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VENTILATION AND ACID–BASE RECOVERY FOLLOWING EXHAUSTING ACTIVITY IN AN AIR-BREATHING FISH

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Summary

The effects of exhausting activity in normoxic (P_{O_2}=20.7 kPa) and hypoxic (P_{O_2}<2.7 kPa) water on ventilatory, metabolic and acid–base variables were examined in spotted gar (*Lepisosteus oculatus*) to determine the role of the air-breathing organ in supporting active metabolism and recovery. The level of aquatic hypoxia used effectively eliminated the gills as a site of O_2 uptake, forcing the fish to respire as a unimodal air-breather. Swimming duration (until exhaustion) was not significantly different in normoxic and hypoxic water. Blood gas, acid–base, cardiovascular and ventilatory variables were monitored at intervals from 15 min to 24 h post-exercise. Fish survived exhaustive exercise using a combination of anaerobic metabolism and increased ventilation (aerial and aquatic), despite respiratory and metabolic acidoses. The cardiovascular effects of exercise (heart rate and dorsal aortic blood pressure) were minor. The metabolic effects of exercise were similar to those in unimodal water-breathing fish; however, even hypoxic animals recovered from exhaustive exercise by 24 h. Thus, the results of this study show that air breathing in *L. oculatus* allows gar to exercise to the same extent in normoxic and hypoxic water and enables them to re-establish blood gas and acid–base balance after exhaustive activity even in hypoxic water.

Key words: gar, *Lepisosteus oculatus*, exercise, control of breathing, metabolism, hypoxia.

Introduction

Aquatic hypoxia has been cited as the primary driving force in the evolution of air breathing in fish (Randall *et al.* 1981). Numerous studies have supported this hypothesis by characterizing the capacity of air-breathing fishes to survive extreme aquatic hypoxia; however, several findings have emerged which call for more holistic interpretations of the ecological and physiological significance of air breathing (bimodal respiration). (1) In many fish species, air breathing serves ancillary, non-respiratory roles, such as buoyancy control (Gee and Graham, 1978; Renfro and Hill, 1970). (2) Air breathing may allow better predator avoidance than other types of hypoxia survival strategies (e.g. aquatic surface respiration; Kramer *et al.* 1983) since air breathers spend less time at the water surface where fish are vulnerable to aerial predation. (3) In some environmental situations, bimodal respiration may be energetically favored over unimodal aquatic respiration because of the density and viscosity differences between air and water (Kramer *et al.* 1978). (4) Aerial respiration may function in normoxic and hypoxic water to help support the metabolic demands of growth (Ponniah and Pandian, 1977), temperature increases (Johansen *et al.* 1970; Rahn *et al.* 1971) and activity (Grigg, 1965; Gee and Graham, 1978; Smatresk and Cameron, 1982*b*). (5) Air-breathing organs in fishes may have evolved to oxygenate the heart (Farmer, 1997). While all of these findings are cited as important selective pressures in the maintenance of air breathing in various species of fish and have been subjected to experimental scrutiny or theoretical analysis, the relationships between air breathing, endurance and recovery after exhaustive exercise have not been addressed. These relationships are potentially significant because they suggest that air breathing may confer a high degree of independence from water quality to the metabolic scope for activity and the ability to recover. Thus, air-breathing fish may not simply survive aquatic hypoxia but may also maintain normal levels of activity when branchial O_2 uptake is limited. This ability would be of considerable adaptive value during times of prolonged hypoxia and hypercapnia, when activity metabolism is compromised in unimodal water-breathing fish. In fact, hypoxia so severely limits the scope for activity in water-breathing fish that it reflexively inhibits swimming (Kutty, 1967).

The first goal of this study is to determine whether air breathing allows spotted gar to exercise to the same extent in normoxic and hypoxic water. In this experiment, the gills were eliminated as a site of O_2 exchange, and the ability of the gar to engage in and recover from exhausting activity supported only by aerial O_2 uptake was tested. This was accomplished by eliciting exhausting activity in gar under two conditions: first, in normoxic water with both the gills and the air-breathing organ available to support activity; and second, in very hypoxic

water (<2.7 kPa), where branchial O_2 uptake was precluded because aquatic P_{O_2} was less than arterial P_{O_2}.

The second goal of this study was to provide a description of the functional repercussions of air breathing on metabolic recovery. The evolutionary transition to air breathing has been accompanied by biochemical and morphological modifications of respiratory structures as well as altered ventilatory control priorities. Morphological changes include a reduction in functional gill surface area coincident with increases in aerial respiratory capacity. Ventilatory control processes have also been modified to allow the selection of the appropriate respiratory mode (aquatic *versus* aerial) (Smatresk *et al.* 1986). The effects of these changes on fundamental aspects of the exercise physiology of air-breathing fish are unknown. To describe the effects of these changes on the pattern of recovery in air-breathing fish, ventilatory, cardiovascular, blood gas and acid–base data were collected before and after activity in normoxic water, where branchial and aerial gas exchange sites were available, and in hypoxic water, where only aerial exchange was possible.

Materials and methods

Animals

Spotted gar, *Lepisosteus oculatus* Winchell, were captured by electroshocking from a series of private lakes near Athens, TX, USA (Henderson Co.). Fish were transported to the University of Texas at Arlington and maintained in 1200 l tanks filled with filtered Arlington tap water at 23±1 °C on a 12 h:12 h light:dark cycle and were fed minnows.

Surgery

Individual gar (N=16) (850–1000 g) were anesthetized in MS-222 (0.01 %) in dechlorinated tap water. Cannulae were inserted into the dorsal aorta (PE 60, 50 cm) and attached to the operculum (PE 160, 50 cm) as described by Smatresk and Cameron (1982*a*). After surgery, the fish were left in the operating sling and their gills were perfused with oxygenated water containing no anesthetic. When swimming motions resumed, fish were transferred to the experimental apparatus and allowed to recover in normoxic water for 24 h.

The surgery for fish used for *in vitro* blood experiments to determine the whole-blood non-bicarbonate buffer curve (N=6) was identical to that described above, with the exception that they did not receive opercular catheters and were placed in 75 l aquaria for recovery.

Water treadmill construction

The water treadmill consisted of two major sections: an experimental chamber and a reservoir. The swimming area was 91 cm wide and 31 cm deep, was filled to a depth of 20 cm with filtered, dechlorinated water and was contained within a watertight experimental chamber 183 cm long. The central space of the chamber was occupied by a large acrylic pylon 51 cm wide and 132 cm long with semicircular ends and straight sides. The sides of this structure served as the inside wall of an oval flume. Curved acrylic baffles, attached to either end of the box, formed the outer curves of the flume, while the straight sides of the chamber formed the outer walls of the straight sections. Two pairs of aluminum grids (1 cm×20 cm×30.25 cm with 1 cm mesh) isolated a section of one straightway, thus defining the 91 cm long swimming area. A stimulating grid supplied by a small (9 V d.c., 3 A) transformer was installed at the rear of the swimming area to ensure consistent swimming effort.

A hinged top covered the chamber except for the swimming area, which was sealed with a separate acrylic canopy. With the top in place, the volume between the water line and the acrylic cover in the swimming area (head space) was physically isolated from the remainder of the chamber and the entire chamber was airtight. This arrangement allowed the P_{O_2} of the water in the swimming chamber to be manipulated independently of the headspace aerial P_{O_2}. Adequate space for gar to air-breathe without interference was confirmed visually. Water oxygen tensions were controlled *via* aerators which consisted of loops of porous pipe distributed throughout the main chamber and reservoir. The aerators were supplied with appropriate gas mixtures from regulated N_2 and O_2 gas cylinders through a pair of flowmeters (Gilmont). Water P_{O_2} was monitored using an oxygen electrode (YSI). Aquatic P_{O_2} ranged from 19.3 to 20.7 kPa during normoxic experiments and from 1.3 to 2.7 kPa during hypoxic experiments.

The swimming current was provided by three 560 W sump pumps located in the reservoir and was delivered to the treadmill flume *via* three flexible hoses that terminated in adjustable nozzles. These nozzles provided directional control of the water coming into the swimming area and were aimed to eliminate ‘dead spots’ in the current where fish could loiter. Water flow within the flume was moderately turbulent, with a velocity range of 1–2 m s^{-1}. Water pumped into the main chamber returned to the reservoir through two large polyvinylchloride lines. A fourth pump (249 W) in the reservoir continuously pumped water into the main chamber to provide circulation when the drive pumps were not in use. Stainless-steel heat-exchange coils combined with a constant temperature controller (Lauda) and a biological filter located in the reservoir controlled temperature and water quality respectively. Temperature was measured downstream from the swimming area and varied between 23 and 24 °C. The entire system contained approximately 350 l of water.

Analytical techniques

Pressure changes in the buccal cavity associated with gill ventilation and air breathing were monitored *via* the water-filled opercular catheter connected to a differential pressure transducer (Validyne DP45-34) and carrier demodulator (Validyne CD15). This signal was recorded on a two-channel chart recorder (Dash 16, Astromed) and yielded a continuous record of gill ventilation frequency (f_G, beats min^{-1}), opercular pressure (P_{Op}, an indication of ventilatory stroke volume) and air-breathing frequency (f_{AB}, breaths h^{-1}).

The dorsal aortic catheter was filled with heparinized Cortland saline and connected to a second channel on the chart recorder *via* a second pressure transducer (Micron MP15D) and associated preamplifier (Coulborne) for continuous recording of dorsal aortic blood pressure (P_{DA}) and heart rate (f_H, beats min^{-1}). Blood samples (0.5 ml) were drawn from the dorsal aortic cannula and analyzed for pH, P_{O_2}, hematocrit (Hct), total CO_2 (C_{CO_2}) and lactate.

pH was measured in 50 µl samples of whole blood using a glass capillary electrode (Radiometer) connected to a digital pH meter (Beckman). Dorsal aortic blood P_{O_2} was measured using an oxygen electrode (Radiometer) connected to a digital meter (Cameron Instruments). Two 50 µl samples of whole blood were centrifuged in glass capillary tubes for 5 min and Hct was recorded as the averaged packed red blood cell fraction. The remaining blood sample volume was centrifuged for 45 s. Duplicate 20 µl plasma samples were used for C_{CO_2} determinations (Capnicon, Cameron Instruments). A sample of plasma (100 µl) was deproteinized with 200 µl of cold 8 % perchloric acid and centrifuged at 3500 ***g*** for 10 min. The protein pellet was decanted and frozen for later enzymatic analysis of lactate concentration (Sigma).

Protocol

Exercise recovery experiments consisted of four parts: chamber acclimation, control measurements, exercise and recovery measurements. Sixteen fish were subjected to each component sequentially in either normoxic (19.3–20.7 kPa P_{O_2}; N=8) or hypoxic (1.3–2.7 kPa P_{O_2}; N=8) water. Aerial P_{O_2} in the headspace was maintained at atmospheric levels at all times in both sets of experiments.

Following a 24 h peroid of recovery from surgery, aquatic P_{O_2} in the treadmill was adjusted to the experimental level. An additional 24 h period was then allowed to pass before control sampling began; however, 12 h into this acclimation period, the opercular and dorsal aortic cannulae were cleared and attached to the appropriate transducers.

Continuous recording of cardiovascular and ventilatory variables began at the end of the acclimation period and continued for 2–4 h. When the fish was judged to be in a steady-state condition (i.e. not struggling), two control blood samples (C1 and C2) were drawn and analyzed 1 h apart.

The dorsal aortic and opercular cannulae were disconnected from the pressure transducers and plugged during the exercise bout to reduce the likelihood of damage to the recording apparatus. The three drive pumps were then activated sequentially, approximately 30 s apart, to increase the water velocity slowly. When the fish was swimming, the electric grid was switched on. Exhaustion was operationally defined as the state at which the fish no longer responded to contact with the shocking grid. When the fish was exhausted, the pumps were turned off and the fish was allowed to recover in the swimming area.

Immediately following the exercise bout, both catheters were reattached to their respective transducers and continuous recording of ventilatory and cardiovascular variables and blood sampling resumed. This point is defined as recovery time zero. Additional blood samples (0.5 ml) were drawn and analyzed 0.25, 0.5, 1, 2, 4, 8 and 24 h following cessation of exercise.

The protocol for *in vitro* determination of whole-blood non-bicarbonate buffer slope is as follows. Periodic checks of blood pH ensured that fish had fully recovered from surgery. Blood (6 ml) was drawn from the dorsal aortic catheter into a heparinized syringe and immediately subdivided evenly into three heparinized flasks. Each flask was then equilibrated with humidified gas mixtures of 0.3, 2 or 4 % CO_2 and air at 23 °C. The blood was shaken gently for 1 h to ensure equilibration at the three CO_2 tensions. pH and C_{CO_2} were measured on samples from each flask as described above.

Data analysis and statistics

Ventilatory variables were measured from the opercular pressure recording. Gill ventilation rate (f_G, beats min^{-1}) was estimated by averaging the rates from three 2 min periods distributed around the predetermined sample time. Opercular pressure amplitude (P_{Op}) was determined as the average amplitude of 12 individual pressure excursions measured every 10 s across each of the 2 min periods used for f_G determinations. Air-breathing frequency (f_{AB}) was counted over 15 min intervals centered around the blood sampling period, multiplied by 4 and reported as breaths h^{-1}.

Cardiovascular variables were derived from the dorsal aortic pressure recording. Heart rate (f_H, beats min^{-1}) was estimated in the same way and over the same intervals as f_G. Dorsal aortic blood pressure (P_{DA}) was calculated as the average of 12 peak deflections measured every 10 s across the same 2 min period.

A two-way repeated-measures analysis of variance (ANOVA) was used to determine whether there were statistically significant effects of oxygen tension (group: hypoxic and normoxic) and activity (time) on cardiovascular and ventilatory variables.

Calculations

Blood P_{CO_2} was calculated from sample pH and C_{CO_2} (mmol l^{-1}) values using the Henderson–Hasselbalch equation and a functional or apparent pK′ calculated according to Smatresk and Cameron (1982*a*):

$$pK' = 0.83pH + 6.911 . \quad (1)$$

Plasma total CO_2 (C_{CO_2}) was converted to bicarbonate equivalents (HCO_3^-; mequiv l^{-1}) using the following equation with a CO_2 solubility coefficient (α_{CO_2}) of 0.0509 l^{-1} (Smatresk and Cameron, 1982*a*):

$$[HCO_3^-] = C_{CO_2} - (\alpha_{CO_2} \times P_{CO_2}) . \quad (2)$$

The non-bicarbonate blood buffer value was calculated from *in vitro* data. A regression analysis of whole-blood pH *versus* plasma $[HCO_3^-]$ at three known P_{CO_2} levels yielded an equation of the form:

$$C_{CO_2} = \beta pH + B , \quad (3)$$

where β is the buffering capacity of whole blood in

μmol pH unit^{-1} (slykes) or the change in [HCO_3^-] in mequiv l^{-1} pH unit^{-1} and B is a constant.

Observed changes in pH and [HCO_3^-] following exercise are the result of several simultaneously occurring processes, including altered internal P_{CO_2} levels (respiratory acidosis), proton efflux from the intracellular compartment (metabolic acidosis) and branchial or renal ion exchange with water (compensation). Alterations in pH and [HCO_3^-] that result from changing P_{CO_2} tensions alone may be predicted by the equation describing the buffer line. When measured [HCO_3^-] deviated from this line for a given pH, the change in [HCO_3^-] is termed ΔH_m^+, the change in metabolic acid load. ΔH_m^+ is a complex variable reflecting the sum of the metabolic or fixed component of the acid–base disturbance as well as any compensation. ΔH_m^+ over any time period (i.e. $t1$ to $t2$) was calculated according to the following equation from McDonald *et al.* (1980):

$$\Delta H_m^+ = [HCO_3^-]_{t1} - [HCO_3^-]_{t2} - \beta(pH_{t1} - pH_{t2}), \quad (4)$$

where values for [HCO_3^-] are calculated from plasma C_{CO_2}, β is the slope of the blood non-bicarbonate buffer line and pH is that of whole blood. Positive values of ΔH_m^+ (also known as base deficit) obtained from this equation indicate the presence of a fixed acid in the blood or compensation involving the net removal of fixed base from the blood or both. A negative ΔH_m^+, or base excess, implies addition of fixed base to the blood, compensation resulting in the net removal of acid or both.

Results

Most fish exhibited spontaneous activity in the swimming chamber while recovering from surgery. Activity decreased as the gar became accustomed to the confined space. When the first swim pump was activated, fish oriented into the current and began to swim. All fish became refractory to contact with the shocking grid after 25–45 min of exercise and generally showed signs of exhaustion (i.e. loss of equilibrium). Swimming duration (length of exercise bout) was variable, but did not differ significantly in normoxic fish (39.4±3.9 min) and hypoxic fish (32.5±4.3 min) (means ± S.E.M., N=16; Student's t-test, t=1.206).

Reactions to the swimming current were variable and unrelated to aquatic oxygen tension. Most fish swam continuously in the strongest current at the front of the swimming area, while others attempted to hold position on the bottom. All fish breathed air during and following the exercise period. Post-exercise activity levels were lower than those observed in the acclimation period. All fish remained upright on the bottom of the chamber. Long periods of immobility were punctuated by brief bouts of struggling.

Ventilatory responses

Sample recordings show dorsal aortic and opercular pressures during aquatic normoxia (Fig. 1A,B) and hypoxia (Fig. 1C,D) in gar before and after exhaustive exercise. f_G was significantly greater in normoxic fish than in hypoxic fish for the duration of the protocol (Fig. 2A). While the pattern of change over time following exercise was complex, f_G did not change significantly over time after exercise during either normoxia or hypoxia. Throughout the protocol, resting P_{Op} was also significantly greater in the normoxic group than in the hypoxic fish (Fig. 2B). However, unlike f_G, the transient increase in P_{Op} during the first 2 h after exercise was statistically significant in both normoxic and hypoxic groups. Air-breathing frequency (f_{AB}, breaths h^{-1}) was significantly greater during hypoxia than during normoxia (Fig. 3). Exercise significantly increased post-exercise f_{AB} in both groups.

Patterns of ventilation

In addition to the quantitative changes in gill ventilation, exercise had an effect on the specific pattern of P_{Op} during normoxia and hypoxia. Resting fish in normoxic water (Fig. 1A) exhibited a ventilatory pattern in which P_{Op} increased steadily during the inter-airbreath interval, peaked immediately prior to each air breath and thereafter fell to its lowest value within 15–20 s. This regular cycle was maintained indefinitely in undisturbed animals. In most fish, exercise abolished the depression of P_{Op} following each air breath (Fig. 1B), resulting in a more or less constant P_{Op} throughout the air-breathing cycle. Another consequence of exercise was an apparent increase in the frequency of coughing: large transient buccal pressure changes distinguishable from air breaths by the absence of any corresponding change in P_{DA}.

Hypoxia significantly depressed P_{Op} except for a few pressure changes usually following the air breaths (Fig. 1C), which were probably associated with the buccal pumping of air into the air-breathing organ. Following exercise in hypoxic water (Fig. 1D), the opercular movements just before an air breath, as noted in normoxic animals, were again present but were greatly exaggerated. P_{Op} increased from essentially zero to levels comparable with those reached during actual air-gulping within 7–9 beats then fell rapidly following the air breath.

Cardiovascular responses

The slight difference in heart rate (f_H, beats min^{-1}) between normoxic and hypoxic fish (Fig. 4A) was not statistically significant. Exercise caused f_H to increase significantly in both groups and, while the percentage change was greater in the normoxic group, the results were not significantly different from those for the hypoxic group.

Aquatic O_2 tension had a significant effect on dorsal aortic blood pressure (P_{DA}, kPa) but exercise did not (Fig. 4B). P_{DA} was significantly greater (0.98–1.08 kPa) in normoxic fish, but did not change significantly over time after exercise in either group.

Blood gas changes

The effects of exercise on dorsal aortic blood P_{O_2} (Pa_{O_2}) (Fig. 5A) were dependent on aquatic oxygen tension. In normoxic fish, Pa_{O_2} rose significantly from a control value of 3.7±0.3 kPa to a peak value of 4.9±0.2 kPa (means ± S.E.M.,

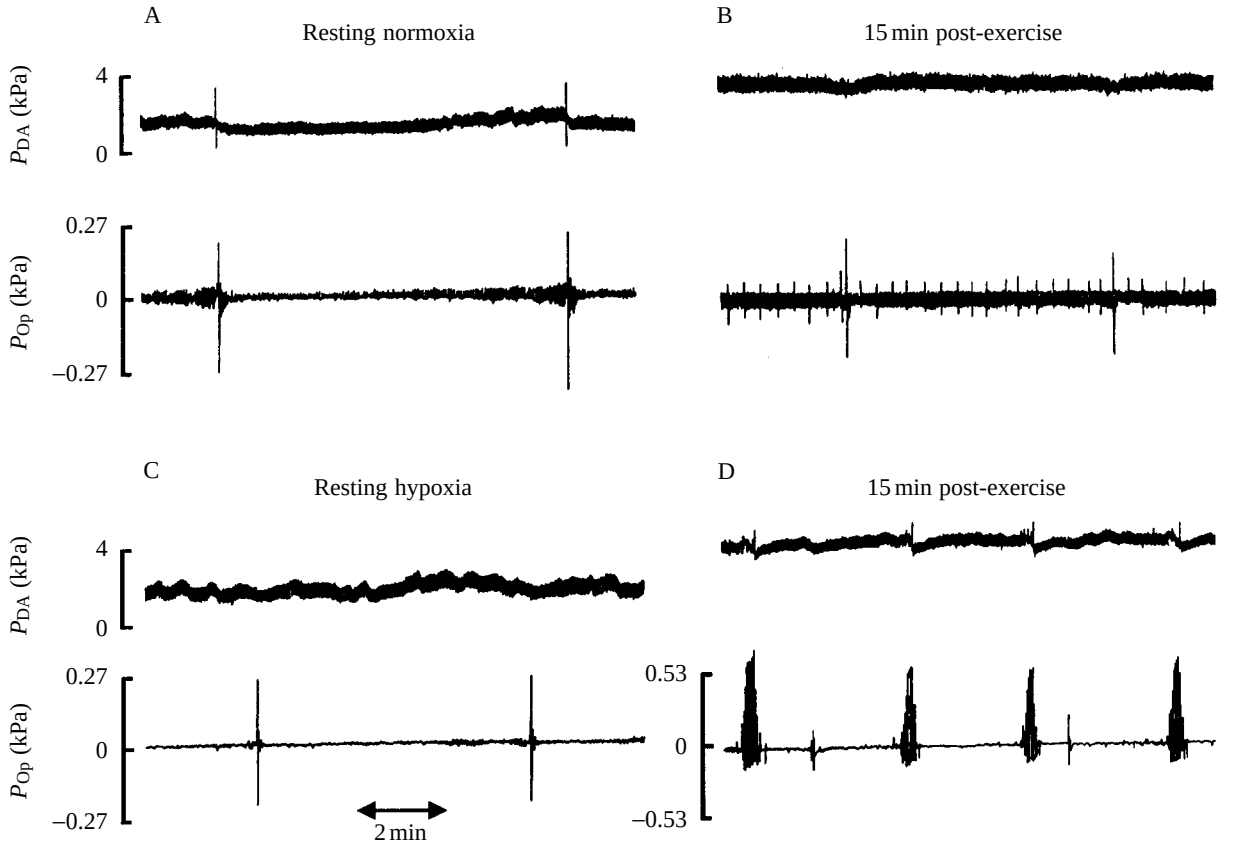

Fig. 1. Representative traces showing changes in the dorsal aortic (P_{DA}; upper trace of each pair) and opercular pressure (P_{Op}; lower trace of each pair) of a gar before and after exercise in normoxic water (A,B) and hypoxic water (C,D). Arterial pressure traces show mean blood pressure and the pressure changes associated with each heart beat. Opercular pressure traces show pressure changes associated with each gill ventilation (small deflections) and air breaths (large deflections). The intermediate-sized pressure spikes in B are coughs, whose stimulus and function are not completely understood. Gill ventilation frequency and opercular pressure are almost completely suppressed by hypoxia (C,D). Traces A, B and C each show two air breaths, which sometimes show up as mechanical artifacts in the dorsal aorta traces (A,D).

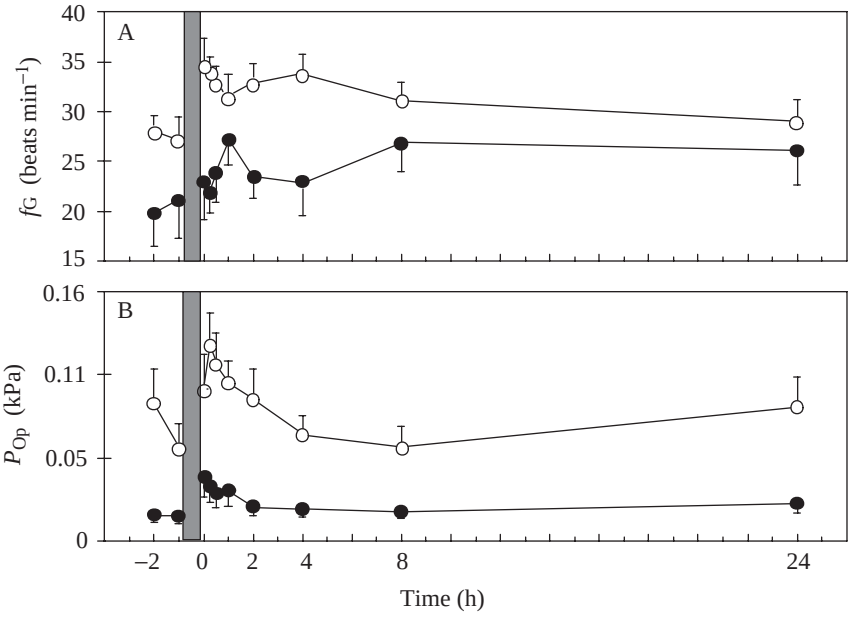

Fig. 2. Gill ventilation frequency (f_G, beats min^{-1}) (A) and opercular pressure (P_{Op}, kPa) (B) before (–2 to 0 h) and after (0–24 h) exhausting exercise in normoxic (open circles) and hypoxic (filled circles) water. f_G was significantly greater in normoxic fish, but did not change significantly over time in either group. P_{Op} was significantly greater in normoxic fish and showed significant changes over time in both groups. Shaded bars indicate the exercise bout. Values are mean ± S.E.M. (N=8).

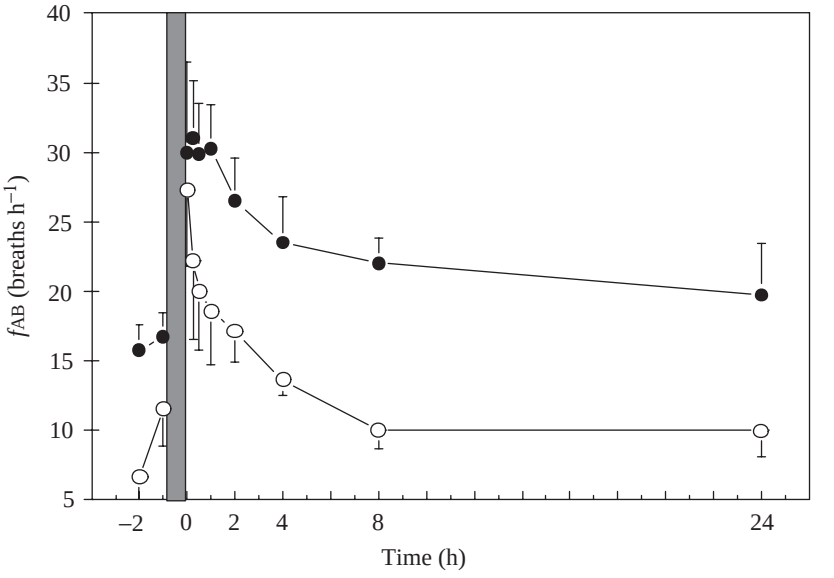

Fig. 3. Air-breathing frequency (f_{AB}, breaths h^{-1}) before (–2 to 0 h) and after (0–24 h) exhausting exercise in normoxic (open circles) and hypoxic (filled circles) water. f_{AB} was significantly greater in hypoxic fish and changed significantly over time post-exercise in both groups. Shaded bars indicate the exercise bout. Values are mean ± S.E.M. (N=8).

N=8) 2 h into recovery and returned to control levels by 24 h. In contrast, Pa_{O_2} of hypoxic fish fell transiently by 1.2 kPa immediately following exercise from a comparatively high control value of 4.5 kPa before returning to control levels after 4 h. One-way repeated-measures analyses of variance (ANOVAs) performed separately for each group against time indicate that the overall increase in Pa_{O_2} in normoxic fish was significant while the overall decrease in hypoxic fish was not.

Dorsal aortic blood P_{CO_2} (Pa_{CO_2}) was significantly greater in hypoxic fish and increased significantly after exercise in both groups (Fig. 5B). The rate of decline in Pa_{CO_2} during post-exercise recovery was much more rapid in normoxic fish, but both groups of fish reached control levels by 4 h post-exercise.

In vitro *determination of non-bicarbonate blood buffer curve*

The calculated regression equation for the non-bicarbonate blood buffer curve was y=68.72–8.04x (r^2=0.99, P<0.001), which is in close agreement with that reported by Smatresk and Cameron (1982*a*,*b*) for oxygenated whole gar blood (y=75.69–8.76x). Thus, the buffer slope (β) was 8.04 μmol pH unit^{-1}.

Acid–base balance

Hypoxic fish were significantly more acidotic than normoxic fish during the control period (Fig. 6A). The pH of both groups decreased significantly immediately following exercise, reaching similar values of 7.24±0.06 during hypoxia and 7.25±0.05 (means ± S.E.M., N=8) during normoxia.

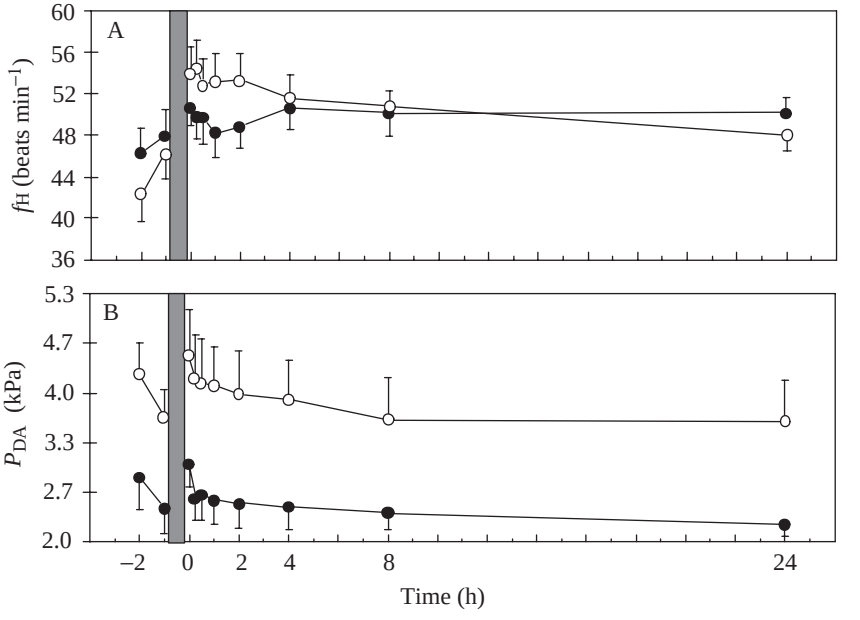

Fig. 4. Heart rate (f_H, beats min^{-1}) (A) and dorsal aortic blood pressure (P_{DA}, kPa) (B) before (–2 to 0 h) and after (0–24 h) exhausting exercise in normoxic (open circles) and hypoxic (filled circles) water. f_H increased significantly after exercise, but values for the normoxic and hypoxic groups were not significantly different. P_{DA} in normoxic fish was significantly greater than in hypoxic fish, and there was no significant change over time in either group. Shaded bars indicate the exercise bout. Values are mean ± S.E.M. (N=8).

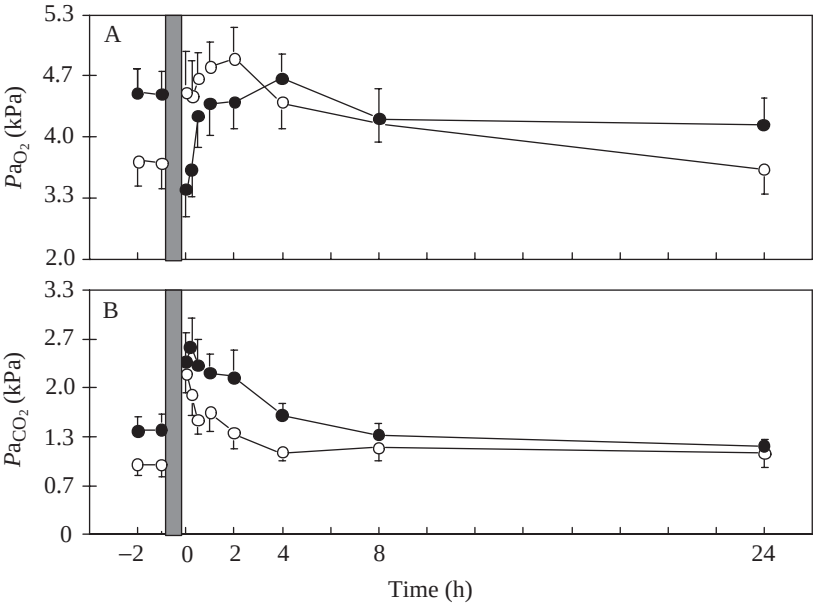

Fig. 5. Dorsal aortic blood P_{O_2} (Pa_{O_2}, kPa) (A) and dorsal aortic blood P_{CO_2} (Pa_{CO_2}, kPa) (B) before (–2 to 0 h) and after (0–24 h exhausting exercise in normoxic (open circles) and hypoxic (filled circles) water. Pa_{O_2} was significantly different in normoxic and hypoxic fish. Pa_{O_2} increased significantly in normoxic fish after exercise, but the changes seen in hypoxic fish were not significant over time. Pa_{CO_2} was significantly different in normoxic and hypoxic fish and increased significantly after exercise in both groups (B). Shaded bars indicate the exercise bout. Values are mean ± S.E.M. (N=8).

However, the significant difference in absolute pH level between the two groups persisted through the 24 h period.

The bicarbonate ion concentration [HCO_3^-] did not differ significantly between normoxic and hypoxic fish (Fig. 6B). There was a significant decrease in [HCO_3^-] in both groups after exercise, and the pattern of recovery as well as the absolute magnitude of change were similar.

Mean absolute lactate concentration at rest was similar in normoxic fish (0.97±0.19 mequiv l^{-1}) and hypoxic fish (0.87±0.30 mequiv l^{-1}) (means ± S.E.M., N=8). The time course of the change in lactate concentration (Δ[lactate]) was plotted separately for the two groups with the associated calculated change in metabolic acid load (ΔH_m^+) (Fig. 7A,B). Immediately after exercise Δ[lactate] was comparable to ΔH_m^+ for both normoxic and hypoxic fish. However, during the first 2 h of recovery, lactate accumulated while ΔH_m^+ fell. Both Δ[lactate] and ΔH_m^+ increased significantly after exercise. ΔH_m^+ was the same in normoxic and hypoxic fish, but the effects of exercise on Δ[lactate] were significantly different between the two groups.

Hematocrit

The absolute magnitude as well as pattern of change in hematocrit (% packed red blood cell volume) was similar for the two groups. Repeated sampling caused hematocrit to decrease significantly over time in both normoxic (from

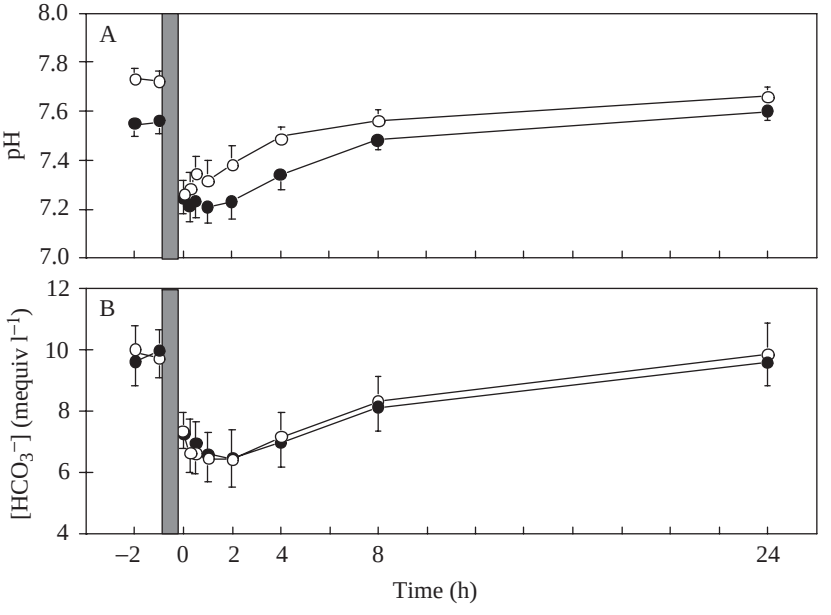

Fig. 6. (A) Dorsal aortic blood pH before (–2 to 0 h) and after (0–24 h) exhausting exercise in normoxic (open circles) and hypoxic (filled circles) water. Dorsal aortic blood pH was significantly lower in hypoxic animals, but was significantly decreased in both groups after exercise. (B) Dorsal aortic blood bicarbonate concentration (mequiv l^{-1}) before and after exhausting exercise in normoxic (open circles) and hypoxic (filled circles) water. Normoxic and hypoxic values were not significantly different, but both showed significant changes over time. Shaded bars indicate the exercise bout. Values are mean ± S.E.M. (N=8).

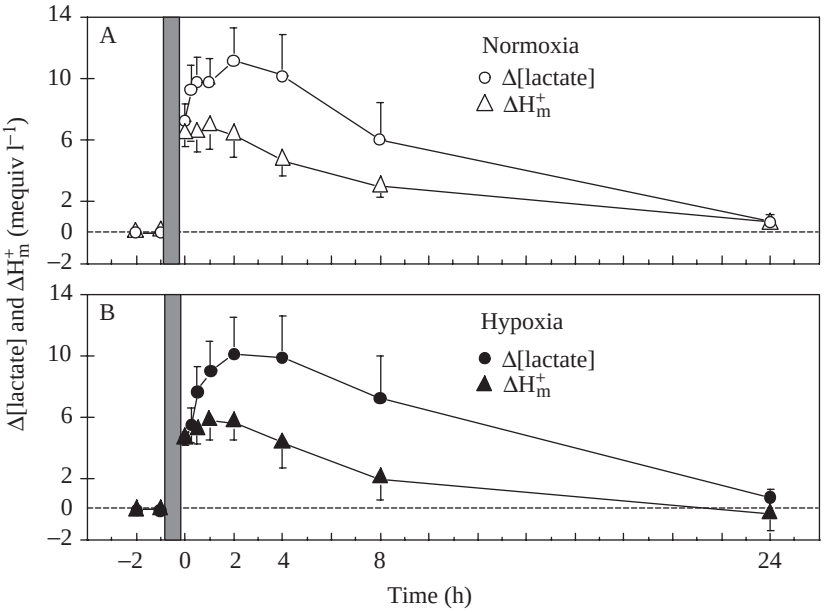

Fig. 7. Post-exercise changes in dorsal aortic blood lactate (Δ[lactate], mequiv l^{-1}) and metabolic acid (ΔH_m^+, mequiv l^{-1}) concentration during normoxia (A, open symbols) and hypoxia (B, filled symbols). Both Δ[lactate] and ΔH_m^+ were significantly elevated after exercise. The effects of exercise on Δ[lactate] were significantly different between the normoxic and hypoxic groups. Values are mean ± S.E.M. (*N*=8).

26.26±2.37 to 18.83±3.04 %) and hypoxic (from 24.20±1.47 to 16.47±2.37 %) (means ± S.E.M., *N*=8) fishes. There was no significant difference between the groups.

Discussion

Exercise and recovery in normoxic water

Resting blood gas values ($P_{a_{O_2}}$ and $P_{a_{CO_2}}$) were greater than previously reported values in the gar (Smatresk and Cameron, 1982*a*). Gill ventilation rate was lower and air-breathing frequency was higher in this study than in the study of Smatresk and Cameron (1982*a*), which could account for the greater $P_{a_{O_2}}$ and $P_{a_{CO_2}}$ values reported here. Also, the overall differences in gill ventilation and air breathing between the two studies suggest that the fish used for these experiments were better acclimated to the experimental chamber (i.e. the fish were freely air-breathing).

Despite the adaptations associated with breathing air, the patterns of ventilatory, cardiovascular and acid–base response to exercise in normoxic water were surprisingly similar to those of unimodal water-breathing fish. Branchial ventilation volume was increased in response to exercise, although less dramatically than in unimodal water breathers, mainly *via* higher ventilation amplitude (P_{O_p}, Fig. 2). Heart rate, and most probably cardiac output, was elevated, which served to match ventilation and perfusion of the gills and air-breathing organ. Dorsal aortic blood pressure, in contrast, did not change significantly, most probably because of the lowered systemic resistance. The most obvious difference between water breathers and gar was post-exercise mortality, which can be as great as 40 % in trout (Wood *et al.* 1983) but was zero in gar. *L. oculatus* relied heavily on aerial, rather than branchial, ventilation to meet the high metabolic demands of exercise. This is significant because air breathing may have allowed the gar to avoid the energetic and osmoregulatory problems associated with aquatic ventilation. In water breathers, branchial ventilation volume may rise sevenfold (aquatic hyperventilation) during exercise (Brett, 1972). Aquatic hyperventilation is energetically unfavorable compared with air breathing because of the differences in density, viscosity and O_2 content per unit volume between air and water. It is possible that the energetic costs of maximal aquatic hyperventilation are an important source of post-exercise stress in water breathers and that this stress, coupled with the acid load and O_2 debt incurred during activity, contributes to high post-exercise mortality rate. Since aquatic ventilation in water-breathing fish is driven primarily by the need for O_2 uptake, critical but ancillary functions of the gills such as osmoregulation and ionoregulation may suffer. However, the combination of air breathing, reduced functional gill surface area and relatively minor increases in ventilation may eliminate this problem in gar. While the proximal causes of post-exercise death are unknown, water-breathing fish appear to be unable to tolerate the severe intracellular acidosis incurred during activity (Wood *et al.* 1983). Gar experience a similar magnitude and pattern of blood acidosis but survive; thus, they may be a good model for future investigations.

The gar's small gills and minor increases in gill ventilation volume following activity appeared to delay both respiratory (branchial CO_2 elimination) and metabolic (branchial ion transport) compensation of the post-exercise acid–base disturbance compared with water-breathing fish. The external gill surface area of gar is not reduced to the same extent as seen in some air-breathing fishes (Landolt and Hill, 1975); however, the unique channeling of blood flow through the lamella of spotted gar reduces the functional gas-exchange surface area (Smatresk and Cameron, 1982*a*), which may have prolonged acid–base recovery. Acid–base recovery followed a pattern similar to that of the salamander *Cryptobranchus alleganiensis*, in which the contribution of P_{CO_2} to the total

acidosis was significant even after 2–4 h of recovery (Boutilier *et al.* 1980). Most water-breathers, in contrast, restore resting P_{CO_2} within 0.5 h post-exercise.

As in other lower vertebrates, lactate accumulated in the blood of *L. oculatus* relatively slowly, reaching a maximum value between 2 and 4 h post-exercise. While the pattern of efflux was similar to that of lactate, estimates of metabolic proton concentration in gar proved to be substantially lower than values for lactate (Fig. 7). Turner *et al.* (1983) offer convincing evidence in trout that this apparent anomaly (H^+_m and lactate are produced at an almost 1:1 ratio *via* anaerobic glycolysis) is the result of differential release rates of lactate and H^+ from exercised muscle to extracellular fluid rather than to differences in rates of clearance from the blood. Therefore, most metabolically produced protons are buffered intracellularly, where buffering capacity is typically 40–200 times that of the extracellular compartment (Heisler, 1984). The relationship between blood lactate and anaerobically produced (metabolic) proton levels (H^+_m) following exhaustive activity separates unimodal water-breathing fish into an 'active' group of pelagic species, which experience a large efflux of lactate post-exercise while H^+_m levels fall, and an 'inactive' group of primarily benthic species, which show higher blood levels of H^+_m than of lactate during recovery (Turner *et al.* 1983). The magnitude of the blood lactate concentration, the pattern of lactate efflux and the discrepancy between lactate and H^+_m concentrations are very similar to those observed in exercised trout (Holeton *et al.* 1983) and hence place gar into the 'active' group of fish species (Turner *et al.* 1983).

Exercise and recovery in hypoxic water

During aquatic hypoxia, P_{aO_2} was approximately 2.5 kPa greater than water P_{O_2}, indicating that oxygen uptake during hypoxia was exclusively *via* the air-breathing organ. The gills are completely ineffective as oxygen uptake organs at the levels of hypoxia used in this study: it has been shown that this species actually loses oxygen from the gills in hypoxic water (Smatresk and Cameron, 1982*a*). Thus, the gar exercised in hypoxic water were functionally unimodal air breathers.

The combination of increased air breathing and anaerobic metabolism sustained a swimming endurance in hypoxic gar similar to that of gar swimming in normoxic water. All fish survived exhausting exercise, and the restoration of acid–base homeostasis was accomplished through continued heavy air breathing, an overall depression of gill ventilation and an alteration in the pattern of gill ventilation. Thus, swimming behavior in *L. oculatus* is not inhibited in hypoxic environments as it is in unimodal water breathers (Kutty, 1967), nor does hypoxia critically affect the ability of *L. oculatus* to survive and recover from exhausting activity.

Despite the depression in gill ventilation, P_{aO_2} decreased from 4.5 to 3.3 kPa immediately after exercise, indicating that gar were probably losing O_2 from the blood to the water when swimming in hypoxic water (Smatresk and Cameron, 1982*a*). Post-exercise acidosis could compromise O_2 delivery through Root and Bohr effects on hemoglobin O_2-affinity. However, there was no indication that lower P_{aO_2} or O_2 content resulted in a greater O_2 debt or metabolic acidosis (i.e. lactate concentration was the same in normoxic and hypoxic fish). In other words, the hypoxic fish were no more anaerobic than the normoxic fish. The major effect of the depressed gill ventilation of the hypoxic fish was that they were significantly acidotic (pre- and post-exercise) compared with the normoxic fish. Since gar in hypoxic water were no more anaerobic than normoxic fish, acidosis was not attributable to increased lactacidosis. Instead, depressed gill ventilation compromised CO_2 exchange, resulting in a respiratory acidosis and, most interestingly, appeared to retard metabolic compensation.

The results of this study show that aquatic hypoxia does not set limits on exercise endurance in spotted gar. Gar can exercise to the same extent in hypoxic water using only the air-breathing organ for oxygen uptake as they can in normoxic water using both the gills and the air-breathing organ. The ventilatory effects of exercise were similar to the effects of hypoxia: increased P_{Op} and air-breathing frequency. Exercise, however, had little effect on cardiovascular variables. Acid–base recovery following exhaustive exercise in normoxic water is more prolonged than in unimodal water-breathing fish and resembles more closely the pattern shown by the aquatic salamander *Cryptobranchus alleganiensis* (Boutilier *et al.* 1980). This may be due, in part, to an increased dependence on aerial O_2 uptake and the concomitant decrease in gill ventilation. Despite a slightly prolonged respiratory acidosis due to inhibited gill ventilation, hypoxic gar were able to re-establish acid–base balance within 24 h after exhaustive exercise. With a lung that can support the metabolic scope equivalent to lungs and gills, gar can maintain activity under hypoxic conditions that incapacitate virtually every other fish in their environment.

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