

**THE AEROBIC PHYSIOLOGY OF THE AIR-BREATHING  
BLUE GOURAMI, *TRICHOGASTER TRICHOPTERUS*,  
NECESSITATES BEHAVIOURAL REGULATION OF AEROBIC  
BREATH-HOLD LIMITS DURING HYPOXIC  
STRESS AND PREDATORY CHALLENGE**

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**EXTENDED ABSTRACT ONLY-DO NOT CITE**

Obligate air-breathing fish are frequent inhabitants of extreme hypoxic environments yet often possess reduced haemoglobin-oxygen affinities and low oxygen transport potentials (Graham, 1997). In the presence of a limited oxygen transport system, these fishes may only maintain aerobic metabolism during aquatic hypoxia by increasing their air-breathing frequency and, hence, rate of aerial (vs. aquatic) respiration (Burggren, 1979). Unfortunately for bimodal breathers the risk of predation increases significantly during air-breathing activities (Wolf and Kramer, 1987). Therefore, a selective trade-off exists between minimizing the risk of mortality and satisfying metabolic demands during hypoxic stress and predatory challenge, especially when fish reduce their air-breathing frequency (Wolf and Kramer, 1987). The potential physiological and behavioural compensations that maintain energy flow in the air-breathing

blue gourami, *Trichogaster trichopterus*, during the interaction of aquatic hypoxia and predatory challenge, were the focus of this study.

The haematology (Hct, Hb, MCHC, NTPs), red cell oxygen affinity ( $P_{50}$ , Bohr effect), muscle buffering capacity and lactate dehydrogenase (LDH) activity of *T. trichopterus* was measured to assess the aerobic capacity of this species.

The behavioural and physiological response of sixty fish (5-25 g) to hypoxic stress and predatory stimuli was assessed in an experimental tank (45 x 20 x 35 cm). Water oxygen pressure ( $PO_2$ ) was controlled continuously by a gas-exchanger positioned above the experimental tank.  $PO_2$  was adjusted to either normoxic (mean  $\pm$  SD.  $19.8 \pm 1.2$  kPa), hypoxic ( $6.7 \pm 0.7$  kPa) or essentially anoxic ( $3.2 \pm 0.92$  kPa) conditions by the regulated flow of air and/or  $N_2$  through the exchanger. "Predatory" visual stimuli were presented, from a 30 cm monitor at one end of the tank, to 50 % of the population at each  $PO_2$  ( $n = 10$ ). The "predatory" stimulus was represented by a horizontal, dual eyespot configuration overlaid on a high contrast oval body form with a randomised movement pattern. Observations of air-breathing behaviour (air-breath frequency and air-breath interval) as well as space use were recorded over 30 min with a digital video camera. Patterns of space-use were recorded by monitoring the vertical and horizontal position of fish every 5 s and allowed us to estimate the rate of spontaneous activity (position change  $h^{-1}$ ). Muscle samples were collected upon completion of the 30-min observation period and analysed for muscle lactate and ATP. The effect of aerial nitrogen (vs. oxygen) on muscle metabolites was also assessed as an experimental reference over the three levels of  $PO_2$  and in the presence/absence of visual stimulation. This was achieved by sealing the aerial space of the experimental tank with polyethylene plastic and rapidly replacing the air with nitrogen 1 min prior to the observation period.

The mean haematocrit and haemoglobin concentration of *T. trichopterus* is not indicative of an exceptionally high oxygen carrying capacity. Molar concentrations of ATP were twice those of GTP and the total nucleotide concentration was relatively high. Both the muscle buffering capacity ( $44.0 \pm 1.9$  slykes) and LDH activity ( $154.3 \pm 60.2$  IU  $g^{-1}$ ) was low. Oxygen equilibrium curves at pH 6.6, 7.0, 7.4, 7.8 and 8.2 suggested that *T. trichopterus* has a low oxygen affinity and the pH-dependence of the blood-oxygen affinity index ( $P_{50}$ ) indicated a relatively low Bohr effect ( $\Phi = -0.35$ ).

Although highly dependent on aerobic pathways, *T. trichopterus* was able to reduce air-breathing frequency in response to predatory stimuli at all levels of PO<sub>2</sub>. However, behavioural observations revealed that spontaneous activity was also reduced. Surprisingly, PO<sub>2</sub> was not found to influence either air-breathing frequency or spontaneous activity. An interesting reversal to the effect of PO<sub>2</sub> and visual stimulus on air-breathing frequency was found following the introduction of spontaneous activity as a covariate (i.e. the analysis of metabolic cost functions: log air-breathing frequency/ spontaneous activity). Visual stimuli alone were no longer found to inhibit air-breathing frequency and decreasing PO<sub>2</sub> stimulated air-breathing frequencies. Therefore, once activity was taken into consideration, decreasing PO<sub>2</sub> did indeed stimulate air-breathing frequency and the apparent inhibition of aerial respiration, following visual stimulation, was the direct result of activity down-regulation rather than breath holding behaviour.

Examination of muscle metabolites indicated that behavioural regulation of energetic demands was sufficient to avoid metabolic costs because neither aquatic hypoxia nor predatory challenge influenced the production of lactate or ATP in the trunk musculature when fish were given an unlimited access to atmospheric O<sub>2</sub>. Interestingly, the presence of aerial nitrogen increased lactate production and suggests that substantial aerobic costs are only imposed when access to atmospheric oxygen is denied.

Although key physiological indicators show that the blue gourami, *T. trichopterus*, is highly dependent upon aerobic pathways, behavioural regulation appears sufficient to offset any metabolic cost that would otherwise be expected during a time of aerobic conflict (i.e. hypoxic stress and predatory challenge).

## References

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