



Full length article

Effects of different light spectra on embryo development and the performance of newly hatched turbot (*Scophthalmus maximus*) larvae

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ABSTRACT

Light is a key environmental factor that synchronizes various life stages from embryo development to sexual maturation in fish. For turbot, light spectra have the most influence at the larval and juvenile stages. In the current study, differences in the development of embryos and the performance of newly hatched turbot larvae exposed to five different spectra: full spectrum (LDF), blue (LDB, peak at 450 nm), green (LDG, peak at 533 nm), orange (LDO, peak at 595 nm) and red (LDR, peak at 629 nm), were examined. At 62.8 h post fertilization, a higher number of embryos exposed to short-wavelengths (LDG and LDB) had developed a heartbeat in comparison with embryos exposed to other wavelengths. Larvae exposed to the green spectrum had higher malformation rates than larvae exposed to the other spectra, indicating that larvae exposed to green light may have significantly reduced survival rates. The results of non-specific immunity parameters showed that the mRNA expression levels of cathepsin D (CTSD), cathepsin F (CTSF), catalase (CAT) and metallothionein (MT) in larvae exposed to LDB were significantly higher than those exposed to other spectra, but CAT activity in larvae exposed to LDB was significantly lower than larvae exposed to the other spectra. There was no significant difference in MT activity in larvae exposed to the five different spectra. The mRNA expression level of lysozyme (LZM) in larvae exposed to LDR was significantly higher than other spectra, while there was no significant difference in LZM activity observed in larvae exposed to LDR, LDG, LDB and LDF. The difference of the enzyme activity of total superoxide dismutase (T-SOD) was not significant among larvae exposed to the five spectra. mRNA expression of the heat shock protein 70 (HSP70) was significantly higher in newly hatched larvae exposed to LDB, LDR and LDG, indicating that larvae exposed to LDB, LDG and LDR exhibited a stress response. The mRNA expression level of the insulin-like growth factor-1 (IGF-1) and growth parameters in the newly hatched larvae exposed to the different spectra were not significantly different. The results of the present study indicate that LDO and LDF should be used for embryo incubation and newly hatched larvae when rearing turbot. This study provides a theoretical basis for optimizing the incubation light environment for fertilized turbot eggs, promoting immunity and reducing stress responses in newly hatched larvae.

1. Introduction

Light intensity, spectrum and photoperiod influence processes including development, metabolism, and reproduction in teleost fish [1,2]. Light is classified into ultraviolet light, visible light, and infrared light according to spectral components. Visible light can be further divided into long wavelength, middle wavelength and short wavelength

spectra. The spectral composition of various water layers in the sea differs due to differences in the absorption of long and short wavelength visible light [3]. Many marine fishes exhibit horizontal or vertical migration in their life cycle, meaning that they experience different light spectra at different life cycle stages. Light spectra can influence the entire life cycle of teleost fish, from embryo development to sexual maturation [2,4]. Previous studies have reported that light spectra can

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influence fish growth and development, energy metabolism and reproductive physiology [5]. It has been found that the green spectrum can significantly stimulate the somatic growth of juvenile barfin flounder (*Verasper moseri*) [6], the blue spectrum can have a significant negative effect on the growth performance of rainbow trout (*Oncorhynchus mykiss*), and the red spectrum can reduce the growth of juvenile gilthead seabream (*Sparus aurata*) [7].

The retina seems to be the primary photoreceptor in teleost fish, with opsin on the cone cell reacting to specific wavelengths [8,9]. The photosensitive pathway of the visual system consists of the retina, the preoptic area and the suprachiasmatic nuclei of the hypothalamus [10]. The pineal gland, an important photoreceptor outside the retina, has also been found to play an important role in detecting different light spectra [4,11], with a similar organizational structure to the retina and a complete photoreceptor pathway.

In poultry, embryos are influenced by light spectra. The red spectrum can significantly increase the maturation level and hatchability of fertilized eggs, and reduce physiological defects of broiler chicks [12]. It has been hypothesized that light irradiance can penetrate the shell, and red light may increase mitochondrial activity, ATP production, and rate of cellular division [13]. Physiological conditions such as internal secretion conditions of the embryo could be changed by light [14]. However, the basis of the photoreceptive mechanism in broiler chick embryos is not yet known. In teleost fish, the influence of light spectra on larvae seems to be species specific [3]. Properties of light have been shown to significantly impact embryonic development and hatching for several cultured species [15]. Sebastian et al. [16] found that the red spectrum could improve the survival rate of newly hatched European eel (*Anguilla anguilla*) larvae. Spectral composition had no influence on the embryonic development and growth performance of newly hatched haddock (*Melanogrammus aeglefinus*) larvae, but significantly affected larval survival rate [2,17]. There was no significant influence of light spectra on embryonic development and growth performance of newly hatched chinook salmon (*Oncorhynchus tshawytscha*) larvae [1]. In addition, studies on zebrafish, have shown that red light and infrared wavelengths accelerated the development of embryos [18]. The influences of light spectra are varied and are explained with the predisposition to adapt to the spectral conditions most frequently encountered in specific ecological niches [19]. Prior to the formation of retina, the pineal gland has been reported to play an important role in photosensitivity, and can mediate physiological performance by secreting melatonin in teleost embryos [20].

A change from original ambient conditions, including the light spectral environment, can lead to a stress response in teleosts [21–23]. It is well documented that stress may induce the generation of reactive oxygen species (ROS) [24], further affecting the antioxidant system that maintains homeostasis in fish [25]. SOD, CAT, peroxidase (POD) and glutathione s-transferase (GST), the biomarkers of antioxidant defense system, play a key role in eliminating ROS [26,27]. Metallothionein (MT), one of the most effective free radical scavengers known to date, also plays an important role in antioxidant activity [28]. Meanwhile, stress responses can result in the alteration of a non-specific immunity response [29]. LZM, a vital enzyme involved in innate immunity, plays an important role in protecting organisms from pathogens prior to the development of an immune system [30,31]. Different spectra have been reported to result in differences in mRNA levels of LZM in zebrafish [32]. Cathepsin maintains cellular homeostasis and is involved in non-specific immunity [33]. Li et al. [34] found that a high flow velocity could produce a stress response and an up-regulation of cathepsin expression in turbot. Previous studies have also demonstrated that long-term stress can negatively influence teleost growth [35]. The growth of teleost fish, including somatic growth, metabolism, and organ development, is regulated by a growth hormone (GH) secreted by the pituitary gland [36]. Most of the biological functions of GH are mediated by IGF-1 [37]. Jia et al. [38] found that a long period of ammonia exposure could reduce the IGF-1 mRNA level and reduce the growth of

juvenile turbot.

Turbot is one of the most important cultured flatfish in China and has a high commercial value. In 2015, global turbot aquaculture production was more than 65,000 tons, of which China produced 55,000 tons, accounting for 85% of global aquaculture production [39]. Optimizing the light environment of fertilized turbot eggs and newly hatched larvae could improve the quality of turbot larvae and have huge economic benefits. While developing, turbot undergoes metamorphosis, moving from the upper water layer to the bottom, experiencing different spectral compositions. The main structure of the retina of turbot is well developed by two days post hatching (dph). The majority of research has focused on the larvae, juvenile and adult stages of turbot [40,41]. To our limited knowledge, there has been no previous research examining the influence of light spectra on the development of fertilized eggs and newly hatched turbot larvae.

The current study aimed to investigate the effects of light spectra on the performance of embryos and newly hatched larvae, including the hatching rate, malformation rate, enzyme activity and gene expression relating to antioxidant defense, non-specific immunity, and gene expression relating to growth. This research provides the theoretical basis for optimizing the light environment for fertilized turbot eggs and newly hatched larvae.

2. Materials and methods

2.1. Experimental set up

LED lights of five different spectra, full spectrum (LDF), blue (LDB, peak at 450 nm, range: 425 nm–772 nm), green (LDG, peak at 533 nm, range: 468 nm–648 nm), orange (LDO, peak at 595 nm, range: 533 nm–654 nm) and red (LDR, peak at 629 nm, range: 573 nm–670 nm) were provided by Shenzhen Fluence Technology PLC (Shenzhen, China). Five experimental tanks (each 1.5 m in diameter and 0.85 m in depth, water volume of approximately 1.5 m³) were located in Shenghang Aquatic Science and Technology Company (Weihai, Shandong Province, China). The LED lights were set over the center of each tank (one spectrum per tank). Photon flux density (PPFD) was set at $1.76 \pm 0.04 \mu\text{mol}/\text{m}^2/\text{s}$ on the water surface in all five tanks. The photoperiod was set as 24 L:0 D. Hatching bowls (1000 mL) with very small holes on the sides and bottom to facilitate a continued exchange of water, while ensuring that the embryo could not escape from the container, were utilized. Nine hatching bowls were placed in each tank (45 bowls in total). The hatching bowls were set around the center of the tank to ensure that the light intensity in the hatching bowls was constant and was reaching the zygotes. Three of bowls in each tank were used to evaluate embryonic development (Group A), three were utilized for calculating hatching rate and body length of newly hatched larvae (Group B), and three were used for assessing deformity rate and measuring enzyme activity and gene expression of larvae (Group C).

2.2. Experimental design and water quality

Male and female turbot brood stock was supplied by Shenghang Aquatic Science and Technology Company (Weihai, Shandong Province, China). In the current study, eggs were collected from ten female turbot and sperm was collected from three male turbot. Sperm motility was checked prior to fertilization. The superior zygotes were selected and 840 ± 90 embryos were transferred to each bowl five minutes after fertilization.

During the entire experimental period, the water temperature in the tanks were maintained at $15.8 \pm 0.2^\circ\text{C}$. Concentrations of ammonia, nitrite, nitrate, chemical oxygen demand (COD), and phosphate were shown in Table 1.

Table 1 Concentrations of ammonia, nitrite, nitrate, chemical oxygen demand (COD), and phosphate of experimental tanks under different spectra.

Table 1
Concentrations of ammonia, nitrite, nitrate, chemical oxygen demand (COD), and phosphate of experimental tanks under different spectra.

Light spectrum	The parameters of water quality				
	COD (mg/L)	Ammonia (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)
Red	0.33 ± 0.05	0.23 ± 0.06	0.0056 ± 0.0016	0.87 ± 0.14	0.044 ± 0.0074
Orange	0.33 ± 0.05	0.15 ± 0.01	0.0032 ± 0.0011	0.72 ± 0.16	0.043 ± 0.0054
Green	0.32 ± 0.05	0.16 ± 0.04	0.0034 ± 0.0010	0.70 ± 0.07	0.039 ± 0.0074
Blue	0.35 ± 0.15	0.13 ± 0.03	0.0049 ± 0.0022	0.82 ± 0.10	0.037 ± 0.0054
Full	0.29 ± 0.10	0.22 ± 0.07	0.0031 ± 0.0003	0.94 ± 0.12	0.041 ± 0.0041

2.3. Fish sampling and analysis

2.3.1. Analysis of embryonic development

Five developmental stages were chosen to analyze embryonic development [42]. These stages were the blastula stage, gastrulation, appearance of dorsal pigmentation, formation of Kupffer's vesicle and heartbeat development.

In the current study, 30 eggs were collected from each of hatching bowls in Group A and a total of 90 fertilized eggs were examined at every period. The conditions of embryonic development were observed and recorded under a stereoscope (Jiangnan Novel Optics Co. Ltd, Nanjing, China). The sampling and observations at each period were carried out within 15 min.

2.3.2. Analyzing and sampling newly hatched larvae

Once the first larva hatched, the number of newly hatched larvae under each light spectrum in Group B was counted every three hours. Subsequently, larvae were collected and anesthetized with a 0.05% solution of MS-222 [43]. The body length of each larvae was measured using a Nikon DS-Fi1 imaging system (H5D) via a Nikon E50i microscope (Nikon, Japan).

In Group C, all newly hatched larvae were collected, anesthetized with a 0.05% solution of MS-222, and frozen in liquid nitrogen to analyze enzyme activity and gene expression at 96 h after hatching. On 2 dph, the remaining one hundred larvae left in Group C was used to record mortality rate. Thirty larvae were collected, anesthetized with a 0.05% solution of MS-222 and observed via a Nikon E50i microscope to check for any malformations.

2.3.3. Oxidative response and enzyme activity of newly hatched larvae

Whole fish tissues were homogenized in an ice-cold 20 mmol/L Tris-HCl buffered solution (containing 10 mmol/L Tris-HCl, 10 mmol/L of saccharose, 0.1 mmol/L of ethylene diamine tetraacetic acid disodium salt (EDTA-2Na), 0.8% NaCl, pH = 7.4). The homogenates were centrifuged at 2500 rpm for 10 min at 4 °C to precipitate large particles. The supernatants were collected and maintained at 4 °C for the evaluation of related enzyme activity in a 1-h period.

T-SOD, CAT and LZM activity were determined using a detection kit (Nanjing Jiancheng Bioengineering Institute, China), following the methods described by Liu et al. [44]. POD activity was also determined using a detection kit (Nanjing Jiancheng Bioengineering Institute, China) as described by Li et al. [45]. MT content was determined using an ELISA detection kit, following to manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China).

2.3.4. Gene expression in newly hatched larvae

An RNA extraction kit (fast pure RNA kit (Fastagen, Shanghai)) was used for total RNA extraction from whole fish tissue homogenates following the manufacturer's instructions. The amount of RNA was measured using GeneQuant 1300 (GE Healthcare Biosciences, Piscataway, NJ) and its quality was checked on an agarose gel. For each sample, 2 µL of RNA was subjected to cDNA synthesis using a Prime Script RT reagent Kit with a gDNA Eraser (Takara, Dalian, China) following the manufacturer's protocol. The reverse transcription reaction (20 mL)

consisted of 1 µg of total RNA, 2 µL of 5 × gDNA eraser buffer, 1 µL of gDNA eraser, and RNase free dH₂O up to a final volume of 10 µL. Once mixed, it was allowed to react at 42 °C for 2 min. The mixture was then cooled at 4 °C. Following this, 10 µL of the resulting liquid, 1 µL of Prime Script RT enzyme Mix, 1 µL of RT Primer Mix, 4 µL of 5 × Prime Script Buffer, and RNase free dH₂O were made up to a final volume of 20 µL. Once mixed, the components were left to react at 37 °C for 15 min, 85 °C for 5 s, and then cooled at 4 °C. cDNA products were stored at –20 °C for quantitative real-time PCR (qRT-PCR).

qRT-PCR reactions were performed following the manufacturer's instructions using a kit (SYBR Premix Ex Taq (Tli RNaseH Plus), Takara, Dalian, China) to detect the expression of differentially expressed genes including IGF-1, GST, HSP70, CTSD, CTSF, CAT, MT, and LZM. The qRT-PCR primer is shown in Table 2. qRT-PCR was performed using a CFX Connect Real-Time System and Bio Rad CFX Manager (version 3.1). The qRT-PCR mixture contained 2 µL diluted cDNA, 10 µL 2 × SYBR Green PCR Mix, 0.4 µL of each gene-specific primer and 7.2 µL RNase-free ddH₂O to a final volume of 20 µL. The cycling parameters were: 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s, 30 s at the specific annealing temperature, followed by a melt curve stage after the cycling stage. The specificity of qRT-PCR was analyzed by agarose gel and melting curve analysis. The expression levels of target genes were calculated using the 2^{–ΔΔCt} method as described by Lin et al. [46]. 18s was used as an internal control gene for mRNA level analysis. For enzyme activity analysis, corresponding kits from Nanjing Jiancheng Bioengineering Institute were used, following to manufacturer's instruction.

2.4. Statistical analysis

Gene expression data (relative fold changes) were represented as mean values ± standard error. Other data were represented as mean values ± standard deviation. All statistical analyses were performed

Table 2
qRT-PCR forward and reverse primer sequence.

Gene		Primer sequence (from 5' to 3')	Reference
18S	F	ATGGCCGTTCTTAGTTGGTG	[47]
	R	CTCAATCTCGTGTGGCTGAA	
IGF-1	F	TGTAAGTGTGGCCTGCAAGACTA	[48]
	R	TGCTGTGCTGCTACGCTCTGT	
GST	F	GGGTTCGCATCGCTTTT	[49]
	R	GGCCTGGTCTCGTCTATGTA	
HSP70	F	CTGTCCCTGGGTATTGAGAC	[49]
	R	GAACACCACGAGGAGCA	
LZM	F	CTCTCAACGTTCCCACTGGTCTA	[50]
	R	GGGTTCATGAAGTGTCTGTAGAT	
MT	F	TGCTCCAAGAGTGGAACTTG	[29]
	R	CGCATGTCTTCCCTTTGCAC	
CTSF	F	GAGGAGTCTGTGGAGCTGTT	[51]
	R	TCAGCTGAGCCITGATCCAA	
CTSD	F	ACTATGGGGACATTGCTCTGGGT	[52]
	R	GGAGTGAGCAGTGAACAGACGGAAC	
CAT	F	CAGTGGGACGAAAGATAA	[53]
	R	CCTGGACGGCTGTAACCG	

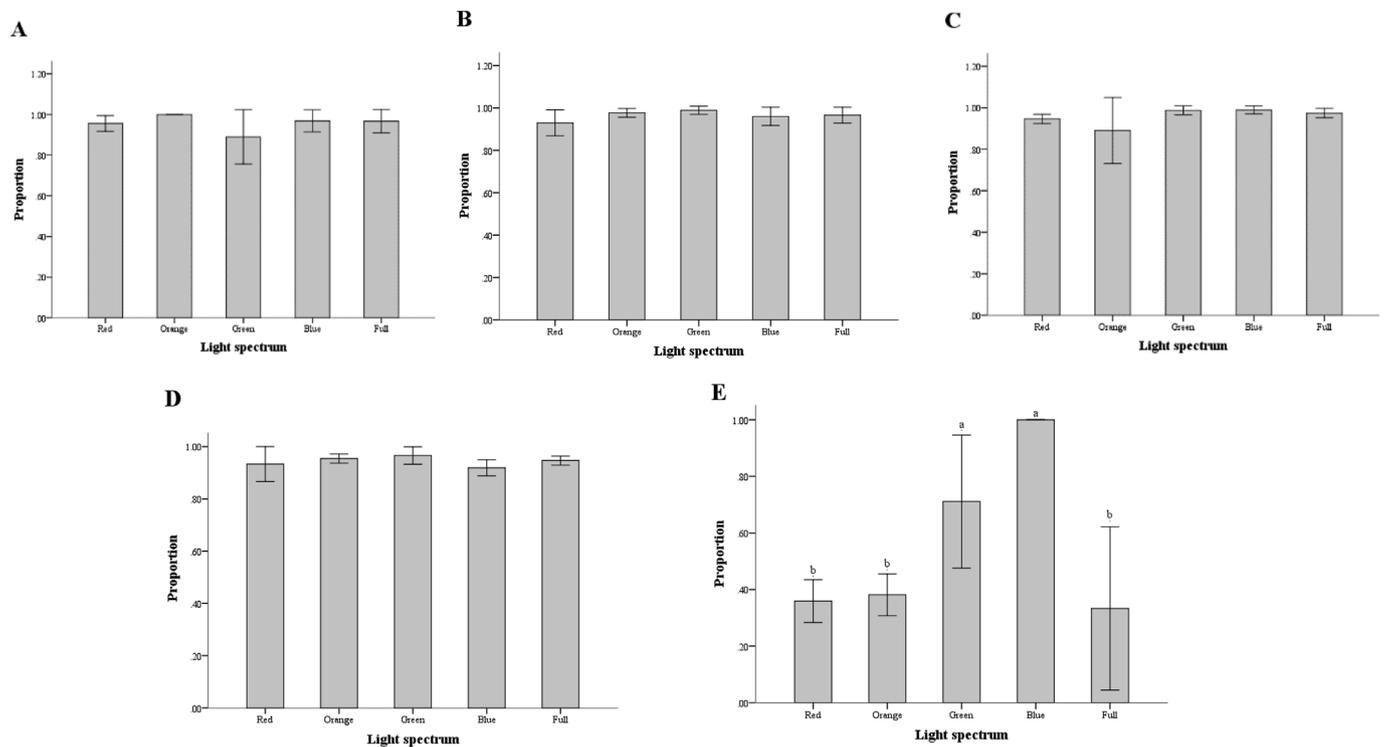


Fig. 1. Proportion of embryos at different stages of embryonic development. (A) blastocyst stage, (B) gastrulation, (C) formation of Kupffer's vesicle, (D) appearance of dorsal pigmentation, (E) development of heartbeat.

using SPSS 19.0 software. All the indexes were compared for each spectrum using one-way ANOVA and Tukey's test. Significance was set at the 0.05 level.

3. Results

3.1. Embryo development at five different spectra

The proportion of embryos reaching blastula stage, gastrulation, appearance of dorsal pigmentation, formation of Kupffer's vesicle and heartbeat development are shown in Fig. 1. There were no significant differences in the proportion of embryos arriving at the blastocyst stage, gastrulation stage, formation of Kupffer's vesicle or the appearance of dorsal pigmentation. At 62.8 h post fertilization, the proportions of embryos recorded with a heartbeat that had been exposed to LDG and LDB was significantly higher than for other light spectra. There was no significant difference between the number of embryos developing a heartbeat when exposed to LDG and LDB.

3.2. Hatching rate of newly hatched larvae and deformity rate, mortality rate of larvae (2dph)

There was no significant difference in hatching rate between the five different spectra (Fig. 2). The malformation rate of larvae exposed to LDG was significantly higher than for the other spectra. There were no significant differences in malformation rate in larvae exposed to LDF, LDR, LDO and LDB (Fig. 3). The mortality rate was shown in Fig. 4. The mortality rate of larvae exposed to LDG was significantly higher than those exposed to LDO and LDF.

3.3. Growth and IGF-1 expression in newly hatched larvae

There were no significant differences observed in the body length of newly hatched larvae exposed to different light spectra (Table 3). In addition, there were no significant differences in the IGF-1 mRNA level

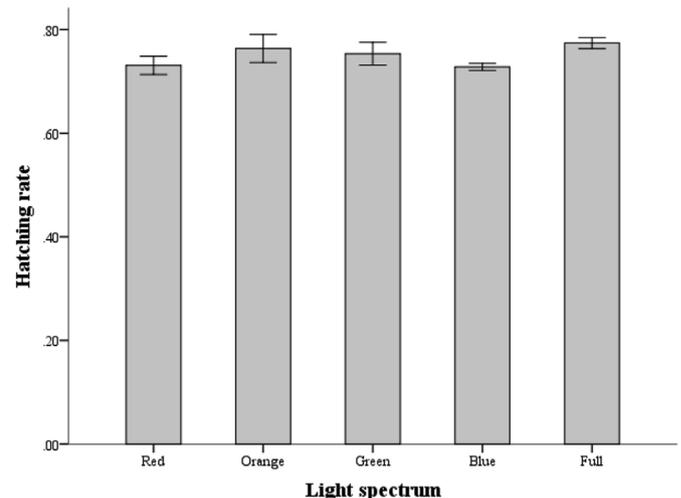


Fig. 2. Hatching rate of fertilized eggs exposed to five different spectra.

of newly hatched larvae exposed to different light spectra (Fig. 5).

3.4. Enzyme activities and gene expression in newly hatched larvae associated with oxidative-stress defense

There were no significant differences in T-SOD activity (Fig. 6 A, Table 4), POD activity (Fig. 6C, Table 4) and MT content (Fig. 6 D, Table 4) in larvae exposed to different light spectra. CAT activity was highest in larvae exposed to LDO, and was significantly higher than for larvae exposed to LDB (Fig. 6 B, Table 4).

The mRNA expression level of HSP70 in larvae exposed to LDO was significantly lower than that those exposed to LDG and LDB (Fig. 7 A, Table 4). There was no significant difference in mRNA levels of HSP70 between LDO, LDR and LDF. The HSP70 mRNA level in larvae exposed to LDB was the highest, but this was not significantly higher than LDR

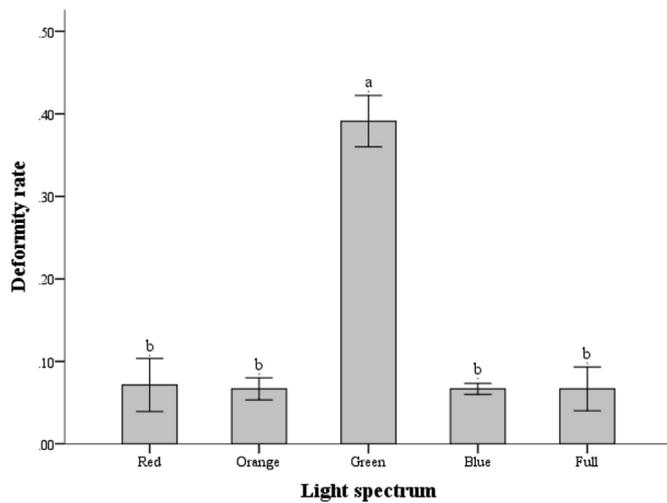


Fig. 3. Malformation rate of larvae exposed to different spectra.

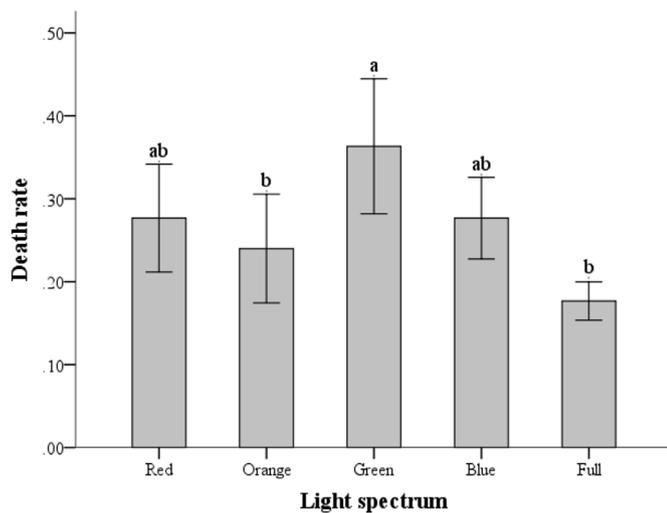


Fig. 4. Mortality rate of newly hatched larvae exposed to different light spectra by 2 dph.

Table 3

Coefficients of body length of newly hatched larvae exposed to different light spectra.

Light spectrum	Mean	Std. Deviation	Amount
Blue	2.7637	0.15082	30
Full	2.7667	0.15212	30
Green	2.7017	0.16779	30
Orange	2.7613	0.1923	30
Red	2.7207	0.17334	30

or LDG. GST mRNA expression levels were highest in larvae exposed to LDR (Fig. 7 B, Table 4). The mRNA expression levels of GST and MT (Fig. 7 D, Table 4) in larvae exposed to LDR and LDB was significantly higher than those exposed to LDO. There were no significant differences between LDB, LDR, LDG and LDF. The CAT mRNA level in larvae exposed to LDB was the highest. This difference was significantly higher than that in larvae exposed to LDO, but not significantly higher than that for larvae exposed to LDR, LDG and LDF. No significant differences were observed between LDO, LDR, LDG and LDG (Fig. 7C, Table 4).

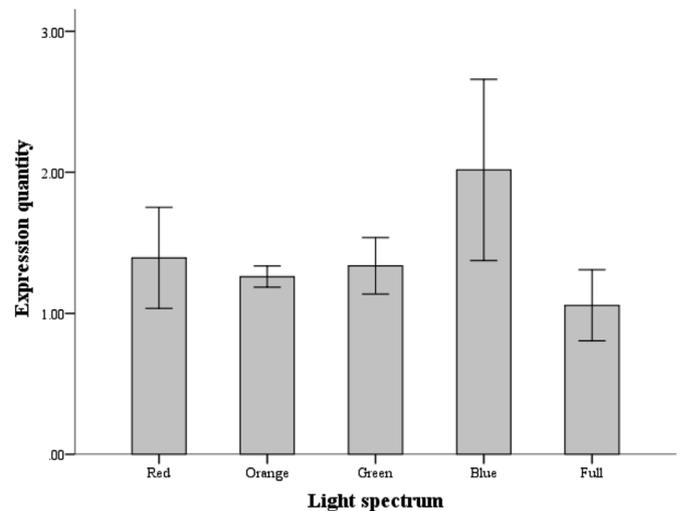


Fig. 5. The mRNA level of IGF-1 in newly hatched larvae exposed to different light spectra.

3.5. Enzyme activities and gene expression of newly hatched larvae associated with non-specific immunity

LZM enzyme activity in larvae exposed to LDR and LDF was significantly higher than that for larvae exposed to LDO. There was no significant difference in LZM activity in larvae exposed to LDO, LDG and LDB. No significant difference in LZM activity was observed in larvae exposed to LDR, LDF, LDG and LDB (Fig. 8, Table 4).

The mRNA expression level of LZM in larvae exposed to LDR was significantly higher than for other light spectra. There were no significant differences in mRNA expression levels of LZM observed in larvae exposed to LDO, LDG, LDB and LDF (Fig. 9 A, Table 4). The CTSE mRNA level in larvae exposed to LDR was highest, and this was significantly higher than that for larvae exposed to LDO and LDF. However, there was no significant difference observed in CTSE mRNA levels in larvae exposed to LDB, LDR and LDG (Fig. 9C, Table 4). Similar with CTSE, the CTSD mRNA level was highest in larvae exposed to LDR, and this was significantly higher than levels in larvae exposed to LDO, LDG and LDF. There was no significant difference in CTSD mRNA levels in larvae exposed to LDB and LDR (Fig. 9 B, Table 4).

4. Discussion

The different absorbance properties of light at different wavelengths in the water column complicates the spectral components in natural waterbodies [3]. The perception of light by fish is mainly dependent on opsin on the cone cells, which has evolved into many variants in the long-term evolution of the organism to adapt to their ambient light spectra composition [54]. Teleosts exhibit great adaptability to the spectral components of their niches [55]. The fertilized eggs of turbot float and larvae live in the upper layer of the water column. During the development process, metamorphosis occurs at around 30 dph, during which the eyes gradually shift to the left side of the body and the juveniles become benthic [56]. Due to the various ambient light spectra experienced by larvae and juvenile turbot, it was speculated that the spectral environment requirements of different life stages of turbot were also different.

The retina of newly hatched larvae is comprised of an outer nuclear layer, the inner nuclear layer and the ganglion cell layer [57]. The retina is well formed by 2 dph, and may not be functional until maturation [57]. Prior to the formation of the retina, the pineal gland, has been shown to be functional [14,15]. It has been found that cerebral lateralization can be affected by light stimulation before the visual system is functional in zebrafish embryos [14]. Light stimuli detected

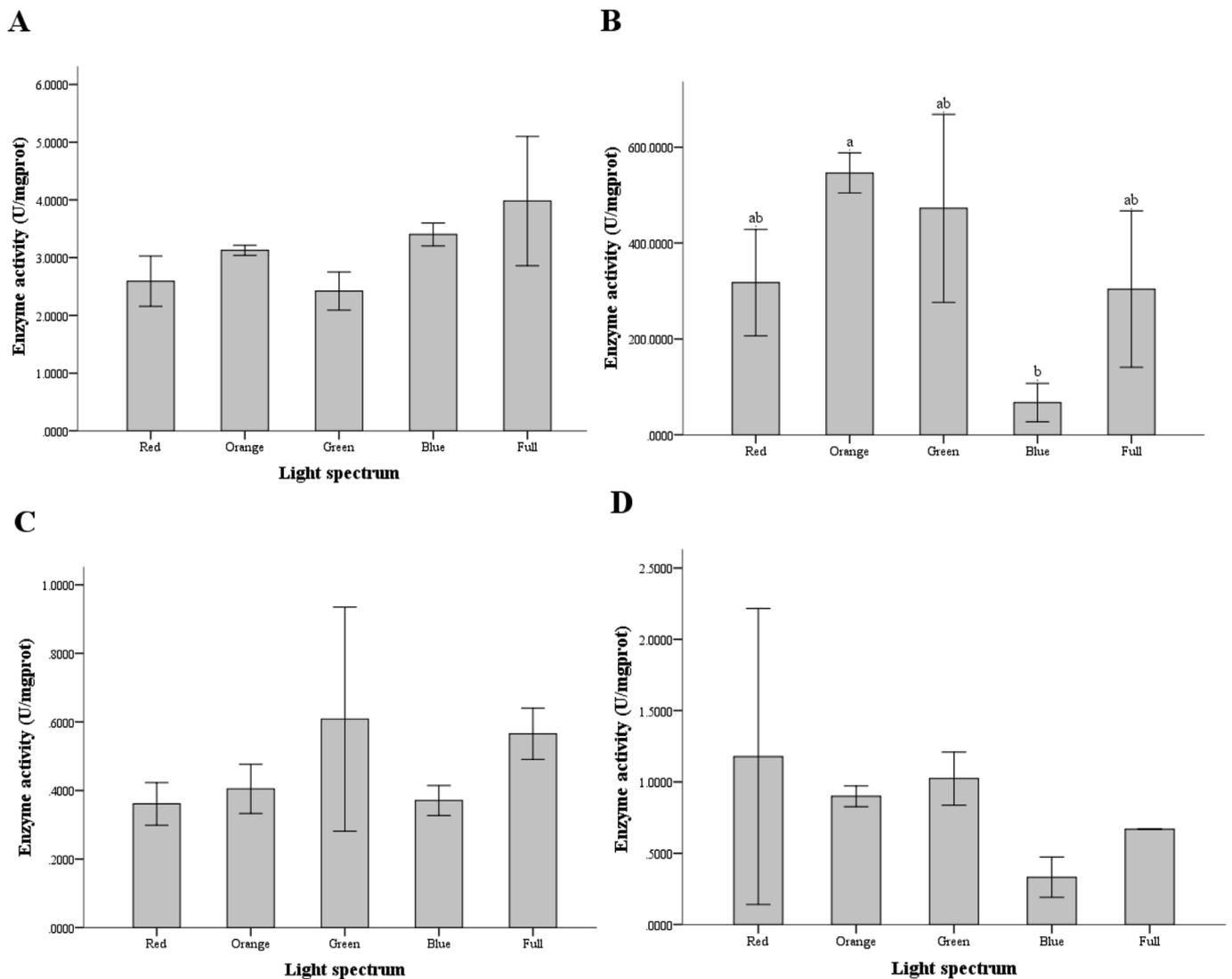


Fig. 6. T-SOD activity (A), CAT activity (B), POD activity (C), and MT content (D) of newly hatched larvae exposed to different light spectra.

Table 4

mRNA level and enzyme activity of genes associated with antioxidant and non-specific immunity in newly hatched larvae exposed to different light spectra in comparison with LDF.

Parameter	mRNA					Enzyme activity				
	Red	Orange	Green	Blue	Full	Red	Orange	Green	Blue	Full
<i>hsp70</i>	↑ (abc)	↓ (c)	↑ (ab)	↑↑ (a)	- (bc)					
CTSD	↑↑ (a)	↓ (c)	↑ (bc)	↑ (ab)	- (bc)					
CTSF	↑↑(a)	↓ (c)	↑ (abc)	↑ (ab)	- (bc)					
GST	↑ (a)	↓ (b)	↑ (ab)	↑ (a)	- (ab)					
SOD						↓ (a)	↓ (a)	↓ (a)	↓ (a)	- (a)
POD						↓ (a)	↓ (a)	↑ (a)	↓ (a)	- (a)
MT	↑ (a)	↓ (b)	↑ (ab)	↑ (a)	- (ab)	↑	↑	↑	↓	-
CAT	↑ (ab)	↓ (b)	↑ (ab)	↑ (a)	- (ab)	↑ (ab)	↑ (a)	↑ (ab)	↓ (b)	- (ab)
LZM	↑↑ (a)	↓ (b)	↑ (b)	↓ (b)	- (b)	↓ (a)	↓↓ (b)	↓ (ab)	↓ (ab)	- (a)

The symbol: “-”: control group. “↓”: lower but not significant. “↓↓”: significantly lower. “↑”: higher but not significant. “↑↑”: significantly higher. “a”, “b”, “c”: signif. codes.

by the pineal gland are transduced via neuro-regulation or humoral-regulation to affect the physiological status of teleosts [15]. However, it is unclear whether and when turbot embryos can detect light stimuli. Previous research elucidated that there were no significant differences in embryonic development between different spectral regimes for haddock embryos [17]. Similarly, in the current study early stage

embryonic development was not significantly affected by light spectra. This may be due to the fact that during these early stages (blastocyst stage, gastrulation stage, formation of Kupffer's vesicle or the appearance of dorsal pigmentation) turbot embryos had no capacity for spectral detection. It could also be attested to the short development time, where a difference may have appeared but was not found to be

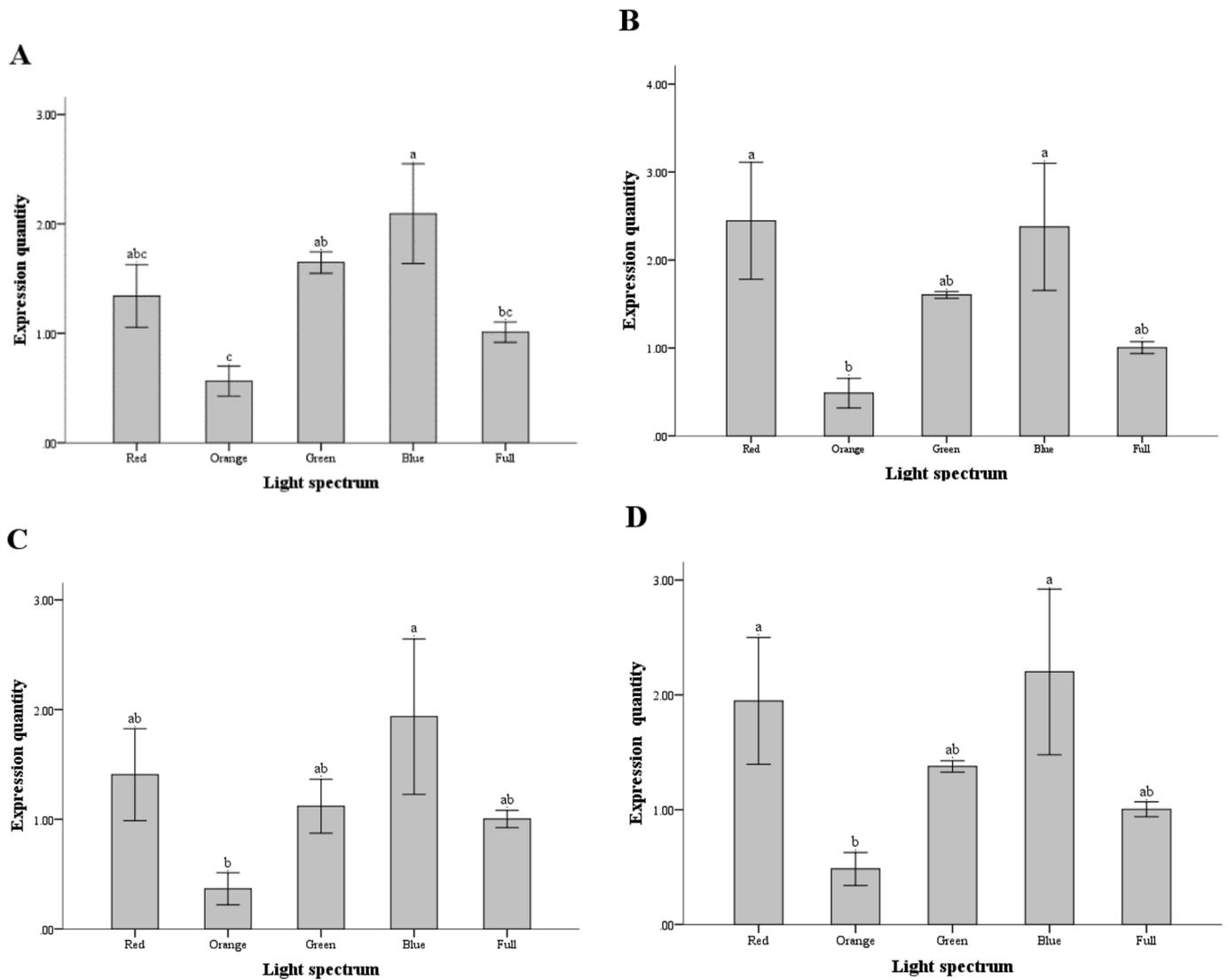


Fig. 7. The mRNA level of HSP70 (A), GST (B), CAT (C) and MT (D) of newly hatched larvae exposed to different light spectra.

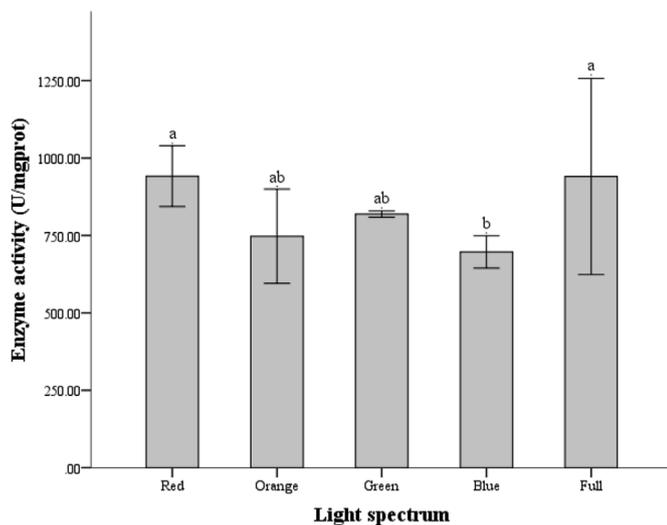


Fig. 8. LZM activity of newly hatched larvae exposed to different light spectra.

significant. However, significant differences were detected after the heartbeat began. The heartbeat of embryos exposed to LDB and LDG began significantly earlier than in larvae exposed to other spectra. It is possible that the pineal gland, which is equipped with a complete photosensitive pathway independent of retinal photoreceptors [10,58], might have been functional prior to hatching. Therefore, it is reasonable to deduce that prior to the heartbeat developing, turbot embryos are capable of sensing light spectra. However, further research is required to determine the exact stage at which turbot embryos have the capacity to sense light spectra.

Skeletal development is critical in aquaculture and is related to the normal external morphology and activity of larvae [59]. Skeletal malformation in larvae can lead to difficulty in movement, growth retardation, and even increased mortality [60–62]. Some studies have revealed that light spectrum is related to calcium absorption [61], bone mineral density [63] and skeletal muscle cell proliferation [64] in birds. Skeletal malformation of teleost larvae is affected by many ambient factors, such as inappropriate incubation temperature or salinity [65], bacterial infection [66], unspecified pollutants [67], nutritional factors [68] and inappropriate light intensity and spectra [69]. Villamizar et al. [70] found that red light could significantly increase the frequency of jaw malformation in European sea bass (*Dicentrarchus labrax*) larvae. Strong illumination and continuous light could also result in a higher malformation rate of European sea bass [71,72]. In the current study,

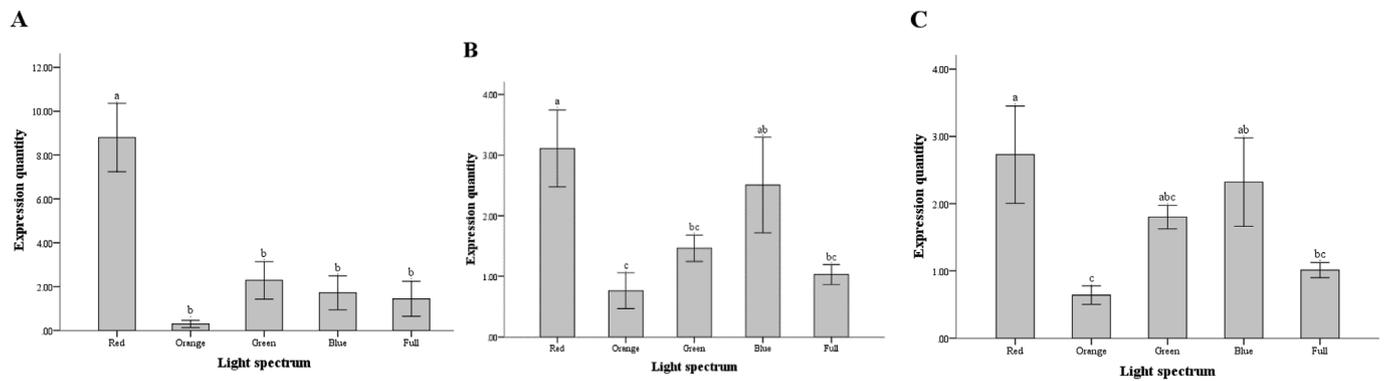


Fig. 9. mRNA expression level of LZM (A), CTSD (B), and CTSF (C) of newly hatched larvae exposed to different light spectra.

light spectrum had no significant influence on the hatching rate of fertilized eggs. However embryos exposed to LDG displayed a higher deformity rate. The mortality rate of larvae exposed to LDG was significantly higher than those exposed to LDO and LDF. In addition, the larvae exposed to LDG were observed to have poor mobility. The highest mortality rate and poor mobility of larvae exposed to LDG were associated with the dramatically higher deformity rate. A further systematic experimental approach will be needed to determine which pathway was influenced by light spectra, affecting the skeletal development of turbot.

IGF-1, a vital component of the growth hormone/insulin-like growth factor-I (GH/IGF-1) axis, plays an important role in the mediation of somatic growth [73,74]. Decreases in IGF-1 levels can inhibit growth and influence other physiological progress [74]. Previous studies have shown that light spectra have a significant effect on melatonin levels in Atlantic salmon (*Salmo salar*) [11]. In addition, melatonin has a bimodal effect on GH production [75]. In the current study, there was no significant difference in the mRNA expression level of IGF-1 and average body length in newly hatched larvae exposed to five different light spectra.

HSP70, which belongs to the heat shock protein family, is a protein used for synergistic immunity. Heat shock proteins perform the most basic physiological functions in cells, such as protein folding, stretching, transport, oligomer formation and depolymerization, maintaining cell survival and function, and improving cell resistance under adverse stress conditions. Therefore, HSP70 is often used as an indicator of stress response [76–79]. Lee et al. [78] reported that high culture density, a stressor, could lead to high levels of HSP70. In the current study, high levels of HSP70 expression in larvae exposed to LDB, LDR and LDG may indicate that these spectra could induce a stress response in larvae.

SOD belongs to a family of metalloenzymes that are omnipresent and vital for protecting individuals from ROS [80]. CAT and POD are also important catalysts for eliminating hydrogen peroxide and maintaining redox balance in the body. POD also displays significant antimicrobial properties [81,82]. MT belongs to a group of nonenzymatic proteins, which play a role in antioxidant activity and heavy metal detoxification [29,83,84]. Due to its highly efficient heavy metal detoxification and antioxidant capacity, MT also plays an important role in repairing nucleic acid damage and anti-stress responses [85]. Further studies have shown that the mRNA level of SOD and CAT were up-regulated in juvenile cinnamon clownfish (*Amphiprion melanopus*) when exposed to LDR [86]. Carlo et al. [87] found that different photoperiods had a significant influence on POD activity in juvenile Nile tilapia (*Oreochromis niloticus*). In the current study, mRNA expression levels of CAT and MT were significantly higher in larvae exposed to LDR, LDB and LDG than in larvae exposed to other spectra. However, CAT activity was the lowest in larvae exposed to LDB, and there was no significant difference in the enzyme activity of MT among larvae exposed to the

five different spectra. There was also no significant difference in SOD and POD activity in larvae exposed to different spectra. In contrast to Zheng et al. [32], who attributed the mismatch between gene expression and enzyme activity to the time-lag between transcription and translation, the results of the current study indicate that the stress response of the newly hatched larvae exposed to LDR, LDB and LDG produced more ROS, causing high mRNA expression, and increased enzyme consumption to neutralize ROS. The function of GST is to alleviate the toxicity of diverse endogenous and exogenous compounds by binding nucleophilic glutathione to various electrophilic exogenous chemicals [88]. Lee et al. [78] reported that a high stocking density resulted in the up-regulation of the mRNA level of GST in cyclopoid copepods (*Paracyclops nana*). The significantly higher GST mRNA levels in larvae exposed to LDR and LDB in the current study, in comparison with those exposed to LDO, indicates that LDB and LDR are stressors for newly hatched turbot larvae.

Cathepsins belong to the papain family and are mainly distributed in lysosome. They are aspartic proteinases [33] and their main role is to maintain cellular homeostasis [89]. Cathepsin D is an endopeptidase whose major functions include protein degradation, antigen processing, and involvement in peptide activation, hormones and growth factors [90]. In addition, Cathepsin D also participates in apoptosis [91], a mechanism of self-protection and development [92]. Chen et al. [93] showed that, CTSD expression in the kidney and spleen was significantly up-regulated following exposure to the megalocytivirus RBIV-C1. Cathepsin F is well known for its role in oocyte maturation, retina development and embryogenesis [94–96]. Some studies have also demonstrated that it plays a role in non-specific immunity [51]. Gao et al. [51] found that bacterial infection could cause a high level of CTSF. The results of the current study showed that the mRNA level of CTSD and CTSF in larvae exposed to LDO was significantly lower than that in larvae exposed to LDR and LDG. It is possible that LDO causes less stress for newly hatched larvae. LZM is a vital lytic protein that defends against Gram-positive bacteria [30]. LZM plays a broad role in antiviral, antibacterial and anti-inflammatory defense [97]. Baekelandt et al. [22] reported that LZM activity in juvenile pikeperch (*Sander lucioperca*) at 42 dph was significantly inhibited by red light. In the current study, the mRNA expression level in larvae exposed to LDR was highest, while there was no significant difference in enzyme activity in larvae exposed to LDR, LDB, LDG and LDF. LDR, LDG and LDB might cause more stress to newly hatched turbot larvae, as indicated by other parameters (HSP70, SOD, CAT, MT, CTSD, CTSF, GST). The up-regulation of LZM at the mRNA level and the increase in LZM enzyme activity in larvae exposed to LDR in the current study indicates that LDR caused stress and stimulates immune expression and enzyme biosynthesis in turbot larvae.

5. Conclusion

This study confirmed that light spectra have a significant effect on embryonic development and performance of newly hatched turbot larvae. The turbot embryo may experience photosensitivity during early ontogeny before the retina is well developed, which may affect the physiology of the embryo and the newly hatched larvae. No significant difference was observed in terms of hatching rate embryos exposed to different light spectra. However, it was found that LDR, LDB and LDG could induce a stress response in newly hatched larvae exhibited by levels of HSP70, antioxidant related genes (CAT, MT, GST), non-specific genes (LZM, CTSF, and CTSD), antioxidant related enzyme activity (T-SOD, POD, and CAT), MT content, and non-specific enzyme activity (LZM). Larvae exposed to LDG were found to have a high rate of malformation. The results of the current study indicated that exposure to LDR, LDB and LDG was not suitable for turbot eggs and newly hatched larvae. LDO and LDF are recommended for the hatching of fertilized eggs and newly hatched turbot larvae.

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References

- D.B. Dey, D.M. Damkaer, Effects of spectral irradiance on the early development of chinook salmon, *Progressive Fish-Culturist* 52 (3) (1990) 141–154.
- G. Downing, The effect of light intensity and spectrum on the incidence of first feeding by larval haddock, *J. Fish Biol.* 59 (6) (2001) 1566–1578.
- N. Villamizar, B. Blanco-Vives, H. Migaud, A. Davie, S. Carboni, F.J. Sánchez-Vázquez, Effects of light during early larval development of some aquaculture teleosts: a review, *Aquaculture* 315 (1–2) (2011) 86–94.
- H. Migaud, A. Davie, J.F. Taylor, Current knowledge on the photoneuroendocrine regulation of reproduction in temperate fish species, *J. Fish Biol.* 76 (1) (2010) 27–68.
- M.A.J. Bapary, M.N. Amin, Y. Takeuchi, A. Takemura, The stimulatory effects of long wavelengths of light on the ovarian development in the tropical damselfish, *Chrysiptera cyanea*, *Aquaculture* 314 (1–4) (2011) 188–192.
- T. Yamanome, K. Mizusawa, E.I. Hasegawa, A. Takahashi, Green light stimulates somatic growth in the barfin flounder *Verasper moseri*, *J. Exp. Zool. Part A-Ecological and Integrative Physiology* 311A (2) (2009) 73–79.
- N. Karakatsouli, S.E. Papoutsoglou, G. Pizzonia, G. Tsatsos, A. Tsopelakos, S. Chadio, D. Kalogiannis, C. Dalla, A. Polissidis, Z. Papadopoulou-Daifoti, Effects of light spectrum on growth and physiological status of gilthead seabream *Sparus aurata* and rainbow trout *Oncorhynchus mykiss* reared under recirculating system conditions, *Aquacult. Eng.* 36 (3) (2007) 302–309.
- J.A.B. Velmurugu Puvanendran, Foraging, growth and survival of Atlantic cod, *Gadus morhua*, larvae reared in different light intensities and photoperiods, *Aquaculture* (214) (2002) 131–151.
- S. Julia, D. Wayne L, T. Nicole, B. Lois, C. Jill A, P. Marie, C. Livia S, T. Ann E O, C. Shaun P, B. Lyn D, The influence of ontogeny and light environment on the expression of visual pigment opsins in the retina of the black bream, *Acanthopagrus butcheri*, *J. Exp. Biol.* 211 (9) (2008) 1495–1503.
- J. Falcon, L. Besseau, S. Sauzet, G. Boeuf, Melatonin effects on the hypothalamo-pituitary axis in fish, *Trends Endocrinol. Metabol.* 18 (2) (2007) 81–88.
- L.M. Vera, A. Davie, J.F. Taylor, H. Migaud, Differential light intensity and spectral sensitivities of Atlantic salmon, European sea bass and Atlantic cod pineal glands *ex vivo*, *Gen. Comp. Endocrinol.* 165 (1) (2010) 25–33.
- G.S. Archer, Effect of two different commercially available white light LED fixtures on broiler hatchability and chick quality, *Br. Poult. Sci.* 59 (3) (2018) 251–255.
- N.N. Houreld, Shedding light on a new treatment for diabetic wound healing: a review on phototherapy, *Sci. World J.* 2014 (2014) 398412.
- R.J. Andrew, D. Osorio, S. Budaev, Light during embryonic development modulates patterns of lateralization strongly and similarly in both zebrafish and chick, *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364 (1519) (2009) 983–989.
- J. Falcón, H. Migaud, J.A. Muñoz-Cueto, M. Carrillo, Current knowledge on the melatonin system in teleost fish, *Gen. Comp. Endocrinol.* 165 (3) (2010) 469–482.
- S.N. Politis, I.A.E. Butts, J. Tomkiewicz, Light impacts embryonic and early larval development of the European eel, *Anguilla anguilla*, *J. Exp. Mar. Biol. Ecol.* 461 (2014) 407–415.
- G. Downing, M.K. Litvak, Effects of light intensity, spectral composition and photoperiod on development and hatching of haddock (*Melanogrammus aeglefinus*) embryos, *Aquaculture* 213 (1) (2002) 265–278.
- V.I. Yusupov, N.B. Simonova, G.M. Chuiko, E.I. Golovkina, V.N. Bagratashvili, Regulatory action of low intensity radiation in the near infrared region on the early development of zebrafish (*Danio rerio*), *Biophysics* 63 (1) (2018) 144–151.
- C. Kusmic, P. Gualtieri, Morphology and spectral sensitivities of retinal and extra-retinal photoreceptors in freshwater teleosts, *Micron* 31 (2) (2000) 183–200.
- P. Ekstrzm, H. Meissl, The pineal organ of teleost fishes, *Rev. Fish Biol. Fish.* 7 (2) (1997) 199–284.
- T.M. Houslay, R.L. Earley, A.J. Young, A.J. Wilson, Habituation and individual variation in the endocrine stress response in the Trinidadian guppy (*Poecilia reticulata*), *Gen. Comp. Endocrinol.* 270 (2019) 113–122.
- S. Baekelandt, S.N.M. Mandiki, M. Schmitz, P. Kestemont, Influence of the light spectrum on the daily rhythms of stress and humoral innate immune markers in pikeperch *Sander lucioperca*, *Aquaculture* 499 (2019) 358–363.
- K. Eslamloo, S.R. Akhavan, A. Eslamifar, M.A. Henry, Effects of background colour on growth performance, skin pigmentation, physiological condition and innate immune responses of goldfish, *Carassius auratus*, *Aquacult. Res.* 46 (1) (2015) 202–215.
- C.E. Trenzado, A.E. Morales, J.M. Palma, M. de la Higuera, Blood antioxidant defenses and hematological adjustments in crowded/uncrowded rainbow trout (*Oncorhynchus mykiss*) fed on diets with different levels of antioxidant vitamins and HUFA, *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 149 (3) (2009) 440–447.
- C.M. Caipang, M.F. Brinchmann, V. Kiron, Short-term overcrowding of Atlantic cod, *Gadus morhua*: effects on serum-mediated antibacterial activity and transcription of glucose transport and antioxidant defense related genes, *Comp. Biochem. Physiol. Mol. Integr. Physiol.* 151 (4) (2008) 560–565.
- D. Abele, S. Puntarulo, Formation of reactive species and induction of antioxidant defence systems in polar and temperate marine invertebrates and fish, *Comp. Biochem. Physiol. Mol. Integr. Physiol.* 138 (4) (2004) 405–415.
- O. Milagrosa, J.V. Juan, G. Carlos, G. Lucia, G.R.O. María, Oxidative stress biomarkers in Senegal sole, *Solea senegalensis*, to assess the impact of heavy metal pollution in a Huelva estuary (SW Spain): seasonal and spatial variation, *Ecotoxicol. Environ. Saf.* 75 (1) (2012) 151–162.
- Q. Wei, M.P. Waalkes, Metallothionein blocks oxidative DNA damage induced by acute inorganic arsenic exposure, *Toxicol. Appl. Pharmacol.* 282 (3) (2015) 267–274.
- R. Jia, B.L. Liu, W.R. Feng, C. Han, B. Huang, J.L. Lei, Stress and immune responses in skin of turbot (*Scophthalmus maximus*) under different stocking densities, *Fish Shellfish Immunol.* 55 (2016) 131–139.
- S. Saurabh, P.K. Sahoo, Lysozyme: an important defence molecule of fish innate immune system, *Aquacult. Res.* 39 (3) (2010) 223–239.
- J.G. O'Neill, Ontogeny of the lymphoid organs in an antarctic teleost, *Harpagifer antarcticus* (Notothenioidei: perciformes), *Dev. Comp. Immunol.* 13 (1) (1989) 25–33.
- J.L. Zheng, S.S. Yuan, W.Y. Li, C.W. Wu, Positive and negative innate immune responses in zebrafish under light emitting diodes conditions, *Fish Shellfish Immunol.* 56 (2016) 382–387.
- K. Brix, A. Dunkhorst, K. Mayer, S. Jordans, Cysteine cathepsins: cellular roadmap to different functions, *Biochimie* 90 (2) (2008) 194–207.
- X. Li, L. Ji, L. Wu, X. Gao, X. Li, J. Li, Y. Liu, Effect of flow velocity on the growth, stress and immune responses of turbot (*Scophthalmus maximus*) in recirculating aquaculture systems, *Fish Shellfish Immunol.* 86 (2018) 1169–1176.
- B. Sadoul, M.M. Vijayan, Stress and growth, *Fish Physiol.* (2016) 167–205 Elsevier.
- A.A.B. Lakeh, H. Farahmand, A. Mirvaghefi, W. Kloas, B.C. Peterson, S. Wuertz, GH and IGF-I induction by passive immunisation of rainbow trout *Oncorhynchus mykiss* (Walbaum) using a somatostatin-14 antibody, *Aquaculture* 316 (1) (2011) 99–103.
- C. Duan, Nutritional and developmental regulation of insulin-like growth factors in fish, *J. Nutr.* 128 (2 Suppl) (1998) 306S.
- R. Jia, B.-L. Liu, C. Han, B. Huang, J.-L. Lei, Effects of ammonia exposure on stress and immune response in juvenile turbot (*Scophthalmus maximus*), *Aquacult. Res.* 48 (6) (2017) 3149–3162.
- L. Liu, X. zhu, M. Zhao, Development course and trends of turbot industry in China, *Agric. Outlook* (9) (2018) 51–59.
- R. Sierra-Flores, A. Davie, B. Grant, S. Carboni, T. Atack, H. Migaud, Effects of light spectrum and tank background colour on Atlantic cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*) larvae performances, *Aquaculture* 450 (2016) 6–13.
- G. Boeuf, P.Y.L. Bail, Does light have an influence on fish growth? *Comp. Biochem. Physiol., A* 126 (1–4) (1999) 15 15..
- X. Tong, D. Ma, S. Xu, Q. Liu, C. Zhao, Z. Xiao, Y. Xiao, J. Li, Morphological and histological development of the embryo of turbot *Scophthalmus maximus*, *Oceanol. Limnol. Sinica* 42 (6) (2011) 844–849.
- F. Lin, S. Xu, D. Ma, Z. Xiao, C. Zhao, Y. Xiao, L. Chi, Q. Liu, J. Li, Germ line specific expression of a vasa homologue gene in turbot (*Scophthalmus maximus*): evidence for vasa localization at cleavage furrows in euteleostei, *Mol. Reprod. Dev.* 79 (11) (2012) 803–813.
- Y. Liu, D. Ma, C. Zhao, W. Wang, X. Zhang, X. Liu, Y. Liu, Z. Xiao, S. Xu, Y. Xiao, Q. Liu, J. Li, Histological and enzymatic responses of Japanese flounder (*Paralichthys olivaceus*) and its hybrids (*P. olivaceus* female symbol x *P. dentatus* male symbol) to chronic heat stress, *Fish Physiol. Biochem.* 40 (4) (2014) 1031–1041.
- H.-X. Li, Y. Xiao, L.-L. Cao, X. Yan, C. Li, H.-Y. Shi, J.-W. Wang, Y.-H. Ye, Cerebroside C increases tolerance to chilling injury and alters lipid composition in wheat roots, *PLoS One* 8 (9) (2013).
- F. Lin, C.Y. Zhao, S.H. Xu, D.Y. Ma, Z.Z. Xiao, Y.S. Xiao, C.A. Xu, Q.H. Liu, J. Li, Germ-line specific and sexually dimorphic expression of a dead end gene homologue in turbot (*Scophthalmus maximus*), *Theriogenology* 80 (6) (2013) 665–672.
- Q. Fu, N. Yang, C. Gao, M. Tian, S. Zhou, X. Mu, F. Sun, C. Li, Characterization, expression signatures and microbial binding analysis of cathepsin A in turbot,

- Scophthalmus maximus* L.(SmCTSA), Fish Shellfish Immunol. 81 (2018) 21–28.
- [48] B.T. Hermann, T.B.H. Reusch, R. Hanel, Effects of dietary purified rapeseed protein concentrate on hepatic gene expression in juvenile turbot (*Psetta maxima*), Aquacult. Nutr. 22 (1) (2016) 170–180.
- [49] S. Reiser, S. Wuerztz, J.P. Schroeder, W. Kloas, R. Hanel, Risks of seawater ozonation in recirculation aquaculture—effects of oxidative stress on animal welfare of juvenile turbot (*Psetta maxima*, L.), Aquat. Toxicol. 105 (3) (2011) 508–517.
- [50] E. Muñoz-Atienza, C. Araújo, S. Magadán, P.E. Hernández, C. Herranz, Y. Santos, L.M. Cintas, In vitro and in vivo evaluation of lactic acid bacteria of aquatic origin as probiotics for turbot (*Scophthalmus maximus* L.) farming, Fish Shellfish Immunol. 41 (2) (2014) 570–580.
- [51] C. Gao, Q. Fu, B. Su, H. Song, S. Zhou, F. Tan, C. Li, The involvement of cathepsin F gene (CTSF) in turbot (*Scophthalmus maximus* L.) mucosal immunity, Fish Shellfish Immunol. 66 (2017) 270–279.
- [52] A. Jia, X.H. Zhang, Molecular cloning, characterization and expression analysis of cathepsin D gene from turbot *Scophthalmus maximus*, Fish Shellfish Immunol. 26 (4) (2009) 606–613.
- [53] H. Ren, Molecular Cloning and Expression Analysis of CAT Gene from *Scophthalmus maximus*, (2017) unpublished, Accession number: MG253621.
- [54] M.D. Mark, M. Donner, D. Eickelbeck, J. Stepien, M. Nowrousian, U. Kuck, F. Paris, J. Hellinger, S. Herlitze, Visual tuning in the flashlight fish *Anomalops katoptron* to detect blue, bioluminescent light, PLoS One 13 (7) (2018) e0198765.
- [55] J.K. Bowmaker, Visual Pigments of Fishes, Visual System of Fish, (1990).
- [56] j. Lei, a. Ma, s. Liu, q. Men, Study on the development of embryo, larval and juvenile of turbot *Scophthalmus maximus*, Oceanol. Limnol. Sinica 34 (1) (2003) 9–18.
- [57] J. Che, J. Chen, M. Hu, Histological structure of retina and visual characteristics of turbot *Scophthalmus maximus* fry, Prog. Fish. Sci. 37 (2) (2016) 25–32.
- [58] P. Ekstrom, T. Vanveen, Pineal neural connection with the brain in 2 teleosts, the crucian carp and the European eel, J. Pineal Res. 1 (3) (1984) 245–261.
- [59] G. Koumoundouros, P. Divanach, M. Kentouri, Osteological development of *Dentex dentex* (Osteichthyes: Sparidae): dorsal, anal, paired fins and squamation, Mar. Biol. 138 (2) (2001) 399–406.
- [60] C. Huang, X.Y. Tan, K. Wu, Q.L. Chen, M.Q. Zhuo, Y.X. Pan, Y.F. Song, Osteological development and anomalies in larval stage of hatchery-reared yellow catfish *Pelteobagrus fulvidraco*, Aquacult. Res. 47 (4) (2016) 1125–1140.
- [61] J.Y. Park, K.H. Han, J.K. Cho, J.I. Myeong, J.M. Park, Early osteological development of larvae and juveniles in red spotted grouper, *Epinephelus akaara* (pisces: Serranidae), Dev. Reprod. 20 (2) (2016) 87–101.
- [62] T.D.S. Lopes, T.M. De Freitas, R.K. Jomori, D.J. Carneiro, Eacute, M.C. Portella, Skeletal anomalies of pacu, *Piaractus mesopotamicus*, larvae from a wild-caught broodstock, J. World Aquacult. Soc. 45 (1) (2014) 15–27.
- [63] R. Hassan, S. Sultana, H.S. Choe, A comparison of monochromatic and mixed LED light color on performance, bone mineral density, meat and blood properties, and immunity of broiler chicks, J. Poult. Sci. 51 (2) (2014) 195–201.
- [64] O. Halevy, I. Biran, I. Rozenboim, Various light source treatments affect body and skeletal muscle growth by affecting skeletal muscle satellite cell proliferation in broilers, Comp. Biochem. Physiol. Part A Molecular & Integrative Physiology 120 (2) (1998) 317–323.
- [65] O.H. Ottesen, S. Bolla, Combined effects of temperature and salinity on development and survival of Atlantic halibut larvae, Aquacult. Int. 6 (2) (1998) 103–120.
- [66] C.M. Morrison, C.A. Macdonald, Normal and abnormal jaw development of the yolk-sac larva of atlantic halibut "*Hippoglossus Hippoglossus*, Dis. Aquat. Org. 22 (3) (1995) 173–184.
- [67] G. Koumoundouros, F. Gagliardi, P. Divanach, C. Boglione, S. Cataudella, M. Kentouri, Normal and abnormal osteological development of caudal fin in *Sparus aurata* L. Fry, Aquaculture 149 (3–4) (1997) 215–226.
- [68] J.M. Cobcroft, P.M. Pankhurst, J. Sadler, P.R. Hart, Jaw development and malformation in cultured striped trumpeter *Latris lineata*, Aquaculture 199 (3–4) (2001) 267–282.
- [69] H.W. Liu, R.R. Stickney, W.W. Dickhoff, D.A. Mccaughran, Effects of environmental factors on egg development and hatching of pacific halibut *Hippoglossus stenolepis*, J. World Aquacult. Soc. 25 (2) (2010) 317–321.
- [70] N. Villamizar, A. García-Alcazar, F.J. Sánchez-Vázquez, Effect of light spectrum and photoperiod on the growth, development and survival of European sea bass (*Dicentrarchus labrax*) larvae, Aquaculture 292 (1–2) (2009) 80–86.
- [71] M.H. Barahona-Fernandes, Some effects of light intensity and photoperiod on the sea bass larvae (*Dicentrarchus labrax* (L.)) reared at the Centre Oceanologique de Bretagne, Aquaculture 17 (4) (1979) 311–321.
- [72] V.R. Cerqueira, B. Chatain, P. Lavens, E. Jaspers, F. Ollevier, Photoperiodic Effects on the Growth and Feeding Rhythm of European Seabass, *Dicentrarchus labrax*, Larvae in Intensive Rearing, Eas Special Publication, 1991.
- [73] A.W. Wood, C.H. Duan, Insulin-like growth factor signaling in fish, Int. Rev. Cytol. 243 (243) (2005) 215–285.
- [74] A. Saeravila, J.A. Calduchginer, P. Prunet, J. Pérezsánchez, Dynamics of liver GH/IGF axis and selected stress markers in juvenile gilthead sea bream (*Sparus aurata*) exposed to acute confinement: differential stress response of growth hormone receptors, Comp. Biochem. Physiol. Part A Molecular & Integrative Physiology 154 (2) (2009) 197–203.
- [75] J. Falcon, Y. Gothilf, S.L. Coon, G. Boeuf, D.C. Klein, Genetic, temporal and developmental differences between melatonin rhythm generating systems in the teleost fish pineal organ and retina, J. Neuroendocrinol. 15 (4) (2003) 378–382.
- [76] G.K. Iwama, P.A. Ackerman, Heat shock proteins and physiological stress in fish, Am. Zool. 39 (6) (1999) 901–909.
- [77] J.-H. Kim, H.-U. Dahms, K.-N. Han, Biomonitoring of the river pufferfish, *Takifugu obscurus* in aquaculture at different rearing densities using stress-related genes, Aquacult. Res. 44 (12) (2013) 1835–1846.
- [78] K.-W. Lee, J.-S. Rhee, J. Han, H.G. Park, J.-S. Lee, Effect of culture density and antioxidants on naupliar production and gene expression of the cyclopid copepod, *Paracyclops nana*, Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol. 161 (2) (2012) 145–152.
- [79] M. Ni, H. Wen, J. Li, M. Chi, Y. Ren, Z. Song, H. Ding, Two HSPs gene from juvenile Amur sturgeon (*Acipenser schrenckii*): cloning, characterization and expression pattern to crowding and hypoxia stress, Fish Physiol. Biochem. 40 (6) (2014) 1801–1816.
- [80] H. Liu, J. He, C. Chi, Y. Gu, Identification and analysis of icCu/Zn-SOD, Mn-SOD and ecCu/Zn-SOD in superoxide dismutase multigene family of *Pseudosciaena crocea*, Fish Shellfish Immunol. 43 (2) (2015) 491–501.
- [81] F.A. Guardiola, A. Cuesta, M. Arizcun, J. Meseguer, M.A. Esteban, Comparative skin mucus and serum humoral defence mechanisms in the teleost gilthead seabream (*Sparus aurata*), Fish Shellfish Immunol. 36 (2) (2014) 545–551.
- [82] D.A. Elvitigala, H.K. Premachandra, I. Whang, T.T. Priyathilaka, E. Kim, B.S. Lim, H.B. Jung, S.Y. Yeo, H.C. Park, J. Lee, Marine teleost ortholog of catalase from rock bream (*Oplegnathus fasciatus*): molecular perspectives from genomic organization to enzymatic behavior with respect to its potent antioxidant properties, Fish Shellfish Immunol. 35 (4) (2013) 1086–1096.
- [83] Y. Nzengue, R. Steiman, W. Rachidi, A. Favier, P. Guiraud, Oxidative stress induced by cadmium in the C6 cell line: role of copper and zinc, Biol. Trace Elem. Res. 146 (3) (2012) 410–419.
- [84] S.M. Wu, J.H. Liu, L.H. Shu, C.H. Chen, Anti-oxidative responses of zebrafish (*Danio rerio*) gill, liver and brain tissues upon acute cold shock, Comp. Biochem. Physiol. Mol. Integr. Physiol. 187 (2015) 202–213.
- [85] W. Qu, M.P. Waalkes, Metallothionein blocks oxidative DNA damage induced by acute inorganic arsenic exposure, Toxicol. Appl. Pharmacol. 282 (3) (2015) 267–274.
- [86] C.Y. Choi, H.S. Shin, Y.J. Choi, N.N. Kim, J. Lee, G.-S. Kil, Effect of LED light spectra on starvation-induced oxidative stress in the cinnamon clownfish *Amphiprion melanopus*, Comp. Biochem. Physiol. Mol. Integr. Physiol. 163 (3–4) (2012) 357–363.
- [87] C.C. Lazado, P.V. Skov, P.B. Pedersen, Innate immune defenses exhibit circadian rhythmicity and differential temporal sensitivity to a bacterial endotoxin in Nile tilapia (*Oreochromis niloticus*), Fish Shellfish Immunol. 55 (2016) 613–622.
- [88] B. Glisic, I. Mihaljevic, M. Popovic, R. Zaja, J. Loncar, K. Fent, R. Kovacevic, T. Smital, Characterization of glutathione-S-transferases in zebrafish (*Danio rerio*), Aquat. Toxicol. 158 (2015) 50–62.
- [89] J.W. Kim, H.J. Kong, B.H. Nam, J.Y. Park, D.H. Kim, J.M. Jeong, C.I. Park, The first report of cathepsin A gene in a teleost (rock bream, *Oplegnathus fasciatus*): an investigation of immune functions upon infection with several pathogens, Dev. Comp. Immunol. 76 (2017) 262–267.
- [90] S. Patel, A. Homaei, H.R. El-Seedi, N. Akhtar, Cathepsins: proteases that are vital for survival but can also be fatal, Biomed. Pharmacother. 105 (2018) 526–532.
- [91] P. Benes, V. Vetvicka, M. Fusek, Cathepsin D—many functions of one aspartic protease, Crit. Rev. Oncol. Hematol. 68 (1) (2008) 12–28.
- [92] W. Lin, L. Li, J. Chen, D. Li, J. Hou, H. Guo, J. Shen, Long-term crowding stress causes compromised nonspecific immunity and increases apoptosis of spleen in grass carp (*Ctenopharyngodon idella*), Fish Shellfish Immunol. 80 (2018) 540–545.
- [93] L. Chen, M. Zhang, L. Sun, Identification and expression analysis of two cathepsins from half-smooth tongue sole (*Cynoglossus semilaevis*), Fish Shellfish Immunol. 31 (6) (2011) 1270–1277.
- [94] J.W. Lee, Y.M. Lee, H. Yang, J.K. Noh, H.C. Kim, C.J. Park, J.W. Park, I.J. Hwang, S.Y. Kim, J.H. Lee, Expression analysis of cathepsin F during embryogenesis and early developmental stage in olive flounder (*Paralichthys olivaceus*), Dev. Reprod. 17 (3) (2013) 221–229.
- [95] M. Fabra, J. Cerdà, Ovarian cysteine proteinases in the teleost *Fundulus heteroclitus*: molecular cloning and gene expression during vitellogenesis and oocyte maturation, Mol. Reprod. Dev. 67 (3) (2004) 282–294.
- [96] T.S. Vihtelic, J.M. Fadool, J. Gao, K.A. Thornton, D.R. Hyde, G. Wistow, Expressed sequence tag analysis of zebrafish eye tissues for NEIBank, Mol. Vis. 11 (127) (2005) 1083.
- [97] S. Saurabh, P.K. Sahoo, Lysozyme: an important defence molecule of fish innate immune system, Aquacult. Res. 39 (3) (2008) 223–239.