The Influence of Dissolved Oxygen on Winter Habitat Selection by Largemouth Bass: An Integration of Field Biotelemetry Studies and Laboratory Experiments

C. T. Hasler[1,2,*]
C. D. Suski[3]
K. C. Hanson[2]
S. J. Cooke[2]
B. L. Tufts[1]
[1]Department of Biology, Queen's University, Kingston, Ontario K7L 3N6, Canada; [2]Fish Ecology and Conservation Physiology Laboratory, Department of Biology and Institute of Environmental Science, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada; [3]Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, 1102 South Goodwin Avenue, Urbana, Illinois 61801

Accepted 6/13/2008; Electronically Published 1/20/2009

ABSTRACT

In this study, field biotelemetry and laboratory physiology approaches were coupled to allow understanding of the behavioral and physiological responses of fish to winter hypoxia. The biotelemetry study compared dissolved oxygen levels measured throughout the winter period with continually tracked locations of nine adult largemouth bass obtained from a whole-lake submerged telemetry array. Fish habitat usage was compared with habitat availability to assess whether fish were selecting for specific dissolved oxygen concentrations. The laboratory study examined behavioral 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 behavior (i.e., yawning and vertical movement) at <2.0 mg/L of dissolved oxygen but no change in tissue lactate, an indicator of anaerobic metabolism. 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 behavioral 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 behavior.

* Corresponding author; e-mail: chasler@connect.carleton.ca.

Physiological and Biochemical Zoology 82(2):143–152. 2009. 1522-2152/2009/8202-8020$15.00
DOI: 10.1086/591806

Introduction

Aquatic systems are spatially heterogeneous for a number of variables across a range of scales. 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 result in patches of optimal habitat mixed with patches of suboptimal 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, 1965; Fretwell and Lucas 1970).

One important environmental resource that influences fish habitat selection and many physiological processes is dissolved oxygen (Hughes 1973; Kramer 1987). Fish respond to reduced levels of dissolved oxygen in a variety of ways. Typically, chemoreceptors sense a decrease in ambient oxygen, and some physiological responses occur (e.g., increases in ventilation rate and amplitude; Perry and Gilmour 2002). Next, a behavioral response occurs, which may include habitat shifts; it is followed by the use of air-breathing organs (if present) and an increased use of surface respiration (Kramer 1987). After these behavioral responses, fish often exhibit more physiological responses to reduced levels of dissolved oxygen, including a decrease in cardiac output (Furimsky et al. 2003). Finally, if no other response is adequate, fish will switch to relying on anaerobic metabolism to meet energy demands. Anaerobic metabolism produces far less ATP relative to aerobic mechanisms, and negative consequences of anaerobic metabolism include the production of lactate and a decrease in blood pH, both of which must be actively cleared on return to an oxygenated environment (Bennett 1978; Wendelaar Bonga 1997; Furimsky et al. 2003; Martinez et al. 2006). The behavioral, physiological, and biochemical responses of fish to hypoxia during winter conditions are not fully understood, and cold temperature may be an important covariable that influences behavioral and/or physiological outcomes because of its importance in determining rates of reactions.

During winter in lakes located at high latitudes, dissolved oxygen is often less abundant than in summer, and this reduction in dissolved oxygen can influence fish behavior and habitat selection (Suski and Ridgway 2009). Many northern temperate lakes can experience hypoxia (or even anoxia) during winter; 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 (Greenbank 1945; Cooper and Washburn 1949). Winter hypoxia has previously been shown to influence fish behavior, 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, few studies have measured the impact of winter hypoxia on seasonal fish habitat selection or linked field- and laboratory-based responses to winter hypoxia.

To quantify 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 because they allow the isolation 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. Biotelemetry, or the remote monitoring of free-ranging individuals, provides clues about why fish choose particular habitats over others and allows for an assessment of environmental- and individual-level variables (Lucas and Baras 2000; Cooke et al. 2004).

The goal of this study was to use a combined approach involving both biotelemetry- and laboratory-based experiments to better understand the influence of winter hypoxia on the behavior, 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 lakewide dissolved oxygen concentrations. For the laboratory study, the behavioral and physiological responses of fish to progressive hypoxia at winter temperatures were quantified. The combined results from these two studies will improve our understanding of how environmental variables influence habitat selection in fishes and will also allow a direct coupling between field- and laboratory-based observations.

Material 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 (44°31′N, 76°22′W; Fig. 1), is an 8.3-ha freshwater lake with a naturally self-sustaining population of largemouth bass. There is little flow of water into and out of the lake, resulting in virtually no immigration or emigration. Dissolved oxygen and temperature were sampled at 22 sites throughout Warner Lake on seven different occasions from November 2, 2005, to April 12, 2006, using a submersible dissolved oxygen and temperature probe (model 55, YSI, Yellow Springs, OH; Fig. 1). 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 as a result of depth differences; dissolved oxygen concentrations for all depths were combined to generate a single mean oxygen concentration for each site. Dissolved oxygen sampling dates were chosen to ensure that the lake was sampled during the pre–ice cover period (November 2, 2005; December 1, 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 cover period (April 12, 2006). Water temperature was varied between 4.0° and 5.5°C during periods of ice cover and between 4.0° and 6.5°C during non–ice cover periods. 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 the movement of largemouth bass in Warner Lake in relation to dissolved oxygen distributions, a stationary acoustic telemetry array consisting of a code division multiple access (CDMA)-based telemetry system (Lotek MAP_600, Lotek Wireless, Newmarket, Ontario) was used. The system and its accuracy are summarized by Cooke et al. (2005) and Hanson et al. (2007). Briefly, the array consists of 13 hydrophones distributed to allow for submeter 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 regularly collected and downloaded to personal laptop computers for processing and filtering in BioMAP (ver. 2.1; Lotek Wireless), which uses a wavelet analysis to remove uncorrelated noise from time series (Hess-Nielsen and Wickerhauser 1996; Akay and Mello 1997).

Study Animals. For the field study, nine Warner Lake resident largemouth bass (399.0 ± 6.5 mm) were captured using standard hook-and-line angling and surgically implanted with CDMA-enabled acoustic tags that emitted a position signal every 60 s (Lotek CTPM11–18, 11 × 60 mm, repetition rate 59.5 s, life expectancy 3 yr). All transmitters were continuously tracked throughout the study periods. Details of the surgery are given by Cooke et al. (2003). For this, fish were anesthetized until unresponsive using a 60-ppm clove oil bath for 6 min. They were then placed on a foamed surgical platform, and their gills were continuously irrigated with a flowing bath of 30-ppm clove oil. A small incision was made on the fish's abdomen and the tag was inserted into the body cavity. The incision was then sutured closed using PDS-II absorbable sutures (3/0, sterile; Ethicon, Somerville, NJ), and fish were placed in a cooler of fresh lake water to recover (<1 h) before release and the initiation of the tracking study.

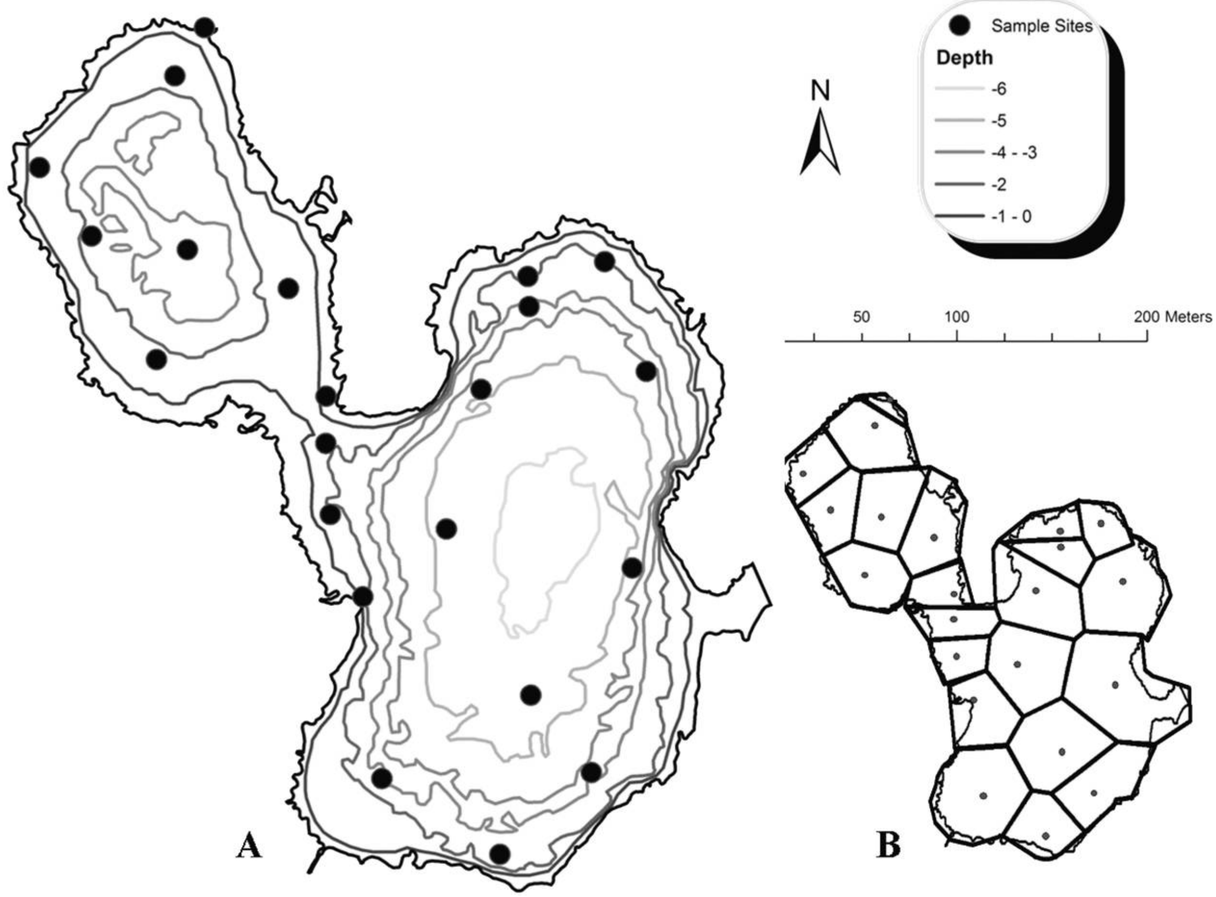

Figure 1. *A*, Dissolved oxygen sample sites (*black circles*). *B*, Thiessen polygons used. Black lines indicated the Warner Lake shoreline, and gray lines correspond to lake depth contours (m).

Data Analysis. To quantify variation in dissolved oxygen concentration across sites and sampling points, a Levene's test was performed across sampling periods to test for unequal variances, followed by a nonparametric Kruskal-Wallis test and a nonparametric post hoc test to identify specific differences (Zar 1984).

When analyzing data from the biotelemetry study, we were interested in comparing ambient dissolved oxygen concentrations with fish location and understanding whether location preference was influenced by dissolved oxygen availability. For this, 500 randomly chosen x and y positions for each fish were chosen for 5 d centered around the dissolved oxygen sampling day and were plotted in ArcGIS (ver. 9.1; ESRI, Redlands, CA; De Solla et al. 1999). Thiessen polygons using the dissolved oxygen sample sites as center points were then created using ArcGIS. When Thiessen polygons are used, all areas of the lake can be assigned to the nearest sample site (center point; therefore a dissolved oxygen concentration; Aurenhammer 1991). Next, the numbers of fish positions (per fish, per day) in each Thiessen polygon were counted, and direct comparisons 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 data set can be affected. 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 comparisons of dissolved oxygen with fish position 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 lower amounts of dissolved oxygen than they likely should have been.

To assess whether fish locations were related to the concentration of dissolved oxygen, χ^2 goodness-of-fit tests were used to compare the number of fish locations documented in the different Thiessen polygons (observed) with the theoretical number of fish locations expected if all fish locations were uniformly distributed across all Thiessen polygons (expcted; Zar 1984). Significantly greater or fewer numbers of fish observed for each polygon would mean that the largemouth bass were either inhabiting or avoiding different areas of the lake on the basis of oxygen availability. If numbers of fish locations were not significantly different from expected values, then fish habitat preferences were not influenced by dissolved oxygen.

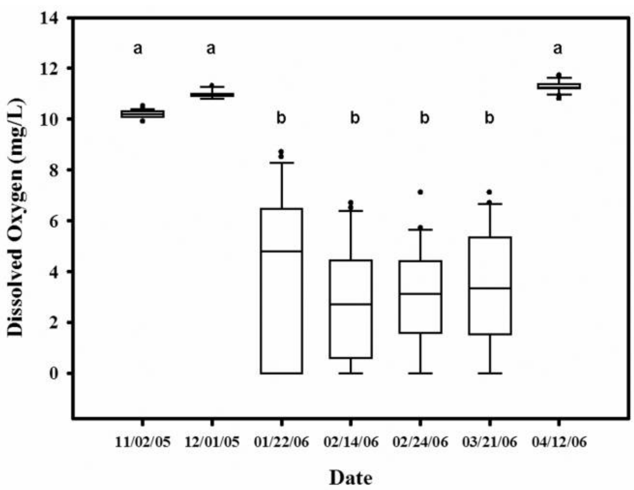

Figure 2. Concentrations of dissolved oxygen (mg/L) sampled at Warner Lake on dates given. Horizontal bars in box plots indicate the tenth, twenty-fifth, fiftieth, seventy-fifth, and ninetieth percentiles, and black circles represent outliers. Dates marked with different letters are statistically different (Kruskal-Wallis test: $\chi^2_6 = 119.98$, $P < 0.0001$; nonparametric post hoc $P < 0.05$).

It is important to note here that during nonlimiting periods, fish locations are distributed throughout the shallow and the deeper basin. However, during the winter period when dissolved oxygen is limiting, fish are typically found only in the deeper basin of Warner Lake (Hasler et al. 2007).

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

Laboratory Study

The laboratory component of this study was designed to quantify both the behavioral responses of largemouth bass to progressive hypoxia and the amount of anaerobic respiration that was occurring, evidenced by lactate production in white muscle. For this, 42 juvenile largemouth bass (size range: 77–110 mm total length) were obtained from Pure Springs Trout Farm (Shannonville, ON) and held (without food) in a tank of dechlorinated 5°C tap water for 2 wk before the experiments. The 42 fish were divided into seven groups of six fish each (one group for behavioral observation and six groups for lethal sampling).

To assess behavioral changes caused by exposure to progressive reductions in dissolved oxygen concentration, we placed six largemouth bass in separate 1-L Erlenmeyer flasks. Graded hypoxia was achieved by first gassing water (5°C) in a central basin with compressed nitrogen gas (99.95% pure); this deoxygenated water (5°C) was 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, and dissolved oxygen concentrations in this circuit were monitored with a dissolved oxygen meter (model 55, YSI). Fish were exposed to different concentrations of dissolved oxygen for 1 h. After 1 h 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; Hughes 1973) and the amount of time spent moving toward the top of the flask (above the midline of the flask) was quantified using a stopwatch. After 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 concentration of dissolved oxygen had been established and stabilized (~30 s), fish were left at this concentration for 1 h 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 six dissolved oxygen concentrations. The dissolved oxygen concentrations generated were as follows: 11.44 ± 0.04, 7.93 ± 0.08, 6.01 ± 0.05, 3.92 ± 0.02, 1.99 ± 0.02, and 1.42 ± 0.03 mg/L ($n = 20$ measurements for each concentration).

To quantify the production of lactate in white muscle during progressive hypoxia, largemouth bass were first exposed to the different dissolved oxygen concentrations for 1 h as described above using the following dissolved oxygen concentrations: 10.83 ± 0.04, 7.94 ± 0.06, 5.96 ± 0.07, 4.11 ± 0.06, 2.00 ± 0.06, and 1.49 ± 0.04 mg/L ($n = 20$ measurements for each concentration). Fish were then anesthetized in a buffered mixture of anesthetic (tricaine methane sulfonate [250 mg/L] and sodium bicarbonate [$NaHCO_3$, 500 mg/L]) by quickly pouring the fish from the Erlenmeyer flask into a container filled with anesthetic (Summerfelt and Smith 1990). Once fish had completely lost equilibrium, most of the epaxial white muscle above the lateral line was excised with a razor blade, freeze clamped in precooled aluminum 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 by Suski et al. (2003) and the enzymatic methods of Lowry and Passonneau (1972) using a 96-well plate spectrophotometer (Spectra MAX Plus; Molecular Devices, Sunnyvale, CA). This sampling protocol was repeated using separate groups of fish until six fish from each dissolved oxygen concentration had been sampled.

Differences in behavioral responses across dissolved oxygen concentrations were assessed using a one-way repeated-measures ANOVA, 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 ANOVA, followed by a Tukey's HSD test (Zar 1984). All tests were performed using JMP 6.0.2 software (SAS Institute). The level of significance (α) for all tests was 0.05, and all means are reported ± 1 SE where appropriate.

Results

Biotelemetry Study

During dates before ice cover (sample dates in November and December), all 22 sample sites had dissolved oxygen concentrations >10 mg/L (Fig. 2). Once complete ice cover occurred (sample dates from January to March), dissolved oxygen concentrations ranged from 0 to 8.5 mg/L across all 22 sites, and the variance in oxygen concentration across sites was significantly different from that during non–ice cover periods (Levene's test; $F_{6,144} = 28.15$, $P < 0.001$; Fig. 2). Once the ice cover melted in April, dissolved oxygen concentrations increased to pre–ice cover values (Fig. 2).

During periods of ice cover when dissolved oxygen concentrations were variable across sample sites, habitat selection was found to be dependent on dissolved oxygen. The observed distributions of fish were significantly different from theoretical expected homogenous distributions (January 20–24, February 12–16, February 22–26, March 19–23: for all χ^2 goodness-of-fit tests, $P < 0.0001$; Fig. 3). Also, telemetered fish consistently inhabited polygons with intermediate dissolved oxygen concentrations (Fig. 3). From January 20 to January 24, 49% of largemouth bass locations were counted in areas with dissolved oxygen between 6 and 7 mg/L, even though portions of the lake contained dissolved oxygen concentrations that were below 2 and above 9 mg/L. From February 12 to February 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 to 7 mg/L (Fig. 3) but not in locales with less than 2 mg/L of dissolved oxygen. Between February 22 and February 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 (Fig. 3). From March 19 to March 23, fish locations were more dispersed across a wider range of dissolved oxygen: fish locations were counted in areas of the lake with no detectable dissolved oxygen (two of nine fish) and in areas measured to have up to 7 mg/L of dissolved oxygen (Fig. 3). During this period, 81% of fish locations were counted in areas with 3–6 mg/L of dissolved oxygen.

Laboratory Study

Juvenile largemouth bass exhibited pronounced behavioral responses when exposed to increasingly anoxic water. After exposure to concentrations of 11.44, 7.93, 6.01, or 3.92 mg/L

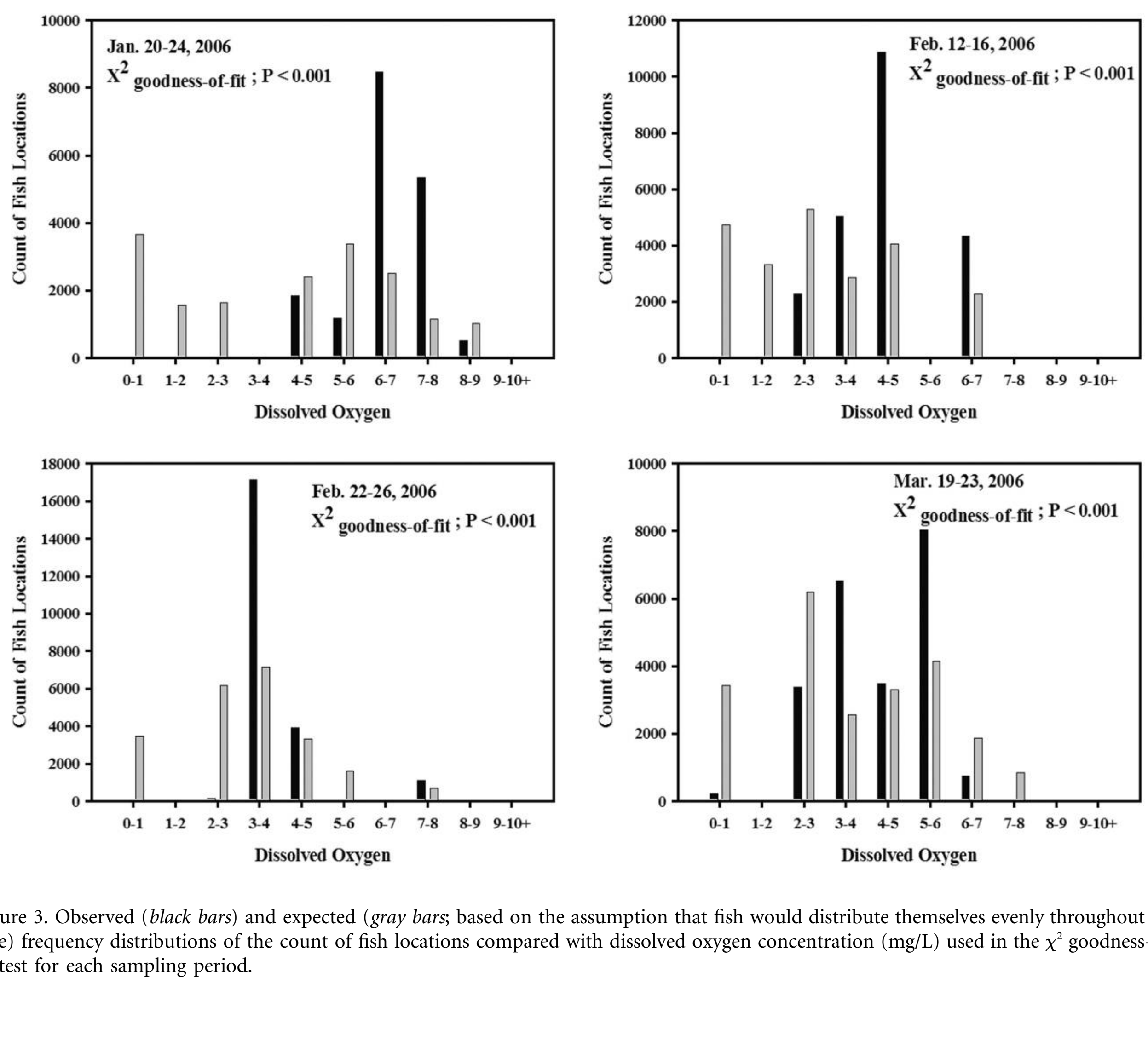

Figure 3. Observed (*black bars*) and expected (*gray bars*; based on the assumption that fish would distribute themselves evenly throughout the lake) frequency distributions of the count of fish locations compared with dissolved oxygen concentration (mg/L) used in the χ^2 goodness-of-fit test for each sampling period.

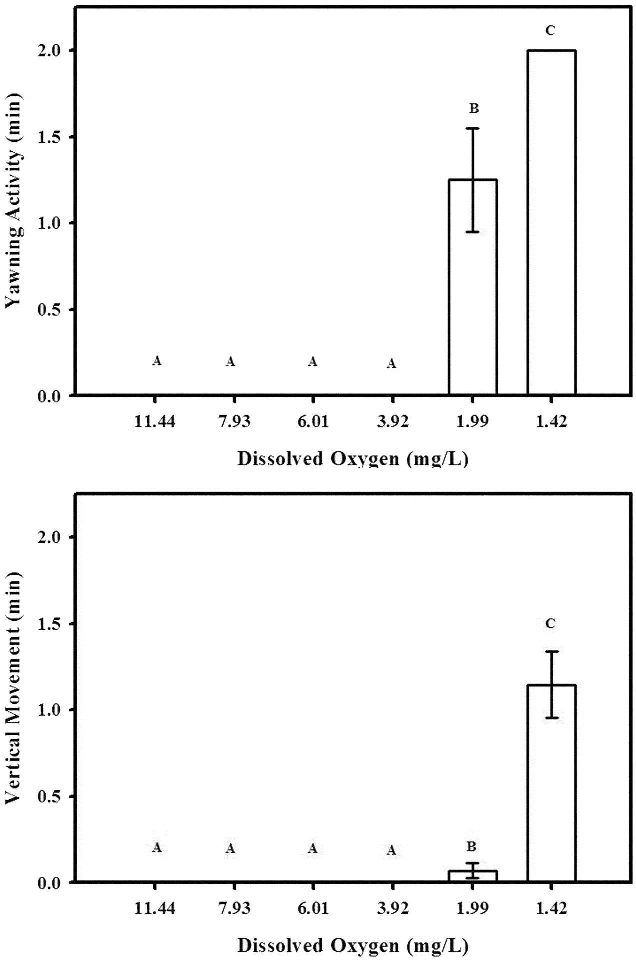

Figure 4. Mean time ± SE (min) spent yawning (*top*) and moving vertically (*bottom*) during a 2-min period for six juvenile largemouth bass exposed to dissolved oxygen concentrations of 11.44 ± 0.04, 7.93 ± 0.08, 6.01 ± 0.05, 3.92 ± 0.02, 1.99 ± 0.02, and 1.42 ± 0.03 mg/L at 5°C. Bars marked with different letters are statistically different (repeated-measures ANOVA; Tukey's HSD test; $P < 0.05$).

dissolved oxygen, yawning activity was not exhibited by any fish (Fig. 4). However, when the same bass were exposed to water with 1.99 mg/L dissolved oxygen, fish yawned for ~60% of the 2-min monitoring period (repeated-measures ANOVA: $F_5 = 50.41$, $P < 0.0001$; Fig. 4, *top*); after exposure to water with 1.42 mg/L dissolved oxygen, the fish yawned for the entire 2-min monitoring period. 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 1.99 mg/L, at which time there was a significant increase in the frequency of vertical movements relative to samples in more oxygenated water (~0% of the 2-min monitoring period; repeated-measures ANOVA; $F_5 = 32.98$, $P < 0.0001$; Tukey's HSD test: $P < 0.05$; Fig. 4, *bottom*). After 1 h of exposure to dissolved oxygen concentrations of 1.42 mg/L, fish spent ~1.2 min moving vertically (Tukey's HSD test; $P < 0.05$).

Juvenile largemouth bass did not demonstrate a significant increase in white muscle lactate concentrations when exposed to increasingly hypoxic water (ANOVA: $P > 0.05$; Fig. 5). Tissue lactate was 2.95 ± 0.28 μmol/g of wet weight of tissue ($n = 36$) for all experimental oxygen treatments, and no significant variation in lactate concentration 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, mean dissolved oxygen concentrations ranged from 10.2 to 11.3 mg/L; the twenty-fifth and seventy-fifth percentiles for dissolved oxygen concentration on these dates were 10.1 and 11.4 mg/L, respectively. Hence, fish were exposed only to water with dissolved oxygen concentrations greater than 10 mg/L. In contrast, during the ice-cover sample dates, mean dissolved oxygen concentrations across all of Warner Lake ranged from 2.7 to 3.8 mg/L, with the twenty-fifth and seventy-fifth percentiles on these dates 0.6 and 6.5 mg/L, respectively. Consequently, fish had access to water with a greater range of concentrations of dissolved oxygen available at these times, and 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 periods of ice cover, is a result of a variety of inherent characteristics of temperate lakes at high latitudes. These include ice cover, organic decomposition, and

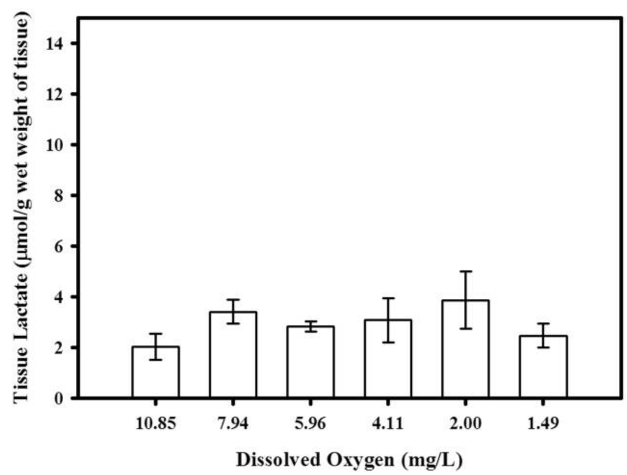

Figure 5. Tissue lactate concentration (μmol/g wet weight tissue) of juvenile largemouth bass exposed to dissolved oxygen concentrations of 10.83 ± 0.04, 7.94 ± 0.06, 5.96 ± 0.07, 4.11 ± 0.06, 2.00 ± 0.06, and 1.49 ± 0.04 mg/L at 5°C for 1 h. No statistical difference was found across treatments, and $n = 6$ fish per treatment (one-way ANOVA; $P > 0.05$).

overcrowding of respiring animals, to name a few (Greenbank 1945; Mathias and Barica 1980; Meding and Jackson 2003).

The combined results of the biotelemetry and the laboratory studies demonstrate that largemouth bass show behavioral changes when exposed to water with dissolved oxygen concentration below 2 mg/L during winter. Sites in Warner Lake that had dissolved oxygen concentrations <2 mg/L contained significantly fewer largemouth bass than would be expected if fish were uniformly distributed with respect to oxygen concentration, while significantly more largemouth bass than expected were found in areas with >2 and <6 mg/L of dissolved oxygen. In addition, during the laboratory study, largemouth bass significantly increased yawning (or gill flaring) and vertical movement behaviors when exposed to water with dissolved oxygen concentrations of 1.99 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 less than 2 mg/L of dissolved oxygen, presumably as they attempt to seek out habitat that is more oxygenated (Scherer 1971; Petrosky and Magnuson 1973). In our laboratory study, yawning activity (or gill flaring) increased at 1.99 mg/L, probably in order to increase water flow across gill lamellae in an effort to increase the amount of dissolved oxygen entering the bloodstream (Hughes 1973; Randall 1982; Perry and Gilmour 2002). In winter field studies, Raibley et al. (1997) measured the dissolved oxygen at specific locations for multiple telemetered largemouth bass 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 concentrations at fish locations, and they did not quantify dissolved oxygen throughout the river system. Largemouth bass avoiding water with poor levels of dissolved oxygen would be advantageous because prolonged exposure to these waters could lead to costly, inefficient anaerobic metabolism, suffocation, and even death (Greenbank 1945; Cooper and 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 below which behavioral changes in overwintering 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 period of ice cover, fish were found to inhabit sites with intermediate levels of dissolved oxygen (concentrations between 6 and 2 mg/L), even though water with 7, 8, and 9 mg/L of dissolved oxygen was available. Previous 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 (Hutchinson 1957, 1965; Chapman 1966; Tracy and Christian 1986). Fry (1971) suggested that there are at least seven important factors in a fish's niche (temperature, dissolved oxygen, toxicity, metabolites, food, salinity, and carbon dioxide), while more recent work suggests that other physical and biotic characteristics (such as cover, water velocity, depth, territories, and aggregations) are equally important to niche generation (Stott and Cross 1973; Suthers and Gee 1986; Kramer 1987; Spoor 1990; Heggenes et al. 1999; Hasler et al. 2007). If some or all of these parameters are assessed by fish in Warner Lake when they make habitat decisions, the choice of intermediate oxygen patches must have benefits that outweigh the advantages associated with inhabiting more oxygenated water. Specifically, in addition to oxygen, factors such as proximity to conspecifics (Breder and Nigrelli 1935; Hasler et al. 2007), proximity to cover, and/or prey abundance may affect selection of habitat by largemouth bass. Fish in this study chose not to inhabit areas with the highest dissolved oxygen concentrations and selected areas with lower amounts of dissolved oxygen, which suggest that fish are using other environmental variables in conjunction with dissolved oxygen to make habitat choices.

Although there was an avoidance of areas with dissolved oxygen <2 mg/L, two of nine fish spent some time in such water during the March sampling period (although only for a few hours per day). Moreover, during the laboratory study, fish did not exhibit increased lactate concentrations in white muscle, despite 1 h 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; two separate studies have concluded that the metabolic rate of largemouth bass during the winter months is greatly reduced and that fish are essentially dormant at that time (Beamish 1970; Crawshaw 1984; Lemons and Crawshaw 1985). In our study, the use of intermediate areas by largemouth bass is not unexpected, since 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 during winter conditions, slightly higher temperatures that are present in areas with low amounts of dissolved oxygen (because of decomposition of organic material) would allow for increased metabolism and activity (Fry 1971; Gee et al. 1978; Burleson et al. 2001). In winter, it may be beneficial for fish to tolerate lower dissolved oxygen concentrations that might be lethal during warmer periods, when the oxygen requirements are relatively higher (Fry 1971). 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. It is evident from the current biotelemetry and laboratory studies that largemouth bass tolerate low levels of dissolved oxygen during winter. In addition, short-term laboratory exposure to low amounts of dissolved oxygen did not facilitate a metabolic change, suggesting that the physiological consequence of winter habitat selection is minimal.

Age and size differences among fish of the same species may influence behavioral and physiological responses to stressors such as exhaustive exercise or low dissolved oxygen (Cech et al. 1979; Petersen and Petersen 1990; Kieffer et al. 1996; Burleson et al. 2001). For example, Kieffer et al. (1996) found a significant positive relationship between the accumulation of muscle lactate, metabolic protons, and body size in brook trout

(*Salvelinus fontinalis*) that were exhaustively exercised (exercise that essentially results in hypoxic conditions in the swimming muscle; Kieffer et al. 1996) but did not find a difference in the anaerobic response to exercise between large and small largemouth bass. In our study, larger fish were used in the biotelemetry study than in the laboratory study because of size limitations related to transmitter implantation. However, regardless of this size difference, both experiments revealed a similar threshold at which a response to hypoxia was generated—approximately 2 mg/L. Specifically, in our laboratory study, yawning and vertical movement began to happen at 1.99 mg/L, while in the telemetry study, fish were rarely found in water with less than 2 mg/L of dissolved oxygen. In a similar result, Burleson et al. (2001) found that regardless of fish size, largemouth bass typically avoided water below 2.4 mg/L during laboratory experiments. Also, they did not find differences in short-term selection for particular areas; larger fish were equally as likely to venture for brief periods of time to areas of low dissolved oxygen concentration when compared to smaller fish (Burleson et al. 2001). Thus, results from previous studies have not documented a size-specific response to anaerobic stressors for largemouth bass (Kieffer et al. 1996; Burleson et al. 2001). Likewise, fish in our biotelemetry and laboratory studies, although different in size, demonstrated similar avoidance responses to cold water with approximately 2 mg/L of dissolved oxygen.

Conclusion

Biotelemetry and laboratory studies conducted to determine the factors affecting behavior and physiology are most often performed independently. In this study, these two approaches were used synergistically to quantify the effect of hypoxia on the behavior and physiology of overwintering largemouth bass. Ambient dissolved oxygen concentration was found to influence not only individual behavioral responses such as increased surface breathing but also habitat selection in the wild. More specifically, largemouth bass telemetered in the field tended to avoid areas with dissolved oxygen concentrations <2.0 mg/L, and laboratory-tested largemouth bass exhibited behavioral responses, such as yawning and vertical movement, when exposed to water with dissolved oxygen levels near 2.0 mg/L. Largemouth bass tended to choose water with intermediate concentrations of dissolved oxygen over the most oxygenated water available, possibly because of multiple abiotic and biotic variables. Overwintering largemouth bass appear to avoid water with less than 2.0 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.

Acknowledgments

The authors would like to thank Chris Moyes, Gerry Barber, Greg Bulté, Rush Dhillon, Vera Li, Connie O'Connor, Andrew Gingerich, and Jane Rodgers, who made comments on an earlier version of this manuscript. David Philipp assisted with fish tagging. Frank Phelan, Floyd Connor, and the staff at the Queen's University Biological Station provided logistical support. This project was supported by Lotek Wireless, and funding was provided by Queen's University, the Illinois Natural History Survey, Canada Foundation for Innovation, Carleton University, and the Natural Sciences and Engineering Research Council.

Literature Cited

Akay M. and C. Mello. 1997. Wavelets for biomedical signal processing. Proc 19th Annu Int Conf IEEE 6:2688–2691.

Aurenhammer F. 1991. Voronoi diagrams: a survey of a fundamental geometric data structure. ACM Comput Surv 23: 345–405.

Beamish F.W.H. 1970. Oxygen consumption of largemouth bass, *Micropterus salmoides*, in relation to swimming speed and temperature. Can J Zool 48:1221–1228.

Bennett A.F. 1978. Activity metabolism of the lower vertebrates. Annu Rev Physiol 40:447–469.

Breder C.M., Jr., and R.F. Nigrelli. 1935. The influence of temperature and other factors on the winter aggregations of the sunfish, *Lepomis auritus*, with critical remarks on the social behaviour of fishes. Ecology 16:33–47.

Burleson M.L., D.R. Wilhelm, and N.J. Smatresk. 2001. The influence of fish size on the avoidance of hypoxia and oxygen selection by largemouth bass. J Fish Biol 59:1336–1349.

Cech J.J., Jr., C.G. Campagna, and S.J. Mitchell. 1979. Respiratory responses of largemouth bass (*Micropterus salmoides*) to environmental changes in temperature and dissolved oxygen. Trans Am Fish Soc 108:166–171.

Chapman D.W. 1966. Food and space as regulators of salmonid populations in streams. Am Nat 100:345–357.

Cooke S.J., B.D.S. Graeb, C.D. Suski, and K.G. Ostrand. 2003. Effects of suture material on incision healing, growth and survival of juvenile largemouth bass implanted with a miniature radio transmitters: case study of a novice and experienced fish surgeon. J Fish Biol 63:1360–1388.

Cooke S.J., S.G. Hinch, M. Wikelski, R.D. Andrews, L.J. Kuchel, T.G. Wolcott, and P.J. Butler. 2004. Biotelemetry: a mechanistic approach to ecology. Trends Ecol Evol 19:334–343.

Cooke S.J., G. Niezgoda, K.C. Hanson, C.D. Suski, R. Tinline, and D.P. Philipp. 2005. Use of CDMA acoustic telemetry to document 3-D positions of fish: relevance to the design and monitoring of aquatic protected areas. Mar Tech Soc J 39: 17–27.

Cooper G.P. and G.N. Washburn. 1949. Relation of dissolved oxygen to winter mortality of fish in Michigan lakes. Trans Am Fish Soc 76:23–33.

Crawshaw L.I. 1984. Low-temperature dormancy in fish. Am J Physiol 246:R479–R486.

De Solla S.R., R. Bonduriansky, and R.J. Brooks. 1999. Eliminating autocorrelation reduces biological relevance of home range estimates. J Anim Ecol 68:221–234.

Farwell M., M.G. Fox, C.D. Moyes, and G. Burness. 2007. Can hypoxia tolerance explain differences in distribution of two co-occurring north temperate sunfishes? Environ Biol Fish 78:83–90.

Fox M.G. and A. Keast. 1991. Effect of overwinter mortality on reproductive life history characteristics of pumpkinseed (*Lepomis gibbosus*) populations. Can J Fish Aquat Sci 48: 1792–1799.

Fretwell S.D. and H.L. Lucas. 1970. On territorial behaviour and other factors influencing habitat distribution in birds. Acta Biotheor 19:16–36.

Fry F.E.J. 1971. Environmental relations and behaviour. Pp. 1–87 in W.S. Hoar and D. J. Randall, eds. Fish Physiology. Vol. 6. Academic Press, New York.

Furimsky M., S.J. Cooke, C.D. Suski, Y. Wang, and B.L. Tufts. 2003. Respiratory and circulatory responses to hypoxia in largemouth bass and smallmouth bass: implications for "live release" angling tournaments. Trans Am Fish Soc 132:1065–1075.

Gee J.H., R.F. Tallman, and H.J. Smart. 1978. Reactions of some Great Plains fishes to progressive hypoxia. Can J Zool 56: 1962–1966.

Gent R., J. Pitlo Jr., and T. Boland. 1995. Largemouth bass response to habitat and water quality rehabilitation in a backwater of the Upper Mississippi River. N Am J Fish Manag 15:784–793.

Greenbank J. 1945. Limnological conditions in ice-covered lakes, especially as related to winterkill of fish. Ecol Monogr 15:343–392.

Hanson K.C., S.J. Cooke, C.D. Suski, G. Neizgoda, F.J.S. Phelan, R. Tinline, and D.P. Philipp. 2007. Assessment of largemouth bass (*Micropterus salmoides*) behavior and activity at multiple spatial and temporal scales utilizing a 3-D whole-lake ecological telemetry observatory. Hydrobiologia 582:243–256.

Hasler C.T., K.C. Hanson, S.J. Cooke, R. Tinline, C.D. Suski, G. Niezgoda, F.J.S. Phelan, and D.P. Phillip. 2007. Frequency, composition and stability of associations among individual largemouth bass (*Micropterus salmoides*) at diel, daily and seasonal scales. Ecol Freshwater Fish 16:417–424.

Heggenes J., J.L. Baglinière, and R.A. Cunjak. 1999. Spatial niche variability for young Atlantic salmon (*Salmo salar*) and brown trout (*S. trutta*) in heterogeneous streams. Ecol Freshwater Fish 8:1–21.

Hess-Nielsen N. and M.W. Wickerhauser. 1996. Wavelets and time-frequency analysis. Proc IEEE 84:523–540.

Hughes G.M. 1973. Respiratory responses to hypoxia in fish. Am Zool 13:475–489.

Hutchinson G.E. 1957. Concluding remarks. Cold Spring Symp Quant Biol 22:415–427.

———. 1965. The niche: an abstractly inhabited hypervolume. Pp. 26–78 in G.E. Hutchinson, ed. The Ecological Theatre and the Evolutionary Play. Yale University Press, New Haven, CT.

Kieffer J.D., A. Ferguson, J.E. Tompa, and B.L. Tufts. 1996. Relationship between body size and anaerobic metabolism in brook trout and largemouth bass. Trans Am Fish Soc 125: 760–767.

Kramer D.L. 1987. Dissolved oxygen and fish behaviour. Environ Biol Fish 18:81–92.

Lemons D.E. and L.I. Crawshaw. 1985. Behavioural and metabolic adjustments to low temperatures in the largemouth bass (*Micropterus salmoides*). Physiol Zool 58:175–180.

Lowry O.H. and J.B. Passonneau. 1972. A flexible system of enzymatic analysis. Academic Press, New York.

Lucas M.C. and E. Baras. 2000. Methods for studying spatial behaviour of freshwater fishes in the natural environment. Fish Fish 1:283–316.

Martinez M.L., C. Landry, R. Boehm, S. Manning, A.O. Cheek, and B.B. Rees. 2006. Effects of long-term hypoxia on enzymes of carbohydrate metabolism in the gulf killifish, *Fundulus grandisi.* J Exp Biol 209:3851–3861.

Mathias J.A. and J. Barica. 1980. Factors controlling oxygen depletion in ice-covered lakes. Can J Fish Aquat Sci 37:185–194.

Meding M.E. and L.J. Jackson. 2003. Biotic, chemical, and morphometric factors contributing to winter anoxia in prairie lakes. Limnol Oceanogr 48:1633–1642.

Niezgoda G., M. Benfield, M. Sisak, and P. Anson. 2002. Tracking acoustic transmitters by code division multiple access (CDMA)-based telemetry. Hydrobiologia 483:275–286.

Nürnberg G.K. 1995. The anoxic factor, a quantitative measure of anoxia and fish species richness in central Ontario lakes. Trans Am Fish Soc 124:677–686.

Perry S.F. and K.M. Gilmour. 2002. Sensing and transfer of respiratory gases at the fish gill. J Exp Zool 293:249–263.

Petersen J.K. and G.I. Petersen. 1990. Tolerance, behaviour and oxygen consumption in the sand goby, *Pomatoschistus minutus* (Pallas), exposed to hypoxia. J Fish Biol 37:921–933.

Petrosky B.R. and J.J. Magnuson. 1973. Behavioral responses of northern pike, yellow perch and bluegill to oxygen concentrations under simulated winterkill conditions. Copeia 1973:124–133.

Pihl L.S., S.P. Baden, R.J. Diaz, and L.C. Schaffher. 1992. Hypoxia-induced structural changes in the diet of bottom-feeding fish and crustacea. Mar Biol 122:349–361.

Rahel F.J. and J.W. Nutzman. 1994. Foraging in a lethal environment: fish predation in hypoxic waters of a stratified lake. Ecology 75:1246–1253.

Raibley P.T., K.S. Irons, T.M. O'Hara, K.D. Blodgett, and R.E. Sparks. 1997. Winter habitats used by largemouth bass in the Illinois River, a large river-floodplain ecosystem. N Am J Fish Manag 17:401–412.

Randall D.J. 1982. The control of respiration and circulation in fish during exercise and hypoxia. J Exp Biol 100:275–288.

Scherer E. 1971. Effects of oxygen depletion and of carbon dioxide buildup on the photic behaviour of the walleye (*Stizostedion vitreum vitreum*). J Fish Res Board Can 28:1303–1307.

Scott W.B. and E.J. Crossman. 1973. Freshwater Fishes of Canada. Fisheries Research Board of Canada, Ottawa.

Shuter B.J. and J.R. Post. 1990. Climate, population viability, and the zoogeography of temperate fishes. Trans Am Fish Soc 119:314–336.

Spoor W.A. 1990. Distribution of fingerling brook trout, *Salvelinus fontinalis* (Mitchell), in dissolved oxygen concentration gradients. J Fish Biol 36:363–373.

Stott B. and D.G. Cross. 1973. The reactions of roach (*Ruilus rutilus*) to changes in the concentration of dissolved oxygen and free carbon dioxide in a laboratory channel. Water Res 7:793–805.

Summerfelt R.C. and L.S. Smith. 1990. Anesthesia, surgery and related techniques. Pp. 213–272 in C.B. Schreck and P.B. Moyle, eds. Methods for Fish Biology. American Fisheries Society, Bethesda, MD.

Suski C.D., S.S. Killen, M.B. Morrissey, S.G. Lund, and B.L. Tufts. 2003. Physiological changes in largemouth bass caused by live-release angling tournaments in southeastern Ontario. N Am J Fish Manag 23:760–769.

Suski C.D. and M.S. Ridgway. 2009. Winter biology of centrarchid fishes. Pp. 264–292 in S.J. Cooke and D.P. Philipp, eds. Centrarchid Fishes: Biology, Diversity, and Conservation. Blackwell-Wiley, Ames, IA.

Suthers I.M. and J.H. Gee. 1986. Role of hypoxia in limiting diel spring and summer distribution of juvenile yellow perch (*Perca flavescens*) in a prairie marsh. Can J Fish Aquat Sci 43:1562–1570.

Tonn W.M. and J.J. Magnuson. 1982. Patterns in the species composition and richness of fish Assemblages in northern Wisconsin lakes. Ecology 63:1149–1166.

Tracy C.R. and K.A. Christian. 1986. Ecological relations among space, time, and thermal niche. Ecology 67:609–615.

Wendelaar Bonga S.E. 1997. The stress response in fish. Physiol Rev 77:591–625.

Zar J.H. 1984. Biostatistical Analysis. Prentice Hall, Englewood Cliffs, NJ.