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Physiological responses of largemouth bass to acute temperature and oxygen stressors

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Abstract Temperature and oxygen gradients exist in nearly every water body, but anthropogenic activities can subject fish to rapid changes in these important environmental variables. These rapid changes in temperature and oxygen (generally referred to as temperature or oxygen shock) may have sub-lethal consequences depending upon the magnitude and the fish species. This study quantified physiological changes in largemouth bass, *Micropterus salmoides* (Lacepède), exposed to two levels of heat and cold shocks and to two levels of hypoxic and hyperoxic shocks. Following a cold shock from 20 °C to 8 °C, plasma cortisol and glucose increased after 1 h and lactate dehydrogenase activity increased after 6 h. Plasma glucose and K^+ concentrations increased 1 h after a heat shock from 20 °C to 32 °C but not after 6 h. Bass subjected to a hypoxic shock from 8 to 2 mg O_2 L^{-1} showed decreased plasma K^+ and increased plasma glucose and white muscle lactate. No changes in physiological parameters were observed in bass subjected up to 18 mg O_2 L^{-1} hyperoxia. Results from this study suggest that largemouth bass can tolerate a wide range of temperature and oxygen shocks, but temperature decreases of 20 to 8 °C and hypoxia as low as 4 mg O_2 L^{-1} should be avoided to minimise physiological perturbations.

KEYWORDS: anthropogenic stressors, cold shock, heat shock, hyperoxia, hypoxia, *Micropterus salmoides*, water quality.

Introduction

Water temperature and dissolved oxygen concentration strongly influence the abundance and distribution of aquatic organisms (Pörtner & Knust 2007). Fish often select a preferred environmental temperature and oxygen concentration to optimise physiological processes (Coutant 1977a, 1985), but the characteristics of their surrounding environment can change frequently. For example, fish can experience rapid changes in temperature or dissolved oxygen (generally referred to as temperature or oxygen shock) of natural and anthropogenic origins. The natural changes in temperature and oxygen are usually only small deviations from ambient conditions and only on occasion become severe and result in mortality (Coutant 1985).

Changes in temperature and oxygen due to anthropogenic activities are often rapid and may present challenges for fishes. Anthropogenic sources of temperature shock include thermal effluents of power plants and factories (Coutant 1977b; Birtwell & Kruzynski 1989), hypolimnetic release of water from upstream reservoirs (Clarkson & Childs 2000) and commercial fishing when by-catch is put on ice prior to release (Hyvärinen *et al*. 2004). Temperature shock can have negative effects for fish by reducing meta-

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bolic rates (Galloway & Kieffer 2003), impairing swimming performance (Hocutt 1973), reducing the ability to capture prey (Donaldson *et al.* 2008), impeding predator avoidance (Ward & Bonar 2003), altering rates of recovery from exercise (Hyvärinen *et al.* 2004; Suski *et al.* 2006) and disrupting physiological homeostasis (Galloway & Kieffer 2003; Suski *et al.* 2006). Anthropogenic sources of hypoxia include cultural eutrophication (Bricker *et al.* 1999) and factory effluents (Birtwell & Kruzynski 1989). Some fish are able to tolerate hypoxia through physiological and behavioural modifications (Wu 2002; Wood *et al.* 2007); but prolonged exposure to hypoxic waters can be challenging for fish and can negatively affect swimming performance (Herbert & Steffensen 2005), reduce growth (Diaz & Rosenberg 1995), impair recovery from exercise (Suski *et al.* 2006) and impact metabolic rate (Hughes 1973). Hyperoxia can occur during times of high photosynthetic activity (Laurent & Perry 1991), such as algal blooms associated with eutrophication (Koray 2004). Negative effects of hyperoxia can include increased susceptibility to diseases (Fridell *et al.* 2007), reduced rate of recovery from exercise (Suski *et al.* 2006) and alterations to ventilation rates that result in acidosis (Gilmour & Perry 1994; Gilmour 2001). Such abrupt changes to aquatic environments are sufficiently problematic that the U.S. Environmental Protection Agency has developed dissolved oxygen and thermal loading (the amount of heat a water body can assimilate) guidelines to ensure protection of aquatic organisms (Environmental Protection Agency 1988). Understanding how fish species respond to an array of temperature and oxygen stressors will provide insight on the impacts of anthropogenic activities on fish and will also provide information that can be used to set thermal and dissolved oxygen guidelines.

When subjected to a stressor, fishes employ a host of physiological adjustments designed to overcome the perceived challenge (Wendelaar Bonga 1997). The magnitude and nature of the deviations from physiological homeostasis can be used to judge the severity of the stressor. As a result, physiological parameters can be used as tools to assess environmental impacts on fishes and provide greater sensitivity than direct measurements of mortality (Heath 1995). Determining the effects of temperature and oxygen stressors on largemouth bass, *Micropterus salmoides* (Lacepède), is crucial because largemouth bass are an economically and ecologically important species (Otis *et al.* 1998; Noble 2002). The objectives of the current study were (1) to quantify the physiological response of resting largemouth bass 1 and 6 h after acute increases and decreases in water temperature (temperature shocks); and (2) to quantify the physiological response of resting largemouth bass 1 and 6 h after acute increases and decreases in dissolved oxygen (oxygen shocks).

Materials and methods

Largemouth bass used in this experiment were collected using alternating current (AC) electric fishing gear from central Illinois lakes between July and early September 2007. The fish ranged from 180 to 326 mm total length [mean 249 ± 3.5 mm SE (standard error)]. After capture, fish were transported to Kaskaskia Biological Station (KBS), Sullivan, IL, USA, and held in aerated tanks in a temperature-controlled laboratory. Water temperatures were maintained at 20.1 ± 0.4 °C, dissolved oxygen concentrations were 7.59 ± 0.17 mg O_2 L^{-1} (YSI 85 temperature and dissolved oxygen metre; YSI Incorporated, Columbus, OH, USA), and tank ammonia concentrations were < 1 mg L^{-1} (Model # 33D; Aquarium Pharmaceuticals Inc., Chalfont, PA, USA). Surface water temperatures during the collection period were 22–26 °C, and fish were held for 7–14 days before use in experiments to allow for recovery from capture. Standard temperature acclimation of fish for laboratory experiments is typically 1 day per degree of temperature change (Brett 1964), so a minimum of 7 days acclimation to 20 °C was appropriate for all fish in this experiment.

Experimental procedure

Twenty-four hours before the start of the experiment, six largemouth bass were placed into individual opaque chambers continuously supplied with 20.0 °C ± 0.1 °C water from a central basin. Water was pumped into the chambers, allowed to overflow and drained back to the central basin forming a closed system similar to that used by Suski *et al.* (2006). The chambers were sufficiently small (approximately 3.5 L) to prohibit large movements of fish. Dissolved oxygen concentrations were maintained at 8.0 ± 0.2 mg O_2 L^{-1} by aeration. Resting largemouth bass were then subjected to one of eight temperature or dissolved oxygen challenges described below and a control treatment where temperature and dissolved oxygen were not altered. This procedure was repeated (using new largemouth bass each time) for all challenges and the control for exposure durations of 1 and 6 h.

Temperature challenges consisted of decreases from 20 to 15 °C and from 20 to 8 °C and increases from 20 to 25 °C and from 20 to 32 °C. Temperature variation was accomplished by chilling or heating water in the

central basin and then pumping it into the chambers. Water temperatures inside the chambers described above took < 5 min to change and the water remained fully oxygenated throughout the experiment. Dissolved oxygen challenges consisted of decreases from 8 to 4 mg O_2 L^{-1} and from 8 to 2 mg O_2 L^{-1} and increases from 8 to 12 mg O_2 L^{-1} and from 8 to 18 mg O_2 L^{-1}. Hyperoxia was created by pumping O_2 gas into chambers and hypoxia challenges were created by pumping N_2 gas into chambers to displace oxygen (Suski *et al.* 2006). Changes in dissolved oxygen usually took < 3 min and were typically within 0.3 mg O_2 L^{-1} of the target concentration; temperature was held constant at 20.0 ± 0.1 °C. Largemouth bass used for controls were held at 20 °C and 8 mg O_2 L^{-1}.

After either 1- or 6-h exposure to the challenge or control conditions, the flow of water to the individual chambers was stopped and fish were euthanised by adding an overdose of anaesthetic [250 mg L^{-1} of 3-aminobenzoic acid ethyl ester methanesulphonate (MS-222) buffered with 500 mg L^{-1} sodium bicarbonate] to each chamber. Following cessation of ventilation, blood was drawn from the gill arch using a 21-gauge hypodermic needle and 1-mL syringe rinsed with lithium heparin (Houston 1990). Immediately after collection, whole blood was centrifuged for 2 min at 2000 ***g*** to separate red cells from plasma, and plasma was stored in 1.5-mL centrifuge tubes in liquid nitrogen or at −80 °C until further processing (Suski *et al.* 2003). A portion of white epaxial musculature (about 5–10 g) posterior to the operculum and above the lateral line was excised, freeze-clamped in aluminium tongs pre-cooled in liquid nitrogen, and stored in liquid nitrogen or at −80 °C until processing (see Suski *et al.* 2003). Six fish were used for each temperature and time combination and for each dissolved oxygen and time combination. The size of fish used did not differ significantly among challenges (temperature: two-way ANOVA, $F_{4,50} = 0.99$, $P = 0.42$; dissolved oxygen: two-way ANOVA, $F_{4,50} = 0.85$, $P = 0.50$).

Laboratory analyses

Analyses of white muscle and plasma parameters are described in detail in Suski *et al.* (2003). Briefly, plasma cortisol and haemoglobin concentrations were determined using commercially available kits (cortisol: Kit # 900-071; Assay Designs, Ann Arbor, MI, USA; haemoglobin: QuantiChrom Haemoglobin Assay Kit, DIHB-250; BioAssay Systems, Hayward, CA, USA). Plasma sodium and potassium concentrations were determined using a flame photometer (Model 2655-00; Cole-Parmer Instrument Company, Chicago, IL, USA), and plasma chloride concentrations were determined using a chloridometer (Model 4435000; Labconco Corporation, Kansas City, MO, USA). Plasma activities of lactate dehydrogenase (LDH; enzyme number 1.1.1.27; IUBMB 1992) were quantified using standard kinetic spectrophotometric techniques based on the methods of Wroblewski and LaDue (1955). White muscle lactate, adenosine triphosphate (ATP), phosphocreatine (PCr) and plasma glucose concentrations were determined enzymatically following the methods of Lowry and Passonneau (1972) in a 96-well microplate read with a commercially available spectrophotometer (Spectra Max Plus 384, Model # 05362; Molecular Devices, Union City, CA, USA). Water content of white muscle was determined by drying pre-weighed tissue in an oven at 80 °C until a constant mass was attained.

Statistical analyses

A two-way ANOVA was used to test for the main effects of time period, temperature or dissolved oxygen concentration and their interaction on plasma and muscle parameters. When the interaction was significant or if the interaction was not significant but at least one of the main effects was significant, a Tukey-Kramer HSD *post hoc* test was used to separate all means. Statistical analyses were performed using SAS Version 8.2 (SAS Institute, Cary, NC, USA), and the level of significance (α) for all tests was 0.05.

Results

Temperature challenges

A significant time-temperature interaction was found for cortisol concentrations ($F_{4,50} = 9.81$, $P < 0.0001$; Fig. 1a). The cortisol concentration at 8 °C after 1 h was six-fold greater than the 1-h control value (Tukey-Kramer HSD, $P < 0.0001$). No other 1-h temperature challenge was significantly different from 1-h controls and cortisol values for the all 6-h temperature challenges were not different from 6-h control levels (Tukey-Kramer HSD, $0.06 < P < 0.99$). No significant differences were observed between any 1-h and 6-h means, except that cortisol was greater after a 1-h exposure to 8 °C than after a 6-h exposure (Tukey-Kramer HSD, $P < 0.001$).

The time–temperature interaction also was significant for plasma glucose concentrations ($F_{4,50} = 7.68$, $P < 0.0001$; Fig. 1b). Plasma glucose concentrations initially (after 1-h exposure) doubled relative to 1-h

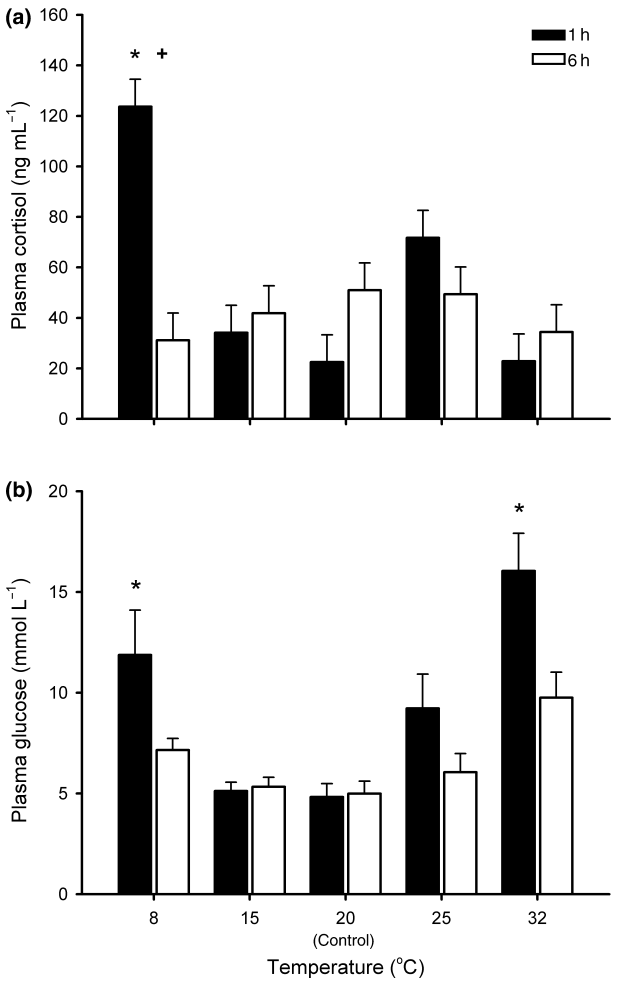

Figure 1. Concentrations of plasma cortisol (a) and glucose (b) of largemouth bass subjected to cold and heat shock (20 °C was the control) for 1 h and 6 h. An asterisk denotes a statistically significant difference from controls for that time period, and a plus sign indicates a statistically significant difference between 1-h and 6-h means at that temperature. Error bars show ±1 standard error (SE). Sample size is 6 fish per challenge or control.

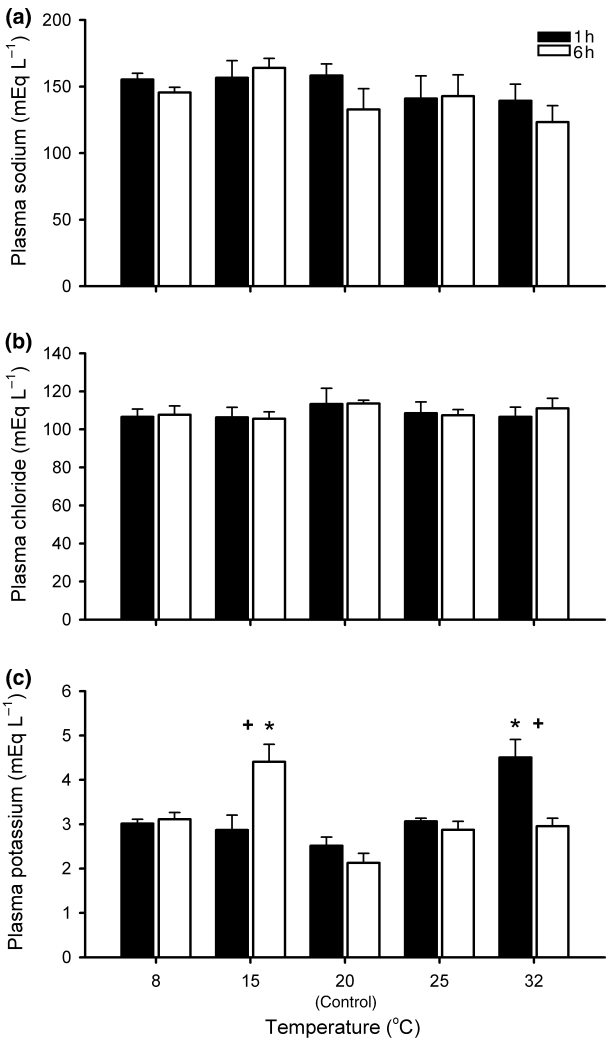

Figure 2. Concentrations of plasma sodium (a), chloride (b) and potassium (c) of largemouth bass subjected to cold and heat shock (20 °C was the control) for 1 h and 6 h. An asterisk denotes a statistically significant difference from controls for that time period, and a plus sign indicates a statistically significant difference between 1-h and 6-h means at that temperature. Error bars show ±1 standard error (SE). Sample size is 6 fish per challenge or control.

controls when temperature decreased from 20 °C to 8 °C and tripled relative to 1-h controls when temperature increased from 20 °C to 32 °C (Tukey-Kramer HSD, $P < 0.007$). No 6-h temperature challenges were significantly different from 6-h controls, and there were no significant differences between 1-h and 6-h means for any temperature (Tukey-Kramer HSD, $0.20 < P < 0.99$).

No significant effects of temperature, time or their interaction were found for plasma sodium (temperature: $F_{4,50} = 1.72$, $P = 0.16$; time: $F_{1,50} = 1.30$, $P = 0.26$; interaction: $F_{4,50} = 0.66$, $P = 0.62$; Fig. 2a) or plasma chloride concentrations (temperature: $F_{4,50} = 0.66$, $P = 0.62$; time: $F_{1,50} = 0.06$, $P = 0.80$; interaction: $F_{4,50} = 0.11$, $P = 0.98$; Fig. 2b). A significant time–temperature interaction was found for concentrations of plasma potassium ($F_{4,50} = 10.56$, $P < 0.0001$; Fig. 2c). Plasma potassium concentrations were nearly two-fold greater than 1-h controls after a 1-h exposure to 32 °C (Tukey-Kramer HSD, $P < 0.0001$) but not different from 6-h controls after a 6-h exposure (Tukey-Kramer HSD, $P > 0.33$). Exposure to 15 °C for 1 h did not cause significant changes in potassium concentrations relative to 1-h controls (Tukey-Kramer HSD, $P > 0.98$), but concentrations more than doubled after 6-h

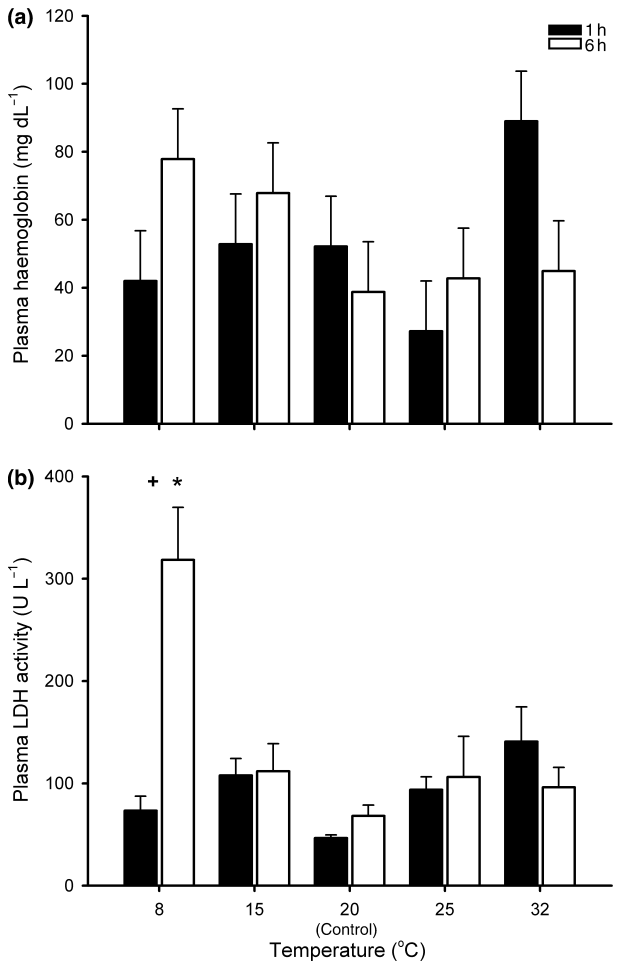

Figure 3. Concentration of plasma haemoglobin (a) and activity of lactate dehydrogenase (LDH; enzyme number 1.1.1.27) (b) of largemouth bass subjected to cold and heat shock (20 °C was the control) for 1-h and 6-h. An asterisk denotes a statistically significant difference from controls for that time period, and a plus sign indicates a statistically significant difference between 1-h and 6-h means at that temperature. Error bars show ±1 standard error (SE). Sample size is 6 fish per challenge or control, except $n = 5$ for LDH activity at 8 °C and 1 h.

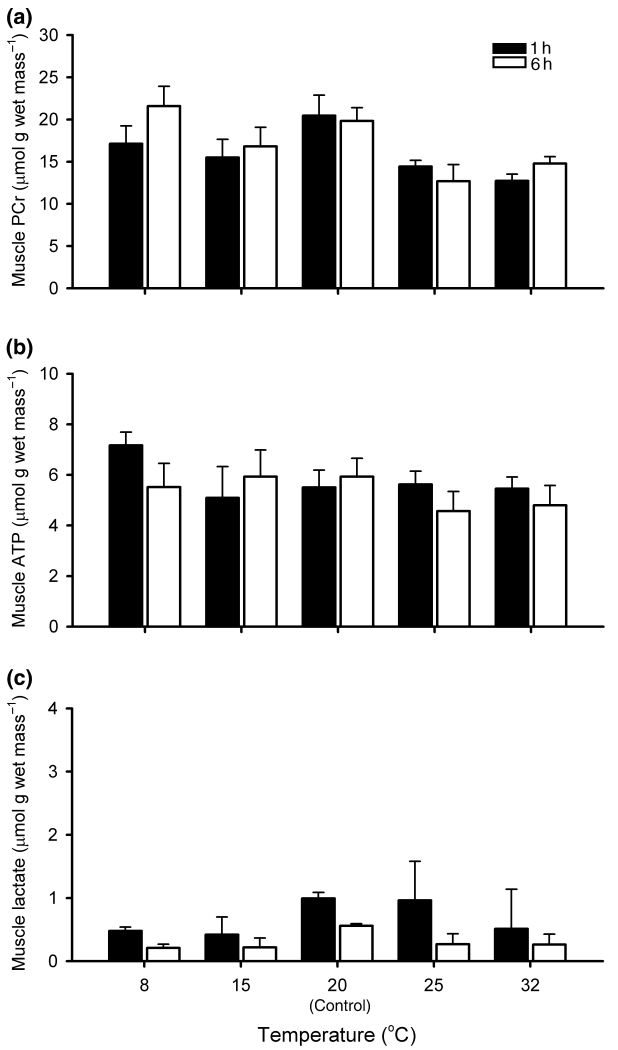

Figure 4. Concentrations of white muscle phosphocreatine (PCr) (a), adenosine triphosphate (ATP) (b) and lactate (c) of largemouth bass subjected to cold and heat shock (20 °C was the control) for 1 h and 6 h. Error bars show ±1 standard error (SE). Sample size is 6 fish per challenge or control, except $n = 5$ for all variables at 20 °C and 1 h.

(Tukey-Kramer HSD, $P < 0.0001$; Fig. 2c). No other 1-h or 6-h temperature challenge was significantly different from controls, and there were no other significant differences between 1-h and 6-h means (Tukey-Kramer HSD, $0.10 < P < 0.98$).

Plasma haemoglobin concentrations were not significantly affected by time ($F_{1,50} = 0.04$, $P = 0.85$) or temperature ($F_{4,50} = 1.56$, $P = 0.20$), and the time–temperature interaction was not significant ($F_{4,50} = 2.22$, $P = 0.08$; Fig. 3a). A significant time–temperature interaction was found for plasma LDH ($F_{4,49} = 8.15$, $P < 0.001$; Fig. 3b). Plasma LDH activity remained constant across all challenges except after a 6-h exposure to a temperature drop from 20 °C to 8 °C, when activity increased four-fold relative to 6-h controls and was greater than the activity at 1-h for the same temperature (Tukey-Kramer HSD, $P < 0.001$). No differences between 1-h and 6-h values were observed at any other temperatures (Tukey-Kramer HSD, $0.97 < P < 0.99$).

White muscle PCr concentration was significantly affected by temperature ($F_{4,49} = 5.64$, $P < 0.001$) but not by time ($F_{1,49} = 0.93$, $P = 0.34$), and the time–temperature interaction was not significant ($F_{4,49} = 0.88$, $P = 0.48$; Fig. 4a). Concentrations at warmer temperatures (25 °C and 32 °C) were significantly lower than at 20 °C (Tukey-Kramer HSD, $0.007 < P < 0.009$), but colder temperatures were not

significantly different from controls (Tukey-Kramer HSD, $0.21 < P < 0.99$). No significant effects of temperature, time or their interaction were found for white muscle ATP (temperature: $F_{4,49} = 0.80$, $P = 0.53$; time: $F_{1,49} = 0.66$, $P = 0.42$; interaction: $F_{4,49} = 0.82$, $P = 0.52$), lactate (temperature: $F_{4,49} = 1.52$, $P = 0.21$; time: $F_{1,49} = 1.24$, $P = 0.27$; interaction: $F_{4,49} = 1.26$, $P = 0.30$; Fig. 4b, c) or water content (temperature: $F_{4,49} = 1.52$, $P = 0.21$; time: $F_{1,49} = 0.61$, $P = 0.44$; interaction: $F_{4,49} = 1.99$, $P = 0.11$).

Dissolved oxygen challenges

Plasma cortisol was not significantly affected by time ($F_{1,49} = 3.19$, $P = 0.08$) or oxygen concentration ($F_{4,49} = 1.77$, $P = 0.15$) and no significant time–oxygen interaction was found ($F_{4,49} = 1.34$, $P = 0.27$; Fig. 5a). By contrast, a significant time–oxygen interaction was found for plasma glucose concentrations ($F_{4,49} = 2.72$, $P = 0.04$; Fig. 5b). Plasma glucose concentrations did not differ from 1-h control values following a 1-h exposure to any oxygen concentration (Tukey-Kramer HSD, $0.97 < P < 0.99$); however, after 6 h at 2 mg O_2 L^{-1}, values were nearly double the 6-h control and 1-h values (Tukey-Kramer HSD, $P = 0.04$). No other 6-h-oxygen concentration combinations were different from controls, and there were no other differences between 1-h and 6-h means (Tukey-Kramer HSD, $0.49 < P < 0.99$).

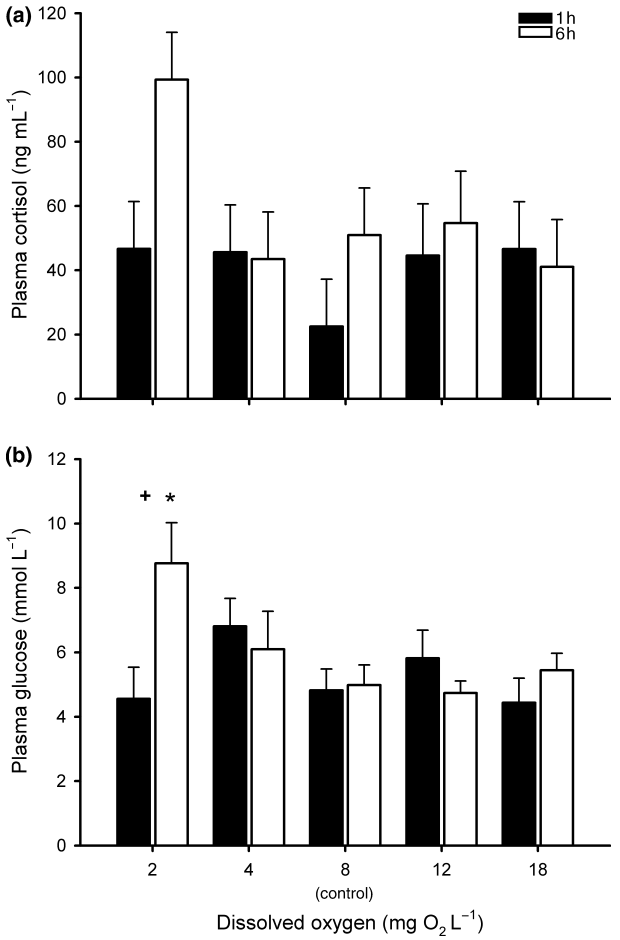

Figure 5. Concentrations of plasma cortisol (a) and glucose (b) of largemouth bass subjected to hypoxic and hyperoxic shock (8 mg O_2 L^{-1} was the control) for 1 h and 6 h. An asterisk denotes a statistically significant difference from controls for that time period, and a plus sign indicates a statistically significant difference between 1-h and 6-h means at that dissolved oxygen concentration. Error bars show ±1 standard error (SE). Sample size is 6 fish per challenge or control except $n = 5$ for both variables at 12 mg O_2 L^{-1} and 1 h.

Plasma sodium and chloride concentrations were not significantly affected by time (sodium: $F_{1,49} = 3.43$, $P = 0.07$; chloride: $F_{1,49} = 1.54$, $P = 0.22$) or oxygen concentration (sodium: $F_{4,49} = 0.63$, $P = 0.64$; chloride: $F_{4,49} = 1.42$, $P = 0.24$), and the time–oxygen interactions were not significant (sodium: $F_{4,49} = 0.75$, $P = 0.56$; chloride: $F_{4,49} = 1.09$, $P = 0.37$; Fig. 6a, b). A significant oxygen–time interaction was found for plasma potassium concentrations ($F_{4,49} = 3.99$, $P = 0.007$; Fig. 6c). Plasma potassium concentrations after 1 h of exposure to 2 mg O_2 L^{-1} were 50% lower than 1-h controls (Tukey-Kramer HSD, $P < 0.05$), but potassium at other levels of oxygen shock were not different from 1-h control levels (Tukey-Kramer HSD, $P = 0.003$). After 6 h of hypoxic exposure, plasma potassium concentrations were at least 50% lower than 6-h controls at both 2 and 4 mg O_2 L^{-1} (Tukey-Kramer HSD, $0.57 < P < 0.98$). Although potassium concentrations following hyperoxic shock of 18 mg O_2 L^{-1} for 6 h were not significantly different from 6-h controls, they were significantly lower than the 1-h value (Tukey-Kramer HSD, $P = 0.04$). Plasma haemoglobin concentrations and LDH activity levels were not significantly affected by time (haemoglobin: $F_{1,49} = 0.26$, $P = 0.61$; LDH: $F_{1,49} = 2.50$, $P = 0.12$) or oxygen concentration (haemoglobin: $F_{4,49} = 0.99$, $P = 0.42$; LDH: $F_{4,49} = 2.43$, $P = 0.06$), and time–oxygen interactions were not significant (haemoglobin: $F_{4,49} = 0.74$, $P = 0.57$; LDH activity: $F_{4,49} = 2.06$, $P = 0.10$; Fig. 7a, b).

White muscle PCr concentrations were significantly affected by oxygen concentration ($F_{4,48} = 6.81$, $P = 0.0002$) but not by time ($F_{1,48} = 2.99$, $P = 0.09$), and the time–oxygen interaction was not significant ($F_{4,48} = 0.35$, $P = 0.84$; Fig. 8a). Concentrations of PCr were only significantly lower than controls at 2 mg O_2 L^{-1} and 4 mg O_2 L^{-1} but not other oxygen levels (Tukey-Kramer HSD, $P < 0.049$). White muscle ATP concentrations were not significantly affected by time

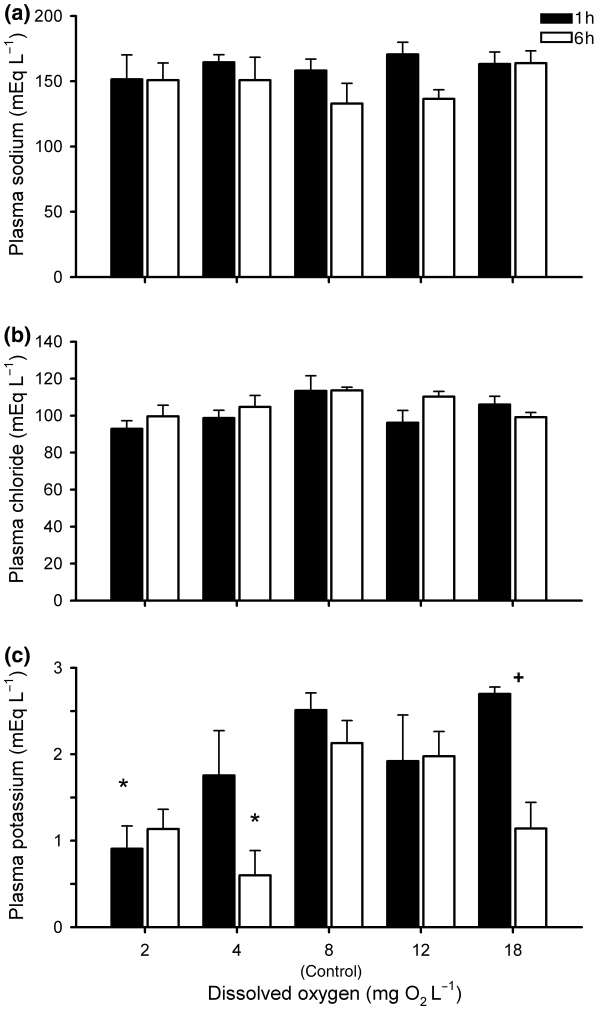

Figure 6. Concentrations of plasma sodium (a), chloride (b) and potassium (c) of largemouth bass subjected to hypoxic and hyperoxic shock (8 mg O_2 L^{-1} was the control) for 1 h and 6 h. An asterisk denotes a statistically significant difference from controls for that time period, and a plus sign indicates a statistically significant difference between 1-h and 6-h means at that dissolved oxygen concentration. Error bars show ±1 standard error (SE). Sample size is 6 fish per challenge or control except $n = 5$ for all variables at 12 mg O_2 L^{-1} and 1 h.

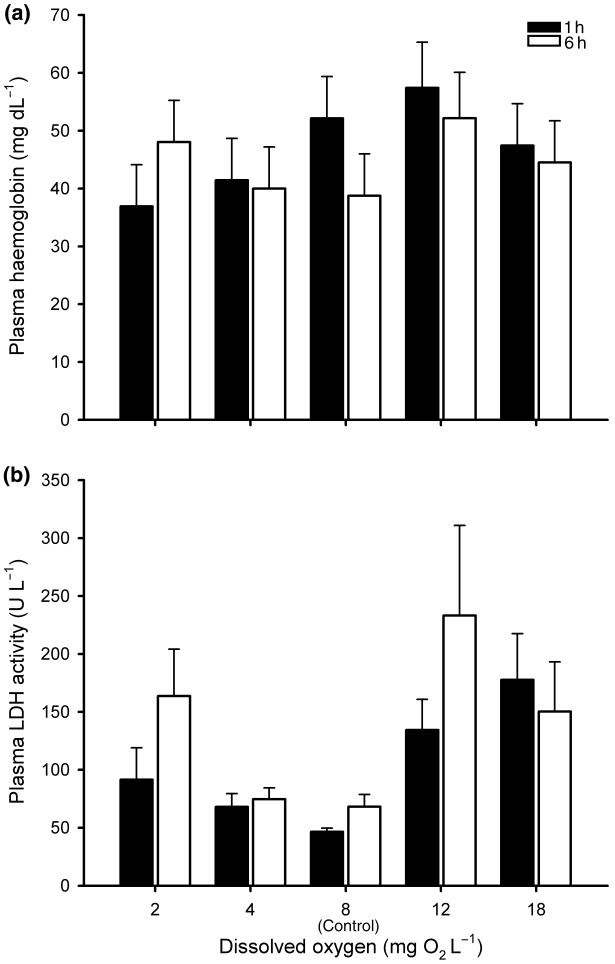

Figure 7. Concentration of plasma haemoglobin (a) and activity of lactate dehydrogenase (LDH; enzyme number 1.1.1.27) (b) of largemouth bass subjected hypoxic and hyperoxic shock (8 mg O_2 L^{-1} was the control) for 1 and 6 h. Error bars show ±1 standard error (SE). Sample size is 6 fish per challenge or control except $n = 5$ for both variables at 12 mg O_2 L^{-1} and 1 h.

($F_{4,48} = 1.01$, $P = 0.41$) or oxygen concentration ($F_{4,48} = 2.07$, $P = 0.10$), and the time–oxygen interaction was not significant ($F_{4,48} = 0.51$, $P = 0.73$; Fig. 8b). By contrast, a significant time–oxygen interaction was found for white muscle lactate ($F_{4,48} = 5.49$, $P = 0.001$; Fig. 8c). Lactate did not differ from controls for any challenge except after a 6-h exposure to 2 mg O_2 L^{-1}, when it was more than three times greater than the 6-h controls (Tukey-Kramer HSD, $P = 0.0008$). No significant effects of oxygen concentration ($F_{4,48} = 2.23$, $P = 0.08$), time ($F_{4,48} = 0.47$, $P = 0.76$) or the time–oxygen interaction ($F_{4,48} = 1.46$, $P = 0.23$) were found for white muscle water content.

Discussion

Changes in temperature and dissolved oxygen resulting from anthropogenic activities can be more rapid than natural changes and potentially have greater consequences for fish. Physiological parameters are sensitive indicators of the impacts of such environmental changes and provide insight into how anthropogenic activities affect fish in ways other than immediate mortality. Large deviations from physiological homeostasis could have negative consequences for largemouth bass, such as reduced growth (Wendelaar

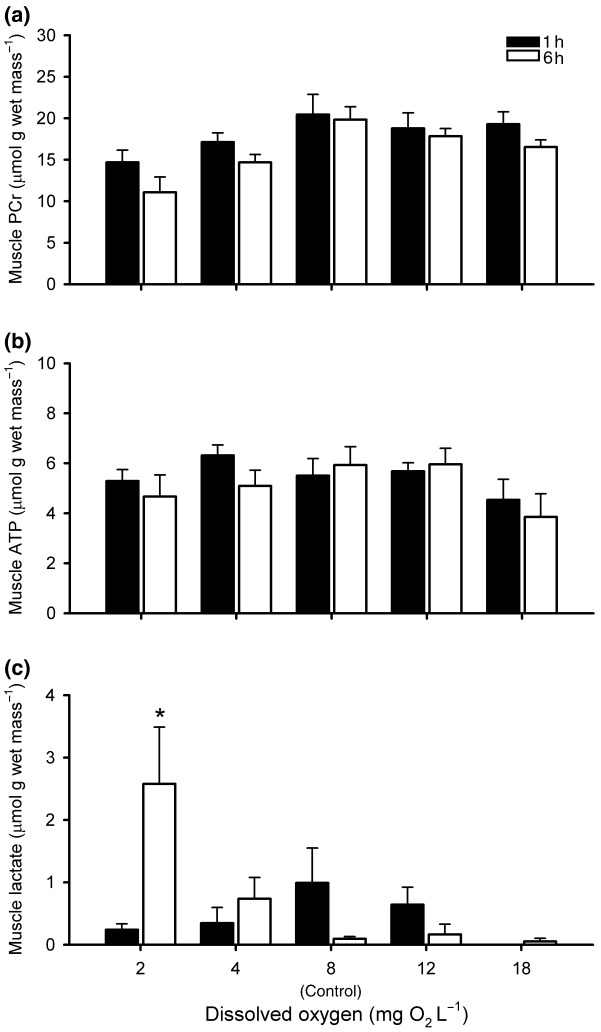

Figure 8. Concentrations of white muscle phosphocreatine (PCr) (a), adenosine triphosphate (ATP) (b) and lactate (c) of largemouth bass subjected to hypoxic and hyperoxic shock (mg O_2 L^{-1} was the control) for 1 h and 6 h. An asterisk denotes a statistically significant difference from controls for that time period. Error bars show ± 1 standard error (SE). Sample size is 6 fish per challenge or control except $n = 5$ for all variables at 12 mg O_2 L^{-1} and 1 h.

Bonga 1997), fitness (Schreck *et al.* 2001), disease resistance (Pickering & Pottinger 1989) and delayed mortality (Wood *et al.* 1983; Kwak & Henry 1995). Cold shock induced significant changes in several parameters after 1-h exposure to the shock, but the magnitude of perturbation depended on the severity of the temperature change. A large (12 °C) cold shock caused a six-fold increase of cortisol and doubling of glucose, but a small (5 °C) cold shock did not affect either of these parameters. Changes in cortisol are of particular importance because they have lethal and sublethal consequences for fish. For example, acute, 2–3-fold increases in cortisol levels 1 h after transport were associated with 10-fold greater 6-day cumulative mortality of Atlantic salmon, *Salmo salar* L., compared to fish displaying no changes in cortisol (Iversen & Eliassen 2009). Sublethal effects of elevated cortisol include suppression of immune function, which has been shown to increase the disease susceptibility of Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), during acute handling stressors (Maule *et al.* 1989). Hyperglycaemia indicates elevated metabolic rate and consumption of energy (Wendelaar Bonga 1997), which can lead to depressed growth rates if chronically elevated (Moon 2001). Although plasma glucose and cortisol concentrations returned to control levels within 6-h, changes in these parameters within the first hour following cold shock could ultimately negatively affect growth, disease resistance and survival of wild populations of largemouth bass if fish are frequently exposed to large, acute cold shocks.

In contrast with cortisol and glucose, LDH activity and potassium concentrations did not change after 1 h of cold shock but instead increased after 6 h, suggesting that these parameters were sensitive only to longer exposures. Elevated plasma LDH activity is indicative of damage to a variety of tissues (e.g. heart, brain, liver, white muscle), as the rupturing of cells releases enzymes (LDH) into the plasma (Morrissey *et al.* 2005). The plasma LDH activity observed at 8 °C after 6 h in this study was similar to LDH activity observed in smallmouth bass, *Micropterus dolomieu* (Lacepède) that experienced severe barotrauma (distension of the swim bladder and haemorrhaging; Morrissey *et al.* 2005), a condition shown to cause mortality of black bass (Feathers & Knable 1983; Gravel & Cooke 2008).

Although greater physiological changes would be expected during larger temperature shocks, plasma potassium increased after a small (5 °C) but not a large (12 °C) cold shock. Temperature-specific rates of ion regulation may explain this pattern. Rates of ion exchange are not only positively related to temperature but are also upregulated via cortisol to combat reduced rates at low temperatures. In common carp, *Cyprinus carpio* L., this results in similar potassium concentrations at high and low temperatures but different ion concentrations at intermediate temperatures (Metz *et al.* 2003). Changes in potassium are of interest because potassium plays an important role in nerve conduction, ion balance and gas transport (Wood & Lemoigne 1991; Nielsen & Lykkeboe 1992; Knudsen & Jensen 1998; Claiborne *et al.* 2002). Ion regulation is costly (up to 20% of resting metabolism; Febry & Lutz 1987) and disturbances to ion balance can reduce growth rates and impair swimming performance (Wilson & Wood 1992; Bury *et al.* 1995), which may

ultimately negatively affect prey capture and predator avoidance (Little & Finger 1990). Clearly, 6 h of cold shock damaged tissues and disrupted potassium balance, suggesting that long-term exposure to reduced temperatures could be problematic for largemouth bass by increasing mortality and reducing growth and swimming performance of impacted individuals.

Similar to the effects of cold shock, largemouth bass showed significant physiological disturbances in several plasma parameters after experiencing acute increases in water temperature. The plasma glucose concentrations 1 h after heat shock from 20 to 32 °C in this study were similar to those of largemouth bass 1 h after exhaustive exercise (anaerobic swimming; Suski *et al.* 2006). Exhaustive exercise differs from aerobic activity by inducing a host of physiological changes, such as depletion of energy stores (glycogen, PCr, ATP), ionic and fluid volume disturbances and acidosis (Wood 1991). In nature, fish typically rely on aerobic swimming activity and only occasionally utilise anaerobic swimming (Beamish 1978; Kieffer 2000). Because of such large physiological changes associated with anaerobic swimming, it is often of short duration, and fish require an extended period of recovery to perform further anaerobic activities (Kieffer 2000). Stressors inducing similar physiological changes to those incurred during exhaustive exercise are of concern, as physiological impairments during exhaustive exercise can lead to immediate and delayed mortality (Wood *et al.* 1983). Although no fish in the current experiment died when subjected to heat shock, elevated glucose levels may also indicate sublethal consequences for largemouth bass, such as reduced growth rates. For instance, a 25% increase in plasma glucose levels of rainbow trout, *Oncorhynchus mykiss* (Walbaum), resulted in a 30% reduction in the concentration of circulating growth hormone, an indicator of growth rate (Farbridge & Leatherland 1992; Johnsson & Björnsson 1994).

Heat shock also affected potassium balance as potassium concentrations increased 1 h after a 12 °C increase in temperature. Previous studies have indicated that increased gill permeability concomitant with the primary stress response should result in a decrease in plasma ion concentrations (Gonzalez & McDonald 1992). In the current study, the exact origin of plasma K^+ following heat shock is not known, but may have originated from erythrocytes or white muscle via lysis, release or leakage from cells (Wood & Lemoigne 1991; Nielsen & Lykkeboe 1992; Knudsen & Jensen 1998; Claiborne *et al.* 2002). A decrease in PCr concentration, an energy store often consumed during exhaustive exercise, was also observed in white muscle at 25 °C and 32 °C, but this change may not be a problem for largemouth bass as it did not reflect the changes that typically occur during exhaustive exercise. Phosphocreatine concentrations during exhaustive exercise typically decrease by 75% relative to resting values (Suski *et al.* 2006), but concentrations did not differ from controls by more than 10% in the current study. Overall, the metrics examined in this study documented a suite of changes occurring 1 h after a heat shock, but these changes were corrected after 6 h. This suggests that prolonged periods of elevated temperatures are not problematic for largemouth bass.

Many physiological systems were impacted by hypoxic shock as glucose, potassium, lactate and PCr concentrations differed from control levels. These changes did not occur until fish were exposed for 6 h or were uncorrected after 6 h, suggesting effects of hypoxia were chronic or not immediately ($\leq$ 1 h) apparent. As glucose levels of largemouth bass typically recover within 2 h of an acute stressor (Suski *et al.* 2006), elevated glucose concentrations after 6 h of hypoxic exposure may indicate chronic elevation, a condition that could lead to depressed growth rates (Moon 2001). Largemouth bass also exhibited potassium loss when exposed to hypoxia of 4 mg O_2 L^{-1} or lower, possibly as a result of modifications to gill structures (Gonzalez & McDonald 1992). Ion loss and ensuing restoration to homeostasis can result in reduced growth rates (Bury *et al.* 1995) and cause disruptions to the circulatory system that can lead to death (Wood & McDonald 1982). The increase in lactate at 2 mg O_2 L^{-1} was likely the product of PCr consumption (Kieffer 2000), as PCr concentrations were lower than controls. Phosphocreatine consumption and lactate accumulation can limit the ability of fish to utilise burst swimming (Kieffer 2000). In addition, recovery of PCr is costly for fish as lipids are oxidised to return this metabolite to homeostasis (Richards *et al.* 2002). Taken together, these results suggest that hypoxic shock is not immediately lethal, but continued exposure to hypoxia may result in physiological changes that negatively impact fish growth rates and burst swimming capacity.

Compared with hypoxic environments, hyperoxic water did not prove to be, based on measured plasma and white muscle parameters, a physiologically challenging environment for largemouth bass, as there were no changes relative to control values in any of the parameters measured. A common response of fish to hyperoxic conditions is decreased ventilation rates, which results in acidosis in rainbow trout (Gilmour & Perry 1994). Hyperoxia causes decreases in chloride and increases in cortisol and potassium concentrations

in Atlantic salmon (Fridell *et al*. 2007). In the current study, white muscle water content, an indicator of acidosis, cortisol and ions did not change relative to baseline levels in any hyperoxia challenge, suggesting largemouth bass are able to tolerate hyperoxia with few consequences.

Implications

The US EPA has insisted that States develop thermal loading and minimum dissolved oxygen standards to ensure adequate water quality for organisms in anthropogenically impacted aquatic systems. For example, the Illinois EPA currently recommends that the maximum change in water temperature from natural temperatures should not exceed 2.8 °C (rate of change not specified in the regulation but assumed to be an acute change) at any time, and dissolved oxygen concentrations should not fall below 5.0 mg O_2 L^{-1} during March through July and 3.5 mg O_2 L^{-1} (or below 4.0 mg O_2 L^{-1} for 7-days average) for most waters from August through February (Illinois Environmental Protection Agency 2008). This and similar regulations may be problematic for fish. During August, water temperatures are high and, therefore, metabolic rates and oxygen demand of fish are elevated. Legally mandated dissolved oxygen concentrations, however, are lowest during this period. Results from the current study suggest that acute changes in water temperature of 2.8 °C should not be a problem for largemouth bass, as only acute changes in water temperature of 12 °C resulted in multiple physiological impairments. Future modifications to dissolved oxygen standards may be needed as dissolved oxygen concentrations of 4 mg O_2 L^{-1} or lower induced deviations from physiological homeostasis that appeared or were uncorrected after 6 h, suggesting chronic exposure to hypoxia may be problematic for largemouth bass. Large temperature shocks and low dissolved oxygen, if not immediately lethal, may have severe consequences for largemouth bass populations by inducing several physiological changes that can negatively impact growth rates, reproduction and disease resistance. Furthermore, temperature and oxygen stressors may be especially detrimental when combined with other stressors and result in mortality of largemouth bass.

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