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Effects of light intensity on metabolism and antioxidant defense in *Haliotis discus hannai* Ino

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ABSTRACT

We examined the effects of light intensity on the metabolism, antioxidant enzymes, and expression of related genes in *Haliotis discus hannai* Ino, to determine the optimal lighting conditions for aquaculture of these animals. Succinate dehydrogenase activity was significantly lower after 48 h in animals exposed to light intensities of 30 and 60 μmol/m^2/s, while lactate dehydrogenase activity and lactic acid content at 96 h were significantly higher compared with animals in the dark or exposed to 5 or 15 μmol/m^2/s (both $P < 0.05$). There was no significant difference in reactive oxygen species (ROS) contents between animals in the dark and those exposed to 5 or 15 μmol/m^2/s ($P > 0.05$) throughout the experiment. However, ROS contents under light intensities of 30 and 60 μmol/m^2/s peaked at 12 h, when expression levels of genes encoding catalase (CAT), thioredoxin peroxidase (TP_X), sigma-glutathione-s-transferase (GST_S), and mu-glutathione-s-transferase (GST_m) also began to increase, and CAT and glutathione peroxidase (GP_X) activities were significantly higher than in animals in the dark or exposed to 5 or 15 μmol/m^2/s ($P < 0.05$). After a further 12 h, the ROS content at 30 μmol/m^2/s began to decrease, and GP_X activity and malondialdehyde (MDA) content also gradually decreased, followed by increases in ROS and MDA 48 h later, to reach respective peaks at 96 h. Gene expression levels of TP_X, GST_S, GST_m, heat shock protein 70 (HSP70), and CAT began to decrease 72 h later, suggesting that excessive accumulation of ROS may have caused oxidative damage to the tissues. At a light intensity of 60 μmol/m^2/s, expression levels of TP_X, GST_S, GST_m, and HSP70 genes, total antioxidant capacity, and GP_X activity peaked at 48 h, and the ROS content was significantly higher than in any other group 48 h later ($P < 0.05$). However, gene expression levels of TP_X, GST_S, GST_m, HSP70, HSP90, and CAT, and reduced glutathione content began to decrease, indicating that the antioxidant system had been unable to perform its normal physiological functions to withstand the long-term stress of adverse environmental factors. These results suggest that the light intensity should be controlled at 5–15 μmol/m^2/s during *H. discus hannai* aquaculture, to maintain the organism's normal physiological metabolism.

Statement of relevance: The disc abalone *Haliotis discus hannai* is an economically important shellfish in China, with demand growing for this high-protein and low-fat seafood. In 2014, 11,5397 tons of *H. discus hannai* were harvested from aquacultural sources, but supply cannot currently meet consumer demand. In recent years, the natural habitat of *H. discus hannai* has declined as a result of overfishing, marine reclamation, water pollution, among others, leading to a sharp decrease in the numbers of *H. discus hannai* in the wild. Thus, the development of an aquacultural system for farming *H. discus hannai*, as well as for its protection in the wild, are a focus of current research.

Light, including photoperiod, quality and intensity, is one of the key environmental factors influencing the growth, culture and survival of aquatic organisms. Over evolutionary time, organisms have evolved both physiological and behavioral mechanisms that enable them to adapt to diurnal fluctuations in light. In Chinese traditional abalone aquaculture, a sunshade net is usually used because of the photophobic nature of abalone. In particular, the aquaculturist usually provides a darkened setting for adult abalones to increase their food intake rate, facilitate their growth and promote gonadal development. For abalones in their natural environment, the light intensity in the water area is relatively stable, suggesting that there is a key regulative effect of light intensity on their growth and development.

Gao et al. (2016a,b) found that a dark environment not only adversely affected the daily aquaculture production, but was also associated with a lower growth rate compared with animals reared under higher light intensities, because of lower food conversion efficiency and greater energy losses through excretion and feces. Physiological metabolism and antioxidant defense systems may therefore be key indicators for measuring the suitability of

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light intensity for aquaculture production. We therefore examined the effects of light intensity on the metabolism and antioxidant defense system, and on the expression of related genes, in *H. discus hannai* using a light-emitting diode (LED) to replace the conventional fluorescent light source. The results of this study will improve our understanding of the physiological tolerance and antioxidant defense characteristics of abalone under different light intensities, and enable the optimization of environmental light conditions in abalone aquaculture.

1. Introduction

Environmental factors such as temperature, salinity, dissolved oxygen, and light may greatly impact on the growth, survival, and behavior of aquatic organisms (Howell and Baynes, 2004). The earth's rotation and its revolution around the sun mean that most organisms inhabit a dynamic environment, featuring gradually changing light conditions (Villamizar et al., 2011). Light is a key environmental factor, comprising light quality, intensity, and cycle. Among these characteristics, light intensity is defined as the luminous flux per unit area of the water body (Cobcroft et al., 2001; Stuart and Drawbridge, 2011). Industrial aquaculture of *Haliotis discus hannai* Ino seeks to create a light environment suitable for the organism's growth and survival by means of artificial regulation, based on its nocturnal, photophobic, and scotophilic characteristics. Gao et al. (2016a,b) found that a dark environment not only adversely affected the daily aquaculture production, but was also associated with a lower growth rate compared with animals reared under higher light intensities, because of lower food conversion efficiency and greater energy losses through excretion and feces. Gao et al. (2016a,b) reported that SOD activity increased immediately when the *H. hannai* were placed in green, purple, blue, and natural light, and CAT activity was higher in the green, purple, blue, and natural light groups than in the red light, orange light, and dark environment. Physiological metabolism and antioxidant defense systems may therefore be key indicators for measuring the suitability of light intensity for aquaculture production.

Previous studies reported that changes in environmental stress factors (pH, temperature, water salinity) might exert physiological effects via oxidation–reduction, resulting in abnormal aerobic metabolism and the accumulation of reactive oxygen species (ROS) and consequent oxidative damage (Bussell et al., 2008; Kim et al., 2007; Karakatsouli et al., 2008). The dynamic balance between the antioxidant system and ROS is a key factor maintaining the animal in a state of health (Seifried, 2007). This internal balance may be more vulnerable to oxidative stress factors in aquatic animals, because of the complexity of their living environment (Almroth et al., 2015; Geret et al., 2002; Hu et al., 2015; Li et al., 2016).

The antioxidant system in animals mainly comprises antioxidant enzymes (Abele and Puntarulo, 2004; Bogdan et al., 2000; Nordberg and Arnér, 2001), heat shock proteins (HSPs) that protect proteins from free radical-induced damage (Limón-Pacheco and Gonsebatt, 2009), and internal small-molecule, non-enzyme antioxidants (e.g., vitamin C, reduced glutathione [GSH]) (Fang et al., 2002; Klaunig and Kamendulis, 2004). The enzyme system, composed of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP_X), and other antioxidant enzymes, plays a critical regulatory role, enhancing the organism's ability to adapt to the external environment and protecting it from oxidative damage (Cetinkaya et al., 2002; Goţia et al., 2001; Muruganandan et al., 2002). Light intensity had a significant impact on the activities of CAT and GP_X in juvenile *Solea senegalensis* (Cañavate et al., 2007), with maximum activities at 200 lx, while high light intensity (800 lx) imposed stress on *Megalobrama amblycephala*, resulting in an increased rate of oxidation in the liver, and decreased immune function (Tian et al., 2015). Activities of acid phosphatase and total superoxide dismutase, and the nitric oxide content in *Epinephelus coioides* were significantly higher at 600–1150 lx than at any other light intensity (Wang et al., 2013). GP_X activity in *Haliotis midae* was significantly higher at 19 °C than at 14 °C, but SOD activity increased with the environmental concentration of dissolved oxygen (Vosloo et al., 2013a,b). Vosloo et al. (2013a,b) found that GPx activity in *H. midae* exposed to low concentrations of dissolved oxygen was significantly lower than at higher concentrations. Kim et al. (2007) showed that increased water temperature significantly increased gene expression levels of Cu/Zn-SOD and Mn-SOD in *H. discus*, by reverse transcription–polymerase chain reaction (PCR). Zhou et al. (2011) reported that bisphenol A interfered with the endocrine regulation of cells and cell cycle progression in *Haliotis diversicolor* by down-regulation of the gene for prohormone convertase 1 and overexpression of those for cyclin-dependent kinase 1 and cyclin B. Expression levels of intracellular Cu/Zn-SOD in *Crassostrea gigas* were increased after 7 days of exposure to hydrocarbon compounds (Boutet et al., 2004). Canesi et al. (2007) reported that low doses of bisphenol A significantly reduced the expression levels of CAT in the hepatopancreas of *Mytilus galloprovincialis* at both the transcriptional and translational levels, and increased the activities of glutathione-s-transferase (GST) and glutathione disulfide reductase. However, reports on the effects of light intensity on the metabolism, antioxidant defense system, and the expression of related genes in *H. discus hannai* Ino are lacking.

We therefore examined the effects of light intensity on the metabolism and antioxidant defense system, and on the expression of related genes, in *H. discus hannai* Ino using a light-emitting diode (LED) to replace the conventional fluorescent light source. The results of this study will improve our understanding of the physiological tolerance and antioxidant defense characteristics of abalone under different light intensities, and enable the optimization of environmental light conditions in abalone aquaculture.

2. Materials and methods

2.1. Source and acclimation of abalone

This study was carried out at the Institute of Oceanology, Chinese Academy of Sciences, Qingdao, Shandong, China, from 18 to 21 December 2015. Juvenile abalones were purchased from Changqing Ocean Science & Technology Co., Ltd. (Weihai, Shandong, China), and all experimental abalones were sourced from the same batch after artificial hatching. Abalones were acclimated in 20 aquaria (60 × 30 × 40 cm, water volume 72 L) for 15 days at a water temperature of 18 °C, salinity 31 ± 1, pH 8.1, concentration of dissolved oxygen >6 mg/L, and a light cycle simulating the natural light cycle. Culture water was added after repeated precipitation and sand filtration. Residual feed and feces were removed and two thirds of the water was replaced with fresh seawater every day at 10:00 h to ensure good water quality. During the acclimation period, *Laminaria japonica* Aresch was thrown into the container to feed the abalone every day at 17:00 h, at a quantity equivalent to 5% of abalone wet body weight, to keep them fully fed.

2.2. Experimental design

Full-spectrum experimental LEDs (Institute of Semiconductors, Chinese Academy of Sciences, Beijing, China) were serially suspended above the aquaria to simulate natural light. The aquaria were divided