Aquaculture 319 (2011) 178–183

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

The measurements of filtering parameters under breathing and feeding of filter-feeding silver carp (*Hypophthalmichthys molitrix* Val.)

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ARTICLE INFO

Article history:
Received 8 May 2010
Received in revised form 29 May 2011
Accepted 13 June 2011
Available online 2 July 2011

Keywords:
Silver carp
Hypophthalmichthys molitrix
Respiration
Feeding
Dissolved oxygen

ABSTRACT

As a typical filter-feeding fish, breathing process of silver carp is combined with its feeding process when it filters plankton in water. The present experiments studied the relationships between breathing and feeding of silver carp in response to the reduction of dissolved oxygen (DO) levels. The results showed that (1) in the clean water without phytoplankton, normal DO levels (5.43–7.73 mg/L) did not affect significantly the respiration of silver carp in terms of respiratory frequency (f_R), oxygen consumption rate (VO_2), respiratory stroke volume ($V_{S.R}$), gill ventilation (V_G) and oxygen extraction efficiency (EO_2); while DO level declined to 4.40 mg/L f_R, $V_{S.R}$ and V_G increased significantly and EO_2 decreases significantly; The VO_2 of silver carp reached the peak when DO levels declined to 2.21 mg/L; The critical oxygen level of silver carp was about 1 mg/L, at which the EO_2 of fish was substantially decreased to the lowest value. (2) f_R, $V_{S.R}$, V_G and VO_2 of silver carp under feeding condition were significantly higher than those in clean water at oxygen levels ranging from 3.37 to 7.73 mg/L; While the EO_2 of the fish under feeding condition was lower significantly than that in clean water; $V_{S.R}$, V_G and V_G/VO_2 of silver carp under feeding condition increased significantly when DO levels declined to 2.21 mg/L. In summary, silver carp fed actively and respired passively when DO levels were above 3 mg/L, i.e., it showed higher V_G, higher FR and stable VO_2. While silver carp fed passively and respired actively when DO were below 3 mg/L, i.e., it showed sharply increased V_G, decreased filtering efficiency (E) and anti-filtering response.

1. Introduction

Dissolved oxygen (DO) is one of the most important environmental factors for various fish species. Those species of which oxygen consumption rate is directly related to DO levels are denominated "conformers" (Shelton, 1970). In response to the decline in DO level, fish show active regulatory mechanism to adapt to the hypoxic conditions. The compensatory mechanisms are different from species to species. Above critical oxygen level, the so-called "regulators" respond to the decrease in DO levels by simultaneously changing both respiratory frequency and respiratory stroke volume so as to maintain the constant oxygen consumption rate regardless of the fluctuation in the DO concentration (Glass et al., 1990; Jensen et al., 1994; Lomholt and Johansen, 1979; Seibel and Childress, 2000).

Generally, oxygen is taken up by fish through breathing movement and the contractions of respiratory muscles consume energy during the process of breathing movement (Alexander, 1970; Fernandes et al., 1995; Lomholt and Johansen, 1979; Rantin et al., 1992; Rantin and Johansen 1984). The cost of breathing for ventilation was estimated in the range of 3–10% of the resting oxygen consumption at normoxia (Farrell and Steffensen, 1987; Rantin et al., 1992). However, this cost might increase up to 50% in hypoxia (Hughes and Saunders, 1970). In addition, feeding process of fish also needs energy, and the oxygen consumption rate increased in the feeding process correspondingly (Jobling, 1981). The breathing process of most fish species is independent of their feeding process; however, the breathing process of filter-feeding fish is combined with their feeding process. In the breathing–feeding process while the filter-feeding fish pumps water through gill cavities, it filters plankton in the water by gill rakers and takes up oxygen by gill filaments simultaneously.

Silver carp (*Hypophthalmichthys molitrix* Val.) as a typical filter-feeding fish (Dong et al., 1992) has drawn much attention worldwide because of its importance as an aquaculture species and its potential for biomanipulation of plankton communities (Dunseth, 1977; Fukushima et al., 1999; Ke et al., 2007; Smith, 1985; Starling, 1993; Starling and Rocha, 1990; Tucker, 2006; Xie and Liu, 2001; Yan et al., 2009). DO contents in silver carp culture ponds and lakes undergo considerable fluctuation daily due to the photosynthesis and respiration of plankton. Therefore, there is an important significance to clarify the relationships between breathing and feeding of silver carp under low DO contents.

Dong et al. (1992) and Dong and Li (1994a, b) have studied the feeding parameters of silver carp, such as respiratory frequency (filtering frequency), suction volume, removal rate and filtering

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doi:10.1016/j.aquaculture.2011.06.020

efficiency to different sizes of food particles under static water condition. Turker et al. (2003) reported the filtration rate of silver carp to phytoplankton in the Partitioned Aquaculture System. In order to examine our hypothesis that there is a strong interaction between breathing and feeding under low DO contents in filtering fish species some biological parameters of breathing and feeding for silver carp were studied and compared in the waters with and without phytoplankton under conditions of various DO levels.

2. Materials and methods

2.1. Experimental fish and phytoplankton

Silver carp with body weight 83.6 ± 6.8 g and standard length 18.7 ± 0.3 cm (mean ± SD), were obtained from Pingdu Fish Breeding Farm, Qingdao, P. R. China. Fish were kept in an outdoor pond (15 m × 20 m × 1.2 m) at least 1 month prior to the experiment. During this period, silver carp filtered natural plankton (the main species were the Chlorophyta; biomass = 10–50 mg/L) in the pond. Water temperature was 22 ± 3 °C, DO was above 5 mg/L, and pH value was about 7.6.

Then fish in apparent good health were collected from the outdoor pond, and were transferred into indoor circular tanks (60 cm in diameter), acclimated for 1 week. During the whole acclimation period, aeration was provided continuously, 90% water (dechlorinated tap water) in the tank was replaced every day, and mixed phytoplankton (biomass = 10 mg/L in the tank) was provided twice every day at 0900 and 1700, respectively. Water temperature was kept at 21 ± 1 °C, DO was maintained above 7 mg/L, and the photoperiod was 12L:12D.

Experimental phytoplankton *Padorina morum* (particle diameter = 20.1 ± 4.6 μm) was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, P. R. China. Phytoplankton was cultured in SE medium. Phytoplankton solution of *P. morum* was filtered through the funken (mesh diameter 9.8 μm) in order to avoid toxicity of the culture medium to the experimental fish. The filtered phytoplankton particles were resuspended in the dechlorinated tap water for later use.

2.2. Experimental methods

Food was suspended 48 h after acclimation, and fish was transferred to an individual perspex flow-through respiratory chamber (according to Dong et al., 1992 revised) for parameter measurements. The fish was acclimatized in the respiratory chamber for 5–6 h before measurements, until the respiratory frequency fell to resting level and apparently stable. During the acclimation period, clean water without phytoplankton flowed through the respiratory chamber continuously with about 9 L/h of flow rate. The differences between the DO concentrations of the inlet and outlet water of the respiratory chamber were kept at about 10–15% (Hughes et al., 1983).

The DO concentrations of the inlet ($C_{in}O_2$ = mg/L) and outlet ($C_{out}O_2$ = mg/L) water of the respiratory chamber were recorded. Similar to the dye dilution technique developed by Jones et al. (1990) and Bushnell et al. (1990), the DO concentrations of inspired (C_iO_2 = mg/L) and expired (C_eO_2 = mg/L) water, which were obtained by fixing polyethylene catheters (3 mm in inner diameter) anterior to the mouth of the fish and the outlet of opercular cavity of the fish, were continuously measured by siphoning the water via polyethylene catheters. The 9 L/h of flow rate flowed through the respiratory chamber was controlled strictly during measurements, and each fish in clean group and feeding group always underwent the same experimental sequence. The respiratory chamber was covered with black cloth to avoid any visual disturbance to the fish during the measurements.

Fish were sequentially exposed to decreasing DO levels of 7.73 mg/L (normoxia), 6.31 mg/L, 5.43 mg/L, 4.40 mg/L, 3.37 mg/L, 2.21 mg/L and 0.97 mg/L obtained by bubbling pure nitrogen, and kept stable at different levels for 60 min before the measurements.

There were 12 replicates in both clean water experiment and feeding experiment. The experimental water with and without phytoplankton *P. morum* (biomass = 20 mg/L) were used, respectively. The water temperature throughout the measurement was kept at 21 ± 1 °C.

DO concentrations were measured by the YSI BOD probe (Model 5010) connected to an YSI oxygen meter (Model 5000-230V, YSI Incorporated, Yellow Springs, OH, USA). The phytoplankton particle concentrations of the inlet and outlet water of the respiratory chamber were counted under a microscope, and the biomass was estimated from approximate geometric volumes of *P. morum*.

2.3. Parameter measurements

Oxygen consumption rate was measured by means of a flow-through respirometry system, concomitantly with the determination of gill ventilation, respiratory stroke volume and dissolved oxygen extraction from water according to the modification by Fernandes and Rantin (1989). The oxygen consumption rate (VO_2 = mg/kg/h) was calculated as:

$$VO_2 = V_R(C_{in}O_2 - C_{out}O_2) / W_t,$$

where V_R was velocity of flow through the respiratory chamber (L/h), W_t was body weight of the fish (kg).

Gill ventilation (V_G = L/kg/min) and oxygen extraction efficiency (EO_2 = %) were calculated as:

$$V_G = VO_2 / 60\,(C_iO_2 - C_eO_2)$$

$$EO_2 = 100\,(C_iO_2 - C_eO_2) / C_iO_2.$$

The respiratory stroke volume ($V_{S.R}$ = mL/kg/breath) was calculated as:

$$V_{S.R} = 1000V_G / f_R,$$

where f_R was respiratory frequency or filtering frequency (breaths/min), which was measured by counting the times of buccal movements of the fish during 5–8 min at each designed DO level. The water convection requirement or ventilatory requirement was the ratio V_G/VO_2 (LH_2O/mgO_2) (Dejours, 1981).

Filtration rate for phytoplankton (FR = mg/kg/h); clearance rate (CR = L/kg/h) is defined as the volume of water cleared of suspended particles per unit time; filtering efficiency (E = %) is defined as the percentage of filtered food particles to total food particles in water, calculated as:

$$FR = V_R(C_{in} - C_{out}) / W_t \quad \text{(Turker et al., 2003)}$$

$$CR = V_R(C_{in} - C_{out}) / C_{in}W_t \quad \text{(Savina and Pouvreau, 2004)}$$

$$E = 100CR / V_G,$$

where C_{in} (mg/L) and C_{out} (mg/L) were the phytoplankton particles concentrations of the inlet and outlet water of the respiratory chamber, respectively.

2.4. Statistical analysis

Statistical analysis was undertaken using SPSS 17.0 for Windows. To analyze possible differences among DO levels, one-way ANOVA