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Different effects of blue and red light-emitting diodes on antioxidant responses in the liver and ovary of zebrafish *Danio rerio*

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Abstract The present study assessed the effects of a white fluorescent bulb (the control) and two different light-emitting diodes (blue LEDs, LDB; red, LDR) on growth, morphology, and oxidative stress in the liver and ovary of zebrafish for 5 weeks. Growth maintained relatively constant under LDB condition, but was reduced under LDR condition. In the liver, hepatosomatic index (HSI) and protein carbonylation (PC) increased under LDR condition, whereas lipid peroxidation (LPO) declined and HSI remained unchanged under LDB condition. The decrease in oxidative damage by LDB could be attributed to the up-regulated levels of mRNA, protein, and activity of Cu/Zn-SOD and CAT. A failure to activate the activity of both enzymes may result in the enhanced PC levels under LDR condition, though both genes were up-regulated at transcriptional and translational levels. In the ovary, although gonadosomatic index sharply increased under LDR condition, LPO and PC dramatically accumulated. The increase in oxidative damage by LDR might result from the down-regulated levels of protein and activity of Cu/Zn-SOD and CAT, though both genes were up-regulated at a transcriptional level. Furthermore, a sharp increase in expression of transcription factor Nrf2 that targets antioxidant genes was observed in the liver but not in the ovary under LDB and LDR conditions. In conclusion, our data demonstrated a positive effect of LDB and negative effect of LDR on fish antioxidant defenses, emphasizing the potentials of LDB as an effective light source in fish farming.

Keywords Reproductive toxicity · Oxidative stress · Light spectrum · Fish

Introduction

The spectral composition of nature light changes differentially underwater because wavelength of light penetrating water varies greatly (Lythgoe 1979). The blue or short spectrum has a strong penetrability and is predominant in deeper waters, whereas the red or long spectrum is rapidly absorbed by water molecules and only penetrates in shallow waters (Migaud et al. 2006). The difference in the light spectra can be detected by fish by retinal and extra-retinal photoreceptors (Vera et al. 2010) and accordingly affects growth and reproductive physiology of fish (Bapary et al. 2011). Light-emitting diodes (LEDs) are the first light source to make available the true spectral composition control to simulate the environmental conditions that match the sensitivity of fish (Migaud et al. 2007; Villamizar et al. 2009). The potentials of LED as an effective light source for studies conducted in behavior, survival,

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growth, and reproduction in fish have been explored to a greater extent (see reviews, Yeh et al. 2014). For example, blue LEDs promote the growth and development of *Dicentrarchus labrax* larvae (Villamizar et al. 2009). Red LEDs stimulate the ovarian development in *Chrysiptera cyanea* (Bapary et al. 2011). However, prior to carrying out these lighting regimes, any potentially negative effects should not be neglected in fish.

There have been several studies on the adverse effects of inappropriate light spectra on the development of skeleton and swim bladder, survival, and growth in fish (Battaglene and Talbot 1990; Bayarri et al. 2002; Trotter et al. 2003; Villamizar et al. 2009). At present, the mechanisms of toxicity of certain LED spectra are not fully known and nonetheless possibly associate with LED-induced oxidative stress (Shin et al. 2011).

Organisms have developed an antioxidant defense system including enzymatic and non-enzymatic mechanisms to cope with oxidative stress (Regoli and Giuliani 2014). Of the antioxidant enzymes, SOD-CAT is considered to be the vital first-line defenses against oxidative stress, since O_2^- is converted to O_2 and H_2O_2, which is subsequently transformed into H_2O by CAT (Yu 1994). The complexity of antioxidant responses reflects a likewise complex mechanism of regulation, which involves different pathways modulating either the catalytic activity and protein level of preexisting enzymes or the de novo synthesis of new molecules. Although there are several studies on the effects of various spectra on the mRNA level and activity of antioxidant enzymes in fish (Choi et al. 2012; Head and Malison 2000; Kim et al. 2014; Shin et al. 2011), investigations on the antioxidant responses of fish to various light spectra are still very limited, especially on a protein level. On the other hand, numerous experimental studies, mainly driven by mammalian models, have demonstrated that antioxidant-related genes are under the control of transcription factor Nrf2 (NF-E2-related factor 2), which represents a critical mechanism of protection against oxidative stress (Baird and Dinkova-Kostova 2011). The regulatory role of Nrf2 in chemicals-induced oxidative stress has recently been demonstrated in fish (Giuliani and Regoli 2014; Jiang et al. 2014, 2015). However, little information is available regarding the role of Nrf2 in LED-induced oxidative stress in fish.

Zebrafish (*Danio rerio*) is a popular vertebrate model system in toxicology. The availability of complete genome sequence facilitates further studies on the mechanisms of toxicology at a molecular level for the fish species. Numerous commercially enzyme-linked immunosorbent assay (ELISA) kits targeting zebrafish have well been developed, which contributes to studies on a protein level. In light of the above, we evaluated the effects of a white fluorescent bulb (the control), blue LEDs, and red LEDs on growth, morphology, and oxidative stress in the liver and ovary of zebrafish for 5 weeks. Growth was evaluated by measuring body weight, body length, condition factor (CF), and specific growth rate (SGR). The morphometric parameters detected included liver weight, ovary weight, hepatosomatic index (HSI), gonadosomatic index (GSI). Oxidative stress was evaluated by determining the levels of lipid peroxidation (LPO) and protein carbonylation (PC), and by investigating the levels of protein, activity, and mRNA of antioxidant enzyme genes including Cu/Zn-SOD and CAT. To understand the regulatory mechanism of antioxidant enzyme genes, the mRNA levels of Nrf2 were also analyzed.

Materials and methods

Light spectra exposure

Female zebrafish (AB strain) about 5 weeks of age were obtained from Institute of Hydrobiology, Chinese Academy of Sciences, China. Prior to the experiment, zebrafish were maintained in 20-L circular fiberglass tanks for the 2-wk acclimatization. At the beginning of the trial, uniform-sized fish (initial body weight: 0.17 ± 0.03 g, mean $\pm$ SEM) were exposed to a white fluorescent bulb (a simulated natural photoperiod; SNP; the control group), blue LED (LDB, peak at 450 nm), and red LED (LDR, peak at 630 nm) for 5 weeks, at an irradiance of 0.9 W/m^2 at the water surface (Fig. 1). The set value of irradiance was according to the study from Bapary et al. (2011). The experiment was conducted in a closed flow-through system. The fish were fed commercial diets (lipid and protein contents of 10.6 and 42.4 % on a dry matter basis, respectively) twice daily at the rate of 5 % of average body weight. Water temperature was maintained at 24.7 ± 4.3 °C with a
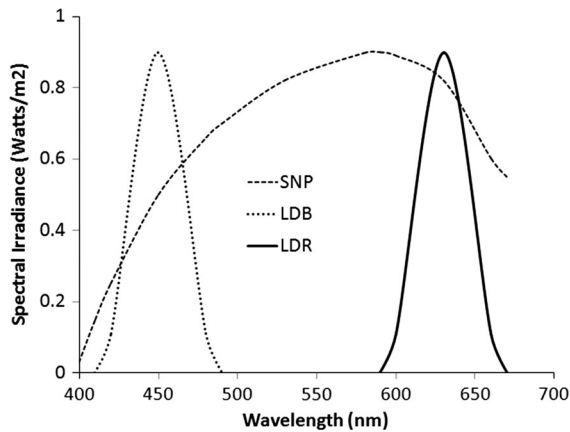
Fig. 1 Spectral profiles of the blue (LDB), red (LDR) LEDs, and a simulated natural photoperiod (SNP) used in tHSI study

12L:12D photoperiod. Dissolved oxygen and pH were 6.76 ± 0.38 and 7.47 ± 0.45 mg L^{-1}, respectively.

At the end of the 5-week period, 24 h after the last feeding, all fish were euthanized with a 0.02 % tricaine methanesulfonate solution (MS-222). Fish body, liver, and ovary were weighed and measured to final body weight, liver weight, ovary weight, body length, SGR, CF (body weight/body length^3), HSI (weight of liver/body weight $\times$ 100 %), and GSI (weight of ovary/body weight $\times$ 100 %). Then, the liver and ovary were immediately frozen in liquid nitrogen and stored at -80 °C until biochemical determinations and RNA extraction. We assured that all experiments, animal care, and protocols followed the ethical guidelines of the Zhejiang Ocean University for the care and use of laboratory animals.

LPO and PC analysis

Tissues were homogenized in an ice-cold 0.1 M phosphate buffered solution (PBS, pH 7.4) containing 1 mM of ethylene diamine tetraacetic acid (EDTA), 0.5 M of saccharose, 0.15 M of KCl, and 1 mM of dithiothreitol (DTT). The homogenates were centrifuged at $500 \times g$ for 15 min at 4 °C to precipitate large particles and centrifuged again at $12,000 \times g$ for 30 min at 4 °C. The supernatants were maintained at 4 °C until being measured for biochemical analysis.

Lipid peroxidation (LPO) was determined by the thiobarbituric acid reactive species (TBARS) assay, which measured the production of malondialdehyde that reacts with thiobarbituric acid, according to the method described by Livingstone et al. (1990). Protein carbonylation (PC) was determined as previously described (Lushchak et al. 2005).

Enzymatic activities and protein levels of Cu/Zn-SOD and CAT

The supernatants from tissue homogenates were obtained according to the method described above. The supernatants were directly applied to the assay of enzymatic activities and protein levels of Cu/Zn-SOD and CAT. Cu/Zn-SOD (EC 1.15.1.1) activity was measured according to the method of Beauchamp and Fridovich (1971) based on aerobic reduction of NBT at 535 nm by superoxide radicals. Catalase activity (CAT, EC 1.11.1.6) was determined by measuring the rate of disappearance of H_2O_2 according to methods described by Beutler (1982). The protein levels of Cu/Zn-SOD and CAT were detected using commercially fish enzyme-linked immunosorbent assay (ELISA) kits (Cusabio, Wuhan, China) in accordance with the manufacturer's instructions. Soluble protein content was determined according to the method of Bradford (1976) using bovine serum albumin (BSA) as a standard. All enzyme activities were expressed as U (units) per mg of soluble protein. All protein levels were expressed as ng per mg of soluble protein. Six biological replicates and two technical replicates were used in the analysis.

Real-time PCR

Extractions of total RNA from liver and ovary and first-strand cDNA synthesis were performed according to the methods in our recent study with slight modification (Zheng et al. 2016). Q-PCR reactions (20 μL) were performed in 96-well plates in an Applied Biosystems Prism 7500 Sequence Detection System (Applied Biosystems, USA) with SYBR® Premix Ex Taq^{TM} Kit (Takara), containing 10 μL SYBR® Premix Ex Taq Master Mix, 2 μL of cDNA, and 0.2 μM of each primer. The primer sequences of each gene used in tHSI analysis are given in Table 1. The thermal program included 1 min at 95 °C, 45 cycles at 95 °C for 5 s, 57 °C for 10 s, and 72 °C for 30 s. Each reaction was verified to contain a single product of the correct size using agarose gel electrophoresis and has no significant differences in amplification efficiencies. The two genes β-actin and GAPDH with the most

Table 1 Primers used for real-time PCR analysis from large yellow croaker (the primers of Cu/Zn-SOD, CAT, and GAPDH from Hou et al. (2014))

Gene	Primer sequences (from 5′ to 3′)	Size (bp)	Accession no.
Cu/Zn-SOD	F: GTCCGCACTTCAACCCTCA	227	BC055516
	R: TCCTCATTGCCACCCTTCC		
CAT	F: CAAGGTCTGGTCCCATAAA	217	BC051626
	R: TGACTGGTAGTTGGAGGTAA		
Nrf2	F: TCGGGTTTGTCCCTAGATG	188	AB081314
	R: AGGTTTGGAGTGTCCGCTA		
β-Actin	F: CCACCACAGCCGAAAGAG	179	AF057040
	R: GGATACCGCAAGATTCCATA		
GAPDH	F: GTAACTCCGCAGAAAAGCC	153	BC095386
	R: CAAAAGAAACTAACACACAC		

stable levels of expression across experimental conditions were selected from a set of four housekeeping genes (EF 1α, β-actin, GAPDH, and Tubulin α), According to the geNorm software (Vandesompele et al. 2002). The relative expression levels were calculated with the "delta–delta Ct" method (Pfaffl 2001), when normalizing to the geometric mean of the best combination of β-actin and GAPDH.

Statistical analysis

Results are presented as mean ± SEM. Prior to statistical analysis, all data were tested for normality of distribution using the Kolmogorov–Smirnov test. The homogeneity of variances among the different treatments was tested using Barlett's test. The variables were subjected to one-way analysis of variance (ANOVA) and Tukey's multiple range test. Analysis was performed using SPSS 18.0 for Windows (SPSS, Michigan Avenue, Chicago, IL, USA), and the minimum significant level was set at 0.05.

Results

Growth and morphology

Growth (body weight, body length, CF, and SGR) remained relatively unchanged, but was inhibited by LDR (Fig. 2). Liver weight, ovary weight, HSI, and GSI were not significantly affected by LDB. In contrast, ovary weight, HSI, and GSI increased under LDR condition (Fig. 3).
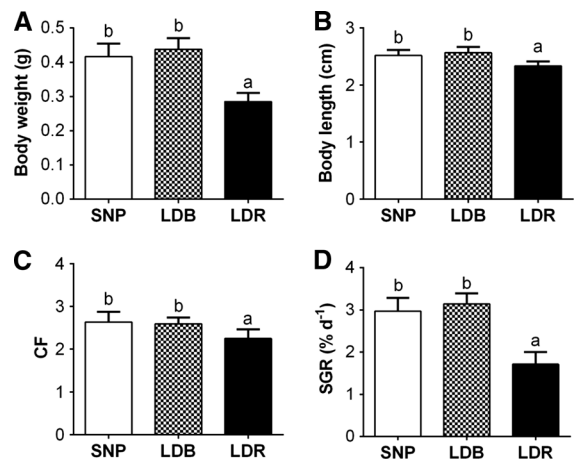

Fig. 2 Changes in body weight (**a**), body length (**b**), condition factor (CF) (**c**), and specific growth rate (SGR) (d) of the female zebrafish under different lighting conditions using LED. Each value represents mean ± SEM ($n = 6$). *Different letters* indicate significant difference at $P < 0.05$

Oxidative damage

Under LDR condition, an increase in PC level in the liver and the levels of LPO and PC in the ovary were observed in zebrafish (Fig. 4). Under LDB condition, the levels of LPO and PC remained unchanged in the ovary and declined in the liver.

mRNA levels of antioxidant genes

LDB and LDR dramatically up-regulated the mRNA levels of Cu/Zn-SOD and CAT in the liver and ovary (Fig. 5). An increase in Nrf2 expression was observed in the liver under LDB and LDR conditions.
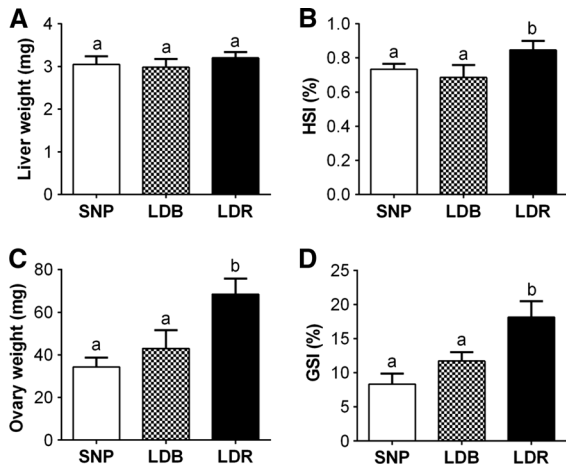
Fig. 3 Changes in liver weight (**a**), hepatosomatic index (HSI) (**b**), ovary weight (**c**), and gonadosomatic index (GSI) (**d**) of the female zebrafish under different lighting conditions using LED. Each value represents mean ± SEM ($n = 6$). *Different letters* indicate significant difference at $P < 0.05$
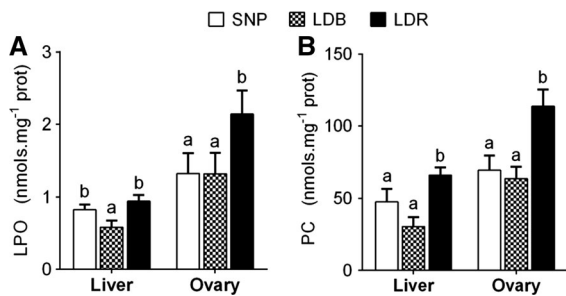

Fig. 4 Changes in the levels lipid peroxidation (LPO) (**a**) and protein carbonylation (PC) (**b**) of the female zebrafish under different lighting conditions using LED. Each value represents mean ± SEM ($n = 6$). *Different letters* indicate significant difference at $P < 0.05$

Contrarily, Nrf2 expression was not significantly affected by LDB and LDR in the ovary.

Protein levels and enzymatic activities of antioxidant genes

In the liver, protein levels and enzymatic activities of Cu/Zn-SOD and CAT were sharply increased by LDB (Fig. 6). Although LDR up-regulated the Cu/Zn-SOD protein level, Cu/Zn-SOD activity still remained unchanged. The protein level and activity of CAT were not significantly affected by LDR.

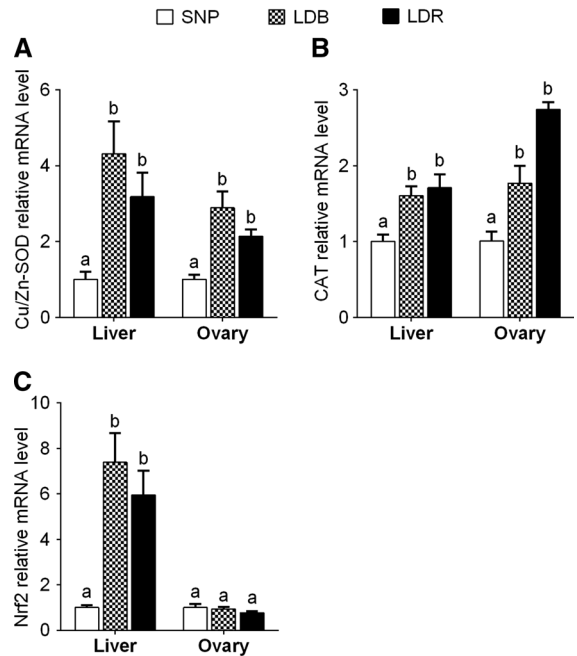In the ovary, protein levels and enzymatic activities of Cu/Zn-SOD and CAT were maintained relatively

Fig. 5 Changes in mRNA levels of Cu/Zn-SOD (**a**), CAT (**b**), and Nrf2 (**c**) in the liver and ovary of the female zebrafish under different lighting conditions using LED. Values are mean ± SEM ($n = 6$), expressed as logarithm of mRNA expression of each gene relative to mRNA expression of the geometric mean of β-actin and GAPDH. *Different letters* indicate significant difference at $P < 0.05$

constant in the LDB group. In contrast, protein level and activity of CAT and Cu/Zn-SOD were significantly inhibited by LDR except that the inhibition of Cu/Zn-SOD protein level was not significant.

Discussion

LEDs have become popular in fish farming because they can be tuned to environmental and species sensitivities through narrow bandwidth outputs. LDB are possibly most practical because of a strong penetrability underwater, whereas LDR are rapidly absorbed by water molecules and cannot be detected by fish (Migaud et al. 2006, 2007). Thus, it may be beneficial that LDB is thought to mimic natural conditions. Several studies have demonstrated growth of fish can be reduced by LDR and cannot be inhibited by LDB (Blanco-Vives et al. 2010; Villamizar et al. 2009; Yamanome et al. 2009), in agreement with our present study. The different effects of LDB and LDR
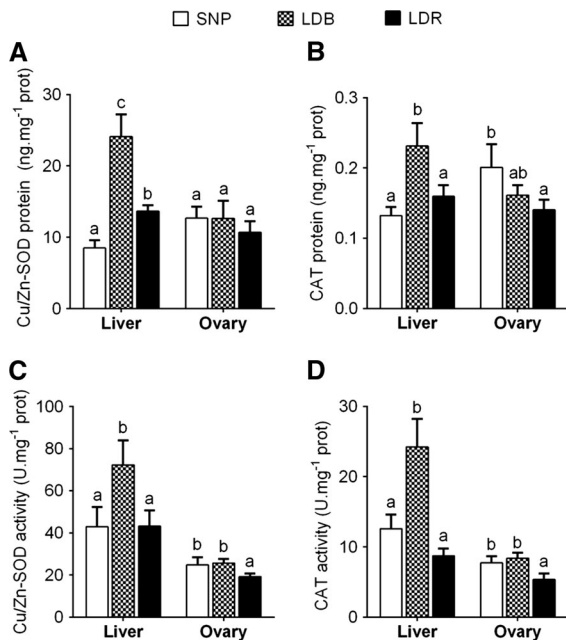
Fig. 6 Changes in protein level and enzymatic activity of Cu/Zn-SOD and CAT in the liver and ovary of the female zebrafish under different lighting conditions using LED. Values are mean ± SEM ($n = 6$). *Different letters* indicate significant difference at $P < 0.05$

on growth were also reflected by the morphology and oxidative damage in the liver. The liver is considered a target organ for many biological and environmental parameters that can alter liver structure and metabolism (Brusle and Anadon 1996). Thus, fish liver is a very interesting model for the study of interactions between environmental factors and hepatic functions. In the present study, HSI remained unchanged in the LDB group but increased in the LDR group. HSI is a morphological index that reflects the status of hepatic energy storage (Foster et al. 1993). The increase in HSI indicates more lipids accumulated into the liver in stressed fish because of mitochondria oxidative function injury, as suggested by Zheng et al. (2013). A substantial portion of cellular reactive oxygen species (ROS) is generated in mitochondria (Martinez-Reyes and Cuezva 2014). Mitochondrial damage would result in the polarization of mitochondrial membrane and increases the generation of ROS (Korshunov et al. 1997). The increase in LPO and PC caused by LDR in the liver further would support the highly production of ROS. In the present study, oxidative damage in the liver was reduced by LDB but was increased by LDR, similar to the reports in *Amphiprion clarkii* (Shin et al.

2011), *Amphiprion melanopus* (Choi et al. 2012), and *Carassius auratus* (Kim et al. 2014). The present results support the hypothesis that the use of LDB would be valuable by reducing oxidative damage in cultured fish.

In the present study, LDR dramatically stimulated the ovarian development, as confirmed by the increased GSI (measure of gonadal development). The level of effectiveness was LDR > LDB > SNP, consistent with the report in *Chrysiptera cyanea* (Bapary et al. 2011). In contrast, the level of effectiveness was LDB > LDR > SNP in *Chrysiptera parasema*. Spectral sensitivity of fish species seems to be different from each other, which may be related to habitat nature (Bapary et al. 2011). Our finding emphasizes the utility of LDR to improve ovarian maturation during early stages of zebrafish development. However, prior to carrying out these lighting regimes, oxidative damage induced by LDR in the ovary should not be neglected in zebrafish. Previous studies have reported that there is a negative relationship between oxidative damage and gonad development in fish subjected to the other stressors such as microcystins (Hou et al. 2016) or cadmium (Pierron et al. 2009), which is contrary to our present results. Our results indicated that there were great differences in the effect of photo- and toxic responses on gonad development. Hou et al. (2016) reported microcystins impaired the development and reproduction of zebrafish by disrupting the transcription of reproduction-related genes. Shin et al. (2014) suggested the light of a specific wavelength up-regulated reproduction-related gene and enhanced sexual maturation in goldfish.

At present, there is limited information about the mechanisms that LDR could induce oxidative damage. It is may be involved in the failure to initiate activation of antioxidant enzyme system. Of the antioxidant enzymes, SOD-CAT is considered to be the vital first-line defenses that directly scavenge (ROS) (Yu 1994). In the present study, a decrease in the activity of Cu/Zn-SOD and CAT may contribute to an increase in the levels of LPO and PC in the ovary. When SOD-CAT system is not able to eliminate or neutralize the excess of ROS, there is an increased risk of oxidative damage due to accumulation of LPO and PC which may, in turn, decrease enzyme activities or even degrade the enzymes (Zhang et al. 2008). In the present study, low protein level paralleled with the increase in enzymatic activity but did not match the sharply increased

mRNA level of Cu/Zn-SOD and CAT in the ovary of fish reared under LDR and LDB conditions, indicating that both antioxidant genes were regulated at a translational level in the ovary. The mismatch between antioxidant gene expression and enzyme protein level may be involved in time-lag effect between transcription and translation, and/or RNA stability (Nam et al. 2005). In contrast, in the liver, the regulation of the antioxidant genes was complicated, depending on spectral composition. For example, under LDB condition, the increase in mRNA level of Cu/Zn-SOD and CAT paralleled with the up-regulation of the levels of protein and activity of both enzymes, suggesting that Cu/Zn-SOD and CAT were regulated by LDB at a transcriptional level; under LDR condition, the increase in mRNA and protein levels of Cu/Zn-SOD and CAT was not consistent with the reduced activity of both enzymes, indicating that Cu/Zn-SOD and CAT were regulated by LDR at a posttranslational level. The mismatch between protein level and enzymatic activity of both antioxidant genes may result from posttranslational modifications (Craig et al. 2007). Martinez-Reyes and Cuezva (2014) suggested the increase in ROS production would in turn inhibit the activities of enzymes through ROS oxidation, which could also explain the mismatch between protein level and enzymatic activity.

Transcription factor Nrf2 is a master regulator of the cellular antioxidant response through binding ARE sequence in the promoter region of antioxidant enzyme genes and activating gene expression (Osburn and Kensler 2008). In particular, Nrf2 is important in protecting the liver, since Nrf2 absence increases hepatic lipid peroxidation (Li et al. 2004). In the present study, an increase in Nrf2 expression level was observed in the liver under LDB and LDR, which could contribute to the reduced oxidative damage. The high mRNA level of Nrf2 paralleled with the increase in expression levels of Cu/Zn-SOD and CAT in the liver, similar to the reports in other fish under stress condition (Regoli and Giuliani 2014; Shi and Zhou 2010; Wu et al. 2014). The result may imply the transcription regulation of antioxidant genes. On the contrary, in the ovary, Nrf2 expression remained constant, though an increase in mRNA level of Cu/Zn-SOD and CAT were detected. The phenomenon could be explained by several reasons. First, antioxidant-related genes may be under the control of the other transcription factors, such as AP-1 (activator protein

1), p53, FoxO (forkhead box O), NF-κB (nuclear factor-kappa B) (Valko et al. 2007). Second, Nrf2 may be mainly activated through posttranscriptional mechanisms such as an increase in protein level and stability of protein and DNA binding instead of the subsequent induction of Nrf2 transcripts (Nguyen et al. 2003; Ning et al. 2010). Although the present study highlights the role of Nrf2 transcription in inducing antioxidant genes in the liver, further analysis at protein and activity levels is still necessary, especially in the ovary.

In conclusion, our study clearly demonstrated a positive effect of LDB and negative effect of LDR on fish antioxidant defenses. LDB could have the potentials of LED as an effective light source to enhance fish growth performance. Thus, LDB might be helpful to maintain fish culture.

References

Baird L, Dinkova-Kostova AT (2011) The cytoprotective role of the Keap1–Nrf2 pathway. Arch Toxicol 85:241–272

Bapary MAJ, Amin MN, Takeuchi Y, Takemura A (2011) The stimulatory effects of long wavelengths of light on the ovarian development in the tropical damselfish, *Chrysiptera cyanea*. Aquaculture 314:188–192

Battaglene SC, Talbot R (1990) Initial swim bladder inflation in intensively reared Australian bass larvae, *Macquaria novemaculeata* (Steindachner) (Perciformes: Percichthyidae). Aquaculture 86:431–442

Bayarri M, Madrid J, Sanchez-Vazquez F (2002) Influence of light intensity, spectrum and orientation on sea bass plasma and ocular melatonin. J Pineal Res 32:34–40

Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44:276–287

Beutler E (1982) Catalase. Red cell metabolism, a manual of biochemical methods, pp 105–106

Blanco-Vives B, Villamizar N, Ramos J, Bayarri MJ, Chereguini O, Sánchez-Vazquez F (2010) Effect of daily thermo-and photo-cycles of different light spectrum on the development of Senegal sole (*Solea senegalensis*) larvae. Aquaculture 306:137–145

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Brusle J, Anadon G (1996) The structure and function of fish liver. Fish Morphol 77–93

Choi CY, Shin HS, Choi YJ, Kim NN, Lee J, Kil GS (2012) Effect of LED light spectra on starvation-induced oxidative stress in the cinnamon clownfish *Amphiprion melanopus*. Comp Biochem Physiol A Physiol 163:357–363

Craig PM, Wood CM, McClelland GB (2007) Oxidative stress response and gene expression with acute copper exposure in zebrafish (*Danio rerio*). Am J Physiol Regul Integr Comp Physiol 293:R1882–R1892

Foster A, Houlihan D, Hall S (1993) Effects of nutritional regime on correlates of growth rate in juvenile Atlantic cod (*Gadus morhua*): comparison of morphological and biochemical measurements. Can J Fish Aquat Sci 50:502–512

Giuliani ME, Regoli F (2014) Identification of the Nrf2–Keap1 pathway in the European eel Anguilla anguilla: role for a transcriptional regulation of antioxidant genes in aquatic organisms. Aquat Toxicol 150:117–123

Head AB, Malison JA (2000) Effects of lighting spectrum and disturbance level on the growth and stress responses of yellow perch *Perca flavescens*. J World Aquacult Soc 31:73–80

Hou J, Li L, Xue T, Long M, Su Y, Wu N (2014) Damage and recovery of the ovary in female zebrafish ip-injected with MC-LR. Aquat Toxicol 155:110–118

Hou J, Li L, Wu N, Su Y, Lin W, Li G, Gu Z (2016) Reproduction impairment and endocrine disruption in female zebrafish after long-term exposure to MC-LR: a life cycle assessment. Environ Pollut 208:477–485

Jiang WD, Liu Y, Hu K, Jiang J, Li SH, Feng L, Zhou XQ (2014) Copper exposure induces oxidative injury, disturbs the antioxidant system and changes the Nrf2/ARE (CuZnSOD) signaling in the fish brain: protective effects of myo-inositol. Aquat Toxicol 155:301–313

Jiang WD, Liu Y, Jiang J, Wu P, Feng L, Zhou XQ (2015) Copper exposure induces toxicity to the antioxidant system via the destruction of Nrf2/ARE signaling and caspase-3-regulated DNA damage in fish muscle: amelioration by myo-inositol. Aquat Toxicol 159:245–255

Kim NN, Choi YJ, Shin HS, Lim JR, Han JM, Cho JH, Lee J, Kil G-S, Choi CY (2014) The effect of LED light spectra on antioxidant system by thermal stress in goldfish, *Carassius auratus*. Mol Cell Toxicol 10:47–58

Korshunov SS, Skulachev VP, Starkov AA (1997) High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. FEBS Lett 416:15–18

Li J, Stein TD, Johnson JA (2004) Genetic dissection of systemic autoimmune disease in Nrf2-deficient mice. Physiol Genomics 18:261–272

Livingstone D, Martinez PG, Michel X, Narbonne J, Ohara S, Ribera D, Winston G (1990) Oxyradical production as a pollution-mediated mechanism of toxicity in the common mussel, *Mytilus edulis* L., and other molluscs. Funct Ecol 4:415–424

Lushchak VI, Bagnyukova TV, Husak VV, Luzhna LI, Lushchak V, Storey KB (2005) Hyperoxia results in transient oxidative stress and an adaptive response by antioxidant enzymes in goldfish tissues. Int J Biochem Cell Biol 37:1670–1680

Lythgoe JN (1979) Ecology of vision. Clarendon Press; Oxford University Press, Oxford

Martinez-Reyes I, Cuezva JM (2014) The H$^+$-ATP synthase: a gate to ROS-mediated cell death or cell survival. BBA-Bioenergetics 1837:1099–1112

Migaud H, Taylor J, Taranger G, Davie A, Cerda-Reverter J, Carrillo M, Hansen T, Bromage N (2006) Pineal gland sensitivity to light intensity in salmon (*Salmo salar*) and sea bass (*Dicentrarchus labrax*): an in vivo and ex vivo study. J Pineal Res 41:42–52

Migaud H, Cowan M, Taylor J, Ferguson HW (2007) The effect of spectral composition and light intensity on melatonin, stress and retinal damage in post-smolt Atlantic salmon, *Salmo salar*. Aquaculture 270:390–404

Nam YK, Cho YS, Choi BN, Kim KH, Kim SK, Kim DS (2005) Alteration of antioxidant enzymes at the mRNA level during short-term starvation of rockbream *Oplegnathus fasciatus*. Fish Sci 71:1385–1387

Nguyen T, Sherratt PJ, Huang HC, Yang CS, Pickett CB (2003) Increased protein stability as a mechanism that enhances Nrf2-mediated transcriptional activation of the antioxidant response element Degradation of Nrf2 by the 26 S proteasome. J Biol Chem 278:4536–4541

Ning JL, Mo LW, Lai XN (2010) Low- and high-dose hydrogen peroxide regulation of transcription factor NF-E2-related factor 2. Chin Med J 123:1063–1069

Osburn WO, Kensler TW (2008) Nrf2 signaling: an adaptive response pathway for protection against environmental toxic insults. Mutat Res, Rev Mutat Res 659:31–39

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Res 29:e45–e45

Pierron F, Baudrimont M, Dufour S, Elie P, Bossy A, Lucia M, Massabuau J-C (2009) Ovarian gene transcription and effect of cadmium pre-exposure during artificial sexual maturation of the European eel (*Anguilla anguilla*). Biometals 22:985–994

Regoli F, Giuliani ME (2014) Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. Mar Environ Res 93:106–117

Shi X, Zhou B (2010) The role of Nrf2 and MAPK pathways in PFOS-induced oxidative stress in zebrafish embryos. Toxicol Sci kfq066

Shin HS, Lee J, Choi CY (2011) Effects of LED light spectra on oxidative stress and the protective role of melatonin in relation to the daily rhythm of the yellowtail clownfish, *Amphiprion clarkii*. Comp Biochem Physiol A: Mol Integr Physiol 160:221–228

Shin HS, Habibi HR, Choi CY (2014) The environmental regulation of maturation in goldfish, *Carassius auratus*: effects of various LED light spectra. Comp. Biochem. Physiol., A: Mol. Integr Physiol 168:17–24

Trotter A, Battaglene S, Pankhurst P (2003) Effects of photoperiod and light intensity on initial swim bladder inflation, growth and post-inflation viability in cultured striped trumpeter (*Latris lineata*) larvae. Aquaculture 224:141–158

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 3:research0034

Vera L, Davie A, Taylor J, Migaud H (2010) Differential light intensity and spectral sensitivities of Atlantic salmon, European sea bass and Atlantic cod pineal glands ex vivo. Gen Comp Endocrinol 165:25–33

Villamizar N, Garcia-Alcazar A, Sanchez-Vazquez F (2009) Effect of light spectrum and photoperiod on the growth, development and survival of European sea bass (*Dicentrarchuslabrax*) larvae. Aquaculture 292:80–86

Wu P, Jiang WD, Liu Y, Chen GF, Jiang J, Li SH, Feng L, Zhou XQ (2014) Effect of choline on antioxidant defenses and gene expressions of Nrf2 signaling molecule in the spleen and head kidney of juvenile Jian carp (*Cyprinus carpio var. Jian*). Fish Shellfish Immunol 38:374–382

Yamanome T, Mizusawa K, Hasegawa EI, Takahashi A (2009) Green light stimulates somatic growth in the barfin flounder *Verasper moseri*. J Exp Zool A Comp Exp Biol 311:73–79

Yeh N, Yeh P, Shih N, Byadgi O, Cheng TC (2014) Applications of light-emitting diodes in researches conducted in aquatic environment. J Renew Sustain Energy 32:611–618

Yu BP (1994) Cellular defenses against damage from reactive oxygen species. Physiol Rev 74:139–162

Zhang XD, Zhu YF, Cai LS, Wu TX (2008) Effects of fasting on the meat quality and antioxidant defenses of market-size farmed large yellow croaker (*Pseudosciaena crocea*). Aquaculture 280:136–139

Zheng JL, Luo Z, Liu CX, Chen QL, Tan XY, Zhu QL, Gong Y (2013) Differential effects of acute and chronic zinc (Zn) exposure on hepatic lipid deposition and metabolism in yellow catfish *Pelteobagrus fulvidraco*. Aquat Toxicol 132–133:173–181

Zheng JL, Zhu QL, Wu CW, Zhu AY, Shen B, Zeng L (2016) Zinc acclimation mitigated high zinc induced oxidative stress by enhancing antioxidant defenses in large yellow croaker *Pseudosciaena crocea*. Aquat Toxicol 172:21–29