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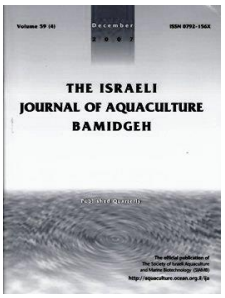

Oxygen Consumption and Lethal Dissolved Oxygen Level of Hybrids of *Siniperca chuatsi*♀ × *S. scherzeri*♂

Jijia Sun[1,2], Jianguo He[1], Guifeng Li[1]*, Li Liu[2], Xiaocui Hou[1], Pengfei Wang[1]

[1] *School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, P.R. China*

[2] *College of Animal Science, South China Agriculture University, Guangzhou 510642, P.R. China*

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Key words: hybrid, *Siniperca chuatsi*, *S. scherzeri*, oxygen consumption rate, lethal dissolved oxygen level, circadian rhythm

Abstract

Oxygen consumption rate (OCR) and lethal dissolved oxygen levels (DO) of the hybrids (225.3 ± 4.6 g) of *Siniperca chuatsi* ♀ × *S. scherzeri* ♂, were determined after acclimating the fish to 15, 20, 26 and 30°C for 20 days. The same parameters were measured for hybrids with different body weight (146.8 ± 6.3 g, 234.3 ± 8.4 g, 273.9 ± 3.3 g, 327 ± 5.1 g) at 26 °C. OCR increased significantly (P <0.05) with increasing acclimation temperature between 20-26°C, and decreased with increasing body weight. Final preferred temperature estimated from the Q_{10} was between 26-30°C. The lethal DO concentration for the hybrids at 30°C was significantly higher (P <0.05) than at other temperatures. OCR over the daily cycle under natural lighting conditions was also determined at 26°C. It showed a circadian rhythm with the lowest point at 0830 and peak rates at 1430. Results show that the hybrid has a higher stress tolerance and higher stocking density than the female parent *S. chuatsi* and indicate their culture potential in subtropical freshwater regions.

* Corresponding author. E-mail: liguif@mail.sysu.edu.cn

Introduction

The measurement of oxygen consumption has been proved to be an efficient method of estimating the metabolic rate of fish, and several different states of respiratory metabolism have been classified for fish as standard, routine, active, and anerobic metabolic rate (Brett et al., 1979; Cech, 1990). Oxygen consumption rate (OCR) of aquatic organisms is one of the physiological responses that correlate with changes in environmental factors, such as temperature, light intensity, dissolved oxygen, and salinity (Salvato et al., 2001). Energy expenditure associated with physiological stress that these factors impose on organisms can also be estimated by measuring OCR (Altinok et al., 2003; Brougher et al., 2005).

Siniperca chuatsi (Basilewsky) and *Siniperca scherzeri* (Steindachner) are the most economically and geographically important sinipercids for aquaculture in China (Zhou et al., 1988). In recent years, studies have shown that the hybrids of *S. chuatsi* ♀ × *S. scherzeri* ♂ combined the rapid growth trait of the female parentand the body surface coloration of the male parent (Wang et al., 2013). Morphological analysis (Mi et al., 2010) and DNA marker (SSR and SNP) studies have been carried out on this hybrid (Wang et al., 2013; Qu et al., 2013; Huang et al., 2013). However, the effects of hybridization on oxygen consumption of this hybrid have not been reported.

In this laboratory study, the effects of temperature and body weight on oxygen consumption of the hybrid were investigated, and the circadian rhythm of OCR and the lethal (DO) levels were measured.

Materials and Methods

Fish. The hybrids of *Siniperca chuatsi* ♀ × *S. scherzeri* ♂ were obtained from Shengsheng Fisheries Ltd. at Foshan in Guangdong Province, China. In the laboratory, 60 fish with wet body weight ranging from 150 to 350 g were placed in a reservoir with constant aeration. Hybrids were starved for 24h before commencing the experiment to reduce associated metabolic responses (Nelson et al., 1985; Beamish et al., 1990).

Experimental facilities. Oxygen consumption rate of the hybrids was determined using a flow-through respirometer (Fig. 1). Hybrids were kept in the respiratory chamber (250×250×300 mm), which was immersed in a 300 L water bath (1500×550×350 mm) for temperature control. The constant temperature treatments of the respirometer were achieved by a mixed temperature control system, which included a thermostat controlling the on/off switch of a 1000 W electric heater, and a manual controller.

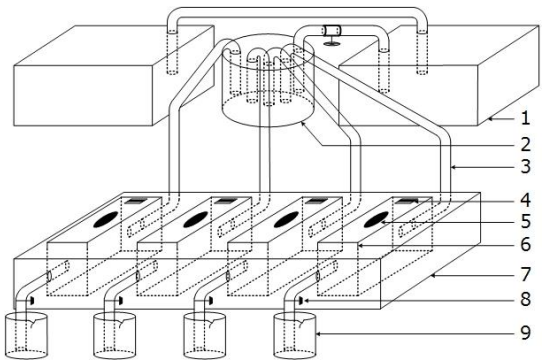

Fig. 1. Diagram of a flow-through respirometer: 1.water tank; 2.water level controller; 3.siphon tube; 4.thermometer; 5.rubber plug; 6.respiratory chamber; 7.water bath; 8.control valve; 9.Sampling vessel.

Determination of oxygen consumption rates. Two OCR measurement experiments were implemented. (1) OCR of hybrids was measured at four acclimation temperatures (15, 20, 26 and 30°C). The mean wet body weight of the fish was 225.3 ± 4.6 g and there were no significant differences in wet weights among the treatments ($P > 0.05$). Fish were maintained at predetermined temperatures for 20 days to ensure full thermal adaptation. Prior to the determination of oxygen consumption, water flow was controlled at 6.0 L/h. The oxygen content of outflow water was ≥ 5 mg/L. All four sides of the chamber were covered with opaque screens to minimize photoperiodic effects on the experimental fishes (fish?). When fish became quiescent after 2 h, oxygen consumption was determined over a period of 6 h and water in the chamber was siphoned off every 2h. Eight fish from each acclimation temperature were introduced one at a time into the chamber. For each treatment, there was a blank control to correct for the respiration of

bacteria in the water. Measurements were made between 0800 and 1200 hours. Oxygen content of water samples was determined using the Winkler method (Strickland et al., 1968). The OCR was calculated using the following formula:

$$OCR\ (mg\ O_2\ /\ kg/h) = (DO_1 - DO_2) \times V/W$$

(1) where DO_1 is the concentration of oxygen (mg O_2/L) in the chamber without fish, DO_2 is the concentration of oxygen in chamber with fish, *V* is the water flow rate (L/h), *W* is the wet body weight of hybrid (Kg).

The temperature coefficients (Q_{10}) were calculated to assess the effect of temperature acclimation on OCR by using the formula (Schmidt-Nielsen, 1997):

$$Q_{10} = \left(Rate_2 / Rate_1\right)^{10/(Temp_2 - Temp_1)}$$

(2) OCRs of four different body weight groups (146.8 ± 6.3 g, 234.3 ± 8.4 g, 273.9 ± 3.3 g, and 327 ± 5.1 g) were measured at 26°C. Six fish from each weight group were individually introduced into the chamber. The procedure followed was then identical to that of the acclimation temperatures.

Measurement of lethal dissolved oxygen (DO) concentration. After the oxygen consumption measurements, the water flow was shut off and the test fish were kept in the sealed respiratory chamber. To determine the lethal DO level, the DO concentration in the chamber was measured for a final time just after the death of each fish. After each lethal DO level was measured, the body weight of dead fish was measured immediately.

Circadian rhythm in the oxygen consumption. After removal of the opaque screens, the chamber was exposed to a natural photoperiod. In this test, the mean temperature was 26 ± 1°C and the body weight of the fish used was 136.8 g, 168.2 g, 203.7 g, and 285.4 g, respectively. Measurements of OCR were made, every 2 h during the 24 period. light period from 0630 to 1630 hours, and dark period was from 1830 to 0430 hours.

Statistical analysis. The data were analyzed by SPSS for Windows (Version 17.0) and expressed as mean±SE. Inter-treatment differences for OCR and lethal DO concentration were analyzed with one-way ANOVA followed by least significant difference (LSD) multiple range tests. Differences were considered statistically significant when $P < 0.05$.

Results

The OCR of the hybrids varied with the acclimation temperature (Fig. 2). The OCR changed significantly ($P < 0.05$) as the acclimation temperature increased from 15°C-26°C, first decreasing to a minimum rate of 92 mg O_2//Kg/h at 20°C and then reaching a peak rate of 136.8 mg O_2/Kg/h at 26°C. However, no significant change ($P > 0.05$) in the OCR was observed between 26°C-30°C. Maximum and minimum temperature quotients Q_{10} were observed between 20°C-26°C and 15°C-20°C as 1.94 and 0.65, respectively. There was a drop in the Q_{10} when the acclimation temperature increased from 26°C-30°C. The OCR decreased with increasing body weight (Fig. 3), and the OCR at mean body weight of 327.0 ± 5.1 g was significantly lower than the others ($P < 0.05$).

The effects of acclimation temperature and body weight on the lethal DO concentration are showed in Fig. 4. Among the four experimental temperature treatments, lethal DO levels increased with increasing temperature. The lethal DO concentration reached a maximum of 1.0648 ± 0.1244 mg/L at 30°C, and this value was significantly higher than at other temperatures ($P < 0.05$) [Fig. 4(a)]. Lethal DO levels decreased in relation to body weight increase, except for a significant increase from 273.9 ± 3.3 g to 327.0 ± 5.1 g ($P < 0.05$) [Fig. 4(b)].

The circadian rhythms in the OCRs of the four different body weight hybrid groups are similar (Fig. 5). The lowest point in OCR was recorded at 0830 hours (the mean OCR 32.1 mgO_2/Kg/h), and the peak rate in OCR at 1430 hours (the mean OCR 178.1 mg O_2/Kg/h). The mean OCRs during the day (148.0, 132.6, 147.3 and 109.9 mg O_2/Kg/h for body weight 136.8, 168.2, 203.7 and 285.4 g respectively) were lower than at night (181.9, 141.1, 163.5 and 125.0 mg O_2/Kg/h), which show a significant circadian rhythm.

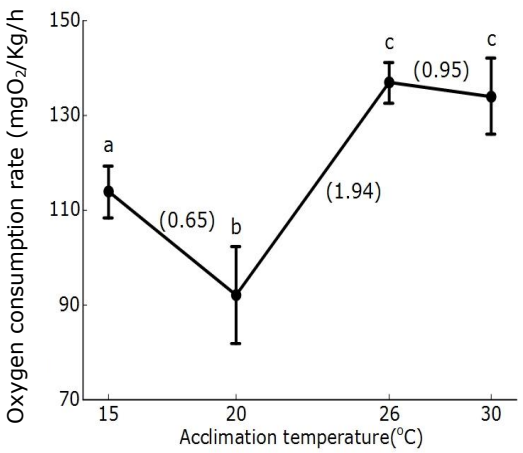

Fig. 2. OCR of hybrids acclimated to 15, 20, 26 and 30°C. Values are expressed as mean ± SE. Different letters on the value indicate significant difference (P < 0.05). Numbers in parenthesis are Q_{10} between acclimation temperatures (15-20, 20-26 and 26-30°C).

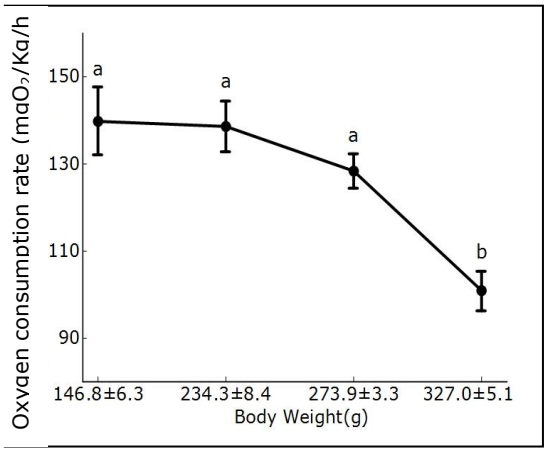

Fig. 3. OCR of hybrids with different body weight. Values are expressed as mean ± SE. Different letters on the value indicate significant difference (P < 0.05).

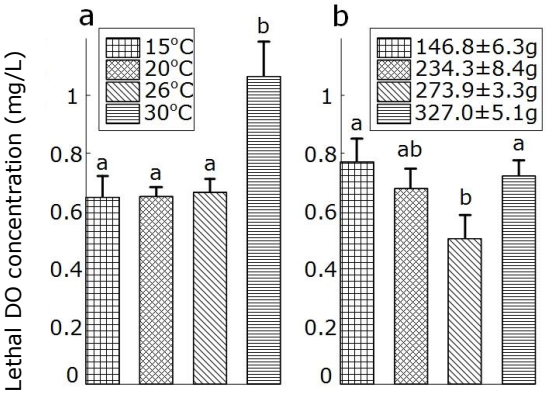

Fig. 4. (a) Lethal DO concentration at different temperature; (b) Lethal DO concentration at different body weight. Different letters over the value indicate significant difference (P < 0.05).

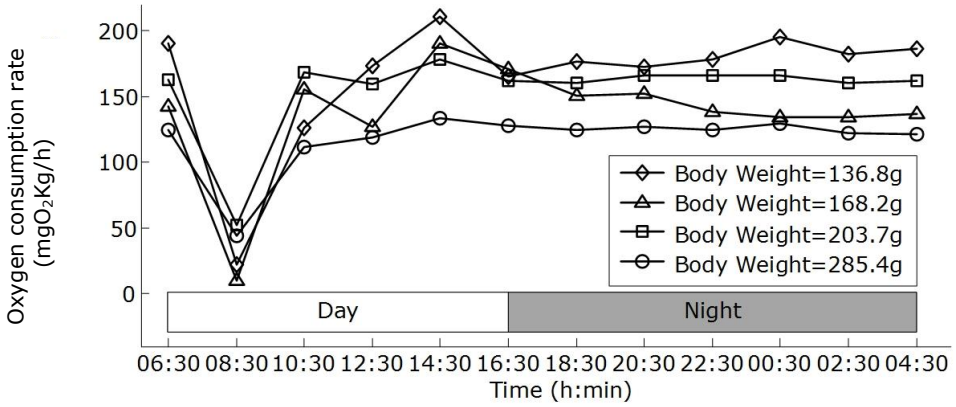

Fig. 5. The circadian rhythm in the OCRs of the four different body weight hybrids

Discussion

In poikilothermic animals, oxygen consumption is often used as an indirect measure of metabolism and is strongly dependent on acclimation temperature (Kita et al., 1996). For many fish, such as *Lepomis macrochirus* and *L. megalotis* (Dent et al., 2003), advanced fingerlings of Indian Major Carps (*Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*) (Das et al., 2004), *Pangasius pangasius* (Debnath et al., 2006), and *Micropterus salmoides* juveniles (Díaz et al., 2007), a linear correlation has been reported between oxygen consumption and acclimation temperature. However, some researchers have suggested that metabolic rates of some fish are negatively related to acclimation temperature (Brown et al., 1989; Walsh et al., 1997).

In the present study, OCR of hybrids showed positive temperature dependence of metabolism from 20°C-26°C and negative temperature dependence from 15°C-20°C and 26°C-30°C. Compared with the OCRs of *Siniperca chuatsi* (average body weight 230±11.79 g) at 20°C (139.0 mgO_2/Kg/h), 26°C (173.7 mgO_2/Kg/h) and 30°C (174.1 mgO_2/Kg/h) (Si et al., 1995), the hybrids had a lower OCR level, and the mean OCR decrease was 41.4 mg O_2/Kg/h. This indicates that the hybrids have a stronger stress tolerance than their parents and could be reared at higher stocking densities than *S. chuatsi*. However, the OCR of the hybrids at 15°C was significantly higher than at 20°C and higher than that of *S. chuatsi*. It seems likely that metabolism of hybrids may be more efficient at lower temperatures than *S. chuatsi*.

A freshwater teleost demonstrates physiological plasticity when it is able to regain or approach its metabolic set-point (e.g. quantitative or qualitative changes in enzyme expression) within the context of thermally fluctuating environments (Dent et al., 2003). This relationship predicts the point where the Q_{10} for oxygen consumption starts to decrease with increasing acclimation temperatures that corresponds to their optimal temperature for growth (Kita et al., 1996). In our study, the optimal temperature for hybrids ranged from 26°C-30°C.

Our study found the OCRs of hybrids decreased with increasing body weight. This negative correlation of OCR and weight was also seen in studies on *S. chuatsi* (Si et al., 1995). Furthermore, the lethal DO levels and the circadian rhythm of the hybrids were similar to those of *S. chuatsi* (Si et al., 1995). These results demonstrated that hybrids had similar respiratory physiological characteristics to their female parent. However, our study also demonstrated that the hybrids had some distinctive oxygen consumption characteristics. The lethal DO concentration for hybrids at 30°C was significantly higher than at other temperatures and nearly 1.7 times higher than that of *S. chuatsi* (Si et al., 1995). The lowest levels in OCR of hybrids occurred at 0830 and 1430, while lowest levels for *S. chuatsi* (female parent) and *S. scherzeri* (male parent) occurred at 0600 and 1200 and 0700 and 1200 (Si et al., 1995; Peng et al., 2005), respectively.

In conclusion, the present study showed that the hybrids had higher stress tolerance and higher stocking density than their female parent *S. chuatsi* from 20°C-30°C. In addition, the final preferred temperature (26°C-30°C) of hybrids based on the Q_{10} value indicates higher culture potential in subtropical freshwater regions. Future researches on various biochemical changes of hybridization, such as metabolic enzymes and heat shock protein, may aid in the understanding of differences in metabolic mechanisms of the hybrid.

Acknowledgements

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