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ORIGINAL PAPER

Continuous measurement of oxygen tensions in the air-breathing organ of Pacific tarpon (*Megalops cyprinoides*) in relation to aquatic hypoxia and exercise

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Abstract The Pacific tarpon is an elopomorph teleost fish with an air-breathing organ (ABO) derived from a physostomous gas bladder. Oxygen partial pressure (PO_2) in the ABO was measured on juveniles (238 g) with fiber-optic sensors during exposure to selected aquatic PO_2 and swimming speeds. At slow speed (0.65 BL s^{-1}), progressive aquatic hypoxia triggered the first breath at a mean PO_2 of 8.3 kPa. Below this, opercular movements declined sharply and visibly ceased in most fish below 6 kPa. At aquatic PO_2 of 6.1 kPa and swimming slowly, mean air-breathing frequency was 0.73 min^{-1}, ABO PO_2 was 10.9 kPa, breath volume was 23.8 ml kg^{-1}, rate of oxygen uptake from the ABO was 1.19 ml kg^{-1} min^{-1}, and oxygen uptake per breath was 2.32 ml kg^{-1}. At the fastest experimental speed (2.4 BL s^{-1}) at 6.1 kPa, ABO oxygen uptake increased to about 1.90 ml kg^{-1} min^{-1}, through a variable combination of breathing frequency and oxygen uptake per breath. In normoxic water, tarpon rarely breathed air and apparently closed down ABO perfusion, indicated by a drop in ABO oxygen uptake rate to about 1% of that in hypoxic water. This occurred at a wide range of ABO PO_2 (1.7–26.4 kPa), suggesting that oxygen level in the ABO was not regulated by intrinsic receptors.

Keywords Fish · Respiration · Air-breathing · Bimodal gas exchange · Oxygen receptors

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Introduction

The only air-breathing fishes that spend their adulthood in the pelagic marine environment are the congeneric Atlantic and Pacific tarpon, *Megalops atlanticus* and *M. cyprinoides* (Graham 1997; Geiger et al. 2000). They use a physostomous gas bladder (swimbladder) as their air-breathing organ (ABO) and exchange gases through four bands of richly-vascular, spongy tissue that run almost the entire length of the organ on the top, bottom and sides (de Beaufort 1909). Tarpon are the only elopomorph teleosts that breathe air, and their ABO is therefore thought to have evolved independently (Graham 1997). This is a curious adaptation because oxygenation at the surface of the sea is usually high, and there would appear to be little selection for air breathing driven by aquatic hypoxia. However, juvenile tarpon reside in freshwater systems that

often become hypoxic, locally or seasonally (Townsend et al. 1992; Wells et al. 2005). They rarely breathe air in normoxic freshwater, but do so in hypoxic water and during exercise (Shlaifer and Breder 1940; Seymour et al. 2004). Clearly, both metabolic oxygen demand and oxygen availability are integrated into their air-breathing behavior and partitioning of oxygen uptake between the gills and ABO.

The signal that triggers air breathing in tarpon has not been studied, although it most likely involves oxygen, since oxygen partial pressure (PO_2) in the water and blood is the principal determinant of gill ventilation in fishes (Smatresk and Cameron 1982; Smatresk et al. 1986; Randall 1990). Most previous approaches to the question of oxygen sensing in the ABO have involved altering the composition of the gas being breathed and observing changes in air-breathing behaviour. The results are mixed, apparently depending on the level of reliance on the ABO or gills. For example, in the obligatory air-breathing South American lungfish *Lepidosiren paradoxa*, breathing hypoxic air causes increases in breathing frequency, but hypoxic water has no effect (Sanchez et al. 2001). Similarly both *Hoplerythrinus unitaeniatus* and *Arapaima gigas*, which also use a modified gas bladder as an ABO, increase air-breathing frequency in response to nitrogen injections into the gas bladder, and decrease it following oxygen injections (Farrell and Randall 1978). On the other hand, air breathing in the gar *Lepisosteus osseus* is unaffected by level of oxygen in the ABO, but frequency increases in hypoxic water (Smatresk and Cameron 1982). Both the gar and the bowfin *Amia calva* use the gas bladder and, like most fish, have outward- and inward-oriented oxygen receptors in the gills that modulate gill ventilation and air breathing (Smatresk et al. 1986; Smatresk 1990; McKenzie et al. 1991). There is no evidence for central oxygen receptors in air-breathing fish (Smatresk 1994), so responses to PO_2 in the ABO might be mediated by branchial receptors via the circulation. In the anabantoid fish *Trichogaster trichopterus*, which uses a labyrinth organ ABO, responses to hypoxia in inspired air occur, but are not immediate, suggesting that some time is required for hypoxic blood to reach the receptors (Burggren 1979).

Our previous work with tarpon dealt with the effects of hypoxia and exercise in juveniles, focussing on the respiratory properties of the blood (Wells et al. 1997, 2003, 2005) and partitioning of respiration between the gills and ABO (Seymour et al. 2004). We measured oxygen exchange by the ABO by analyzing expired gas on a breath-by-breath basis in a flow-through aerial respirometer, and found that aquatic hypoxia was the primary stimulant for air-breathing, but increased activity in hypoxic water resulted in greater aerial oxygen uptake rates (Seymour et al. 2004). However, the measurements integrated oxygen uptake from the ABO into the body between breaths, and the pattern of oxygen uptake during the apnoeic period was unknown. Continuous measurements of PO_2 in the ABO would permit us to answer questions related to the control of blood flow to the ABO during apnoea and regulation of internal PO_2. In particular, is the rate of oxygen removal from the ABO augmented when metabolic rate increases with faster swimming? When tarpon are not air breathing in normoxic water, do they cease oxygen uptake from the ABO by stopping blood flow or by simply allowing the oxygen to be depleted? Finally, is the ABO PO_2 regulated to an extent that indicates intrinsic oxygen-sensitive chemoreceptors?

Historically, measuring PO_2 in the ABO of air-breathing fish has involved either catheterization of the organ for gas sampling or collection of mixed end-expiratory gas. Both of these methods have their limitations, the most important of which is that continuous monitoring of PO_2 during the breath-hold is lacking. Also, good results for single breath-holds are only available during long (5–160 min), sometimes forced, apnoeas during which a large fraction of the oxygen is absorbed (Graham 1997). Another problem with the catheterisation approach is that sampling changes the volume of the organ and potentially affects mechanoreceptors that influence air-breathing behavior (Milsom 1990; Pack et al. 1990). Development of small diameter, flow independent, oxygen-sensitive optodes offers, for the first time, the opportunity to measure PO_2 continuously in the ABO, without changing gas volume. The traces permit measurements of breathing frequency, rate of oxygen uptake from the ABO, maximum and minimum PO_2 during air breathing and effective ventilation volumes. Furthermore, the results have implications concerning perfusion of the ABO and the regulation of ventilation.

Materials and methods

Animals

Pacific tarpon *Megalops cyprinoides* were caught in local billabongs either by hook and line or with seine nets and transported to Charles Darwin University where they were held in recirculating, filtered water at 26°C. The fish were not fed during their 1–2 week captivity.

Oxygen sensors

Oxygen-sensitive, implantable optodes (Precision Sensing GmbH, http://www.presens.de) were used to measure PO_2 in the ABO. Ours consisted of a 140 μm diameter glass fiber encased in two sheaths, 600 and 900 μm diameter.

The manufacturer removed 20 mm of the outer sheath and 2 mm of the inner sheath, leaving the tapered glass tip exposed. Preliminary experiments showed that either the fluorescent dye on the bare optode tip came off after a few minutes in the ABO or the tip punctured gas exchange tissue and became sluggish. Therefore we protected the tip with a custom-made PVC tubing collar that was glued to the end of the outer sheath with cyanmethacrylate adhesive and extended just beyond the end of the glass fiber. The collar was the same outside diameter as the outer optode sheath (900 μm), but the inside diameter was slightly larger than the inner sheath (750 μm). Because the space between the optode tip and the collar contained air, any fluid that entered the collar tended to be sucked away from the tip by capillarity. The optodes had a response time of about 1 s, if the tips were in the free air inside the ABO. Despite the collar, however, the tip sometimes touched the ABO wall or acquired adherent fluid during a run. In these cases, the response was sluggish, but the data were still useful to measure breathing frequencies, mean PO_2 and overall rate of oxygen uptake. They were calibrated before and after each experiment in a stream of pure nitrogen and in water-saturated air at measured atmospheric pressure. Continuous recordings were taken with a data acquisition system (PowerLab, http://www.ADInstruments.com) from both the optode interface (Microx T3, http://www.presens.de) and a Clark oxygen electrode (YSI model 58, http://www.ysi.com) in the flume water.

Instrumentation and recovery

To implant the optode into the ABO, a fish was first anaesthetized (0.13 g l^{-1} MS222 buffered with 0.13 g l^{-1} sodium bicarbonate) and placed on a foam-operating sling where its gills were irrigated with a diluted anesthetic solution (0.08 g l^{-1} MS222 buffered with 0.08 g l^{-1} sodium bicarbonate). A 14G or 15G stainless steel needle was inserted anteriorly under the scales at a point level with the anal fin and midway between the lateral line and the bottom of the fish. The needle then acted as a trochar to introduce the optode into the ABO. Numerous trials and dissections had shown that the trochar could be reliably placed into the posterior region of the ABO where the blood supply was much reduced and could be largely avoided. The placement of the trochar also minimized the likelihood of any of the four bands of gas exchange capillaries being damaged. A cleaning wire was inserted down the lumen of the needle prior to injecting a small amount of pure oxygen into the ABO. These procedures served to clear the needle of debris and provide a high PO_2 signal to show that the optode was correctly placed in the airspace of the ABO. The optode was carefully advanced down the lumen of the needle, which was then withdrawn, leaving the tip of the optode 1–3 cm inside the ABO lumen. The optode lead was sutured to the skin and allowed to trail behind the fish during swimming. There was no evidence of gas leakage around the optode (bubbles escaping the fish or water in the ABO at autopsy). Fish recovered for 2–22 h (mean 11.5 h) in the swim chamber at a slow water velocity (0.06 m s^{-1}). At this speed, fish orientated into the water current but did not have to swim if they rested on the bottom. During this period, air was bubbled into the swim chamber to maintain normoxia and to accustom the fish to the sound of bubbles.

Swim flume

The swim chamber was in an 80-l, recirculating flume, equipped to control current speed and dissolved oxygen level, as described earlier (Seymour et al. 2004), but with some modifications for the present experiments. The swim chamber was 750 mm long, 200 mm wide and 142 mm deep, but in some cases it was divided to provide dual channels 98 mm wide, so that two fish could be tested simultaneously. Water flow was controlled by a 12 VDC trolling motor (Mercury T2400) and a variable 0–25 A power supply (model D3800, Dick Smith, http://www.dse.com.au). Flow rates in the center of the swim chamber were calibrated over a range of 0.06–0.48 m s^{-1} by timing the traverse of entrained particles. Darwin tap water, treated with an aquarium conditioner, was replaced between runs, which were carried out at 27 ± 2°C. To avoid visual disturbance, the flume was covered with black plastic sheeting, except for a breathing hole at the top that was covered with a translucent hood to prevent escape, and a small opening at the rear that permitted observations of gill ventilation.

Protocol

The effect of dissolved oxygen on air-breathing frequency was determined by bubbling nitrogen through the flume water during measurements at a mean swimming speed of 0.65 BL s^{-1} (±0.17, 95% CI). PO_2 was reduced in a stepwise fashion: approximately 20, 16, 12, 8, 6, and 4 kPa. The transition time between steps increased as PO_2 decreased, being about 2, 4, 8, 10, and 12 min, respectively. After equilibration, PO_2 was manually regulated at a reasonably constant level for at least 20 min by bubbling nitrogen when necessary. An aquatic PO_2 of about 6 kPa induced air breathing in all fish. At this level, the velocity of the hypoxic water was increased in a stepwise fashion, nominally 0.1, 0.15, 0.2, and 0.5 m s^{-1}, again for periods lasting 20 min. Therefore, each fish might be exposed to the lowest hypoxia level for about 1 h. Gill ventilation was measured by timing visual observations (20 beats to the nearest second) through a small opening at the back of the flume. In some trials, water PO_2 was reduced to about 2 kPa to examine its

effect on gill ventilation. Opercular movements were not discernable during high-speed swimming; therefore the data presented are from fish swimming at low to moderate speeds (0.10–0.33 m s^{-1}). Following the hypoxic swimming challenge, flume speed was decreased and the fish was returned to normoxic water for a period, then over-anaesthetized and sacrificed by vertebral dislocation or transection. Body mass and fork length were measured. In a separate group of fish, the volume of the ABO (V_{ABO}) was measured by submerging the freshly killed animal in water, surgically opening the ABO and collecting the bubbles in an inverted, water-filled container. The volume was measured at atmospheric pressure by collecting the gas in a 50 ml syringe.

Data analysis and statistics

Respiratory variables were determined on a breath-to-breath basis from records of ABO PO_2, the upward deflections of which were visually related to individual air breaths. Air-breathing frequency was calculated from breathing intervals. The volume of each air breath (V_{ab}) was estimated from the difference in PO_2 just before a breath (PO_{2min}) and just after it (PO_{2max}). We made three assumptions: that V_{ABO} was filled to a constant level with each breath (otherwise buoyancy of the fish would vary, and this did not appear to be the case, based on extensive visual observations during air breathing); that ventilation was a single exhalation occurring before inhalation (tarpon do release a small bubble after inhalation, but this is likely residual air from inside the mouth); and that the PO_2 of inspired air (PO_{2I}) was 20.2 kPa. The equation is $V_{ab} = V_{ABO}\,(PO_{2max} - PO_{2min})/(PO_{2I} - PO_{2min})$. The rate of oxygen uptake by the ABO was calculated from the slope of the PO_2 trace. The decrease in ABO PO_2 during breath-holding was practically linear in records when mean PO_2 was above about 8 kPa, however in two cases where the mean was lower, the trace became somewhat curved. In cases where the trace was curved or when the optode response was sluggish, the rates were measured only in the linear segments. PO_2 prevailing in the ABO was evaluated as the mean value throughout the measurement period.

All reported statistics are means, 95% confidence intervals (CI) and numbers (n) of fish. Because of technical difficulties with the optodes and the cooperation of the animals, not all fish provided data for all variables under every experimental condition. Thus sample size varies.

Results

Body mass of the ten fish used for respirometry ranged between 171 and 362 g, with mean mass of 238 g (±38, 95% CI). Mean fork length was 262 mm (±18). Fork length (FL) was linearly related to body mass (M) according to: FL = 0.49 M + 145 (R^2 = 0.99). Fork length was considered to be body length (BL) for the purpose of determining swimming speed.

ABO volume was measured as 55.6 ml kg^{-1} (±8.1) in six additional fish weighing 176 g (±22). This value was used to estimate the volume of each air breath in experimental fish.

Opercular movements were easily visible in normoxic water, when air breathing was absent. The rate of pumping increased with declining aquatic PO_2 until about 8 kPa, when it began to decrease (Fig. 1). With the decrease in frequency of opercular movements, amplitude also decreased markedly and the movements became arrhythmic, until they ceased entirely in most fish below about 6 kPa.

The aquatic PO_2 threshold when gill ventilation began to decrease corresponded approximately with the onset of ABO ventilation. The mean water PO_2 at which tarpon first breathed air during progressive oxygen depletion at the slowest swimming speed was 8.3 kPa (±2.3; n = 8). Once regular air breathing had commenced, we evaluated ABO variables in fish under the comparable conditions of water PO_2 (6.1 kPa ± 2.9) and a slow mean swimming speed (0.65 BL s^{-1} ± 0.17). There was no significant relationship between air-breathing frequency and aquatic PO_2 over the water PO_2 range of 4–8 kPa, and mean air-breathing frequency (f_{ab}) was 0.73 min^{-1} (±0.27; n = 8). In fish with optodes that responded quickly (for example, see Fig. 3), it was possible to evaluate changes in ABO PO_2 associated with individual breaths. On average, ABO PO_2 varied within a 5 kPa range. Mean PO_{2min} preceding an air breath was 8.4 kPa (±2.9; n = 5), mean PO_{2max} was 13.2 kPa (±2.5; n = 5) and overall mean PO_2 was 10.9 kPa (±1.9; n = 8). The range of aquatic PO_2 we used was too small,

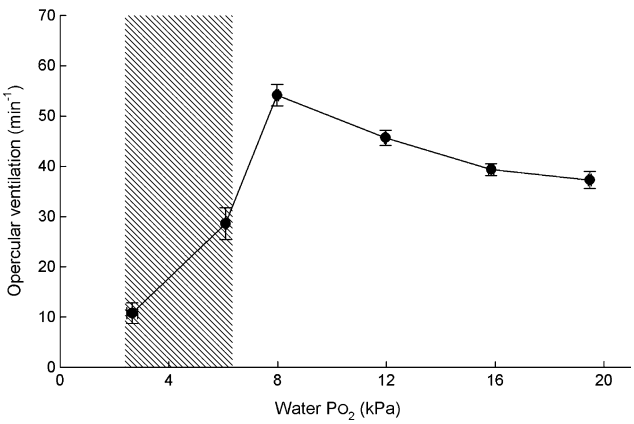

Fig. 1 Relationship between dissolved oxygen and opercular ventilation frequency in tarpon (n = 10). Values are means ± CI. In addition to the decrease in opercular ventilation rate at low water PO_2, the *shaded region* indicates where opercular stroke amplitude became appreciably low and often visibly imperceptible

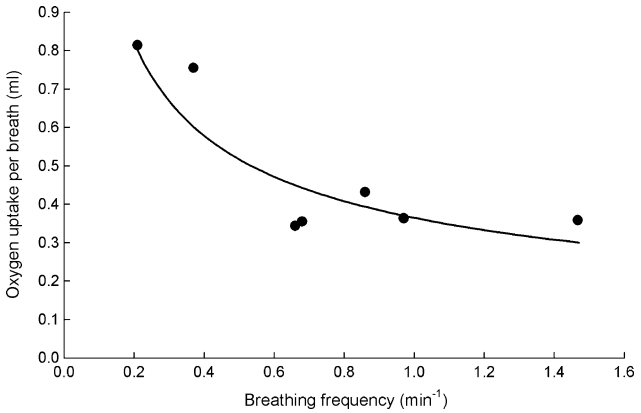

Fig. 2 Relationship between volume of oxygen taken up per breath (VO_{2ab}) and breathing frequency (f_{ab}) in seven tarpon swimming slowly (0.65 BL s^{-1}) in water with a mean PO_2 of 6.1 kPa. Each point is the mean from one fish. The descriptive relationship is $VO_{2ab} = 0.365\ f_{ab}^{-0.50}$ ($R^2 = 0.75$)

and the variability too high, to detect any relationship between aquatic PO_2 and minimum or maximum ABO PO_2. Calculated air-breath volume (V_{ab}) was 23.8 ml kg^{-1} (±3.5; $n = 7$), which corresponded to a tidal volume of 43% of V_{ABO} (55.6 ml kg^{-1}). The mean rate of oxygen uptake from the ABO ($\dot{V}O_{2air}$) was 1.19 ml kg^{-1} min^{-1} (±0.16; $n = 7$). The amount of oxygen taken up with each breath (VO_{2ab}) was 0.49 ml (±0.15; $n = 7$), or 2.32 ml kg^{-1} (±0.79; $n = 7$). Because the exhaled air contains some oxygen, the extraction coefficient becomes (2.32/(23.8 × 0.205)) = 0.48. There was a significant inverse relationship between mean f_{ab} and VO_{2ab} in seven fish (Fig. 2). These measurements of ABO respiratory variables with an internal oxygen optode align well with our previous study on tarpon in which ABO gas exchange was measured by analysis of exhaled gas (Seymour et al. 2004). In that study, involving tarpon of a similar mass (265 g) and at a similar swimming speed (0.4–0.8 BL s^{-1}) in water of a similar oxygen tension (8.4 kPa), mean f_{ab} was 0.55 min^{-1}, $\dot{V}O_{2air}$ was 0.89 ml kg^{-1} min^{-1} and VO_{2ab} was 0.52 ml, values that are not significantly different from the present results.

The pattern of ABO gas exchange changed with exercise, when swimming speed was increased (Fig. 3). Although there was no significant effect of swimming speed on volume of oxygen consumed per air breath (VO_{2ab}, Fig. 4a), volume of air breaths (V_{ab}; Fig. 4b), or frequency of breathing (f_{ab}; Fig. 4c), the overall rate of oxygen uptake from the ABO ($\dot{V}O_{2air}$; Fig. 4d) increased significantly with swimming speed, reaching about 1.90 ml kg^{-1} min^{-1}. Thus individual fish accomplished this increase in different ways, either by consuming more oxygen per breath or by increasing frequency.

Under aquatic normoxia, air breathing ceased and ABO PO_2 quickly plateaued. Such plateaus are apparent at the beginnings and ends of individual runs (e.g., Fig. 3). They could occur over a very wide range of ABO PO_2, between 1.7 kPa (after overnight in normoxic water) to 26.4 kPa (after an inadvertent breath of hyperoxic air following bubbling of oxygen through the flume to raise aquatic PO_2). The apparent plateaus were not absolute, however. $\dot{V}O_{2air}$ decreased to 0.012 ml kg^{-1} min^{-1} (±0.06; $n = 5$), which was about 1% of the rate during air breathing in aquatic hypoxia (1.19 ml kg^{-1} min^{-1}). These low $\dot{V}O_{2air}$ values are evident in continuous recordings from three fish that were left undisturbed overnight in normoxic water flowing at 0.06 m s^{-1} (Fig. 5). Although two fish did not air breathe over the 5-h period and the other fish breathed 14 times, the slopes for PO_2 during the long periods of apnoea were similar to each other. This similarity implies that, during the plateau phase in normoxic water, $\dot{V}O_{2air}$

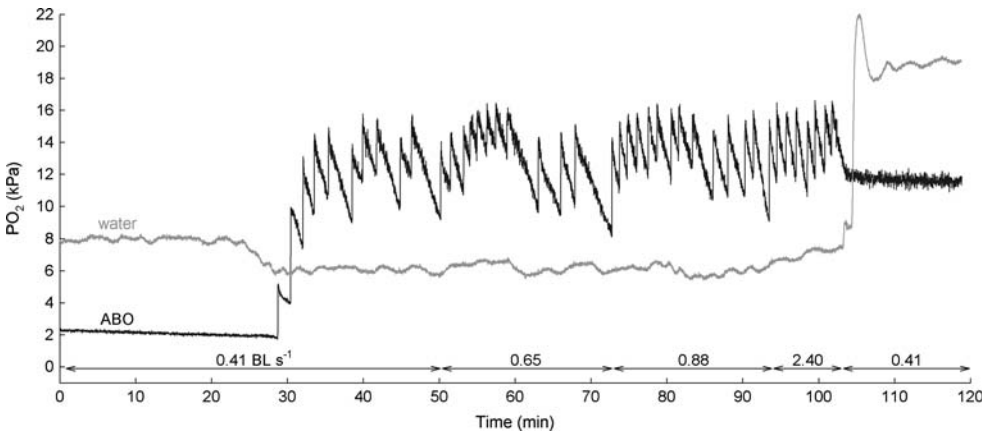

Fig. 3 Oxygen partial pressure (PO_2) in the flume water and the air-breathing organ (*ABO*) of tarpon during an experimental run at selected swimming speeds. Note the first air breath when water PO_2 dropped to about 6 kPa (mean for all individuals = 8.3 ± 2.3 kPa), and the shallow slopes of the ABO trace before the first breath and after the last breath, indicating negligible ABO oxygen uptake. Oscillations in aquatic PO_2 are due to periodic bubbling of N_2, which was sometimes associated with long breath-holds

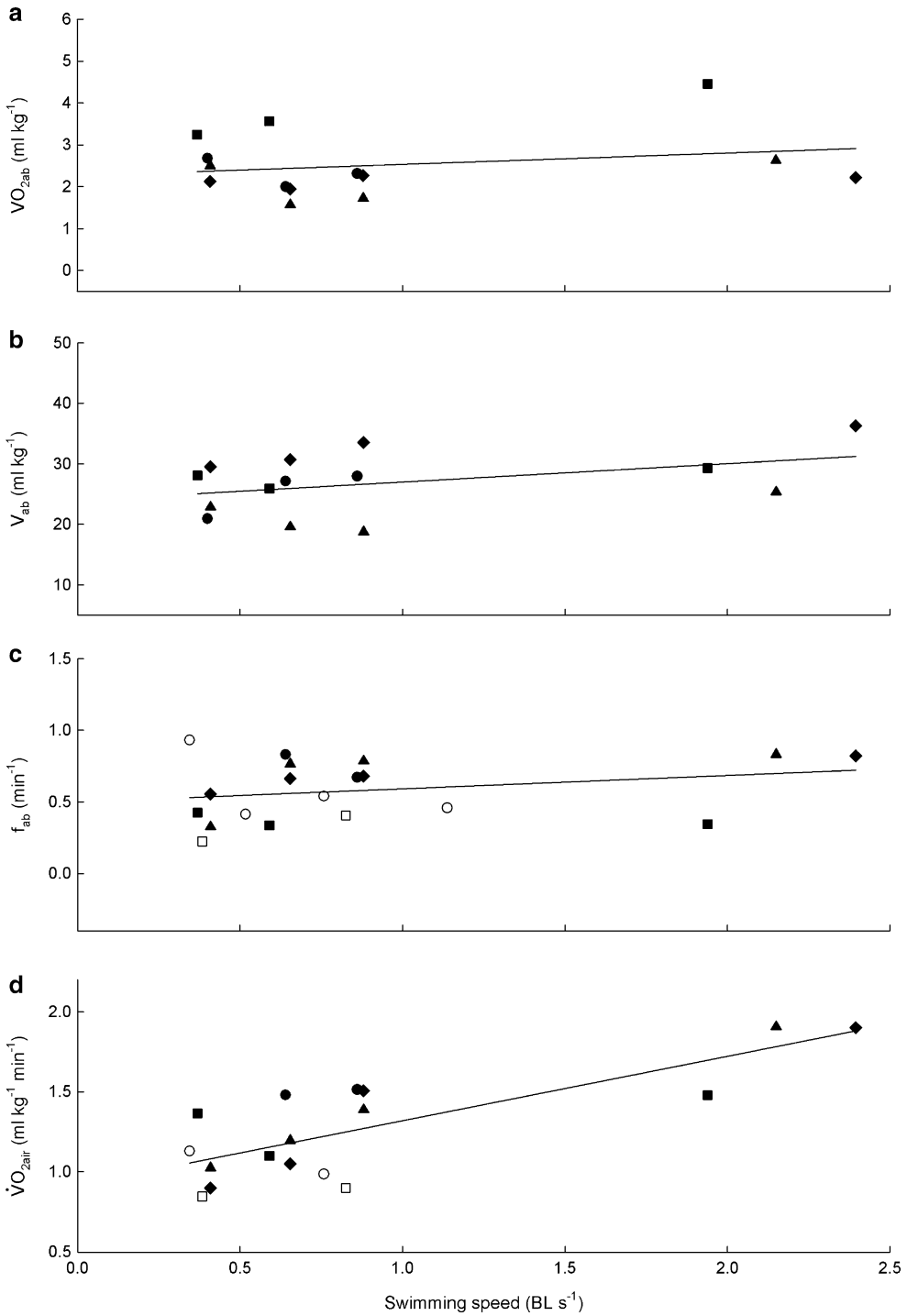

Fig. 4 Effect of swimming speed (U) on respiratory variables in tarpon at an aquatic ambient PO_2 between 5 and 7 kPa. **a** Oxygen uptake per air breath (VO_{2ab}). **b** Volume of air breath (V_{ab}). **c** Frequency of breathing (f_{ab}). **d** Rate of oxygen uptake from the ABO ($\dot{V}O_{2air}$). Each point is a mean from individual fish designated by *different symbols*. $\dot{V}O_{2air}$ is significantly correlated with U ($P < 0.05$), and the descriptive relationship is $\dot{V}O_{2air} = 0.40\,U + 0.92 (R^2 = 0.61)$. The other regression lines have no significant slope

was independent of ABO PO_2. It is also noteworthy that in one fish, there were occasional decreases in ABO PO_2 without preceding increases, indicating that transient increases in $\dot{V}O_{2air}$ could occur without air breaths (Fig. 5).

Discussion

This study clearly shows that the transition from water breathing to air breathing in resting juvenile tarpon begins

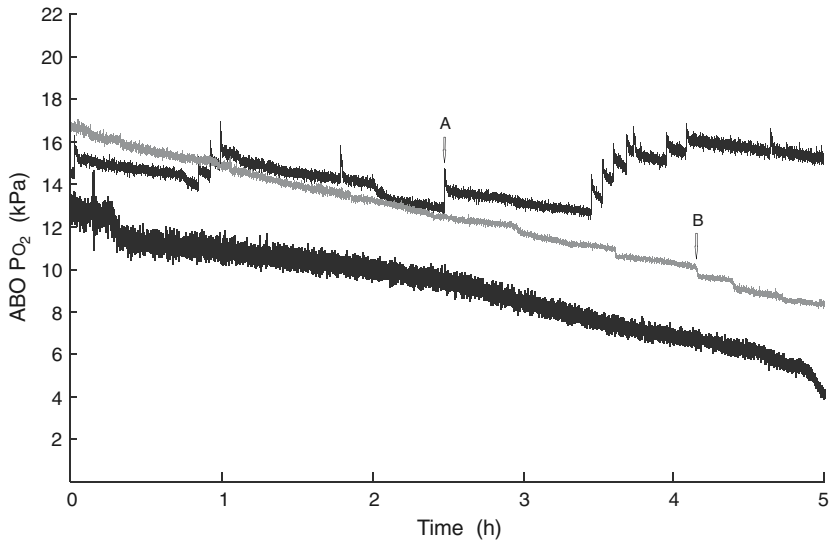

Fig. 5 Three examples of changes in ABO PO_2 during slow or no swimming (0.06 m s^{-1}) in normoxic water for an arbitrary 5-h period for which the data are complete. Gradual decrease in PO_2 indicates a slow oxygen uptake by the ABO. Sharp upward spikes imply single air breaths (**a**), followed by a steep downward slope indicating a high rate of oxygen uptake from the ABO. Relatively rapid downward steps in fish that were not breathing (**b**) are assumed to indicate transient increases in ABO blood perfusion. Note the difference in temporal scale, and therefore oxygen exchange rates, in comparison with Fig. 3

at an aquatic PO_2 below about 8.3 kPa, the mean PO_2 of the first air breath. Below this point gill ventilation rate and amplitude progressively decrease until it is visually imperceptible in most fish below about 6 kPa (Fig. 1). One interpretation of this finding is that branchial oxygen uptake becomes ineffective at this level of hypoxia. The maximum saturation of the blood in equilibrium with an aquatic PO_2 of 6 kPa would be between 60 and 85%, assuming a pH of 7.4–7.8, respectively [Wells et al. 2005; this is a physiologically relevant pH range for tarpon (R.M.G. Wells, personal observation)]. Thus, it is clear that gill ventilation becomes incapable of fully saturating arterial blood at this level of hypoxia. Furthermore, it has been long considered that continued perfusion and ventilation of the gills under hypoxic conditions could result in loss of oxygen from the ABO to the water (e.g., Randall et al. 1981; Graham 2006). Loss of gill surface area and the formation of non-respiratory vascular shunts around the branchial exchange surface in air-breathing fishes are considered adaptations to the problem (Randall et al. 1981; Graham 1997, 2006). Assuming the blood leaving the ABO is in equilibrium with ABO gas, it can have a mean PO_2 as high as 13 kPa during aquatic hypoxia. Because all of the oxygenated blood leaving the ABO flows directly to the heart and then to the gills, there could be a considerable outward PO_2 gradient between the blood and the water. The extent of the gradient, however, depends on the proportion of cardiac output directed to the ABO and the systemic venous PO_2 and pH. At present it is not possible to estimate oxygen gradients across the gills, so this analysis must await direct sampling of blood from the ventral and dorsal aortae.

The relative stability of ABO PO_2 during apnoea in normoxic water, compared with air breathing in hypoxic water (Figs. 3, 5), strongly suggests that blood flow to the ABO exchange surface can be greatly reduced, if not stopped completely. In support of this, we recently documented for similarly sized tarpon, a significant increase in cardiac output with the onset of air breathing, presumably to maintain blood pressure during periods of ABO vasculature perfusion (T. D. Clark et al., unpublished). The lack of PO_2 change in the ABO during apnoea in normoxic water cannot be attributed to normoxic PO_2 in the blood passing from the gills to the ABO, which would prevent oxygen uptake, because the plateaus occurred at ABO PO_2 ranging from 1.7 to 26.4 kPa. It is reasonable to believe that the low residual oxygen uptake (about 1% of the air-breathing rate) may have been due to the metabolic demand of the ABO tissue itself, because the organ represents a similar proportion of the body. Because oxygen can be supplied directly from the ABO gas, there would be no need to perfuse the organ at all. One role of cutting circulation to the ABO during periods when aquatic respiration is sufficient is that it stabilizes ABO volume and hence buoyancy. Because oxygen uptake concentrates the nitrogen in the ABO, it too would tend to dissolve into the blood if the ABO were perfused. Without perfusion, however, oxygen content of the ABO was gradually depleted over many hours in our experiments (Fig. 5). Gradual loss of gas bladder volume in Atlantic tarpon that were prevented from breathing for a week or

more has been implicated in their death, leading to the misleading conclusion that tarpon are obligate air breathers (Shlaifer and Breder 1940; Geiger et al. 2000).

An ability of cardiac output to bypass the ABO exchange surface completely is likely related to the reliance of any individual species on air breathing. Flow in the pulmonary artery of the African lungfish *Protopterus aethiopicus* can decrease to zero when the fish is resting quietly underwater, presumably relying on gills alone (Szidon et al. 1969). On the other hand, the obligate air-breathing electric eel *Electrophorus electricus* can control circulation to the ABO in its mouth, increasing it just after a breath to about 75% of cardiac output and decreasing it gradually during the breath-hold, but it never stops flow entirely (Johansen et al. 1968).

Our results help to address the question of whether oxygen sensors are located in the ABO of tarpon. The wide range of ABO PO_2 (1.7–26.4 kPa) during the virtual plateaus in aquatic normoxia speaks against them. If oxygen receptors exist in the ABO, then they must have been ineffective during aquatic normoxia, permitting ABO PO_2 to become severely hypoxic. In contrast, during aquatic hypoxia, mean ABO PO_2 remained between 8.4 and 13.2 kPa, and the lower value could be viewed as a threshold of ABO oxygen receptors for triggering breathing. However, a simpler explanation for these results is that the receptors that govern both air breathing and ABO blood flow are not in the ABO, but probably reside in the gills (Smatresk 1990, 1994; Burleson et al. 1992; Taylor 1992). Although gill ventilation decreases and appears to stop during hypoxic exposure, the quick cessation of air breathing and ABO oxygen uptake in response to rising aquatic oxygen (Fig. 3) shows that the fish are still able to sense oxygen in the water.

Tarpon responded to the increased oxygen demand of swimming in hypoxic water with variable combinations of changes in air breathing frequency, tidal volume and amount of oxygen taken up per breath (Fig. 4), which is consistent with earlier findings (Seymour et al. 2004). Importantly, while swimming speed increased 3.7-fold, which likely doubled oxygen demand (e.g., Lee et al. 2003; Clark and Seymour 2006), ABO oxygen uptake increased only 1.6-fold. The simplest explanation for this is that there was continued oxygen uptake by the gills, which seems reasonable as the PO_2 of the water was 6.1 (±2.9 kPa) and the P_{50} of tarpon hemoglobin is below 4 kPa (Wells et al. 2005). Furthermore, with exercise in water-breathing fishes, oxygen extraction by the tissues increases, reducing venous PO_2 (Kiceniuk and Jones 1977; Farrell and Clutterham 2003), and in turn enhancing the oxygen gradient between the water and blood at the gills. While there were no significant opercular movements at the highest swimming speeds, the fish may have benefited from ram ventilation of the gills, taking up oxygen from the hypoxic water into even more hypoxic blood.

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