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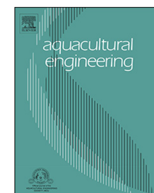
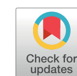


Effects of green wavelength light on antioxidant and non-specific immune responses of the olive flounder *Paralichthys olivaceus* maintained at different stocking densities



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ABSTRACT

In the present study, we investigated the effects of green light illumination on antioxidant systems and immunity in the olive flounder *Paralichthys olivaceus*. Fish at three different stocking densities (900, 1350, and 2250 fish per 8-ton tank) were compared. The effects of green light illumination were assessed by measuring survival rates, mRNA expression activity of antioxidant enzymes (superoxide dismutase and catalase), an oxidative stress-related parameter (hydrogen peroxide, H_2O_2), and immune-related parameters (lysozyme and melatonin). Overall, fish survival rates decreased over the 30-day period of the experiment, but survival rates were significantly higher among the groups of fish exposed to green light. In high stocking densities groups, mRNA levels and activities of antioxidant enzymes and H_2O_2 concentrations had increased at 30 days; however, in fish under green light conditions, significantly lower levels of antioxidant enzyme expression were observed. By contrast, parameters indicating immune responses decreased in high stocking densities groups, although in fish under green light treatment, significantly higher levels of immune response were observed. A comet assay revealed that a high stocking density increased the rate of nuclear DNA damage; however, treatment with green wavelength light reduced the frequency of damage. These results indicate that although high density induces oxidative stress and reduces immune system responses in olive flounder, green wavelength light prevents oxidative stress and boosts the immune system.

1. Introduction

Many environmental factors can cause physiological stress in fish cultures, such as stocking density, salinity, water temperature, photoperiod, and light. Among commercially cultured fish, stocking density is particularly important, as it has a direct influence on fish growth, feed intake, and aquaculture productivity (Beckmann et al., 1990; Rowland et al., 2006; Pierce et al., 2008). Although there may be differences in stocking density depending on the species and stage of growth (Jorgensen et al., 1993; Bjornsson, 1994), high-density culture generally has a detrimental effect on growth and survival by acting as a stress factor (Bjornsson, 1994; Holm et al., 1990).

When fish become stressed, reactive oxygen species (ROS), such as superoxides, $O^{\cdot 2-}$, hydrogen peroxide (H_2O_2), peroxyl radicals, and hydroxyl radicals, are produced in the body (Yang and Yeo, 2004; Choi et al., 2016; Kim et al., 2016). Organisms protect themselves from oxidative stress by inducing antioxidant defense systems that reduce cell damage caused by ROS (Pandey et al., 2003; George et al., 2004). The defense systems comprise enzymes that have antioxidant effects, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) (Hansen et al., 2006). The first step in the elimination of ROS is mediated by SOD, which converts intracellular oxygen free radicals (O_2^-) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). Thereafter, CAT and GPX break down H_2O_2 into molecular oxygen (O_2) and water (H_2O), thus reducing its toxic effects (Shull et al., 1991; Choi et al., 2016). The elevation of ROS by stress causes a decrease in the activity of lysozyme and melatonin and has a negative effect on immune function (Wang et al., 2008; Choi et al., 2016).

Recently, it has been reported that light of specific wavelengths enhances the immune system of fish or reduces their stress levels (Choi et al., 2016; Jung et al., 2016; Kim et al., 2016). These previous studies examined various physiological effects in fish exposed to light of specific wavelengths produced using light-emitting diodes (LEDs) (Villamizar et al., 2009; Shin et al., 2011; Choi et al., 2016). The results

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of these studies indicate that it should be possible to combine the use of LEDs, which have low power consumption, with the positive effects of specific wavelengths on the health of fish to improve aquaculture efficiency.

In general, aquaculture systems for the olive flounder *Paralichthys olivaceus* use stocking densities of breeding fish that are determined by the likely mortality and the required number of juveniles to meet planned production volume shipments. Although the olive flounder is resistant to disease in culture, it does have high mortality rates at high densities due to stress caused by the culture environment.

In this study, we investigated the possibility of improving health levels in olive flounder cultures using specific light wavelengths. Fish were maintained at different stocking densities and exposed to white or green light for 30 days. The effects of the different stocking densities and light treatments were assessed by comparing rates of survival and growth, expression and activity of the antioxidant system, and changes in immune responses, and by measuring DNA damage via a comet assay.

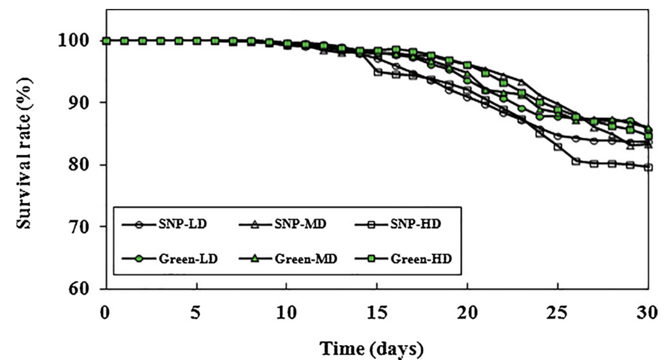

Fig. 1. Changes in survival rates (%) of olive flounder cultured under white (SNP, simulated natural photoperiod) or green light and at different stocking densities (LD, low stocking density; MD, medium stocking density; HD, high stocking density). Survival rate was measured at daily intervals (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

2. Materials and methods

2.1. Experimental fish and stocking densities

Olive flounder (length, 7.25 ± 1.1 cm; mass, 3.41 ± 0.5 g) were obtained from the Jeju Fisheries Research Institute, National Institute of Fisheries Science. On April 28, 2016, the fish were stocked in eighteen 8-ton filter tanks (1.5 m in depth) at three different stocking densities [900 (low stocking density group), 1350 (medium stocking density group), and 2250 (high stocking density group)] at the Future Aquaculture Research Center (Jeju, Korea). Triplicate cultures at each stocking density were exposed to either white light from a fluorescent bulb or green light from an LED for 30 days. Fish in all treatment groups were maintained in outdoor tanks supplied with a continuous flow of seawater, at ambient temperature (15 ± 1.5 °C), and under simulated natural photoperiod (SNP: 27 W white light bulb under a 12-h light:12-h dark photoperiod; lights on at 07:00 and off at 19:00). The fish were fed twice daily (09:00 h and 17:00 h) with a commercial diet (crude protein content ≥52%, crude fat ≥8%, crude ash ≤15%, and crude fiber ≤4%). Fish exposed to the white light (wavelength range 350–650 nm) were used as the control group. Those exposed to the green light (LED with a peak at 530 nm under a 12-h light:12-h dark photoperiod; lights on at 07:00 and off at 19:00) (S-tech LED Co., Kyunggi, Korea) are termed the treatment group here. Exposure to white or green light lasted for 30 days (April 28, 2016 to May 29, 2016). The LEDs were positioned 1.5 m above the water surface of experimental tanks, and irradiance at the water surface was maintained at approximately 0.5 W/m^2 (Fig. 1). In addition, we also installed a 3-m-high square barrier around the tank to block external light. Spectral analysis of the lights was performed using a FieldSpec spectroradiometer (ASD Inc., Boulder, CO, USA).

2.2. Survival rate and growth parameters analysis

In each experiment, the flounders were grouped according to stocking density and light source, and the numbers of dead and living individuals were counted each day at 13:00. Survival rates were determined at daily intervals for 30 days and were calculated as follows: survival rate (%) = number of fish at the assessment time/number of fish at the start of the experiment × 100.

For the fish in each experimental group, we measured body length, body depth, weight, and specific growth rate (SGR). For each parameter, we compared values obtained at the start and after 30 days of treatment. SGR was calculated as SGR (%) = (final mean body weight - Initial mean body weight)/experimental period (days) × 100.

2.3. Tissue sampling

We collected liver and blood samples from fish in each group at 14:00 h on day 0 (*n* = 30) and 30 (*n* = 30) at the end of the experimental period. Immediately after collection, the liver tissues were frozen in liquid nitrogen and stored at −80 °C until used for RNA extraction. Blood samples were obtained from the caudal vasculature using a 1-mL syringe coated with heparin. After centrifugation (4 °C, 10,000 × *g*, 5 min), the plasma was stored at −80 °C until analysis.

2.4. Quantitative real-time PCR (qPCR)

Total RNA was extracted from liver tissue using TRI Reagent (Molecular Research Center, Inc., USA) according to the manufacturer's instructions. Reverse transcription was performed using M-MLV reverse transcriptase (Sigma, St Louis, MO, USA) according to the manufacturer's instructions. The resulting cDNA was diluted and stored at 4 °C for use PCR amplification.

The relative expression levels of SOD (GenBank Accession No. EF681883) and CAT (GenBank Accession No. GQ229479) were determined using qPCR. The primers for qPCR were based on known sequences of olive flounder: SOD: forward (5′-CGT TGG AGA CCT GGG GAA TGT G-3′) and reverse (5′-ATC GTC AGC CTT CTC GTGGAT C-3′); CAT: forward (5′−CCA AAC TAC TAT CCC AAC AGC-3′) and reverse (5′−CCA CAT CTG GAG ACA CCT T-3′); and β-actin (GenBank Accession No. HQ386788): forward (5′-GGA CCT GTA TGC CAA CAC TG-3′) and reverse (5′-TGA TCT CCT TCT GCA TCC TG-3′). PCR amplification was conducted using a Bio-Rad CFX96 Real-time PCR Detection System (Bio-Rad, Hercules, CA, USA) and iQ SYBR Green Supermix (Bio-Rad) according to the manufacturer's instructions. The following protocol was used for qPCR: one cycle of denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 20 s, annealing, and extension at 55 °C for 20 s. All analyses were based on the cycle threshold (ΔCt) values of the PCR products. Ct was defined as the PCR cycle at which the fluorescence signal crossed a threshold line that was placed in the exponential phase of the amplification curve. After the PCR program, qPCR data from three replicate samples were analyzed using Bio-Rad software to estimate transcript copy numbers for each sample. In addition, to ensure that the primers amplified a specific product, we performed a melting curve analysis, which showed that the products of each primer pair had a single melting point. β-actin was used as the internal control, and all data are expressed relative to the corresponding β-actin-calculated ΔCt levels. The calibrated ΔCt value (ΔΔCt) for samples (SOD and CAT) and internal control (β-actin) was calculated as $\Delta\Delta Ct = 2\hat{}\text{-}(\Delta Ct_{sample} - \Delta Ct_{internal\ control})$.

2.5. Antioxidant activity analysis

Plasma samples were centrifuged at 5000 × *g* for 5 min at 4 °C. The supernatant was removed, and the remaining pellet was used for further analyses. SOD and CAT activities were determined using a fish superoxide dismutase (SOD) ELISA kit (catalog no. MBS282055; MyBioSource, USA) and fish catalase (CAT) ELISA kit (catalog no. MBS705697; MyBioSource), respectively.

2.6. Plasma analysis

Plasma samples were centrifuged (4 °C, 10,000 × *g* for 5 min) and the pellets used for analysis of H_2O_2, lysozyme, and melatonin levels. Plasma H_2O_2 levels were measured using a PeroxiDetect kit (Sigma) following a modified version of the method described by Nouroozzadeh et al. (1994). Absorbance was read at 560 nm and the concentrations of H_2O_2 were interpolated from a standard curve.

Plasma lysozyme and melatonin were analyzed by immunoassays using the ELISA kits (catalog nos. MBS099538 and MBS013211, respectively; MyBioSource). ELISA was performed according to the manufacturer's instructions. The optical density of each well of the detection microplate was determined within 10 min using a microplate reader set to 450 nm.

2.7. Comet assay

The comet assay is a relatively simple and sensitive technique for quantitatively measuring DNA damage in eukaryotic cells (Bajpayee et al., 2005). Here, we applied the technique to liver cells (1×10^5 cells/mL) using a CometAssay Reagent kit for single cell gel electrophoresis assay (Trevigen Inc., Maryland, USA), following the method described by Singh et al. (1988) with some modifications. Cells were immobilized on a slide coated with agarose gel and immersed in a freshly prepared alkaline unwinding solution for 20 min. An electrophoretic current was run through the gel (15 V for 30 min), followed by staining with SYBR Green (Trevigen Inc.) for 30 min in the dark. Fluorescence levels were determined using a fluorescence microscope (excitation filter 465–495 nm; Eclipse C*i*, Nikon, Japan). At least 100 cells from each slide were assessed. For a quantitative analysis of the comet assays, we used comet assay IV image analysis software (version 4.3.2; Perceptive Instruments Ltd., UK) to analyze the comet tail lengths (distance of DNA migration from head) and percentage of DNA in the tails (tail intensity/total intensity in tail).

2.8. Statistical analysis

All data were analyzed using the SPSS statistical package (version 19.0; SPSS Inc., USA). A two-way ANOVA followed by Tukey's post hoc test was used to compare differences in the data ($P < 0.05$). Data are expressed as means ± standard error (SE).

3. Results

3.1. Changes in survival rate and growth parameters

In all control and treatment groups, the survival rate had decreased by 30 days (Fig. 1) in higher stocking density groups. There were no differences in the survival rates among all groups until day 15 after the start of treatment. Thereafter, however, the survival rates were higher under green light conditions. In particular, the survival rate of fish in the high stocking group under SNP conditions was lower than that of fish exposed to green light (by approximately 84.7%). However, the survival rate of fish under green light conditions and low stocking density was higher than that in other experimental groups (by approximately 85.6%).

After 30 days, total length, body depth, and body weight had increased in all treatment groups (Table 1: by approximately 1.48-, 1.78-, and 2.56-fold, respectively, at day 30 compared with day 0 in fish under SNP conditions). In fish cultured under green light conditions and low stocking density, total length, body depth, and body weight were higher than those in the other experimental groups (by approximately 1.16-, 1.07-, and 1.24-fold, respectively). In addition, SGR was highest (21.1% ± 5.4%) in fish cultured under green light conditions and low stocking density. In particular, SGR was higher under green light conditions (12.2% ± 4.6%) than in SNP (9.5% ± 2.0%) and at low stocking density.

Table 1
Changes in total lengths, body depths, body weight and Specific growth rate (SGR) of olive flounder cultured for 0 or 30 days under white (SNP) or green light and at different stocking densities.

Stocking density	0 days		30 days	
	SNP	Green	SNP	Green
	Total length (mm)			
LD	72.46 ± 3.9^{a}	72.55 ± 4.3^{a}	102.03 ± 4.0^{b}	114.47 ± 4.2^{c*}
MD	72.36 ± 3.9^{a}	72.61 ± 3.1^{a}	103.27 ± 2.9^{b}	112.02 ± 5.3^{bc}
HD	72.69 ± 3.6^{a}	71.91 ± 2.7^{a}	102.93 ± 5.2^{b}	109.43 ± 4.8^{bc}
	Body depth (mm)			
LD	23.88 ± 1.5^{a}	23.78 ± 1.3^{a}	38.84 ± 1.2^{b}	41.45 ± 1.4^{c*}
MD	23.98 ± 1.2^{a}	22.54 ± 1.4^{a}	38.23 ± 1.3^{b}	40.98 ± 0.9^{bc}
HD	24.12 ± 1.1^{a}	23.82 ± 1.0^{a}	36.13 ± 1.4^{b}	38.58 ± 2.0^{bc}
	Body weight (g)			
LD	3.42 ± 0.8^{a}	3.28 ± 0.5^{a}	7.11 ± 1.6^{b}	9.61 ± 2.6^{c*}
MD	3.32 ± 0.5^{a}	3.44 ± 0.7^{a}	7.01 ± 1.7^{b}	7.13 ± 1.6^{b}
HD	3.36 ± 0.6^{a}	3.14 ± 0.6^{a}	6.22 ± 1.9^{b}	6.81 ± 2.5^{b}
			SGR (%)	
LD			12.3 ± 2.7^{a}	21.1 ± 5.4^{c*}
MD			12.3 ± 1.7^{a}	12.3 ± 3.0^{b}
HD			9.5 ± 2.0^{a}	12.2 ± 4.6^{ab}

Differences in superscript letters indicate data showing significant differences between the two light wavelengths or different densities ($P < 0.05$). Asterisk (*) indicates a significant difference between different wavelengths within the same stocking density ($P < 0.05$). All values are means ± SE ($n = 10$). SNP, simulated natural photoperiod (white fluorescent bulb); LD, low stocking density; MD, medium stocking density; HD, high stocking density.

3.2. Expression and activity of antioxidant enzymes (SOD and CAT)

The patterns of SOD mRNA expression in liver tissue and plasma were found to have increased between 0 and 30 days in the high stocking group [by approximately 2.26-fold (HD), respectively]. Similar results were obtained in the pattern of SOD activity (Fig. 2). In particular, expression and activity in the high stocking density group were significantly greater than those in low and medium stocking density groups. By contrast, in fish cultured under green light and at high stocking densities, the levels of antioxidant enzymes were significantly lower than those under SNP conditions [by approximately 0.84-fold (HD), respectively].

Similar to the results for SOD, mRNA expression and activity of CAT increased by approximately 1.70-fold (HD) by 30 days in the high stocking group compared 0 days (Fig. 3). In particular, expression and activity in the high stocking density group were significantly higher than those in the low and medium stocking density groups. By contrast, in fish cultured under green light and a high stocking density, the levels of antioxidant enzymes were significantly lower compared to those under SNP conditions (by approximately 0.85-fold).

3.3. Plasma H_2O_2 levels

The initial plasma H_2O_2 level of 4.91 ± 0.32 mol/peroxide/mL increased to 7.96 ± 0.48 mol/peroxide/mL (approximately 1.62-fold higher than at 0 days under SNP conditions) after 30 days in the high stocking group (Fig. 4). In fish cultured under green light conditions

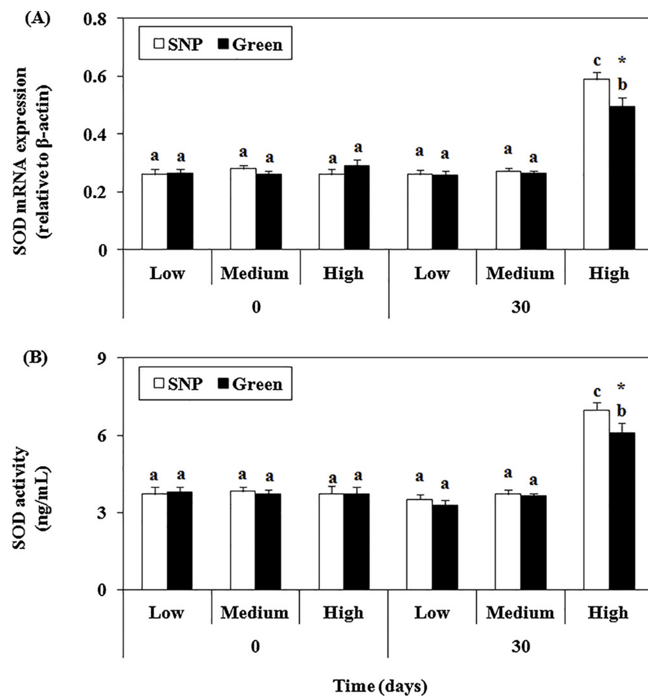

Fig. 2. Expression and activity of superoxide dismutase (SOD) in the liver and plasma of olive flounder cultured under white (SNP) or green light and at different stocking densities. (A) SOD mRNA levels relative to $\beta-$actin mRNA levels in the same sample. (B) SOD plasma activity was also analyzed using a plate reader. Differences in superscript letters indicate data showing significant differences between the two light wavelengths or different densities ($P < 0.05$). An asterisk (*) indicates a significant difference between different wavelengths within the same stocking density ($P < 0.05$). All values are means ± SE (n = 10) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

and high stocking density, the level of H_2O_2 increased significantly to 6.40 ± 0.84 mol/peroxide/mL after 30 days (a 0.79-fold increase compared with 30 days in SNP groups).

3.4. Plasma lysozyme and melatonin levels

Plasma lysozyme and melatonin levels were 31.13 ± 1.74 U/mL and 11.16 ± 0.69 pg/mL, respectively, at the start of the experiment (Fig. 5). These levels had decreased after 30 days to 26.14 ± 0.91 U/mL (approximately 1.12-fold lower than at 0 days in fish under SNP conditions) and 8.55 ± 0.26 pg/mL (approximately 1.31-fold lower than at 0 days under SNP conditions), respectively. Although, compared with the initial values, the levels of lysozyme and melatonin decreased under exposure to green light and a high stocking density, after 30 days, the levels (30.30 ± 0.84 U/mL and 10.13 ± 0.35 pg/mL, respectively) were significantly higher than those under the SNP conditions (by approximately 1.09- and 1.19-fold, respectively).

3.5. Comet assay

DNA damage in liver cells was analyzed using 100 randomly selected cells from tissues collected at the end of the 30-day experiment. For the comet assay quantification, we analyzed tail lengths and % DNA in the tail. Both parameters increased had significantly at the end of the experiment (Fig. 6). After 30 days, both tail lengths and % DNA in tails of the group exposed to green LED at low-socking density were significantly lower than those in other experimental groups.

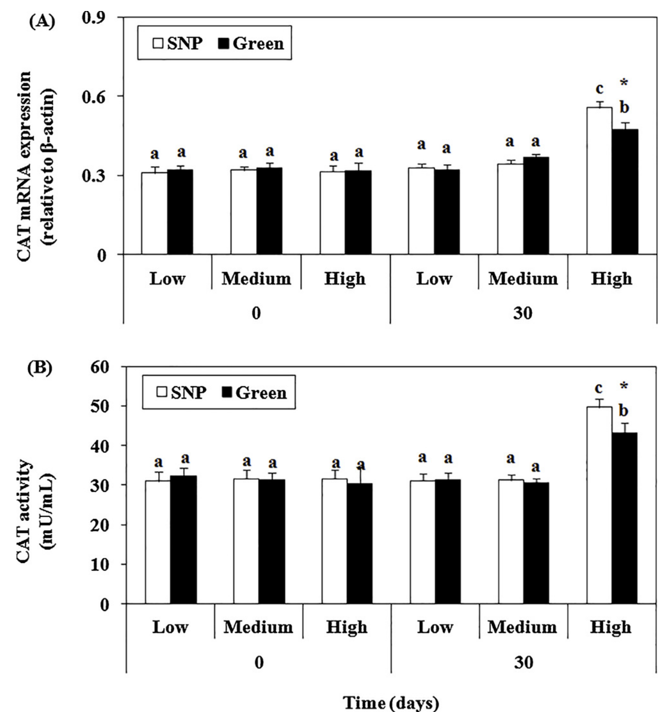

Fig. 3. Expression and activity of catalase (CAT) in the liver and plasma of olive flounder cultured under white (SNP) or green light and at different stocking densities. (A) CAT mRNA levels relative to $\beta-$actin mRNA levels in the same sample. (B) The plasma activity of CAT was also analyzed using a plate reader. Differences in superscript letters indicate data showing significant differences between the two light wavelengths or different densities ($P < 0.05$). An asterisk (*) indicate a significant difference between different wavelengths within the same stocking density ($P < 0.05$). All values are means ± SE (n = 10) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

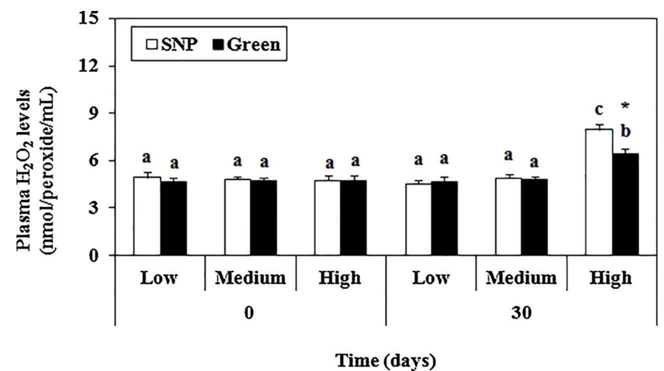

Fig. 4. Plasma activity of H_2O_2 of olive flounder cultured under white (SNP) or green light and at different stocking densities. Differences in superscript letters indicate data showing significant differences between the two light wavelengths or different densities ($P < 0.05$). An asterisk (*) indicates a significant difference between different wavelengths within the same stocking density ($P < 0.05$). All values are means ± SE (n = 10) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

4. Discussion

The present study was conducted to determine whether culturing juvenile olive flounder under green wavelength LED light would enhance their production of antioxidants and promote immune function at a high stocking density. We initially compared survival rates among fish cultured under SNP conditions and under green light and observed no

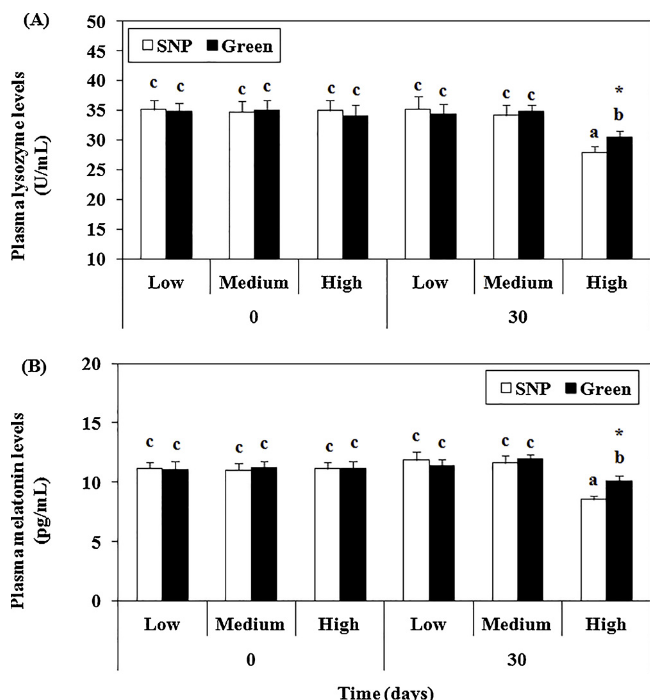

Fig. 5. Plasma activity of lysozyme (A) and melatonin (B) of olive cultured under white (SNP) or green light and at different stocking densities. Differences in superscript letters indicate data showing significant differences between the two light wavelengths or different densities ($P < 0.05$). An asterisk (*) indicates a significant difference between different wavelengths within the same stocking density ($P < 0.05$). All values are means ± SE ($n = 10$) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

significant differences until 15 days after the start of treatment. Thereafter, however, survival rates were higher under green light conditions. On the basis of these results, we suggest that as the fish grew in size during the experiment, there was a concomitant increase in density-related stress. In particular, after 30 days, the difference in survival rates between SNP + high stocking density group and other treatment groups increased. The survival rate at 30 days was lowest in the SNP + high stocking density group and highest in the green LED + low stocking density group. Our comparison of growth parameters in fish maintained under different stocking densities and under SNP or green light conditions identified significantly increased growth parameters in the all density groups exposed to green light compared with SNP.

In a similar study, Shin et al. (2013) reported that the growth rate (total length) of yellowtail damselfish *Chrysiptera parasema* under green wavelength light was significantly higher than that under SNP conditions over a prolonged culture period (4 months), suggesting that green wavelength light had a positive effect on the growth of yellowtail damselfish. The results of this earlier study are consistent with our conclusion that green wavelength light plays a positive role in the growth of the olive flounder.

We also compared the levels of antioxidant enzymes and ROS in fish at different stocking densities and under SNP or green light conditions. Our analyses showed that SOD and CAT mRNA expression and enzyme activities increased and that H_2O_2 concentrations increased significantly increased only at high stocking densities; however, these increases were less pronounced in fish under green light conditions than under SNP conditions. In particular, the decreases in antioxidant enzyme activity and H_2O_2 concentration in the plasma were the highest in experimental group (green LED + high stocking density).

Kim et al. (2016) reported that olive flounder showed induced stress after exposure to a high temperature environment (30 °C) and that, under these conditions, plasma H_2O_2 concentrations and antioxidant gene expression rates and enzyme activity levels were significantly higher under green wavelength light. Choi et al. (2016) examined the

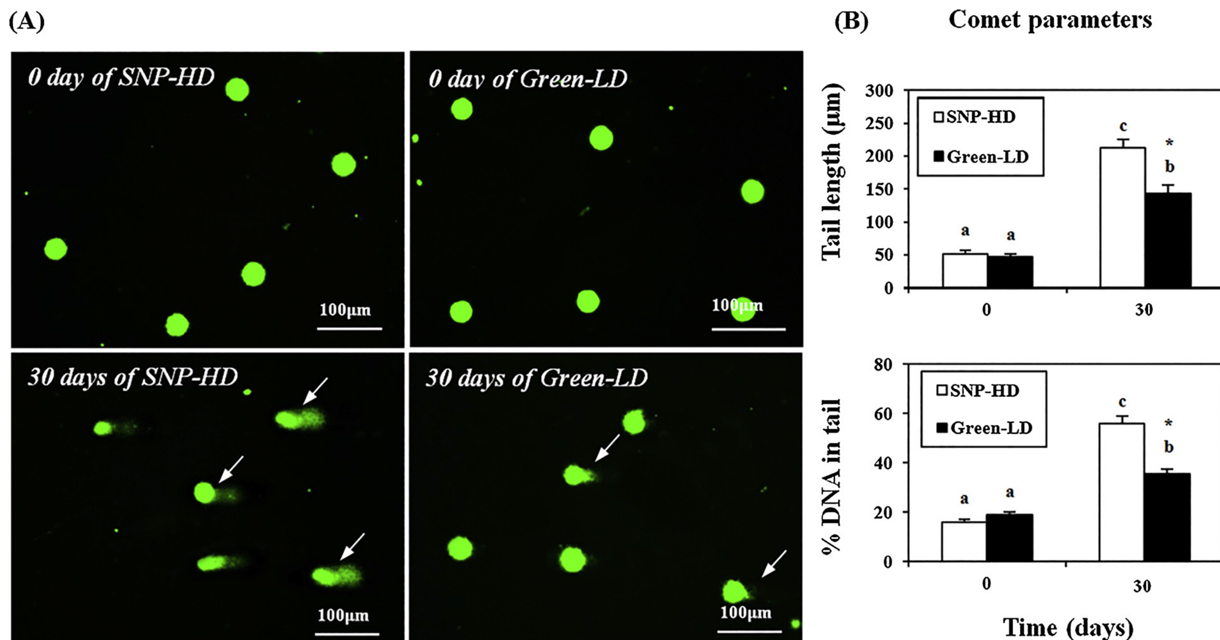

Fig. 6. Comet assay images (A) and comet assay parameters of tail length and % DNA in tail (B) of olive flounder cultured under white (SNP) or green light and at different stocking densities (LD, low stocking density; HD, high stocking density), as measured by fluorescence microscopy. White arrows in (a) indicate damaged nuclear DNA (DNA breaks) in hepatocytes stained with SYBR Green. Scale bar = 100 µm. Differences in superscript letters indicate data showing significant differences between the two light wavelengths or different densities ($P < 0.05$). An asterisk (*) indicates a significant difference between different wavelengths within the same stocking density ($P < 0.05$). All values are means ± SE ($n = 10$) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

effects of different light spectra (produced by red and green LEDs) on induced oxidative stress in gold-striped amberjack *Seriola lalandi* over 4 months, and showed that antioxidant enzyme levels and H_2O_2 concentrations in fish exposed to green light were significantly lower than those under control conditions (white light) or red LED. The results of these studies indicate that green light is effective in reducing oxidative stress in fish. Likewise, our findings here indicate that green wavelength light increased the antioxidant capacity of olive flounder, and contributed to reducing oxidative stress in olive flounder due to high stocking density.

In the present study, plasma concentrations of lysozyme and melatonin were measured to monitor changes in immunity under different stocking densities and light wavelengths. We found that plasma levels of lysozyme and melatonin in fish under green wavelength light conditions were significantly higher than those in fish under SNP conditions at high stocking density, but no significant differences were observed at low or medium stocking densities.

Choi et al. (2012) reported that green wavelength light significantly increased the concentration of plasma lysozyme in cinnamon clownfish after artificial induction of oxidative stress by starvation. Subsequently, Choi et al. (2016) reported that the levels of lysozyme and melatonin in the plasma of goldfish increased significantly after 4 months exposure to green wavelength light, suggesting that green wavelength light enhanced the immune system of the fish. The results of the present study are consistent with those of the published studies in suggesting that green wavelength light enhances immunity and growth compared with SNP (white light) conditions, and in particular reduces the effects of stress resulting from high stocking density.

Our comet assay showed that the amount of nuclear DNA damage in hepatocytes increased between the start and completion of the experiment. However, after 30 days, DNA damage was significantly lower in fish in the low stocking density + green light treatment compared with the high stocking density + white light group.

Kim et al. (2016) similarly reported an increased level of nuclear DNA damage in hepatocytes after exposing olive flounder to a high temperature environment, presumably due to increased stress in the fish. However, under different light wavelengths, DNA damage was significantly lower under green wavelength light. The results of the present study are consistent with those of Kim et al. (2016) and show that green wavelength light effectively reduces oxidative stress in flounder due to high stocking density, and thereby prevents nuclear DNA damage in hepatocytes and protects cells.

In conclusion, the results of our analyses indicate that green wavelength light from LEDs is effective in reducing oxidative stress in olive flounder caused by high stocking density. Thus, the use of this wavelength of light offers the prospect of culturing olive flounder at higher stocking densities due to the effective improvement in antioxidant capacity, enhanced immunity, and higher survival rates of juveniles.

In future studies, it will be necessary to further investigate variations in light wavelength and intensity to identify the optimal conditions that enhance physiological activity and immune responses of fish at different developmental stages in order to reduce stress caused by high stocking densities.

Conflict of interest

The authors declare that they have no conflict of interests.

Acknowledgments

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