

## Relevance of Light Spectra to Growth of the Rearing Tiger Puffer *Takifugu rubripes*

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**ABSTRACT** : In fish, light (photoperiod, intensity and spectra) is main regulator in many physiological actions including growth. We investigate the effect of light spectra on the somatic growth and growth-related gene expression in the rearing tiger puffer. Fish was reared under different light spectra (blue, green and red) for 8 weeks. Fish body weight and total length were promoted when reared under green light condition than red light condition. Expression of somatostatins (*ss1* and *ss2*) in brain were showed higher expression under red light condition than green light condition. The *ss3* mRNA was observed only higher expression in blue light condition. Expression of growth hormone (*gh*) in pituitary was detected no different levels between experimental groups. However, the fish of green light condition group was showed more high weight gain and feed efficiency than other light condition groups. Our present results suggest that somatic growth of tiger puffer is induced under green light condition because of inhibiting *ss* mRNA expression in brain by effect of green wavelength.

**Key words** : light spectra, somatic growth, somatostatin, growth hormone, tiger puffer

### INTRODUCTION

In fish, light (photoperiod, intensity and spectra) is one of main environmental conditions on the regulation of numerous physiological actions including growth, feeding activity, migration and reproduction (Boeuf & Le Bail, 1999; Migaud et al., 2010; Kim et al., 2013). Studies were conducted to find a suitable light conditions to promote the growth in farmed fish (Simensen et al., 2000; Kissil et al., 2001; Henne & Watanabe, 2003; Taylor et al., 2005). In natural sea water, light spectra were distributed along the depth of water

because water column act as a chromatic filter (Villamizar et al., 2011). Generally, red spectrum (long wavelength) is absorbed in the shallow water and blue spectrum (short wavelength) was predominated to deeper depth. According to presence of light wavelength with depth, fish physiological functions and habitat are adapted. However, in general aquaculture system, fish is reared under the shallow water depth and natural light or artificial white light conditions. Therefore, study is required on the appropriate light spectra conditions in fish aquaculture system. Recent studies were noticed that the light spectra affects in somatic

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growth of teleost fish (Villamizar et al., 2009; Karakatsouli et al., 2010; Hyedamejad et al., 2013). However, these results of fish growth effect by light spectra were always not synchronized and it appeared the species specificity.

The tiger puffer *Takifugu rubripes* is a commercially valuable species in aquaculture industry of South Korea, China and Japan. However, effect of main environmental condition including light spectra on growth of tiger puffer is no information until now. The aim of the present study is to suggest an effective rearing conditions in tiger puffer aquaculture farm using the ability of light spectra adaptation. We investigate the impact of artificial different light spectra on the somatic growth the rearing tiger puffer and analyze the expression of growth-related genes in each brain and pituitary.

## MATERIALS & METHODS

### 1. Fish and experimental design

Tiger puffer for the present study were obtained from Tham-Ra Fishery located in Seogwipo, Jeju, South Korea and kept in rearing tank at Marine Science Institute, Jeju National University. Fish were reared under natural photoperiod and water temperature conditions in indoor tank and fed commercial pellets (Daehan co., MP3, Busan, South Korea) twice a day before starting experiment. After sufficiently adapted, fish (n=120, body weight 124.2±1.5 g, total length 18.1±0.1 cm) were divided into three groups; blue (480 nm), green (520 nm) and red (590 nm) wavelength conditions. The light intensity of the blue, green and red were adjusted 150 lx and 15.2±1 ft-cd according to the previous methods (Hur, 2011). Experimental fish were reared during 8 weeks under natural water temperature (10.8–12.5°C) and photoperiod was equated with natural photoperiod using a timer. The fish were fed commercial pellets twice a day during experimental period. Fish body weight (BW) and total length (TL) measured every 4 weeks. In during experimental periods, body weight gain, feed consumption and feed

efficiency were calculated. Fish sampling was performed initial five fish and final six fish for each light condition groups. Sampled brain and pituitary tissues were immediately stored –80°C until the analysis.

### 2. Total RNA extraction and real-time qPCR

Collected brain and pituitary tissues of the tiger puffer were absolutely homogenized with RiboEx™ (GeneAll, Seoul, Korea) reagent for total RNA extraction and total RNA was extracted following the manufacturer's protocol. Extracted total RNA with treated using RQ1 RNase-Free DNase (Promega, Madison, WI, USA). RNA concentration measured using the Nano Vue (GEHealthcare, Ver.1.0.1, UK) and 500 ng of total RNA used in cDNA synthesis with PrimeScript RT reagent Kit (Takara Bio Inc, Otsu, Japan).

Synthesized cDNA 20 ng used for Real-time qPCR with Evagreen premix PCR kit (abm Inc, Canada). For real-time qPCR, primer sets of each genes were designed by isolated somatostatins (*ss1*, *ss2* and *ss3*) and growth hormone (*gh*) of *Takifugu rubripes* from National Center for Biotechnology Information (NCBI, Table 1). Real-time qPCR was conducted by CFX™ Real-time System (Bio-Rad, Hercules, CA, USA)

**Table 1. Primer sets used in this study**

Primers	Sequence(5'-3')	GeneBank accession No.
<i>ss1</i> -F	TCTGCTACTGCAGTCCAACG	XM_003968318
<i>ss1</i> -R	CCTTCAGCCAGAGCAAGGTT	
<i>ss2</i> -F	TGCATTTCAGGGTGTGTCTC	XM_003968317
<i>ss2</i> -R	CAATCAGGTCCTCCACAGCA	
<i>ss3</i> -F	ATGCAGCTTCTGGTGGTGTT	XM_003963129
<i>ss3</i> -R	GAATCAGCTCCTTGCGCTCT	
<i>gh</i> -F	CTCATCAAGGCCAGTCAGGAT	U63807
<i>gh</i> -R	CTCCACCTTGTGCATGTCCT	
<i>ef1-a-f</i>	GGCCTTCTCAAACCAAGCAT	AB193485
<i>ef2-a-f</i>	CCTTTGGTGGGTCGTTCTTG	

and amplification performed on the conditions: initial denaturation at 95°C for 10 min, 40 cycles of 95°C for 15 sec, 60°C for 1 min and last 60°C for 1 min. Growth-related genes expression were normalized to the amount of the internal control *efl-α* (elongation factor 1-α) gene (Table 1).

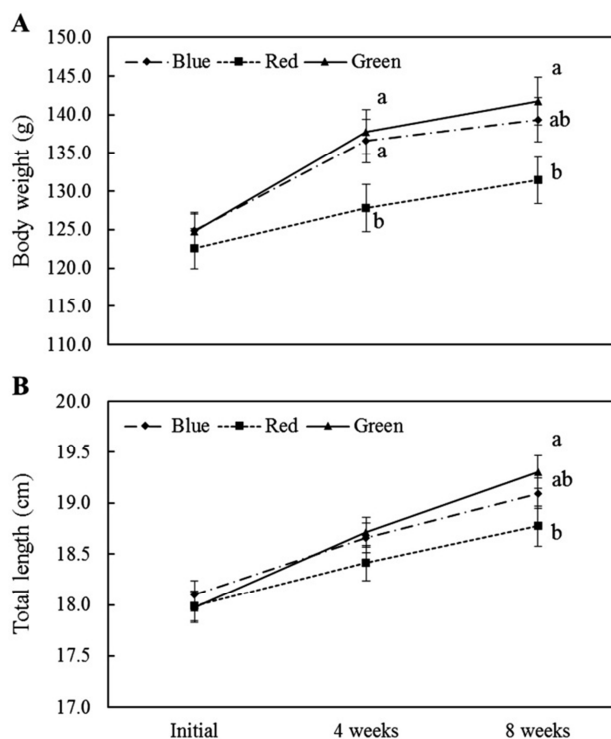
### 3. Statistical analysis

Real-time qPCR expression data was presented as mean  $\pm$  standard error (SEM) and considered significantly different at  $P < 0.05$ . Statistical differences among means were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test.

## RESULTS

### 1. Fish growth

We have measurement of fish BW and TL between experimental groups in every 4 weeks (Fig. 1). Initial fish BW was no different between three groups, but BW of green and blue light conditions groups were more increased than red light condition after 4 weeks. After that, BW of green light condition was steadily maintained significantly more high levels than red light condition until final weeks. The TL of fish was no differed between experimental groups after 4 weeks, but in final weeks, TL of green light condition was significantly increased than red light condition. In during experimental period, change of survival rate, weight gain and feed efficiency were shown in Table 2. The fish



**Fig. 1. Change of BW (A) and TL (B) during tiger puffer rearing under different light spectra (blue, red and green) conditions.** Means represented by different letters are significant ( $P < 0.05$ ). Values are mean  $\pm$  SEM.

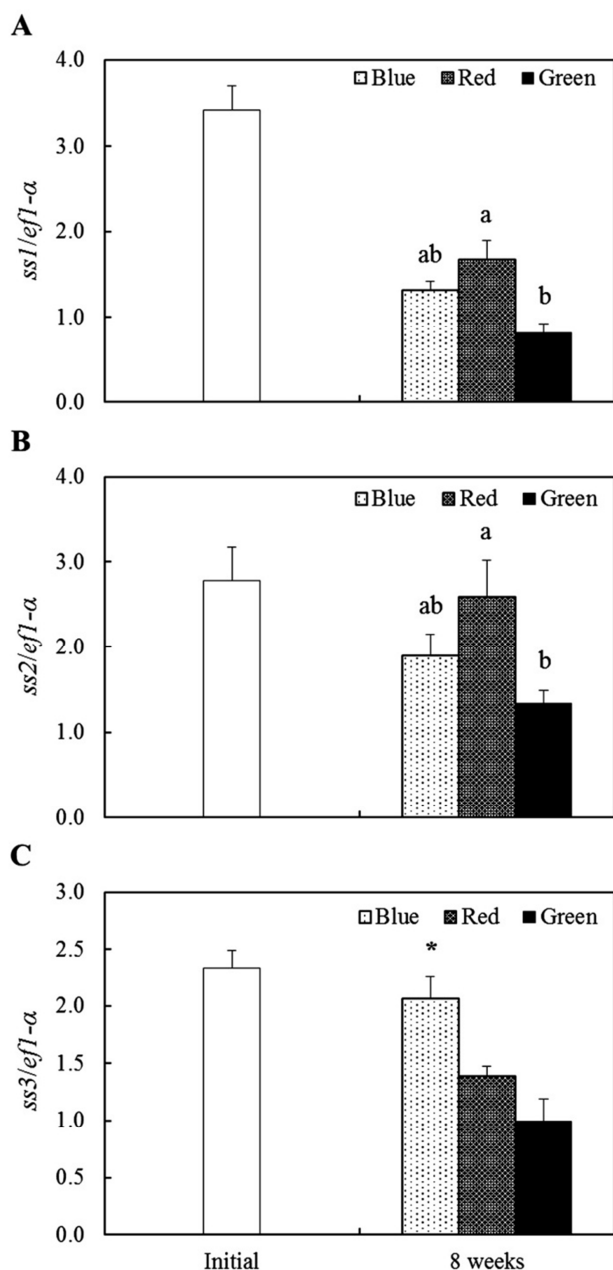
in each light condition groups did not die during the experiment. The fish of green light condition group was showed more high weight gain and feed efficiency than other light condition groups.

### 2. The mRNA expression of growth-related genes

We profiled somatostatins (*ss1*, *ss2* and *ss3*) and growth hormone (*gh*) mRNA expression conducted by different light

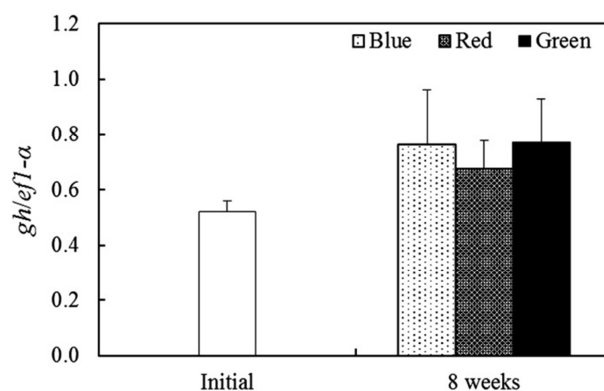
**Table 2. Growth performance and feed efficiency of tiger puffer rearing under different light spectra (blue, red and green) conditions for 8 weeks**

Experimental group	Initial		8 week		Survival rate (%)	Weight gain (g)	Feed efficiency (%)
	Fish No.	Sum weight(g)	Fish No.	Sum weight(g)			
Blue	40	4,994	40	5,574	100	580	37.0
Red	40	4,901	40	5,257	100	356	29.8
Green	40	4,992	40	5,670	100	678	41.0



**Fig. 2.** *ss1*, *ss2* and *ss3* mRNA levels in the brain of tiger puffer under different light spectra conditions (blue, red and green). Means represented by different letters are significant ( $P < 0.05$ ). Values are mean  $\pm$ SEM.

wavelength in tiger puffer. After 8 weeks, the *ss1* mRNA of red light condition was shown significantly ( $P < 0.05$ ) high expression than other experimental groups and *ss1* mRNA expression of green light condition was lowest than



**Fig. 3.** GH mRNA levels in the pituitary of tiger puffer under different light spectra conditions (blue, red and green). Means represented by different letters are significant ( $P < 0.05$ ). Values are mean  $\pm$ SEM.

other groups at 8 weeks (Fig. 2A). The *ss2* mRNA was showed similar expression pattern with *ss1* mRNA and red light condition was observed higher expression than other groups at 8 weeks (Fig. 2B). The *ss3* mRNA was observed more increased expression in blue light condition than other experimental groups at 8 weeks (Fig. 2C). In pituitary, *gh* mRNA was showed no specific expression between the three groups at 8 weeks (Fig. 3).

## DISCUSSION

Recently, characteristics of adaptive physiology of fish in rearing environmental factors apply to aquaculture. We discuss about information of adaptive physiology of fish in light spectra. Effect of light spectra on the physiological actions (growth, feeding, stress response and reproduction) have been studied in teleost fish (Volpato & Barreto, 2001; Blanco-Vives et al., 2010; Bapary et al., 2011; Volpato et al., 2013). Especially, effect of light spectra on the somatic growth were announced from some fish. For instance, BW and TL of barfin flounder were increased when reared under green or blue (short wavelength) light conditions and fish growth of red group was remain behind than other groups (Yamanome et al., 2009). Also, green or blue wavelength

was reported to the positive effect on the fish growth via haddock (Downing, 2002), crucian carp, Chinese sleeper (Ruchin, 2004), olive flounder (Ko & Park, 2012) and rock fish (Shin et al., 2015). Based on these results, we performed study of light spectra effect on the growth of tiger puffer. Tiger puffer was reared in different light conditions (blue, green and red) for 8 weeks and showed better somatic growth when reared under green light condition. However, reared tiger puffer under red light condition was shown slower growth than other experimental groups. In contrast to our results, rainbow trout showed negative impact on growth performance when rearing under blue light condition (Karakatsouli et al., 2007) and yellow light was efficiently induced the mean weight (Heydarnejad et al., 2013). These different results were suggest that the light spectra represents a species specific effect in fish growth, because preferred wavelength was different for each fish species.

Nevertheless, physiological mechanism of light spectra with growth of fish is still unclear and hardly study. Therefore we investigated changes of weight gain, feed efficiency and growth-related genes expression (*ss1*, *ss2*, and *ss3*) under different light spectra conditions. In generally, SS is well known growth hormone-inhibiting hormone (GHIH) and released in hypothalamus (Sheridan & Kittilson, 2004; Klein & Sheridan, 2008). This hormone is inhibited GH secretion in pituitary gland and shows a negative effect on the growth in goldfish (Cook et al., 1984) and orange-spotted grouper (Wang et al., 2014). Furthermore, SS is related to feeding behavior and digestion in fish. In rainbow trout, food conversion was significantly reduced by SS implantation (Very et al., 2001). In contrast with SS, GH is released hormone in pituitary and it has ability of somatic growth control with many biological actions including appetite, osmoregulation and reproduction in teleost fish (Tatsuta & Hirano, 1993; Mingarro et al., 2002; Kim et al., 2015). In our presents, weight gain and feed efficiency were higher in green light condition, but in red light condition weight gain and feed efficiency were low. The *ss1* and *ss2* genes

of brain were showed high expression when reared under red light condition and its expression were low when reared under green light condition. Therefore, we present results suggest that light spectra were acts as controller of *ss* gene expression regulation and this *ss* gene expression will have an effect on the feeding behavior and digestion in fish. However, retardation of *gh* expression in pituitary was not observed and showed no different expression between experimental groups. In different light conditions (blue, green and red), yellowtail clownfish *gh* mRNA expression was more higher under green and blue light conditions than under red light condition and induced higher growth rate (Shin et al., 2012). These data were indicate that light spectra affects in endocrine system associated with growth. Although, our present was not confirmed difference of *gh* expression in pituitary, but *gh* expression in red light condition was tended to lower than blue and green light conditions. We suggest that *ss* genes are affect directly or indirectly on the *gh* expression and further studies are required to relationship of *ss* with *gh* genes in tiger puffer.

In this study, green light condition induces higher somatic growth and reducing expression *ss1* and *ss2* in the brain, but, red light condition is conflicting results with somatic growth and *ss1* and *ss2* expression. It suggest that red wavelength affects in growth retardation by promoting activation of *ss* expression. Therefore, artificial green light wavelength is suggest more effective on the growth stimulation than red light wavelength in tiger puffer aqua-farm system, but further studies are needed to clarify the mechanism of light spectra with growth endocrine system.

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