Fish Sci (2012) 78:549–556
DOI 10.1007/s12562-012-0482-8

ORIGINAL ARTICLE

Biology

Effects of LED light spectra on the growth of the yellowtail clownfish *Amphiprion clarkii*

Hyun Suk Shin · Jehee Lee · Cheol Young Choi

Received: 1 October 2011 / Accepted: 13 February 2012 / Published online: 11 March 2012

Abstract Growth hormone (GH) is an essential polypeptide required for the normal growth and development of vertebrates. We have studied the effects of light-emitting diodes (LEDs) emitting different spectra (red, green, and blue) on the GH of yellowtail clownfish *Amphiprion clarkii*. Full-length GH cDNA from the pituitary of the yellowtail clownfish was first cloned and then the expression of GH mRNA under different light spectra was measured. GH mRNA expression was significantly higher under green and blue light than under red light spectra. These results indicate that in yellowtail clownfish, short-wavelength LED enhances growth more than long-wavelength LED, and that LED lights are more effective for enhancing growth than white fluorescent bulbs. Injection of melatonin resulted in significantly higher expression levels of GH mRNA compared to the control. We therefore conclude that green and blue light enhance GH levels and that melatonin plays a role in modulating growth of the yellowtail clownfish.

Keywords Light-emitting diodes · Light spectra · Melatonin · Growth · Yellowtail clownfish

H. S. Shin · C. Y. Choi (✉)
Division of Marine Environment and Bioscience,
Korea Maritime University, Yeongdo-gu, Busan 606-791,
Republic of Korea
e-mail: choic@hhu.ac.kr

J. Lee
Division of Marine Life Sciences, Jeju National University,
Jeju, Jeju Special Self-Governing Province 690-756,
Republic of Korea
e-mail: jehee@jejunu.ac.kr

Introduction

The influence of environmental factors on the growth and reproduction of fish has been studied extensively for more than 10 years. Salinity, pH, and oxygen availability are known to play a major role on the capacity to develop and grow, but light is not only essential to almost all life, both plants and animals, on earth, it also modulates a number of physiological and behavioral changes that occur within a 24-h period as part of the circadian rhythm [1]. Specifically, daily light conditions affect the survival and growth of fish [2, 3]. Under lighting regimes, the somatic growth of organisms, including fish, is an integral biological process, and measures of growth may serve as indicators of organismal fitness, since growth is related to the maintenance of homeostasis [4].

Growth, food intake, and digestion are related to specific behavioral rhythms, including reproduction, with pineal hormones (such as melatonin) being the assumed regulators of these mechanisms [5, 6]. For example, the administration of melatonin to goldfish maintained under a short (but not long) photoperiod for several days caused an accelerated weight gain and growth [7], and melatonin implants increased the body weight of Atlantic salmon parr *Salmo salar* [8].

Growth hormone (GH), which is required for the normal growth of vertebrates, is synthesized in the pituitary gland and functions to control growth through the complex modulation of various metabolic processes [9]. GH also promotes growth in vertebrates, including fish, through various endocrine and environmental factors (i.e., salinity, pH, oxygen availability, light) that play a role in the physiological environment [10]. Another hormone that promotes growth is melatonin [11]. This hormone modulates GH synthesis, and it is primarily controlled by light.

Melatonin may also modulate the central neural pathways involved in the regulation of GH synthesis, as well as control the circadian rhythm of organisms [12]. It has also been shown to affect the physiological processes involved in the growth and development of goldfish *Carassius auratus* [13].

In some cases, color is added to rearing environments by painting the tanks or using colored lights in an attempt to increase the visibility of food and thereby increase growth rates by increasing food intake [14]. For example, the survival rate of haddock larvae *Melanogrammus aeglefinus* L. is higher under blue and green light conditions [15], and the growth rate of silver carp larvae *Hypophthalmichthys molitrix* Val. and young carp *Cyprinus carpio* L. increases under green light conditions [16, 17].

Light-emitting diodes (LEDs) represent a new form of lighting technology that is still being developed. LEDs can be manufactured to output specific wavelengths [18]. Consequently, by emitting narrow bandwidth light, especially short high-energy wavelengths, LEDs might provide a much more efficient lighting system than those currently used by the fish farming industry because they can be adjusted to the environmental sensitivity of a given species [19]. The spectral composition of incident light changes differentially in underwater environments, with the short or blue end of the visible spectrum becoming predominant in deeper waters and the long or red end of the visible spectrum predominantly penetrating shallow waters [20, 21]. From a practical perspective, because there is a rapid attenuation in light intensity with increasing depth, a particular LED wavelength can be applied according to the species of interest and the water environment. Yamanome et al. [22] reported that barfin flounder *Verasper moseri* reared under light consisting of long-wavelengths, such as green and blue light (fluorescent lamp), showed rapid growth, while in Nile tilapia *Oreochromis niloticus*, the blue light spectrum was found to prevent stress [23]. In contrast, compared to short wavelengths, certain long wavelengths, such as red light, have been found to induce stress in yellowtail clownfish [24]. However, LED studies have only recently begun to address the effects of photoperiod and light intensity in fish [1, 19, 25], and the number of investigations carried out to date on the effects of LEDs on the growth of fish is limited [19, 26].

The clownfish is a popular ornamental seawater fish [27]. The rapid growth of ornamental fish aquaculture, such as clownfish production, is an important component of aquaculture in Korea. To understand the effects of different LED spectra on growth and GH mRNA expression levels, we reared yellowtail clownfish under different LED lights (i.e., red, green, and blue) and investigated growth and diurnal changes in GH mRNA expression levels under the different light conditions. We also examined the effects of LED lights on GH mRNA expression levels under different LED spectra following melatonin injection in order to understand the growth-promoting effect of melatonin.

Materials and methods

Experimental fish and conditions

Yellowtail clownfish ($n = 300$, length 3.5 ± 0.3 cm, weight 2.1 ± 0.5 g) were purchased from the Center of Ornamental Reef and Aquarium (CCORA, Jeju, Korea) and then allowed to acclimate for 2 weeks in twelve 300-l circulation filter black-frosted tanks in the laboratory. The fish were exposed to a simulated natural photoperiod (SNP). Fish exposed to a white fluorescent bulb (27 W) constituted the control group; light intensity near the water surface of the tanks was approximately 0.96 W/m^2. The water temperature and photoperiod were 27 ± 1 °C and 12/12 h (light/dark), respectively, with light supplied between 0700 hours and 1900 hours. The fish were fed a commercial feed twice daily (0900 hours and 1700 hours) at a rate of 4–5 % of wet body weight per day. pH was 7.8–7.9, and ammonia was not detected (0 ppm) in the water. For the experimental groups, the fish were exposed to either blue (peak at 450 nm), green (530 nm), or red (630 nm) LEDs (Daesin LED Co. Kyunggi, Korea) (Fig. 1). The LEDs were set 50 cm above the water surface, and the irradiance at the water surface was maintained at approximately 0.9 W/m^2. The fish were reared under these conditions for 6 months. Prior to each experiment, the fish were anesthetized with 200 mg/l tricaine methanesulfonate (MS-222; Sigma, St. Louis, MO) and then euthanized by spinal transection at 4-h intervals in order to collect the pituitary under dim light.

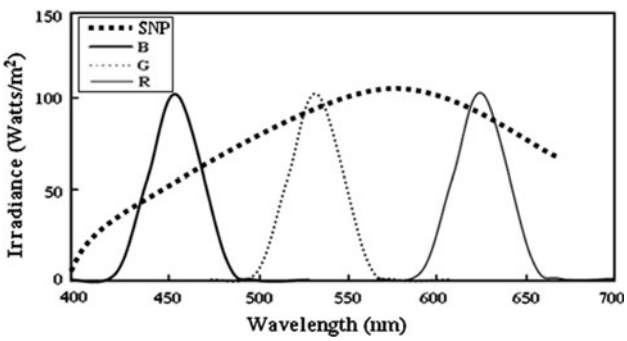

Fig. 1 Spectral profiles of blue (*B*), green (*G*), and red (*R*) light-emitting diodes (LEDs) used in this study. The *filled square–dotted line* Spectral profile of white fluorescent light (*SNP* simulated natural photoperiod)

Isolation of GH cDNA

Primers for GH were designed using highly conserved regions from fire clownfish *Amphiprion melanopus* GH (GenBank accession no. ADJ57589) and green sunfish *Lepomis cyanellus* GH (AAS20461) sequences. GH forward (5′-TTT GCA GAC GGA GGA ACA AC-3′) and reverse (5′-GGC GAC AGT CGA CAT TTA GC-3′) primers were used. Total RNA was extracted from the pituitary gland using a TRIzol kit (Gibco/BRL, Gaithersburg, MD). Reverse transcription (RT) was performed using M-MLV reverse transcriptase (Bioneer, Daejeon, Korea) according to the manufacturer's instructions. PCR amplification was performed using a Takara Taq DNA polymerase (Takara, Tokyo, Japan) with the following cycling conditions: initial denaturation at 95 °C for 2 min; 40 cycles of denaturation at 95 °C for 20 s, annealing at 56 °C for 40 s, and extension at 72 °C for 1 min; a final extension at 72 °C for 7 min. The PCR product was separated in 1% agarose gels, purified, and ligated into a pGEM-T Easy Vector (Promega, Madison, WI). A colony that formed after transformation was cultivated in DH5α (RBC Life Sciences, Seoul, Korea), and plasmid DNA was extracted using a LaboPass Plasmid DNA Purification kit (Cosmo, Seoul, Korea). The GH cDNA sequence data were analyzed using an ABI 3100 DNA Sequencer (Applied Biosystems, Foster City, CA).

GH rapid amplification of cDNA 3′ and 5′ ends

For the GH rapid amplification of cDNA ends (RACE) reaction, total RNA was extracted from the pituitaries using a TRIzol kit (Gibco/BRL). Using 2.5 μg of total RNA as a template, 3′ RACE cDNA and 5′ RACE cDNA were synthesized using a CapFishing™ full-length cDNA Premix kit (Seegene, Seoul, Korea). First-strand cDNA synthesis was conducted using an oligo (dT) anchor primer (5′-CTG TGA ATG CTG CGA CTA CGA $T(T)_{18}$-3′) and a CapFishing™ adaptor (Seegene, Seoul, Korea).

GH-specific primers were designed based on the sequences of the PCR product amplified by the reverse transcription (RT)-PCR. For 3′ RACE, the PCR reaction mixture (50 μl) contained 5 μl of 3′ RACE cDNA, 1 μl of 10 mM 3′ RACE target primer (5′-CTG TGA ATG CTG CGA CTA CGA T-3′), 1 μl of 10 mM 3′ RACE GH-specific primer (5′-CAC ATC CGA TCA CGG TGG AAA CAT GTA C-3′), and 25 μl of SeeAmp Taq Plus Master Mix (Seegene). The PCR was performed as follows: initial denaturation at 94 °C for 5 min; 40 cycles of denaturation at 94 °C for 40 s, annealing at 62 °C for 40 s, and extension at 72°C for 1 min; a final extension at 72 °C for 5 min.

For 5′ RACE, the PCR reaction mixture (50 μl) contained 5 μl of 5′ RACE cDNA, 1 μl of 10 mM 5′ RACE target primer (5′-GTC TAC CAG GCA TTC GCT TCA T-3′), 1 μl of 10 mM 5′ RACE GH-specific primer (5′-TGA ACG TGG CTG CAG CGT TCT CTC TC-3′), and 25 μl of SeeAmp Taq Plus Master Mix. The PCR was carried out as follows: initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 40 s, annealing at 62°C for 40 s, and extension at 72°C for 1 min; a final extension at 72°C for 5 min. The PCR product was processed by electrophoresis in 1% agarose gels. Transformation and sequencing were performed as described above.

Sequence comparisons

The BLAST algorithm (Blastp) of the National Center for Biotechnology Information was used to compare sequences between the yellowtail clownfish and other teleosts.

Quantitative PCR

Quantitative PCR (QPCR) was conducted to determine the relative expression levels of GH mRNA using total RNA extracted from the pituitary. The following QPCR primers were designed with reference to known sequences of the yellowtail clownfish: GH forward (5′-CTC CAG TTG GCT CCT TAT G-3′) and reverse (5′-CAC CGT CAA GTA TGT CTC C-3′) primers; β-actin forward (5′-CCA ACA GGG AGA AGA TGA C-3′) and reverse (5′-TAC GAC CAG AGG CAT ACA-3′) primers. The GH forward primer position was at 448–466 and the reverse position was at 555–573; the β-actin forward primer position was at 128–146 and the reverse position was at 212–229. PCR amplification was conducted using a Bio-Rad iCycler iQ Multicolor Real-time PCR Detection System (Bio-Rad, Hercules, CA) and an iQ™ SYBR Green Supermix (Bio-Rad), according to the manufacturer's instructions. The cycling program for the QPCR was: 95°C for 5 min, followed by 35 cycles each of 95°C for 20 s and 55°C for 20 s. As an internal control, the experiments were duplicated with β-actin, with all the data expressed as the change relative to the corresponding calculated β-actin threshold cycle (Ct) levels. The efficiencies were found to be 95.3 % for β-actin 97.0 % for GH. All data were expressed as change with respect to the corresponding calculated β-actin cycle threshold (ΔC_t) levels. The calibrated ΔC_t value ($\Delta\Delta C_t$) for each sample and internal control (β-actin) was calculated [$\Delta\Delta C_t = 2\text{^}-(\Delta C_t$ sample $- \Delta C_t$ internal control)]. In addition, to ensure that the primers amplified a specific product, we performed a melt curve and found melting at only one temperature.

Melatonin injection

Melatonin (Sigma) was dissolved and diluted in 0.9% physiological saline. After being anesthetized, the

yellowtail clownfish (3.5 ± 0.3 g) were intraperitoneally injected with 200 μg melatonin/g body weight (BW) at a volume of 10 μl/g BW. We injected the fish at 0700 hours, with five fish subsequently being sacrificed by decapitation at 1100 hours (Zeitgeber time, ZT4), 1500 hours (ZT8), 1900 hours (ZT12), 2300 hours (ZT16), 0300 hours (ZT20), 0700 hours (ZT24), and 1100 hours (ZT28), respectively. After injection, the pituitary was sampled from five fish at 4-h intervals from ZT4 to ZT28, respectively. All of the fish survived the experimental period.

Statistical analysis

All data were analyzed using the SPSS statistical package (ver. 10.0; SPSS, Chicago, IL). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to assess statistically significant differences among the different time-points of daily and circadian variation and the different light spectra. A value of $P < 0.05$ was considered to be statistically significant. All values are presented as means ± standard deviation ($n = 5$).

Results

Identification of full-length GH cDNA

A single PCR product of the expected size (428 bp) was obtained by RT-PCR. A PCR-based cloning strategy (RT-PCR followed by 3′ and 5′ RACE) was then used to clone the full-length cDNA encoding GH. The full-length GH cDNA contained a reading frame that was predicted to encode a protein of 204 amino acids (JN008015). On comparing the amino acid sequence of yellowtail clownfish GH with those deduced from the GH cDNAs of other teleost species, we determined that the yellowtail clownfish GH sequence contained the conserved signal peptide (Met^{1}-Ser^{17}) and the three putative N-glycosylation sites (Asn^{201}-Cys^{202}-Thr^{203}). The four cysteine (Cys) residues in yellowtail clownfish GH are located at conserved positions (69, 177, 194, and 202). The yellowtail clownfish GH amino acid sequence showed 99% similarity with the fire clownfish GH (ADJ57589), 94% with the gilthead seabream GH (AAA03329), 93% with the green sunfish GH (AAS20461), and 93% with the ballyhoo GH (AAV48597) (Fig. 2).

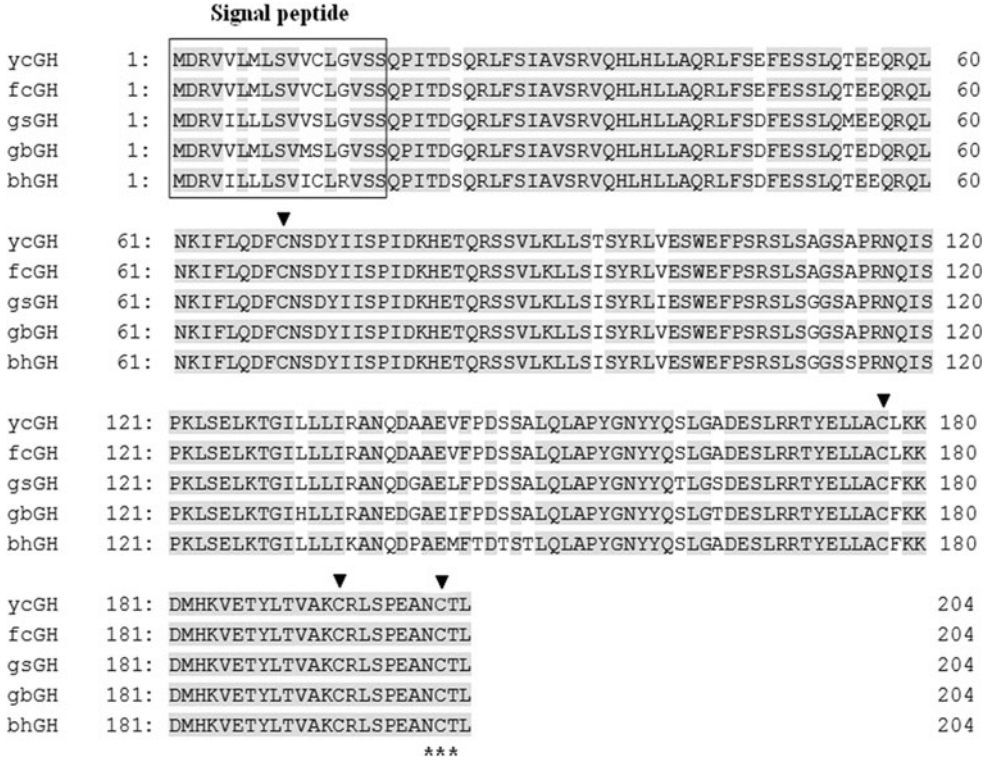

Fig. 2 Comparison of the growth hormone (GH) amino acid sequence of yellowtail clownfish *Amphiprion clarkii*, fire clownfish *Amphiprion melanopus*, green sunfish *Lepomis cyanellus*, gilthead seabream *Sparus aurata*, and ballyhoo *Hemiramphus brasiliensis* optimally aligned to match identical residues, as indicated by the *shaded box*. *Line-box* Signal peptide of the GH-encoding sequence. Putative N-glycosylation sites (Asn-X-Ser/Thr) are marked by *asterisks*, cysteine (Cys) residues are marked by *closed inverted triangles*. The sequences were taken from the GenBank/EMBL/DDBJ sequence database. The GenBank accession numbers for the GH sequences used for alignment are as follows: yellowtail clownfish (*ycGH*, JN008015), fire clownfish (*fcGH*, ADJ57589), green sunfish (*gsGH*, AAS20461), gilthead seabream (*gsbGH*, AAA03329), and ballyhoo (*bhGH*, AAV48597)

Expression of GH mRNA in the pituitary during the daily rhythm

We examined the effect of the different LED light spectra on the expression of GH mRNA in the pituitary by QPCR (Fig. 3). GH mRNA was expressed at significantly higher levels during the scotophase than during the photophase for all light spectra. In addition, the expression of GH mRNA was significantly higher in the pituitary of fish in the green and blue LED groups than in those in the red and SNP groups (Fig. 3a).

Following the injection of melatonin into living fish, the GH expression patterns of the different LED groups during the daily rhythm were similar to those of the control (i.e., without injection) LED groups. However, the expression levels of the GH gene in the melatonin-injected LED groups were significantly higher than that of the control LED groups (Fig. 3b).

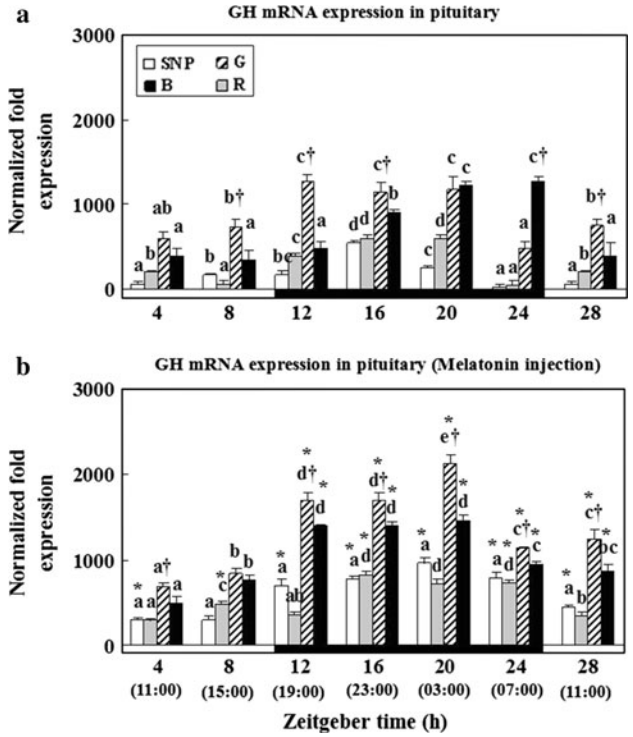

Fig. 3 Changes in the expression levels of GH mRNA in the pituitary before (**a**) and after injection with melatonin (**b**) under lighting conditions using red (*R*), green (*G*), and blue (*B*) LEDs and a simulated natural photoperiod (SNP), as measured by quantitative real-time PCR. The fish were reared under a light:dark (LD) cycle (12:12 h). Total pituitary RNA (2.5 μg) was reverse-transcribed and amplified. The results are expressed as normalized fold expression levels with respect to the *β*-actin levels in the same sample. *White bar* Photophase, *black bar* scotophase. The *dagger symbol* indicates significant differences between different light spectra within the same Zeitgeber time (ZT) ($P < 0.05$), *asterisks above the letters* indicate significant differences between the control (i.e., without melatonin injection) and melatonin-injected group ($P < 0.05$). All values are presented as the mean ± standard deviation ($n = 5$)

Total length

The total lengths of the fish under green and blue LED conditions were significantly higher compared to those under other light conditions (Table 1). At the end of the experiment (6 months), the green and blue LED groups had the greatest total body length (6.1 ± 0.4 and 6.2 ± 0.2 cm, respectively), while the red LED and SNP groups had the shortest total body length (4.9 ± 0.3 and 4.8 ± 0.5 cm, respectively).

Discussion

In this study, we examined the growth of yellowtail clownfish under different LED spectra. We also determined the effect of melatonin on the growth effects induced by specific LED wavelengths by examining diurnal changes in GH mRNA expression levels following the intraperitoneal injection of melatonin.

Analysis of the full-length GH cDNA isolated from the pituitary of yellowtail clownfish revealed that it had a high identity (93–99%) with those of other species (Fig. 2). Li et al. [28] reported that the first 17 amino acid residues (Met^1-Ser^{17}) at the N terminus are highly hydrophobic in GH of the orange-spotted grouper *Epinephelus coioides*

Table 1 Changes in the total length of yellowtail clownfish reared under a simulated natural photoperiod and red, blue, and green light-emitting diode lights

Months reared until LEDs	Total length of yellowtail clownfish (cm)			
	SNP	Red	Blue	Green
0	3.5 ± 0.3^{a1}	3.5 ± 0.3^{a1}	3.5 ± 0.3^{a1}	3.5 ± 0.3^{a1}
2	3.7 ± 0.2^{a1}	3.8 ± 0.4^{ab1}	4.2 ± 0.3^{b2}	4.1 ± 0.2^{ab1}
4	3.9 ± 0.3^{a1}	4.0 ± 0.3^{a1}	5.2 ± 0.4^{b3}	5.3 ± 0.3^{b2}
6	4.8 ± 0.5^{a2}	4.9 ± 0.3^{a2}	6.2 ± 0.2^{b4}	6.1 ± 0.4^{b2}

Different lowercase letters indicate significant differences between lights of different wavelengths; different numbers indicate significant differences between the start of the experiment and after 6 months of rearing ($P < 0.05$)

SNP Simulated natural photoperiod, *LEDs* light-emitting diodes

and that that this sequence has a high degree of homology to the signal peptide sequence of other fish GHs. The positions of four Cys residues in yellowtail clownfish GH (Cys^{69}, Cys^{177}, Cys^{194}, and Cys^{202}) were nearly the same as those in other vertebrate GHs, whereby two disulfide bonds were formed to contribute to the tertiary structure/hormone binding/hormone receptor interaction [28]. One possible site for N-glycosylation (Asn-X-Ser/Thr motif) is present at Asn^{201} in the predicted amino acid sequence of the yellowtail clownfish GH [29]. Therefore, we examined yellowtail clownfish GH with respect to the GHs of other teleosts for the presence of the signal peptide and the putative N-glycosylation sites and found them to be present.

We observed that the expression levels of GH RNA in the pituitary of yellowtail clownfish were significantly higher in the green and blue LED groups than in the red LED and control groups during the diurnal rhythm (Fig. 3a). In addition, yellowtail clownfish reared for 6 months under these different light regimes showed a significantly rapid growth rate about 74 and 77 % under the green and blue LED conditions, respectively, whereas the growth rates were 37 and 40% under control and red LED conditions, respectively (Table 1). Blanco-Vives et al. [26] reported that the exposure of Senegal sole *Solea senegalensis* larvae to red light resulted in a delay in growth, with the yolk sac remaining visible, whereas under the blue light treatment the yolk sac was completely absorbed. Based on these results, blue light appeared to be the most efficient light spectrum for the development of Senegal sole larvae. Using a similar experimental design for European sea bass *Dicentrarchus labrax*, Villamizar et al. [19] observed that the growth rate and feeding performance of larvae were significantly higher under the blue treatment than under to red or white treatments. These researchers suggested that blue wavelengths provide sufficient stimulus to the visual system for the larvae to feed well. The difference in photoperiodic response under the same intensity of light at different wavelengths is due to a difference in the number of photons received by the photoreceptors [30]. In another study, Migaud et al. [18] reported that LEDs of the blue–green spectrum might be the most suitable for fishes because these wavelengths generally penetrate seawater more efficiently. For example, the maximal growth rate of crucian carp and rotan occurs under both green and blue light [31]. This process is primarily regulated by the eyes and pineal organ because only these organs can detect color [32, 33]. It is known, for example, that crucian carp are able to detect colors at an early stage of development. However, it is more difficult to distinguish between blue and green colors than green and red colors. In other words, if there is greater contrast between colors, detection is easier. Ruchin [31] suggested that the negative effect of red light on growth rate was induced through changes in energy metabolism, endocrinology, and/or physiology. These various findings support our results that the rapid growth rate was rapid under green and blue LED spectra, leading to the conclusion that short wavelengths, such as green and blue, effectively enhance the growth of adult fish as well as larvae.

In this study, although changes in the GH mRNA expression patterns were similar between melatonin-injected and non-injected fish, the GH mRNA expression levels were significantly higher in the melatonin-injected group (Fig. 3a). These results support the findings of John et al. [12], who reported the stimulation of GH secretion by melatonin in Japanese quail *Coturnix japonica*. The cause of these changes is still unclear, but de Vlaming [7] reported a concomitant increase in growth with increasing doses of melatonin in goldfish, leading him to theorize a functional relationship between growth and melatonin. In addition, the localization of 2-[^{125}I]-iodomelatonin binding sites (melatonin binding site) in the brain revealed high levels of melatonin binding within the preoptic area, which has also been shown to contain neurons that are immunoreactive for GH-releasing factor [34, 35]. The effects of melatonin on growth may thus result from the differential impact of the hormone on GH, and perhaps on other pituitary hormones. In addition to melatonin having a direct effect on the pituitary, it might also modulate fish feeding and growth through controlling the production of releasing and inhibiting factors by neurons from the preoptic area and hypothalamic nuclei, as well as by directly targeting peripheral tissues [36]. Furthermore, salmonid fish are susceptible to certain wavelengths at which the pineal photoreceptor cell integrates the light signal, which allows melatonin production to be controlled in an on/off manner [37–39]. Melatonin also contributes to the control of GH secretions [40]. These findings led us to hypothesize that light of different wavelengths controls melatonin secretion, with melatonin production subsequently affecting GH.

Shin et al. [24] reported that green and blue LED spectra reduce stress relative to red LED spectra in yellowtail clownfish, while Schlenk and Rice [41] reported that stress inhibits the growth of fish. These results indicate that the stress-reducing effect of short wavelengths, such as green and blue LEDs, promotes the growth of fish.

In conclusion, we showed a component of the relationship among different light wavelengths, GH expression, and melatonin in ornamental yellowtail clownfish by using LEDs, which are a new form of lighting technology. This study indicated that green and blue spectra promote growth in yellowtail clownfish and that melatonin stimulates GH secretion, consequently playing a role in promoting fish growth. Therefore, our study results might be applied to an

artificial photic system aimed at effectively enhancing the growth of fish reared under captive conditions.

Acknowledgments This research was supported by Technology Development Program for Fisheries, Ministry for Food, Agriculture, Forestry and Fisheries, Korea, and by the MKE, under the ITRC support program supervised by the NIPA (2012-C1090-1221-0015).

References

1. Pierce LX, Noche RR, Ponomareva O, Chang C, Liang JO (2008) Novel function for period 3 and exo-rhodopsin in rhythmic transcription and melatonin biosynthesis within the zebrafish pineal organ. Brain Res 1223:11–24
2. Barahona-Fernandes MH (1979) Some effects of light intensity and photoperiod on the sea bass larvae (*Dicentrarchus labrax*) reared at the Centre Oceanologique de Bretagne. Aquaculture 17:311–321
3. Tandler A, Helps S (1985) The effects of photoperiod and water exchange on growth and survival of gilthead sea bream (*Sparus aurata*, Linnaeus; Sparidae) from hatching to metamorphosis in mass rearing systems. Aquaculture 48:71–82
4. MacKenzie DS, Vanputte CM, Leiner KA (1998) Nutrient regulation of endocrine function in fish. Aquaculture 161:3–25
5. Underwood H (1989) The pineal and melatonin: regulators of circadian function in lower vertebrates. Experientia 45:914–922
6. Zachmann A, Falcón J, Knijff SCM (1992) Effects of photoperiod and temperature on rhythmic melatonin secretion from the pineal organ of the white sucker (*Catostomus commersoni*) in vitro. Gen Comp Endocrinol 86:26–33
7. de Vlaming VD (1980) Effects of pinealectomy and melatonin treatment on growth in the goldfish, *Carassius auratus*. Gen Comp Endocrinol 40:245–250
8. Porter MJR, Duncan NJ, Mitchell D, Bromage NR (1998) The use of cage lighting to reduce plasma melatonin in Atlantic salmon (*Salmo salar*) and its effects on the inhibition of grilsing. Aquaculture 176:237–244
9. Canosa LF, Chang JP, Peter RE (2007) Neuroendocrine control of growth hormone in fish. Gen Comp Endocrinol 151:1–26
10. Chen TT, Marsh A, Shamblott M, Chan KM, Tang YL, Cheng CM, Yang BY (1994) Structure and evolution of fish growth hormone and insulin-like growth factors genes. In: Sherwood NM, Hew CL (eds) Molecular endocrinology of fish, fish physiology. Academic Press, Bethesda, pp 179–209
11. Zeman M, Výboh P, Juráni M, Lamošová D, Koštál B, Bilčík B, Blažíček p, Jurániová E (1993) Effects of exogenous melatonin on some endocrine, behavioral and metabolic parameters in Japanese quail *Coturnix coturnix japonica*. Comp Biochem Physiol 105A:323–328
12. John TM, Viswanathan M, George JC, Scanes CG (1990) Influence of chronic melatonin implantation on circulating levels of catecholamines, growth hormone, thyroid hormones, glucose, and free fatty acids in the pigeon. Gen Comp Endocrinol 79:226–232
13. Bromage N, Porter M, Randall C (2001) The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. Aquaculture 197:63–98
14. Ullmann JFP, Gallagher T, Hart NS, Barnes AC, Smullen RP, Collin SP, Temple SE (2011) Tank color increases growth, and alters color preference and spectral sensitivity, in barramundi (*Lates calcarifer*). Aquaculture 322–323:235–240
15. Downing G (2002) Impact of spectral composition on larval haddock, *Melanogrammus aeglefinus* L., growth and survival. Aquacult Int 33:251–259
16. Radenko VN, Alimov IA (1991) Importance of temperature and light to growth and survival of larval silver carp, *Hypophthalmichthys molitrix*. Vopr Ikhtiol 31:655–663
17. Ruchin AB, Vechkanov VS, Kuznetsov VA (2002) Growth and feeding intensity of young carp *Cyprinus carpio* under different constant and variable monochromatic illuminations. J Ichthyol 42:191–199
18. Migaud H, Cowan M, Taylor J, Ferguson HW (2007) The effect of spectral composition and light intensity on melatonin, stress and retinal damage in post-smolt Atlantic salmon, *Salmo salar*. Aquaculture 270:390–404
19. Villamizar N, García-Alcazar A, Sánchez-Vázquez FJ (2009) Effect of light spectrum and photoperiod on the growth, development and survival of European sea bass (*Dicentrarchus labrax*) larvae. Aquaculture 292:80–86
20. Lythgoe JN, Muntz WRA, Partridge JC, Shand J, Williams DM (1994) The ecology of visual pigments of snappers (Lutjanidae) on the Great Barrier Reef. J Comp Physiol 174A:461–467
21. Myrberg AA Jr, Fuiman LA (2002) The sensory world of coral reef fishes. In: Sale PF (ed) Coral reef fishes. Academic Press, San Diego, pp 123–148
22. Yamanome T, Mizusawa K, Hasegawa E, Takahashi A (2009) Green light stimulates somatic growth in the barfin flounder, *Verasper moseri*. J Exp Zool 311A:73–79
23. Volpato GL, Barreto RE (2001) Environmental blue light prevents stress in the fish Nile tilapia. Braz J Med Biol Res 34:1041–1045
24. Shin HS, Lee JH, Choi CY (2011) Effects of LED light spectra on oxidative stress and the protective role of melatonin in relation to the daily rhythm of the yellowtail clownfish, *Amphiprion clarkii*. Comp Biochem Physiol 160A:221–228
25. Boeuf G, Le Bail PY (1999) Does light have an influence on fish growth? Aquaculture 177:129–152
26. Blanco-Vives B, Villamizar N, Ramos J, Bayarri MJ, Chereguini O, Sánchez-Vázquez FJ (2010) Effect of daily thermo- and photo-cycles of different light spectrum on the development of Senegal sole (*Solea senegalensis*) larvae. Aquaculture 306:137–145
27. Tissot BN, Best BA, Borneman EH, Bruckner AW, Cooper CH, D'Agnes H, Fitzgerald TP, Leland A, Lieberman S, Amos AM, Sumaila R, Telecky TM, McGilvray F, Plankis BJ, Rhyne AL, Roberts GG, Starkhouse B, Stevenson TC (2010) How U.S. ocean policy and market power can reform the coral reef wildlife trade. Mar Policy 34:1385–1388
28. Li WS, Chen D, Wong AOL, Lin HR (2005) Molecular cloning, tissue distribution, and ontogeny of mRNA expression of growth hormone in orange-spotted grouper (*Epinephelus coioides*). Gen Comp Endocrinol 144:78–89
29. Law MS, Cheng KW, Fung TK, Chan YH, Yu KL, Chan KM (1996) Isolation and characterization of two distinct growth hormone cDNAs from the goldfish, *Carassius auratus*. Arch Biochem Biophys 330:19–23
30. Vriend J, Lauber JK (1973) Effects of light intensity, wavelength and quanta on gonads and spleen of the deer mouse. Nature 244:37–38
31. Ruchin AB (2004) The effect of light regime on feeding intensity and growth rate in fishes (in Russian). Hydrobiologichesky Zhurn (Kiev) 40:48–52
32. Levin J, McNicol E (1982) Color vision in fishes. Sci Am 246:108–117
33. Ekstrom P, Meissl H (1997) The pineal organ of teleost fishes. Rev Fish Biol Fish 7:199–284
34. Porter MJR, Randall CF, Bromage NR, Thorpe JE (1998) The role of melatonin and the pineal gland on development and smoltification of Atlantic salmon (*Salmo salar*) parr. Aquaculture 168:139–155

35. Falcón J, Migaud H, Muñoz-Cueto JA, Carrillo M (2010) Current knowledge on the melatonin system in teleost fish. Gen Comp Endocrinol 165:469–482
36. Schreck CB (1993) Glucocorticoids: metabolism, growth and development. In: Schreibman MP, Scanes CG, Pang PKT (eds) The endocrinology of growth, development, and metabolism in vertebrates. Academic Press, San Diego, pp 367–392
37. Falcón J (1999) Cellular circadian clocks in the pineal. Prog Neurobiol 8:121–162
38. Bayarri MJ, Madrid JA, Sánchez-Vázquez FJ (2002) Influence of light intensity, spectrum and orientation on sea bass plasma and ocular melatonin. J Pineal Res 32:34–40
39. Iigo M, Abe T, Kambayashi S, Oikawa K, Masuda T, Mizusawa K, Kitamura S, Azuma T, Takagi Y, Aida K, Yanagisawa T (2007) Lack of circadian regulation of in vitro melatonin release from the pineal organ of salmonid teleosts. Gen Comp Endocrinol 154:91–97
40. Falcón J, Besseau L, Fazzari D, Attia J, Gaildrat P, Beauchaud M, Boeuf G (2003) Melatonin modulates secretion of growth hormone and prolactin by trout pituitary glands and cells in culture. Endocrinology 144:4648–4658
41. Schlenk D, Rice CD (1998) Effects of zinc and cadmium treatment on hydrogen peroxide mortality and expression of glutathione and metallothionein in a teleost hepatoma cell line. Aquat Toxicol 43:121–129