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Hypoxia tolerance and partitioning of bimodal respiration in the striped catfish (*Pangasianodon hypophthalmus*)

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ABSTRACT

Air-breathing fish are common in the tropics, and their importance in Asian aquaculture is increasing, but the respiratory physiology of some of the key species such as the striped catfish, *Pangasianodon hypophthalmus* Sauvage 1878 is unstudied. *P. hypophthalmus* is an interesting species as it appears to possess both well-developed gills and a modified swim bladder that functions as an air-breathing organ indicating a high capacity for both aquatic and aerial respiration. Using newly developed bimodal intermittent-closed respirometry, the partitioning of oxygen consumption in normoxia and hypoxia was investigated in *P. hypophthalmus*. In addition the capacity for aquatic breathing was studied through measurements of oxygen consumption when access to air was denied, both in normoxia and hypoxia, and the critical oxygen tension, P_{crit}, was also determined during these experiments. Finally, gill ventilation and air-breathing frequency were measured in a separate experiment with pressure measurements from the buccal cavity. The data showed that *P. hypophthalmus* is able to maintain standard metabolic rate (SMR) through aquatic breathing alone in normoxia, but that air-breathing is important during hypoxia. Gill ventilation was reduced during air-breathing, which occurred at oxygen levels below 8 kPa, coinciding with the measured P_{crit} of 7.7 kPa. The findings in this study indicate that the introduction of aeration into the aquaculture of *P. hypophthalmus* could potentially reduce the need to air-breathe. The possibility of reducing air-breathing frequency may be energetically beneficial for the fish, leaving more of the aerobic scope for growth and other activities, due to the proposed energetic costs of surfacing behavior.

1. Introduction

Air-breathing fish are abundant in tropical waters, where hypoxia is common and where high water temperatures decrease oxygen solubility and increase metabolism (Diaz, 2001; Graham and Wegner, 2010). The striped catfish (*Pangasianodon hypophthalmus*, Sauvage 1878) is a widespread and economically important teleost in southeast Asia that uses a modified swim-bladder for gas exchange (Browman and Kramer, 1985; Danguy and Lenglet, 1988; Podkowa and Goniakowska-Witalinska, 1998). It has been classified as a continuous obligate air-breather (Browman and Kramer, 1985), but the seemingly well-developed gills (personal observation, see Fig. 1) indicate a high capacity for aquatic oxygen uptake. The partitioning of oxygen consumption has, however, not been quantified. *P. hypophthalmus* is migratory in nature (So et al., 2006) and its stream-lined appearance, almost reminiscent of sharks points to an active lifestyle, where a high capacity for aquatic breathing may be beneficial, because frequent surfacing, while increasing the aerobic scope in hypoxic water, also reduces the time available for other activities and increase the risk of aerial predation (Kramer, 1983; 1987).

To study the partitioning of oxygen uptake and the effects of aquatic hypoxia in *P. hypophthalmus*, we developed an intermittent-closed respirometer for simultaneous measurements in both air and water. The relatively short but frequent measurement intervals used in intermittent-closed respirometry make it possible to identify periods of spontaneous activity that must be excluded when determining standard metabolic rate (SMR). Also, intermittent-closed respirometry minimizes problems of waste-product accumulation in the chamber and the necessity to correct for washout time (Steffensen, 1989). The critical oxygen tension (P_{crit}), defined as the oxygen partial pressure of the water (PO_{2w}) where the SMR can no longer be maintained was measured to provide a functional characterization of the capacity for branchial gas exchange. In addition to the metabolic measurements, changes in gill ventilation and air-breathing frequency were measured during exposure to stepwise hypoxia. This was done to investigate whether changes in gill ventilation and the initiation of air-breathing were correlated with P_{crit}. It was hypothesized that gill ventilation would be reduced during air-breathing when PO_{2w} was decreased. Furthermore, the survival and the ability to maintain buoyancy were assessed in animals, both with and without access to air, in order to investigate the importance of air-breathing for survival and in maintaining

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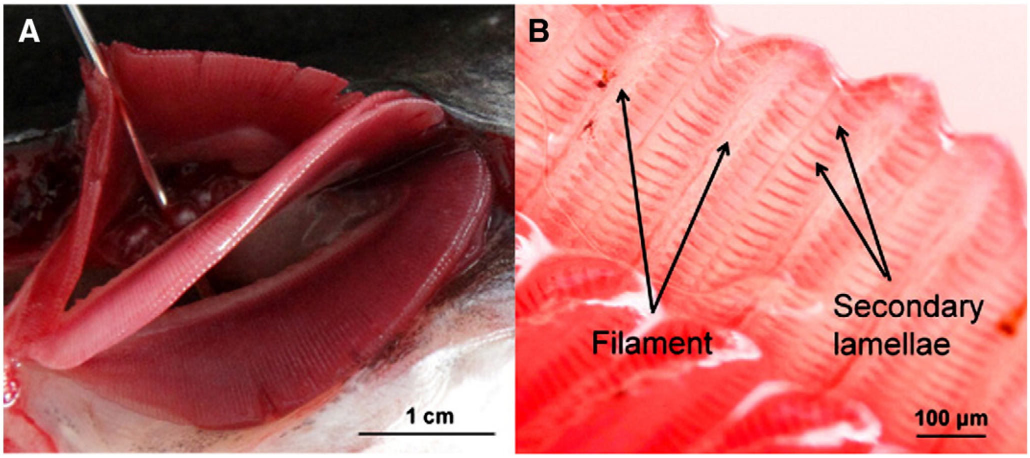

Fig. 1. (A) Whole gills from *P. hypophthalmus*. The individual was 18 cm long and weighed 80 g. None of the gill filaments are reduced, and the filaments appear to be the same length on all 4 gill arches. (B) Close-up of the tip of several gill filaments. This individual was 40 cm long and weighed 1 kg. Filaments and secondary lamellae have been pointed out.

buoyancy. We hypothesized that *P. hypophthalmus* has a high capacity for both aquatic and aerial respiration and an associated low dependence on aerial respiration during aquatic normoxia.

2. Methods and materials

2.1. Animals

Juvenile *Pangasianodon hypophthalmus* were obtained from a local fish farmer in Vietnam and transported to Can Tho University where they were kept at 27 ± 1 ° C in a 500-L tank with continuous aeration (PO_2 approximately 20 kPa). Water was changed every second day to keep levels of NH_3, NO_2^-, and NO_3^- low (0, < 0.2, and < 403 μmol L^{-1}, respectively). Fish were fed commercial floating pellets (30% protein, 5% lipid, 2800 kcal kg^{-1}) to satiation once a day, and uneaten food was removed after 1–2 h. Food was withheld for 2 days before measurements. All experiments were performed in accordance with national guidelines for the protection of animal welfare in Vietnam and the Danish guidelines for animal welfare in LBK726 of 9 September 1993.

2.2. Forced submergence

Forty-five fish (120–180 g) were randomly divided into 3 50-L tanks maintained at 6.1, 12.3, or 20.4 kPa O_2 (27 °C) using an oxygen control system (MPA-48, InsiteIG, CA, USA). Air-breathing was prevented for 24 h by placing a net 5 cm under the surface, and the fish were inspected every 8 h. Forced submergence in normoxic water for 24 h caused no apparent discomfort and the effects of prolonged submergence on survival and buoyancy in normoxic water were therefore investigated. Eighteen fish (120–180 g) were fasted for 3 days and submerged for 6 days in normoxic water as described above. The fish were inspected twice a day, and activity levels and posture were noted to assess buoyancy. Eighteen control fish were kept in a separate 500-L tank with access to air.

2.3. Aquatic oxygen consumption, SMR, and P_{crit}

2.3.1. Intermittent-closed respirometry

Oxygen consumption without access to air (MO_2) was measured using intermittent-closed respirometry for at least 20 h at 27.0 ± 0.1 °C (Steffensen et al., 1984; 1989). The respirometer, which was submerged in water in a large tank (~30 L), consisted of a chamber containing the fish and a closed loop where water passed a galvanic oxygen electrode that continuously measured the oxygen partial pressure of the water (PO_{2w}) within the respirometer. In addition, an open loop could be activated to flush the respirometer with fresh water from the tank containing the system. The opening and closure of the flush pump as well as the logging of the PO_{2w} measurements were automated by a software system (Respirometer 2.0) developed at the Zoophysiology section at Aarhus University. Each individual MO_2 measurement lasted 15 min, where the respirometer was closed for 3 min, so the decline in PO_{2w} could be used to calculate MO_2, followed by 12 min of flush to renew oxygen levels. This setup provided independent measures of MO_2 at 15-min intervals. MO_2 for all points was calculated as:

$$MO_2 = \frac{\left|\frac{\Delta PO_2}{\Delta t}\right| \cdot \beta O_2 \cdot V_{sys}}{M_b}$$

where $\Delta PO_2/\Delta t$ is the rate at which PO_{2w} declined during the closed period, βO_2 is the solubility of oxygen in water at 27 °C (1.617 μmol mm Hg^{-1} L^{-1}), V_{sys} is the volume of water in the system corrected for the volume of the fish (density assumed to be 1 kg L^{-1}), and M_b is body mass. Values for MO_2 were omitted when the R^2 of the linear regression for the decline in PO_{2w} was less than 0.985. At the completion of the experiment, measurements were continued for 0.5–2 h after removing the fish from the respirometer to determine background MO_2. Background MO_2 was subtracted from the measured MO_2, to control for bacterial oxygen uptake (7 ± 4 mg O_2 kg^{-1} h^{-1}). All parts of the respirometer system were carefully cleaned after each experiment and re-filled with chlorine free tap water, and the PO_{2w} was allowed to reach equilibrium before proceeding with the next fish. The O_2 electrode was calibrated before each measurement.

2.3.2. Experimental protocol

Since *P. hypophthalmus* survived forced submergence in normoxic water for 6 days, we measured standard and routine metabolic rates (SMR and RMR, respectively) in 13 fish (136 ± 24 g) denied access to air in normoxic water. SMR was determined as the average of the 5 lowest MO_2 determinations during the 24-h period. The 10% quantile was also used as a measure of SMR for comparison. RMR was calculated as the average MO_2 during the entire measurement period.

In a further 7 fish (117 ± 21 g), we measured SMR and RMR during mild hypoxia (12–13 kPa) since preliminary studies showed that mild hypoxia reduced the variability in MO_2, presumably as a result of reduced spontaneous activity, and therefore allowed for a more reliable determination of SMR. This oxygen level was well above the critical oxygen partial pressure (P_{crit}) that was measured after 8 h of mild hypoxia (12–13 kPa) during preliminary trials. P_{crit} was determined as the PO_{2w}, where MO_2 was reduced below the SMR determined prior to hypoxia (Affonso and Rantin, 2005; Thuy et al., 2010). This method was chosen because fish were seldom at rest when the measurement of P_{crit} was initiated, in which case the point at which SMR cannot be