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Effects of flow velocity on growth, food intake, body composition, and related gene expression of *Haliotis discus hannai* Ino

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ABSTRACT

This study investigated the effects of different flow velocities in a circulating aquaculture system on growth, food intake, and related gene expression of the abalone *Haliotis discus hannai* Ino. Abalones (shell length: 41.39 ± 2.85 mm, body weight: 8.19 ± 0.66 g) were cultured at three flow velocities; high-velocity (400 L/h), medium-velocity (300 L/h), and low-velocity (200 L/h) during the course of the experiment. Four repeats of each flow velocity were conducted over an experimental cycle of 90 days. Results showed that the survival and specific growth rate of abalones in the 200 L/h group were significantly lower than in any other group at the end of the experiment, while total ammonia nitrogen and NO_2-N concentration in the water was significantly higher than that in any other group ($P < 0.05$). Food intake, food conversion efficiency, protein content, pepsin, and α-amylase activity of abalones in the 300 L/h group were significantly higher than in the 200 and 400 L/h groups ($P < 0.05$), but there was no significant difference identified between 200 and 400 L/h groups ($P > 0.05$). Although no significant difference was identified between 300 and 400 L/h groups with respect to cellulase activity or the expression levels of *Hdaly*, both were significantly higher than in the 200 L/h group ($P < 0.05$). In the 400 L/h group, hexokinase and pyruvate kinase activity, and lactic acid content were significantly higher compared with in the 300 L/h group ($P < 0.05$). The ash and fat contents of abalones in the 200 L/h group were significantly lower than in any other group, but moisture content was significantly higher ($P < 0.05$). At Day 90, the expression levels of *Hdamyl*, *Hdlam*, and *Hdcel* in the 300 L/h group were significantly higher than in any other group ($P < 0.05$), and compared with Day 45, the expression levels of were *Hdamyl* significantly increased ($P < 0.05$). Although no significant difference was identified between 200 and 400 L/h groups with respect to the expression levels of *Mn-SOD* and *CAT*, both were significantly higher than those in the 300 L/h group ($P < 0.05$). Therefore, control of flow velocity at 300 L/h will not only stimulate the food intake and growth of abalones, but also reduce energy consumption to resist against water flow impact and avoid oxidative damage due to water quality deterioration. This will be beneficial for abalone health and will improve aquaculture production.

1. Introduction

The abalone *Haliotis discus hannai* Ino is one of the important marine economic shellfish species in China; its aquaculture production reached 128,000 t in 2015, equivalent to > 90% of the total world output that year (China Fishery Statistics Yearbook, 2016). However, numerous issues have emerged alongside the rapid development of the aquaculture industry, such as an increase in offshore water pollution, frequent occurrence of natural disasters including typhoon and red tide, space limitation of mariculture, and annual increase in mortality due to high summer temperatures (Wu and Zhang, 2016). Therefore, there is an urgent need to transform the conventional extensive, resource-dependent aquaculture system to a resource-saving, environmentally friendly recirculating aquaculture system.

In China, the closed recirculating aquaculture of abalones remains in an early stage of development, and progress is limited compared with developed countries. In contrast with the conventional flowing aquaculture model, the advantages of recirculating aquaculture include small land coverage, a high degree of intensiveness, low labor cost, stable and controllable water environment, and fulfilled purposes of resource conservation, and ecological and environmental protection (Dalsgaard et al., 2013; McKenzie et al., 2012; Suhr and Pedersen,

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2010). The cubic drawer-type recirculating aquaculture system designed by Wu et al. (2012) increases the stocking density of *H. discus hannai* up to 6–9 times that of the flowing aquaculture system, and the energy consumption for seawater heating is only 1/7 of the flowing aquaculture system. Based on abalone physiological and ecological characteristics, the environmental factors of the recirculating aquaculture system are suitable for the optimal control of abalone growth, survival, and reproduction.

Flow velocity is a critical and complex ecological factor in the aquatic environment; it can stimulate the sensory organs of aquatic organisms for generation of corresponding movement and response mechanisms. It has multiple ecological effects that directly or indirectly affect the behavior and physiological state of aquatic organisms (Lupandin, 2005). There are many factors influencing the growth and survival rate of abalones such as water quality (James and Barr, 2012; Naylor et al., 2013), food quality and quantity (Viera et al., 2005), and stocking density (Ahmed et al., 2013; Capinpin et al., 1999). Wassnig et al. (2010) found that the aquaculture output and food conversion rate of 2-yr-old hybrid abalone (*Haliotis laevigata* and *Haliotis rubra*) increases linearly with flow velocity under high-density stocking conditions (11,400/tank), with an optimal flow velocity of 246 L/min. Wells et al. (1998) showed that there was no significant difference between *Haliotis iris* cultured in an inner bay with steady water flow and in a high wave area with respect to muscle, muscle composition, hemocyanin concentration, and growth rate. The activity of glycolytic pyruvate reductase enzyme and tauropine dehydrogenase of *H. iris* cultured in the high wave area increased significantly, while the glycogen content. Flow velocities may produce different movement states and swimming speeds of aquatic organisms; as the movement speed accelerates, the O_2 consumption rate also increases. Wardle et al. (1996) determined the metabolic rate of *Trachurus trachurus* at different swimming speeds, when the swimming speed reached 0.87 m/s, the O_2 consumption rate increased to 5.2 times that of the control group. As the flow velocity increased, the movement of *Oncorhynchus keta* increased, and its O_2 consumption rate also increased significantly (Lee et al., 2003a, 2003b). The effects of flow velocity on the growth of *Salmo salar* L. and *Oncorhynchus mykiss* have been reported (Fivelstad et al., 2004; Good et al., 2009); however, both species were cultured in an open flowing aquaculture system. To the best of the authors' knowledge, the effects of flow velocity on the growth and food intake of *H. discus hannai* in a closed recirculating aquaculture system have not yet been reported.

Therefore, this study examined the effects of different flow velocities on the growth and food intake of *H. discus hannai* in a proprietary multi-layer, cubic recirculating aquaculture system, and further revealed the physiological adaptation mechanism of *H. discus hannai* at different flow velocities by means of molecular biological analysis. This was conducted in an attempt to provide a theoretical basis for aquatic environment regulation and optimization of the industrialized recirculating aquaculture system.

2. Materials and methods

2.1. Experimental facility and acclimation of abalones

This experiment was carried out at the Institute of Oceanology, Chinese Academy of Sciences, Qingdao of Shandong, China. Four sets of the multi-layer and cubic recirculating aquaculture system were used as the experimental facility. Each facility set comprised the aquaculture system and water treatment system. The aquaculture system had three layers comprising the aquaculture tank, perforated partition, inlet pipe, drain pipe, and wave maker. The water treatment system consisted of a filter tank, tilted partition, sewage tank, temperature-regulated chamber, heat exchanger, cooler, water pump, UV disinfection device, foam separator, jet pump, disk aerator, biotank, air pump, oxygen cone, and oxygen cylinder (Fig. 1). The upper part of filter tank (volume: 0.13 m^3) comprised filter tilted plates that were laid with biochemical cottons of different pore sizes, the biochemical cottons laid on two pieces of tilted plates provided the layered filtering by descending order of pore size. The drain outlet was provided at the bottom of the other end of the tank, which was directly connected to the sewage tank. The food residues and feces were deposited in the bottom part of the sewage tank, and the food residues were collected by opening the bottom drain valve.

In this experiment, *H. discus hannai* were obtained from the same batch of seeds through artificial hatching, then brought to the laboratory, transferred at a specific density (800 ind/m^2) to different aquaculture tanks with four sets of recirculating system, and acclimated for 15 days. Water temperature was maintained at 17 °C, salinity at 29 ± 1, pH at 7.8, and the concentration of dissolved O_2 at > 6 mg/L. A natural light cycle was set. Aquaculture water comprised natural seawater that had undergone sedimentation and sand filtration. In the period of acclimation, *Laminaria japonica* Aresch was added to the tanks to feed the abalone once daily at 18:00 h, with the feeding quantity equivalent to 6% of the wet body weight of abalone, in order to ensure satiation.

2.2. Experimental design

Three flow velocity groups were established in this experiment, the flow velocity of each layer in a set of the aquaculture system was controlled at 200, 300, and 400 L/h using a flowmeter. This was equivalent to 13, 20, and 27 daily cycles, four repeats were provided for each group, with an experimental cycle of 90 days. The experimental abalones had mean ± standard error shell length of 41.39 ± 2.85 mm and weight of 8.19 ± 0.66 g. Abalone were fed fresh L. *japonica* once daily at 18:00 h, and the feeding amount was set at 6% of the wet weight of abalone to ensure satiation. The fresh kelp was added to the three aquariums at the same time to determine potential food loss. At 10:00 h the next morning, the residual food in the aquariums was collected and weighed, and calculated for the correction of food intake values. UV light was set through the microcomputer control system to operate from 22:00 h until 03:00 h the next day, and the water was treated for 5 h every day. Three pieces of “W-shaped” substrates (height: 15 cm) were evenly placed in each aquaculture tank to enlarge the attachment area and avoid the adverse effect of wastes deposited at the bottom. The filter cottons and the bottom part of sewage tank were cleaned every 6 days, and 3% seawater was added to counteract evaporation.

The water temperature, salinity, pH, dissolved O_2 concentration, and dissolved O_2 saturation in the four sets of the recirculating aquaculture system were determined daily using a YSI-556MPS portable multi-parameter water quality measuring instrument (Yellow Springs Instruments Inc., Yellow Springs, OH, USA). Water sampling was performed at the water inlet and outlet of the different flow velocity groups, and the concentration of total ammonia nitrogen (TAN), NO_2^-N, and NO_3^-N, and the chemical oxygen demand (COD) in the water were measured once every 6 days. Three water samples were collected from each layer, and the TAN, NO_2-N, and NO_3-N concentration, and COD was measured using Nessler's reagent colorimetry, naphthyl diamine hydrochloride spectrophotometry, ultraviolet spectrophotometry, and alkaline potassium permanganate method (Li et al., 2015).

2.3. Sample collection

All abalones were starved for 24 h before the start of experiment, and prior to the experiment, 20 abalones were randomly selected and used as the initial samples for later analysis of enzyme activity and biochemical composition. After the start of experiment, 100 abalones were randomly selected from each flow rate group every 45 days for the measurement of shell length and weight, and the survival rate of