Fish Sci (2017) 83:997–1006
https://doi.org/10.1007/s12562-017-1136-7

ORIGINAL ARTICLE

Biology

Effects of various wavelengths of light on physiological stress and non-specific immune responses in black rockfish *Sebastes schlegelii* subjected to water temperature change

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Received: 24 June 2017 / Accepted: 17 September 2017 / Published online: 23 October 2017

Abstract We investigated the effects of light-emitting diodes (LEDs) on physiological stress and immunity in black rockfish *Sebastes schlegelii* subjected to water temperature change [20 (normal temperature) → 14 → 20 °C] using progressive stepwise decline [experiment (Exp.) I; 2 °C/day] and direct decline (Exp. II; immediate exposure) in water temperature. We measured stress-related parameters such as cortisol, glucose, heat shock protein 70 and Na^+/K^+-ATPase, and immune parameters such as immunoglobulin M and lysozyme. We confirmed nuclear DNA damage using a comet assay. Physiological stress indicators were significantly higher, immune parameters were significantly lower, and the amount of nuclear DNA damage was higher after exposure to low temperatures, but these effects were mitigated by exposure to green or blue LEDs. The direct decline in temperature induced more physiological stress and nuclear DNA damage than the progressive stepwise change. Green and blue LEDs aid recovery from physiological damage in fish.

Keywords Cold pool · Cold shock · Light-emitting diodes · Physiological stress · Specific wavelengths

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Introduction

Sources of stress for fish include temperature, salinity, and breeding density [1]. In particular, changing water temperature induces oxidative stress in teleosts and is known to have negative effects such as the reduction of antioxidant regulation and immune function [2, 3].

In general, when a fish is exposed to an acute temperature change, a large amount of stress is induced in the fish's body [4, 5]. The organism has a stress defense mechanism to maintain in vivo homeostasis during stress [6, 7]. This response to stress is regulated by the hypothalamic-pituitary-interrenal axis. First, corticotropin-releasing hormone is secreted in the hypothalamus, then it acts on the anterior pituitary to stimulate the secretion of adrenocorticotropic hormone (ACTH) [6]. ACTH, which is derived from the pro-opiomelanocortin precursor protein, then stimulates the synthesis and release of cortisol in the interrenal cells of the head kidney [6, 8]. Cortisol has also been reported to be the direct cause of the increase in plasma glucose [9, 10].

In the bodies of fish exposed to stressors such as rapid water temperature changes, heat shock proteins (HSPs) are produced in large quantities. HSPs repair the proteins damaged by these external environmental factors and allow the cells to maintain their normal functions [8, 11, 12].

The stress caused by changing water temperature causes increased activity of Na^+/K^+-ATPase (NKA) in gills. NKA is an enzyme involved in maintaining homeostasis, including gas exchange, regulation of osmotic pressure, and acid–base balance, by activating chloride cells in the gills [13, 14]. Kang et al. [15] reported that acute water temperature changes (28 → 18 °C) significantly decreased the activity of NKA in juvenile milkfish *Chanos chanos*. Mitrovic and Perry [16] also reported that exposure to an acute water temperature change

(25 → 7 °C) decreased the activity of NKA significantly in goldfish *Carassius auratus*.

Another effect of stress on organisms is a negative effect on maintaining immunity. In particular, immunoglobulin M (IgM) and lysozyme have been used as immune indicators [7, 17, 18]. Recent studies have shown that levels of these two immune indicators significantly change in fish exposed to specific wavelengths of light [19, 20].

In other words, light has effects on physiological responses such as immune control and stress [19, 21, 22]. Studies have been conducted on the effects of specific wavelengths on a variety of physiological responses using light-emitting diodes (LEDs), which can be manufactured to output specific wavelengths [19, 23, 24]. LEDs have various advantages, including low power consumption, long life, and high efficiency. Thus, the positive effects of specific wavelengths on organisms can be positively associated with aquaculture.

The black rockfish, *Sebastes schlegelii*, is an ovoviviparous fish belonging to the order Scorpaeniformes and family Scorpaenidae, and is known to have an optimum temperature of 18–22 °C. This species is sensitive to light and has been reported to react sensitively to various wavelengths of visible light, especially the blue-green part of the spectrum [25]. It is an important aquaculture species because it grows faster than other species [26, 27]. However, at aquaculture sites on the southern coast of Korea, cold pools occur frequently, leading to mass mortality of black rockfish. Cold pools occur mainly in the summer season and are known to create temperature zones about 5 °C colder than the surrounding sea [28].

Therefore, in the present study, we investigated the endocrine changes caused by physiological stress in black rockfish exposed to acute temperature changes such as those in cold pools, and we tested whether specific-wavelength light could adjust the induced stress response. The rearing environment was established as one of two types: a progressive stepwise change or a direct decline in water temperature. We examined the stress responses (cortisol, glucose, HSP70, and NKA), immunoregulatory responses (IgM and lysozyme), and the changes in hormone levels and enzyme activities of rockfish exposed to the low-temperature environment, while irradiating the fish with various light sources. In addition, a comet assay was performed to determine whether specific wavelengths are effective in reducing the nuclear DNA damage that is expected to be caused by rapid temperature change.

Materials and methods

Experimental fish and conditions

Black rockfish, *Sebastes schlegelii* (n = 135, length 18.5 ± 0.5 cm, mass 27.2 ± 0.8 g), were provided by the Korea Institute of Ocean Science & Technology (Tongyeong, Korea), and were allowed to acclimate for 1 week in nine 300-L circulation filter tanks in the laboratory (15 individuals per tank). The tanks were equipped with automatic temperature regulation systems (JS-WBP-170RP; Johnsam, Seoul, Korea). The fish were divided into two experimental groups, one with a progressive stepwise change in water temperature [experiment (Exp.) I] and the other a direct decline in water temperature (Exp. II), each starting at 20 °C water temperature (normal temperature). In Exp. I groups, the water temperature was decreased from 20 to 14 °C in daily decrements of 2 °C, and then increased to 20 °C by daily increments of 2 °C. Exp. II groups were moved into 14 °C tanks immediately; 24 h later, they were returned to 20 °C water. The control groups were exposed to 20 °C and no change in temperature during the experiment. Fish were allowed to acclimate to each experimental temperature [20 → 14 → 20 °C] for 24 h before samples were taken. In this study, no fish died in any group. Each group was divided into three subgroups (n = 5 each): two experimental groups that were exposed to green (peak at 530 nm) or blue (peak at 450 nm) light-emitting diodes (LEDs; Daesin LED, Kyunggi, Korea); and a control group irradiated with a white fluorescent bulb (stimulated natural photoperiod; SNP) (Fig. 1). The photoperiod was a 12-h light:12-h dark cycle (lights on at 07:00 hours and off at 19:00 hours). The LEDs were placed 50 cm above the water surface, and the irradiance at the surface was maintained at approximately 0.5 W/m^2. This protocol was used because a light intensity of 0.5 W/m^2 has the effect of causing a physiological change [24]. Spectral analysis of the lights was performed using a Photo-Radiometer HD 2102.1 (Delta OHM, Padova, Italy). The fish were reared under these conditions with a daily feed of commercial feed until the day prior to the sampling. Fish were anaesthetized with 200 µL/L 2-phenoxyethanol (Daejung, Kyunggi, Korea) to minimize stress prior to blood and tissue collection. Blood was collected rapidly from the caudal vein using a 1-mL syringe coated with heparin. Plasma samples were separated by centrifugation (4 °C, 1000 g, for 10 min) and stored at − 80 °C until analysis. Tissues were collected, immediately frozen in liquid nitrogen, and stored at − 80 °C until total RNA was extracted for analysis.

Total RNA extraction and complementary DNA synthesis

Total RNA was extracted from each sample using TRI Reagent (Molecular Research Center, Cincinnati, OH), according to the manufacturer's instructions. Then, 2 µg of total RNA was reverse-transcribed in a total reaction volume of 20 µL, using an oligo-(dT)$_{15}$ anchor and M-MLV reverse transcriptase (Promega, Madison, WI), according to the manufacturer's protocol. The resulting complementary DNA

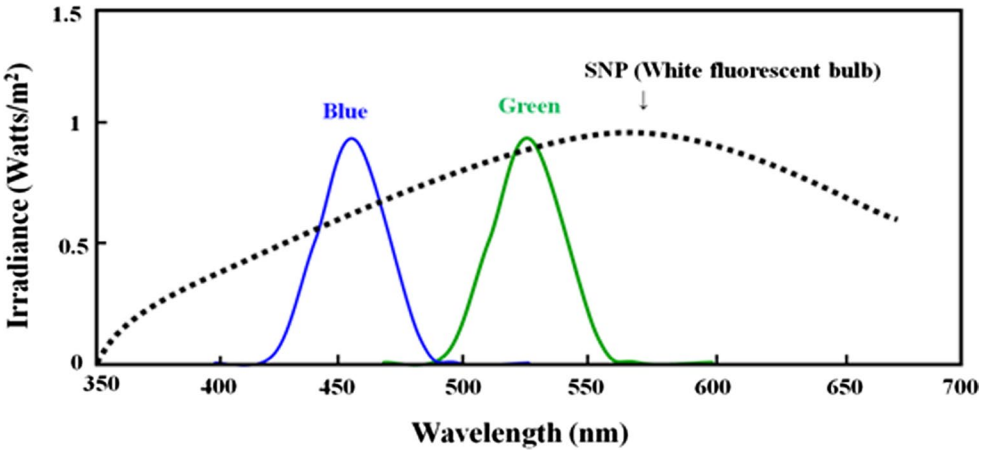

Fig. 1 Spectral profiles of light-emitting diodes (LEDs; blue, 450 nm; green, 530 nm) and white fluorescent bulb (control) in this study. LEDs were set to an intensity of 0.5 W/m^2. *SNP* Stimulated natural photoperiod

(cDNA) was diluted and stored at 4 °C for use in polymerase chain reaction (PCR) and real-time quantitative PCR (qPCR) analysis.

Quantitative PCR

The qPCR analysis was conducted to determine the relative expression levels of the antioxidant enzymes HSP70 and NKA using total RNA extracted from the livers and gills of the black rockfish. The primers for qPCR were designed using known black rockfish sequences (Table 1). We conducted qPCR amplification using iCycler iQ Multicolor Real-Time PCR Detection System (Bio-Rad, Hercules, CA) and the iQ SYBR Green Supermix (Bio-Rad), according to the manufacturer's instructions. β-actin was also amplified as a control for each sample, and all data were expressed as the change with respect to the corresponding calculated β-actin threshold cycle (Ct) levels. All analyses were based on the Ct values of the PCR products. The Ct was defined as the PCR cycle in which the fluorescence signal crossed a threshold during the exponential phase of the amplification curve. The calibrated ΔCt value (ΔΔCt) of each sample and the internal control (β-actin) were calculated [$\Delta\Delta Ct = 2^{\wedge} -$ ($\Delta Ct_{sample} - \Delta Ct_{internal\ control}$)]. After PCR was completed, the qPCR data from three replicate samples were analyzed using Bio-Rad to estimate the transcript copy numbers of each sample.

Table 1 Primers used for quantitative polymerase chain reaction amplification

Gene (accession no.)	Primer	DNA sequences
HSP70 (KC172645)	Forward	5′-AGGGATAAAGTCTCT GCC AAG-3′
	Reverse	5′-TCAATCACC GTC TTC TCGTC-3′
NKA (HQ6655051)	Forward	5′-TTAGCGGTCAGGGTCAGA-3′
	Reverse	5′-GGTGTCTCCTTCTTCGTC C-3′
β-actin (JN226153)	Forward	5′-GACCACCTACAACAGCAT CAT-3′
	Reverse	5′-TACCTCCAGACAGCACGG-3′

HSP70 Heat shock protein 70, *NKA* Na^+/K^+-ATPase

Plasma parameter analysis

Plasma samples were separated by centrifugation (4 °C, 1000 *g*, for 10 min). Cortisol, IgM, and lysozyme levels were analyzed using an immunoassay with an ELISA kit (cortisol, MBS704055; IgM, MBS700823; lysozyme, MBS099538; Mybiosource, San Diego, CA). Absorbance was read at 450 nm, and the concentration was interpolated from a standard curve.

Plasma glucose levels were measured using a dry multilayered analytic slide method in a biochemistry auto analyzer (Fuji Dri-Chem 4000; Fujifilm, Tokyo).

NKA analysis

Gill tissue NKA was extracted from gill tissues of each sample by homogenization and centrifugation (4 °C, 1000 *g*, for 15 min) in ice-cold phosphate-buffered saline. The NKA concentration was analyzed using an immunoassay with an ELISA kit (MBS084723; NAK, Mybiosource). Absorbance was read at 450 nm, and the concentration was interpolated from a standard curve.

Comet assay

The comet assay is a relatively simple, sensitive technique for quantitatively measuring DNA damage in eukaryotic cells [29]. Liver cells (1×10^5 cells/mL) were examined using a Comet Assay Reagent Kit for single-cell gel electrophoresis (Trevigen, Gaithersburg, MD), according to the methods described by Singh et al. [30]. Cells were immobilized in agarose gel on Comet Assay slides and immersed in freshly prepared alkaline unwinding solution for 20 min.

Slides were then electrophoresed at 15 V for 30 min. Samples from fish exposed to SNP and green LED at 20 (control), 14, and 20 °C were stained with SYBR Green (Trevigen) for 30 min in the dark, and read using a fluorescence microscope (excitation filter 465–495 nm; Eclipse Ci, Nikon, Japan). At least 100 cells from each slide were analyzed. For quantification, we analyzed the tail length (distance of DNA migration from the head) and % DNA in the tail (tail intensity/total intensity × 100) using the Comet Assay IV image analysis software (version 4.3.2; Perceptive Instruments, UK).

Statistical analysis

All data were analyzed using the SPSS statistical package (version 10.0; SPSS, Chicago, IL). A two-way ANOVA followed by Tukey's post hoc test was used to compare differences in the data ($P < 0.05$). The values are expressed as the mean ± SE.

Results

Change in concentrations of cortisol and glucose

The plasma cortisol concentrations in all experimental groups increased significantly due to a change of temperature to 14 °C, and then decreased significantly as the temperature rose again to 20 °C. However, the cortisol concentrations in Exp. II were generally higher than those in Exp. I. The cortisol levels in the green and blue LED treatments were significantly lower than in SNP (Fig. 2a, b).

The plasma glucose concentrations in all experimental groups increased significantly due to a change of temperature to 14 °C, and then decreased significantly as the temperature rose again to 20 °C. However, the glucose concentrations in Exp. II were generally higher than in Exp. I. The glucose levels in the green and blue LED treatments were significantly lower than those in SNP (Fig. 2c, d).

Change in messenger RNA expression of HSP70

The change of messenger RNA (mRNA) expression of HSP70 in black rockfish exposed to low water temperatures under the different wavelengths of LEDs are shown in Fig. 3a, b. The change of mRNA expression increased significantly with temperature decrease in all experimental groups. In addition, the levels of mRNA expression of HSP70 showed significant decreases with exposure to green or blue LED. In particular, the levels in the green LED treatment groups were significantly lower than those observed in the other light groups.

Changes in mRNA expression and concentrations of NKA

The changes in NKA mRNA expression and concentrations in gill tissue of black rockfish exposed to low water temperature under the different wavelengths of LEDs are shown in Fig. 4a, b. The mRNA concentrations in all experimental groups decreased significantly due to a change of temperature to 14 °C, and then increased significantly as the temperature rose again to 20 °C. The NKA mRNA expression and concentrations in Exp. II were generally lower than those in Exp. I. The NKA concentrations in the green and blue LED groups were significantly higher than those in the white fluorescent bulb (SNP) group (Fig. 4c and d).

Changes in plasma concentrations of IgM and lysozyme

The plasma concentrations of IgM in all experimental groups decreased significantly due to a change of temperature to 14 °C, and then increased significantly as the temperature rose again to 20 °C. The IgM concentrations in Exp. II were generally lower than those in Exp. I. The IgM concentrations in the green and blue LED treatments were significantly higher than those in SNP (Fig. 5a, b). The variations in the plasma lysozyme concentrations were similar to the variations in the plasma IgM concentrations (Fig. 5c, d).

Comet assay

The comet assay is shown in Fig. 6. The DNA content in the tail and tail length both increased significantly at 14 °C. Nuclear DNA tail and tail length decreased significantly as the temperature rose again to 20 °C. Nuclear DNA content and tail length in the green and blue LED treatments were significantly lower than those in SNP.

Discussion

This study was conducted to investigate whether the effects of stress can be mitigated and immunity enhancement obtained by irradiating black rockfish with LEDs while exposing them to a low-temperature environment. The fish were divided into two groups, then exposed to a low-temperature environment using either a progressive stepwise change (Exp. I) or a direct decline (Exp. II) in water temperature. We observed physiological responses using molecular biological methods.

The plasma cortisol and glucose levels, which are physiological stress indicators, were significantly increased following the water temperature decrease. An acute change in water temperature induced oxidative stress in the fish, and caused an increase in plasma cortisol concentration.

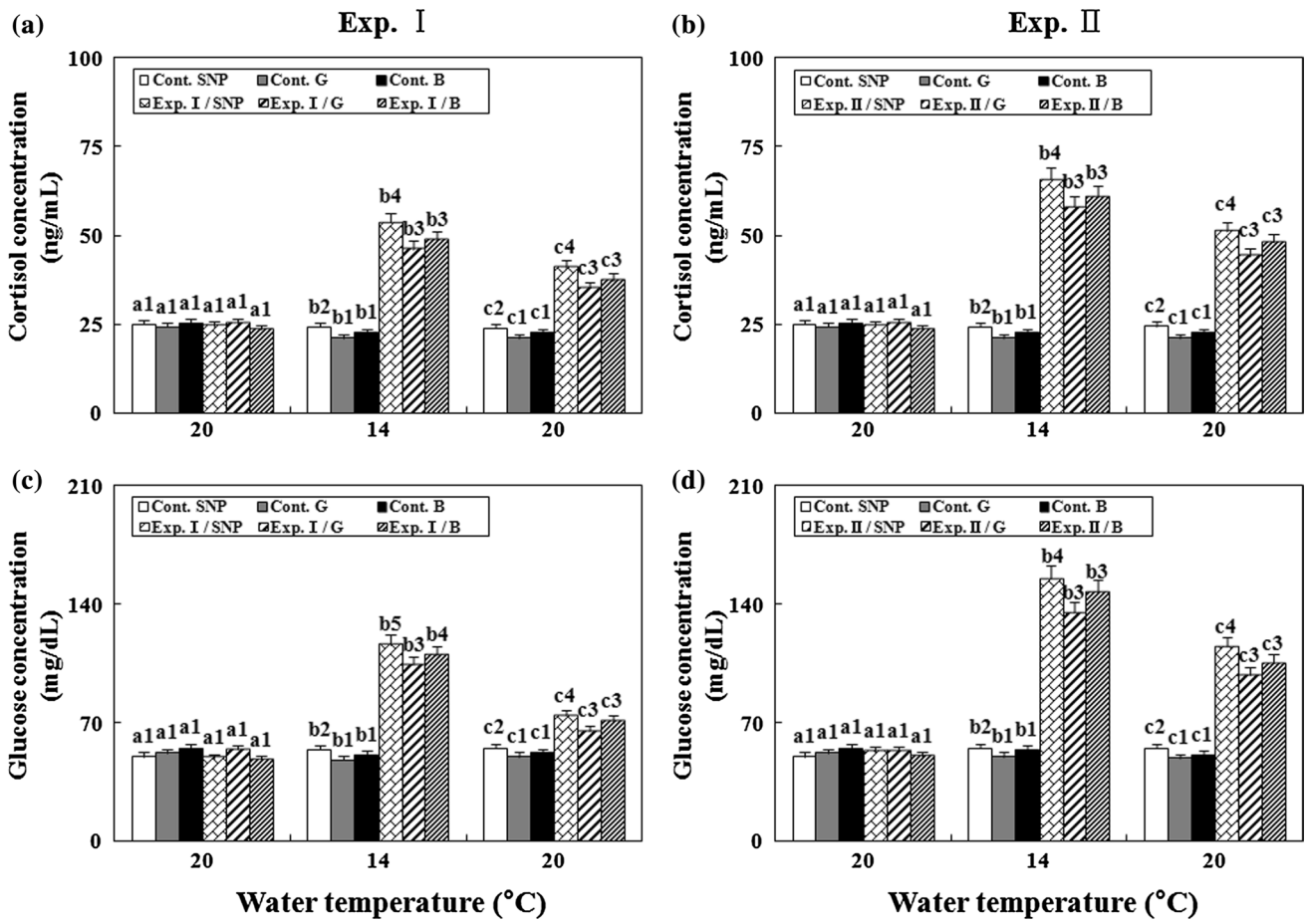

Fig. 2 Changes in plasma cortisol concentrations of experiment (Exp.) I (**a**) and Exp. II (**b**) groups, and plasma glucose concentrations of Exp. I (**c**) and Exp. II (**d**) groups during water temperature changes [20 → 14 → 20 °C] under light conditions using green, blue light-emitting diode (LED) and SNP, as measured using a microplate reader. The lowercase letters indicate the temperatures (*a* 20 °C, *b* 14 °C, *c* return to 20 °C) and the different numbers indicate significant differences within a temperature ($P < 0.05$). All values are represented as mean ± SE ($n = 5$). *Exp. I* Progressive stepwise decline in water temperature, *Exp. II* direct decline of water temperature, *Cont.* control, *G* green LED, *B* blue LED

At this time, the fish's energy requirement increased to compensate for the energy consumed by oxidative stress. For that reason, gluconeogenesis was promoted and the glucose level increased [31, 32]. After the black rockfish were recovered to their initial (normal) water temperature (20 °C) after exposure to a low-temperature (14 °C) environment, the plasma levels of cortisol and glucose remained higher in Exp. II than in Exp. I. However, in the low-temperature groups irradiated with green or blue wavelengths, the plasma cortisol and glucose concentrations were significantly decreased when compared to the SNP control.

Based on a similar study, Kim et al. [24] reported that oxidative stress was significantly increased when flatfish, *Paralichthys olivaceus*, were exposed to high-water-temperature environments. However, as a result of irradiation with green and blue wavelengths, oxidative stress was significantly decreased.

In the present study, similar to previous studies, the black rockfish showed significant increases in cortisol and glucose concentrations due to a large amount of stress in the body when exposed to a low-temperature environment. However, we suggest that short-wavelength lights such as green and blue LEDs are effective in reducing stress.

In this study, HSP70 mRNA expression was significantly increased following water temperature decrease in both experimental groups (Exp. I and Exp. II). However, despite the water's return to the initial temperature, HSP70 mRNA expression was maintained at significantly higher levels in Exp. II compared to Exp. I. Green and blue LEDs significantly reduced HSP70 mRNA expression in the low-water-temperature groups. HSP70, which is a protein particularly sensitive to environmental stress factors such as water temperature [33], seems to play a role in maintaining the normal function of liver cells in fish exposed to water temperature stress, because it plays a role in stabilizing the

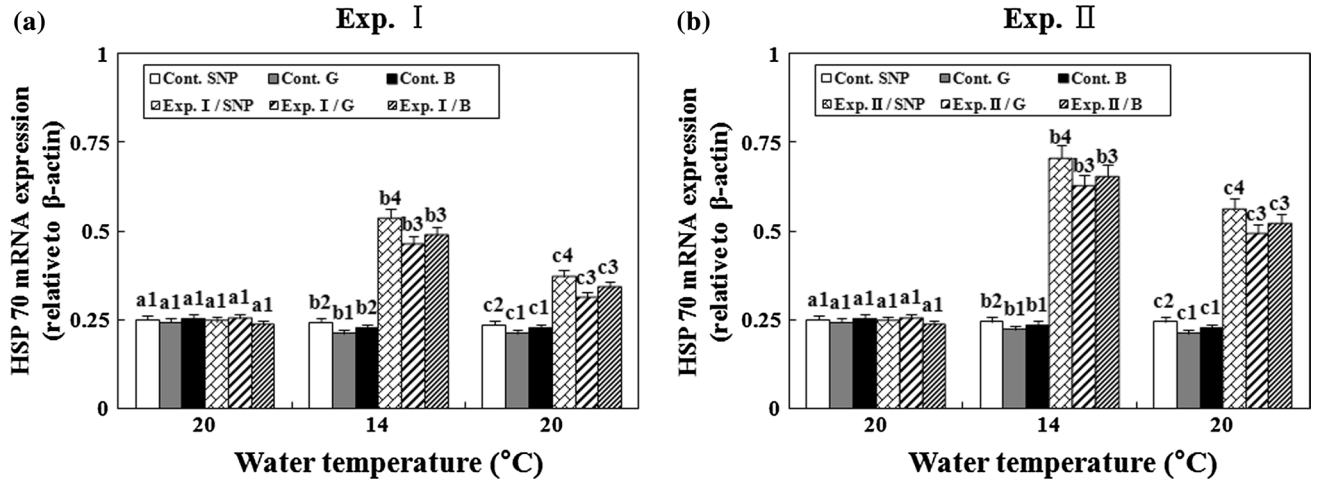

Fig. 3 Change in heat shock protein 70 (*HSP70*) messenger RNA (*mRNA*) expression of Exp. I (**a**) and Exp. II (**b**) groups during water temperature changes under light conditions using green LED, blue LED, and SNP, as normalized fold expression levels with respect to the β-actin levels in each sample. The lowercase letters indicate the temperatures (*a* 20 °C, *b* 14 °C, *c* return to 20 °C) and the different numbers indicate significant differences within a temperature ($P < 0.05$). All values are represented as mean ± SE ($n = 5$). For other abbreviations, see Figs. 1 and 2

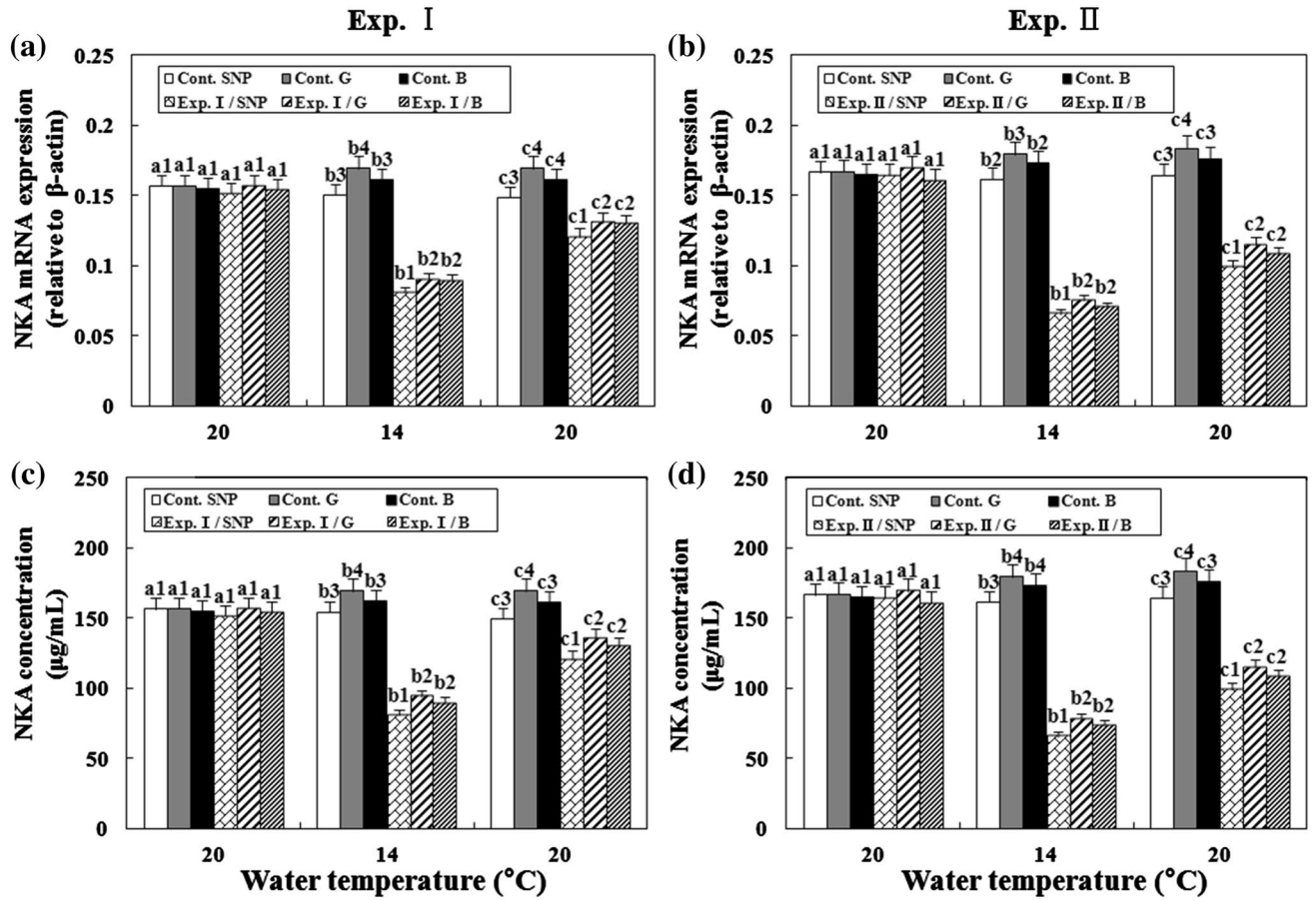

Fig. 4 Change in Na^+/K^+-ATPase (*NKA*) messenger RNA (*mRNA*) expression of Exp. I (**a**) and Exp. II (**b**) groups and concentration of Exp. I (**c**) and Exp. II (**d**) groups during water temperature changes under light conditions using green LED, blue LED, and SNP, as normalized fold expression levels with respect to the β-actin levels and as meaured using a microplate reader in each parameter. The lowercase letters indicate the temperatures (*a* 20 °C, *b* 14 °C, *c* return to 20 °C) and the different numbers indicate significant differences within a temperature ($P < 0.05$). All values are represented as mean ± SE ($n = 5$). For other abbreviations, see Figs. 1 and 2

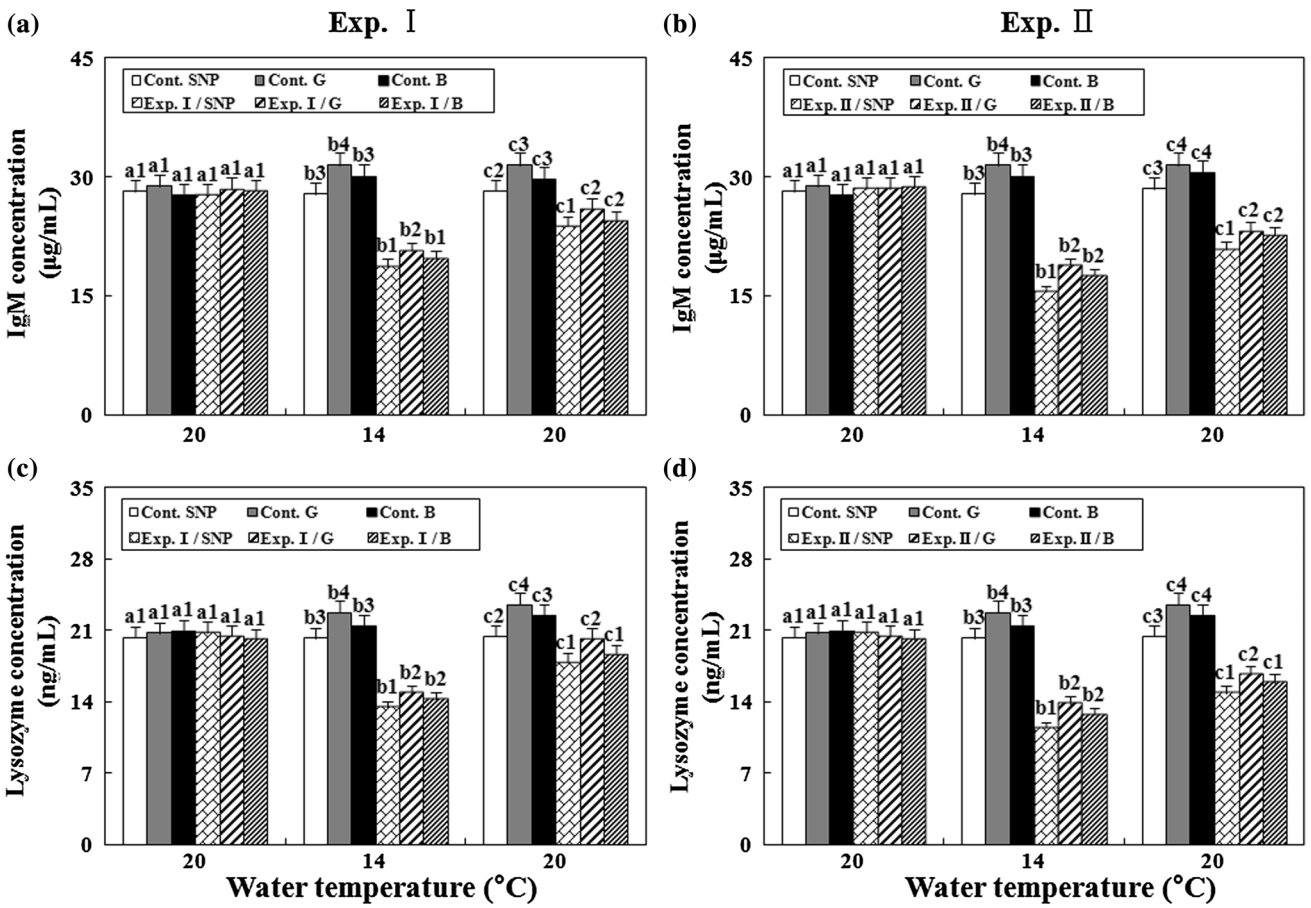

Fig. 5 Changes in plasma immunoglobulin M (*IgM*) concentration of Exp. I (**a**) and Exp. II (**b**) groups, and plasma lysozyme concentration of Exp. I (**c**) and Exp. II (**d**) groups during water temperature changes using green, blue LED and SNP, as measured using a microplate reader. The lowercase letters indicate the temperatures (*a* 20 °C, *b* 14 °C, *c* return to 20 °C) and the different numbers indicate significant differences within a temperature ($P < 0.05$). All values are represented as mean ± SE ($n = 5$). For other abbreviations, see Figs. 1 and 2

tertiary structure of the synthesized protein, thus maintaining normal cell function [11, 34].

Kim et al. [24] reported that HSP mRNA expression was significantly increased when goldfish were exposed to low water temperature. However, as a result of irradiation with green or blue wavelengths, it was significantly decreased.

Similarly, HSP70 mRNA expression was significantly increased in black rockfish exposed to water temperature shock with changing water temperature. It appears that black rockfish can counter the stress in the body and reduce damage to liver cells because of the stress-reducing effect of green and blue LEDs.

To investigate the effects of stress induced by a direct decline in water temperature on the function of gills, we analyzed NKA mRNA expression and concentration in black rockfish gills. In both of the low-temperature groups (Exp. I and Exp. II), NKA mRNA expression and concentration significantly decreased; however, they tended to increase when the fish were recovered to a normal water temperature (20 °C). After irradiating the low-temperature groups (Exp. I and Exp. II) with green or blue LEDs, NKA mRNA expression and concentration significantly increased. In general, morphological changes of gill filaments occur when fish are exposed to temperatures below the habitat temperature [35]. Sardella et al. [36] reported that the activity of NKA decreased in tilapia exposed to low water temperatures. Mitrovic and Perry [16] reported that the NKA concentration in gills was significantly decreased when goldfish were exposed to a rapidly decreasing temperature environment (25 → 7 °C). Acutely changing water temperature significantly decreased the NKA mRNA expression and concentration in gills. Thus, acutely changing conditions lead to changes in morphology and function of gills, and it is thought that the activity of NKA decreased as a result. Green and blue LEDs play a role in mitigating the negative effects of an acute change in water temperature.

In addition, we investigated the effects of acute changes in water temperature on the immune parameters of black

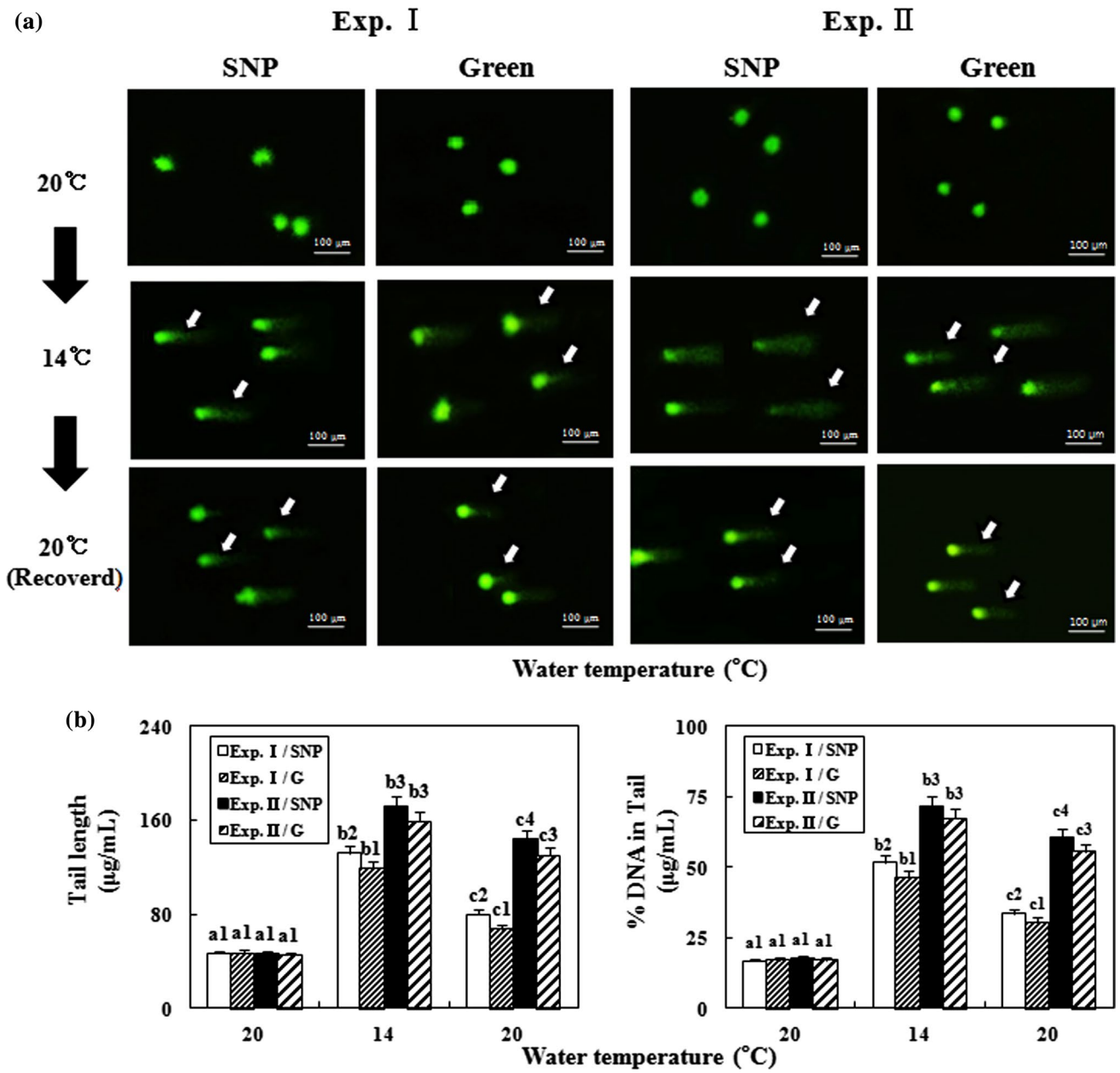

Fig. 6 Comet assay images (**a**) and comet assay parameter tail length and % DNA in tail (**b**). Comet assay of liver cell nuclear DNA damage under light conditions of green LED, blue LED, and SNP during temperature change. White arrows in **a** indicate damaged nuclear DNA (DNA breaks) in liver cells, which have been stained with SYBR Green. Scale bars = 100 μm. The lowercase letters indicate the temperatures (*a* 20 °C, *b* 14 °C, *c* return to 20 °C) and the different numbers indicate significant differences within a temperature ($P < 0.05$). All values are represented as mean ± SE ($n = 5$). For abbreviations, see Figs. 1 and 2

rockfish. We observed changing levels of IgM and lysozyme, immunity indicators, in plasma. In both of the low-water-temperature experimental groups, the levels of IgM and lysozyme were significantly decreased. When the water was restored to its initial temperature, they tended to increase again. However, despite returning to normal water temperature, the levels of IgM and lysozyme were not recovered fully in all experimental groups. After fish were exposed to low water temperature and green or blue LED irradiation, the levels of IgM and lysozyme in plasma tended to significantly decrease with the return to normal water temperature.

In support of our results, Park et al. [37] reported that H_2O_2, an indicator of stress in the body, was significantly increased when clownfish *Amphiprion melanopus* were exposed to a low-water-temperature environment (28 → 20 °C). On the other hand, the plasma concentration

of lysozyme decreased significantly, suggesting that a large number of free radicals are generated in response to acute water temperature changes, directly decreasing immune parameters.

Kim et al. [24] and Jung et al. [3] reported that the levels of melatonin and lysozyme were significantly decreased when fish were exposed to acute changes of water temperature (low and high temperature). They were significantly increased under green-wavelength LEDs. Green LEDs seem to have a positive effect, enhancing immune function, because green-wavelength LED light effectively reduces oxidative stress.

Similar to results of previous studies, in this study, we found that green and blue wavelength light significantly decreased the generated stress in the body by playing a key role in improving immune efficiency despite the stress induced by an acute change in water temperature which significantly decreased the immune response.

In this study, we also conducted a comet assay to investigate the effects of low-temperature conditions on nuclear DNA of black rockfish. The liver cells in control groups showed normal nuclear DNA. However, tail length and % DNA in the tail in liver cells were significantly increased in both low-temperature experimental groups, which means that the damage to nuclear DNA was caused by water temperature. After recovery to normal water temperature, the nuclear DNA damage in liver cell in Exp. II was higher than that in Exp. I. Irradiation with green or blue LEDs tended to reduce the nuclear DNA damage in liver cells of fish.

In a similar study of nuclear DNA damage of liver cells, Kim et al. [24] reported that nuclear DNA damage significantly increased with exposure to a high-water-temperature environment, but the nuclear DNA damage in liver cells significantly decreased with irradiation with green-wavelength LEDs.

Therefore, in this study, similar to previous studies, the stress caused by exposure to acute changes in water temperature led to nuclear DNA damage in liver cells of black rockfish, but green-wavelength light seemed to have a function in protecting the cells while decreasing the nuclear DNA damage to liver cells.

In summary:

1. Black rockfish, when exposed to low water temperatures similar to those of cold pools, showed differences in stress and physiological recovery responses following different rates of change in water temperature.
2. We suggest that green and blue wavelengths play roles in stress reduction and have positive effects on immunity.
3. When cold pools affect aquaculture fish, irradiation with a short wavelength such as green might be effective in not only reducing the time required for the fish to recover normal function, but also their ability to restore physiological function at all.

LEDs seem to play a positive role in physiological stress and immunity in our study. Nevertheless, there is still little research on the mechanisms of the effects of LEDs on each stress and immunity indicator. Physiological changes may occur through complex mechanisms by interaction with photoreceptors (red opsin, blue opsin, green opsin, and others) in the retina. Furthermore, it is possible that light at a specific wavelength is more effective in the aquaculture of fish normally exposed to limited wavelengths of light, and studies on the interactions of fish photoreceptors with physiological indicators at specific wavelengths are needed.

Acknowledgements This research was supported by the project titled 'Long-term change of structure and function in marine ecosystems of Korea' and 'Innovative marine production technology driven by LED-ICT convergence photo-biology', and grant from the National Institute of Fisheries Science (R2017020) funded by the Ministry of Oceans and Fisheries, Korea.

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