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Respiratory responses to progressive hypoxia in the Amazonian oscar, *Astronotus ocellatus*

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ABSTRACT

This study determined the respiratory responses to progressive hypoxia in oscar, an extremely hypoxia-tolerant Amazonian cichlid. Oscar depressed oxygen consumption rates ($\dot{M}_{O_2}$), beginning at a critical O_2 tension (P_{crit}) of 46 Torr, to only 14% of normoxic rates at 10 Torr. Total ventilation ($\dot{V}_w$) increased up to 4-fold, entirely due to a rise in ventilatory stroke volume (no change in ventilatory frequency), and water convection requirement ($\dot{V}_w/\dot{M}_{O_2}$) increased substantially (up to 15-fold). Gill O_2 extraction fell steadily, from 60% down to 40%. Although O_2 transfer factor (an index of gill O_2 diffusion capacity) increased transiently in moderate hypoxia, it decreased at 10 Torr, which may have caused the increased expired–arterial P_{O_2} difference. Venous P_{O_2} was always very low (≤7 Torr). Anaerobic metabolism made a significant contribution to ATP supply, indicated by a 3-fold increase in plasma lactate that resulted in an uncompensated metabolic acidosis. Respiration of isolated gill cells was not inhibited until below 5 Torr; because gill water P_{O_2} always exceeded this value, hypoxic ion flux arrest in oscars [Wood et al., Am. J. Physiol. Reg. Integr. Comp. Physiol. 292, R2048-R2058, 2007] is probably not caused by O2 limitation in ionocytes. We conclude that metabolic depression and tolerance of anaerobic bi-products, rather than a superior capacity for O_2 supply, allow oscar to thrive in extreme hypoxia in the Amazon.

1. Introduction

The Amazonian oscar (acará-açu; *Astronotus ocellatus*) is a hypoxia-tolerant cichlid that lives in the floodplains and flooded jungles of Brazil. This species can experience drastic daily fluctuations of environmental oxygen in its natural habitat, due to the combination of photosynthetic macrophytes, stagnant water, and decaying organic matter. Nevertheless, oscar thrive in the extreme hypoxia that can exist in these areas, where they continue to feed and reproduce. In fact, adult oscar can survive severe hypoxia (<10 Torr) for at least 20 h and complete anoxia for up to 6 h at 28 °C (Muusze et al., 1998; Almeida-Val et al., 2000; Richards et al., 2007). Oscar are therefore unlike many other hypoxia-tolerant vertebrates, like carp (Nilsson and Renshaw, 2004), frogs (Boutilier, 2001b), and turtles (Lutz and Milton, 2004; Warren and Jackson, 2008), which experience hypoxia at much cooler temperatures.

Metabolic depression is the most pervasive strategy for surviving hypoxia, which reduces O_2 demands through coordinated regulation of individual cells and whole physiological control systems (Hochachka et al., 1996; Guppy and Withers, 1999; Boutilier, 2001a; Bickler and Buck, 2007). The most ATP demanding cellular processes are protein synthesis and ion pumping, and there is evidence that both of these are downregulated during severe hypoxia in oscar. Protein synthesis declines 30–60% in the brain, liver, heart, and gills of oscar (Lewis et al., 2007). The activity of the dominant ion-motive ATPase in the gills and kidney, Na^+,K^+-ATPase, is greatly reduced during hypoxia (Richards et al., 2007; Wood et al., 2007). In the gills, these responses are accompanied by a reduction of active ion absorption from the surrounding freshwater, but ionic homeostasis is maintained due to a parallel decline in passive ion leak across the gills (Wood et al., 2007). In addition

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to reducing ATP demand, the capacity of aerobic metabolic pathways of ATP supply are reduced during hypoxia in a congeneric species of oscar (*A. crassipinnis*), while that of anaerobic pathways increase (Chippari-Gomes et al., 2005). Reducing O_2 demands by depressing ATP turnover and by recruiting anaerobic forms of ATP production thus allow oscar to maintain cellular [ATP] and redox balance (Richards et al., 2007).

Enhancing O_2 supply in hypoxia could be an effective strategy for circumventing cellular O_2 lack. Some of the earliest work on rainbow trout (*Oncorhynchus mykiss*) in hypoxia demonstrated that several physiological systems respond to help maintain O_2 transport, including increases in ventilation, gill diffusion capacity, blood pressure, and haemoglobin O_2 affinity (Holeton and Randall, 1967a,b; Randall et al., 1967; Nikinmaa and Soivio, 1982). The exceptional ability of oscar to match cellular O_2 supply and demand, and thus survive prolonged periods of severe hypoxia, could be partly due to an enhanced O_2 supply capacity. The objective of the present study was to test this possibility by determining how the respiratory system of oscar responds to hypoxia. O_2 consumption, breathing, gill gas exchange, blood gases and acid–base status, and plasma lactate were measured during exposure to progressive hypoxia. Because gill ion flux (both active influx and passive efflux) is partially arrested during hypoxia (Wood et al., 2007), we also measured respiration of isolated gill cells to determine if cellular O_2 shortage (i.e., ATP lack) contributes to this response.

2. Materials and methods

2.1. Experimental animals

Oscars (*Astronotus ocellatus*) (115–470 g; 310 ± 42 g) were obtained from Sítio dos Rodrigues (Km 35, Rod. AM-010, Brazil), and were moved to the Ecophysiology and Molecular Evolution Laboratory of the Instituto Nacional de Pesquisas da Amazônia (INPA), in Manaus, Brazil. Fish were held for at least 1 month in 500 l tanks on a partially recirculating water filtration system at 28 ± 3 °C. The holding and experimental water was typical Amazonian soft water taken from a well on the INPA campus (concentrations in μM: Na^+ 35, Cl^- 36, K^+ 16, Ca^{2+} 18, Mg^{2+} 4; dissolved organic carbon, 0.6 mg C/l; pH 6.5). Fish experienced a natural photoperiod and were fed daily with commercial pellets, but were starved for at least 2 days before experiments. All experimental procedures complied with Brazilian and INPA animal care regulations.

2.2. Experiment one: responses to hypoxia in oscar

2.2.1. Surgical procedures

Oscar were deeply anaesthetized in tricaine methanesulfonate anaesthetic (0.5 g/l MS-222, neutralized with 1.0 g/l $NaHCO_3$) and transferred to a surgery table. A lateral incision was made just rostral of the caudal peduncle to expose the spinal column, caudal artery, and caudal vein. Either the artery (4 fish) or vein (5 fish) was cannulated with a flexible polyethylene cannulae (PE-50) filled with 100 IU/ml heparinized Cortland saline (in mM: NaCl 124, KCl 5.1, $CaCl_2{\cdot}2H_2O$ 1.6, $MgSO_4{\cdot}7H_2O$ 0.9, $NaHCO_3$ 12, $NaH_2PO_4{\cdot}H_2O$ 2.9, glucose 5.6, pH 7.7) (Wolf, 1963) and the incision was sutured closed. Additional PE-50 cannulae were implanted through the rostrum, positioned to sample water in the buccal cavity (i.e., inspired water), and through the operculum, positioned to sample water coming off the gills halfway along the arch (i.e., expired water). Fish were moved to experimental chambers to recover overnight from surgery.

2.2.2. Experimental procedures

Experimental chambers were 2.5 l (3 smaller fish, 115–150 g) or 5.5 l (7 larger fish, 310–470 g) plastic containers sealed with parafilm. The chambers were half submerged in a large water bath to maintain the experimental temperature of 28 ± 1.5 °C, and were fitted with flow through water lines and aeration stones. Dissolved O_2 concentration of the water (measured with a portable D_{O_2} meter; WTW Oxi325 Oximeter) and ventilatory frequency (fR), monitored visually, were determined in normoxia at the start of each experiment, and samples were taken from water and blood cannulae. Water lines and aeration stones were then removed and the chamber was sealed. Dissolved O_2 (D_{O_2}) and fR were measured every 5 min thereafter until water D_{O_2} reached 0.5 mg/l (~10 Torr). Samples from water and blood cannulae were taken at 4, 2, 1, and 0.5 mg O_2/l (~140, 75, 40, 20, and 10 Torr). Cannulae were submerged throughout the experiment to minimize O_2 diffusion between air and the samples. Water samples were slowly drawn into glass syringes to not disturb the normal ventilatory flows of the fish. All oscar survived the experiments, which lasted 1.5–2 h, and were subsequently recovered in normoxia.

2.2.3. Measurements and calculations

After being drawn, water and blood samples (0.4 ml) were immediately analyzed. Oxygen tensions (P_{O_2}) of inspired water ($P_{I_{O_2}}$), expired water ($P_{E_{O_2}}$), and blood, as well as blood pH, were determined using Radiometer blood gas/pH electrodes maintained at experimental temperature. Plasma was isolated by centrifugation, and then total CO_2 content (C_{CO_2}) was measured using the method of Cameron (1971) and lactate was measured with a portable lactate meter (Accutrend-Lactate, TYP3012522).

Oxygen consumption rates ($\dot{M}_{O_2}$) were calculated every 5 min from the change in D_{O_2} (multiplied by chamber volume), and are reported relative to body mass at 140, 75, 40, 20, and 10 Torr water P_{O_2}. Critical O_2 tensions (P_{crit}), the P_{O_2} when fish began O_2 conforming, were determined from the 5 min measurements of $\dot{M}_{O_2}$ and water D_{O_2} using the computer program described by Yeager and Ultsch (1989). Total ventilation ($\dot{V}_w$) was calculated by the Fick principle:

$$\dot{V}_w = \frac{\dot{M}_{O_2}}{(P_{I_{O_2}} - P_{E_{O_2}})\alpha_{O_2}} \quad (1)$$

where α_{O_2} is the water O_2 solubility (in mmol/l/Torr). Ventilatory stroke volume (V_v) is the quotient of $\dot{V}_w$ and fR, and water convection requirement (WCR) is the quotient of $\dot{V}_w$ and $\dot{M}_{O_2}$. Gill O_2 extraction (E_{O_2}) is the difference of $P_{I_{O_2}}$ and $P_{E_{O_2}}$, divided by $P_{I_{O_2}}$. Oxygen transfer factor (T_{O_2}), a measure of the relative ability of the respiratory surface to exchange O_2 (Randall et al., 1967), is defined as $\dot{M}_{O_2}$ relative to the average P_{O_2} driving force for diffusion across the gas exchange surface:

$$T_{O_2} = \frac{\dot{M}_{O_2}}{1/2(P_{I_{O_2}} + P_{E_{O_2}}) - 1/2(P_{a_{O_2}} + P_{v_{O_2}})} \quad (2)$$

Blood P_{CO_2} and [HCO_3^-] were calculated from measured values of C_{CO_2} and pH using two equations:

$$pH = pK + \log\frac{[HCO_3^-]}{\alpha_{CO_2}P_{CO_2}} \quad (3)$$

$$[HCO_3^-] = C_{CO_2} - \alpha_{CO_2}P_{CO_2} \quad (4)$$

where pK is the apparent pK (pK_{app}) for teleost plasma, and α_{CO_2} is the plasma CO_2 solubility, both at the experimental temperature. Values for α_{O_2}, α_{CO_2}, and pK were obtained from Boutilier et al. (1984). Water P_{CO_2} was estimated by assuming that CO_2 accumulation in the water was related to $\dot{M}_{O_2}$ by a respiratory quotient of 0.85 (i.e., the product of the amount of O_2 consumed and the respiratory quotient), using α_{CO_2} obtained from Boutilier et al. (1984).

2.3. Experiment two: respiration of isolated gill cells during hypoxia

Six oscar were rapidly stunned by cephalic blow and sacrificed by pithing. The pericardial cavity was then opened and the gills were perfused with 20 ml of ice-cold heparinized (100 IU/ml) phosphate-buffered saline (PBS, in mM: 137 NaCl, 2.7 KCl, 8.1 Na_2HPO_4, 1.5 KH_2PO_4; pH 7.7) through the bulbous arteriosus. Gill arches were then immediately excised, rinsed in cold PBS, and blotted lightly. Arches were placed in cold PBS, and isolation of gill cells was then performed by methods similar to those of Kelly et al. (2000). Filaments were cut from gill arches and transferred to 5 ml PBS containing 9 IU trypsin and 5.3 mM EDTA, and shaken at 300 rpm on a rotator. Filaments were mechanically agitated and strained through nylon stocking (Trifil, Modelo Europeu) into 20 ml of PBS containing 8% albumin to saturate the trypsin. The remaining strained filaments were transferred to fresh trypsin solution, then shaken and strained as before. Cells in 8% albumin were subsequently centrifuged at 500 × *g* for 10 min at 4 °C, and the supernatant was aspirated. The pellet containing gill cells was suspended in 10 ml distilled water for 30 s to lyse any remaining erythrocytes, and 30 ml PBS was added. Cells were centrifuged as before, the supernatant was aspirated, and cells were resuspended in 10 ml PBS. This was then repeated and cells were resuspended in 10 ml Cortland saline.

Respiration of isolated gill cells in Cortland saline was measured in a Tucker chamber maintained at 25 °C. The chamber was filled with the cell suspension, thus removing air and closing the chamber, and P_{O_2} was measured every 5 min thereafter with a Radiometer electrode. Oxygen consumption rate was determined from the change in P_{O_2} over time, using the appropriate α_{O_2} for physiological saline at 25 °C from Boutilier et al. (1984), and was expressed relative to total protein concentration in the cell suspension (determined using the Bradford assay with bovine serum albumin standards; Sigma–Aldrich). O_2 consumption by the electrode reduced the P_{O_2} of Cortland saline alone by 0.12 Torr/min, so cell respiration was corrected for this background rate of consumption. Cell densities were determined after each experiment with a haemocytometer, and erythrocyte contamination was minimal. Expressing data relative to cell density (data are not shown) yielded very similar results to data that were expressed relative to protein concentration. Cells were healthy and intact, displaying no eosin uptake in eosin exclusion tests.

2.4. Statistical analyses

Data are expressed as means ± S.E.M. Repeated measures ANOVA was used to ascertain overall differences, with Holm–Sidak post hoc tests for pairwise comparisons to normoxic controls. All statistical analyses were conducted using Sigmastat (version 4, Systat Software Inc.) and a significance level of $p < 0.05$ was used throughout.

3. Results

3.1. Respiratory responses of oscar to progressive hypoxia

Oscar depressed aerobic metabolism during hypoxia, reflected by decreases in O_2 consumption rates at water O_2 tensions of 40 Torr and lower (Fig. 1). When exposed to the lowest P_{O_2} (10 Torr), O_2 consumption was only 14% of the normoxic rate. Depression of aerobic metabolism began at a critical O_2 tension of 46.4 ± 6.9 Torr when all fish were grouped together, but there were inter-individual differences in P_{crit} between fish of different sizes.

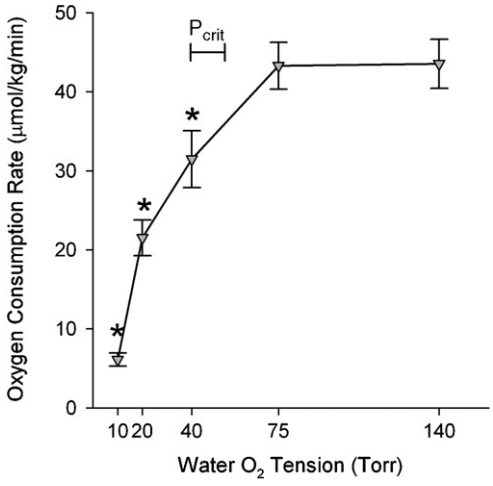

Fig. 1. Oxygen consumption rate decreased in oscar during hypoxia, with the onset of depression occurring at an average critical oxygen tension (P_{crit}) of 46.4 ± 6.9 Torr ($n = 10$). *Significant difference from normoxic controls ($p < 0.05$).

The P_{crit} of large fish (388 ± 20 g) was 34.3 ± 4.1 Torr, while that of smaller fish (129 ± 11 g) was substantially higher, at 74.4 ± 3.5 Torr.

Ventilation increased during hypoxia in oscar, particularly at a water P_{O_2} of 40 Torr and lower (Fig. 2). The increase in $\dot{V}_W$ was 4-fold above the normoxic value at 20 Torr, and this increase was entirely accounted for by a rise in ventilatory stroke volume (V_V). At 10 Torr, $\dot{V}_W$, V_V, and fR all appeared to decline compared to values at 20 Torr. However, water convention requirements, which indicate the relationship of total ventilation to aerobic metabolism, increased monotonically to nearly 15-fold above normoxic values in deep hypoxia (Fig. 3). In contrast, the percentage of O_2 extracted by the gills from inspired water decreased from just over 60% in normoxia to around 40% in deep hypoxia. This was not caused by a reduction in the ability of the gills to exchange gases during moderate hypoxia, reflected by a transient increase of the oxygen transfer factor (quotient of $\dot{M}_{O_2}$ and the average P_{O_2} driving force for O_2 diffusion; see Section 2). However, the oxygen transfer factor decreased considerably in severe hypoxia (Fig. 3).

Blood P_{O_2} of oscar declined during hypoxia (Fig. 4). Arterial P_{O_2} ($P_{a_{O_2}}$) was extremely similar to expired water P_{O_2} in normoxia (55 Torr) and moderate hypoxia. $P_{a_{O_2}}$ diverged below $P_{E_{O_2}}$ during severe hypoxia, reaching values of 18, 9, and 4 Torr at the three lowest water P_{O_2} (40, 20, and 10 Torr), when $P_{E_{O_2}}$ was 23, 15, and 8.5 Torr, respectively. Venous P_{O_2} was always very low, declining from 7 Torr in normoxia to less than 2 Torr in severe hypoxia. Inspired P_{O_2} was always very similar to bulk water P_{O_2}, but was slightly elevated above the latter in severe hypoxia, suggesting that oscar were searching out the most oxygenated water in the respirometer.

Acid–base balance was disturbed in oscar during hypoxia, reflected by decreases in blood pH (0.4–0.6 units), total CO_2 concentration, and bicarbonate concentration (Fig. 5). Total CO_2 and bicarbonate declined earlier in venous blood (40 Torr) than in arterial blood (10 Torr). Furthermore, pH was maintained longer in the blood (10 Torr), than venous CO_2 and bicarbonate contents. There was a slight rise in arterial and venous P_{CO_2} during hypoxia, but at least in arterial blood this could be explained by a rise in water P_{CO_2} in the closed respirometer (grey dashed line in Fig. 5). Hypercarbia was unlikely to have been entirely responsible for the blood acidosis, however, because (1) the pattern of acidosis was typical of an uncompensated metabolic acidosis, with only a slight respiratory acidosis in severe hypoxia (Fig. 6), and (2) plasma lactate concentrations increased over 3-fold during severe hypoxia (Fig. 7). Overall,

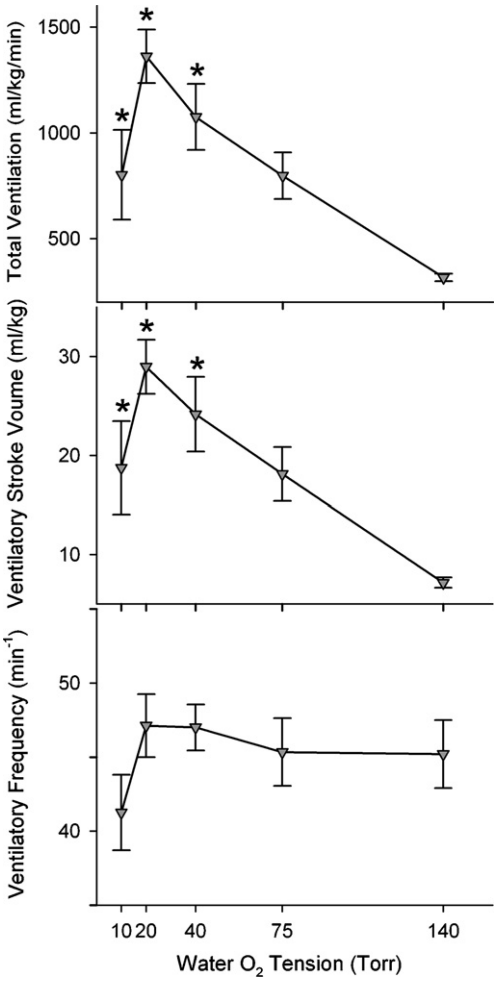

Fig. 2. Total ventilation (top) and ventilatory stroke volume (middle) of oscars increased substantially during hypoxia. Ventilatory frequency (bottom) did not change significantly during hypoxia ($n \geq 7$). *Significant difference from normoxic controls ($p < 0.05$).

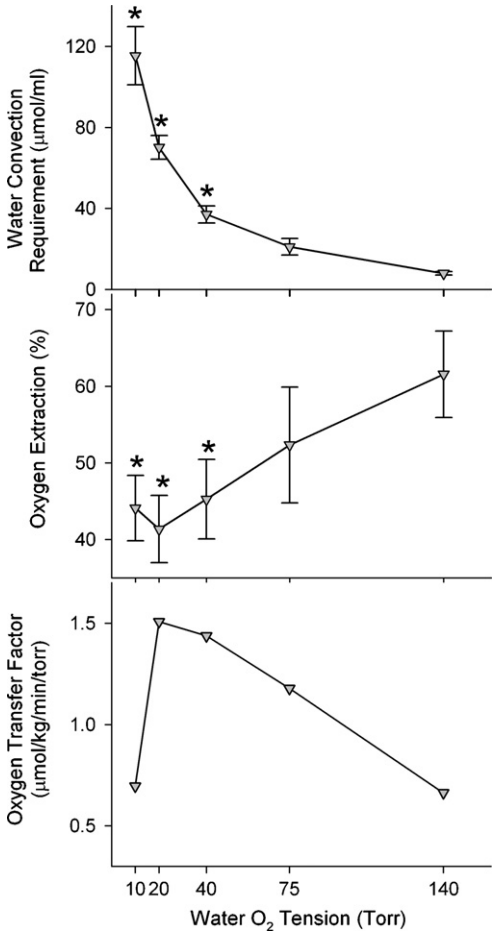

Fig. 3. Water convection requirement (quotient of total ventilation and oxygen consumption rate) increased (top), gill O_2 extraction (middle) decreased, and the average oxygen transfer factor (which indicates the relative ability of the gills to exchange gases, see Section 2) increased transiently (bottom) in oscars during hypoxia ($n \geq 7$). *Significant difference from normoxic controls ($p < 0.05$).

acid–base imbalance and lactate accumulation did not occur until water P_{O_2} was less than P_{crit}.

3.2. Respiration of isolated oscar gill cells during hypoxia

Respiration of isolated gill cells was not inhibited by ambient hypoxia until P_{O_2} was extremely low (Fig. 8). Surprisingly, respiration increased as P_{O_2} declined between 5 and 30 Torr compared to the O_2 consumption rates of cells at high P_{O_2}. The reason for this transient increase is unclear, but it is possible that cold exposure from cell isolation had residual suppressive effects on respiration in the early stages of this experiment. Respiration declined somewhere between 0 and 5 Torr, but the average rate in this interval was still equivalent to the O_2 consumption rates of cells at high P_{O_2}. Our cell respirometer lacked the resolution necessary to closely estimate the P_{O_2} when respiration first became O_2 limited, but our results nevertheless demonstrate that this does not occur until near anoxia. Lactate concentrations measured in the cells and media at the end of the experiment were below detection levels.

4. Discussion

Along with an incredible diversity of fish species in the Amazon are numerous examples of morphological adaptations to O_2

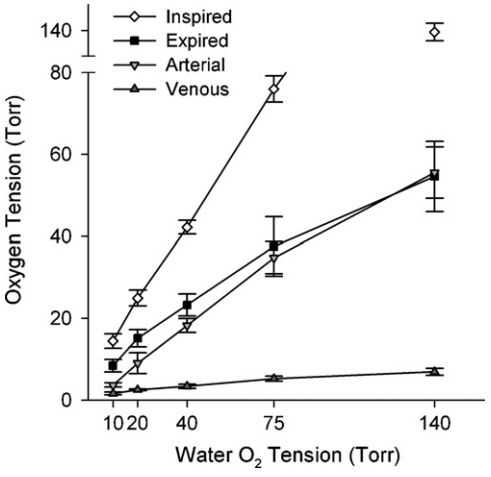

Fig. 4. Inspired water (diamonds), expired water (squares), arterial blood (downward triangles), and venous blood (upward triangles) O_2 tensions (P_{O_2}) decreased during hypoxia ($n \geq 4$). Each progressive decrease of bulk water P_{O_2} significantly reduced inspired, expired, arterial, and venous P_{O_2} ($p < 0.05$), except that there was no significant difference between venous P_{O_2} measured at 40 and 20 Torr.

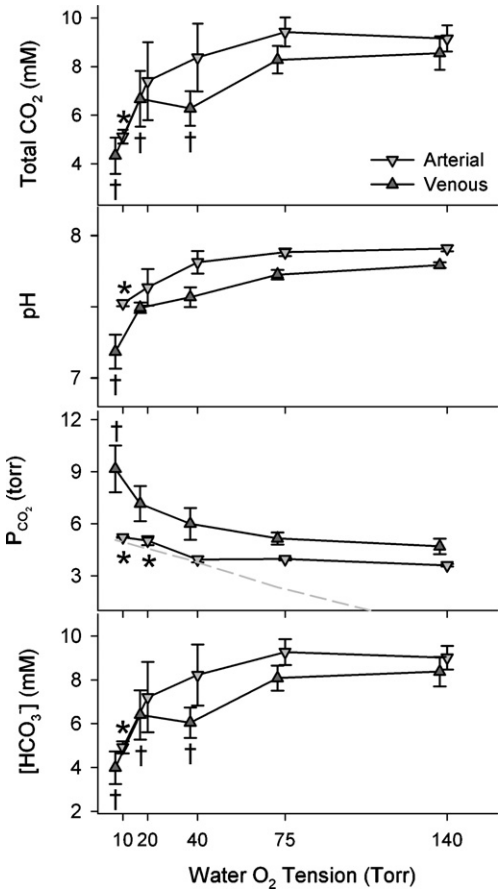

Fig. 5. Total CO_2 concentration, pH, and bicarbonate concentration ($[HCO_3^-]$) decreased in arterial (downward triangles) and venous (upward triangles) blood during hypoxia in oscars ($n \geq 4$). The slight increase in blood CO_2 tension (P_{CO_2}) could be explained by progressive hypercarbia in the closed respirometer (grey dashed line). * and †, Significant difference from normoxic controls for arterial or venous blood, respectively ($p < 0.05$).

poor environments (Val, 1995; Brauner and Val, 2006). Accessory air-breathing organs or adaptations to skim the O_2-rich water surface can help maintain O_2 supply when waters become hypoxic. For obligate water breathing fish however, particularly those that

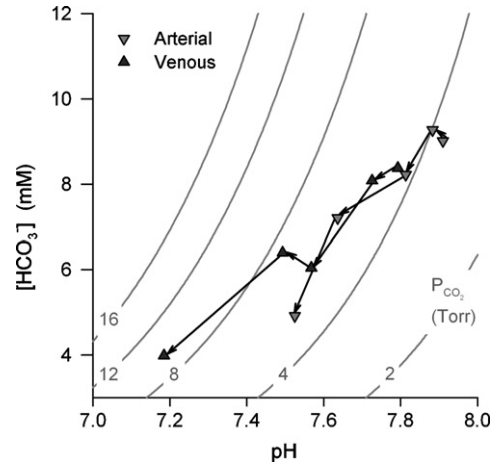

Fig. 6. Oscar experience a primarily uncompensated metabolic acidosis in arterial (downward triangles) and venous (upward triangles) blood during progressive hypoxia. Average values at each level of hypoxia are shown (see Fig. 5 for standard errors) and arrows indicate the direction of increasing severity of hypoxia.

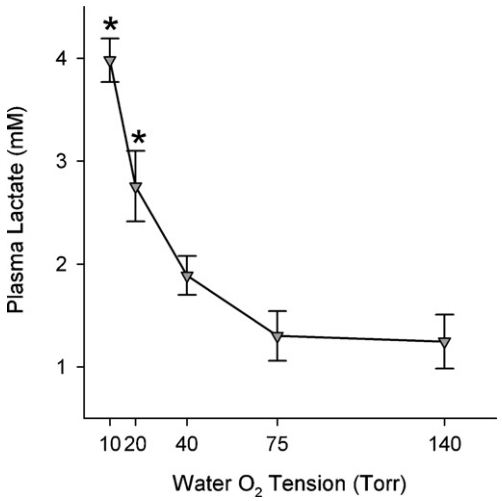

Fig. 7. Plasma lactate increased during hypoxia in oscars ($n \geq 6$). *Significant difference from normoxic controls ($p < 0.05$).

are subject to predation at the water surface, O_2 supply from their hypoxic environment must meet cellular O_2 demands. Physiological and biochemical adjustments then become essential to survive. Although O_2 supply appears to be enhanced in oscar during hypoxia (Figs. 2 and 3), our results demonstrate that ventilation and O_2 extraction under hypoxic conditions are not markedly better than in other fish species. The remarkable hypoxia tolerance of Amazonian oscar probably relies instead on pronounced suppression of aerobic metabolism (Fig. 1) and tolerance of the bi-products of anaerobic metabolism (Figs. 5–7). As a consequence of these physiological responses, oscar can survive complete anoxia for up to 6 h at 28 °C (Muusze et al., 1998; Almeida-Val et al., 2000), and can thrive in the often extreme conditions of the Amazon.

4.1. Ventilation and gas exchange in oscar during hypoxia

The hypoxic ventilatory response of oscar (Figs. 2 and 3) was similar in magnitude to that of many other Amazonian and neotropical species (Rantin et al., 1992; Sundin et al., 1999; de Salvo

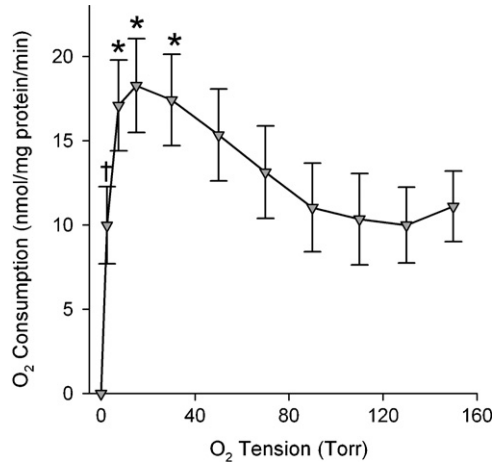

Fig. 8. Respiration of isolated oscar gill cells during hypoxia ($n = 6$). Data are shown over 5–10 Torr intervals of O_2 tension in the medium. *Significant difference from normoxic controls ($p < 0.05$). †Significant decline compared to data for the 20–30, 10–20, and 5–10 Torr intervals. Respiration during normoxia or hypoxia was always significantly higher than respiration in complete anoxia, when O_2 consumption was zero.

Souza et al., 2001; Sakuragui et al., 2003; Oliveira et al., 2004; Leite et al., 2007). However, in these and many other fish species (Holeton and Randall, 1967a,b; Glass et al., 1990; Greco et al., 1995; Burleson et al., 2002; Stecyk and Farrell, 2002; MacCormack et al., 2003; Vulesevic et al., 2006), which respond to hypoxia by increasing both ventilatory stroke volume and ventilatory frequency, the hypoxic ventilatory response of oscar is exclusively due to the former (Fig. 2). The reason for this difference is uncertain, but may relate to differences in the metabolic costs of breathing (Rantin et al., 1992).

Oxygen extraction by the gills decreased in oscar during hypoxia (Fig. 3) and was less than in other hypoxia-tolerant species (Steffensen et al., 1982; Rantin et al., 1992). The O_2 transfer factor (an index of gill O_2 diffusion capacity) increased transiently during moderate hypoxia, but then declined in severe hypoxia. When also considering the parallel divergence between $P_{E_{O_2}}$ and $P_{a_{O_2}}$ (Fig. 4), these observations might suggest that lamellar recruitment decreases in severe hypoxia in oscar. In contrast, both lamellar perfusion and the O_2 transfer factor increase during hypoxia in trout (Randall et al., 1967; Booth, 1979), in accordance with the general view (Nilsson, 2007). Decreased lamellar recruitment could have contributed to the reduction of passive ion loss in oscar during severe hypoxia (Wood et al., 2007), and in this way may serve to facilitate gill metabolic depression (see Section 4.2 below) rather than O_2 uptake.

Larger ventilatory stroke volumes (V_v) may have reduced the proportion of water that contacts the gas exchange surface, and thus also contributed to the decrease of $P_{a_{O_2}}$ relative to $P_{E_{O_2}}$. However, this contradicts findings in the genus *Hoplias* during hypoxia, when the more hypoxia-tolerant traíra, *H. malabaricus*, maintains higher O_2 extraction and preferentially increases V_v compared to the less tolerant trairão, *H. lacerdae* (Rantin et al., 1992).

Arterial and venous P_{O_2} were relatively low in oscar during normoxia (Fig. 4), which is typical of less active and/or hypoxia-tolerant species (Wood et al., 1979; Burggren, 1982) and unlike more active species (Holeton and Randall, 1967b; Bushnell and Brill, 1992). The low venous P_{O_2} we observed in normoxia (7 Torr), and the further decline in hypoxia, could imply that oscar have a high haemoglobin O_2 affinity. Haemoglobin saturation of venous blood in normoxia at rest is typically between 40% and 70% in teleosts (Wood et al., 1979; Burggren, 1982). If venous haemoglobin saturation is ~50% or higher in normoxic oscar, our $P_{v_{O_2}}$ measurements would suggest that their haemoglobin P_{50} (P_{O_2} at half saturation) is quite low (<10 Torr). High haemoglobin O_2 affinities are characteristic of many hypoxia-tolerant fish, such as carp (Burggren, 1982; Albers et al., 1983), jeju, pacu (*Piraractus mesopotamicus*), traíra (Perry et al., 2004), jaraqui (*Semaprochilodus* spp.) (Val et al., 1986), and tambaqui (Gonzalez et al., 2005), and is often related to P_{crit} (Mandic, M., Todgham, A.E., Richards, J.G., Unpublished). A low P_{50} is beneficial because it helps keep arterial saturation and O_2 concentration high when $P_{a_{O_2}}$ falls, and reduces the $P_{a_{O_2}}$ necessary to fully saturate arterial blood in normoxia, which could reduce ventilatory requirements and the associated metabolic costs. However, the disadvantage of a low P_{50} is that it can impair unloading of O_2 from haemoglobin at the tissues in normoxia (Brauner and Val, 2006; Scott and Milsom, 2006). If the P_{50} of oscar is indeed as low as it appears, their moderately high P_{crit} (Fig. 1) may not be as strongly influenced by P_{50} as in other species.

Overall, the oxygen supply capacity of oscar was not enhanced compared to other fish species during hypoxia (except for the potentially lower P_{50}). Their remarkable hypoxia tolerance probably relies instead on a suppression of O_2 demands and a tolerance of anaerobic bi-products, as discussed below.

4.2. Metabolic depression in oscar during hypoxia

Oxygen consumption rates of oscar were low in normoxia, as observed in other hypoxia tolerant species (Rantin et al., 1992). O_2 consumption fell drastically in severe hypoxia, to only 14% of the normoxic rate (Fig. 1), a magnitude of depression that rivals many other hypoxia tolerant vertebrates (Guppy and Withers, 1999). The critical O_2 tension for O_2 conformance (P_{crit}) averaged 46 Torr, but was higher in small oscar than in large oscar, as previously observed for un-instrumented individuals of this species (Muusze et al., 1998; Sloman et al., 2006). This P_{crit} value is similar to many other Amazonian species (Saint-Paul, 1984; Oliveira et al., 2004; Affonso and Rantin, 2005), to some other neo-tropical species (Rantin et al., 1992; de Salvo Souza et al., 2001), and to certain hypoxia-intolerant species (Ultsch et al., 1978), all measured between 20 and 30 °C. In contrast, the P_{crit} of oscar is higher than that of some other Amazonian fish, like traíra (*H. malabaricus*, 22 Torr at 25 °C; Sakuragui et al., 2003), as well as other hypoxia-tolerant species, like mormyrid (*Petrocephalus catostoma*, 9 Torr at 20 °C; Chapman and Chapman, 1998) and tilapia (*Oreochromis niloticus*, 18 Torr at 25 °C; Fernandes and Rantin, 1989). While the P_{crit} of oscar is at or above that of many other fish, their hypoxia/anoxia tolerance is exceptional (Muusze et al., 1998; Almeida-Val et al., 2000), which suggests that P_{crit} is not a reliable sole indicator of hypoxia tolerance in fishes.

Depression of ATP turnover (i.e., total metabolism) is probably substantial in oscar during hypoxia, but is likely not as dramatic as the pronounced decline in aerobic metabolism. Lactate increased substantially in the plasma during hypoxia (Fig. 7), as previously observed in plasma, muscle, and liver (Chippari-Gomes et al., 2005; Richards et al., 2007; Wood et al., 2007). ATP supply from anaerobic metabolism may therefore make a substantial contribution to total metabolism during hypoxia in oscar; however, future measurements of this contribution in its entirety are necessary to determine the overall decline in ATP turnover.

Our recent work in oscar has revealed a novel response to hypoxia in fish that likely has an important role in hypoxic metabolic arrest. Oscar decrease active ion absorption during hypoxia (Wood et al., 2007), which should reduce the costs of gill ion pumping (Wood et al., 1978). Ion influx decreases at and below P_{crit} (Fig. 1), as does a concurrent reduction of passive ion loss that helps maintain ion balance (Wood et al., 2007). This 'ion flux arrest' is analogous to channel arrest in hypoxic cells (Boutilier, 2001a), but occurs as a coordinated event that alters the function of an entire epithelium. Two observations in the current study suggest that flux arrest does not occur due to cellular O_2 lack in the ionocytes. Firstly, expired P_{O_2} (which should best represent the P_{O_2} in contact with ionocytes) is high (23 Torr) at a water P_{O_2} of 40 Torr, and is still considerable (8.5 Torr) at a water P_{O_2} of 10 Torr. Secondly, respiration of gill cells is not inhibited by hypoxia until well below 5 Torr (Fig. 8). Hypoxic flux arrest therefore appears to be a regulated event that occurs to reduce whole-animal metabolic demands, rather than a consequence of a mismatch between supply and demand of ATP or O_2 in ionocytes.

4.3. Acid–base responses to hypoxia in oscar

The uncompensated metabolic acidosis in hypoxia (Figs. 5 and 6), likely caused by an increase in anaerobic metabolism (Fig. 7), must undoubtedly be tolerated by oscar. Juvenile oscar do not compensate severe respiratory acidosis (water P_{CO_2} of 30 Torr) for at least 24 h, unlike other fish that typically retain HCO_3^- in exchange for Cl^- to partially restore blood pH (D.W. Baker and C.J. Brauner, unpublished). Furthermore, intracellular pH, at least in the muscle, changes in parallel to blood pH in hypoxia (Richards et al.,

2007), so oscar may not avoid intracellular acidification like some other Amazonian fish (Brauner et al., 2004). Continued metabolic function, albeit at a reduced rate, may therefore be resilient to the bi-products of anaerobic metabolism in this species. Metabolic enzymes are markedly tolerant of intracellular acidosis in some anoxia-tolerant turtles (Storey and Hochachka, 1974), and it is possible that protein function in oscar is similarly tolerant of pH change.

4.4. Conclusions

The exceptional hypoxia tolerance of oscar is undoubtedly facilitated by a pronounced metabolic depression that involves a coordinated response in many tissues. O_2 supply along the O_2 transport pathway appears to be maintained in hypoxia to the extent that it can, but O_2 extraction may be sacrificed during severe hypoxia to help reduce passive ion leak across the gills, and thus reduce the costs of gill ion pumping. Despite a probable reduction in ATP turnover during hypoxia, anaerobic metabolism is recruited by oscar to help maintain ATP supply. The associated increase in H^+ and lactate production must be tolerated by this species, which may contribute to their prolonged survival in anoxia.

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References

Affonso, E.G., Rantin, F.T., 2005. Respiratory responses of the air-breathing fish *Hoplosternum littorale* to hypoxia and hydrogen sulfide. Comp. Biochem. Physiol. C: Pharmacol. Toxicol. 141, 275–280.

Albers, C., Manz, R., Muster, D., Hughes, G.M., 1983. Effect of acclimation temperature on oxygen transport in the blood of the carp, *Cyprinus carpio*. Respir. Physiol. 52, 165–179.

Almeida-Val, V.M., Val, A.L., Duncan, W.P., Souza, F.C., Paula-Silva, M.N., Land, S., 2000. Scaling effects on hypoxia tolerance in the Amazon fish *Astronotus ocellatus* (Perciformes: Cichlidae): contribution of tissue enzyme levels. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 125, 219–226.

Bickler, P.E., Buck, L.T., 2007. Hypoxia tolerance in reptiles, amphibians, and fishes: life with variable oxygen availability. Annu. Rev. Physiol. 69, 145–170.

Booth, J.H., 1979. The effects of oxygen supply, epinephrine, and acetylcholine on the distribution of blood flow in trout gills. J. Exp. Biol. 83, 31–39.

Boutilier, R.G., 2001a. Mechanisms of cell survival in hypoxia and hypothermia. J. Exp. Biol. 204, 3171–3181.

Boutilier, R.G., 2001b. Mechanisms of metabolic defense against hypoxia in hibernating frogs. Respir. Physiol. 128, 365–377.

Boutilier, R.G., Heming, T.A., Iwama, G.K., 1984. Physicochemical parameters for use in fish respiratory physiology. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology, vol. 10A. Academic Press, London, UK, pp. 403–430.

Brauner, C.J., Val, A.L., 2006. Oxygen transfer. In: Val, A.L., Almeida-Val, V.M.F., Randall, D.J. (Eds.), The Physiology of Tropical Fishes. Fish Physiology, vol. 21. Academic Press, London, UK, pp. 277–306.

Brauner, C.J., Wang, T., Wang, Y., Richards, J.G., Gonzalez, R.J., Bernier, N.J., Xi, W., Patrick, M., Val, A.L., 2004. Limited extracellular but complete intracellular acid–base regulation during short-term environmental hypercapnia in the armoured catfish, *Liposarcus pardalis*. J. Exp. Biol. 207, 3381–3390.

Burggren, W.W., 1982. "Air gulping" improves blood oxygen transport during aquatic hypoxia in the goldfish *Carassius auratus*. Physiol. Zool. 55, 327–334.

Burleson, M.L., Carlton, A.L., Silva, P.E., 2002. Cardioventilatory effects of acclimatization to aquatic hypoxia in channel catfish. Respir. Physiol. Neurobiol. 131, 223–232.

Bushnell, P.G., Brill, R.W., 1992. Oxygen transport and cardiovascular responses in skipjack tuna (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*) exposed to acute hypoxia. J. Comp. Physiol. (B) 162, 131–143.

Cameron, J.N., 1971. Rapid method for determination of total carbon dioxide in small blood samples. J. Appl. Physiol. 31, 632–634.

Chapman, L.J., Chapman, C.A., 1998. Hypoxia tolerance of the mormyrid *Petrocephalus catostoma*: implications for persistence in swamp refugia. Copeia 1998, 762–768.

Chippari-Gomes, A.R., Gomes, L.C., Lopes, N.P., Val, A.L., Almeida-Val, V.M., 2005. Metabolic adjustments in two Amazonian cichlids exposed to hypoxia and anoxia. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 141, 347–355.

de Salvo Souza, R.H., Soncini, R., Glass, M.L., Sanches, J.R., Rantin, F.T., 2001. Ventilation, gill perfusion and blood gases in dourado, *Salminus maxillosus* Valenciennes (teleostei, characidae), exposed to graded hypoxia. J. Comp. Physiol. (B) 171, 483–489.

Fernandes, M.N., Rantin, F.T., 1989. Respiratory responses of *Oreochromis niloticus* (Pisces, Cichlidae) to environmental hypoxia under different thermal conditions. J. Fish Biol. 35, 509–519.

Glass, M.L., Andersen, N.A., Kruhøffer, M., Williams, E.M., Heisler, N., 1990. Combined effects of environmental Po_2 and temperature on ventilation and blood gases in the carp *Cyprinus carpio* L. J. Exp. Biol. 148, 1–17.

Gonzalez, R.J., Wilson, R.W., Wood, C.M., 2005. Ionoregulation in tropical fish from ion-poor, acidic blackwaters. In: Val, A.L., Almeida-Val, V.M.F., Randall, D.J. (Eds.), The Physiology of Tropical Fish. Fish Physiology, vol. 22. Academic Press, San Diego, CA, pp. 397–437.

Greco, A.M., Gilmour, K.M., Fenwick, J.C., Perry, S.F., 1995. The effects of softwater acclimation on respiratory gas transfer in the rainbow trout *Oncorhynchus mykiss*. J. Exp. Biol. 198, 2557–2567.

Guppy, M., Withers, P., 1999. Metabolic depression in animals: physiological perspectives and biochemical generalizations. Biol. Rev. 74, 1–40.

Hochachka, P.W., Buck, L.T., Doll, C.J., Land, S.C., 1996. Unifying theory of hypoxia tolerance: molecular metabolic defense and rescue mechanisms for surviving oxygen lack. Proc. Natl. Acad. Sci. U.S.A. 93, 9493–9498.

Holeton, G.F., Randall, D.J., 1967a. Changes in blood pressure in the rainbow trout during hypoxia. J. Exp. Biol. 46, 297–305.

Holeton, G.F., Randall, D.J., 1967b. The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. J. Exp. Biol. 46, 317–327.

Kelly, S.P., Fletcher, M., Pärt, P., Wood, C.M., 2000. Procedures for the preparation and culture of 'reconstructed' rainbow trout branchial epithelia. Methods Cell Sci. 22, 153–163.

Leite, C.A., Florindo, L.H., Kalinin, A.L., Milsom, W.K., Rantin, F.T., 2007. Gill chemoreceptors and cardio-respiratory reflexes in the neotropical teleost pacu, *Piaractus mesopotamicus*. J. Comp. Physiol. (A) 193, 1001–1011.

Lewis, J.M., Costa, I., Val, A.L., Almeida-Val, V.M., Gamperl, A.K., Driedzic, W.R., 2007. Responses to hypoxia and recovery: repayment of oxygen debt is not associated with compensatory protein synthesis in the Amazonian cichlid, *Astronotus ocellatus*. J. Exp. Biol. 210, 1935–1943.

Lutz, P.L., Milton, S.L., 2004. Negotiating brain anoxia survival in the turtle. J. Exp. Biol. 207, 3141–3147.

MacCormack, T.J., McKinley, R.S., Roubach, R., Almeida-Val, V.M.F., Val, A.L., Driedzic, W.R., 2003. Changes in ventilation, metabolism, and behaviour, but not bradycardia, contribute to hypoxia survival in two species of Amazonian armoured catfish. Can. J. Zool. 81, 272–280.

Muusze, B., Marcon, J., Van den Thillart, G., Almeida-Val, V., 1998. Hypoxia tolerance of Amazon fish Respirometry and energy metabolism of the cichlid *Astronotus ocellatus*. Comp. Biochem. Physiol. A: Mol. Integr. Physiol. 120, 151–156.

Nikinmaa, M., Soivio, A., 1982. Blood oxygen transport of hypoxic *Salmo gairdneri*. J. Exp. Zool. 219, 173–178.

Nilsson, G.E., 2007. Gill remodeling in fish- a new fashion or an ancient secret? J. Exp. Biol. 210, 2403–2409.

Nilsson, G.E., Renshaw, G.M., 2004. Hypoxic survival strategies in two fishes: extreme anoxia tolerance in the North European crucian carp and natural hypoxic preconditioning in a coral-reef shark. J. Exp. Biol. 207, 3131–3139.

Oliveira, R.D., Lopes, J.M., Sanches, J.R., Kalinin, A.L., Glass, M.L., Rantin, F.T., 2004. Cardiorespiratory responses of the facultative air-breathing fish jeju, *Hoplerythrinus unitaeniatus* (Teleostei, Erythrinidae), exposed to graded ambient hypoxia. Comp. Biochem. Physiol. A: Mol. Integr. Physiol. 139, 479–485.

Perry, S.F., Reid, S.G., Gilmour, K.M., Boijink, C.L., Lopes, J.M., Milsom, W.K., Rantin, F.T., 2004. A comparison of adrenergic stress responses in three tropical teleosts exposed to acute hypoxia. Am. J. Physiol. Reg. Integr. Comp. Physiol. 287, R188–R197.

Randall, D.J., Holeton, G.F., Stevens, E.D., 1967. The exchange of oxygen and carbon dioxide across the gills of rainbow trout. J. Exp. Biol. 46, 339–348.

Rantin, F.T., Kalinin, A.L., Glass, M.L., Fernandes, M.N., 1992. Respiratory responses to hypoxia in relation to mode of life of two erythrinid species (*Hoplias malabaricus* and *Hoplias lacerdae*). J. Fish Biol. 41, 805–812.

Richards, J.G., Wang, Y.S., Brauner, C.J., Gonzalez, R.J., Patrick, M.L., Schulte, P.M., Choppari-Gomes, A.R., Almeida-Val, V.M.F., Val, A.L., 2007. Metabolic and ionoregulatory responses of the Amazonian cichlid, *Astronotus ocellatus*, to severe hypoxia. J. Comp. Physiol. (B) 177, 361–374.

Saint-Paul, U., 1984. Physiological adaptation to hypoxia of a neotropical characoid fish *Colossoma macropomum*, Serrasalmidae. Environ. Biol. Fish. 11, 53–62.

Sakuragui, M.M., Sanches, J.R., Fernandes, M.N., 2003. Gill chloride cell proliferation and respiratory responses to hypoxia of the neotropical erythrinid fish *Hoplias malabaricus*. J. Comp. Physiol. (B) 173, 309–317.

Scott, G.R., Milsom, W.K., 2006. Flying high: A theoretical analysis of the factors limiting exercise performance in birds at altitude. Respir. Physiol. Neurobiol. 154, 284–301.

Sloman, K.A., Wood, C.M., Scott, G.R., Wood, S., Kajimura, M., Johannsson, O.E., Almeida-Val, V.M.F., Val, A.L., 2006. Tribute to R. G. Boutilier: the effect of size on the physiological and behavioural responses of oscar, *Astronotus ocellatus*, to hypoxia. J. Exp. Biol. 209, 1197–1205.

Stecyk, J.A.W., Farrell, A.P., 2002. Cardiorespiratory responses of the common carp (*Cyprinus carpio*) to severe hypoxia at three acclimation temperatures. J. Exp. Biol. 205, 759–768.

Steffensen, J.F., Lomholt, J.P., Johansen, K., 1982. Gill ventilation and O_2 extraction during graded hypoxia in two ecologically distinct species of flatfish, the flounder (*Platichthys flesus*) and the plaice (*Pleuronectes platessa*). Environ. Biol. Fish. 7, 157–163.

Storey, K.B., Hochachka, P.W., 1974. Enzymes of energy metabolism in a vertebrate facultative anaerobe, *Pseudemys scripta*. Turtle heart pyruvate kinase. J. Biol. Chem. 249, 1423–1427.

Sundin, L.I., Reid, S.G., Kalinin, A.L., Rantin, F.T., Milsom, W.K., 1999. Cardiovascular and respiratory reflexes: the tropical fish, traira (*Hoplias malabaricus*) O_2 chemoresponses. Respir. Physiol. 116, 181–199.

Ultsch, G.R., Boschung, H., Ross, M.J., 1978. Metabolism, critical oxygen tension, and habitat selection in darters (*Etheostoma*). Ecology 59, 99–107.

Val, A.L., 1995. Oxygen transfer in fish: morphological and molecular adjustments. Braz. J. Med. Biol. Res. 28, 1119–1127.

Val, A.L., Schwantes, A.R., de Almeida-Val, V.M.F., 1986. Biological aspects of Amazonian fishes-VI. Hemoglobins and whole blood properties of *Semaprochilodus species* (Prochilodontidae) at two phases of migration. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 83, 659–667.

Vulesevic, B., McNeill, B., Perry, S.F., 2006. Chemoreceptor plasticity and respiratory acclimation in the zebrafish *Danio rerio*. J. Exp. Biol. 209, 1261–1273.

Warren, D.E., Jackson, D.C., 2008. Lactate metabolism in anoxic turtles: an integrative review. J. Comp. Physiol. (B) 178, 133–148.

Wolf, K., 1963. Physiological salines for fresh-water teleosts. Prog. Fish Cult. 25, 135–140.

Wood, C.M., Kajimura, M., Sloman, K.A., Scott, G.R., Walsh, P.J., Almeida-Val, V.M.F., Val, A.L., 2007. Rapid regulation of Na^+ fluxes and ammonia excretion in response to acute environmental hypoxia in the Amazonian oscar, *Astronotus ocellatus*. Am. J. Physiol. Reg. Integr. Comp. Physiol. 292, R2048–R2058.

Wood, C.M., McMahon, B.R., McDonald, D.G., 1978. Oxygen exchange and vascular resistance in the totally perfused rainbow trout. Am. J. Physiol. Reg. Integr. Comp. Physiol. 234, R201–R208.

Wood, C.M., McMahon, B.R., McDonald, D.G., 1979. Respiratory gas exchange in the resting starry flounder, *Platichthys stellatus*: a comparison with other teleosts. J. Exp. Biol. 78, 167–179.

Yeager, D.P., Ultsch, G.R., 1989. Physiological regulation and conformation: a BASIC program for the determination of critical points. Physiol. Zool. 62, 888–907.