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ORIGINAL ARTICLE

Aquaculture

An evaluation of the growth-promoting effects of green light on spotted halibut for its practical application in aquaculture

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Abstract

We examined the effects of tank color and stocking density on the growth of spotted halibut (*Verasper variegatus*) under a green light-emitting diode (LED). Fish reared for 40 days under a green LED (10 µmol m^{-2} s^{-1} at the water surface) grew faster than those reared under a white LED, irrespective of tank color (white or black). Green light promoted higher growth than indoor room light when fish were kept for 32 days under both the tested stocking densities, 4.3 kg m^{-2} and 13 kg m^{-2}, but stocking density alone did not affect fish growth. These results suggest that a growth-promoting effect of green light can be expected within the range of stocking densities applied here for fish housed in both black and white tanks.

Keywords Feed · Green light · Growth · Light-emitting diode · Spotted halibut · Stocking density · Tank color

Introduction

The rapid growth of fish is one of the most important aims of aquaculture. The somatic growth of fish is affected by a variety of factors associated with rearing conditions, including light conditions, water temperature and quality, stocking density, and diet quality (Kestemont and Baras 2001). Photoperiod and intensity and quality of light have been shown to affect the growth of fish (Boeuf and Le Bail 1999; Villamizar et al. 2011). Although the effects of different regions of the spectrum have received less attention than the effects of photoperiod on feeding in fish (Kestemont and Baras 2001), several authors—including us—have recently reported the positive effects of light quality, specifically chromatic light, or wavelength, on the growth of juvenile and adult fish, such as common carp *Cyprinus carpio* (Karakatsouli et al. 2010), blunt snout bream *Megalobrama amblycephala* (Tian et al. 2015), rainbow trout *Oncorhynchus mykiss* (Karakatsouli et al. 2008), Pacific bluefin tuna *Thunnus orientalis* (Tsutsumi et al. 2014), barfin flounder *Verasper moseri* (Takahashi et al. 2016, 2018), spotted halibut *Verasper variegatus* (Shimizu et al. 2019), Japanese flounder (olive flounder) *Paralichthys olivaceus* (Shimizu et al. 2019), and yellow perch *Perca flavescens* (Head and Malison 2000).

We previously revealed that the growth of three species of flatfish is intimately linked with the quality of light by using LED lamps with a peak of a particular wavelength (Takahashi et al. 2016, 2018; Shimizu et al. 2019). Barfin flounder exposed to green and blue light grew faster than when exposed to red light, and at a low temperature (6.6 °C), exhibited highest growth under green light (Takahashi et al. 2016). Barfin flounder also showed a light intensity-dependent increase in growth rate under green light in a dark room. However, no light intensity-dependent effect on growth was observed when the fish were exposed to green light under indoor room lighting (Takahashi et al. 2018). Among the types of chromatic light tested (blue, blue-green, green, and red), green light showed the strongest growth-promoting effects in spotted halibut (Shimizu et al. 2019). Similar effects to those observed for spotted halibut were seen in Japanese flounder (Shimizu et al. 2019). Finally, the growth-promoting effects of green light on spotted halibut were observed within a wide range of water temperatures, i.e., between 12 and 21 °C (Shimizu et al. 2019).

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The growth-promoting effects of green light suggest its possible use for the improvement of aquaculture of spotted halibut, a promising species for both aquaculture and stock enhancement in Japan (Wada et al. 2010). However, effective methods for irradiation with green light in association with other rearing conditions still require elucidation. The influence of abiotic or biotic factors on fish growth is sometimes masked, buffered, or amplified by other factors, such as different combinations of light intensity and tank color modifying the feeding behavior of fish larvae (Villamizar et al. 2011). However, knowledge on the effects of the interaction of environmental factors—excluding water temperature—with green light on the growth of spotted halibut is limited (Shimizu et al. 2019).

In research evaluating the growth parameters of fish, one common criterion is to use tank replicates to avoid the tank effect. Hence, approximately four tanks per treatment are used and data from each tank are treated as a single biological replicate (Ruohonen et al. 2001). However, this is not always practical when relatively large sized fish, such as pre-commercial- or commercial-sized fish, are the subjects of research. Therefore, the accumulation of evidence from different types of experimental design is thought to be useful to overcome this practical difficulty. In this study, we conducted a series of experiments designed to elucidate the effects of tank color and stocking density on the growth of fish exposed to green light. Fish rearing was performed in a conventional indoor aquarium, not in a dark room, for practical reasons. In experiments using relatively large fish, their slow growth rate and a limited sample size make it difficult to detect growth differences. Individual-based rearing experiments are useful for revealing environmental responses in the feeding and growth of juvenile flatfish (Kusakabe et al. 2017). However, the effects of stocking density cannot be investigated in individual-based experiments. In this study, we used electronic tags to identify individual fish to exclude individual variability, which compensates for the reduced statistical power of using relatively large fish.

Materials and methods

Experimental fish

Spotted halibut (*V. variegatus*) were raised at the Miyako Laboratory, Japan Fisheries Research and Education Agency (henceforth “Miyako”). All experiments were conducted in accordance with the Guidelines for the Care and Use of Animals of Kitasato University.

Procedures common to experiments 1 and 2

A series of rearing experiments were conducted in the indoor aquarium at Miyako. Tanks were equipped with green or white LED lamps (LLM0200A; Stanley Electric, Tokyo) with peak wavelengths of 518 nm (green) or 447 and 550 nm (white) [see Shimizu et al. (2019) for the full spectrum]. The photon flux density of the light from the LED lamps was 10 μmol m^{-2} s^{-1} at the water surface. Fish were exposed to both controlled LED lighting under a 10:14-h light–dark cycle (light phase 0700–1700 hours) and indoor room light, which comprised natural daylight from the windows and white LED light from the ceiling. The photon flux density of indoor room light was 0.2 μmol m^{-2} s^{-1} at the water surface, from 0700 to 1700 hours. Fish were discriminated by implantation with electronic tags (Pit Tag; Biomark, Boise, ID). Implantation of electronic tags and measurements of total length (TL) and body weight (BW) were conducted after anesthetization using 0.05% 2-phenoxyethanol. Fish were fed commercial pellets (Otohime EP series; Marubeni Nisshin Feed, Tokyo) from 0800 to 1600 hours using automatic feeders (3 kg-12 h; FIAP Clockwork feeder; Nichimo, Japan). Growth parameters were calculated as follows:

$$Specific\ growth\ rate\ of\ BW\left(SGR\ of\ BW,\ \%\ day^{-1}\right) = \left[\left(InBW_f - InBW_i\right)/t\right] \times 100,$$

$$Specific\ growth\ rate\ of\ TL\left(SGR\ of\ TL,\ \%\ day^{-1}\right) = \left[\left(InTL_f - InTL_i\right)/t\right] \times 100,$$

$$Condition\ factor\ (CF,\ \%) = BW_f/TL_f^3 \times 100.$$

where BW_i, BW_f, TL_i, TL_f, CF, and t are initial BW, final BW, initial TL, final TL, and time (days), respectively.

Experiment 1: rearing of spotted halibut in white or black tanks under a LED

We reared fish in two black and two white round indoor tanks (diameter, 96 cm; height, 75.5 cm) containing approximately 400 l of running seawater with a water exchange rate of 23 ml s^{-1} at natural water temperature. During the rearing period, the water temperature increased from 14.2 to 17.8 °C. One black and one white tank were exposed to white LED light, and the two other tanks were exposed to green LED light. The initial BW and TL were 123.5 ± 1.9 g and 21.0 ± 0.1 cm, respectively ($n = 32$). Each tank contained eight fish implanted with an electronic tag. Fish were reared for 40 days from 15 June (day 0) to 25 July 2019. Fish were fed with Otohime EP-3 intermittently (40 g $tank^{-1}$ day^{-1}).

Residual feed was collected from the bottom of the tanks every day after feeding (1600–1700 hours) to calculate the amount of food intake in each tank. BW and TL were measured in anesthetized fish on day 0 and on the last day of the study.

Experiment 2: rearing of spotted halibut at different densities

We reared fish in blue square tanks (bottom surface area, 6 m^2; height, 100 cm) containing approximately 3000 l of running seawater with a water exchange rate of 173 ml s^{-1} at a controlled temperature (12.0–15.0 °C). Two of the tanks contained 80 fish (low-density groups), while the other two tanks contained 240 fish (high-density groups). The initial stocking densities of the low-density and high-density groups were 4.3 kg m^{-2} and 13 kg m^{-2}, respectively. One each of the low-density and high-density groups was exposed to green LED light. The other two tanks, which were only exposed to indoor room light, were used as controls. Twenty fish from each tank were implanted with an electronic tag. The initial BW and TL of the implanted fish were 323.6 ± 4.6 g and 30.6 ± 0.1 cm, respectively ($n = 80$). The fish were reared for 32 days from 20 December (day 0) to 21 January 2020. Fish were fed with Otohime EP-8 intermittently (300–500 g $tank^{-1}$ day^{-1} for the low-density group and 900–1500 g $tank^{-1}$ day^{-1} for the high-density group) from 0800 to 1600 hours. Tanks were cleaned daily in the afternoon. The BW and TL of the implanted fish were measured on day 32 under anesthesia.

Statistics

All the data are presented as mean ± SE. The differences in BW, TL, and CF were assessed using two-way repeated-measures ANOVA. The difference in specific growth rate (SGR) of BW and SGR of TL was assessed using two-way ANOVA. Statistical significance was accepted at P-values < 0.05. The analyses were carried out using R version 3.6.1 (R Core Team, Vienna). Two-way repeated-measures ANOVA was conducted using the R function anovakun, version 4.8.5 (Iseki 2016.

Results

Combination of white or green LED light with a white or black tank

The effects of light source (white LED or green LED) and tank color (white or black) on growth are shown in Table 1 and Fig. 1. During the 40-day rearing period, light source had a significant effect on BW, TL, SGR of BW, and SGR of TL, but tank color did not; no significant interactions between light source and tank color were found (Table 1). In comparison to white light, green light promoted somatic growth (BW, TL, SGR of BW, and SGR of TL), irrespective of tank color (Fig. 1). No significant effects of light source, tank color, or an interaction between them were detected for CF (Table 1). Moreover, no significant difference in CF was observed between the fish exposed to white and green light in both the white and black tanks (Table 3). The average daily food intake of fish that were fed for 39 days and exposed to green light was 23.9 ± 0.8 and 25.0 ± 1.0 g $tank^{-1}$ day^{-1} in the white and black tanks, respectively, while that of fish under the same conditions but exposed to white light was, respectively, 20.0 ± 0.7 and 19.8 ± 0.6 g $tank^{-1}$ day^{-1}.

Table 1 Results of the ANOVA for experiment 1 ($n = 8$, $df = 1$)

Factors	F-value	P-value
Two-way repeated-measures ANOVA for BW		
Light × time	13.3481	0.0011**
Tank color × time	0.0087	0.9263
Light × tank color × time	0.2088	0.6512
Two-way repeated-measures ANOVA for TL		
Light × time	22.3725	0.0001***
Tank color × time	1.2255	0.2777
Light × tank color × time	0.0101	0.9206
Two-way ANOVA for SGR of BW		
Light × time	14.6031	0.0007***
Tank color × time	0.0118	0.9142
Light × tank color × time	0.2820	0.5996
Two-way ANOVA for SGR of TL		
Light × time	23.1068	0.0000***
Tank color × time	1.2497	0.2731
Light × tank color × time	0.0051	0.9438
Two-way repeated-measures ANOVA for CF		
Light × time	2.3451	0.1369
Tank color × time	2.7166	0.1105
Light × tank color × time	1.6516	0.2093

BW Body weight, *TL* total length, *SGR* specific growth rate, *CF* condition factor

**$P < 0.01$, ***$P < 0.001$

Combination of green light and different stocking densities

Stocking densities on day 32 in the low-density groups, estimated from the BW of samples, were 6.7 and 7.2 kg m^{-2} under indoor room light and green LED, respectively (Table 2; Fig. 2). Those of the high-density groups under indoor room light and green LED were 20 and 22 kg m^{-2}, respectively (Table 2; Fig. 2). Light source had a significant

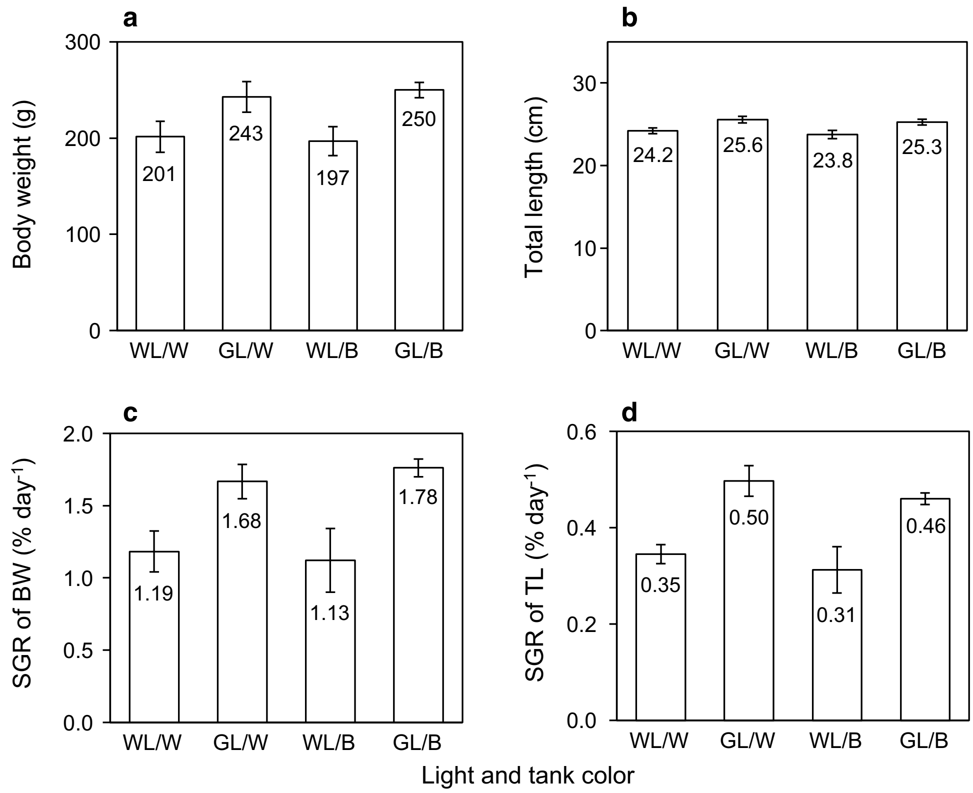

Fig. 1 Body weight (*BW*) (**a**), total length (*TL*) (**b**), specific growth rate (*SGR*) of BW (**c**), and SGR of TL (**d**) of spotted halibut in white tanks (*W*) or black tanks (*B*) irradiated for 40 days with white light (*WL*) or green light (*GL*) from a light-emitting diode (LED). All data are presented as means ± SE ($n = 8$)

Table 2 Results of the ANOVA for experiment 2 ($n = 20$, $df = 1$)

Factors	*F*-value	*P*-value
Two-way repeated-measures ANOVA for BW		
Light × time	5.5735	0.0208*
Stocking density × time	0.0610	0.8056
Light × stocking density × time	0.0465	0.8299
Two-way repeated-measures ANOVA for TL		
Light × time	8.3534	0.0050**
Stocking density × time	0.0011	0.9734
Light × stocking density × time	0.2990	0.5861
Two-way ANOVA for SGR of BW		
Light × time	6.0432	0.0162*
Stocking density × time	0.1314	0.7180
Light × stocking density × time	0.0411	0.8399
Two-way ANOVA for SGR of TL		
Light × time	8.5933	0.0045**
Stocking density × time	0.0099	0.9210
Light × stocking density × time	0.2013	0.6549
Two-way repeated-measures ANOVA for CF		
Light × time	2.7250	0.1029
Stocking density × time	0.4425	0.5079
Light × stocking density × time	1.2532	0.2665

For abbreviations, see Table 1

*$P < 0.05$, **$P < 0.01$

effect on BW, TL, SGR of BW, and SGR of TL after the 32-day rearing period, while stocking density did not; there was no significant interaction between light source and stocking density (Table 2). Compared to indoor room light, green light promoted somatic growth (BW, TL, SGR of BW, and SGR of TL), irrespective of stocking density (Fig. 2). No significant effects of light source, stocking density, or their interaction, were detected for CF (Table 2). Moreover, no significant difference in CF was observed between the fish under indoor room light and those under green light in both the low- and high-density groups (Table 3).

Discussion

The influence of tank color on green light-induced growth

Green light stimulated the growth of spotted halibut in both the white and black tanks. The average SGR of BW and SGR of TL in the green light group were more than 40% higher than those of the control group, irrespective of tank color. These results, together with those previously reported (Shimizu et al. 2019; Takahashi et al. 2016, 2018) suggest that chromatic light (peak wavelength 518 nm) is an effective stimulator of growth in flatfish such as spotted halibut, barfin founder, and Japanese flounder. Because no difference in the

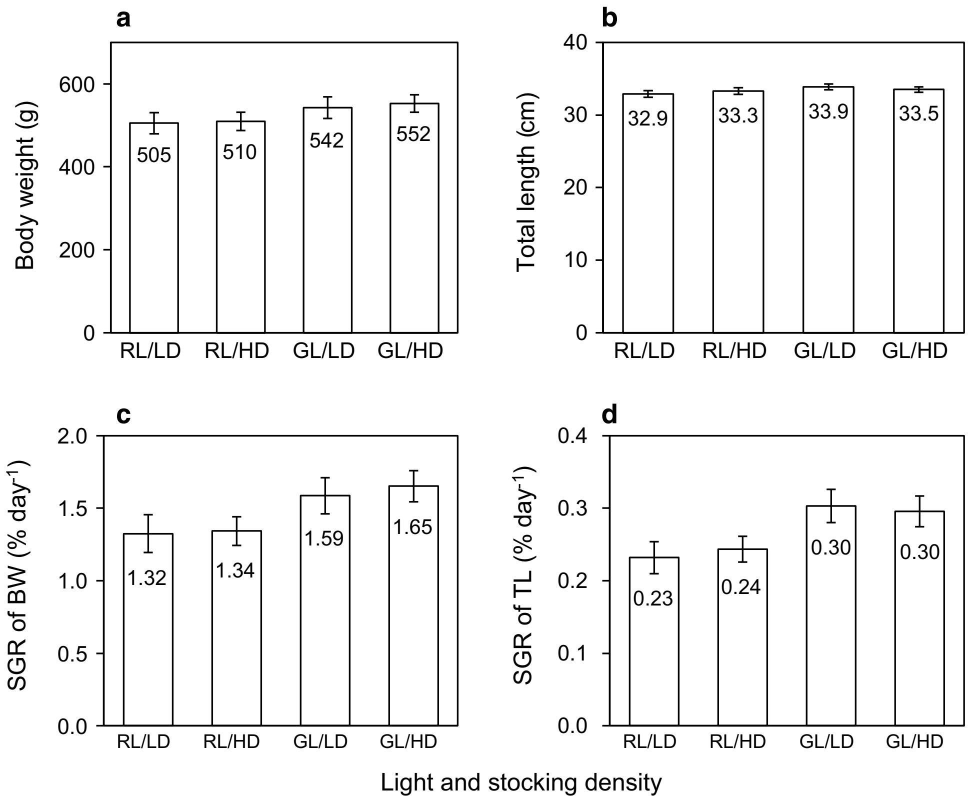

Fig. 2 BW (**a**), TL (**b**), SGR of BW (**c**), and SGR of TL (**d**) of spotted halibut under green LED light and control fish (indoor room light) maintained for 32 days at different stocking densities. All data are presented as means ± SE ($n = 20$). *RL* Room light, *GL* green light, *LD* low density, *HD* high density; for other abbreviations, see Fig. 1

Table 3 CF of spotted halibut reared under green light

Experiment/rearing condition	CF (%)
Experiment 1 (40 days)	
White LED/white tank ($n = 8$)	1.40 ± 0.05
Green LED/white tank ($n = 8$)	1.44 ± 0.02
White LED/black tank ($n = 8$)	1.45 ± 0.03
Green LED/black tank ($n = 8$)	1.55 ± 0.02
Experiment 2 (32 days)	
Room light/low density ($n = 20$)	1.39 ± 0.03
Room light/high density ($n = 20$)	1.36 ± 0.03
Green LED/low density ($n = 20$)	1.37 ± 0.03
Green LED/high density ($n = 20$)	1.45 ± 0.02

All data are presented as means ± SE. No statistically significant differences were observed ($P > 0.05$)

LED Light-emitting diode

SGR of BW and the SGR of TL was observed under green light between the fish housed in the white and black tanks, it is conceivable that different tank colors, at least white and black, do not influence the effect of green light. The similar patterns of enhanced SGR of BW, SGR of TL, and food intake under green light indicate that green light may stimulate growth through increased food intake.

In contrast to the similar growth seen here in spotted halibut reared in the white and black tanks, in other studies barfin flounder housed in white tanks fed more and grew faster than those in black tanks (Amiya et al. 2005, 2008; Sunuma et al. 2009; Takahashi et al. 2004; Yamanome et al. 2005). A growth-promoting effect of white tanks has also been observed in Japanese flounder (Kang and Kim 2013a, b). Thus, different tank colors, at least where white and black are concerned, do not seem to influence the growth of spotted halibut, in contrast to barfin flounder and Japanese flounder. We previously observed growth-promoting effects of green light on spotted halibut that were reared in a blue tank (Shimizu et al. 2020) in addition to in white and black tanks. Thus, green light is expected to promote the growth of flatfish even if conventional tanks are used, as tank color per se does not appear to influence its growth-enhancing effect.

Melanin-concentrating hormone (MCH), which regulates skin pigmentation in teleosts, acts as a neuropeptide that enhances feeding in mammals (Qu et al. 1996). The expression of MCH genes in the brain was higher in barfin flounder reared in a white tank than in a black tank (Amiya et al. 2005, 2008; Takahashi et al. 2004; Yamanome et al. 2005). These results lead to the hypothesis that MCH might be involved in the growth-promoting effect of white tanks for barfin flounder. In the present study, tank color did not affect the growth rate of spotted halibut. It remains unknown if white tanks induce an increase in MCH expression in spotted halibut or whether MCH has a feeding-enhancement effect in this flatfish. There may be differences between barfin flounder and spotted halibut in the expression of MCH or its function in the regulation of feeding.

Effect of stocking density on the impact of green light

Stocking density did not affect the growth-promoting effect of green light. No significant effects of stocking densities were observed for any of the growth parameters examined (BW, TL, SGR of BW, and SGR of TL). However, exposure to green light significantly affected these parameters, with more than 20% higher average SGR of BW and SGR of TL in the green light group compared to the control group. Although we did not monitor aggressive behavior in this study, there were no injuries or deaths in any group during the experiment, suggesting that the stocking densities used are suitable for the rearing of spotted halibut. In a previous study, we observed an increase in the growth of fish in the first 15 days of a 60-day rearing period under the same light conditions as those used in the present study, with the fish stocked in blue-colored square tanks identical to those used in the present study (Shimizu et al. 2020). In that study, the stocking density was approximately 1.8 kg m^{-2} (15 fish; 730 g individual BW), i.e., one-third of the lowest stocking density in the present study, and the water temperature fluctuated between 17.8 °C and 20.8 °C, which was higher than the temperature range in the present study (12.0–15.0 °C). Thus, we consider that even with the lowest density used here, the fish were too crowded for green light to induce a clear growth-promoting effect within the 32-day rearing period. Longer exposure to green light, in combination with a higher water temperature, should improve its effect on the growth of spotted halibut.

Effect of green light on the CF

In a previous study, we found that spotted halibut irradiated by green light at 15 °C or 18 °C had a higher CF than the controls under indoor room light (Simizu et al. 2019). This result contradicts those of the present study, even though the experimental design was almost identical. The reason for this inconsistency remains unknown. An increase in CF due to green light exposure has also been observed in barfin flounder (Takahashi et al. 2016, 2018). The results of the latter study suggested that green light might enhance anabolism. However, there is no clear evidence to support this hypothesis. The plasma levels of insulin and insulin-like growth factor I were not always altered by green light irradiation in studies undertaken by Simizu et al. (2019) and Takahashi et al. (2016, 2018). Considering that green light exposure did not increase CF in the present study, the hypothesis that green light enhances anabolism may not hold.

Conclusions

The results of the present study suggest that green light could be used to increase the production of flatfish, at least spotted halibut, in conventional indoor aquariums. An increase in growth under green light is likely to be achieved irrespective of whether the rearing tanks are white or black. Regarding the stocking density, a density of at least 13 kg m^{-2} should be acceptable; exposure to green light caused, on average, 20% greater SGR of BW and SGR of TL compared to the control group. Longer exposure to green light is expected to improve growth. Our series of recent studies support the growth-promoting effect of green light on spotted halibut under several rearing conditions, including a range of water temperatures (Shimizu et al. 2019). In contrast, although the neuroendocrine and endocrine systems involved in the growth of flatfish are evoked by green light, and MCH is a candidate hormone linking green light to somatic growth (Shimizu et al. 2019; Takahashi et al. 2016, 2018), little is known about the physiological mechanisms underlying the effect of green light on growth. The biological processes related to an increase in growth which are influenced by green light have yet to be clarified.

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