Molluscan Research

ISSN: 1323-5818 (Print) 1448-6067 (Online) Journal homepage: https://www.tandfonline.com/loi/tmos20

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To cite this article: Jin Ah Song, Yong Seok Lee, Young-Ung Choi & Cheol Young Choi (2020) Effects of light-emitting diodes on thermally-induced oxidative stress in the bay scallop *Argopecten irradians*, Molluscan Research, 40:2, 130-141, DOI: 10.1080/13235818.2020.1712040

To link to this article: https://doi.org/10.1080/13235818.2020.1712040

Published online: 16 Jan 2020.

MOLLUSCAN RESEARCH
2020, VOL. 40, NO. 2, 130–141
https://doi.org/10.1080/13235818.2020.1712040

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Effects of light-emitting diodes on thermally-induced oxidative stress in the bay scallop *Argopecten irradians*

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ABSTRACT

Water temperature is an important stressor that affects the physiological and biochemical responses of scallops. In this study, we investigated the effect of different light-emitting diodes (LEDs; red, green and blue) on oxidative stress in *Argopecten irradians*. PCR revealed *MnSOD* mRNA expression in the digestive diverticula, gill, adductor muscle and eye. *CAT* and *HSP70* mRNA were expressed in the digestive diverticula, gill and adductor muscle. Additionally, we measured the changes in the expression of *HSP70*, *MnSOD* and *CAT* as well as H_2O_2 levels during thermal/laboratory stress. In the digestive diverticula, gill and adductor muscle, the mRNA expressions and activities and H_2O_2 levels significantly increased in response to thermal changes. The gene expressions and activities and H_2O_2 levels were significantly lower in scallops that received green LED light than in those that received no mitigating treatment. A comet assay revealed that thermal change groups had increased rates of nuclear DNA damage; however, treatment with green LED reduced the frequency of damage. The results indicated that low or high water temperature conditions induced oxidative stress in *A. irradians* but that green LED significantly reduced this stress.

ARTICLE HISTORY
Received 13 December 2018
Final version received 3 December 2019

KEYWORDS
Antioxidant; bivalves; short wavelength light; water temperature change

Introduction

Scallops are major aquaculture species worldwide and of high commercial value (Hannam et al. 2009). *Argopecten irradians* (Lamarck, 1819) is a particularly important commercial shellfish species in the Atlantic, as well as in China and Korea (Heffernan et al. 1988; Oh et al. 2003). In general, *A. irradians* live in shallow water less than 10 m deep and are often exposed to rapid fluctuations in water temperature in estuarine ecosystems (Barber and Davis 1997).

In addition to habitat distribution, water temperature is one of the most limiting factors for the stable culture of *A. irradians*. In the Atlantic coasts of Canada and the United States, *A. irradians* can suffer mass mortalities due to low temperatures in winter (Barber and Davis 1997; Couturier et al. 1995). In Atlantic coastal regions, such as the Baie des Chaleurs, St. Lawrence Gulf, Nova Scotia, where *A. irradians* has been actively cultured, thermal stratification occurs due to the increase in water temperature in summer. Water temperature fluctuations of up to 15°C or more can suddenly occur in each water layer according to the movement of the thermocline (Côté et al. 1993; Pearce et al. 2004). Depending on the movement of the water layer, cultured *A. irradians* are frequently exposed to rapid temperature changes, i.e., from the cold water temperature of the lower layer to the warm water temperature of the surface layer and vice versa (Lafrance et al. 2002).

The gills of the scallop are directly exposed to factors in the cultured environment (water temperature, toxicity, etc.). The digestive diverticula are not only responsible for digestion in the body, but also play a variety of roles, such as detoxification and adaptation (Pan et al. 2006). In addition, the haemocytes of the scallop affect physiological responses and play important roles in wound repair and phagocytosis (Chen et al. 2007; Prado-Alvarez et al. 2013).

Exposure to stress environments, such as rapid water temperature changes, results in excessive amounts of reactive oxygen species (ROS) in the tissues of scallops, which can lead to apoptosis by damaging unsaturated lipids while moving through the haemolymph (Hannam et al. 2009; Jiang et al. 2016). As an important defence mechanism against ROS, scallops activate superoxide dismutase (SOD), which primarily clears ROS (SOD: $2O_2^- + H^+ \rightarrow H_2O_2 + O_2$; Kashiwagi et al. 1997). H_2O_2, a ROS produced by SOD, is converted to nontoxic H_2O and O_2 by catalase (CAT) ($2H_2O_2 \rightarrow 2H_2O + O_2$; Kashiwagi et al. 1997). These enzymes used in the antioxidant process are, therefore, commonly used as stress indicators in many aquatic organisms, including scallops (Frantzen et al. 2016).

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Supplemental data for this article can be accessed at https://doi.org/10.1080/13235818.2020.1712040

Published online 16 Jan 2020

Heat shock proteins (HSPs) can act as molecular chaperones, modulating stress response and decreasing the cellular damage induced by environmental stressors. They also play a role in immune responses (Taldone et al. 2014; Yang et al. 2014; Zhou et al. 2014). Recently, there has been an increase in the number of studies on HSP responses in bivalves. The adaptability of an individual to a new environment may be controlled by the expression of genes, e.g., the production of HSPs (Brown et al. 2004; Sgrò et al. 2008). Various aquatic organisms, including shellfish, show increased HSP expression in response to stress caused by water temperature fluctuations in the laboratory (from control to high/low temperature) (Brun et al. 2008; Hu et al. 2015) and the synthesised HSPs play a variety of roles to protect cells against lethal damage by responding to early detection in molluscs (Brown et al. 2004; Cheng et al. 2016; Ding et al. 2018). To prevent the tissue damage caused by heat stress, scallops have developed numerous defensive strategies, such as inducing HSP70 synthesis. For example, Giannetto et al. (2017) suggested that *Mytilus galloprovincialis* Lamarck, 1819 responds to oxidative stress through cytoprotective mechanisms by increasing HSP expression.

Speiser et al. (2011) reported the presence of a proximal retina and distal retina in the eyes of *A. irradians*, which have been reported to absorb maximally at wavelengths of 506 nm and 535 nm, respectively. Recent studies have demonstrated relationships between various light sources and stress in fish (Volpato and Barreto 2001; Shin et al. 2011; Choi et al. 2012). Studies have also shown that crustaceans respond to light at certain wavelengths and that specific wavelengths influence the stress levels of shrimps (Caves et al. 2016; Choe et al. 2018; Choi et al. 2018). However, research on scallops is limited and other than the study by Speiser et al. (2011), who confirmed that scallops absorb light maximally at a certain band of wavelengths, researchers have only speculated that scallops may respond to specific wavelengths.

Based on the results of previous marine biology research (i.e., on fish and crustaceans), we hypothesised that *A. irradians,* which has a green-sensitive retina, would have stress reduced in green light. In the present study, we investigated the possibility of controlling oxidative stress (induced by high water temperature fluctuations) in *A. irradians* through exposure to specific light-emitting diode (LED) wavelengths. We examined the effect of a white fluorescent light (control) and three LED wavelengths (red, green and blue) after exposing *A. irradians* to various temperature/laboratory changes. We observed that the assessment of *SOD*, *CAT* and *HSP70* mRNA expression levels in the tissues of *A. irradians* is a basic procedure to identify the characteristic physiological and biochemical responses of the tissues and an essential process to explore the physiological function of each gene. We also measured the change in antioxidant enzymes (*SOD* and *CAT*) and *HSP70* mRNA expression and the changes in antioxidant enzymes and H_2O_2 activity in the haemolymph. The effect of stress on apoptosis and the extent of cell death at different light wavelengths were tested using comet assay to evaluate the degree of nuclear DNA damage.

Materials and methods

Experimental animals

A. irradians (shell length, 65 ± 10 mm; total weight, 48.81 ± 4.01 g) was purchased from a commercial market (Tongyoung, Korea) and maintained in four 400-L recirculating filter tanks that contained filtered and aerated seawater, prior to laboratory experiments. Four experimental conditions were established in duplicate and 40 *A. irradians* were maintained per tank. Study animals were allowed to acclimate to the experimental conditions for one week. Half of the total volume of seawater was changed daily. *A. irradians* were reared with automatic temperature regulation systems (JS-WBP-170RP; Johnsam Co., Buchoen, Korea), with the water temperature and salinity maintained at 17°C and 30‰, respectively.

The control group was exposed to a white fluorescent bulb and the experimental groups were exposed to red (630 nm), green (520 nm) or blue (460 nm) LED lights (Daesin LED Co. Kyunggi, Korea) (Figure 1). The photoperiod was maintained at 12 h light: 12 h dark (lights on 07:00 h and lights off 19:00 h). The LEDs were placed 40 cm above the surface of the water and the irradiance at the surface of the water was maintained at ~1.0 W/m^2. The spectral analysis of the lights was performed using a spectroradiometer (FieldSpec®, ASD, CO, USA). All scallops were initially exposed to 17°C water temperature. The water temperature was then increased from 17°C to 25°C or decreased from 17°C to 11°C in daily

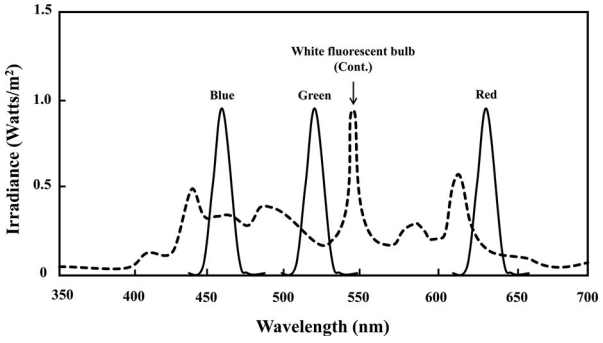

Figure 1. Spectral profiles of the red (630 nm), green (520 nm) and blue (460 nm) LEDs with light intensities of 1.0 W/m^2. The dotted line shows the spectral profile of the white fluorescent bulb (Control).

increments of 1°C. Sampling was performed after scallops had been exposed to experimental temperatures (11, 13, 15, 17, 19, 21, 23 or 25°C). This experimental design results in each temperature treatment group having spent a different length of time in the laboratory when measurements were made. Consequently, the results reported below reflect the interactive effects of both temperature and duration of exposure to laboratory conditions.

Five *A. irradians* from each experimental condition were randomly selected for analysis. Haemolymph (1 mL) was collected from the adductor muscle using a 1 mL syringe within two min of removing the scallops from the tank. Tissues, i.e., digestive diverticula, gill, adductor muscle and eye, were collected from each scallop. The haemolymph was separated by centrifugation (4°C, 750 × *g*, 3 min) and stored at −80°C until further analysis.

Total RNA extraction and complementary DNA synthesis

Total RNA was extracted from the digestive diverticula, gill, adductor muscle and eye using the TRI reagent® (Molecular Research Center, Inc., Ohio, USA), according to the manufacturer's instructions. Total RNA (2 µg) was reverse-transcribed in a total volume of 20 µL, using an oligo-d$(T)_{15}$ anchor and M-MLV reverse transcriptase (Promega, Madison, WI, USA), according to the manufacturer's protocol. Synthesised complementary DNA (cDNA) was stored at −20°C until further use.

Reverse transcription PCR

The samples used were from the controlled-temperature group (17°C). PCR was conducted using cDNA reverse-transcribed from the total RNA extracted from the digestive diverticula, gills, adductor muscle and eye. PCR was used to amplify the *MnSOD*, *CAT* and *HSP70* cDNA using the following primers: *MnSOD* forward primer, 5′-TCT GCA GAC ATC ATG CAA CT-3′; *MnSOD* reverse primer, 5′-TCA GCT CTG TCT TCA TGG CT-3′; *CAT* forward primer, 5′-GAG GTC ACT CAT GAC ACT AC-3′; *CAT* reverse primer, 5′-TGG ATG AAG ACT GAC CAG GA-3′; *HSP70* forward primer, 5′-ATG ACG AAG TCT TGT GCA CC-3′; *HSP70* reverse primer, 5′-GCG AGT CGT TGA AGT AGG CT-3′; *β-actin* forward primer, 5′-CAA ACA GCA GCC TCC TCG TCA-3′; and *β-actin* reverse primer, 5′-CTG GGC ACC TGA ACC TTT CGT T-3′. For PCR, the Perfect Premix Kit Ver. 2.0 (Takara, Japan) was used with an initial denaturation for 2 min at 94°C followed by 35 cycles of denaturation for 30 s at 94°C; annealing for 30 s at 54.3 (*MnSOD*), 52.6 (*CAT*) and 55.0°C (*HSP70*), extensions for 1 min in 72°C; and a final extension for 7 min at 72°C. The amplified PCR products were processed by electrophoresis in a 3% agarose gel containing 5 µg /mL ethidium bromide. The quantification of RT–PCR-amplified fragments was conducted using a high-resolution scanner (Chemi-Doc™ XRS+, Bio-Rad, Hercules, CA, USA).

Quantitative PCR (qPCR)

Quantitative PCR (qPCR) was conducted using cDNA reverse-transcribed from the total RNA extracted from the digestive diverticula, gills and adductor muscle to determine the relative expression levels of *MnSOD*, *CAT*, *HSP70* and *β-actin* mRNA. The primers used for qPCR are shown in Table 1. PCR amplification was conducted using a Bio-Rad CFX96™ Real-time PCR Detection System (Bio-Rad) and iQ™ SYBR Green Supermix (Bio-Rad) with the following conditions: 0.5 µL of cDNA, 0.26 µM of each primer, 0.2 mM dNTP, SYBR Green and Taq polymerase in buffer (10 mM Tris–HCl [pH 9.0], 50 mM KCl, 1.4 mM $MgCl_2$ and 20 nM fluorescein) in a total volume of 25 µL. The qPCR protocol was as follows: 95°C for 5 min, followed by 40 cycles of 95°C for 20 s and 55°C for 20 s. As internal controls, experiments were duplicated with *β-actin* and all data were expressed relative to the corresponding *β-actin* threshold cycle (ΔCt) levels. The calibrated ΔCt values (ΔΔCt) for each sample and the internal controls (*β-actin*) were calculated using the $2^{-\Delta\Delta Ct}$ method: $\Delta\Delta Ct = 2\text{^-}(\Delta Ct_{sample} - \Delta Ct_{internal\ control})$. We measured mRNA levels in the digestive diverticula, gills and adductor muscle of *A. irradians*.

Analysis of haemolymph parameters

Centrifuged haemolymph samples were assayed for SOD, CAT and H_2O_2 activities using assay kits (SOD, BM-SOD200; CAT, BM-CAT400; H_2O_2, BO-PER500; BIOMAX Co., Ltd., Korea), according to the manufacturer's instructions. In the SOD assay, oxidation of xanthine was monitored by measuring absorbance at 450 nm. One unit of SOD activity was defined as 50% inhibition of the oxidation process (U/mL of haemolymph). One unit of CAT activity was defined as 50% H_2O_2 consumption at 1 min, pH 7.0 (U/mL of haemolymph).

Comet assays

The comet assay is a relatively simple and sensitive technique for quantitatively measuring DNA damage in eukaryotic cells (Bajpayee et al. 2005). Digestive diverticula cells (1×10^5 cells/mL) were examined using a CometAssay Reagent kit with single-cell gel electrophoresis assays (Trevigen Inc., Maryland, USA), according to the method described by Singh et al. (1988), with some modifications. Cells were immobilised in agarose gels on CometAssay comet slides and immersed in freshly prepared alkaline unwinding solution for 20 min. Next, slides were electrophoresed at 18 V for 30 min. The samples were stained with SYBR

Table 1 Primers used for QPCR amplification

Genes	Primer	DNA sequences	PCR Efficiency (%)
MnSOD (**EU137676**)	Forward	5′- TAG GGA TTT TGG CTC GTT TG -3′	99.7
	Reverse	5′- GTA GGC ATG CTC CCA AAC AT -3′	
CAT (**GQ265925**)	Forward	5′- TAC TGC AAG GCC AAG CTT TT -3′	102.5
	Reverse	5′- TTG GGA AAA ATA TGG GGT CA -3′	
HSP70 (**AY485261**)	Forward	5′- AAG AGC ACT GGC AAG GAA AA -3′	97.4
	Reverse	5′- CAT AGC TCT CCA GGG CAT TC -3′	
β-actin (**AY335441**)	Forward	5′- CAA ACA GCA GCC TCC TCG TCA -3′	100.4
	Reverse	5′- CTG GGC ACC TGA ACC TTT CGT T -3′	

Green (Trevigen Inc.) for 30 min in the dark and then read using a fluorescence microscope (excitation filter 465–495 nm; Eclipse C*i*, Nikon, Japan). At least 100 cells from each slide were analysed. For quantification of comet assay results, we analysed the tail length (distance of DNA migration from the head), percentage of DNA in the tail (tail intensity/total intensity) and tail moment (amount of DNA damage, product of tail length and percentage of DNA in tail) using comet assay IV image analysis software (version 4.3.2; Perceptive Instruments Ltd., UK).

Statistical analysis

All data were analysed using the SPSS statistical package (version 10.0; SPSS Inc., USA). The normality of the data and the homogeneity of variances were tested using Shapiro–Wilk test (SW test) and paired t- test. A one-way ANOVA followed by a Tukey's post-hoc test was used to compare differences in the data ($P < 0.05$; Supplementary Table S1). Values were expressed as means ± standard deviation (SD).

Results

Tissue distribution analysis of MnSOD, CAT *and* HSP70 *mRNA*

The results of the RT–PCR for *MnSOD*, *CAT* and *HSP70* are shown in Figure 2. The mRNAs were expressed in three of the examined tissues (digestive diverticula, gill and adductor muscle). However, only low expression of *MnSOD* mRNA was observed in the eye. There was a strong expression of *MnSOD* mRNAs in the digestive diverticula, gill and adductor muscle. Expression of *CAT* mRNA was strong in the digestive diverticula, gill and adductor muscle, but no band

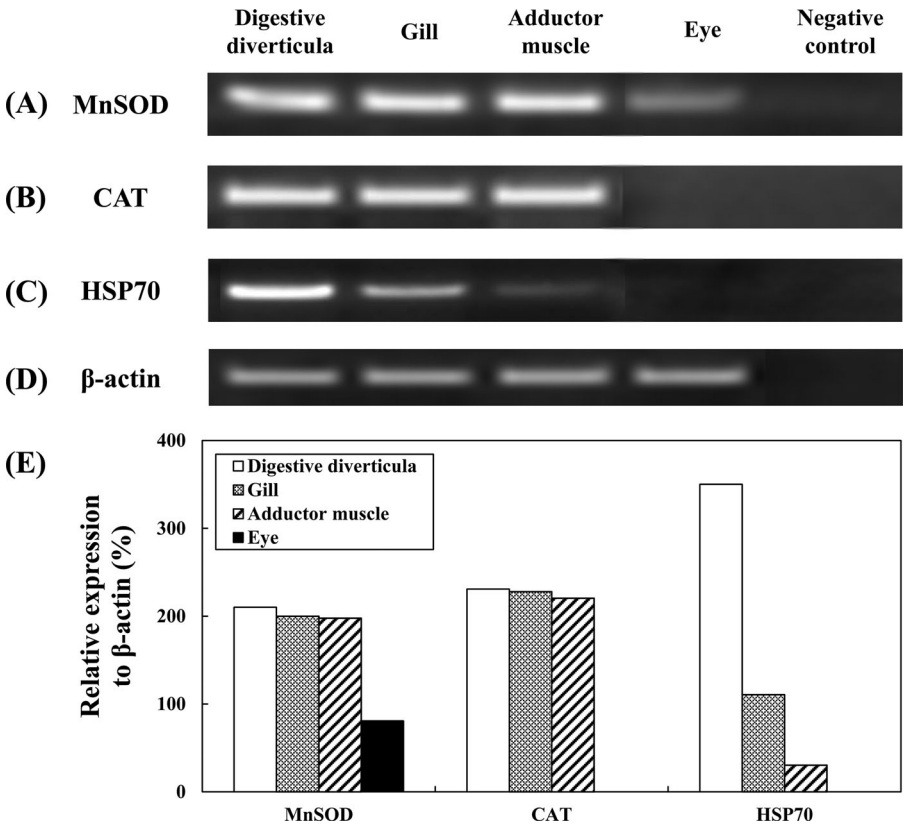

Figure 2. Representative RT-PCR gels showing *MnSOD* (**A**), *CAT* (**B**), *HSP70* (**C**) and *β-actin* (D) mRNA's expression in the digestive diverticula, gill, adductor muscle and eye of *A. irradians*. N.C. represents negative control. (E) is the mRNA's expression relative to *β-actin*.

was detected in the eye. The expression level of *HSP70* mRNA ranged from high to low, as follows: digestive diverticula > gill > adductor muscle and no band was detected in the eye.

Expression of HSP70, MnSOD *and* CAT *mRNA in the digestive diverticula, gill and adductor muscle*

The different expressions of *HSP70*, *MnSOD* and *CAT* mRNA in each of the three tissues indicated that the genes [from the controlled-temperature group (17°C)] responded differently to changes in water temperature (Figures 3–5). *HSP70* mRNA expression was found to be temperature/laboratory-dependent, with the highest level of expression occurring at 25°C. Similarly, the expressions of *MnSOD* and *CAT* mRNA were also found to be temperature/laboratory-dependent. In the digestive diverticula, gill and adductor muscle, *HSP70*, *MnSOD* and *CAT* mRNA expression increased significantly with thermal variation. The mRNA expression in the green LED group was lower than that of the other spectra.

Activities of SOD and CAT

The activities of SOD and CAT in the haemolymph were found to be temperature/laboratory-dependent (Figure 6) and significantly increased with thermal variation, but those in the green LED group were lower than those of the other spectra. It was either the same or higher than the expression seen at 17°C, for temperatures ranging from 19–25°C and 11–15°C.

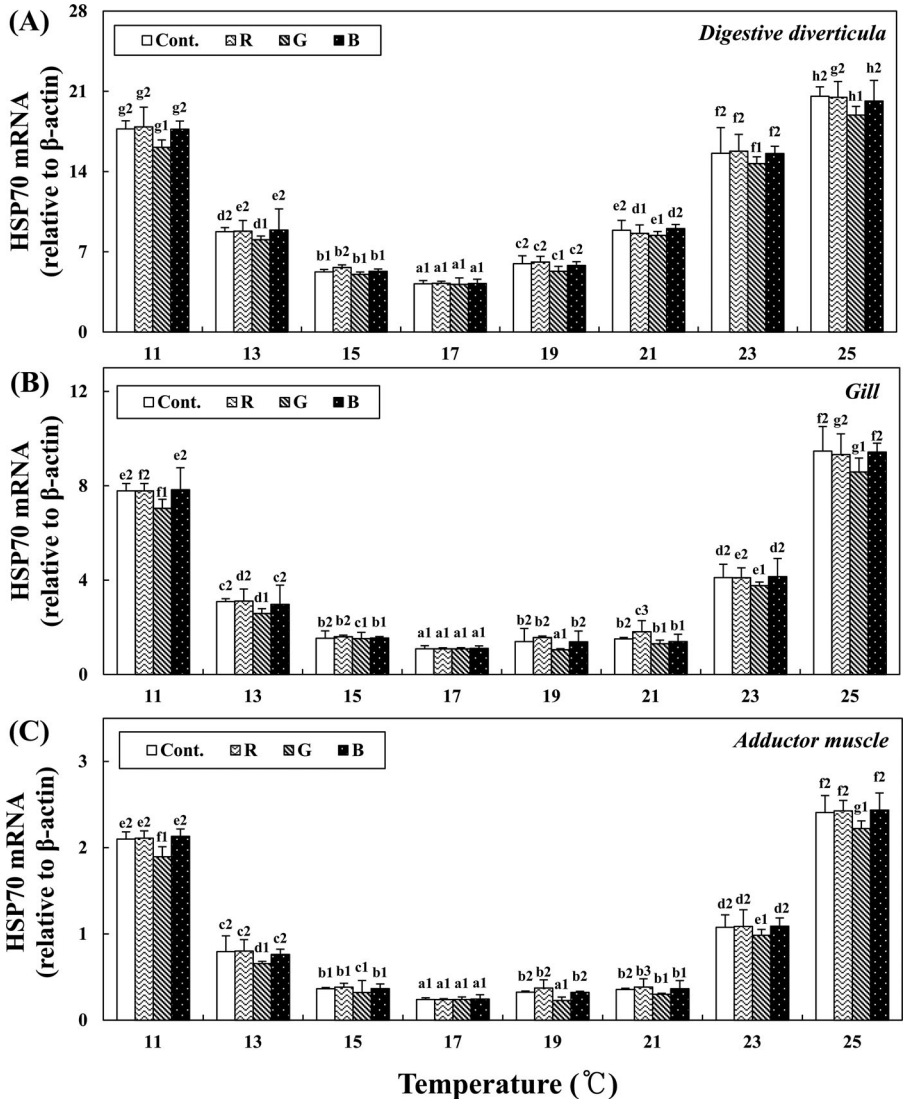

Figure 3. Changes in the expression levels of *HSP70* mRNA in the digestive diverticula (**A**), gill (**B**) and adductor muscle (**C**) of *A. irradians* relative to *β-actin* after exposure to red (R), green (G) and blue (B) LEDs and a white fluorescent bulb (Control), as measured by quantitative PCR. Values with different characters indicate significant differences after exposure to different water temperature within the same LED spectra ($P < 0.05$). Numbers indicate significant differences between the LED spectra within the same temperature ($P < 0.05$). All values are means ± SD ($n = 5$).

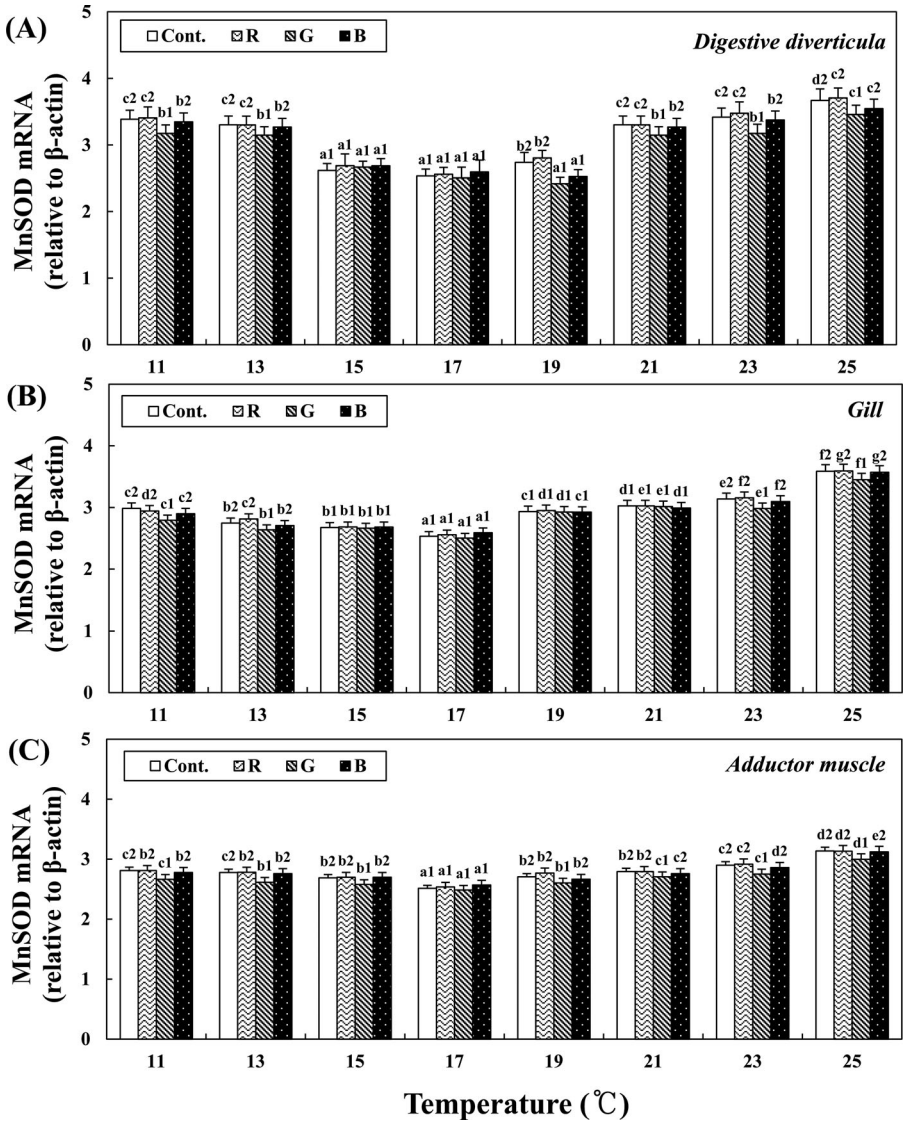

Figure 4. Changes in the expression levels of *MnSOD* mRNA in the digestive diverticula (**A**), gill (**B**) and adductor muscle (**C**) of *A. irradians* in response to thermal changes relative to *β-actin* after exposure to red (R), green (G) and blue (B) LEDs and a white fluorescent bulb (Control), as measured by quantitative PCR. Values with different characters indicate significant differences after exposure to different water temperature within the same LED spectra ($P < 0.05$). Numbers indicate significant differences between the LED spectra within the same temperature ($P < 0.05$). All values are means ± SD ($n = 5$).

Concentration of H_2O_2

The H_2O_2 concentration increased significantly in the haemolymph after thermal fluctuations (Figure 7). The H_2O_2 concentration in the green LED group was lower than that of the other spectra. It is either the same or higher than the expression seen at 17°C, for temperatures ranging from 19–25°C and 11–15°C.

Analysis of DNA damage

Digestive diverticula tissue DNA damage following exposure to various temperatures (11, 17 and 25°C) was analysed using 100 randomly selected cells. Both the DNA content in the tail and tail length significantly increased with thermal variation (Figure 8). Within experimental groups at the same variant temperature, significantly less DNA damage was induced in the green LED group as compared to the control light.

Discussion

This study was conducted to investigate the effects of various LEDs (red, green and blue) on the antioxidative and stress defence mechanisms in *A. irradians* after exposure to high or low temperatures. *A. irradians* was expected to absorb light at certain wavelengths.

We observed the expression of antioxidant enzymes, i.e., *MnSOD*, *CAT* and *HSP70* (a stress related gene) in three tissues (digestive diverticula, gill and adductor muscle) that had been reported to express stress-related genes (Huang et al. 2014; Liu et al. 2015). In all three genes, a high level of expression was observed in the digestive diverticula and gill but only *MnSOD* mRNA expression was observed in the eyes. The expression of *CAT* and *HSP70* mRNAs was not detected in the eye (Figure 2).

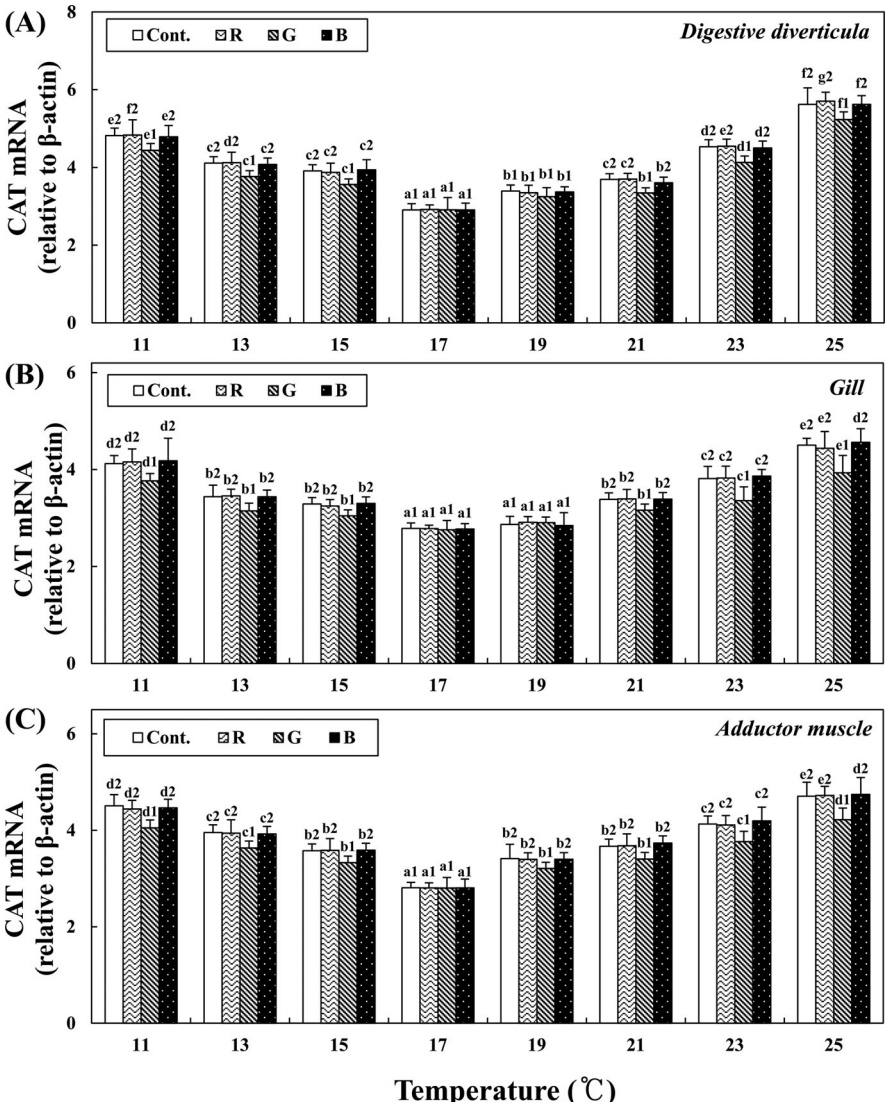

Figure 5. Changes in the expression levels of *CAT* mRNA in the digestive diverticula (**A**), gill (**B**) and adductor muscle (**C**) of *A. irradians* in response to thermal changes relative to *β-actin* after exposure to red (R), green (G) and blue (B) LEDs and a white fluorescent bulb (Control), as measured by quantitative PCR. Values with different characters indicate significant differences after exposure to different water temperature within the same LED spectra ($P < 0.05$). Numbers indicate significant differences between the LED spectra within the same temperature ($P < 0.05$). All values are means ± SD ($n = 5$).

The results of the present study are consistent with those of Guerra et al. (2013), which reported on activities in scallop tissues. They reported that the enzymatic activities of *SOD* and *CAT* were significantly higher in the gills than in the adductor muscle in *Argopecten ventricosus* (G. B. Sowerby II, 1842).

Additionally, the expression level of *HSP* mRNA was similar to that reported in previous studies, e.g., it was highly expressed in the digestive diverticula and gill in *Haliotis diversicolor* Reeve, 1846 (Huang et al. 2014). The *HSP* expression levels were ranked in the following order: digestive diverticula > gill > adductor muscle in *Ruditapes philippinarum* Adams & Reeve, 1850 (Liu et al. 2015).

The results of this study suggested that the changes in water temperature/laboratory induced stress in *A. irradians* triggered the antioxidant mechanism in *A. irradians* and that the stress response was most active in the digestive diverticula and gill tissues.

To further elucidate the mechanisms for coping with water temperature/laboratory stress, we measured *SOD* and *CAT* mRNA and activities as well as the H_2O_2 levels in the digestive diverticula, gills and adductor muscle of *A. irradians*. The *SOD* and *CAT* mRNA expression level and activity tended to gradually increase in the low temperature (11°C) and high temperature (25°C) environments. However, the expression levels and activities of SOD and CAT in the green LEDs group were lower than those of the control and other light groups in both the low and high temperature experimental conditions.

Oxidative stress is caused by the excessive production of ROS via the oxidant mechanism (Kashiwagi et al. 1997). In a previous study, the mussel *Mytilus coruscus* Gould, 1861 was exposed to water temperature changes and the SOD activity was found to increase in the gills and digestive diverticula, in such a manner that the greater the temperature/laboratory change, the greater the

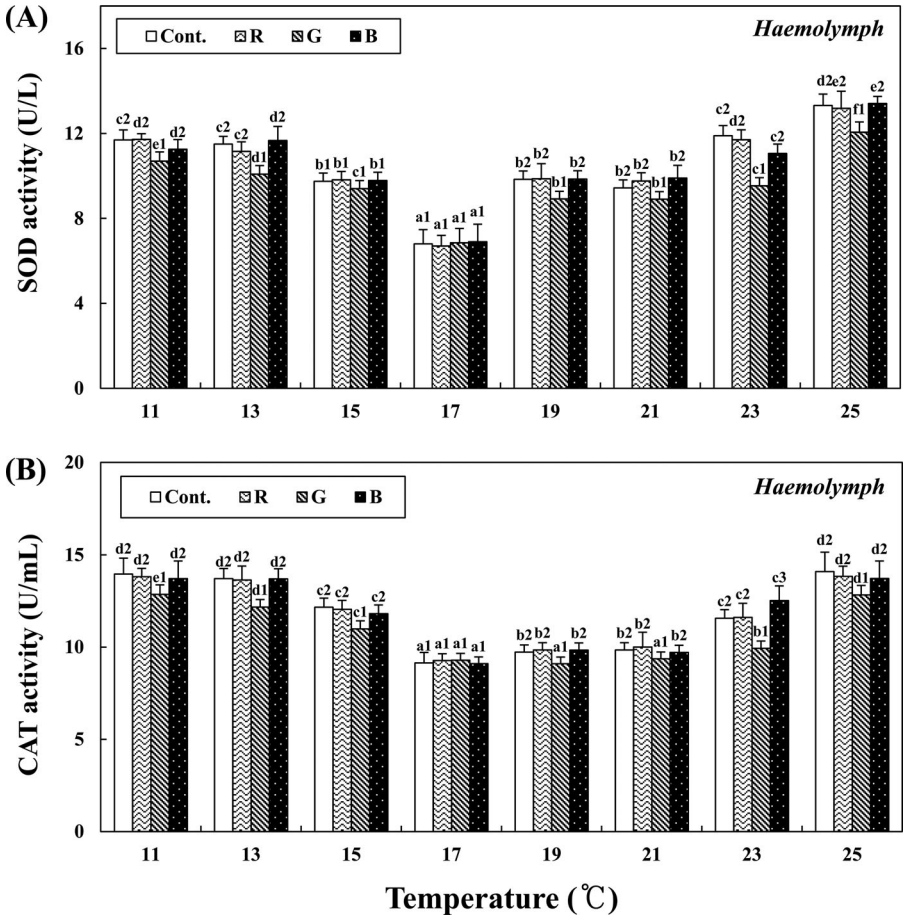

Figure 6. Changes in the activities of haemolymph SOD (**A**) and CAT (**B**) of *A. irradians* in response to thermal changes relative to the *β-actin* after exposure to red (R), green (G) and blue (B) LEDs and a white fluorescent bulb (Control). Values with different characters indicate significant differences after exposure to different water temperature within the same LED spectra ($P < 0.05$). Numbers indicate significant differences between the LED spectra within the same temperature ($P < 0.05$). All values are means ± SD ($n = 5$).

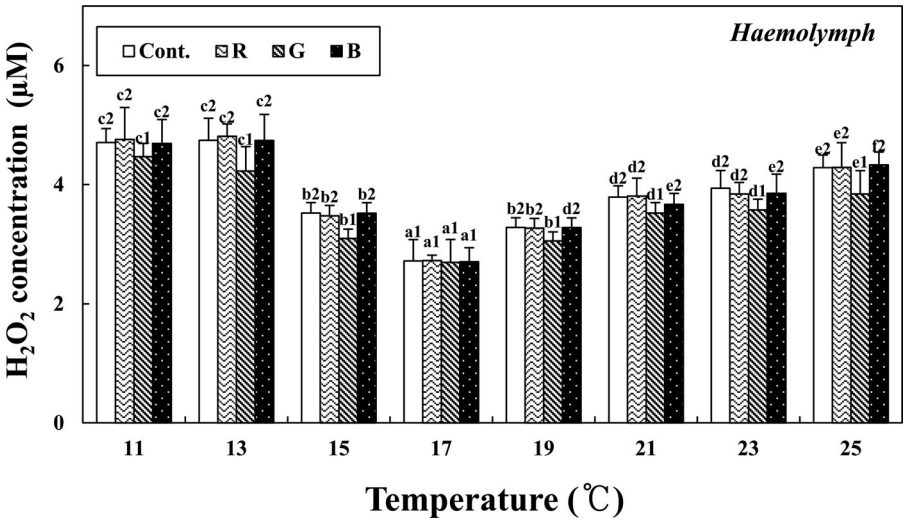

Figure 7. Concentration of H_2O_2 in haemolymph of *A. irradians* after exposure to red (R), green (G) and blue (B) LEDs and a white fluorescent bulb (Control). Values with different characters indicate significant differences after exposure to different water temperature within the same LED spectra ($P < 0.05$). Numbers indicate significant differences between the LED spectra within the same temperature ($P < 0.05$). All values are means ± SD ($n = 5$).

activity (Hu et al. 2015). An increase in SOD activity was also reported in the digestive diverticula and gill tissues of *Perna viridis* (Linnaeus, 1758), in response to the increase in water temperature (Verlecar et al. 2007). Our findings were, therefore, with those of previous studies. *A. irradians* showed an increase in oxidative stress in

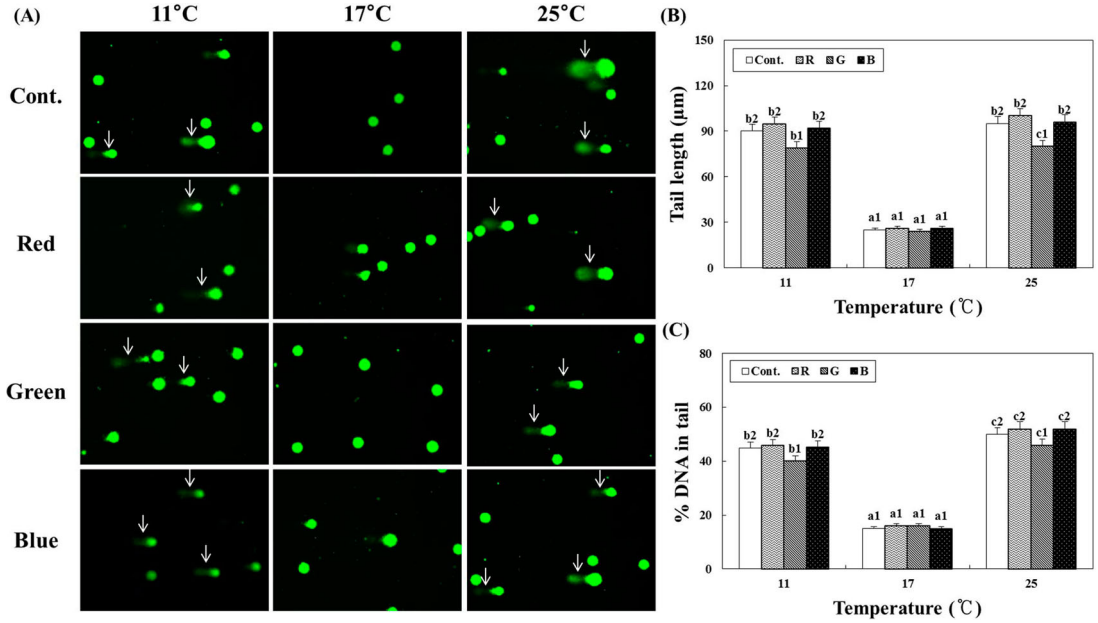

Figure 8. Comet assay images (**A**) and comet assay parameters (**B**) tail length and percentage DNA in tail after exposure to red (R), green (G) and blue (B) LEDs and a white fluorescent bulb (Control) at different temperature (11, 17 and 25°C). White arrows (in **A**) indicate damaged nuclear DNA (DNA breaks) of digestive diverticula cells which are stained with SYBR-green. The lowercase letters (in **B** and **C**) with different characters indicate a significant difference between different water temperatures at the same LED spectra ($P < 0.05$). The numbers above the bars indicate a significant difference between different LED spectra within the same temperatures ($P < 0.05$). All values are means ± SE ($n = 5$).

response to the increase or decrease in water temperature, which is considered to be a defence strategy for each tissue to suppress the damaging effects of the accumulated O_2^-.

The expression of SOD and CAT increases to remove H_2O_2, which is a product of thermal stress. Jiang et al. (2016) reported that *Mizuhopecten yessoensis* (Jay, 1857) showed an increase in the expression of both SOD and CAT as water temperature increased. Regoli et al. (1997) also reported that *Adamussium colbecki* Smith, 1902 showed an increase in CAT activity due to the production of H_2O_2. The results of the present study are consistent with these previous studies in that we observed increases in SOD and CAT expressions and H_2O_2 activity in *A. irradians* in response to increased and decreased water temperature. SOD and CAT were presumably expressed in an interdependent manner as defence mechanisms against the large quantities of ROS produced in the scallop body due to exposure to thermal/laboratory stress.

We observed *HSP70* mRNA expression in tissues (digestive diverticula, gill and adductor muscle) to determine the degree of stress in response to environmental changes in temperature detected by the scallop. *HSP70* mRNA expression was found to gradually increase with an increase in variation of water temperature. However, in the green LED group, the expression level of *HSP70* mRNA was lower than that of the control and other light groups under the same experimental conditions.

Heat shock stress causes protein denaturation and aggregation and inhibits important processes, such as mRNA translation and transcription (Richardson et al. 2011). Ding et al. (2018) reported that when the scallop *M. yessoensis* was exposed to changing water temperature, *HSP* mRNA expression increased in the digestive diverticula, gill and adductor muscle. Jiang et al. (2017) reported that when *Chlamys farreri* [= *Azumapecten farreri* (Jones & Preston, 1904)] was exposed to changing water temperature, *HSP70* mRNA expression increased in the digestive diverticula and gill and when *R. philippinarum* was exposed to changing water temperature, *HSP60* mRNA expression increased in the gill (Ding et al. 2017).

The findings of the present study were similar to those of these previous studies, whereby the expression level of *HSP* increased in the digestive diverticula, gill and adductor muscle in response to changes in water temperature; indicating that HSP was involved in the adaptive response to thermal/laboratory stress.

In addition, to determine the effective wavelength for controlling the thermal/laboratory stress, *A. irradians* exposed to the thermal change environment was investigated with control light and three wavelengths. The degree of nuclear DNA damage in the hepatopancreas cells of the scallop was also observed. DNA damage increased with thermal variation, although comparisons of the various light treatments at the same temperature shows that the DNA damage was significantly less induced in the green LED group.

Generally, in aquatic organism ROS, including H_2O_2, induces apoptosis and promotes DNA damage (Chen et al. 2007; Istomina et al. 2011). Slobodskova et al. (2012) reported that when *M. yessoensis* was exposed to an anoxic environment, DNA damage was significantly increased. The results of the present study are consistent with those studies, whereby we observed increases in DNA damage in *A. irradians* in response to increased and decreased water temperature. Therefore, oxidative stress is considered to be associated with DNA damage, and increased oxidative stress of *A. irradians* due to thermal change is thought to have affected DNA damage levels. Furthermore, it is suggested that green light can reduce DNA damage in *A. irradians*.

In summary, *A. irradians* exhibited responses in enzyme levels and H_2O_2 concentration to green light wavelengths when exposed to a thermal/laboratory stress environment. It may be noted, however, that there were no significant differences in the expression and activity of the antioxidative enzymes SOD, CAT and HSP70, and in H_2O_2 activity in response to red and blue LED exposure.

To date, no studies have assessed the effect of light on the thermal/laboratory stress response in shellfish, however, numerous studies have been conducted on fish and crustaceans (Shin et al. 2011; Choi et al. 2012; Choe et al. 2018). Previous studies have also reported that the absorption of wavelengths is species-specific and that there is an effective wavelength for stress control (Volpato and Barreto 2001; Choi et al. 2012; Caves et al. 2016; Choe et al. 2018). In addition to responding to the green light wavelength among the various wavelengths that they were exposed to, *A. irradians* was able to effectively control the oxidative stress in its body when exposed to the corresponding wavelength, thereby increasing its adaptability to stress by defending against ROS.

In conclusion, the expression and activities of SOD, CAT, HSP70 and H_2O_2 in *A. irradians* were found to decrease significantly in the experimental group that was exposed to the green LED wavelength. This study suggests that light of a green wavelength is effective to reduce stress in *A. irradians* and plays a positive role when the scallops are exposed to a stressful environment, such as transportation for aquaculture. Additionally, this study presents basic data on the optical reaction of *A. irradians* and is the first study on the physiological response to light exposure of a definite wavelength in a scallop species exposed to fluctuating water temperatures. Further studies are needed to clarify the way in which the light absorbed through the retina of the scallop regulates the physiological stress reaction mechanisms as well as to investigate the limit line of the defence mechanism controlled by light.

Acknowledgments

This research was supported by the project titled 'Development and commercialization of high density low temperature plasma based seawater sterilization purification system' funded by the Ministry of Oceans and Fisheries, Republic of Korea. We thank two anonymous reviewers who have made helpful comments on previous versions of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was supported by the project titled 'Development and commercialization of high density low temperature plasma based seawater sterilization purification system' funded by the Ministry of Oceans and Fisheries, Republic of Korea.

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