Aquaculture 479 (2017) 479–486

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Gill remodeling in response to hypoxia and temperature occurs in the hypoxia sensitive blunt snout bream (*Megalobrama amblycephala*)

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ARTICLE INFO

Keywords:
Megalobrama amblycephala
Gill
Hypoxia
Temperature
Lamella
Gill remodeling

ABSTRACT

In this study, we found that blunt snout bream (*Megalobrama amblycephala*) had a relatively high critical oxygen tension at which it lost its equilibrium (LOE_{crit}), confirming that it is a hypoxia sensitive fish. Blunt snout bream has the ability to remodel its gill structure in response to oxygen levels. When blunt snout bream were exposed to 4- or 7-days of hypoxia, the average protruding lamella heights and mean lamellar area of gills were significantly ($P < 0.01$) larger than those of normoxic controls. These changes resulted in reduced average interlamellar cell mass (ILCM) height and volume under hypoxia. After 1 week of normoxic recovery, gill lamellae were reversibly embedded with ILCM cells. Irrespective of dissolved oxygen concentration, the average protruding lamella height of fish gills at 25 °C was significantly ($P < 0.01$) larger than that of fish cultured at 10 °C, suggesting that blunt snout bream also have the ability to modify gill structure in response to water temperature. In response to hypoxia, blood erythrocyte count and haemoglobin (Hb) concentration increased significantly ($P < 0.01$) under hypoxia. Furthermore, the plasma chloride concentration ($[Cl^-]$) was significantly ($P < 0.01$) reduced. Our results are the first to show that blunt snout bream, as a hypoxia sensitive fish, have the capacity to alter respiratory surface area in response to hypoxia and temperature.

1. Introduction

Fish are often challenged to survive in variable environments, with fluctuating dissolved oxygen (O_2) concentrations (Yamanaka et al., 2007; Fu et al., 2014). The gills of many fish species are capable of extensive remodeling in response to changes in O_2 (Matey et al., 2008; Dhillon et al., 2013; Dabruzzi and Bennett, 2014). Under hypoxic conditions, both crucian carp (*Carassius carassius*) and goldfish (*C. auratus*) respond by reducing the size of their interlamellar cell mass (ILCM), exposing the lamella and increasing the functional surface area of the gill (Sollid et al., 2003; Mitrovic et al., 2009). Gill remodeling in crucian carp is also found after a sustainable swimming experience (Brauner et al., 2011). This hypoxia-induced gill remodeling is fully reversible as the lamellae become embedded again if the fish is returned to normoxic water (Sollid and Nilsson, 2006). These strategies serve to either enhance O_2 uptake from the O_2-depleted water or prolong survival when environmental O_2 tensions are below levels where routine metabolic rate can be maintained (Nilsson, 2007).

Compared to typical hypoxia-tolerant cyprinid species such as crucian carp and goldfish, several freshwater bream species including blunt snout bream (*Megalobrama amblycephala*), thick jaw bream (*M. pellegrini*) and Chinese bream (*Parabramis pekinensis*) are generally considered to be hypoxia-sensitive fish species. The oxygen tension threshold for loss of equilibrium (LOE_{crit}), which represents the oxygen tension at which the fish can no longer maintain body balance due to systemic disorganization, is considered as an ecological index of lethality (Coutant et al., 1969; Chapman et al., 1995; Currie et al., 2004). Both Chinese bream and thick jaw bream exhibited significantly higher LOE_{crit} than crucian carp, suggesting that they are hypoxia-sensitive species (Dhillon et al., 2013; He et al., 2015). Additionally, gill remodeling in response to changing respiratory requirements could be an ancient mechanism occurring in many teleosts (Nilsson, 2007). However, a recent study showed that thick jaw bream has lost the ability to increase mass-specific gill surface area through reductions in the ILCM volume under hypoxia (Dhillon et al., 2013).

The blunt snout bream is a herbivorous fish, and is widely favored as a delicacy (Li et al., 1993). Since 1960, it has been accepted as a principal species in the Chinese freshwater fish polyculture systems (Ke, 1965). In 2015, aquaculture production of blunt snout bream totaled > 700,000 tons (FBMA, 2016). Blunt snout bream is also a

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http://dx.doi.org/10.1016/j.aquaculture.2017.06.020
Received 18 April 2017; Received in revised form 14 June 2017; Accepted 15 June 2017
Available online 16 June 2017

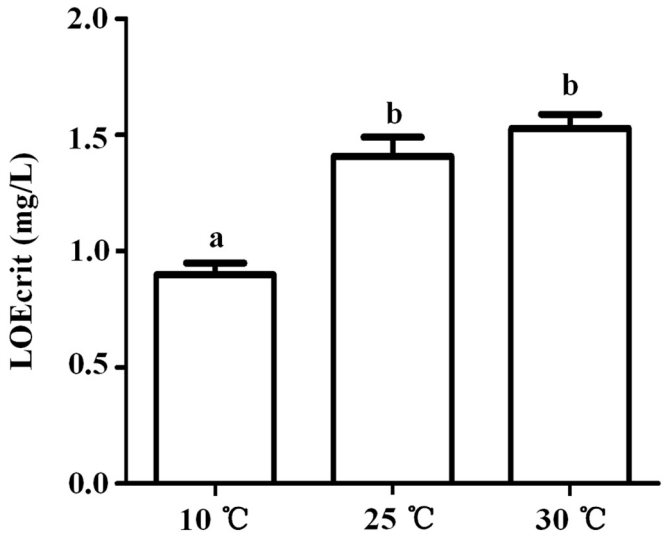

Fig. 1. The LOE_{crit} at which blunt snout bream lost equilibrium. Data are represented as mean ± SE for separate fish (n = 15). Letters that differ indicate statistically significant differences at 10 °C, 25 °C, 30 °C ($P < 0.01$).

hypoxia-sensitive fish species. It exhibits rapid mortality (< 2 h) under acute hypoxia (< 0.5 mg·L^{-1} O_2) (Shen et al., 2010; Tian et al., 2014; Li et al., 2015). The present study was conducted in order to determine whether, like its hypoxia-sensitive relative the thick jaw bream, the blunt snout bream has lost the ability to remodel its gills under hypoxia.

2. Materials and methods

2.1. Experimental fish

'Pujiang No.1' strain blunt snout bream were obtained from the Bream Genetics and Breeding Center (BGBC) of Shanghai Ocean University. One year old juvenile fish were transported to the laboratory, where they were maintained in aquaria supplied with circulating dechlorinated tapwater, and fed daily with commercial food (Ningbo Tech-Bank, Ningbo, China). The photoperiod was kept constant at light (6 am to 18 pm): dark (18 pm to 6 am). Fish were held at water temperatures of 10 ± 0.1 °C, 25 ± 0.1 °C and 30 ± 0.1 °C for 8 d, and exhibited normal feeding behavior over the acclimation period.

2.2. Oxygen tension threshold for LOE (LOE_{crit})

Fifteen juvenile fish (~30 g) were transferred from the holding tank to 20-L glass tanks and left to acclimate overnight at temperatures of 10 (DO = 11 ± 0.5 mg·L^{-1}), 25 (DO = 8.5 ± 0.5 mg·L^{-1})and 30 °C (DO = 7.9 ± 0.5 mg·L^{-1}) flow-through water. At the start of the experiment, a mesh screen was placed 5 cm below the water surface to prevent the fish from accessing the air-water interface. Then nitrogen gas was introduced to the tank by a gas disc connected with the nitrogen cylinder to rapidly decrease $[O_2]$ from normoxia to 6 mg·L^{-1}. After this point, the $[O_2]$ was held at 6 mg·L^{-1} for 1 h, then decreased to 3 mg·L^{-1} over 30 min, held at 3 mg·L^{-1} for 30 min, and then decreased to 1.5 mg·L^{-1} over 30 min, and held at this new $[O_2]$ for an additional 30 min. Subsequently, the $[O_2]$ was decreased in a similar stepwise manner (decreased over 30 min and then held at the new level for 30 min) in increments of 0.5 mg·L^{-1}, to a final level of 0 mg·L^{-1}. An oxygen electrode (YSI, ProODO, Germany) was used to monitor the oxygen level. When a fish showed LOE, the $[O_2]$ and time were recorded. The LOE_{crit} was calculated for individual fish using Brett's (1964) equation:

$$LOE_{crit} = [O_2]_{2i} - \left(\frac{t_i}{t_{ii}}\right)[O_2]_{2ii}$$

where $[O_2]_{2i}$ is the lowest level of oxygen at which the fish could maintain equilibrium for the full duration; $[O_2]_{2ii}$ is the decrease in oxygen tension at each increment (0.5 mg·L^{-1}), t_i is the time required for the fish to lose equilibrium at the final $[O_2]_2$, and t_{ii} is the time held at each $[O_2]_2$. The process was repeated three times at each temperature.

2.3. Hypoxic treatment

Twenty juvenile fish (~30 g) were kept in 20-litre glass tanks of dechlorinated tapwater bubbled with nitrogen gas (N_2). An oxygen electrode (YSI, ProODO, Germany) was used to monitor $[O_2]$ and control the rate of N_2 bubbling to achieve a $[O_2]$ of 2.0 mg·L^{-1}. The experimental fish were exposed to hypoxia for up to 7 days at temperatures of 10 and 25 °C. Five fish were sampled from each treatment at 0, 4 and 7 days of exposure. Thereafter, remaining fish were returned to normoxia for one week. The control groups at 10 and 25 °C in normoxic flow-through water.

2.4. Light microscopy (LM) and scanning electron microscopy (SEM)

Fish were rapidly euthanized (< 1 min) with 0.5 g·L^{-1} tricaine methanesulfonate (MS 222). For histology, the 3rd gill arches were removed from the left side of each fish and the 5 filaments selected for analysis (Bowden et al., 2014). Gill tissues were fixed in Bouin's solution for 24 h and then transferred to 70% ethanol. After dehydration in a graded ethanol series to absolute ethanol, samples were embedded in paraffin. The paraffin-embedded tissues were sectioned at 5 μm-thick using a microtome (Leica, RM2125RT, Germany). Slices were then routinely stained with HE and microphotographs were taken under a

Table 1
Morphometric characteristics of the blunt snout bream gills under hypoxia conditions (DO = 2.0 mg·L^{-1}).

	Gill morphometry					
	10 °C			25 °C		
	Normoxia	4 days of hypoxia	7 days of hypoxia	Normoxia	4 days of hypoxia	7 days of hypoxia
Protruding lamella height (μm)	19.4 ± 0.8	32.4 ± 2.8*	41.7 ± 1.7*	26.1 ± 0.3	38.1 ± 1.5*	56.4 ± 1.3*
Protruding lamella basal length (μm)	126.7 ± 8.5	131.2 ± 4.2	137.2 ± 7.1*	133.7 ± 6.4	137.2 ± 5.2	149.2 ± 6.3*
Protruding lamella thickness	9.6 ± 0.2	9.7 ± 0.1	9.5 ± 0.3	9.4 ± 0.3	9.8 ± 0.1	9.6 ± 0.2
Distance between lamella	28.4 ± 0.2	28.6 ± 0.3	28.4 ± 0.2	28.7 ± 0.1	27.6 ± 0.3	28.5 ± 0.2
ILCM height (μm)	38.4 ± 0.2	28.1 ± 1.4*	20.7 ± 0.5*	30.4 ± 0.4	22.5 ± 0.3*	15.6 ± 0.4*

Note: ILCM = interlamellar cell mass. Values are means ± SE, n = 15.
* $P < 0.01$.