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Effects of photoperiod and light Spectrum on growth performance, digestive enzymes, hepatic biochemistry and peripheral hormones in spotted sea bass (*Lateolabrax maculatus*)

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ABSTRACT

Growth performance, digestive and metabolic activities, and contents of peripheral hormones of spotted sea bass (*Lateolabrax maculatus*) juveniles were evaluated under natural light and three different light spectrums (white, blue and red) in combination with three photoperiods (light: dark cycle, 12: 12-h, 18: 6-h and 24: 0-h). Bass in 18-h blue light environment displayed the best growth performance and digestive enzyme activities, while red light environment significantly impeded growth and digestive enzyme activities. Altered contents of melatonin, cortisol, thyroid hormones (T_3 and T_4), and testosterone (T) were observed in bass reared in red light, suggesting that red light could disturb endocrine homeostasis associated with biological rhythm (melatonin), stress coping (melatonin and cortisol), growth and development (T_3 and T_4), and aggressive behavior or hyperactivity (T_3, T_4 and T). Impaired growth performance might be due to energy used to cope with stress. We concluded that the red spectrum environment was stressful to spotted bass and the selection of appropriate light conditions (such as 18-h blue light) might lead to a beneficial outcome for spotted sea bass culture.

1. Introduction

Light is one of the multiple important environmental factors that regulate growth, development, maturity and other physiological processes from humans to teleosts. Intensity, spectrum and photoperiod are three fundamental characteristics of light that impact physiology. Light intensity regulates human circadian rhythm and change in light intensity may affect alertness, body temperature, heart rate and melatonin secretion (Cajochen et al., 2005). In rodent, increased light intensity prevents loss of vasopressin-expressing neurons in aged rat (Lucassen et al., 1995). In chickens, high intensity light promotes weight gain with lower percentage of body fat and higher percentage of body protein (Charles et al., 1992). Blue spectrum significantly decreases heart rate and heart rate variability in human, indicating a significant alteration of the emotional state (Litscher et al., 2013). Light spectrum regulates endocrine glands including pineal gland, adrenal cortex and thyroid gland in birds (Vriend and Lauber, 1973). Syrian hamsters (*Mesocricetus auratus*) in red or yellow spectrum undergo reproductive atrophy (Brainard et al., 1986). In domestic animals, photoperiod affects growth, lactation, and immune functions in cattle (reviewed in (Collier et al., 2006)), and long photoperiod enhances lamb growth rate (Schanbacher, 1984). Photoperiod may also influence human reproduction (Roenneberg and Aschoff, 1990).

Light can also elicit physiological responses in teleosts. Previous study reported that growth performance of barfin flounder (*Verasper moseri*) changes with light intensity (Takahashi et al., 2018). Light from multiple blue and green wavelengths in the spectrum, especially green spectrum, can decrease oxidative stress and apoptosis caused by thermal stress in olive flounder (*Paralichthys olivaceus*) (Kim et al., 2016), while red spectrum contributes to increased oxidative stress of yellowtail clownfish (*Amphiprion clarkii*) (Shin et al., 2011). Moreover, green light is more effective in stimulating somatic growth of barfin flounder (Takahashi et al., 2016). Regarding photoperiod, Giannetto et al. studied the influence of continuous light on transcriptional levels

Abbreviations: AGR, absolute growth rate; Ctrl, control group; SGR, specific growth rate; T, testosterone; T_3, triiodothyronine; T_4, thyroxine; W12/18/24, white light with 12-h light: 12-h dark/18-h light: 6-h dark/24-h light: 0-h dark photoperiod; B12/18/24, blue light with 12-h light: 12-h dark/18-h light: 6-h dark/24-h light: 0-h dark photoperiod; R12/18/24, red light with 12-h light: 12-h dark/18-h light: 6-h dark/24-h light: 0-h dark photoperiod

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of stress-related and antimicrobial peptide genes, demonstrating that continuous light leads to significant transcriptional changes of these genes in juvenile Atlantic cod (*Gadus morhua*) (Giannetto et al., 2014).

Light acts as the exogenous stimulus that cause an endogenous rhythmic secretion of melatonin, a "time-keeping" hormone, which regulates rhythmic physiological functions (Bromage et al., 2001). Melatonin is primarily synthesized and released by photo-neuroendocrine system, such as the pineal gland and retina, and usually peaks at night (Cassone, 1998). Endogenous melatonin rhythm transduces light signal into neuroendocrine signal, driving enduring changes in the endocrine axes, the autonomic nervous system, and the immune system (Walton et al., 2011). Rhythmic melatonin circulation provides information about the time and season, mediating the reproductive cycle, seasonal migration, feeding strategies, growth trajectories and other seasonal or time-dependent physiological functions (Bayarri et al., 2003; Bromage et al., 2001; Reiter, 1993; Villamizar et al., 2009). For example, when juvenile rabbitfish (*Siganus guttatus*) is exposed to continuous light or continuous darkness, growth is stunted, as the rhythm of growth hormone secretion disappears and somatolactin mRNA expression becomes irregular (Ayson and Takemura, 2006). Long photoperiod (18 L: 6D) suppresses circulating melatonin levels and significantly affects the daily diel rhythm of pituitary luteinizing hormone release, resulting in dyshomeostasis of gonadotropin secretion and large numbers of immature fishes (Bayarri et al., 2004).

In contrast to terrestrial conditions, incident light can be dramatically altered in aquatic conditions since the water column acts as a chromatic filter (Jerlov, 1968). In aquatic conditions, with increasing depth, longer wavelengths of red-yellow spectrum are rapidly attenuated (Migaud et al., 2006) and shorter wavelengths of blue-green spectrum become predominant (Jerlov, 1968; Villamizar et al., 2009). Most marine fish larvae are visual feeders (Puvanendran and Brown, 1999; Puvanendran et al., 2002). Consequently, fish develop specific pineal photoreceptors sensitive to blue and green spectra, enabling their larvae to capture food and escape predator (Marchiafava and Kusmic, 1993; Villamizar et al., 2009). European sea bass (*Dicentrarchus labrax*) larvae display the highest wet weight gain and rapid development of fins, teeth and swim bladders under the blue spectrum, and the lowest wet weight gain and slowest absorption of yolk oil and globule under the red spectrum (Villamizar et al., 2009).

Spotted sea bass (*Lateolabrax maculatus*), which belongs to the genus *Lateolabrax* and family Serranidae, is a newly re-described teleost species that usually inhabits estuary and/or freshwater. It is a high-valued euryhaline fish that can be reared in a wide range of areas including coastal waters as well as inland salty/freshwater ponds. We investigated growth performance, digestive enzyme activity, and triglyceride and glucose contents of spotted sea bass after exposure to red, white, or blue light with 12L:12D (12-h light: 12-h dark), 18L:6D, and 24L:0D photoperiods. We also measured contents of peripheral hormones including melatonin, cortisol, thyroid hormone (triiodothyronine (T_3) and thyroxine (T_4)) and testosterone (T). The results from this study will guide the development of light-assisted protocol for spotted sea bass culture.

2. Materials and methods

2.1. Ethics statement

All experiments were conducted in conformity to the Guidelines of Animal Research and Ethics Committees of Ocean University of China. Bass experiments also complied with National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). No endangered or protected species were involved in our studies. In this study, spotted sea bass juveniles were immature, and the influence of sex was not considered.

Table 1
Light conditions in different groups.

Light	White[a,b]	Blue[b]	Red[b]
12 L: 12D	W12	B12	R12
18 L: 6D	W18	B18	R18
24 L: 0D	W24	B24	R24

[a] White light contains all visible spectrums.
[b] The peak wavelengths of the blue and red light are around 460 and 625 nm, respectively, and light intensity (irradiance) of white, blue and red were adjusted to ~0.43, 0.41 and 0.41 $W\,m^{-2}\,s^{-1}$, respectively.

2.2. Experimental design

Spotted sea bass (2.45 ± 0.15 g) were acquired from Spotted Sea Bass Elite Breeding Farm (Dongying, Shandong, China). These fingerlings were spawned on the same day from the same batch of broodstock, thus all larvae were in same day-age and had synchronized development. Before the experiments, bass were maintained under natural light in 1000-l tanks with low population density (around 0.1225 kg/m^3) for fourteen days. Bass were fed *Artemia* twice at 8 a.m. and 5 p.m. everyday. Culture conditions were under the standards of the Spotted Sea Bass Elite Breeding Farm. Water temperature, dissolved oxygen, salinity and pH fluctuated in a minor range: 15.7 ± 0.7 °C, 7.52 ± 0.15 mg/l, 30 ± 0.8‰ and 7.7 ± 0.4, respectively.

After a 14-day acclimation, fish were exposed to natural light (control group) or one of nine different light manipulation treatments. Each group had three replicates and each replicate had 50 fish. The light manipulation was three by three design, three photoperiods applied to three different spectra, giving nine groups: white (W), blue (B) and red (R) spectra with 12-h light, 18-h light or 24-h light.

Light spectrum and intensity were measured by a hand-hold spectroradiometer (Table 1). Villamizar et al. used light intensity of 0.42 $W\,m^{-2}\,s^{-1}$ in their study of European sea bass (Villamizar et al., 2009). In our study, light intensity was adjusted by a variable resistor (0–5500 Ω) to ~0.43, 0.41 and 0.41 $W\,m^{-2}\,s^{-1}$ for white, blue and red light, respectively. Waterproof light emitting diodes (LEDs) were hung approximately 20 cm under the water surface and 20 cm above the tank bottom. All the LEDs worked with variable resistors to maintain a consistent light intensity. All tanks (top, bottom and side) were tightly covered with black screen to prevent light pollution.

2.3. Sampling

Three to four bass per tank (nine to twelve bass per group) were sampled every 15 days with tricaine methane sulfonate (MS 222, 200 mg/l). Body weight (± 0.01 g) were recorded and specific growth rate (SGR) and absolute growth rate (AGR) were calculated according to the formula described previously by Nytrø et al. (2014): SGR (%/day) = 100 × (Exp(g) − 1) and AGR (g/day) = $(W_2 - W_1)/(t_2 - t_1)$, where Exp is natural constant (e) and g is the instantaneous growth coefficient defined as $g = (\ln W_2 - \ln W_1)/(t_2 - t_1)$ (Nytrø et al., 2014). Liver, gastrointestinal tract, or total visceral mass were collected and stored in liquid nitrogen.

2.4. Digestive activities of gastrointestinal tract

Gastrointestinal samples were rinsed with cold distilled water and then homogenized in 10-fold dilution (1 g per 10 ml) phosphate buffer at 4 °C. The homogenate was then centrifuged at 4 °C at 12,000 × *g* for 10 min. Supernatant was collected after centrifugation and stored at 4 °C. All enzymatic assays were conducted within 12 h after extraction and performed as described previously (Shan et al., 2008).

2.5. Triglyceride, glucose and peripheral hormones

Tissues were homogenized as described above. Liver triglyceride and glucose in visceral mass excluding liver (designated as non-hepatic glucose) were determined with a Mindray Automated Biochemistry Analyzer (Ni et al., 2016). Head (including brain and pituitary) and eyes were homogenized for melatonin measurement using a commercially available ELISA kit (IBL, Hamburg, Germany). This kit has been validated previously in Atlantic salmon (*Salmo salar*) and European sea bass (Bayarri et al., 2004; Migaud et al., 2007). Visceral mass triiodothyronine (T_3), thyroxine (T_4), testosterone (T), and cortisol contents were determined by radioimmunoassay using commercially available kits (Tianjin Nine Tripods Medical and Bioengineering Co., Ltd. Tianjin, China) (Wen et al., 2014).

Melatonin contents at night were evaluated at the same time (3 h after sunset). To avoid the influence of light on night melatonin, one day before sampling, we separated the bass to be sampled to a new tank under the same light manipulation as the bass in the original tank. When sampling, we added MS 222 (200 mg/l) into the new tank in the dark; when bass stopped swimming and sank, they were immediately transferred into liquid nitrogen and 24-h later, head (including brain and pituitary) and eyes were separated and homogenized for melatonin measurement.

2.6. Statistical analysis

Statistical analysis was conducted with SPSS 16.0. Student's *t*-test was used to compare Day 0 with Day 45 and 60 in control group; and control group and the nine light manipulation groups. Two-way ANOVA was used to investigate the effects of photoperiod and light spectrum, and their interaction on the growth performance, digestion and metabolism, and hormone profile. Where significant differences were found, means among different light conditions were compared using Duncan's multiple range test at $P < 0.05$ significance level. Results are expressed as mean ± standard deviation (means ± S.D.).

3. Results

3.1. Growth performance

During this experiment, natural light and dark ratio (in hours) was approximately 14.36: 9.24 h per day (Supplementary Fig. S1). In natural light, bass had significantly higher body weights on Day 30, 45 and 60 when compared to Day 0 (Fig. 1A). Two-way ANOVA showed that both light spectrum and photoperiod had significant effects on body weight, 60-day SGR and AGR (Table 2). In comparison to bass reared in natural light, on Day 45 and 60, bass of R12 and R24 showed significant decrease in body weight, whereas bass of B18 had significantly higher body weight (Fig. 1B & C). Bass reared in red light had significantly lower 60-day SGR than bass in B18 (Fig. 1D). Bass of B18 showed significantly higher 60-day AGR than all other groups (Fig. 1E). Heat map showed that bass in W18 and B18 displayed highest 60-day weight gain (Fig. 1F). When compared to those of W18, bass of B18 had lower individual differences within treatment (Fig. 1F).

Bass increased body weights in all groups during the experiment (Fig. 2A–J) but the rate of the increase was different. In natural light, the slope of the linear regressive equation that described body weight is 0.1528 (Fig. 2A). Bass in B18 displayed the highest growth rate with a slope of 0.1895 (Fig. 2F), while the slowest growth rate was observed in bass in R12 with a slope of 0.0978 (Fig. 2H).

3.2. Digestion and metabolism

Bass in natural light displayed no significant changes in trypsin, lipase and amylase activity during the experiment (Fig. 3A, D & G). However, bass in B18 had significantly increased trypsin and lipase activity on Day 45 and 60 (Fig. 3B, C, E & F), significantly higher than all other groups. The bass in W12 and all red light treatments had significant decrease in trypsin activity on Day 45 and 60 (Fig. 3B & C). Spectrum, photoperiod and their interaction contributed to the significant differences of trypsin and lipase activities of bass in different treatments (Table 3). Light treatment did not affect amylase activity (Fig. 3G, H & I).

Hepatic triglyceride and glucose as well as non-hepatic glucose of bass in natural light had no significant change on Day 0, 45 and 60 (Fig. 4A, D & G). Bass in red light displayed significant decrease in hepatic triglyceride and glucose, and significant increase in non-hepatic glucose (Fig. 4B, C, E, F, H & I). Bass in B18 displayed significantly higher hepatic glucose content than those of bass in natural light on Day 45 and 60 (Fig. 4H & I). Spectrum, photoperiod and their interaction led to significant differences of hepatic glucose contents, whereas only spectrum affected hepatic triglyceride and non-hepatic glucose contents (Table 3).

3.3. Hormone profile

Melatonin contents during the day and at night did not change during the experiment in bass in natural light (Fig. 5A & D). Light conditions had no effects on day-time melatonin contents. Bass in red light had significantly higher night melatonin contents than all other groups (Fig. 5E & F). Two-way ANOVA showed that spectrum might contribute to the significantly increased night melatonin contents of bass in red light (Table 4).

In natural light, bass showed significantly higher T_3 and T_4 contents on Day 45 and 60 when compared to Day 0 (Fig. 6A & D). Red light led to significant T_3 increase and T_4 decrease of bass on Day 60 (Fig. 6C & F). Two-way ANOVA showed that red spectrum might lead to significantly decreased T_3 on Day 60, and spectrum and photoperiod might contribute to the significant decrease of T_4 on Day 60 (Table 4).

In natural light, bass had decreased cortisol contents on Day 45 and 60 when compared to Day 0 (Fig. 7A). Red light caused a significantly higher cortisol content in bass when compared to that of bass in natural light, and W18, W24, B18 and B24 on Day 45 (Fig. 7B). Spectrum might lead to significant difference in cortisol content (Table 4). The T content of bass in natural light had no change during the experiment (Fig. 7D). In comparison to bass in natural light and other light condition groups, bass in red light displayed significantly higher T contents (Fig. 7E & F) and two-way ANOVA showed that spectrum might be responsible for the significantly increased T contents of bass in red light (Table 4). Bass in red light exhibited more attack behaviors (Supplementary Fig. S2).

4. Discussion

In a natural setting, photoperiod is altered by seasonal changes and plays an important role in the regulation of growth, metabolism and endocrine functions of teleosts. In comparison to terrestrial environment, aquatic environment is more complicated, as light characteristics are influenced by water, and light of shorter wavelengths becomes more predominant with increasing depth (Jerlov, 1968; Villamizar et al., 2009). In this study, we evaluated growth performance, metabolism and peripheral hormone contents of spotted sea bass cultured at three light spectrums with three photoperiods.

We observed that longer photoperiods and shorter spectrums were beneficial in promoting bass growth (Fig. 1). For example, blue light was more effective than white light in stimulating bass growth. Previous studies showed that in several teleosts, such as sea bream (*Sparus aurata*; (Chatain and Ounais-Guschemann, 1990)), flounder (*Rhombosolea tapirina*; (Hart et al., 1996)), snapper (*Pagrus auratus*; (Fielder et al., 2002)), and Atlantic cod ((Puvanendran and Brown, 2002)), long photoperiod can enhance growth rate. It was also reported that shorter wavelengths can improve fish growth, as shown in European sea bass (Villamizar et al., 2009), Senegal sole (*Solea senegalensis*; (Blanco-Vives

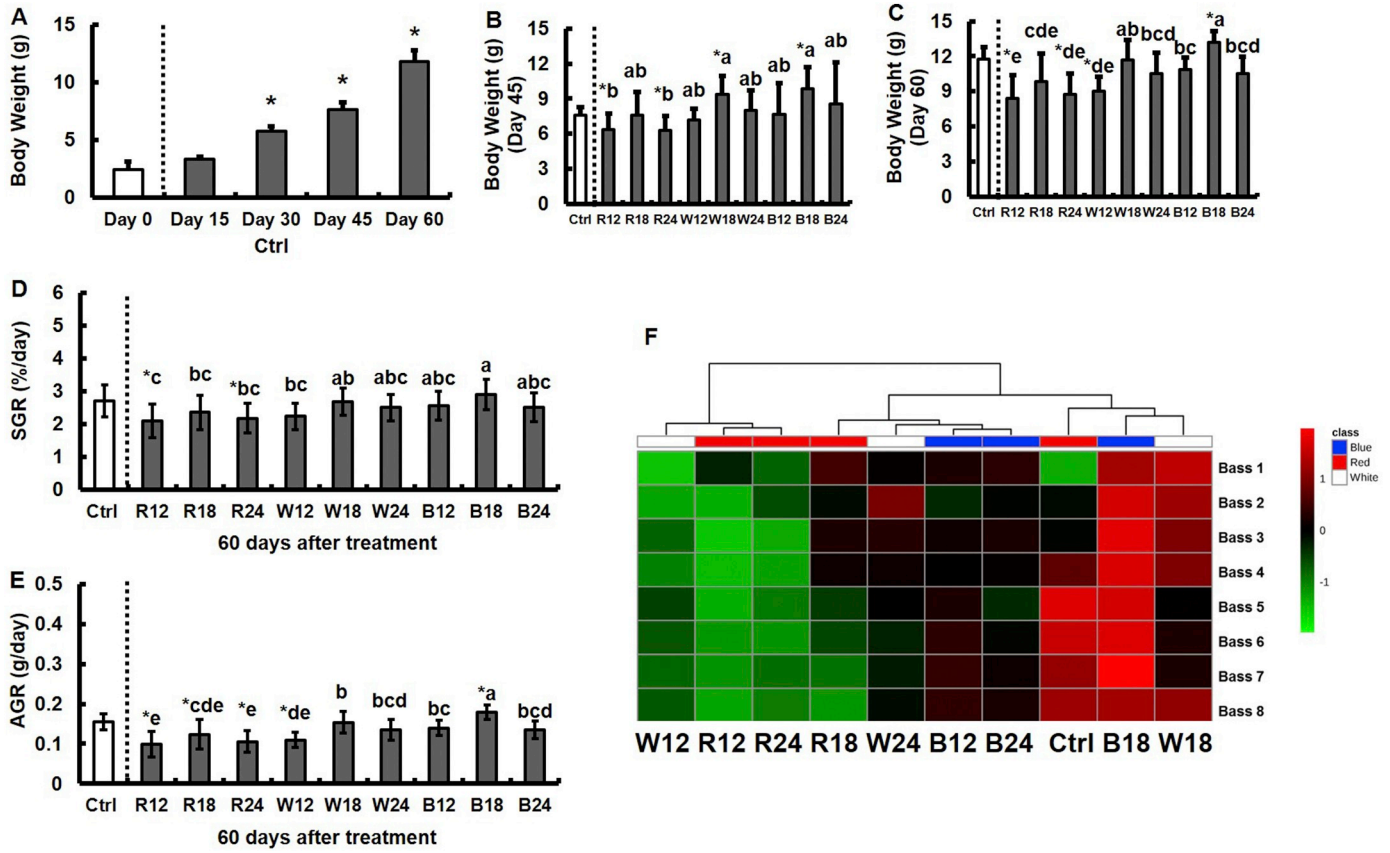

Fig. 1. Growth performance of bass in different light conditions. Body Weight (A–C), 60-day SGR (D) and AGR (E), and heat map of 60-day body weight gain (F) of bass are shown. Ctrl represents control group; W, B, and R represents white, blue, and red light, respectively; 12, 18, and 24 represents 12L:12D, 18L:6D, and 24L:0D photoperiods, respectively. In heat map, after data normalization, red color represents higher 60-day weight gain and green color represents lower 60-day weight gain. Data are presented as means ± S.D., n = 8. Asterisk indicates significant difference when compared to Day 0 (A) or Ctrl (B, C, D, E) ($P < 0.05$, Student's *t*-test). Different letters indicate significant differences among R12/18/24, W12/18/24 and B12/18/24 ($P < 0.05$, Duncan's multiple range test). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Growth performance of bass in different light conditions.

Time		Two-way ANOVA		
		Spectrum	Photoperiod	Interaction
45	Body	0.005	0.007	0.955
60	Weight	< 0.001	< 0.001	0.386
60	SGR	0.004	0.029	0.863
60	AGR	< 0.001	< 0.001	0.327

P value obtained by two-way ANOVA is listed.

et al., 2010)) and barfin flounder larvae (Takahashi et al., 2016). As there is a rapid attenuation of red light in water, diminished photons may fail to stimulate the visual system of bass larvae, affecting feeding and resulting in lower growth rate (Villamizar et al., 2009), similar to a previous study in Atlantic cod (Migaud et al., 2009). This might contribute to the significant impairment in bass growth when exposed to red light.

Levels of pancreatic enzymes (amylase, trypsin and lipase) are important indicators for evaluating the maturation and function of the digestive system (Infante and Cahu, 2001; Ribeiro et al., 1999). Multiple studies have investigated the effects of environmental factors on digestive enzymes activities (reviewed in (Shan et al., 2008)). However, the impacts of light on digestive enzymes in fish is not well studied. Cuvier-Péres et al. indicated that trypsin activity is extremely dependent on light intensity in European sea bass larvae (Cuvier-Péres et al., 2001). However, Suzer et al. reported conflicting results in pandora (*Pagellus erythrinus*, (Suzer et al., 2006)). Shan et al. indicated that lipase activity of miiuy croaker (*Miichthys miiuy*) larvae and juveniles increases significantly with long photoperiods, while trypsin and amylase activities are independent of photoperiod (Shan et al., 2008). In our study, we identified that in blue light environments, 18L:6D photoperiod increased trypsin and lipase activities, while 24L:0D photoperiod was ineffective (Fig. 3B, C, E & F). There is no information in the literature about the impact of spectrum on the activities of digestive enzymes. We observed that red light could significantly reduce trypsin activity (Fig. 3B & C).

Cortisol is released from interrenal tissue (analogous to the adrenal cortex in mammals) and acts as the primary glucocorticoid in response to stressful challenges in teleosts (Aluru and Vijayan, 2009). In goldfish, green light decreases and red light increases cortisol levels (Song et al., 2016). We also showed that red light significantly increased cortisol content in bass (Fig. 7B). Stress is an energy-demanding process, requiring fish to mobilize energy substrates to fuel cellular processes. Cortisol stimulates glucose production by increasing glycogenolysis and enhances lipid utilization by increasing gluconeogenesis (Barton et al., 1987; Mommsen et al., 1999). We observed that accompanying the high cortisol content, red light decreased hepatic triglyceride (Fig. 4B & C), increased non-hepatic glucose and decreased hepatic glucose contents (Figs. 4E, F, H, I & 7B). These results suggested that bass might mobilize hepatic energy stores to cope with stress under red light.

There is mounting evidence indicating that light pollution at night is an environmental endocrine disruptor, disturbing circadian rhythms and melatonin secretion (Russart and Nelson, 2017). We observed that bass in R18 showed significantly increased night melatonin content

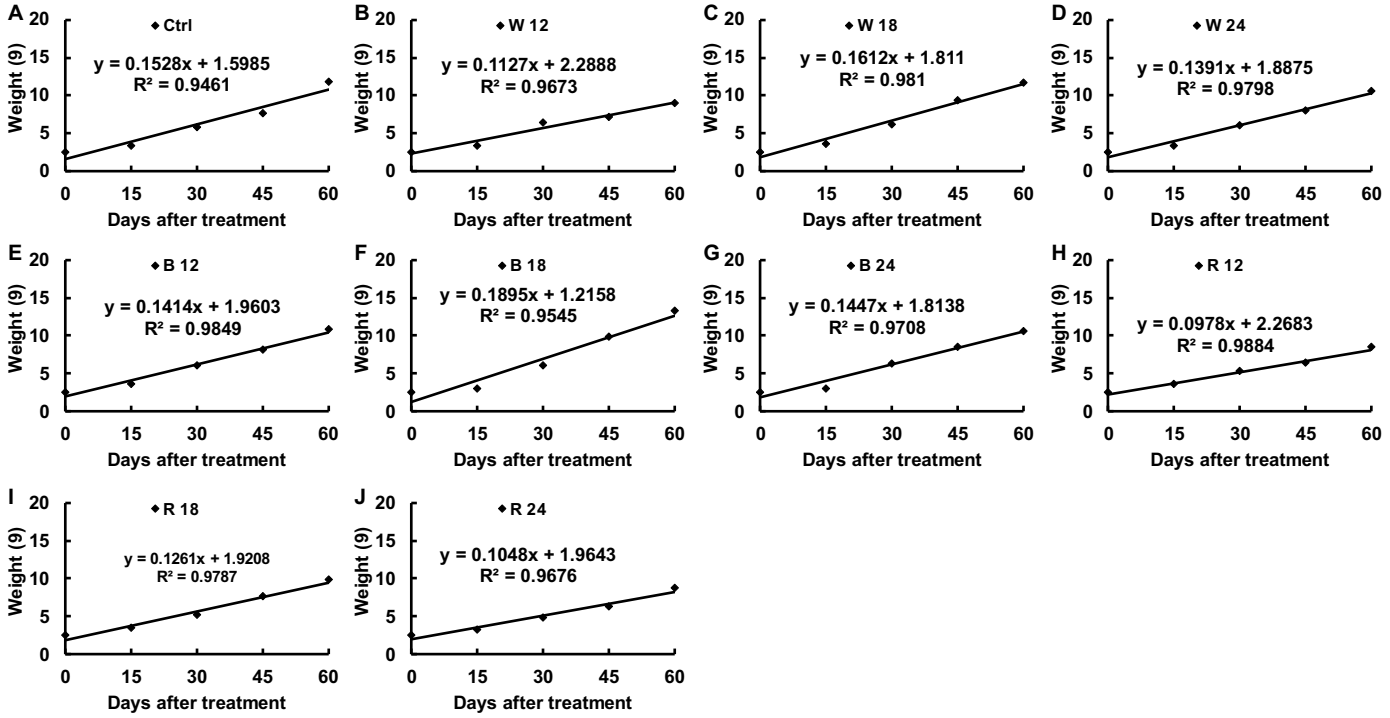

Fig. 2. Linear regressive analysis of bass body weight in different light conditions. Ctrl represents control group; W, B, and R represents white, blue, and red light, respectively; 12, 18, and 24 represents 12L:12D, 18L:6D, and 24L:0D photoperiods, respectively. Data were means of 8 fish.

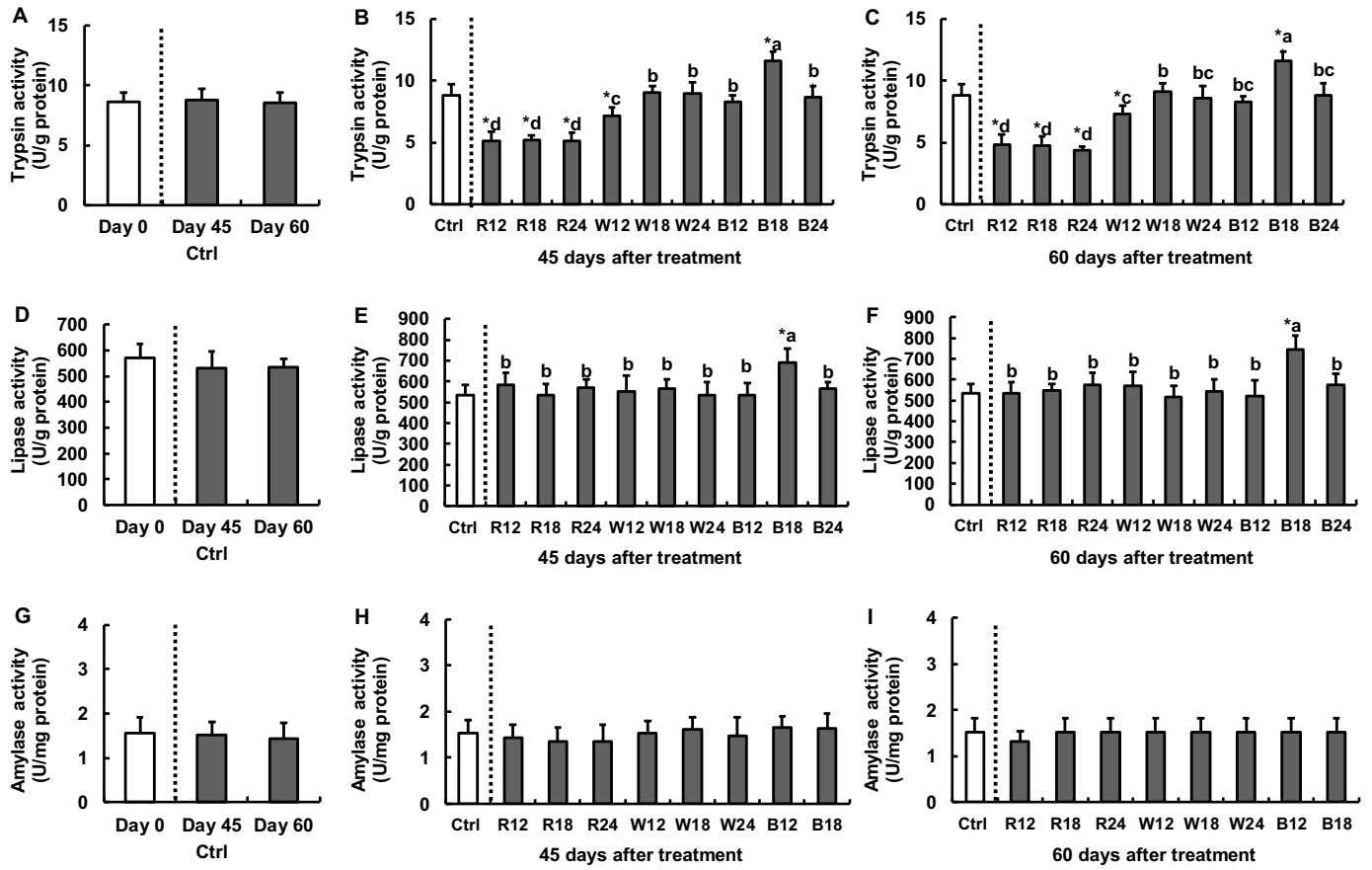

Fig. 3. Activities of digestive enzymes in bass reared in different light conditions. Trypsin activity (A–C), lipase activity (D–F), and amylase activity (G–I) of bass are shown. Data are presented as means ± S.D., n = 8. Asterisk indicates significant difference when compared to Day 0 (A, D, G) or Ctrl (B, C, E, F, H, I) ($P < 0.05$, Student's *t*-test). Different letters indicate significant differences among R12/18/24, W12/18/24 and B12/18/24 ($P < 0.05$, Duncan's multiple range test).

Table 3
Digestive enzyme activity, triglyceride and glucose of bass in different light conditions.

Time		Two-way ANOVA				Two-way ANOVA		
		Spectrum	Photoperiod	Interaction		Spectrum	Photoperiod	Interaction
45	Trypsin	< 0.001	< 0.001	< 0.001	Hepatic Triglyceride	< 0.001	0.655	0.814
60		0.016	< 0.001	< 0.001		< 0.001	0.536	0.629
45	Lipase	0.017	0.021	< 0.001	Hepatic Glucose	< 0.001	< 0.001	< 0.001
60		< 0.001	0.003	< 0.001		< 0.001	< 0.001	< 0.001
45	Amylase	0.016	0.875	0.939	Non-Hepatic Glucose	< 0.001	0.151	0.484
60		0.777	0.671	0.631		< 0.001	0.104	0.193

P value obtained by two-way ANOVA is listed.

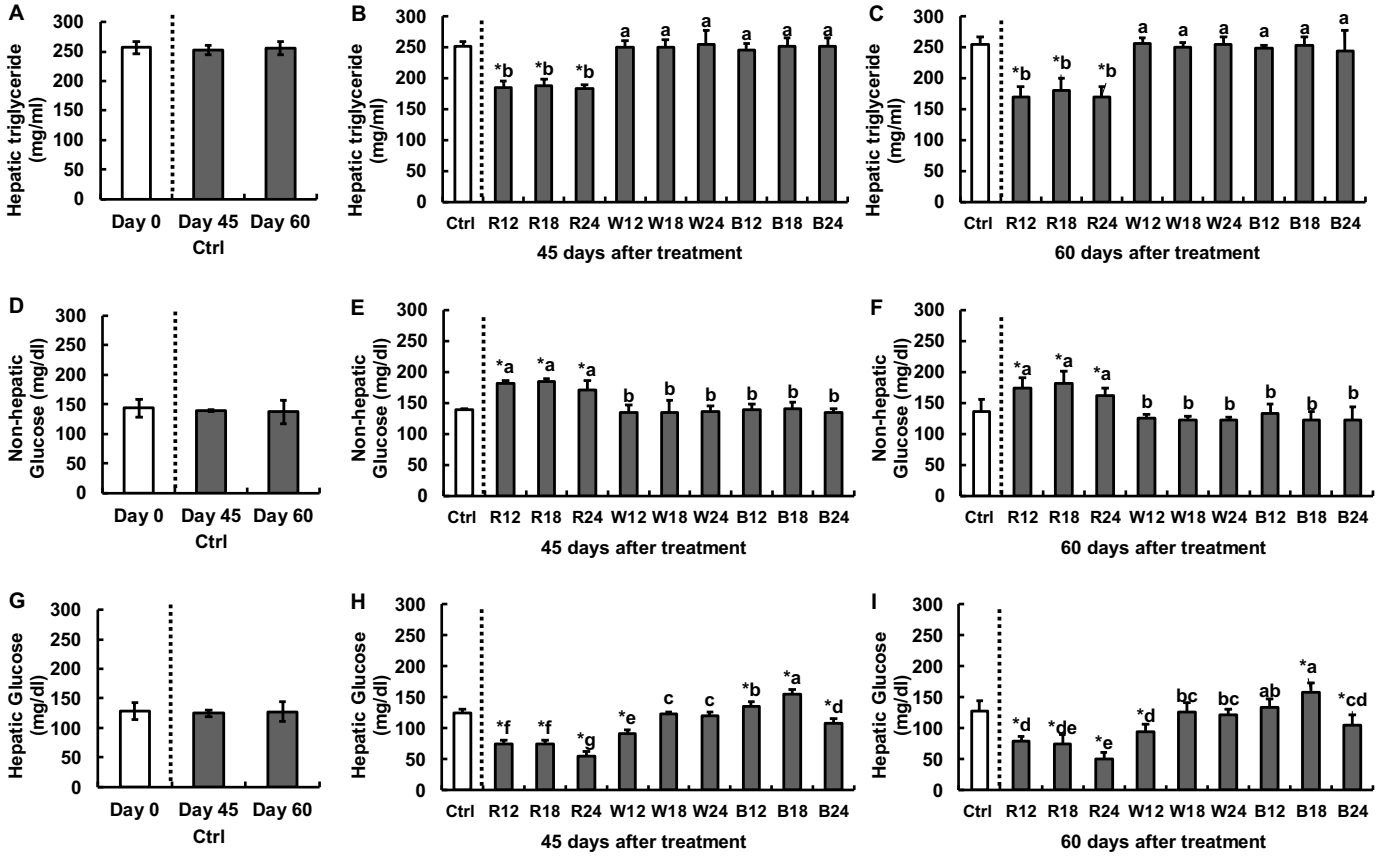

Fig. 4. Triglyceride and glucose content of bass in different light conditions. Hepatic triglyceride (A–C), non-hepatic glucose (D–F), and hepatic glucose (G–I) of bass are shown. Data are presented as means ± S.D., n = 8. Asterisk indicates significant difference when compared to Day 0 (A, D, G) or Ctrl (B, C, E, F, H, I) ($P < 0.05$, Student's *t*-test). Different letters indicate significant differences among R12/18/24, W12/18/24 and B12/18/24 ($P < 0.05$, Duncan's multiple range test).

(Fig. 5E & F), suggesting in red light environment, long photoperiod might disturb circadian rhythms. Melatonin acts as an antioxidant to protect organisms against oxidative stress (Reiter et al., 2000). In yellowtail clownfish, high melatonin levels as well as increased superoxide dismutase and catalase activities are observed in red light (Shin et al., 2011). In this study, red light, but not blue or white light, with 12L:12D photoperiod significantly increased night melatonin content (Fig. 5E & F). We propose that red light might cause oxidative stress in bass.

In teleosts, thyroid hormones play a vital role in the regulation of growth, development, energy utilization and reproduction (Blanton and Specker, 2007; Power et al., 2001). Fish display faster and healthier growth with adequate thyroid hormones (Schnitzler et al., 2011). In natural light, we observed increased T_3 and T_4 contents on Day 60 (Fig. 6C & F), suggesting bass was in favorable growth performance. T_3, converted from T_4, exhibits most biological effects in target cells. We observed that red light led to decreased T_4 and increased T_3 contents (Fig. 6C & F), indicating an increased T_3 conversion rate from T_4. Thyroid hormones over-secretion leads to decreased triglycerides level, intermittent problems of gastrointestinal tract and increased weight loss, which was consistent with our results of poor growth, modest digestive performance, and decreased hepatic triglyceride content of bass in red light.

In humans, higher T level is linked to risk-taking and sensation-seeking behaviors, and in animals such as birds and rodents, T is associated with aggression, stress coping and exploration (reviewed by (Caramaschi et al., 2013)). In adult zebrafish (*Danio rerio*), exposure 17α-ethinylestradiol (EE_2), a synthetic derivative of estradiol (E_2) that decreases T level, to males reduces aggressive behavior in dominant fish (Colman et al., 2009), providing indirect evidence that T is linked to aggression. An early study of Striped trumpeter (*Latris lineata*) revealed that a higher percentage of abnormal behavior (swim into the walls) is observed in the red tank (Cobcroft and Battaglene, 2009). Consistent with these studies, we observed bass exposed to red light had higher T content and increased attack behaviors (Fig. 7E & F and Supplementary Fig. S2).

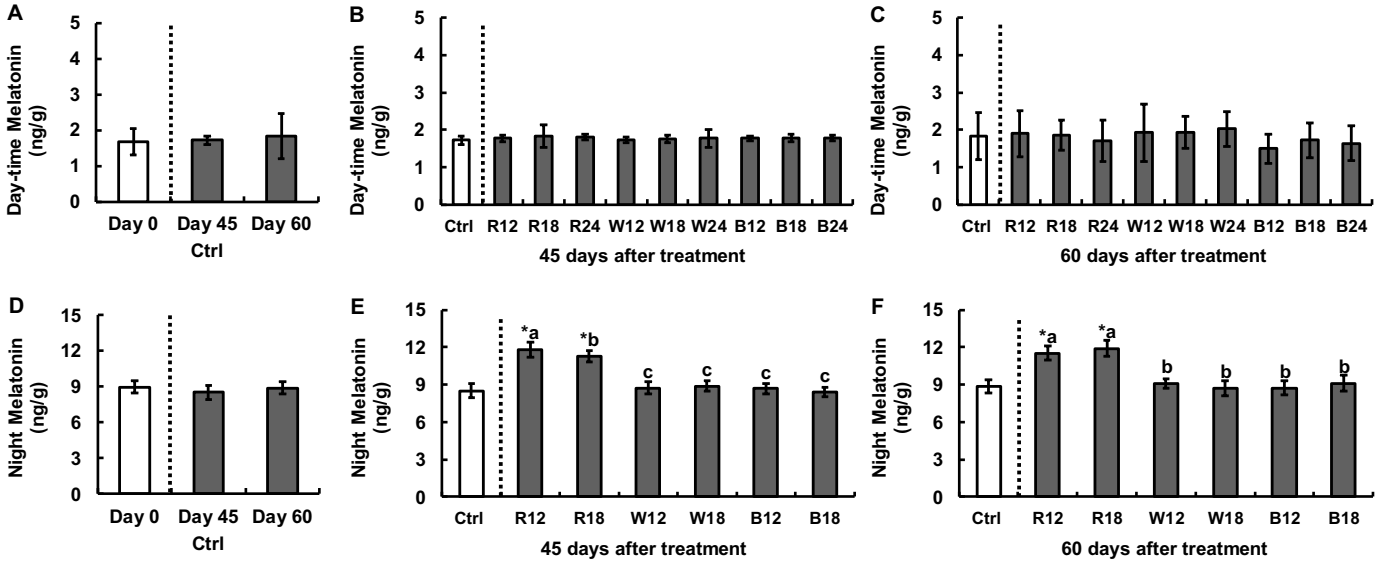

Fig. 5. Melatonin content of bass in different light conditions. Day-time melatonin (A–C) and night melatonin (D–F) of bass are shown. Data are presented as means ± S.D., n = 8. Asterisk indicates significant difference when compared to Day 0 (A & D) or Ctrl (B, C, E & F) ($P < 0.05$, Student's *t*-test). Different letters indicate significant differences among R12/18/24, W12/18/24 and B12/18/24 ($P < 0.05$, Duncan's multiple range test).

Table 4
Endocrine profiles of bass in different light conditions.

Time		Two-way ANOVA				Two-way ANOVA		
		Spectrum	Photoperiod	Interaction		Spectrum	Photoperiod	Interaction
45	Day-Time Melatonin	0.503	0.719	0.985	Night Melatonin	< 0.001	0.122	0.122
60		0.078	0.898	0.857		< 0.001	0.508	0.098
45	T_3	0.043	0.596	0.741	T_4	< 0.001	0.003	0.969
60		< 0.001	0.519	0.408		< 0.001	< 0.001	0.365
45	T	< 0.001	0.555	0.931	Cortisol	< 0.001	0.637	0.597
60		< 0.001	0.511	0.731		< 0.001	0.616	0.827

P value obtained by two-way ANOVA is listed.

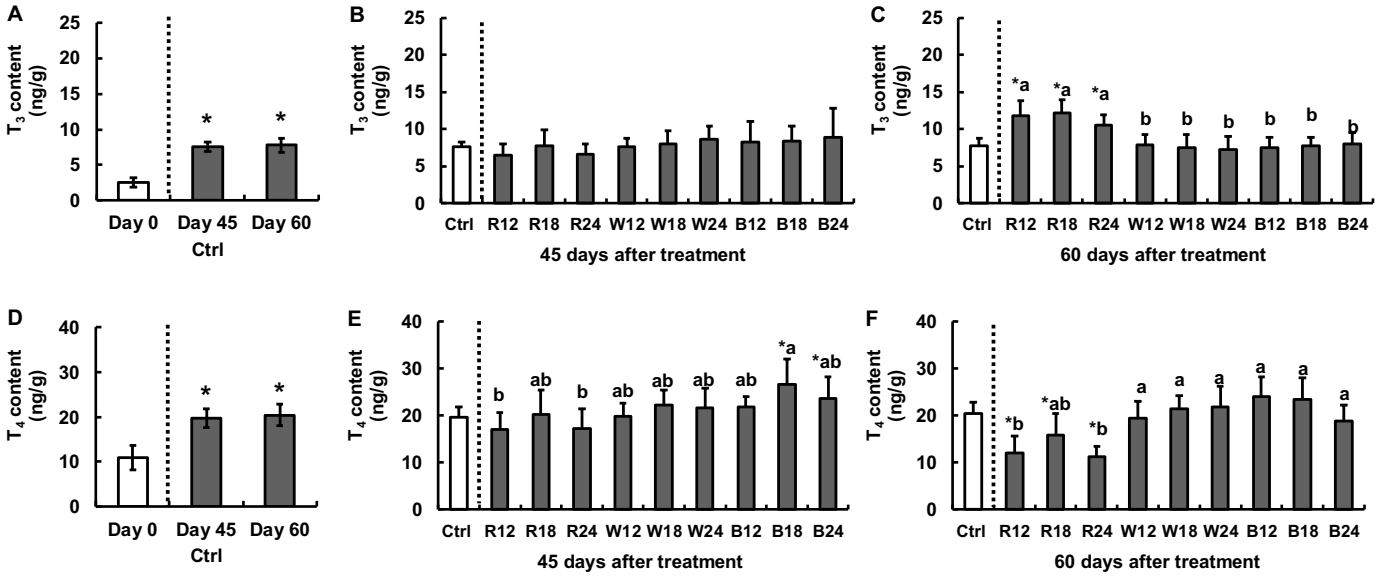

Fig. 6. Triiodothyronine (T_3) and thyroxine (T_4) content of bass in different light conditions. T_3 (A–C) and T_4 (D–F) of bass are shown. Data are presented as means ± S.D., n = 8. Asterisk indicates significant difference when compared to Day 0 (A & D) or Ctrl (B, C, E & F) ($P < 0.05$, Student's *t*-test). Different letters indicate significant differences among R12/18/24, W12/18/24 and B12/18/24 ($P < 0.05$, Duncan's multiple range test).

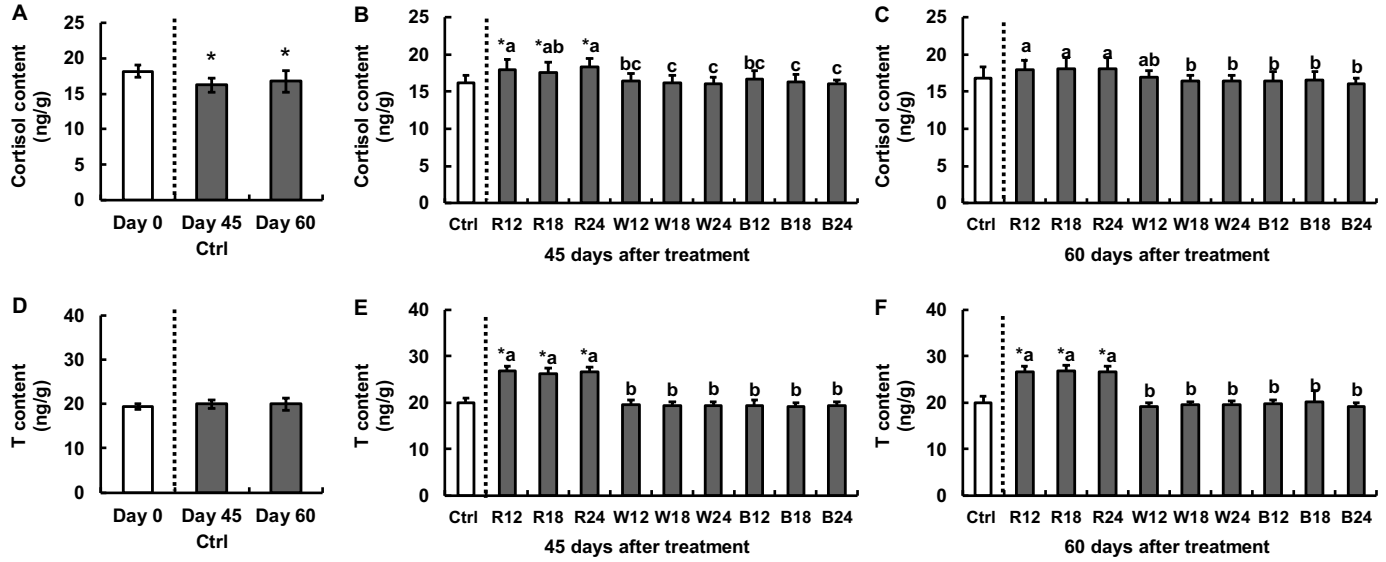

Fig. 7. Cortisol and testosterone (T) content of bass in different light conditions. Cortisol (A–C) and T (D–F) of bass are shown. Data are presented as means ± S.D., n = 8. Asterisk indicates significant difference when compared to Day 0 (A & D) or Ctrl (B, C, E & F) ($P < 0.05$, Student's *t*-test). Different letters indicate significant differences among R12/18/24, W12/18/24 and B12/18/24 ($P < 0.05$, Duncan's multiple range test).

5. Conclusion

In this study, we showed that light spectrum and photoperiod had a dramatic influence on growth performance, digestive and metabolic functions, and peripheral hormones of spotted sea bass, a newly re-described economically valuable species in Perciformes. Bass in 18-h blue light displayed higher growth rate and digestive activities with increased hepatic glucose accumulation. Growth rate, trypsin activity, and content of hepatic triglyceride and glucose of bass were significantly decreased when reared in red light. Increased melatonin, T_3, cortisol and T contents of bass in red light indicated that red light might act as an endocrine disruptor. Red light might also cause stress and increased aggression. The poor growth performance of bass in red light might be due to a trade-off in energy balance between stress coping and growth because bass mobilized energy to cope with stressors. Therefore, research on ideal light conditions have important implications for prompting welfare and increasing the production of spotted sea bass.

Competing interests

All authors declare that they have no competing interest.

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Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2019.04.029.

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