



Effects of Red LED Spectra and Different Photoperiods on the Circadian Rhythm of Abalones (*Haliotis discus hannai*)

Jin A Kim¹, Min Ju Kim², Jun-Hwan Kim^{3*}, and Cheol Young Choi^{1,2*}

¹Department of Convergence Study on the Ocean Science and Technology,
Korea Maritime and Ocean University, Busan 49112, Korea

²Division of Marine BioScience, Korea Maritime and Ocean University, Busan 49112, Korea

³Department of Aquatic Life Medicine, Jeju National University, Jeju 63243, Korea

Abstract : Light is an external environmental factor that affects the biorhythms of aquatic organisms and is involved in their behavior, reproduction, and various biological responses. The biorhythm (24-h cycle) of organisms is controlled internally by clock genes, such as period (*Per*), cryptochrome (*Cry*), and melatonin receptor (*MT-R*), and is externally controlled by light, food, and temperature. In this study, the experimental organisms were divided into photoperiod and LED groups. The photoperiod group was exposed to two photoperiods (LD, 12 h light: 12 h darkness; DD, constant darkness), and the LED group was exposed to a red LED light (wavelength, 630 nm; 12 h LED light: 12 h darkness). *Per* and *Cry* mRNA expression levels increased during the bright phase and decreased during the dark phase. *MT-R* mRNA expression decreased during the bright period and increased during the dark period. The trend in clock gene expression in the RD group was similar to that in the DD group but not in the LD group. Red LED irradiation induced gene expression similar to the 24 h memorization condition, suggesting that long-wavelength light conditions can change the biorhythm of abalones. This study provides academic information on the biorhythm cycle of abalone (a marine gastropod), which is less well known than that of fish and can aid in predicting the physiological response of abalones to changes in light wavelength.

Key words : circadian rhythm, disk abalone, light cycle, melatonin, red wavelength

1. Introduction

Light is an external environmental factor that influences the circadian rhythm of aquatic organisms, impacting their behavior, reproduction, and various biological responses (Pierce et al. 2008). All organisms exhibit different circadian rhythms in response to exposure to light. Circadian rhythms can be altered by seasonal changes, and artificial light not only modifies the circadian cycle but also alters the spectral composition of the light to which organisms are exposed, thereby affecting the fundamental 24 h circadian rhythm of aquatic organisms (Jin et al. 2009).

Circadian rhythms are internally regulated by clock genes and are often externally influenced by light, food, and temperature (Ripperger et al. 2011; Mata-Sotres et al. 2016). The prominent clock genes, period (*Per*) and cryptochrome (*Cry*), are regulated by light and enable organisms to perceive a 24 h cycle (Kim et al. 2018). *Per* forms a heterodimer with timeless genes and acts as a negative feedback loop that controls transcriptional activation and repression of clock genes (Landskron et al. 2009; Kim et al. 2012). *Cry* is rapidly induced in response to light stimulation by causing structural changes and autophosphorylation of Timeless through a direct interaction (Besharse et al. 2004). In addition to these clock genes, melatonin regulate the circadian rhythm in organisms. In invertebrates, melatonin is primarily expressed in the

*Corresponding author. E-mail: junhwan1982@hanmail.net
choic@kmou.ac.kr

cerebral ganglia and retina (Gao et al. 2021). It is synthesized by arylalkylamine-N-acetyltransferase, which is inhibited by light, from the neurotransmitter 5-hydroxytryptamine and is released into the hemolymph at night, resulting in higher melatonin levels during the night than in the daytime (Iuvone et al. 2005). Melatonin plays a role in neuroendocrine transmission by regulating circadian rhythms and participates in immune regulation and antioxidant defense mechanisms (Petrovsky 2001; Maciel et al. 2010).

Although extensive research has been conducted on light-regulated clock genes and melatonin in terrestrial organisms (Terazono et al. 2003; Johnston et al. 2006), studies focusing on the physiological aspects of light responses in aquatic organisms, particularly invertebrates, remain relatively limited. Most existing research in this field has been conducted on fish species (Choi et al. 2019; Ma et al. 2023). Particularly, few studies on light wavelength-related physiology have focused on gastropods (invertebrates), such as abalones (Schnytzer et al. 2018), and studies on the expression characteristics of circadian rhythm-related genes are sparse (Gao et al. 2021; Botté et al. 2023).

Previous studies on nocturnal species, including abalones, have reported typical nocturnal behavioral patterns. They inhabit rocky reefs, hide in crevices during the day, and emerge to feed and engage in activities at night (Gao et al. 2021). Regarding nocturnal abalones, research conducted under controlled conditions showed that their survival and feeding rates were higher under constant darkness (DD), where 24 h darkness was maintained, compared to conditions with a natural light-dark cycle of 12 h light and 12 h darkness (12L:12D) (García-Esquivel et al. 2007; Gorrostieta-Hurtado et al. 2009).

Abalones, predominantly found in shallow coastal areas, are generally more light-sensitive than other fish species (Gao et al. 2022). Species inhabiting shallow depths are more responsive to long-wavelength light than those inhabiting deeper waters, and their spectral sensitivity peaks vary according to habitat depth, aligning with the spectral composition of their respective habitats (Gao et al. 2022). A study exposing abalones to various light wavelengths revealed that abalones exhibit photopositive behavior in the long-wavelength range (red and orange wavelengths), while avoidance behavior is observed in the short-wavelength range (blue and green wavelengths) (Gao

et al. 2016). Furthermore, compared to the red and orange long-wavelength ranges, abalones exhibit significantly lower growth rates, survival rates, and feed conversion efficiencies in the blue and green short-wavelength ranges (Xiaolong et al. 2016).

These findings suggest that organisms perceive wavelengths in the long wavelength range, such as red light, as darker environmental conditions. This phenomenon indicates a close association between the perception of light wavelengths and various physiological responses related to circadian rhythms in abalones.

Therefore, this study aimed to investigate the effects of changes in the light-dark cycle and specific wavelengths of light on the circadian rhythms of abalones. Specifically, we examined changes in *Per*, *Cry*, and melatonin receptor (*MT-R*) mRNA expressions in abalones exposed to two light cycle conditions (12L:12D and DD) and red LED light. By examining the temporal expression patterns of these genes, particularly focusing on red wavelength light, which falls within the long wavelength range, we aimed to elucidate their impact on the circadian rhythm of abalones.

2. Materials and Methods

Experimental organism

Disk abalones (*Haliotis discus hannai*) (average length 6.35 ± 0.52 cm, average weight 42.07 ± 6.14 g) were purchased at an abalone farm (Wando, Jeollanam-do, Korea) and transferred to the laboratory for stability at laboratory tank conditions (salinity 34 ± 0.7 psu, temperature 20.2 ± 1.2 °C, light cycle 12L:12D). The experiment was conducted over 24 h, and each experiment was conducted three times. During the adaptation period for two weeks, sufficient food (raw seaweed) was supplied once daily, and feeding was stopped 24 h before commencing the experiment. No organism died during the acclimatization and experimental periods.

Light wavelength and photoperiod conditions

During the experiment, the water quality conditions were maintained at 22°C, pH 8.0, and 35 psu under white fluorescent light (07:00 on, 19:00 off) and a thermostat (JS-WBP-170RP; Johnsam Co., Seoul, Korea) was used. The photoperiod group was illuminated with a white fluorescent bulb, while the red LED light-irradiated group

received light from a red LED (Total LED Co., Seoul, Korea). Before starting the experiment, the experimental organisms were divided into photoperiod and LED groups (Fig. 1). The photoperiod group was exposed to two photoperiods (LD, 12L:12D; DD, constant darkness), and the LED group was exposed to a red LED (wavelength, 630 nm; 12L:12D). The experimental Zeitgeber time (ZT; 12L:12D) was determined based on natural light conditions. LD and LED groups lit at 07:00 and went off at 19:00 at an intensity of 0.5 W/m^2 at the bottom of the water tank. Sampling was performed six times daily [ZT2 (09:00), ZT6 (13:00), ZT10 (17:00), ZT14 (21:00), ZT18 (01:00), and ZT22 (05:00) h]. A spectrometer (MR-16; Rainbow Light Technology, Pingzhen, Taoyuan, Taiwan) and an optical radiometer (HD 2102.1; Delta OMH Co., Caselle, Padova, Italy) were used. Retinal and hepatopancreatic tissues were collected and stored at -80°C until analysis.

Total RNA extraction and cDNA synthesis

Total RNA was extracted from each retina and hepatopancreas sample using TRI Reagent[®] (TR 118; Molecular Research Center Inc., Cincinnati, OH, USA) according to the manufacturer's instructions. Two micrograms of total RNA were reverse-transcribed in a total reaction volume of $20 \mu\text{L}$ using an oligo-(dT)₁₅ anchor and M-MLV reverse transcriptase (M1701; Promega, Madison, WI, USA) according to the manufacturer's protocol. The cDNA

product was diluted and stored at 4°C for use in Reverse Transcription polymerase chain reaction (PCR) and real-time PCR analyses.

Real-time Polymerase Chain Reaction (real-time PCR)

Real-time PCR was conducted to determine the relative expression levels of *Per* and *Cry* associated with circadian rhythm genes using total RNA extracted from the abalone retina. Relative expression levels of *MT-R* were examined in the retina and hepatopancreatic tissues. Primers were designed using known disk abalone sequences (Table 1). PCR amplification was performed using a Bio-Rad iCycler iQ multicolor real-time PCR detection system (1708740; Bio-Rad Laboratories Inc., Hercules, CA, USA) and the iQ[™] SYBR[®] green supermix (1708880; Bio-Rad Laboratories Inc., Hercules, CA, USA), following the manufacturer's instructions. As a control, the β -actin gene was amplified from each sample. All data were expressed as the difference from the corresponding calculated β -actin threshold cycle (Ct) levels. The Ct values of the PCR products formed the basis for all analyses. These were defined as PCR cycles in which the fluorescence signal crossed a threshold during the exponential phase of the amplification curve. The calibrated ΔCt value ($\Delta\Delta\text{Ct}$) per sample and the internal control (β -actin) were calculated as follows: $[\Delta\Delta\text{Ct} = 2^{-(\Delta\text{Ct}_{\text{sample}} - \Delta\text{Ct}_{\text{internal control}})}]$. After the PCRs, real-time PCR data from three replicate samples were analyzed using the Bio-Rad system to estimate the

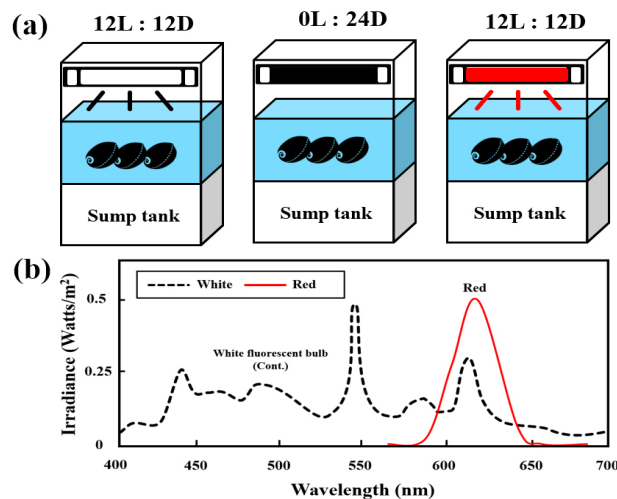


Fig. 1. (a) Experimental water tank setup and (b) spectral profiles of light-emitting diode (red, 640 nm) and a white fluorescent bulb (Cont.) used in the present study. Intensity in LED treatment was 0.5 W/m^2

transcript copy numbers for each sample.

In situ hybridization

The *MT-R* sequence, which was used as an *in situ* hybridization probe, was initially designed (Table 1) and then amplified using PCR. Subsequently, the plasmid was inserted into the pGEM-T Easy Vector (A137A; Promega, Madison, WI, USA). After confirming the antisense sequence via sequencing, the plasmid DNA underwent further amplification using PCR, employing antisense, sense, and T7 primers (5'-TAA TAC GAC TCA CTA

TAG GG-3'). DIG-labeled RNA probes were synthesized using a DIG RNA labeling mix (Merck, Darmstadt, Germany), with PCR products obtained from the antisense primers and T7 RNA polymerase (Merck, Darmstadt, Germany) serving as antisense labeling probes.

To prepare frozen section tissue slides, we collected hepatopancreatic tissues from disk abalones across all experimental groups, with some groups sampled at 6 h (in the LD and RD groups) and others at 18 h (in the RD and DD groups). The collected tissues were then fixed overnight at 4°C in a 4% paraformaldehyde solution. After

Table 1. Primers used for qPCR amplification and *in situ* hybridization

Genes (Accession no.)	Primer	DNA sequences	E (%)
<i>For qPCR</i>			
<i>Per</i> (MN547619)	Forward	5'-CGG TCC TGT ACC AGA TCC AC-3'	102.77
	Reverse	5'-CTG TAC CTC GAT CCA CTC GC-3'	
<i>Cry</i> (MW481327)	Forward	5'-GCT GCY ACA AAC AAC CCG TG-3'	98.48
	Reverse	5'-CAT GAT GGC GTC GAT CCA TG-3'	
<i>MT-R</i> (MW861452)	Forward	5'-GTG TCA TCA TCG GAA CCA TC-3'	101.14
	Reverse	5'-GTC AAG GTG ATA GTC TGG AC-3'	
β -actin (MW387000)	Forward	5'-GAT AGT GCG AGA CAT CAA GG-3'	95.38
	Reverse	5'-CGA TAG TTA TCA CAG ACC CG-3'	
<i>For in situ hybridization</i>			
<i>MT-R</i> (MW861452)	Forward	5'-ATA TGT ACG TCA CGA CCT TG-3'	
	Reverse	5'-CAT GCG CAG ATA ACA GAT TC-3'	

E: qPCR Efficiency, E was calculated as $E = (10^{-1/\text{slpoc}} - 1) \times 100$

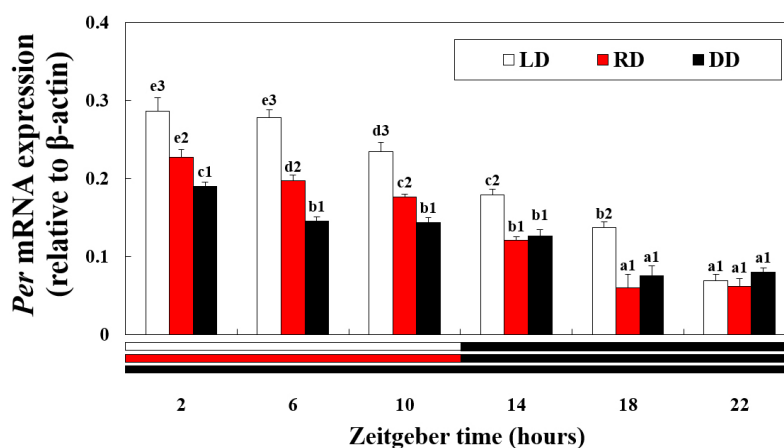


Fig. 2. Expression of period (*Per*) mRNA in the retina of disk abalone exposed to different wavelength and light cycle treatments. The letters indicate significant difference according to the same light treatments at the different zeitgeber time ($P < 0.05$). The numbers indicate significant differences according to the different light treatments at the same zeitgeber time ($P < 0.05$). All values are expressed as means \pm standard deviation ($n = 5$)

fixation, the samples were washed with phosphate-buffered saline (PBS) and stored in a 30% sucrose solution to prevent the formation of ice crystals. Next, the sections were subjected to hybridization using a hybridization buffer comprising deionized formamide, 20× saline sodium citrate, 0.1% Tween-20, 1 M citric acid, and yeast tRNA (in a 1:100 ratio with the hybridization buffer). The RNA probe was included simultaneously, and hybridization was carried out for 18 h at 65°C.

To detect the hybridization signal, the tissue sections were first exposed to a blocking solution containing calf serum in PBS/Tween (PBST) for 1 h at 20°C. Subsequently, the sections were incubated at 4°C with an anti-digoxigenin antibody conjugated to alkaline phosphatase (at a 1:2000 dilution in the blocking solution; Roche, Basel, Switzerland). After multiple PBST washing steps, the sections were rinsed in alkaline Tris buffer consisting of 1 M Tris at pH 9.5, 1 M MgCl₂, 5 M NaCl, and 10% Tween-20. Color development was achieved using a labeling mix of alkaline Tris buffer, nitro blue tetrazolium, and 5-bromo-4-chloro-3-indolyl phosphate disodium salt. Finally, the sections were washed with PBST, mounted with Aqua Polymount (Polysciences, Warrington, PA, USA), and observed under a stereomicroscope (Nikon Eclipse CI, Tokyo, Japan) for image capture.

Statistical analysis

All data were analyzed using SPSS v. 24.0 (IBM Corp.,

Armonk, NY, USA). A two-way ANOVA and Tukey's post hoc test were used to compare differences in the data ($P < 0.05$). Values are expressed as mean ± standard deviation (SD).

3. Results

Per mRNA expression

Per mRNA expression peaked at 2 h of exposure, regardless of the light source conditions, and *Per* expression significantly decreased over time (Fig. 2) ($P < 0.05$). The expression decreased in the RD group compared to the LD group, and the expression level decreased the most in the DD group. From 14 to 22 h, there was no significant difference in *Per* mRNA expression between the RD and DD groups ($P > 0.05$).

Cry mRNA expressions

The *Cry* mRNA expression peaked at 6 h and increased after 2 h of exposure (Fig. 3). *Cry* mRNA expression decreased significantly over time ($P < 0.05$) and was maintained from 18 h. *Cry* mRNA expression was significantly higher in the LD group than in the RD group, and the expression level decreased the most in the DD group ($P < 0.05$). From 14 h to 22 h, there was no significant difference in mRNA expression levels between the RD and DD groups.

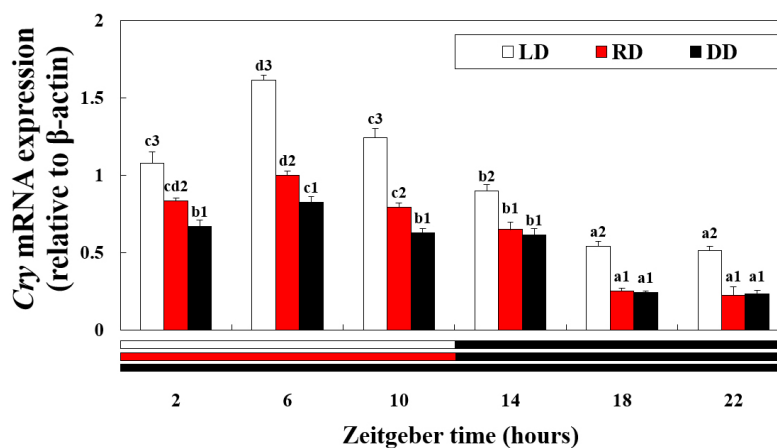


Fig. 3. Expression of cryptochrome (*Cry*) mRNA in the retina of disk abalone exposed to different wavelength and light cycle treatments. The letters indicate significant difference according to the same light treatments at the different zeitgeber time ($P < 0.05$). The numbers indicate significant differences according to the different light treatments at the same zeitgeber time ($P < 0.05$). All values are expressed as means ± standard deviation ($n = 5$)

MT-R mRNA expressions

Except for the RD group, retinal *MT-R* mRNA expression was maintained at a constant level from 2 h to 10 h of exposure and then increased over time from 14 h (Fig. 4a). Thereafter, a peak was observed at 18 h, and

MT-R mRNA expression significantly decreased at 22 h ($P < 0.05$). *MT-R* mRNA expression was significantly higher in the DD group than in the RD group, and the expression level decreased the most in the LD group ($P < 0.05$). From 10–22 h, there was no significant difference

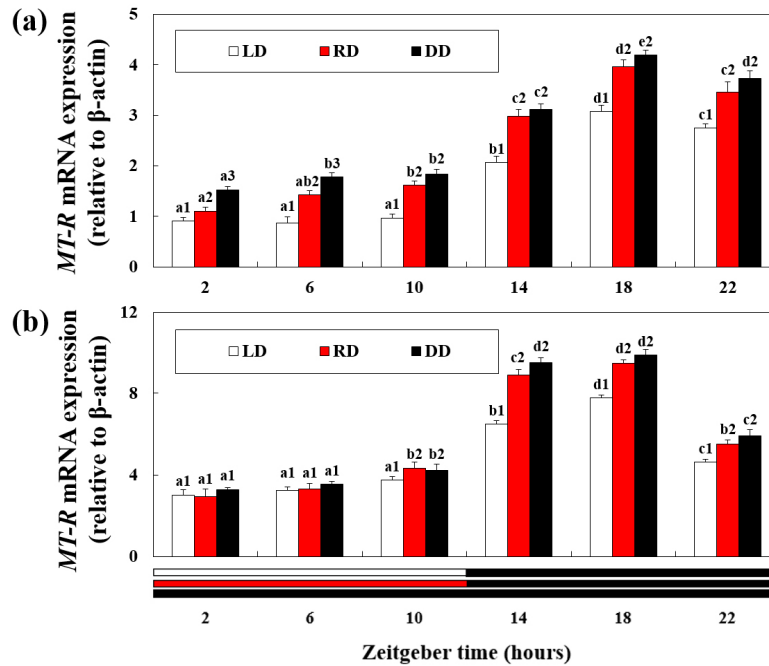


Fig. 4. Expression of melatonin receptor (*MT-R*) mRNA in the (a) retina and (b) hepatopancreas of disk abalone exposed to different wavelength and light cycle treatments. The letters indicate significant difference according to the same light treatments at the different zeitgeber time ($P < 0.05$). The numbers indicate significant differences according to the different light treatments at the same zeitgeber time ($P < 0.05$). All values are expressed as means \pm standard deviation ($n = 5$)

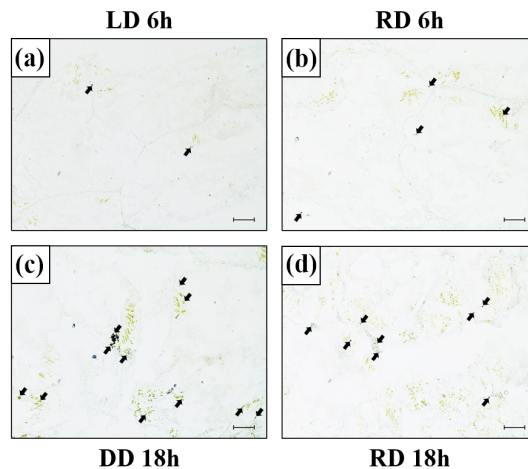


Fig. 5. Expression of the melatonin receptor (*MT-R*) mRNA in the hepatopancreas of disk abalone detected by *in situ* hybridization (a–d). (a) LD group at 6 h, (b) RD group at 6 h, (c) DD group at 18 h, and (d) RD group at 18 h. Dark areas (black arrow) indicate the mRNA expression of *MT-R* in the hepatopancreas. Scale bar = 50 μ m

in mRNA expression levels between the RD and DD groups.

In the hepatopancreas, *MT-R* mRNA expression peaked at 18 h and decreased significantly at 22 h (Fig. 4b) ($P < 0.05$). *MT-R* mRNA expression was significantly higher in the DD group than in the LD group in 14–22 h ($P < 0.05$). However, there were no significant differences in the mRNA expression levels between the LD and RD groups from 6–10 h and between the RD and DD groups from 14–22 h.

Confirmation of *MT-R* mRNA expression site

To confirm *MT-R* mRNA expression in the hepatopancreas, *in situ* hybridization was performed using an *MT-R* mRNA probe (Fig. 5). *MT-R* mRNA signal intensity was observed in the cytoplasm of the hepatopancreatic tissue, consistent with *MT-R* mRNA expression. When comparing the expression levels between the LD and RD groups at 6 h when light was provided, the *MT-R* probe signal was notably higher in the RD group than in the LD group. Conversely, when comparing expression levels between the RD and DD groups during the dark period of 18 h, a significantly stronger signal was observed in the DD group.

4. Discussion

The circadian rhythm is a key factor influencing various physiological functions in organisms, including growth, maturation, and behavior (Patke et al. 2020). Changes in the external natural environment, such as alternating light conditions between day and night, can lead to physiological changes in organisms.

In our study, exposure to decreased light intensity during the transition from the light-to-dark period led to a significant decrease in the expression levels of *Per* and *Cry* mRNA in abalones. Kong et al. (2023), who investigated the expression of *Per* and *Cry* for 24 h in razor clams (*Sinonovacula constricta*), a nocturnal shellfish similar to abalones, reported that the expression levels of *Per* and *Cry* significantly increased during the light period and started decreasing at the onset of darkness. This indicates a rhythmic expression pattern influenced by the light response. The increased expression of *Per* and *Cry* observed in this study also indicates light-responsive regulation, suggesting that the presence or absence of light influences

the expression of circadian clock genes. In contrast, the *MT-R*, known to be expressed at night, showed a significant increase in expression during the transition from day to night in our study. *In situ* hybridization results confirmed a high level of expression at night (18 h). This finding is consistent with the results of Balzer et al. (1997), who conducted a study on crayfish (*Procambarus clarkii*), an aquatic invertebrate species, and reported increased *MT-R* expression during the dark period. These results collectively suggest that a decrease in light intensity leads to increased melatonin synthesis under dark conditions, resulting in the upregulation of *MT-R* expression. Increasing melatonin levels enhances antioxidant capabilities and reduces oxidative damage by eliminating reactive oxygen species in the body (Zhang et al. 2019). Additionally, it promotes the expression of immune proteins, thereby enhancing the organism's immune system (Huang et al. 2020).

Since the abalones in this study were exposed to DD conditions without light, owing to the expression characteristics of each gene, it was expected that *MT-R* would consistently show an extremely high expression level and *Per* and *Cry* would consistently show an extremely low expression level for 24 h. Despite the absence of light, the expression levels of *MT-R*, *Per*, and *Cry* in DD-exposed abalones were similar to those observed in abalones exposed to LD conditions. It is worth noting that *Per* and *Cry*, which are the key regulators of circadian rhythms, are typically regulated by external factors such as light. However, even in the absence of light, these genes are regulated to some extent by internal negative feedback mechanisms, allowing for a certain degree of rhythmicity following a 24 h cycle (Fonken and Nelson 2014). The presence of circadian rhythms in clock genes has been reported in bivalve species such as oysters (*Crassostrea gigas*) (Perrigault and Tran 2017) and mussels (*Mytilus edulis*) (Chapman et al. 2020). However, rhythmicity has not been demonstrated in marine gastropods, such as sea snails (*Bulla gouldiana*) (Constance et al. 2002) and limpets (*Cellana rota*) (Schnytzer et al. 2018).

Therefore, based on the observed consistent gene expression patterns, abalones may have their intrinsic circadian rhythms, distinguishing them from other marine gastropods, even under conditions without light (DD). However, further research on the molecular mechanisms and functions of clock genes is needed to clarify and

better understand circadian rhythms in invertebrates.

Another noteworthy finding in this study was the decrease in *Per* and *Cry* expression and the increase in *MT-R* expression in the RD experimental group (red LED exposure group) compared to the control group (LD group). This pattern was partially similar to the expression trends observed in the DD experimental group, where darkness was maintained. Additionally, there was no significant difference in the expression of clock genes between the RD and DD experimental groups, starting from the onset of darkness at 14 h. Considering these two results, it can be inferred that abalones perceive red wavelengths as darkness, leading to similar circadian rhythms under RD and DD conditions. A study by Jang et al. (2017) on mud shrimps (*Upogebia major*) also reported that exposure to a 650 nm red wavelength did not induce light-stimulated responses similar to the DD condition. Based on the similarity between the circadian rhythms observed in the RD and DD conditions in this study, it is expected that effects such as growth and maturation promotion reported under DD conditions (Xiaolong et al. 2018) may also occur under RD conditions.

In summary, in this study, we investigated the effects of two light cycle conditions (12L:12D, DD) and the red wavelength in the light environment on the expression pattern of clock genes in abalones. We found that red LED light induced gene expression patterns closer to the 24 h memorization condition. These results suggest that long-wavelength red light can alter the circadian rhythm in abalones, in contrast to the effects of general light exposure. The findings of this study provide valuable scientific information on the circadian rhythm of abalones (marine gastropods), which is less understood than that of fish, and contribute to our understanding of the physiological responses of nocturnal abalones to changes in light wavelengths.

Acknowledgments

This research was supported by the National Research Foundation of Korea Grant funded by the Korean Government (2021R1F1A1046310).

References

Balzer I, Espínola IR, Fuentes-Pardo B (1997) Daily variations

of immunoreactive melatonin in the visual system of crayfish. *Biol Cell* **89**:539–543

- Besharse JC, Zhuang M, Freeman K, Fogerty J (2004) Regulation of photoreceptor *Per1* and *Per2* by light, dopamine and a circadian clock. *Eur J Neurosci* **20**:167–174
- Botté A, Payton L, Tran D (2023) Artificial light at night at environmental intensities disrupts daily rhythm of the oyster *Crassostrea gigas*. *Mar Pollut Bull* **191**:114850
- Chapman EC, Bonsor BJ, Parsons DR, Rotchell JM (2020) Influence of light and temperature cycles on the expression of circadian clock genes in the mussel *Mytilus edulis*. *Mar Environ Res* **159**:104960
- Choi CY, Kim TH, Oh YH, Min TS, Choi JY, Song JA (2019) Effects of various LED light spectra on circadian rhythm during starvation in the olive flounder (*Paralichthys olivaceus*). *Biol Rhythm Res* **50**:355–365
- Constance CM, Green CB, Tei H, Block GD (2002) Bulla gouldiana period exhibits unique regulation at the mRNA and protein levels. *J Biol Rhythm* **17**:413–427
- Fonken LK, Nelson RJ (2014) The effects of light at night on circadian clocks and metabolism. *Endocr Rev* **35**:648–670
- Gao X, Lin S, Zhang M, Lyu M, Liu Y, Luo X, You W, Ke C (2022) Review: Use of electrophysiological techniques to study visual functions of aquatic organisms. *Front Physiol* **13**:798382
- Gao X, Pang G, Luo X, You W, Ke C (2021) Effects of light cycle on motion behaviour and melatonin secretion in *Haliotis discus hannai*. *Aquaculture* **532**:735981
- Gao X, Zhang M, Zheng J, Li X, Chi L, Song C, Liu Y (2016) Effect of LED light quality on the phototaxis and locomotion behaviour of *Haliotis discus hannai*. *Aquac Res* **47**:3376–3389
- García-Esquivel Z, Montes-Magallón S, González-Gómez MA (2007) Effect of temperature and photoperiod on the growth, feed consumption, and biochemical content of juvenile green abalone, *Haliotis fulgens*, fed on a balanced diet. *Aquaculture* **262**:129–141
- Gorrostieta-Hurtado E, Searcy-Bernal R, Anguiano-Beltrán C, García-Esquivel Z, Valenzuela-Espinoza E (2009) Effect of darkness on the early postlarval development of *Haliotis corrugata* abalone fed different diatom densities. *Cienc Mar* **35**:113–122
- Huang Z, Aweya JJ, Zhu C, Tran NT, Hong Y, Li S, Yao D, Zhang Y (2020) Modulation of crustacean innate immune response by amino acids and their metabolites: inferences from other species. *Front Immunol* **11**:574721
- Iuvone PM, Tosini G, Pozdeyev N, Haque R, Klein DC, Chaurasia SS (2005) Circadian clocks, clock networks,

- arylalkylamine N-acetyltransferase, and melatonin in the retina. *Prog Retin Eye Res* **24**:433–456
- Jang JC, Chung JK, Hur YS, Song JH, Kim JM (2017) Behavioral monitoring system for mud shrimp *Upogebia major* and the photoresponse to illumination with different wavelength LEDs. *Korean J Fish Aquat Sci* **50**:413–420
- Jin Y, Chen R, Sun L, Liu W, Fu Z (2009) Photoperiod and temperature influence endocrine disruptive chemical-mediated effects in male adult zebrafish. *Aquat Toxicol* **92**:38–43
- Johnston JD, Tournier BB, Andersson H, Masson-Pévet M, Lincoln GA, Hazlerigg DG (2006) Multiple effects of melatonin on rhythmic clock gene expression in the mammalian pars tuberalis. *Endocrinology* **147**:959–965
- Kim M, De La Pena JB, Cheong JH, Kim HJ (2018) Neurobiological functions of the period circadian clock 2 gene, *per2*. *Biomol Ther* **26**:358–367
- Kim NN, Shin HS, Lee J, Choi CY (2012) Diurnal gene expression of *Period2*, *Cryptochrome1*, and arylalkylamine N-acetyltransferase-2 in olive flounder, *Paralichthys olivaceus*. *Anim Cells Syst* **16**:27–33
- Kong F, Shang J, Ran Z, Zhang M, Liao K, Xu J, Yan X (2023) Light entrainment and molecular regulation of the circadian clock in razor clam *Sinonovacula constricta*. *Aquaculture* **564**:739072
- Landskron J, Chen KF, Wolf E, Stanewsky R (2009) A role for the PERIOD: PERIOD homodimer in the Drosophila circadian clock. *PLoS Biol* **7**:e1000003
- Ma H, Liu S, Wei P, Fei F, Ren J, Dai M, Ma Z, Liu Y (2023) Effects of LED spectrum on circadian rhythmic expression of clock genes and *Aanat2* in the brain of juvenile European seabass (*Dicentrarchus labrax*). *Front Mar Sci* **9**:1005352
- Maciel FE, Ramos BP, Geihs MA, Vargas MA, Cruz BP, Meyer-Rochow VB, Vakkuri O, Allodi S, Monserrat JM, Nery LEM (2010) Effects of melatonin in connection with the antioxidant defense system in the gills of the estuarine crab *Neohelice granulata*. *Gen Comp Endocr* **165**:229–236
- Mata-Sotres A, Yufera J, Manuel JM, Martinez-Rodriguez G (2016) Daily rhythms of digestive enzyme activity and gene expression in gilthead seabream (*Sparus aurata*) during ontogeny. *Comp Biochem Physiol Part A* **197**:43–51
- Patke A, Young MW, Axelrod S. (2020) Molecular mechanisms and physiological importance of circadian rhythms. *Nat Rev Mol Cell Bio* **21**:67–84
- Perrigault M, Tran D (2017) Identification of the molecular clockwork of the oyster *Crassostrea gigas*. *PLoS One* **12**:e0169790
- Petrovsky, N (2001) Towards a unified model of neuroendocrine-immune interaction. *Immunol Cell Biol* **79**:350–357
- Pierce LX, Noche RR, Ponomareva O, Chang C, Liang JO (2008) Novel functions for *Period 3* and *Exo-rhodopsin* in rhythmic transcription and melatonin biosynthesis within the zebrafish pineal organ. *Brain Res* **1223**:11–24
- Ripperger JA, Jud C, Albrecht U (2011) The daily rhythm of mice. *FEBS Lett* **585**:1384–1392
- Schnytzer Y, Simon-Blecher N, Li J, Waldman Ben-Asher H, Salmon-Divon M, Achituv Y, Hughes ME, Levy O (2018) Tidal and diel orchestration of behaviour and gene expression in an intertidal mollusc. *Sci Rep*, **8**:4917
- Terazono H, Mutoh T, Yamaguchi S, Kobayashi M, Akiyama M, Udo R, Ohdo S, Okamura H, Shibata S (2003) Adrenergic regulation of clock gene expression in mouse liver. *P Natl A Sci* **100**:6795–6800
- Xiaolong G, Mo Z, Xian L, Ce S, Changbin S, Ying L (2016) Effects of LED light quality on the growth, metabolism, and energy budgets of *Haliotis discus discus*. *Aquaculture* **453**:31–39
- Xiaolong G, Mo Z, Xian L, Fucun W, Changbin S, Ying L (2018) Light cycle effects on *Haliotis discus hammai* Ino growth, energy budget, and related gene expression. *Aquaculture* **483**:213–222
- Zhang C, Zhang Q, Pang Y, Song X, Zhou N, Wang J, He L, Lv J, Song Y, Cheng Y, Yang X (2019) The protective effects of melatonin on oxidative damage and the immune system of the Chinese mitten crab (*Eriocheir sinensis*) exposed to deltamethrin. *Sci Total Environ* **653**:1426–1434

Author's Information

Jin A Kim

Student, Korea Maritime and Ocean University

Min Ju Kim

Student, Korea Maritime and Ocean University

Jun-Hwan Kim

Professor, Jeju National University

Cheol Young Choi

Professor, Korea Maritime and Ocean University

Received Dec. 27, 2023

Revised Mar. 11, 2024

Accepted Mar. 12, 2024

Copyright © 2024 Ocean and Polar Research

This is an open access article distributed under the terms of the
Creative Commons Attribution Non-Commercial License ([\[creativecommons.org/licenses/by-nc/3.0/\]\(http://creativecommons.org/licenses/by-nc/3.0/\)\), which permits unrestricted
educational and non-commercial use, provided the original work is
properly cited.](http://</p></div><div data-bbox=)