Scatter plot of dissolved oxygen (mg/L) and the count of fish locations during January 20-24, February 12-16, February 22-26, and March 19-23.

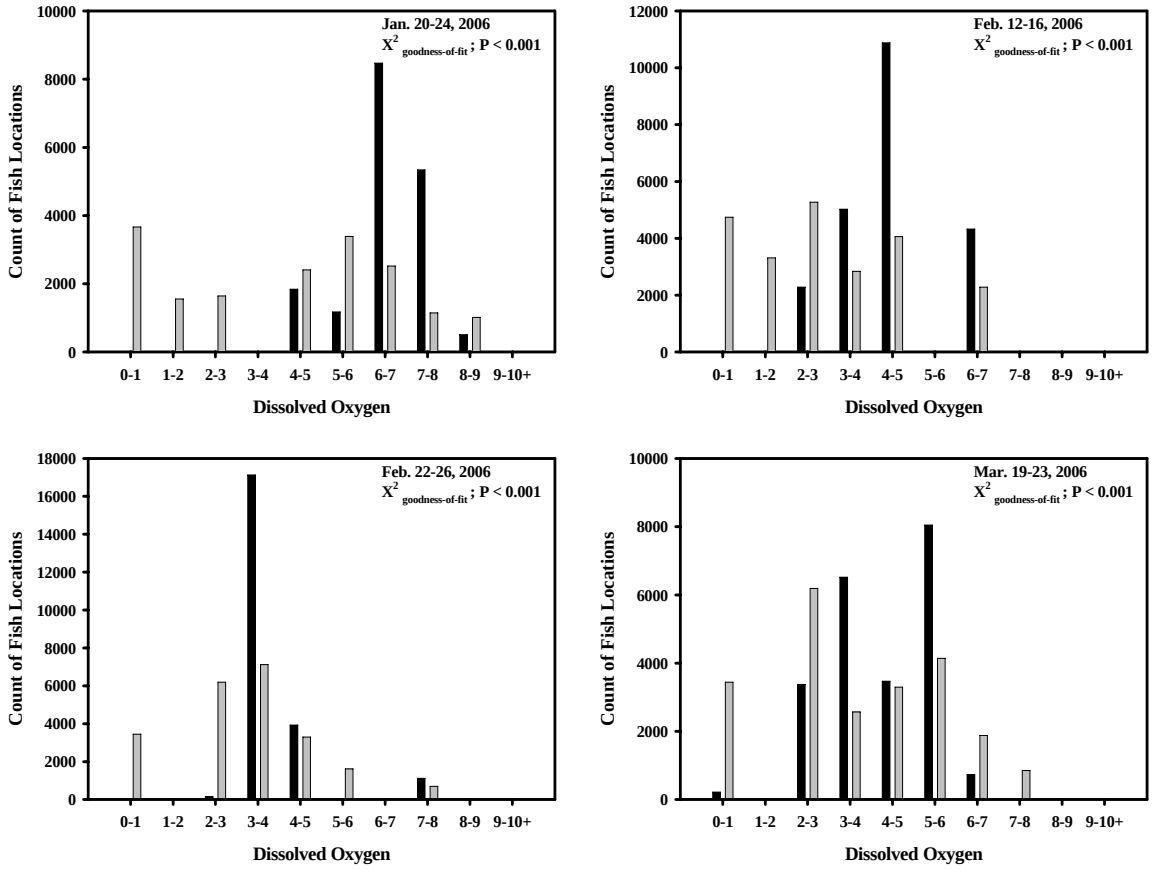


Figure 3.4. Observed and expected (theoretical uniform) frequency distributions of the count of fish locations compared to dissolved oxygen (mg/L) used in the chi-square goodness-of-fit test for each sampling period.

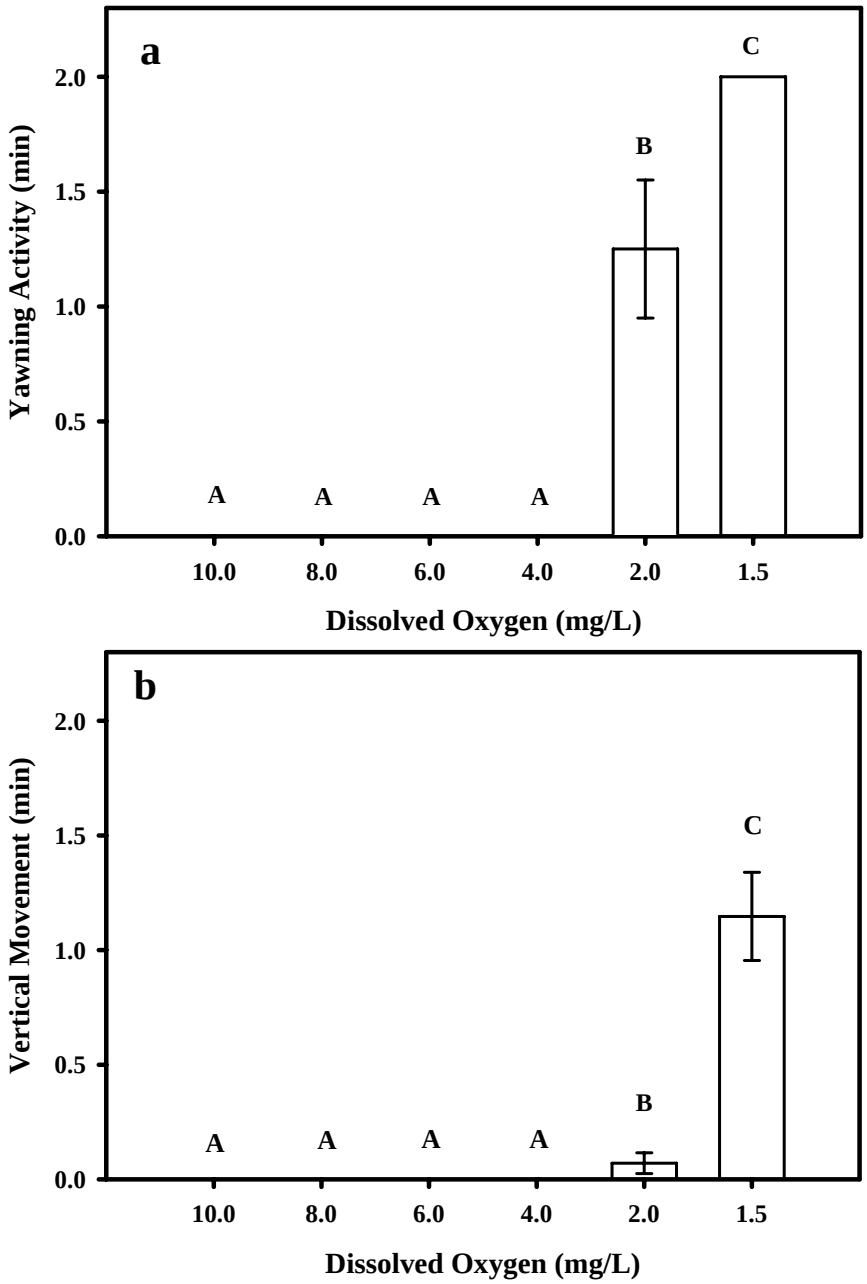


Figure 3.5. Mean time (minutes) (± SE) spent yawning (**a**) and moving vertically (**b**) during a two minute period for 6 juvenile largemouth bass exposed to 10.0, 8.0, 6.0, 4.0, 2.0 and 1.5 mg/L concentrations of dissolved oxygen at 5°C. Bars not sharing the same letter are statistically different (repeated-measures ANOVA; Tukey's HSD test; $P < 0.05$).

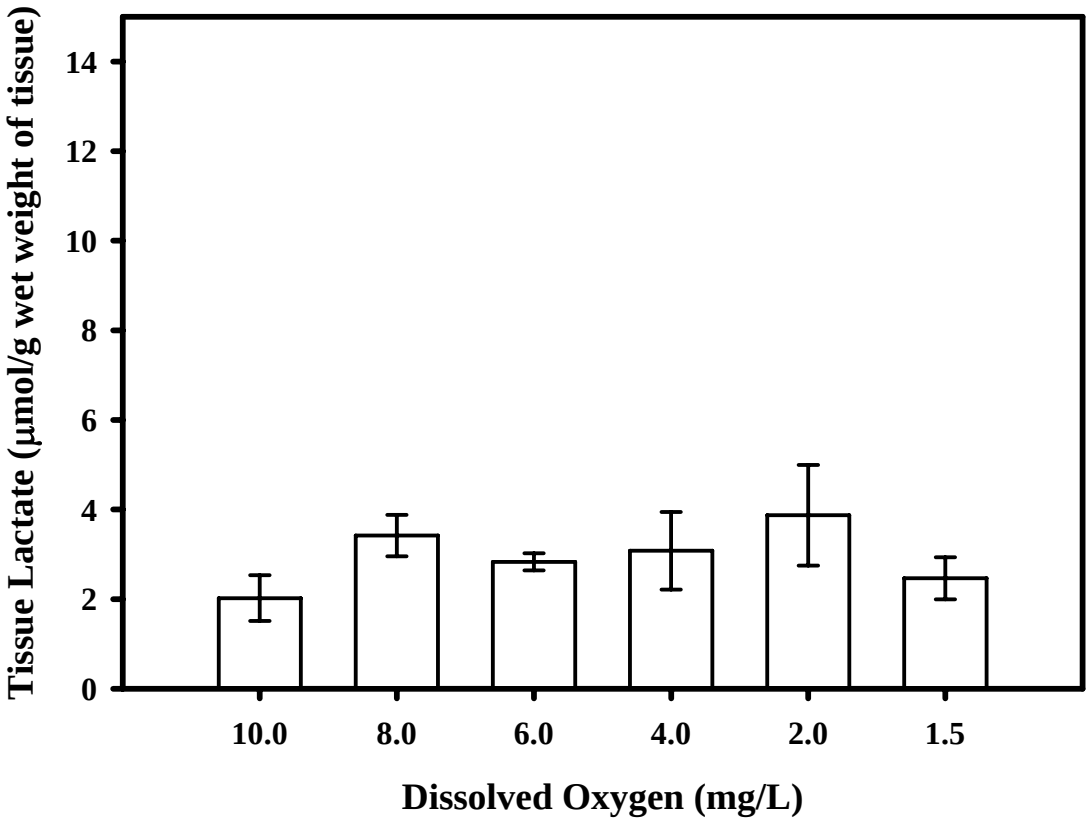
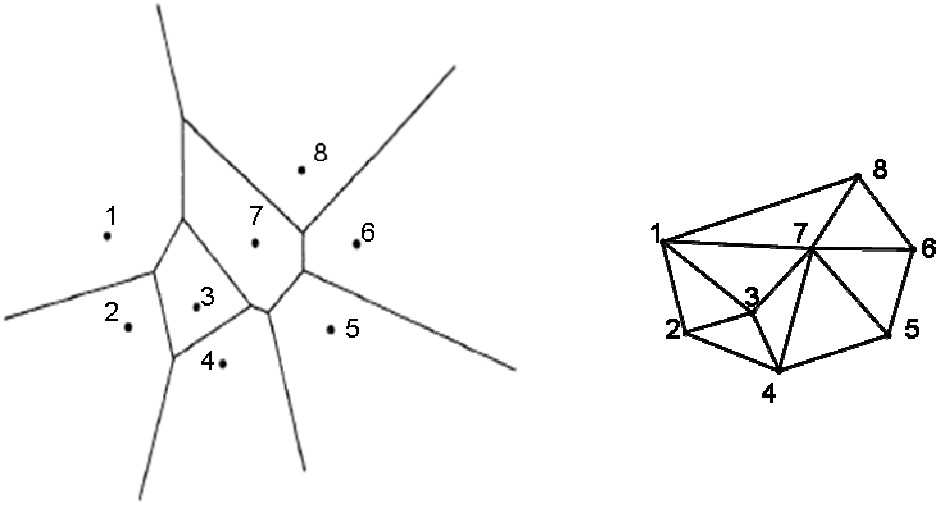


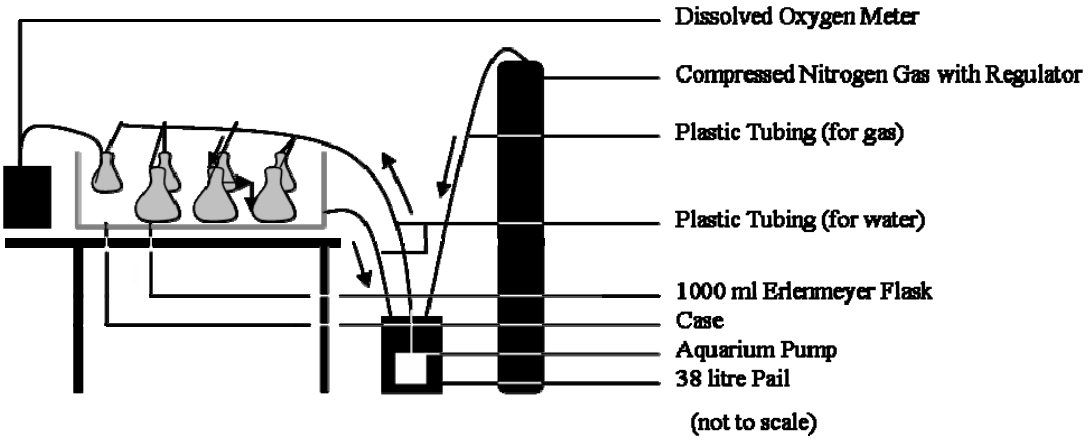
Figure 3.6. Tissue lactate concentration (µmol/g wet weight of tissue) of juvenile largemouth bass exposed to 10.0, 8.0, 6.0, 4.0, 2.0 and 1.5 mg/L concentrations of dissolved oxygen at 5°C for one hour. No statistical difference was found across treatments, and n = 6 fish per treatment (one-way ANOVA; $P > 0.05$).

Appendix

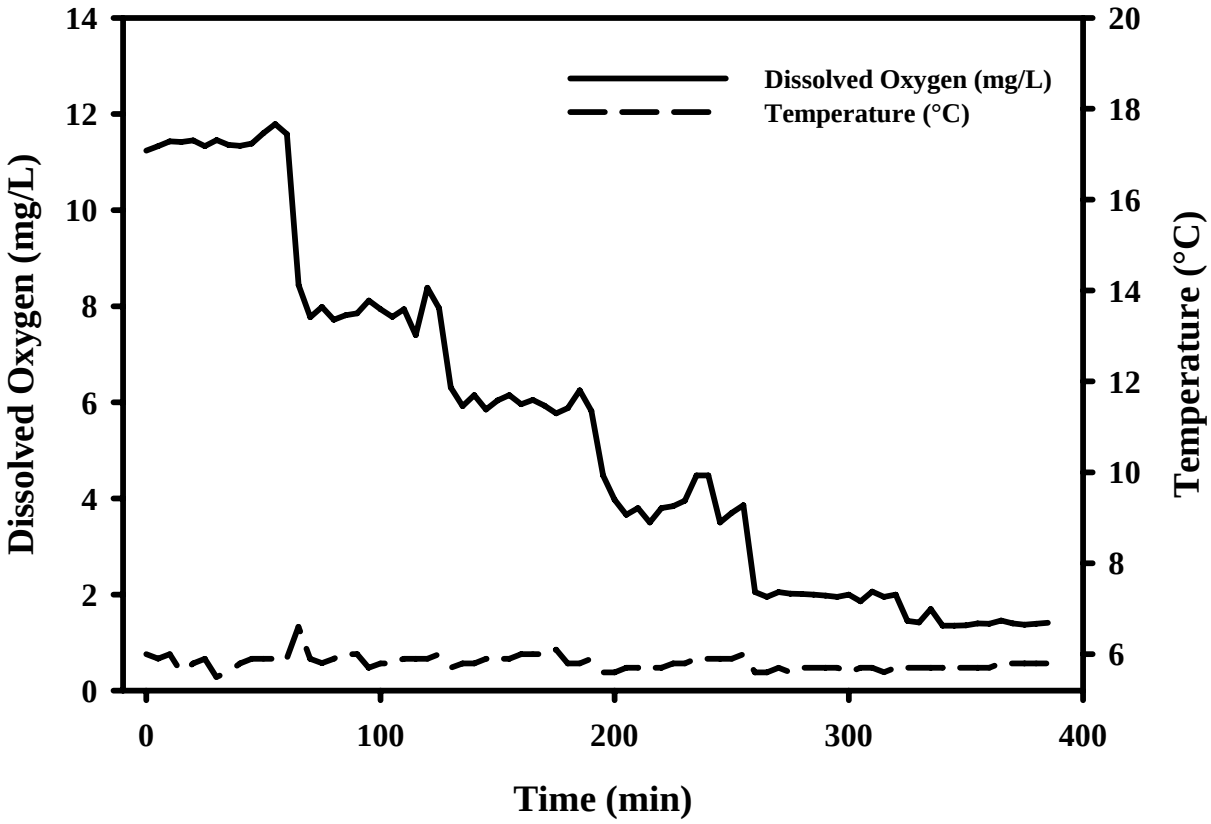
Appendix 3.1. Thiessen Polygons

Given a set of distinct points in a plane, we can assign a portion of the plane or a "territory" which consists of all areas in the plane that are closest to each point. These convex regions are known as *Voronoi* or *Thiessen* polygons. These areas will share boundaries and be convex. The construction of these polygons is straightforward. The lines joining each set of contiguous points (those that will share a boundary) are connected. The perpendicular bisectors of these points will always meet other perpendicular bisects in groups of three, or will intersect the boundary of the region. It is a characteristic of the construction that the triangles produced by connected neighbouring points are as close to equilateral as possible. An example is provided in the figure (adapted and modified from Aurenhammer 1991) below.

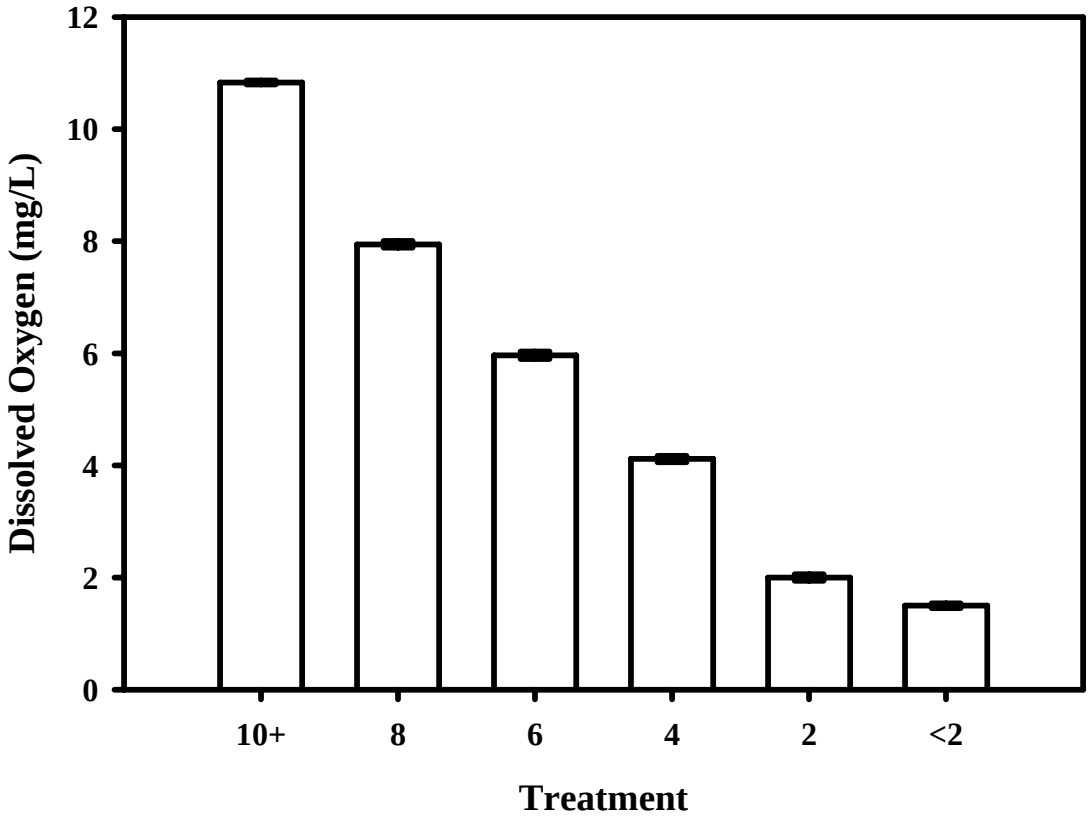




Appendix 3.2. Experimental apparatus (not to scale) used to expose 6 largemouth bass per experiment to deoxygenated water. Water, deoxygenated by the compressed nitrogen gas was pumped from the pail to each Erlenmeyer flask. Water spilled through the Erlenmeyer flasks into the case and was returned to the pail via a plastic tube attached to a hole in the case. Dissolved oxygen was monitored using a dissolved oxygen meter (YSI Inc., Yellow Springs, Ohio) and dissolved oxygen concentrations in the circulating water were adjusted accordingly using a regulator attached to the compressed nitrogen gas cylinder.



Appendix 3.3. Dissolved oxygen (mg/L) and temperature (°C) of water exposed to 6 juvenile largemouth bass during the 6 hour behavioural experiment.



Appendix 3.4. Dissolved oxygen (mg/L) of water used during the exposure to 6 juvenile largemouth bass per treatment during the physiological experiment.

Chapter 4. General Discussion

Recently, new developments in biotelemetry and laboratory techniques have begun to reveal winter's effect on freshwater fishes. It is important to link these two types of study because they both offer insight into organismal biology, which can in turn be scaled to entire populations and communities. It was the purpose of this thesis to complete both biotelemetry and laboratory studies to examine the winter ecology of a freshwater fish. Specifically, I examined the relationship between cold temperature and both swimming activity and performance, as well as the relationship between winter hypoxia and habitat selection using largemouth bass as a model.

Findings and Implications

In chapter 2, activity and three types of swimming speeds were analyzed across seasons using both biotelemetry and swim tunnels. Previous studies have measured swimming performance using either swim tunnels or biotelemetry, with most of the research being completed on salmoides (e.g. Gregory & Wood 1998; Hinch & Bratty 2000). Before now, juvenile largemouth bass have been assessed in swim tunnels (Farlinger & Beamish 1977; Kolok 1992) and using gross scale biotelemetry (Raibley et al. 1997). However, in this study, by using fine scale biotelemetry and assessing swimming performance in a swim tunnel across seasons I was able to compare the effect of seasonal temperature changes on three different swimming types and assess possible effects of seasonal acclimatization. Activity decreased as ambient water temperatures decreased (2.5 cm/s @ 25.0°C to 0.6 cm/s @ 4.0°C), but recovered slightly late in the winter period, possibly indicating acclimatization to winter conditions (0.6 cm/s increased to 0.8 cm/s). Likewise, prolonged swimming, or U_{crit}, decreased as water temperature

cooled (61.1 cm/s @ 25.0°C to 32.9 cm/s @ 7.5°C) and so did burst swimming at 3.0 body length/s (19.7 s to 2.7 s), which were assessed only in the swim tunnel. My unique telemetry findings indicate that swimming activity decreases with temperature, and winter acclimatization should be considered when future studies examine *in situ* swimming activity.

In chapter 3, I demonstrated that largemouth bass behavioural responses to winter hypoxia in the lab can be equated to winter habitat selection. Previous research in laboratory settings demonstrated marked avoidance of water with below 2 mg/L of dissolved oxygen (Burleson et al. 2001), but no studies have demonstrated that laboratory dissolved oxygen thresholds are applicable in the wild, with the exception of one gross scale telemetry study (Raibley et al. 1997). For the first time, I documented that individual largemouth bass during the winter possibly select their habitat using dissolved oxygen, but spend considerable time in habitat with intermediate levels of dissolved oxygen: more fish were found in areas of the lake with higher dissolved oxygen and not in areas below 2 mg/L. In the laboratory portion of the study I found typical avoidance behaviours (yawning, 0.00 to 2.00 per two minutes of observation, n = 6; vertical movement, 0.00 to 1.15 per two minutes of observation, n = 6) increased when water was below 2 mg/L, but lactate did not build up (2.02 to 3.87 μmol/g wet weight of tissue, n = 6 per treatment). These findings lend support to the fundamental niche concept; as well they suggest that physiological consequences of intermediate habitat in the wintertime are minimal (Hutchinson 1957; Huey 1991), as long as complete anoxia is not reached.

In general, I found that largemouth bass decrease winter activity more as a behavioural change than as a physiological change and dissolved oxygen is a much more important environmental factor during the winter than in other seasons. These findings

are a result of combining biotelemetry and laboratory methods and are strongly supported by previous studies investigating the winter ecology of freshwater fishes. Also, these results will further research on the winter ecology of freshwater fishes because it is still unclear to what extent winter acclimatization influences locomotor capacity in the wild and to what extent physiology influences habitat selection during the winter.

Future Research

In this thesis, I presented data combining biotelemetry and laboratory studies to assess winter ecology of fish, specifically largemouth bass. Recent work has substantiated the importance of understanding the winter ecology of fish because of growing concern about global climate change (Carpenter et al. 1992; Somero 2005; Suski & Ridgway in press). Global climate change could increase winter hypoxia because of increased eutrophication and will alter aquatic thermal regimes (Eaton & Scheller 1996). More research is needed to understand 1) the relationship between over-wintering habitat and reproductive success, as well as 2) the effect of changing water flow rates to fish during the winter. Another important need is 3) the linking of individual physiological changes calculated in the laboratory to large scale biotelemetry studies. By integrating biotelemetry and laboratory techniques to understand the behavioural and physiological responses of fish to changing climate, solutions to the damage climate change could have on fish can be made.

Currently, there are a number of new developments which could benefit from the integration of biotelemetry and laboratory techniques. In terms of biotelemetry, the Canadian Foundation for Innovation has committed $35 million to the installation of a

global ocean tracking network. The project is led by Dr. Ron O'Dor and an international consortium of scientists and industry partners and is an extension of the Pacific Ocean Shelf Tracking (POST) array led by Dr. David Welch. The initiative of the project is to track all types of marine life ranging from salmon to turtles to whales. The five themes of the project are: ocean physics and modeling; the biology and behaviour of highly migratory marine living resources; the impact of climate change; resource management; and the international social and legal framework for oceans. This project would be more beneficial to the oceans if scientists using the eco-physiology "tool-box" were involved, as eco-physiological correlates could be made with environment, animal movement, migration, life span, and fitness (Wikelski & Cooke 2006).

Biotelemetry might also prove beneficial if coupled with new developments in laboratory techniques. Stable isotopes, endocrinology, physiological genomics, and environmental toxicology could all be incorporated with biotelemetry. Stable isotopes can be used to assess diet and movement patterns and have only recently been used with respect to fish; this new tool is revealing the importance of habitat during different life stages of fishes and if fish can be tracked to specific locations, more accurate assessments of life stages and habitat links can be made (Cunjak et al. 2005). Endocrinology is being used to assess the impact of recreational disturbances on terrestrial mammals and could be used to assess similar stress (i.e. boat traffic) on free-ranging fishes (Creel et al. 2002); fully submerged hydrophone arrays (e.g., the one used in this thesis) could track boat traffic and fish movement with relative ease. Physiological genomics linked with biotelemetry offers the possibility of investigating the function of gene products in the context of whole organisms in natural environments (Ammar et al. 2000; K. Miller, Department of Fisheries and Oceans, personal communication). Lastly, environmental

toxicology studies could use biotelemetry to link loading of contaminants to movement patterns and post-exposure survival (Ben-David et al. 2002). Linking biotelemetry with new developments in laboratory techniques will greatly benefit conservation and management strategies of all fishes.

Summary and Conclusions

1. **Studying the winter ecology of centrarchids has been a challenge in the past because of obvious constraints associated with winter field work.** Many studies have attempted to simulate winter conditions in laboratory settings to gain further insights into changes in fish behaviour and physiology that are associated with cold temperature and hypoxia. However, few studies have documented any *in situ* behavioural responses of fish faced with winter conditions.

2. **Results from biotelemetry and swim tunnel experiments show that swimming activity and performance decrease as ambient water temperatures decrease.** Using telemetered largemouth bass, I found that both activity and swimming performance decreased as temperature decreased. Likewise, swim tunnel assessments showed that largemouth bass swimming performance also decreased with ambient water temperature. Surprisingly, swimming activity increased as stable cold temperatures persisted throughout the winter, indicating that seasonal timing may be an important factor when assessing swimming performance. Because I combined both studies, I was able to examine all three types of

swimming performances (sustained, prolonged, and burst) across changing temperatures.

3. **When biotelemetry and laboratory studies were coupled, findings indicated overwintering largemouth bass avoided habitats with less than 2.0 mg/L of dissolved oxygen, but used intermediate habitats.** Using telemetered fish and measurements of dissolved oxygen across an entire lake throughout the winter period, I found that fish avoided areas with less than 2 mg/L of dissolved oxygen. Likewise, laboratory fish demonstrated avoidance responses (i.e., yawning and vertical movement) when exposed to water with dissolved oxygen concentrations near 2 mg/L. These data indicate that 2 mg/L is close to the behavioural threshold for largemouth bass. Also, fish were found to inhabit intermediate habitats during the late winter period and to use depths considered close to anoxic. These data support the *n-dimensional* niche concept but not the concept of physiological consequences associated with habitat selection, as no change to tissue lactate was found when fish were exposed to a range of dissolved oxygen (10 to 1.5 mg/L). This study represents– for the first time– the combination of biotelemetry and laboratory data to assess habitat selection in fish.

4. **Coupling biotelemetry and laboratory studies on the winter ecology of freshwater fishes is important for the field of conservation physiology and the use of laboratory techniques to study conservation.** Field and laboratory assessments of the effect of the environment on individual organisms will help

wildlife managers and shareholders mitigate the problems of a changing environment and increased anthropogenic stresses.

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