Chemosphere 184 (2017) 907–915

Contents lists available at ScienceDirect

Chemosphere

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

Effects of multi-environmental factors on physiological and biochemical responses of large yellow croaker, *Larimichthys crocea*

Qian-Feng Wang [a, b], Wei-Liang Shen [b], Cheng Liu [a, b], Dan-Li Mu [a], Xiong-Fei Wu [b], Nian-Gang Guo [b], Jun-Quan Zhu [a, *]

[a] Key Laboratory of Applied Marine Biotechnology of Ministry of Education, College of Marine Sciences, Ningbo University, Ningbo, Zhejiang, 315211, China
[b] Ningbo Academy of Oceanology and Fishery, Zhejiang, 315012, China

HIGHLIGHTS

- This page studied effects of multi-environment factors on physiological and biochemical parameters of *Larimichthys crocea.*
- Oxygen consumption and ammonium excretion rates increased first and then decreased with elevated temperature and salinity.
- The activities of ATPase and SDH in myocardium decreased at first and then increased under hypoxia.
- The LDH activity and HIF-1α mRNA expression in myocardium increased at first and then decreased under hypoxia.

ARTICLE INFO

Article history:
Received 6 May 2017
Received in revised form
7 June 2017
Accepted 12 June 2017
Available online 13 June 2017

Handling Editor: David Volz

Keywords:
Temperature
Salinity
Oxygen consumption and ammonium excretion
Hypoxia
Enzymatic activities
Hypoxia-inducing factor 1

ABSTRACT

Land-based recirculating aquaculture systems (RAS) and cage culture are important methods of *Larimichthys crocea* production. The effects of environmental factors on physiological and biochemical aspects of *L. crocea* require clarification. Temperature and salinity are controlled in RAS and directly affect *L. crocea* growth and survival. To explore optimal parameters, the oxygen consumption rate (R_O), ammonium excretion rate (R_N), and O/N ratio at different temperatures (8, 14, 20, 26, and 32 °C) and salinities (5, 15, 25, and 35‰) were determined. R_O, R_N, and O/N first increased and then decreased with elevated temperature and salinity, peaking at 26 °C and 25‰, respectively. This suggests that the metabolism of *L. crocea* was maximal at 26 °C and 25‰ salinity, which promote its growth and survival. Additionally, hypoxia affects cage culture, and has significant effects on enzymatic activities and stress-inducible gene expression. To accelerate the selective breeding of hypoxia-tolerant *L. crocea* in cage culture, we measured adenosine triphosphatase (ATPase), lactate dehydrogenase (LDH), and succinate dehydrogenase (SDH) activities, and hypoxia-inducing factor 1 (HIF-1) mRNA expression in the myocardium under hypoxia (2.5, 3.5, and 4.5 mg L^{-1}). ATPase and SDH activities first decreased and then increased under hypoxia, whereas LDH activity and HIF-1α expression first increased and then decreased. Thus, under hypoxia, the myocardial mitochondria switched from being susceptible to being resistant to injury induced by energy metabolism, and respiration in *L. crocea* likely converted from aerobic to anaerobic during adaptation. Furthermore, the upregulation of HIF-1α mRNA suggests it has an active role in protection against anoxic damage.

1. Introduction

Larimichthys crocea has high commercial and nutritional value, and aquaculture in the coastal areas of southeast China is an increasingly important source of its production (Cai et al., 2016). However, in recent years, the scale of cage culture has expanded and the space for coastal cages has become limiting. In addition, the present situation of crowed culture areas and increased culture density permits fish diseases and pests to spread frequently,

Abbreviations: RAS, Recirculating Aquaculture Systems; DO, dissolved oxygen; LDH, lactate dehydrogenase; SDH, succinate dehydrogenase; ATPase, adenosine triphosphatase; ROS, reactive oxygen species; LPO, lipid peroxide; TCA, tricarboxylic acid cycle; HIF-1α, hypoxia inducible factor-1α; RT-PCR, reverse-transcription polymerase chain reaction.

* Corresponding author.
E-mail address: zhujunquan@nbu.edu.cn (J.-Q. Zhu).

http://dx.doi.org/10.1016/j.chemosphere.2017.06.043
0045-6535/

representing a major bottleneck that restricts aquaculture development (Beveridge et al., 2002; Sharma et al., 2012). Thus, a growing proportion of *L. crocea* is cultured in land-based recirculating aquaculture systems (RAS), although the overall proportion of RAS is still lower than that of cage culture in China.

Successful RAS requires proper knowledge on the physiological aspects of *L. crocea* under different water parameters to provide optimal conditions for their survival and growth. In RAS processes, temperature and salinity have long been recognized as two of the most important environmental factors affecting the biological metabolism of aquatic organisms (Moser and Hettler, 2010; Zheng et al., 2008). Temperature and salinity changes have significant effects on all biological processes, such as behavior, survival, growth rate, food intake, osmotic regulation, metabolism, and energy acquisition and utilization (Brucet et al., 2012; Macdonald et al., 1991; Scherr et al., 2010). Respiratory metabolism is an important aspect of bioenergetics, with energy expenditure being used to maintain life processes. Respiratory metabolism is a useful and sensitive indicator of daily energy consumption, which can reflect not only the physiological condition and nutritional status but also the effect of the environmental conditions on physical activity (Barbieri and Paes, 2011). Oxygen consumption is a direct indicator of metabolic rate and an indirect indicator of growth capacity (Brett, 1979, 2011). Therefore, in aerobic organisms, quantifying the rate of oxygen consumption will provide an indication of the amount of energy released from the oxidation of food substratum (Jobling, 1981). Ammonia is the main nitrogenous compound excreted by teleosts; however, it is toxic to most aquatic animals and therefore, can limit production in aquaculture (Russo and Thurston, 1991). Ammonia has adverse effects on osmoregulation, cell function, tissue structure, blood biochemistry, growth, and reproductive capacity (Dolomatov et al., 2011; Jeney et al., 1992; Tilak et al., 2007). Thus, preventing the accumulation of toxic waste products, such as ammonia, is required to maintain the health of aquatic animals in intensive aquaculture. Rates of oxygen consumption and ammonium excretion over a certain time period can be used to indicate the energy consumed by an organism for the maintenance of biogenic activities (Gnaiger, 1983). Both rates are affected by biological factors (e.g., body size, hunger, feeding, and activity) and non-biological factors (e.g., temperature, salinity, dissolved oxygen [DO], heavy metal pollution, pH, and diurnal rhythm) (Barbieri and Paes, 2011; Caia and Summerfeltb, 1992; Faruq et al., 2008; Rosas et al., 1999; Zheng et al., 2008). Moreover, the ratio of oxygen consumption to ammonium excretion (O/N) can be used to determine which food substrates (carbohydrate, lipid, and protein) the organisms use to support their life (Bayne and Widdows, 1978).

In addition, although the DO level is not an important water parameter in RAS, because the seawater is constantly and manually aerated, hypoxia is still an important environmental factor in marine cage culture. The DO level is a key factor affecting fish welfare and development. In general, the DO level inside cages in offshore seawater is lower than that in the surrounding seawater. This is because with increasing farming density, the activity of bacteria decomposing organic matter (e.g., redundant fish feeds) increases, which occurs especially in summer and is more frequent in red tides in the littoral zone and in nets that have accumulated long-term fouling (Braithwaite and Mcevoy, 2005; Burt et al., 2012; Feng et al., 2004; Herbert and Steffensen, 2005). Moreover, the photosynthetic capacity within the cage is insufficient to supply the oxygen demand of the fish biomass (Wildish et al., 2011); therefore, oxygen requirements must be met by physical transport such as winds and tides (Johansson et al., 2007). For the adult large yellow croaker, DO levels below the critical point of 5 mg L^{-1} are considered hypoxic and insufficient to support optimal fish growth, causing loss of appetite. However, DO levels are only lethal when they fall below 3 mg L^{-1} (2 mg L^{-1} for juvenile *L. crocea*) (Zhang and Wang, 2007).

In this study, we attempted to elucidate the effects of three environmental factors (temperature, salinity, and hypoxia) on *L. crocea*. First, the effects of water temperature (8, 14, 20, 26, and 32 °C) and salinity (5, 15, 25, and 35‰) on physiological activities of *L. crocea* of two sizes were assessed. Physiological parameters, such as oxygen consumption rate, ammonium excretion rate, and O/N ratio were examined to improve our understanding of optimal rearing conditions in RAS for this species. Second, the effects of hypoxia (2.5, 3.5, and 4.5 mg L^{-1}) on enzymatic activities (adenosine triphosphatase [ATPase], lactate dehydrogenase [LDH], succinate dehydrogenase [SDH]) and hypoxia-inducible gene [hypoxia-inducing factor 1α, HIF-1α]) expression in the myocardium were investigated to determine the mechanisms underlying hypoxia acclimation. The aim was to supplement knowledge on individual physiological ecology with that on culture physiological ecology and provide data to support enhancements in marine ranching.

2. Materials and methods

2.1. Animals and rearing conditions

L. crocea of 1 year (91.7 ± 13.7 g) and 2 years (353.2 ± 35.3 g) of age with mixed females and males were obtained commercially from a local farm in 2016 and acclimatized for 1 month in two 2000-L rearing tanks containing filtered and aerated seawater (DO 8.5 ± 0.5 mg L^{-1}, Tm 19.5 ± 0.5 °C, salinity 24.5 ± 0.5‰). During this period, approximately half of the seawater in the tank was exchanged with fresh seawater every day. The fish were fed daily during the rearing period. To ensure that fish were fasted upon initiation of experiments, feed was withheld for 2 days prior to oxygen consumption and ammonia excretion measurements.

2.2. Experimental design and methods

2.2.1. Salinity experiment

The temperature experimental setup consisted of 8.5-L resting respirometers immersed in a 150-L sealed experimental tank (Tm 19.5 ± 0.5 °C) and seawater was recirculated over a trickle filter. *Larimichthys crocea* of two sizes were transferred randomly from the rearing tanks directly to four 150-L sealed experimental tanks (20 fish/tank) with salinity of 5, 15, 25, and 35‰ for 48 h to test the effects of an acute salinity change on oxygen consumption and ammonia excretion. The seawater was not aerated during the salinity experiment to avoid oxygen super-saturation.

2.2.2. Temperature experiment

The temperature experimental setup was the same as that described for the salinity experiment. The seawater (salinity 24.5 ± 0.5‰) was recirculated over a trickle filter. *Larimichthys crocea* of two sizes were randomly divided into five experimental tanks (20 fish/tank) and acclimated to the experimental conditions by progressively increasing or decreasing seawater temperature (2 °C/day) to reach the desired experimental temperature of 8, 14, 20, 26, and 31 °C. Fish were allowed to acclimatize to the experimental temperature for 1 week prior to experiments. The seawater was continuously aerated until 6 h before the temperature experiment to avoid oxygen super-saturation and keeping for 48 h to measure oxygen consumption and ammonia excretion rates.

2.2.3. Determination of oxygen consumption rate and ammonia excretion rate

At the end of the temperature and salinity experiments, each