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Respiratory and Circulatory Responses to Hypoxia in Largemouth Bass and Smallmouth Bass: Implications for "Live-Release" Angling Tournaments

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Abstract.—Arterial blood respiratory variables, ventilation rate, and cardiac output were examined in largemouth bass *Micropterus salmoides* and smallmouth bass *M. dolomieu* to compare their physiological responses to graded levels of hypoxia. Reduction in water P_{O_2} (P_{wO_2}) from 150 to 45 torr (1 torr ≈ 133.3 Pa) caused similar decreases in arterial P_{O_2} (P_{aO_2}) in both species, although total blood O_2 content was markedly higher in largemouth bass at the lower oxygen tensions. Curves for in vitro O_2 dissociation in normoxic fish indicated that largemouth bass blood had a higher affinity for O_2 than smallmouth bass blood. Severe hypoxia caused a significant increase in blood concentrations of catecholamines in smallmouth bass but not in largemouth bass. Increases in ventilation rate (54%) and decreases in cardiac output (27%) during hypoxia were also more pronounced in smallmouth bass than in largemouth bass. Arterial blood pH did not change during hypoxia exposure in largemouth bass but decreased significantly during severe hypoxia in smallmouth bass. The results of this study indicate that smallmouth bass are more sensitive to hypoxia than largemouth bass are. These results have important implications for situations in which these two bass species may be exposed to periods of hypoxia, such as during live-release angling events.

Largemouth bass *Micropterus salmoides* and smallmouth bass *M. dolomieu* are both extremely popular species for recreational anglers in North America. In recent years, these black basses have also become the principal target species for competitive angling events, which annually number in the tens of thousands on this continent (Shupp 1979; Duttweiler 1985; Schramm et al. 1991).

Although closely related, these species display very different lifestyles and habitat preferences (Demers et al. 1996). Largemouth bass are generally viewed as ''sit and wait'' predators that prefer shallower, warmer, weedy areas (Heidinger 1975). In contrast, smallmouth bass are more ''active'' predators that prefer deeper and colder open waters (Coble 1975). Presumably, these ecological differences may also be associated with different physiological adaptations that permit each of these two species to better exploit their respective environments through habitat partitioning. To our knowledge, however, limited information is available about the physiological differences between them.

Indirect evidence of significant physiological differences between these two species of black bass has arisen from observations at live-release competitive angling events. During these events, fish may be subjected to several physiological stressors, including strenuous exercise (angling), confinement and crowding, acute air exposure, and aquatic hypoxia. Although the goal is to release the fish alive at the conclusion of each tournament day, some degree of fish mortality is frequently encountered at these events (Wilde 1998). Our observations during several years of research at tournaments, as well as earlier evidence provided by other investigators (Bennett et al. 1989; Hartley and Moring 1995), suggest that largemouth bass are much more tolerant of tournament stressors than smallmouth bass are and exhibit lower rates of mortality at these events. At present, however, the underlying physiological reasons for the ap-

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parently different tolerances of these two species for tournament conditions are unknown.

One of the most common and potentially serious stressors that fish may be exposed to during live-release tournaments is hypoxia. Unless adequate precautions are taken, aquatic hypoxia can easily occur at several locations within a tournament, including the live wells in boats, the bags used to transfer fish to the weigh-in scales, and the tanks of the release boats used to disperse the fish at the end of the tournament day (Hartley and Moring 1993). Because the gills of most fish species collapse when the fish are removed from water, the air exposure the fish experience during the weigh-in is probably another source of hypoxia for tournament fish. Despite the wealth of information concerning the general effects of hypoxia on fish and the regulatory mechanisms involved in their responses to this disturbance, relatively little is known about the physiological responses of centrarchid fish to different levels of hypoxia. Moreover, no previous studies have compared the physiological responses to hypoxia in species such as largemouth and smallmouth bass, which may frequently encounter this type of disturbance during live-release angling events.

Given this background, the purpose of the present study was to compare the effects of hypoxia on arterial blood variables, ventilation rates, and cardiac output in largemouth bass and smallmouth bass. We hypothesize that these two species may show important differences in their physiological responses to different levels of hypoxia and that these differences may partially explain why smallmouth bass appear to be less tolerant of live-release angling events.

Methods

Experimental animals.—Largemouth bass and smallmouth bass were collected by angling from various lakes in southeastern Ontario, Canada. Fish were transported to the Biology Department at Queen's University, Kingston, Ontario, in oxygenated water and held in freshwater aquaria containing Lake Ontario water at 20 ± 2°C for at least 48 h before the experiments. Fish were maintained in a 12 h: 12 h light: dark photoperiod and were fed a diet of live worms.

In vivo experiments.—For the initial series of experiments, largemouth bass (422 ± 23 g, N = 6) and smallmouth bass (389 ± 38 g, N = 7) were first anesthetized in water containing 0.2 g/L ethyl–*m*-aminobenzoate (MS-222; Sigma Chemical Co.) and 0.4 g/L $NaHCO_3$. After anesthetization, the fish were placed on a surgical table and their gills were continuously irrigated with water containing half the concentration of the anesthetic used above. A polyethylene cannula (Clay Adams, PE 50) was inserted into either the caudal artery or dorsal aorta (Soivio et al. 1975). Fish were then allowed to recover from the surgical procedure in black Perspex boxes supplied with flowing aerated water for 24 h before further experimentation.

Graded hypoxia was achieved by manipulating the percentages of air and N_2 with a gas mixer (Cameron Instruments). Water in a central basin was gassed with the gas mixtures and recirculated through the black Perspex boxes by using a submersible pump. During the course of experimentation, sequential samples of arterial blood were periodically taken through the cannulae to determine the partial pressure of oxygen (Pa_{O_2}), arterial pH (pHa), hematocrit, hemoglobin (Hb) concentration, and O_2 content (Ca_{O_2}). Another portion of each blood sample was centrifuged immediately (10 000 × g for 1 min at room temperature). After centrifugation, the resulting plasma supernatant liquid was collected and then immediately frozen in liquid N_2 before storage at −80°C for subsequent analyses of lactate and catecholamines. The red blood cells were resuspended in physiological saline and returned to the circulation by way of the cannulae in an effort to reduce the effects of sampling. Blood samples were collected before hypoxia exposure, at 2 h after exposure to Pw_{O_2} of 90 torr (1 torr ≈ 133.3 Pa), at 2 h after exposure to 60 torr, at 1 h after exposure to 45 torr, and at 12 h after return to normoxia.

For this initial series of experiments, the sample sizes for smallmouth bass at the deeper levels of hypoxia and recovery were reduced because some mortality was observed for this species in the latter stages of these experiments. Since the combined results in this study were very consistent, we felt that it was appropriate to limit the number of wild bass used in these experiments and did not attempt to further increase our numbers of smallmouth bass to be tested at the deeper levels of hypoxia.

Analytical techniques.—During the in vivo experiments, Pa_{O_2} and Pw_{O_2} were monitored by using P_{O_2} electrodes (Radiometer) connected to a blood gas monitor (PHM 73 pH/Blood Gas Monitor; Radiometer). Arterial pH (pHa) was monitored by using a capillary pH electrode (BMS 3 Mk 2 Blood Microsystem; Radiometer) and a PHM Research pH meter (Radiometer). The Po_2 electrodes were calibrated with a solution containing 1 mg of sodium sulfite (Na_2SO_3) in 5 mL of a 0.01

M sodium borate ($Na_2B_4O_7$) solution (Tucker 1967). The P_{O_2} of air-saturated water was calculated by using the local barometric pressure on each experimental day. The pH electrodes were calibrated with precision buffers (Radiometer). All experiments were performed at room temperature (20°C).

Plasma lactate concentration was determined on perchloric-acid-extracted samples (neutralized with $KHCO_3$) in a 96-well plate spectrophotometer (Spectra MAX Plus; Molecular Devices Corp., Sunnyvale, California) with a commercially available lactate assay (Sigma). Catecholamines were separated from plasma by extraction with alumina and measured by high-performance liquid chromatography (HPLC) with electrochemical detection. Dihydroxybenzylamine was used as an internal standard in all samples. Hemoglobin concentrations were determined with a commercially available Hb assay kit (Sigma).

In vitro blood oxygen dissociation curves.—Blood samples were collected from largemouth bass (487 ± 46 g, $N = 6$) and smallmouth bass (458 ± 44 g, $N = 6$) by caudal puncture, washed three times with Cortland saline, and stored in a refrigerator overnight before experimentation. Blood samples were then placed in intermittently rotating tonometer vessels (incubated at 20°C) and gassed with humidified air. Total O_2 saturation of the blood was achieved by allowing the cells to equilibrate with 100% of a 0.5% CO_2/air mixture for 1 h. Different oxygen tensions were then achieved by controlling the relative percentages of 0.5% CO_2 in air and 0.5% CO_2 in N_2 with a Wöstoff gas mixing pump. At each of the different gas tensions, the blood was equilibrated for 30 min before sampling. Blood was sampled with a gastight Hamilton syringe, and the blood O_2 content was determined by the method of Tucker (1967). Six oxygen dissociation curves were generated for each species by using animals that were not subject to any other experiments.

Cardiac output experiments.—Surgical procedures and the cardiac output apparatus are described elsewhere in detail (Cooke et al. 2001; Schreer et al. 2001). Briefly, largemouth bass (264 ± 43 g, $N = 8$) and smallmouth bass (216 ± 21 g, $N = 8$) were anesthetized with 60 mg/L clove oil (emulsified with a 9:1 solution of ethanol : clove oil by volume) until the fish had lost equilibrium and were nonresponsive. Water containing a maintenance concentration of anesthetic (30 mg/L clove oil) was pumped over the gills during surgery. A flexible silicone cuff-type Doppler flow probe (subminiature 20 MHz piezoelectric transducer; Iowa Doppler Products, Iowa City, Iowa), sized to match the diameter of the vessel, was placed around the ventral aorta. The lead wire from the probe was then sutured to the side of the fish in six locations to prevent shifting of the cuff. The Doppler flow probe was attached to a flowmeter (545C-4 Directional Pulsed Doppler Flowmeter; Bioengineering Department, The University of Iowa, Iowa City, Iowa) and a digital strip-chart recorder (LabVIEW, Version 4.0.1; National Instruments Corporation, Austin, Texas) to monitor cardiac output, stroke volume, and heart rate. For both species, this entire surgical procedure was completed within 10 min.

After surgery, fish were placed immediately into individual black Perspex fish boxes and monitored until they had regained equilibrium. Previous studies have found that full recovery of cardiac variables is complete within 12 h in these species after this type of surgery (Schreer et al. 2001; Cooke, unpublished data). Fish were therefore permitted to recover from surgery and to acclimate to the boxes for 18 to 24 h before the experiments. The experimental boxes were continuously supplied with water at temperatures within ±0.3°C of desired experimental temperatures. Access to the laboratory was restricted during the resting and recovery period to prevent external disturbance. Cardiac output was sequentially monitored during normoxia, at 2 h after a decrease to 90 torr, at 2 h after a decrease to 60 torr, at 1 h after a decrease to 45 torr, and then after a 12-h recovery period at normoxia. Each recording period consisted of a 10-min interval at the end of the desired treatment after cardiac parameters and oxygen levels had stabilized at each desired oxygen tension.

After experimentation, the fish were euthanatized with an overdose of anesthetic (180 ppm clove oil), and a postmortem calibration was conducted to convert Doppler shift (in volts) to actual blood flow (mL/min). Pig blood perfused through the ventral aorta was used to calibrate the probes over a range of flow rates encompassing those recorded during the trials. Reference flow rates were analyzed by linear least-squares regression.

Ventilation rate.—High-resolution black and white video cameras with infrared illumination (AU 401; J. J. Communications, Inc., Englewood, New Jersey) were used to noninvasively monitor ventilation rates. The cameras were positioned inside the Perspex boxes at the influent end, facing the effluent. Ventilation rates were discretely monitored during manipulations by viewing ventila-

tory activity on a small monitor. Although we attempted to monitor ventilation rates from all of the fish used in the cardiac output series of experiments ($N = 8$ for both species), on numerous occasions the position of the fish in black boxes precluded our measurements of this variable. Thus, the sample sizes at each sampling time in the ventilation experiments ($N = 4–7$ for largemouth bass, $N = 5–6$ for smallmouth bass) are less than those for the associated cardiac output series of experiments.

Statistical analyses.—For all of the in vivo hypoxia experiments, where individual fish were repeatedly sampled over time, the results were analyzed by using a repeated-measures two-way analysis of variance (ANOVA) with species and oxygen tension as the main effects, followed by a Student's *t* post hoc test. As explained above, for logistical reasons the ventilation rates of individual fish in these in vivo experiments could not be repeatedly sampled at all oxygen tensions. The ventilation results were therefore analyzed using a two-way ANOVA (main effects: species and oxygen tension) followed by a Student's *t* post hoc test. For statistical comparisons of P_{50} values for smallmouth and largemouth bass obtained in the in vitro experiments we used an unpaired *t*-test. All data are means ± SEs, and the level of significance (α) for all tests was 0.05.

Results

The effects of graded levels of hypoxia on the blood oxygen variables in largemouth bass and smallmouth bass during the first series of in vivo experiments are shown in Figure 1. The P_{O_2} gradient ($Pw_{O_2} - Pa_{O_2}$) was not statistically different between species, and Pa_{O_2} decreased directly with Pw_{O_2} (Figure 1). Total O_2 content ($[O_2]/[Hb]$) of largemouth bass blood did not change from normoxic values, even at the lowest levels of hypoxia. In contrast, significant decreases in $[O_2]/[Hb]$ occurred at Pw_{O_2} values of 60 and 45 torr in smallmouth bass (Figure 1B). Moreover, the species differed significantly in $[O_2]/[Hb]$ values at each level of hypoxia. During the recovery period, the Pa_{O_2} and $[O_2]/[Hb]$ in smallmouth bass remained significantly different from normoxic values.

Oxygen dissociation curves obtained from in vitro experiments provided further evidence that the blood oxygen transport characteristics were different between these two species of bass (Figure 2). Largemouth bass blood had a P_{50} value (the oxygen tension at which Hb is 50% saturated with O_2) that was 8.6 torr lower than that of smallmouth bass.

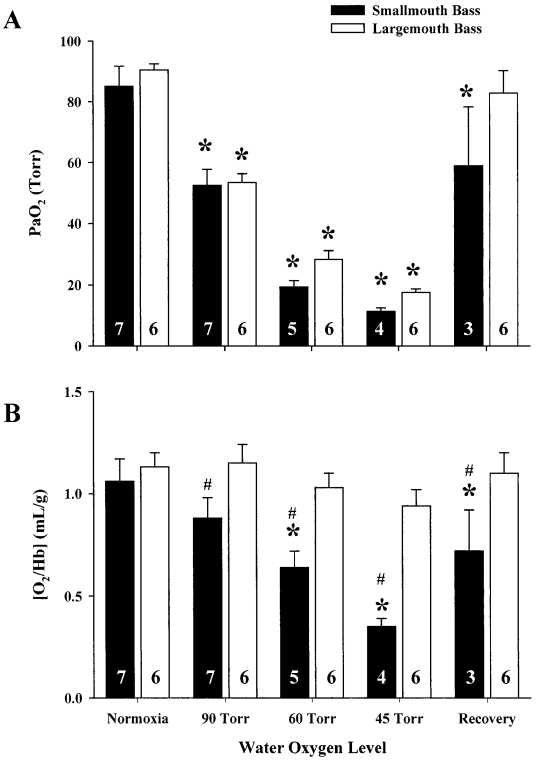

FIGURE 1.—(**A**) Partial pressure of oxygen in arterial blood (Pa_{O_2}) and (**B**) blood oxygen content per gram of hemoglobin (O_2/Hb) in largemouth and smallmouth bass at different water oxygen levels. Values are means ± SEs; 1 torr ≈ 133.3 Pa. Asterisks indicate significant differences from control (normoxia) values, and pound signs indicate significant differences between species. Sample sizes for each treatment are shown on the individual bars.

Hematocrit values did not significantly differ from control values during hypoxic exposure in largemouth bass (Table 1). Smallmouth bass, however, had a significant increase in red blood cell concentration at $Pw_{O_2} = 45$ torr. This increase resulted in significantly greater hematocrit values in smallmouth bass than in largemouth bass at this level of hypoxia (Table 1). During recovery, the hematocrit values in smallmouth bass returned to levels not significantly different from the initial normoxic values in this species or from largemouth bass recovery values.

The plasma catecholamine profiles also differed substantially between these two species during hypoxia (Table 1). At $Pw_{O_2} = 45$ torr, plasma adrenaline concentrations in smallmouth bass in-

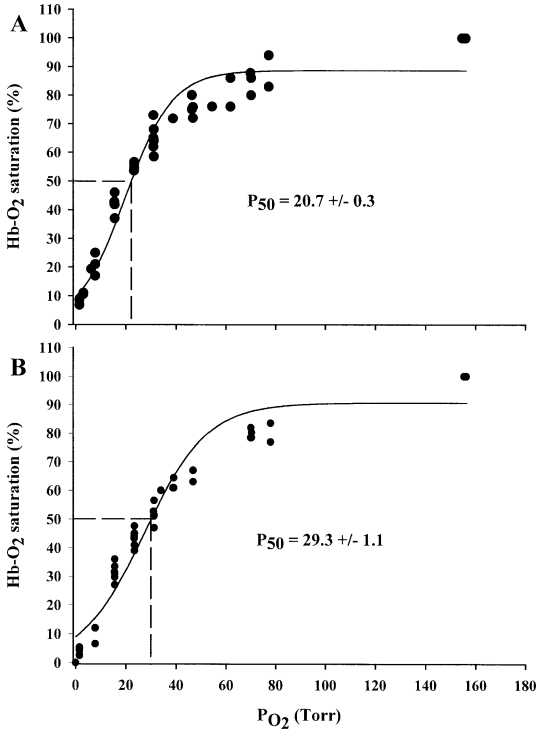

FIGURE 2.—In vitro O_2 dissociation curves for the blood of (**A**) largemouth bass and (**B**) smallmouth bass. Data were fitted to a sigmoidal curve to represent the Hb–O_2 binding properties of the blood. (P_{50}) values were obtained from individual curves from each species (N = 6).

creased by about two orders of magnitude and plasma noradrenaline concentrations were about 70 times greater than those in normoxic control individuals. In contrast, largemouth bass exhibited no significant increases in plasma catecholamine concentrations at any point during the hypoxic exposure. During recovery, plasma adrenaline and noradrenaline concentrations in smallmouth bass returned to levels that were no longer significantly different from those during the initial normoxia period.

Ventilation rate increased by about 25% at the first level of hypoxia (PwO_2 = 90 torr) in smallmouth bass and remained elevated as PwO_2 was decreased (Figure 3). Ventilation rate also increased in largemouth bass at PwO_2 values of 60 and 45 torr, in comparison with control normoxic levels in this species, but it was significantly lower than that in smallmouth bass at all sampling times during the hypoxic exposure. Ventilation rates were not monitored after the 12-h recovery period.

Cardiac output dropped by about 20% in smallmouth bass exposed to moderate hypoxia (PwO_2 = 90 torr) and remained significantly lower than control values at all sampling times during the hypoxic exposure (Figure 4A). In contrast, largemouth bass did not exhibit any significant changes in cardiac output relative to their normoxic control values during these experiments. The decrease in cardiac output in smallmouth bass was mainly due to a significant decrease in heart rate (bradycardia) that was prevalent at all levels of hypoxia (Figure

TABLE 1.—Hematocrit values, noradrenaline concentrations, adrenaline concentrations, blood lactate concentrations, and arterial pH (pHa) values for smallmouth and largemouth bass during exposure to graded levels of external hypoxia. Values are means ± SEs, and sample sizes are given in parentheses; 1 torr ≈ 133.3 Pa. Pound signs indicate significant differences between species, and asterisks indicate significant differences from normoxia within a species.

Species	Normoxia	90 torr	60 torr	45 torr	Recovery
			Hematocrit (%)		
Smallmouth bass	19.2 ± 2.4 (7)	21.0 ± 2.3 (7)	21.9 ± 3.2 (5)	26.8 ± 3.4#* (4)	17.5 ± 4.8 (3)
Largemouth bass	16.7 ± 2.0 (6)	15.5 ± 1.8 (6)	16.8 ± 2.0 (6)	16.5 ± 1.8# (6)	14.4 ± 1.6 (6)
			Noradrenaline (nmol/L)		
Smallmouth bass	2.4 ± 0.6 (7)	20.1 ± 16.1 (6)	42.3 ± 16.7 (4)	185.2 ± 142.7#* (4)	4.3 ± 2.7 (3)
Largemouth bass	5.96 ± 5.2 (6)	1.0 ± 0.4 (6)	1.2 ± 0.6 (6)	6.5 ± 2.7# (6)	2.0 ± 0.6 (6)
			Adrenaline (nmol/L)		
Smallmouth bass	0.85 ± 0.07 (7)	2.4 ± 1.2 (6)	3.3 ± 0.50 (4)	87.2 ± 29.3#* (4)	1.2 ± 0.28 (3)
Largemouth bass	1.07 ± 0.56 (6)	0.67 ± 0.2 (6)	0.67 ± 0.1 (6)	1.22 ± 0.5# (6)	0.69 ± 0.24 (6)
			Lactate (mmol/L)		
Smallmouth bass	0.5 ± 0.1 (7)	1.6 ± 0.5 (6)	4.3 ± 1.7#* (5)	9.4 ± 2.4#* (5)	0.9 ± 0.1 (3)
Largemouth bass	0.4 ± 0.1 (6)	0.6 ± 0.1 (6)	1.4 ± 0.2# (6)	4.1 ± 1.0#* (6)	0.7 ± 0.3 (6)
			pHa		
Smallmouth bass	8.1 ± 0.03 (7)	8.0 ± 0.03# (6)	8.1 ± 0.05 (4)	7.8 ± 0.07#* (4)	7.9 ± 0.12#* (3)
Largemouth bass	8.0 ± 0.03 (6)	8.2 ± 0.04# (6)	8.3 ± 0.05* (6)	8.2 ± 0.08# (6)	8.1 ± 0.06# (6)

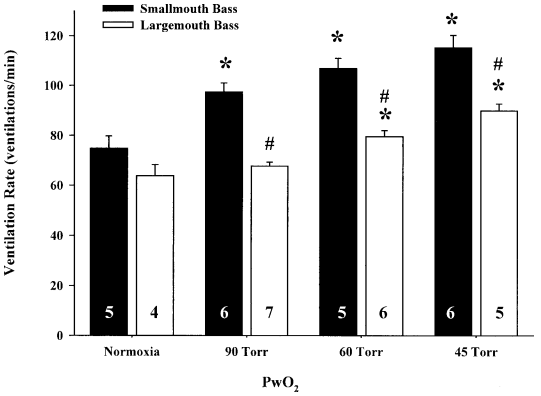

FIGURE 3.—The effects of graded levels of hypoxia on ventilation rates in largemouth and smallmouth bass. Values are means ± SEs, and sample sizes are shown on individual bars. Asterisks indicate ventilation rate values that are significantly different from those for the same species during normoxic conditions; pound signs indicate significant differences between species at a given sample time.

4B). A bradycardia was also observed in largemouth bass but only at the most extreme level of hypoxia (PwO_2 = 45 torr). At the deepest level of hypoxia, smallmouth bass also experienced a significant increase in stroke volume (Figure 4C). During the recovery period, all of the cardiac variables monitored in smallmouth bass remained significantly different from both the control normoxic values for this species and the largemouth bass recovery values. In addition, some of the heartbeats in smallmouth bass appeared to be erratic during the recovery period (traces not shown).

In smallmouth bass, plasma lactate concentrations increased significantly at PwO_2 values of 60 and 45 torr (Table 1). In contrast, plasma lactate concentrations in largemouth bass were not significantly different from control normoxia values until P_wO_2 = 45 torr. At the deepest level of hypoxia, the plasma lactate concentrations in smallmouth bass were also approximately double those in largemouth bass. During recovery, plasma lactate concentrations in both species returned to levels that were not significantly different from the initial normoxia levels.

The effect of hypoxia on arterial blood pH is also shown in Table 1. In largemouth bass, the only significant change in arterial blood pH during hypoxia was an alkalinization at PwO_2 = 60 torr. In contrast, smallmouth bass exhibited a significant acidosis in arterial blood at the deepest level of hypoxia (PwO_2 = 45 torr). At the hypoxia levels of 90 and 45 torr, blood pH in smallmouth bass was also significantly lower than that in largemouth bass. Blood pH in smallmouth bass remained significantly depressed during the recovery period, compared with both the normoxic control values in this species and the largemouth bass recovery values.

Discussion

The physiological consequences of exposure to acute hypoxia in fish have been examined in several studies. The general mechanisms involved in the regulatory responses to this environmental stress in fish are therefore well understood and have been the subject of previous reviews (Fritsche and Nilsson 1993; Jensen et al. 1993). The main purpose of the present study, however, was to determine whether two closely related centrarchid species, the largemouth bass and the smallmouth bass, have different levels of hypoxia tolerance. The results of this study provide strong evidence that this is indeed the case.

The first indication that these two bass species have different tolerances for aquatic hypoxia is seen when the effects of changing water PO_2 on the blood oxygen transport properties are compared between these two species in vivo (Figure 1). Although PaO_2 declines in parallel with PwO_2 in largemouth bass, no significant changes in $[O_2]$/[Hb] occur, even at the deepest levels of hypoxia. In smallmouth bass, the effect of changing water PO_2 on PaO_2 shows a trend similar to that in largemouth bass, but the impact of these changes on $[O_2]$/[Hb] is markedly different. In smallmouth bass, exposure to the most severe levels of hypoxia also causes a profound decrease in $[O_2]$/[Hb]. These results suggest the likelihood of important differences in the oxygen transport characteristics of the red blood cells between these two species.

We therefore conducted an additional series of in vitro experiments to examine the oxygen transport properties of the red blood cells in these two species of bass. The P_{50} value indicates the oxygen tension (PO_2) at which Hb is 50% saturated with O_2 and can provide important insights about the ability of a fish to maintain aerobic respiration during hypoxia (Jensen et al. 1993). A lower P_{50} value, for example, indicates that blood has an increased ability to bind oxygen at lower arterial oxygen tensions, thereby increasing an animal's ability to tolerate ambient hypoxia. In general, species that are relatively tolerant of environmental hypoxia, such as the common carp *Cyprinus carpio* and American eel *Anguilla rostrata*, often have relatively low P_{50} values (Takeda 1990; Perry and

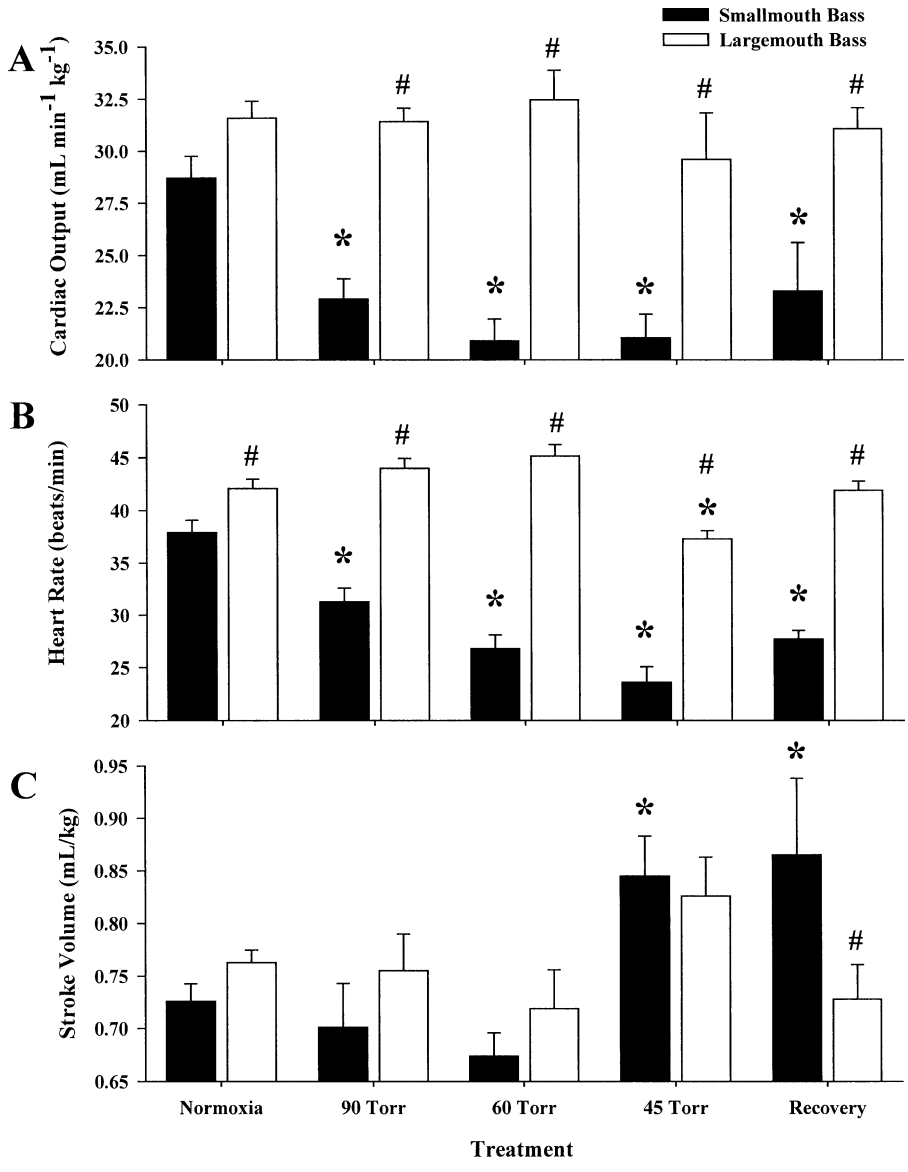

FIGURE 4.—The effects of graded levels of hypoxia on (**A**) cardiac output, (**B**) heart rate, and (**C**) stroke volume in largemouth and smallmouth bass. Values are means ± SEs (N = 8 for both species). Asterisks indicate values that are significantly different from those for the same species during normoxic conditions. Pound signs indicates significant differences between species at a given sample time.

Reid 1992; Jensen et al. 1993). In contrast, species that are less able to tolerate hypoxia, such as the rainbow trout *Oncorhynchus mykiss*, tend to have higher P_{50} values (Perry and Reid 1992; Jensen et al. 1993). As might be anticipated from our in vivo observations, the P_{50} value obtained for largemouth bass blood in the present study was significantly lower than that obtained for smallmouth bass blood (Figure 2). These in vitro results indicate that the large differences in $[O_2]$/[Hb] between these two species at low environmental oxygen tensions in vivo (Figure 1) can be mainly attributed to the different oxygen transport characteristics of their red blood cells. The oxygen transport characteristics of intact red blood cells were examined in the present study, and several characteristics of red blood cells could contribute to these differences in oxygen transport. These dif-

ferent findings may indicate significant differences in the properties of Hb between these two closely related species. Alternatively, there also could be species differences in important modulators of hemoglobin oxygen binding, such as red blood cell pH or organic phosphate concentrations. The relative importance of these factors will require further investigation. The P_{50} values in the present study were derived from blood obtained from normoxic fish. Future studies should probably also examine the P_{50} values for blood from hypoxic smallmouth bass and largemouth bass, which may be somewhat different from those in present study.

Further evidence that smallmouth bass are more sensitive to environmental hypoxia than largemouth bass was obtained from the species differences in catecholamine release during progressive hypoxia. Catecholamines are released during severe hypoxia in fish to initiate a variety of physiological mechanisms that will enhance blood O_2 transport (Perry and Wood 1989; Ristori and Laurent 1989; Perry and Reid 1992; Julio et al. 1998; Reid et al. 1998). For example, these stress hormones promote the release of additional red blood cells from the spleen, increase the gill surface area to enhance O_2 uptake, and activate red blood cell membrane ion transporters that increase intracellular pH and enhance Hb–O_2 binding (Nikinmaa 1983, 1990; Fievet et al. 1990). In the present experiments, smallmouth bass showed a relatively large increase in plasma catecholamine concentrations at the lowest level of hypoxia, but similar changes were not observed in largemouth bass (Table 1). These findings can be explained by the profound decrease in blood oxygen content in smallmouth bass at the lowest level of hypoxia, which necessitates the recruitment of additional catecholamine-sensitive mechanisms to enhance blood oxygen transport. In smallmouth bass, the increase in hematocrit at the lowest level of hypoxia provides further evidence that this is indeed the case. In contrast, similar catecholamine-sensitive mechanisms are probably not recruited in largemouth bass at the lowest level of hypoxia because blood oxygen content is already largely maintained by the greater inherent blood oxygen affinity of this species (Figure 2). Interestingly, the differences in the catecholamine release profiles between the two closely related bass species are as dramatic as those found between two species of teleosts that have very different hypoxia tolerance, the rainbow trout and the American eel (Perry and Reid 1992).

Smallmouth bass were also found to have a lower threshold for the initial perception of hypoxic stress and recruitment of regulatory mechanisms. Fish are thought to perceive hypoxia through both external and internal oxygen receptors (Perry and Gilmour 2002). Water oxygen levels (P_{WO_2}) are monitored by way of external gill O_2 receptors, which are involved in the control of both ventilation rate and cardiac activity (Daxboeck and Holeton 1978; Fritsche and Nilsson 1989, 1993; Perry and Gilmour 2002). Ventilatory responses to hypoxia are also mediated by internal receptors that respond to changes in either blood oxygen content (Randall 1982; Smith and Jones 1982) or the partial pressure of oxygen in the blood (Glass et al. 1990). Even at the most moderate level of hypoxia (P_{WO_2} = 90 torr), smallmouth bass increased ventilation rate (Figure 3) and decreased heart rate and cardiac output (Figure 4). In combination with lamellar recruitment and decreased blood–water diffusion distances, these physiological responses are thought to facilitate oxygen uptake by increasing the amount of water flowing over the branchial lamellae and by delaying the transit time of the blood in the gills. In the present study, however, we saw no evidence that these early physiological responses to hypoxia provided any enhancement of the blood oxygen transport status in smallmouth bass in comparison with that in largemouth bass (Figure 1). In contrast to the situation in smallmouth bass, a hyperventilatory response occurred in largemouth bass only at a P_{WO_2} of 60 torr (Figure 3). Moreover, the only change in cardiac function that was observed during hypoxic exposure in largemouth bass was a significant bradycardia at the most severe level of hypoxia (Figure 4). Taken together, these results may indicate that the internal and external O_2 receptors of largemouth bass have a greater threshold of sensitivity than those of smallmouth bass, which leads to a delayed onset of the regulatory mechanisms for hypoxia in largemouth bass. Alternatively, the present results could also be largely explained by the fact that the O_2 receptors in these species are primarily responding to the oxygen content of the blood, rather than to the P_{O_2}.

Hypoxic exposure in fish can also have significant consequences for their acid–base status. During the early phases of hypoxia, hyperventilation may lead to an alkalinization of the blood as a result of the increase in CO_2 (acid) excretion (Jensen et al. 1993). When hypoxic exposure becomes severe, the anaerobic production of lactic acid can also cause a metabolic acidosis in both the intracellular and extracellular space (Jensen et al.

1993). In the present study, we monitored the lactate concentrations and acid–base status in the blood (Table 1) and found that blood lactate concentrations became significantly greater at a higher water oxygen tension in smallmouth bass than in largemouth bass. At the deepest level of hypoxia, the magnitude of the increase in blood lactate concentration in smallmouth bass was also about twice that in largemouth bass. In terms of blood acid–base status, significant alkalinization was evident in the blood of largemouth bass during the P_{WO_2} = 60 torr treatment, but a similar response was not observed in smallmouth bass. The differences in acid–base status of these two species at the most severe level of hypoxia also provided another piece of evidence that smallmouth bass are less tolerant of hypoxia than largemouth bass. At the lowest ambient P_{O_2}, smallmouth bass had a significant acidosis in their blood, but there was no significant change in the blood acid–base status of largemouth bass. As these results indicate, any potential metabolic acidosis that might be anticipated because of the modest increase in blood lactate during severe hypoxia in largemouth bass must have been offset by other factors (e.g., hyperventilation and alkanization) or acid–base regulatory mechanisms (e.g., buffering or ion transport processes). In contrast, the acidosis associated with anaerobic metabolism in smallmouth bass appears to have exceeded the capacity of this species for acid–base regulation by similar mechanisms. At present, we do not know whether the physiological mechanisms involved in acid–base regulation also differ between these two bass species, and this may be an interesting avenue for future studies.

Some important interspecific differences were also observed in these experiments during the posthypoxia recovery period. After the 12-h recovery period, all of the monitored variables in largemouth bass had returned to levels that were not significantly different from control normoxia values. In contrast, many of the physiological variables monitored in smallmouth bass, such as arterial P_{O_2}, $[O_2]$/[Hb], and pH, as well as all of the cardiac variables, remained significantly different after the recovery period. Moreover, all of the largemouth bass used in this study survived, whereas four of the smallmouth bass from the first series of in vivo experiments did not survive the hypoxia exposure. In the second series of in vivo experiments, some of the smallmouth bass exhibited erratic heartbeats during the recovery period, but this was never observed in largemouth bass. Taken together, these recovery results provide further evidence of important differences between largemouth bass and smallmouth bass in terms of their abilities to tolerate hypoxia.

In summary, the present experiments provide strong evidence that smallmouth bass are more sensitive to hypoxia than largemouth bass are. Progressive reductions in water oxygen levels had a much greater impact on blood oxygen transport properties, acid–base status, ventilation rates, and cardiac variables in smallmouth bass than in largemouth bass. The more severe hypoxia exposure in these studies also caused significant catecholamine release, as well as some mortalities, in smallmouth bass but not in largemouth bass. These physiological differences in hypoxia tolerance correspond to the different lifestyles and habitat preferences of these two species and probably result from very different selective pressures on their respective oxygen transport systems. These findings also indicate that centrarchid species such as bass may be useful models for examining the evolution of physiological traits in closely related species. In this regard, further studies may be warranted to determine what other physiological differences exist between these two species of bass.

The results of the present study also have important implications for fisheries management. In our experience, hypoxia is probably one of the most significant factors contributing to fish mortality during live-release angling tournaments. When adequate precautions are not taken, hypoxia may occur at any of several different stages at these events, including live well holding, bag confinement, weigh-in air exposure, and the holding tanks of the live-release vessels used to disperse the fish at the end of the event. A reduced tolerance for hypoxia would therefore largely explain why smallmouth bass often appear to be less tolerant of tournament procedures than largemouth bass. Since angling tournaments normally target the largest fish in a given system, tournaments that include smallmouth bass should take extra precautions to ensure that sufficient oxygen levels are provided at each stage of the event. In the future, tournament organizers and fisheries managers should probably develop guidelines for appropriate oxygen thresholds based on the needs of smallmouth bass, rather than those of largemouth bass, in regions where these two species coexist.

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References

Bennett, D. H., L. K. Dunsmoor, R. L. Rohrer, and B. E. Rieman. 1989. Mortality of tournament-caught largemouth and smallmouth in Idaho lakes and reservoirs. California Fish and Game 75:20–26.

Cooke, S. J., D. P. Philipp, K. M. Dunmall, and J. F. Schreer. 2001. The influence of terminal tackle on injury, handling time, and cardiac disturbance of rock bass. North American Journal of Fisheries Management 21:265–274.

Coble, D. W. 1975. Smallmouth bass. Pages 21–33 *in* R. H. Stroud and H. Clepper, editors. Black bass biology and management. Sport Fishing Institute, Washington, D.C.

Daxboeck, C., and G. F. Holeton. 1978. Oxygen receptors in the rainbow trout, *Salmo gairdneri*. Canadian Journal of Zoology 56:1254–1259.

Demers, E., R. S. McKinley, A. H. Weatherley, and D. J. McQueen. 1996. Activity patterns of largemouth and smallmouth bass determined with electromyogram biotelemetry. Transactions of the American Fisheries Society 125:434–439.

Duttweiler, M. W. 1985. Status of competitive fishing in the United States: trends and state fisheries policies. Fisheries 10(5):5–7.

Fievet, B., J. Caroff, and R. Motais. 1990. Catecholamine release controlled by blood oxygen tension during deep hypoxia in trout: effect on red blood cell Na^+/H^+ exchanger activity. Respiration Physiology 79:81–90.

Fritsche, R., and S. Nilsson. 1989. Cardiovascular responses to hypoxia in the Atlantic cod, *Gadus morhua*. Experimental Biology 48:153–160.

Fritsche, R., and S. Nilsson. 1993. Cardiovascular and ventilatory control during hypoxia. Pages 183–201 *in* J. C. Rankin and F. B. Jensen, editors. Fish ecophysiology. Chapman and Hall, London.

Glass, M. L., N. A. Andersen, M. Kruhøffer, E. M. Williams, and N. Heisler. 1990. Combined effects of environmental P_{O2} and temperature on ventilation and blood gasses in the carp *Cyprinus carpio* L. Journal of Experimental Biology 148:1–17.

Hartley, R. A., and J. R. Moring. 1993. Observations of black bass (Centrarchidae) confined during angling tournaments: a cautionary note concerning dissolved oxygen. Aquaculture and Fisheries Management 24:575–579.

Hartley, R. A., and J. R. Moring. 1995. Differences in mortality between largemouth and smallmouth bass caught in tournaments. North American Journal of Fisheries Management 15:666–670.

Heidinger, R. C. 1975. Life history and biology of the largemouth bass. Pages 11–20 *in* R. H. Stroud and H. Clepper, editors. Black bass biology and management. Sport Fishing Institute, Washington, D.C.

Jensen, F. B., M. Nikinmaa, and R. E. Weber. 1993. Environmental perturbations of oxygen transport in teleost fishes: causes, consequences and compensations. Pages 161–179 *in* J. C. Rankin and F. B. Jensen, editors. Fish ecophysiology. Chapman and Hall, London.

Julio, A. E., C. J. Montpetit, and S. F. Perry. 1998. Does acid-base status modulate catecholamine secretion in the rainbow trout (*Oncorhynchus mykiss*)? Journal of Experimental Biology 201:3085–3095.

Nikinmaa, M. 1990. Vertebrate red blood cells. Springer-Verlag, Berlin.

Nikinmaa, M. 1983. Adrenergic regulation of haemoglobin oxygen affinity in rainbow trout red cells. Journal of Comparative Physiology 152:67–72.

Perry, S. F., and K. M. Gilmour. 2002. Sensing and transfer of respiratory gases at the fish gill. Journal of Experimental Zoology 293:249–263.

Perry, S. F., and S. D. Reid. 1992. Relationship between blood O_2 content and catecholamine levels during hypoxia in rainbow trout and American eel. American Journal of Physiology 263:R240–R249.

Perry, S. F., and C. M. Wood. 1989. Control and coordination of gas transfer in fishes. Canadian Journal of Zoology 67:2961–2970.

Randall, D. 1982. The control of respiration and circulation in fish during exercise and hypoxia. Journal of Experimental Biology 100:275–288.

Reid, S. G., N. Bernier, and S. F. Perry. 1998. The adrenergic stress response in fish: control of catecholamine storage and release. Comparative Biochemistry and Physiology 120A:1–27.

Ristori, M. T., and P. Laurent. 1989. Plasma catecholamines in rainbow trout (*Salmo gairdneri*) during hypoxia. Experimental Biology 48:285–290.

Schramm, H. L., Jr., M. L. Armstrong, N. A. Funicelli, D. M. Green, D. P. Lee, R. E. Manns, Jr., B. D. Taubert, and S. J. Waters. 1991. The status of competitive sport fishing in North America. Fisheries 16(3):4–12.

Schreer, J. F., S. J. Cooke, and R. S. McKinley. 2001. Cardiac response to variable forced exercise at different temperatures: an angling simulation for smallmouth bass. Transactions of the American Fisheries Society 130:783–795.

Shupp, B. D. 1979. 1978. Status of bass fish tournaments in the United States. Fisheries 4(6):11–19.

Smith, F. M., and D. R. Jones. 1982. The effects of changes in blood oxygen carrying capacity on ventilation volume in the rainbow trout (*Salmo gairdneri*). Journal of Experimental Biology 97:325–335.

Soivio, A., K. Nynolm, and K. Westman. 1975. A technique for repeated sampling of the blood of indi-

vidual resting fish. Journal of Experimental Biology 62:207–217.

Takeda, T. 1990. Ventilation, cardiac output and blood respiratory parameters in the carp, *Cyprinus carpio*, during hyperoxia. Respiratory Physiology 81:227–239.

Tucker, V. A. 1967. Method for oxygen content and dissociation curves on microliter blood samples. Journal of Applied Physiology 23:410–414.

Wilde, G. R. 1998. Tournament-associated mortality in black bass. Fisheries 23(10):12–22.