

VENTILATORY FLOW RELATIVE TO INTRABUCCAL AND INTRAOPERCULAR VOLUMES IN THE SERRASALMID FISH *Piaractus mesopotamicus* DURING NORMOXIA AND EXPOSED TO GRADED HYPOXIA

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ABSTRACT

Ventilation volume (\dot{V}_G – $\text{mlH}_2\text{O}\cdot\text{min}^{-1}$), respiratory frequency (f_R – $\text{breaths}\cdot\text{min}^{-1}$) and tidal volume (V_T – $\text{mlH}_2\text{O}\cdot\text{breath}^{-1}$) were measured in a group of *Piaractus mesopotamicus* (650.4 ± 204.7 g; $n = 10$) during normoxia and in response to graded hypoxia. The f_R was maintained constant, around 100 $\text{breaths}\cdot\text{min}^{-1}$, from normoxia until the O_2 tension of the inspired water (PiO_2) of 53 mmHg, below which it increased progressively, reaching maximum values (157.6 ± 6.3 $\text{breaths}\cdot\text{min}^{-1}$) at 10 mmHg. The V_T rose from 1.8 ± 0.1 to 6.0 ± 0.5 and 5.7 ± 0.4 $\text{mlH}_2\text{O}\cdot\text{breath}^{-1}$ in the PiO_2 of 16 and 10 mmHg, respectively. The \dot{V}_G increased from 169.3 ± 11.0 (normoxia) to 940.1 ± 85.6 $\text{mlH}_2\text{O}\cdot\text{min}^{-1}$ at the PiO_2 of 16 mmHg, below which it also tended to decrease. A second group of fish (29 to 1510.0 g, $n = 34$) was used for the evaluation of allometric relationships concerning ventilation and dimensions of the buccal and opercular cavities. At maximum \dot{V}_G , the V_T corresponded to $93.2 \pm 2.4\%$ of the buccal volume and $94.9 \pm 2.3\%$ of the opercular volume, suggesting that the \dot{V}_G of *P. mesopotamicus* is limited by the volumes of buccal and opercular cavities in severe hypoxia.

Key words: hypoxia, normoxia, ventilatory parameters, buccal and opercular volumes, *Piaractus mesopotamicus*.

RESUMO

Fluxo ventilatório relativo aos volumes intrabucal e intraoperculares no serrasalmídeo *Piaractus mesopotamicus* durante a exposição à normóxia e à hipóxia gradual

Em um grupo de *Piaractus mesopotamicus* ($Wt = 650,4 \pm 204,7$ g, $n = 10$) foram determinados a ventilação branquial (\dot{V}_G – $\text{mlH}_2\text{O}\cdot\text{min}^{-1}$), a frequência respiratória (f_R – $\text{ciclos respiratórios}\cdot\text{min}^{-1}$) e o volume ventilatório (V_T – $\text{mlH}_2\text{O}\cdot\text{ciclo respiratório}^{-1}$) em normóxia e em resposta à hipóxia gradual. A f_R foi mantida constante, em torno de 100 $\text{ciclos respiratórios}\cdot\text{min}^{-1}$, de normóxia até a tensão de O_2 da água inspirada (PiO_2) de 53 mmHg, abaixo da qual aumentou progressivamente, atingindo seus valores máximos ($157,6 \pm 6,3$ $\text{ciclos respiratórios}\cdot\text{min}^{-1}$) em 10 mmHg. O V_T aumentou de $1,8 \pm 0,1$ para $6,0 \pm 0,5$ e $5,7 \pm 0,4$ $\text{mlH}_2\text{O}\cdot\text{ciclo respiratório}^{-1}$ nas PiO_2 de 16 e 10 mmHg, respectivamente. A \dot{V}_G aumentou de $169,3 \pm 11,0$ (normóxia) para $940,1 \pm 85,6$ $\text{mlH}_2\text{O}\cdot\text{min}^{-1}$ na PiO_2 de 16 mmHg, abaixo da qual também tendeu a diminuir. Um segundo grupo de peixes (29 to 1510,0 g, $n = 34$) foi utilizado para a avaliação das relações alométricas entre a ventilação e as dimensões das cavidades bucal e

operculares. Na \dot{V}_G máxima, o V_T correspondeu a $93,2 \pm 2,4\%$ do volume bucal e $94,9 \pm 2,3\%$ de volume opercular, sugerindo que a \dot{V}_G de *P. mesopotamicus* é limitada pelos volumes dessas cavidades durante hipóxia severa.

Palavras-chave: hipóxia, normóxia, parâmetros ventilatórios, volumes bucal e operculares, *Piaractus mesopotamicus*.

INTRODUCTION

Respiratory homeostasis in teleosts depends on an oxygen-oriented control of gill ventilation which normally increases during environmental hypoxia (Holeton & Randall, 1967; Hughes & Saunders, 1970; Lomholt & Johansen, 1979; Steffensen *et al.*, 1982; Rantin & Johansen, 1984; Kalinin *et al.*, 1996). This response is nearly immediate, reflecting the action of O_2 chemoreceptors (Satchell, 1991; Milsom, 1993). The exact sites of these receptors have been considerably discussed and the early literature is not entirely consistent. Branchial receptors were well documented by Milsom & Brill (1986) and these appear to screen both water and arterial blood.

In many fish species the ventilatory responses to hypoxia are considerable and maintain the arterial PO_2 relatively constant during moderate hypoxia (for a review see Milsom, 1993). To attenuate the effects of hypoxia, different species adopt distinct strategies to increase gill ventilation by enhancing tidal volume and/or respiratory frequency.

The relationship between differential pressure of the ventilatory apparatus and the water volume pumped across the gills is determined by the morphology of buccal and opercular chambers and the performance of the ventilatory muscles (Ballintijn, 1969a,b, 1972). These morphological features are species-specific, but may change with development in the individual. Moreover, short term adaptations to the environment may modify the morphology of the chambers (Hughes & Saunders, 1970).

The respiratory physiology in relation to the environment of the teleost fish depends on the mechanisms described above. Rantin *et al.* (1992) and Kalinin *et al.* (1996) studied the respiratory responses to hypoxia in two ecologically distinct water-breathing erythrinids, *Hoplias malabaricus*, typical of stagnant oxygen poor environments of

tropical and sub-tropical regions of South America, and *Hoplias lacerdae*, that inhabits well-oxygenated rivers of central and southern regions of Brazil.

Both species substantially increased ventilation in response to hypoxia. As a difference, *H. malabaricus* mainly increased tidal volume whereas respiratory frequency changed little. Conversely, *H. lacerdae* predominantly increased ventilation by means of respiratory frequency.

In this context, we studied the serrasalmid fish *Piaractus mesopotamicus* (Holmberg), known in Brazil as pacu, a migratory species distributed in South America from the Amazon, in the north, to the Paraná-Paraguay basin, in the south (Severi, 1991). This species is an important food fish and highly suitable for aquaculture (Saint-Paul, 1986). Within the Central Brazilian Pantanal, pacu also occurs in shallow waters and in floodplain lakes that are often subjected to temporary hypoxia or even anoxia.

The aim of this study was to assess ventilation volume (\dot{V}_G – $mlH_2O \cdot min^{-1}$), respiratory frequency (f_R – $breaths \cdot min^{-1}$) and tidal volume (V_T – $mlH_2O \cdot breath^{-1}$) of *Piaractus mesopotamicus*. In addition, tidal volume was evaluated in relation to the intraopercular and intrabuccal volumes, which allows quantification of the morphological limits to increase of tidal volume.

MATERIAL AND METHODS

Specimens of *Piaractus mesopotamicus* ($n = 10$; $Wt = 650.4 \pm 204.7$ g, mean SE) were obtained from the Center of Research in Tropical Fish (CEPTA/IBAMA) – Pirassununga, SP, Brazil. Fish were acclimated to $25^\circ C$ in 250-l holding tanks with continuous flow of dechlorinated and well-aerated water (oxygen water tension – $PwO_2 > 135$ mmHg) for at least 3 weeks prior to experimentation. The fish were fed with commercial food pellets *ad libitum* but were fasted for two days prior to experimentation.

Ventilation volume (\dot{V}_G) was measured applying the method of Rantin *et al.* (1992). Fish were housed in a flow-through respirometer while inlet and outlet water PO_2 ($PinO_2$, $PoutO_2$, respectively) were continuously recorded. PE-catheters were inserted into the roof of the mouth as well as through both opercular cleithra to perform continuous measurements of inspired (PiO_2) and expired (PeO_2) water O_2 tensions. The \dot{V}_G was calculated according to the equation:

$$\dot{V}_G = \dot{V}_R \cdot (PinO_2 - PoutO_2) / (PiO_2 - PeO_2)$$

where \dot{V}_R represents the flow through the respirometer. All water tensions ($PinO_2$, $PoutO_2$, PiO_2 and PeO_2) were measured simultaneously by siphoning the water through the catheters to O_2 electrodes (FAC 001- O_2) that were housed in temperature-controlled cuvettes and connected to oxygen analyzers (FAC-204A, FAC – São Carlos, SP, Brazil).

Respiratory frequency (f_R) was recorded connecting the buccal catheter to a pressure transducer (Spectramed P10EZ) that was coupled to a LP-02 amplifier. The resulting signals were displayed on a Linseis 7065 recorder. Ventilatory tidal volume (V_T) was calculated by dividing \dot{V}_G by f_R .

After surgery the fish were placed into the experimental chamber to recover for 12 hours or more in well-aerated water ($PO_2 > 130$ mmHg) at 25°C. Subsequently, $PinO_2$ was stepwise lowered from the normoxic value (140 mmHg) to 100, 70, 50, 40, 30, 20 and 10 mmHg by bubbling the water with N_2 or compressed air at controlled rates. Each tension was maintained for a 40 min. period, and the measurements were taken over the last 10 min.

The buccal and opercular volumes were determined according to the method of Kalinin (1996). The buccal and opercular cavities of anaesthetized fish (benzocaine 0.1%) were completely filled with an alginate (JelPrint Dentsply Ind. Com. Ltd. Brazil). After hardening, a precision electronic scale (Micronal B-360) was used to weight the casts. The corresponding volumes were derived from a calibration curve of volume (ml) versus weight (g). The morphometric characteristics of buccal ($VBuc$) and opercular (VOp) volumes were

plotted against total length (Lt – cm) and compared to the plots of weight (Wt – g) versus Lt for 34 specimens of *P. mesopotamicus* (29 to 1,510 g).

Statistical analyses

One-way analysis of variance (ANOVA) followed by Bartlett's test for homogeneity of variances and Tukey-Kramer multiple comparisons test were employed to evaluate the data. Additionally, the Mann-Whitney test was employed to compare tidal volume in percentage of buccal and opercular volumes.

RESULTS

Changes in \dot{V}_G , f_R , and V_T of *P. mesopotamicus* during progressive hypoxia are presented in Fig. 1 (upper, middle and lower panel, respectively). The \dot{V}_G increased by about 5.6-fold with the reduction of PiO_2 from normoxia to 16 mmHg. This was accomplished by a 3.3-fold increase in V_T and a 1.6-fold increase in f_R . The f_R increased progressively until the PiO_2 of 10 mmHg was reached, while the \dot{V}_G and V_T tended to decrease below 16 mmHg.

The Fig. 2 compiles the data for the intrabuccal and intraopercular volumes in relation to body length. This figure also include the length-weight relationships. Combining the data for tidal volume (Fig. 1) and the morphometric information (Fig. 2) it becomes possible to express ventilatory responses in terms of percentage of the maximum volume possible (Fig. 3). When gill ventilation reached a maximum, V_T corresponded to $93.2 \pm 2.4\%$ of the buccal volume and $94.9 \pm 2.3\%$ of opercular volume.

DISCUSSION

The results obtained in the present study are in agreement with those reported by Kalinin *et al.* (1996) to the erythrinid *H. malabaricus*, which maintained a larger V_T whereas f_R changed little. The responses of *P. mesopotamicus* and *H. malabaricus* seems advantageous in terms of energy expenditure, assuming that a constant velocity of muscular contraction is energy saving, whereas a higher frequency of contraction occurs on the cost of work against a high internal viscosity of the muscle and of the water (Rantin *et al.*, 1992).

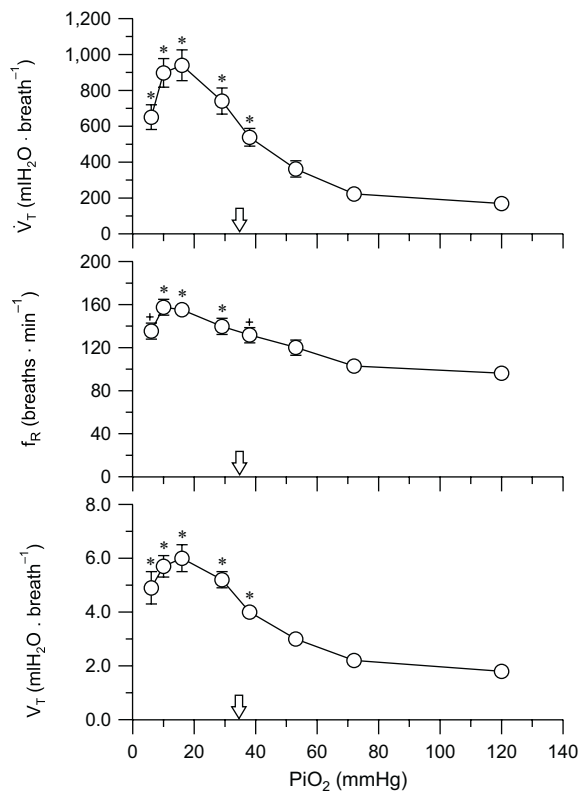


Fig. 1 — Ventilatory variables of *Piaractus mesopotamicus*: Upper panel: Gill ventilation. Middle panel: Respiratory frequency. Lower panel: Tidal volume. Mean values \pm SE, $n = 10$. Significance levels are indicated as follow: * - $p < 0.001$, + - $p < 0.01$. Arrows indicate the critical oxygen tension (P_{cO_2}) of the species.

Hughes & Saunders (1970) and Smith & Jones (1982) reported that trout (*Oncorhynchus mykiss*) mainly increases ventilation by adjustments of V_T . In trout only the highest levels of \dot{V}_G correlate with any substantial elevation of f_R . The relative importance of these two components is species-dependent. As an example, frequency adjustments play a larger role in carp, *Cyprinus carpio* (Glass *et al.*, 1990). In any event, the studies indicate that ventilatory responses in fish cannot be evaluated without some measurement of tidal volume.

Previous studies (Rantin *et al.*, 1998) have determined the critical oxygen tension (P_{cO_2}) for *P. mesopotamicus* as 34 mmHg, a high value compared with those presented by typical hypoxia-tolerant tropical teleost. It is important to emphasize that, when subjected to environmental hypoxic conditions, this species become dependent on aquatic surface respiration (ASR), when the fish rise to the surface to continue branchial respiration by positioning their mouths to skim the air/water

interface which is richer in oxygen due to diffusion from the atmosphere (Saint-Paul & Bernardino, 1988). This behavior was also observed in experimental conditions by Rantin *et al.* (1998). These authors observed that the performance of ASR in *P. mesopotamicus* occurs just below the P_{cO_2} when fish was allowed to access the water surface, compensating the high P_{cO_2} value. In the present study, the highest values of \dot{V}_G , f_R and V_T occurred below the P_{cO_2} and the inability to increase these parameters in the more hypoxic tensions coincided with the increased time spent in ASR recorded by Rantin *et al.* (1998).

An evaluation of the ventilatory capacity can be obtained by comparing the tidal volume to the morphometric data for the ventilatory apparatus. The values obtained in the present study show that the maximum responses to hypoxia correlate with V_T that approach the upper limit set by the morphology of the opercular chamber and this probably explains the lack of any increase in V_T

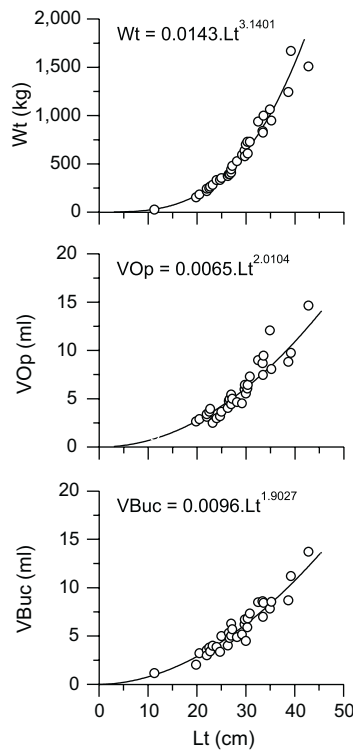


Fig. 2 — The relationships between the total body weight (Wt – upper panel), opercular volume (VOp – middle panel), buccal volume (VBuc – lower panel) and the total body length of *Piaractus mesopotamicus* (n = 34). Regression equations are shown for the respective figures.

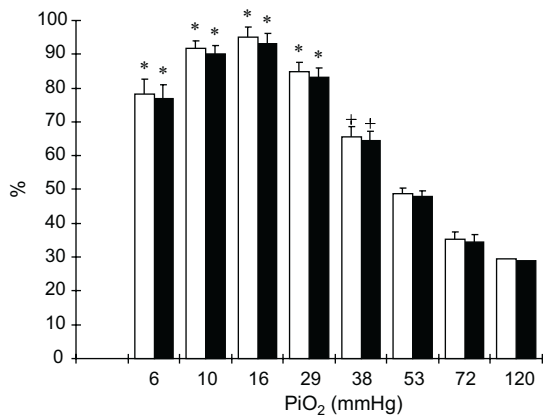


Fig. 3 — Tidal volume in percentage of buccal (■) and opercular (□) volumes as a function of oxygen tensions of inspired water in *Piaractus mesopotamicus* (n = 10). Bars represent mean values ± SE. Significance levels are indicated as follow: * – p < 0.001, + – p < 0.01.

below 16 mmHg. The same pattern of response was described by Kalinin *et al.* (1996) for *H. lacerdae*.

This method for expressing ventilatory responses adds information on the species-specific conditions for responding to hypoxia and on the

adaptation of the species to environments that may be subjected to temporary or long-terms depletion of dissolved O₂. Moreover, the method might explain some responses such as transitions to an increase of respiratory frequency during severe hypoxia.

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