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Effects of different green light intensities on the growth performance and endocrine properties of barfin flounder *Verasper moseri*

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ABSTRACT

We previously reported that the somatic growth of barfin flounder, *Verasper moseri*, was effectively stimulated by the green light compared to the blue and red lights. Herein, we report the effects of different green light intensities on the growth and endocrine system of the fish. Fish were reared in a dark room with light from a light-emitting diode (LED) at a peak wavelength of 518 nm under controlled photoperiod (10.5:13.5 h, light:dark cycle; 06:00–16:30, light) with three levels of photon flux density (PFD)—2 (low), 7 (medium), or 21 (high) $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the water surface. The average water temperature was 10.2 °C, and the fish were fed until satiety. The fish reared under high PFD of green light showed the highest specific growth rates, followed by the medium PFD group. Under high PFD, the fish showed the highest amount of melanin-concentrating hormone mRNA in their brains and insulin in plasma, while the lowest amount of growth hormone was observed in their pituitary glands. These results suggest that the green light stimulated the growth of barfin flounders in a light intensity-dependent manner in association with their central and peripheral endocrine systems. However, when the fish were reared in an ordinary room where they received both ambient and green LED lights, the fish under LED and ambient light grew faster than those under ambient light only (control). Moreover, no difference was observed in the specific growth rate of the fish reared under the three different green LED light intensities, suggesting that the growth was equally stimulated by the green light within a certain range of intensities under ambient light.

1. Introduction

Barfin flounder (*Verasper moseri*) is a large Pleuronectiform fish inhabiting the cold sea areas around the northeastern coast of Japan. The production and release of the barfin flounder juveniles are carried out in this area for resource enhancement and aquaculture. The photic environmental factors, such as the background colors and light wavelengths used in rearing tanks, have been shown to modulate various physiological processes, such as growth, body color, and hormone production in barfin flounders (Yamanome et al., 2005, 2009; Amiya et al., 2005, 2008; Takahashi et al., 2004, 2016).

Compared to a barfin flounder growing in a black tank, the one growing in a white tank exhibits a paler body color and higher melanin-concentrating hormone (MCH) content in its hypothalamus, pituitary, and plasma (Amiya et al., 2005, 2008; Takahashi et al., 2004). This suggests that MCH, which is produced in the hypothalamus and released from the neurohypophysis, participates in the body color changes by aggregating pigments in skin and scale chromatophores of barfin flounders (Takahashi et al., 2014). Moreover, they show more stimulated feed intake when reared in white than in black tanks (Sunuma et al., 2009). MCH is a neuropeptide that enhances the feed intake in mammals (Qu et al., 1996), and high MCH production has been observed in the barfin flounders showing high growth performance (Amiya et al., 2005, 2008; Takahashi et al., 2004; Yamanome et al., 2005). It has, therefore, been suggested that the promotion of somatic growth on a white background is the result of increased feed

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intake, probably induced by the enhanced production of MCH in the brain (Takahashi et al., 2014).

We previously showed that the barfin flounders reared under a green filter grew faster than the control fish reared under ambient light (Yamanome et al., 2009). Recently, when we investigated the effects of the particular light wavelengths on the growth of barfin flounders using light-emitting diodes (LEDs; blue, green, and red), the blue and green lights had greater effects on their growth than the red light (Takahashi et al., 2016). Notably, the green light induced the highest growth-promoting effects at low water temperature. Under low temperature—6.6 °C on average—the fish reared in ambient light showed a decrease in their body weight because of the low feed intake. Under the same temperature, the fish irradiated with green light showed relatively higher plasma levels of insulin and insulin-like growth factor-I (IGF-I). Although no significant difference was observed in the expression levels of the growth hormone (*gh*) gene in the pituitaries of the fish grown under three different lights, the expression levels of the *mch* gene in the brains of the fish under green light were lower than those under blue and red light (Takahashi et al., 2016).

Like many other fishes, barfin flounders have well-developed vision systems as demonstrated by the presence of six functional opsin genes (Kasagi et al., 2015). Three of the six opsins are green-sensitive and the other three are single ultraviolet-, blue-, and red-sensitive opsins, respectively. The presence of the three green-sensitive opsins seems to be linked to the relatively greater effects of the green light on the growth of barfin flounder (Takahashi et al., 2014).

In our previous study, we evaluated the effects of the particular light wavelengths on the growth performance and endocrine functions of barfin flounder at a single light intensity for each color—blue, green, and red (Takahashi et al., 2016). The previous study was performed under a water temperature so low that the fish did not grow at all under ambient light. Therefore, the present study was undertaken to evaluate the intensity-dependent effects of green light on the growth performance of barfin flounder at a temperature at which they grow under ambient light. We chose to use green light because in general, it had a greater effect on growth than the blue and red light, in the previous experiments (Takahashi et al., 2016). Here, we performed experiment 1 in a dark room to evaluate the potential effects of green LED light irradiation on growth and the expression of genes related to feed intake and plasma levels of metabolism-associated hormones and biochemical parameters such as glucose and FFAs. Factors evaluated were the same as those described in a previous experiment (Takahashi et al., 2016). Furthermore, we explored the potential benefits of green light on aquaculture in a room with ambient light (experiment 2), because rearing fish in dark rooms is impracticable in many facilities.

2. Materials and methods

2.1. Barfin flounder, rearing under LED lighting, and tissue collection

Barfin flounder, *V. moseri*, were bred and used in experiments at the Akkeshi Laboratory, Japan Fisheries Research and Education Agency, Hokkaido, Japan, and all experiments were conducted according to the Guidelines for the Care and Use of Animals of Kitasato University, Tokyo, Japan. Prior to running the experiments, fish were reared in conventional indoor tanks with routine feeding and without controlling water temperature and photoperiod. All fish used for the present experiment were implanted with an electric tag (Pit Tag; Biomark, Boise, ID, USA) for individual identification. Round white tanks (100 cm diameter) containing approximately 400 L of running seawater (ventilation rate: 400 L/h) were used for fish rearing. Each tank contained 50 fish. These tanks were equipped with LEDs (Model LLM0200A; Stanley Electric Co., Ltd., Tokyo, Japan) with a peak wavelength of 518 nm (green) under controlled photoperiod (10.5:13.5, light:dark cycle; 06:00–16:30, light) according to our previous experiments (Takahashi et al., 2016). The fish in the white tanks were irradiated with the LED-emitted green light at the photon flux density (PFD) of 2 (low), 7 (medium) or 21 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (high) at the surface of water. Experiment 1 was conducted in a dark room. As a reference to evaluate the growth performance and endocrine functions under common rearing conditions in the laboratory, the fish were also reared in an indoor tank under ambient light, in which the direct sunlight was blocked by covering the window with a black cloth to avoid unexpected effects of direct sunlight such as increased water temperature. The fish reared under ambient light were excluded from the statistical analyses, because they were under natural photoperiod. Fish were reared for 21 days under controlled water temperature at 8.1–12.1 °C (average 10.2 °C) from November 26, 2013 to December 16, 2013. In experiment 2, the tanks were placed in an ordinary fish rearing room wherein the experimental fish received both the ambient and LED lights simultaneously, while the control fish received only the ambient light. The fish were reared for 28 days under controlled water temperature at 9.9–10.9 °C (average 10.3 °C) from January 16, 2014 to February 13, 2014. Experiments 1 and 2 were carried out in separate rooms, because there was limited space to set up four tanks that would receive ambient light in the building with the dark room. In both experiments, the total length (TL) and body weight (BW) were measured every week without anesthesia, except for the terminal sampling of experiment 1. These fish were returned to their respective tanks after measuring TL and BW. The fish were fed commercial pellets (EP-1, Higashimaru, Kagoshima, Japan) once daily from 09:00 to 10:00 until satiety. The unconsumed diet was collected 3 h after feeding and the number of remaining pellets was calculated to estimate the amount of diet consumed. In experiment 1, several tissues were collected from the fish anesthetized by using 0.05% 2-phenoxyethanol at the end of the experiment between 10:00 and 15:00. No diet was provided to the fish on a sampling day. The brains, pituitary glands, and livers were immediately dissected and snap-frozen on dry ice. Blood was collected using heparinized needles and syringes, and then plasma was separated by centrifugation (3000 *g* for 15 min at 4 °C). The samples were stored at −80 °C until analysis.

2.2. Evaluation of growth performance

Growth parameters were calculated as follows:

$$\text{specific growth rate (SGR,\% day}^{-1}) = [(\ln W_f - \ln W_i)/t] \times 100$$

$$\text{voluntary feed intake (VFI,\% body weight day}^{-1}) = [\text{Feed}_{\text{consumed (dry matter)}}/(W_{mean} \times t)] \times 100$$

$$\text{feed efficiency ratio (FER)} = W_{gain}/\text{Feed}_{\text{consumed (dry matter)}}$$

$$\text{condition factor (CF, \%)} = (W_f/TL_f^3) \times 100$$

In the above equations, W_i, W_f, W_{mean}, W_{gain}, t, $\text{Feed}_{\text{consumed (dry matter)}}$, and TL_f are the initial weight, final weight, mean weight, weight increment (g), time period (day), consumed feed (g), and final total length (cm), respectively.

2.3. cDNA for mRNA quantification

The cDNA used as a template to synthesize cRNA for quantitative reverse transcription polymerase chain reaction (qRT-PCR)

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