



Physiological and oxidative stress response of goldfish *Carassius auratus* induced by a light dimming system

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Abstract Light is an essential factor for organisms and affects the endocrine and stress regulation of fish in nature. However, sudden changes in light and dark conditions in artificial environments can negatively impact fish. In the present study, to evaluate the physiological and oxidative stress responses of goldfish (*Carassius auratus*) exposed to two different light conditions, sudden light changes and slowly dimming light changes for 24 h, we analyzed the mRNA expression and activity of stress indicators [corticotropin-releasing hormone (CRH) and pro-opiomelanocortin (POMC)], levels of plasma cortisol and glucose, mRNA expression of glucocorticoid receptor (GR), and activity of plasma oxidative stress indicators (superoxide dismutase and catalase). Consequently, the mRNA expressions and activities of CRH and POMC, plasma levels of cortisol and glucose, and mRNA expression of GR were found to be significantly increased during the light changes, particularly in the control group. Additionally, plasma levels of cortisol and glucose in the control group were significantly higher than those in the dimming group

during the light changes. However, no significant differences in mRNA expression levels and activities of antioxidant enzymes both in the control and dimming groups were observed. These results indicate that dimming light induces less stress than sudden changes in light.

Keywords *Carassius auratus* · Goldfish · HPIaxis · Fish rearing · Light exposure

Abbreviations

| | |
|------|-----------------------------------|
| ACTH | Adrenocorticotropin hormone |
| CAT | Catalase |
| CRH | Corticotropin-releasing hormone |
| GR | Glucocorticoid receptors |
| HPI | Hypothalamic–pituitary–interregal |
| LED | Light-emitting diode |
| POMC | Pro-opiomelanocortin |
| ROS | Reactive oxygen species |
| SOD | Superoxide dismutase |

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Introduction

Light is an important environmental factor that affects fish throughout their entire lifetime and also regulates their circadian rhythm. Light affects not only physiological and biochemical functions but also behavioral traits (Boeuf and Le Bail 1999; Marchesan et al. 2005; Choi et al. 2014). Therefore, light is an important component of aquaculture technologies, and techniques comprising light

intensity and photoperiod regulation have been widely used for inducing or suppressing physiological responses such as growth and maturation in fish (Biswas et al. 2005).

Unlike natural sunlight, light sources in anthropogenic environments such as aquaculture farms with recirculation or indoors facilities are generally abruptly controlled, with conditions rapidly changing from light to dark. This rapid change in light and dark conditions has been found to negatively affect fish (McHugh and Heidinger 1978; Jørgensen 1983).

To reduce the stress experienced by fish due to rapid changes in light and dark, a dimming system, which involves gradually changing the light intensity over time, may be useful (Heinen 1998). Since a fluorescent lamp is typically used in aquaculture farms, it is not possible to gradually change the light intensity. However, the recent emergence of light-emitting diodes (LED) has enabled the development of a dimming system, in which the brightness of light can be changed by adjusting the electric current intensity.

Therefore, in conditions where natural light does not exist, it is possible to adjust the intensity and period of artificial indoor light based on the optical characteristics of a breeding species by using the LED-based dimming system. Since dimming systems can adjust the light intensity to reflect the times of sunrise and sunset, using these systems in aquaculture may positively affect the growth and survival of fish in an eco-friendly manner (Drummond and Dawson 1970; Delabbio 2015).

Rapid and abrupt changes in light and dark conditions not only reduce brain activity in fish (Laming and Bullock 1991), but also increase oxygen consumption by adversely affecting heartbeat and respiratory control (Pouliot and Noüe 1988). Additionally, it has been reported that abrupt light changes alter fish behaviors such as causing sudden movements or avoidance of fish, which can cause stress (Manci et al. 1992).

Fish maintain homeostasis through defense mechanisms against various stress factors including sudden light changes. Among these defense mechanisms, the hormone activation system at the hypothalamic–pituitary–interrenal (HPI) axis is the major mechanism that controls stress hormones in fish (Mommsen et al. 1999). Corticotropin-releasing hormone (CRH), which is released from the hypothalamus, stimulates the secretion of pro-opiomelanocortin (POMC) in the anterior pituitary (Alsop and Vijayan 2009). Adrenocorticotrophic hormone (ACTH) and α -melanocyte-stimulating hormone are derived from the precursor protein of POMC

on interrenal cells at the head kidney that synthesize and release cortisol (Bonga 1997; Flik et al. 2006; Tsalafouta et al. 2017). As a stress indicator, cortisol directly affects cells by specifically binding to glucocorticoid receptors (GR) in the cell membrane. Additionally, cortisol is secreted into the plasma to control various physiological phenomena (Beato and Sánchez-Pacheco 1996). This cascade system, the primary step for the stressor-mediated upregulation of cortisol, is known as the HPI axis (Barton 2002; Schreck et al. 2016).

Meanwhile, stress induces redox reactions in the body and the production of reactive oxygen species (ROS), which cause oxidative stress in cells and tissues (Shin et al. 2011). Owing to these toxic oxidative effects of ROS, organisms have developed antioxidant defense mechanisms comprising antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) that convert ROS into nontoxic substances (McFarland et al. 1999).

The present study aimed to analyze the physiological and oxidative stress response of goldfish in a dimming system. For this purpose, a control group of goldfish (*Carassius auratus*) was exposed to a light source commonly used in aquaculture with abrupt light and dark changes. In the dimming group of goldfish, a dimming system that gradually changed the light and dark conditions in a manner reflective of natural sunrise and sunset times was used. The stress levels of the fish during the experimental period were examined by evaluating endocrine factors. We analyzed CRH, POMC, and GR mRNA expression in the diencephalon, plasma CRH and POMC activities, and plasma cortisol and glucose levels to determine the physiological stress levels during the experiment. Additionally, SOD and CAT activities were analyzed to evaluate the differences in oxidative stress induction during the experiment.

Materials and methods

Experimental fish

Common goldfish *C. auratus* (length 7.5 ± 0.9 cm; mass 13.4 ± 2.8 g) were purchased from a Choryang aquarium (Busan, Korea) and were allowed to acclimatize for 2 weeks in twelve 300-L circulation filter tanks in the laboratory. The fish were exposed to a white LED with 12-h light/12-h dark photoperiods before the experiment. The water temperature was maintained at 22 ± 0.5 °C. For all experiments, the fish were given

commercial feed daily until the day prior to sampling. The fish were anesthetized with 100 µg/L of 2-phenoxyethanol (Sigma, St. Louis, MO, USA) to minimize stress prior to the collection of blood, diencephalon, and liver tissues. Blood was collected from the caudal vein using a 1-mL syringe coated with heparin. Plasma samples were separated by centrifugation (4 °C, 1000×g, for 15 min) and stored at – 80 °C until analysis. The tissues were removed from the fish, immediately frozen in liquid nitrogen, and stored at – 80 °C until analysis.

Light conditions

All fishes were exposed to light from a white LED (Daesin LED Co., Kyunggi, Korea) placed 20 cm above the water surface. For the control group, the light was turned on at 07:00 after sampling and turned off at 19:00 after sampling (12-h light/12-h dark). The light intensity for the control group was approximately 0.5 W/m². We also established a dimming group in which the light was turned on from 6:30 to 7:30 and turned off from 18:30 to 19:30. The light intensity for the dimming group was gradually increased to 0.5 W/m² an hour after starting to turn on the light. Additionally, the light intensity in the dimming group was gradually decreased to dark an hour before starting to turn off the light. We sampled five fishes from each group at 06:30, 7:00, 07:30, 08:00, 08:30, 10:00, 13:00, 16:00, 18:30, 19:00, 19:30, 20:00, 20:30, 22:00, 01:00, and 04:00 (Fig. 1). Sampling at 6:30 and 19:30 was performed before the light changes.

Total RNA extraction and cDNA synthesis

Total RNA was extracted from each sample using TRI Reagent® (Molecular Research Center, Inc., Cincinnati, OH, USA) according to the manufacturer's instructions. Next, 2 µg of total RNA was reverse-transcribed in a reaction volume of 20 µL using an oligo-(dT)₁₅ anchor and M-MLV reverse transcriptase (Promega, Madison, WI, USA) according to the manufacturer's protocol. The resulting cDNA was diluted and stored at 4 °C for use in polymerase chain reaction (PCR) and real-time quantitative PCR (qPCR) analysis.

qPCR analysis

qPCR analysis was conducted to determine the relative expression levels of CRH, POMC, and

GR using total RNA extracted from the diencephalon of goldfish. The qPCR primers were designed using known goldfish sequences (Table 1). We conducted qPCR amplification using a Bio-Rad iCycler iQ multicolor real-time PCR detection system (Hercules, CA, USA) and SYBR green supermix (Bio-Rad) following the manufacturer's instructions. As a control, the β-actin gene was amplified for each sample, and all data were expressed as the difference compared to the corresponding calculated β-actin threshold cycle (Ct) levels. The Ct values of the PCR products were used in all analyses. The Ct level is 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) per sample and internal control (β-actin) were calculated as follows: $[\Delta\Delta Ct = 2^{-(\Delta Ct_{\text{sample}} - Ct_{\text{internal control}})}]$. After the PCRs were complete, the qPCR data from three replicate samples were analyzed using Bio-Rad software to estimate the transcript copy numbers of each sample.

Plasma parameter analysis

Plasma, collected using heparin as an anticoagulant, was separated from the whole blood by centrifugation (4 °C, 1500×g, for 15 min) and immediately stored at – 20 °C. The levels of CRH, POMC, cortisol, SOD, and CAT were estimated by immunoassay using ELISA kits (CRH, MBS031034; POMC, MBS017228; cortisol, MBS704055; SOD, MBS705758; CAT, MBS038818; Mybiosource, Inc., San Diego, CA, USA). Additionally, glucose levels were determined with a dry multiplate analytic slide method using a biochemical automatic analyzer (Fuji Dri-Chem 4000, Fujifilm, Tokyo, Japan).

Statistical analysis

All data were analyzed using SPSS version 19.0 software (SPSS Inc., Chicago, IL, USA). One-way analysis of variance followed by Tukey's post hoc test was used to compare differences in the data ($P < 0.05$). The values are expressed as mean ± standard error (SE).

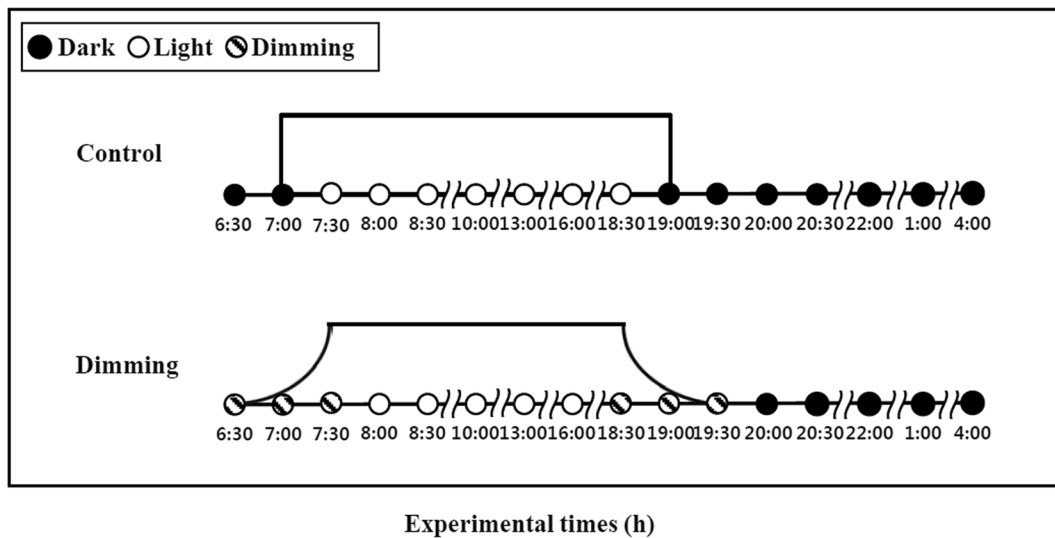


Fig. 1 Light system and sampling schedule for goldfish in the control and dimming groups. Black circles indicate dark, white circles indicate light, and hatched circles indicate dimming period

Results

CRH mRNA expression and activity

Similar trends in CRH mRNA expression and activity were observed during the experiment. Plasma CRH activity was significantly increased from 7:00, peaked at 7:30, and decreased to 10:00, followed by a significant increase again at 18:00 and decrease at 19:30 in the control group (Fig. 2). Additionally, CRH activity was significantly increased and peaked at 7:00 and then decreased at 08:00 in the dimming group. Similar tendencies in CRH mRNA expression were observed in the control and dimming groups. CRH mRNA expression and activity during the light changes in the control group were significantly higher than those in the dimming group. These values were much higher when changing

from dark to light (from 6:30 to 7:30) than when changing from light to dark (from 18:30 to 19:30).

POMC mRNA expression and activity

POMC mRNA expression and activity showed similar tendencies. Plasma POMC activity was significantly increased from 7:00, peaked at 7:30, decreased at 8:30, and significantly increased again at 18:30, followed by a decrease at 20:00 in the control group (Fig. 3). Additionally, POMC activity was significantly increased and peaked at 7:00 and then decreased at 7:30 in the dimming group. POMC mRNA expression showed similar tendencies in the control and dimming groups, but these values during the light changes in the control group were significantly higher than those in the dimming group. CRH mRNA expression and activity were much

Table 1 Primers used for qPCR amplification

| Genes (accession no.) | Primer | DNA sequences |
|---------------------------|---------|----------------------------------|
| CRH (AF098629) | Forward | 5'-CCC GAG ACA TCC CAG TAT T-3' |
| | Reverse | 5'-GTA ATT GCC ATC CAA GCG A-3' |
| POMC (AJ431209) | Forward | 5'-GTC TAC CTT GGA GAA AGC CA-3' |
| | Reverse | 5'-GCG GAA ATG CTC CAT GGA GT-3' |
| GR (HQ656017) | Forward | 5'-CTC CTG GCT CTT CCT CAT-3' |
| | Reverse | 5'-TCT GGT TAC ACT GGT CAC T-3' |
| β -actin (AB039726) | Forward | 5'-ATT TGG CAT CAC ACC TTC T-3' |
| | Reverse | 5'-TTC TCC CTG TTG GCT TTG-3' |

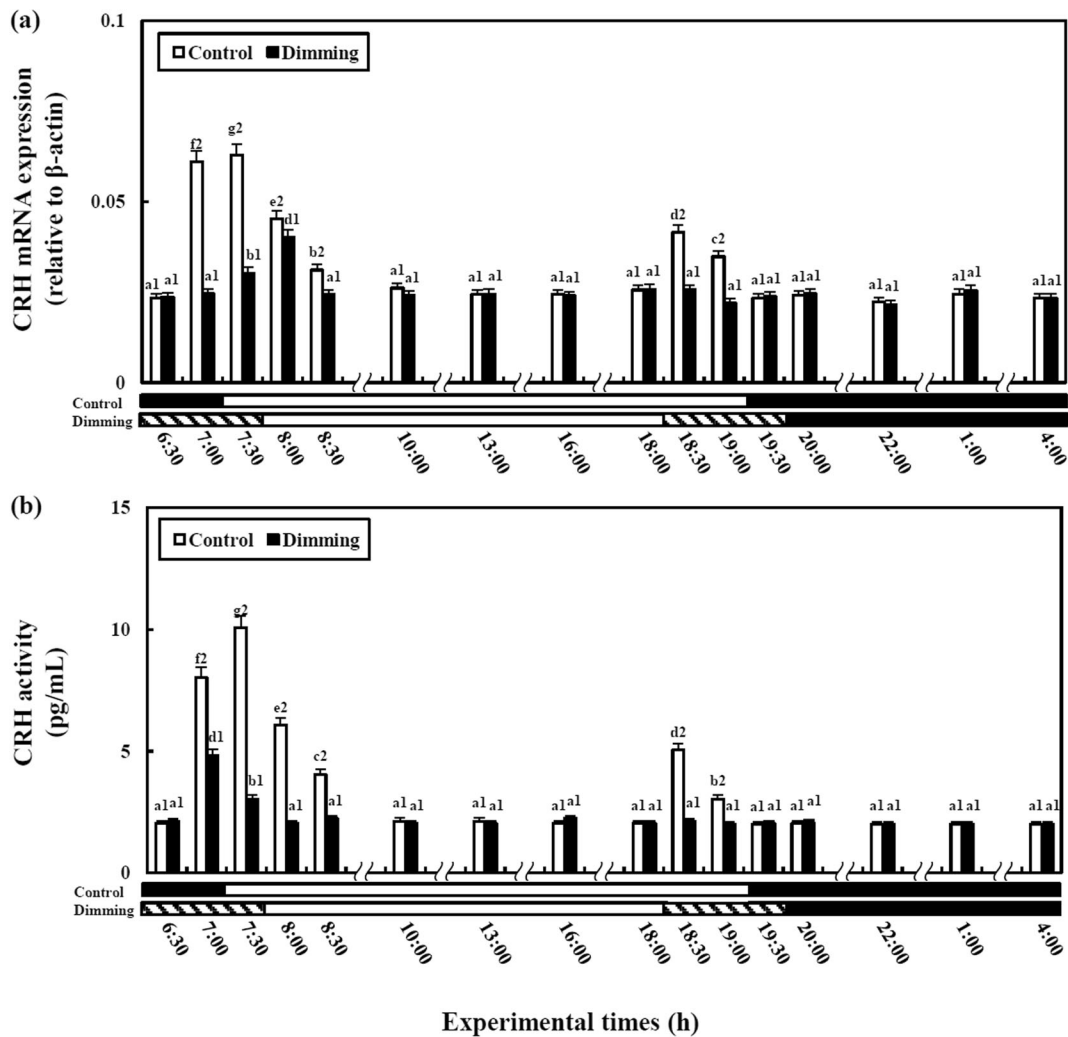


Fig. 2 Changes in the expression of corticotropin-releasing hormone (CRH) mRNA in diencephalon (a) and plasma CRH activity (b) in goldfish exposed to two different light conditions (control and dimming). White bar represents the light period, black bar represents the dark period, and hatched bar represents the dimming

period. Values with different numbers indicate significant differences among different experimental periods ($P < 0.05$). The lowercase letters indicate significant differences between different light conditions ($P < 0.05$). All values are shown as the means \pm SE ($n = 5$)

higher when changing from dark to light (from 6:30 to 7:30) than when changing from light to dark (from 18:30 to 19:30).

Plasma cortisol and glucose level

Plasma cortisol and glucose levels showed similar tendencies; these were significantly increased at 7:00, peaked at 7:30, and decreased at 8:30 (Fig. 4). They also increased at 18:30 and 19:30, respectively, and decreased at 20:00. However, in

the dimming group, the plasma glucose level was significantly increased and peaked at 7:30 and then decreased at 8:30. There was no significant difference in the cortisol plasma level in the dimming group. We found that plasma cortisol and glucose levels during the light changes in the control group were significantly higher than those in the dimming group. Additionally, these levels were markedly higher when changing from dark to light (from 6:30 to 7:30) than when changing from light to dark (from 18:30 to 19:30).

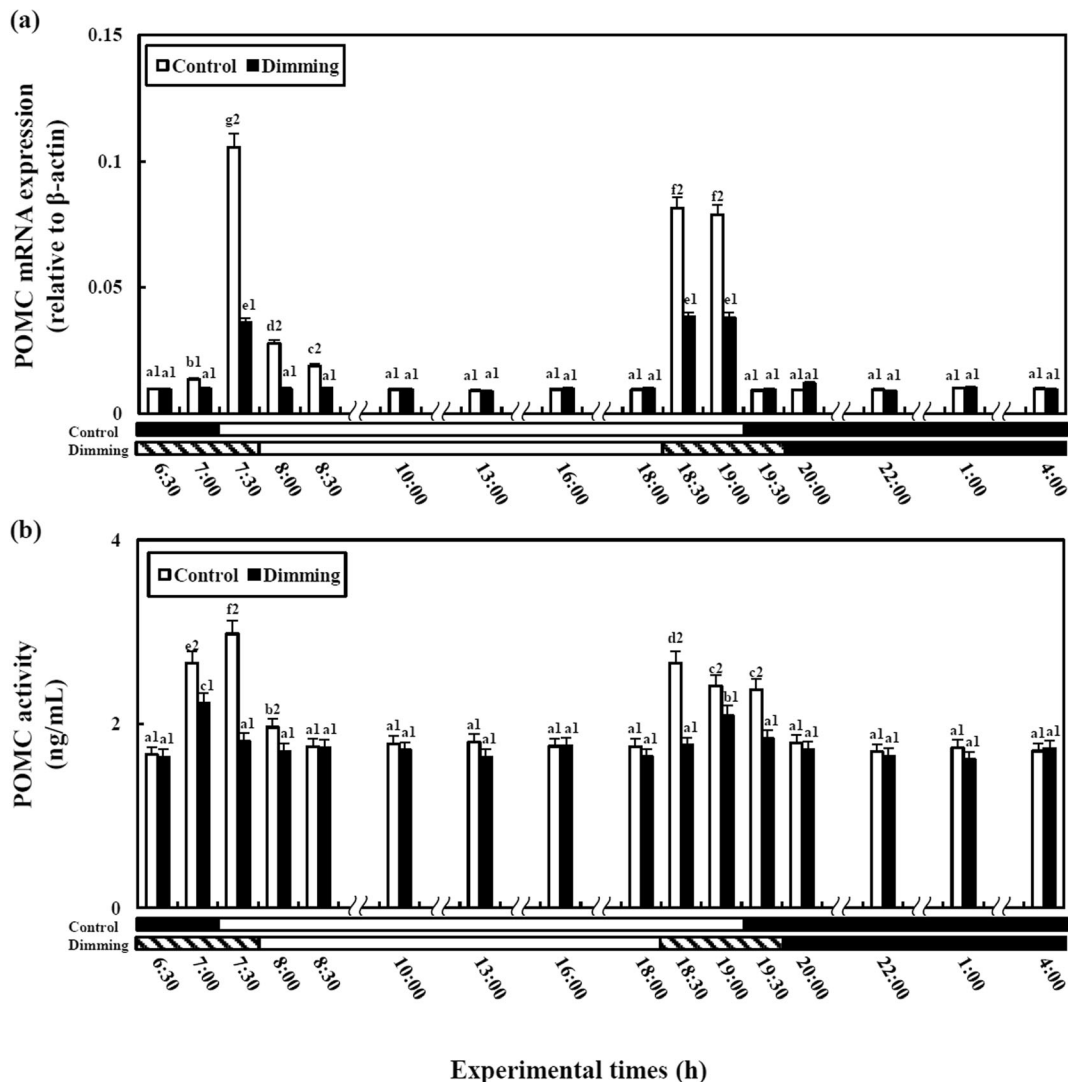


Fig. 3 Changes in the expression of pro-opiomelanocortin (POMC) mRNA in diencephalon (a) and plasma POMC activity (b) in goldfish exposed to two different light conditions (control and dimming). White bar represents the light period, black bar represents the dark period, and hatched bar represents the dimming

period. Values with different numbers indicate significant differences among different experimental periods ($P < 0.05$). The lowercase letters indicate significant differences between different light conditions ($P < 0.05$). All values are shown as the means \pm SE ($n = 5$)

GR mRNA expression

In the control group, GR mRNA expression was significantly increased at 7:30, peaked at 8:00, and decreased to 10:00 (Fig. 5). The level also increased at 19:00 and decreased at 19:30. In the dimming group, GR mRNA expression was significantly increased and peaked at 7:30 and decreased at 8:00. These levels during the light changes in the control group were significantly higher than those in the dimming group. Additionally, GR

mRNA expression was much higher when changing from dark to light (from 6:30 to 7:30) than when changing from light to dark (from 18:30 to 19:30).

SOD and CAT activity

There were no significant changes in SOD and CAT activities in the plasma over time in either the control or dimming group (Fig. 6). There were also no significant differences between the control and dimming groups.

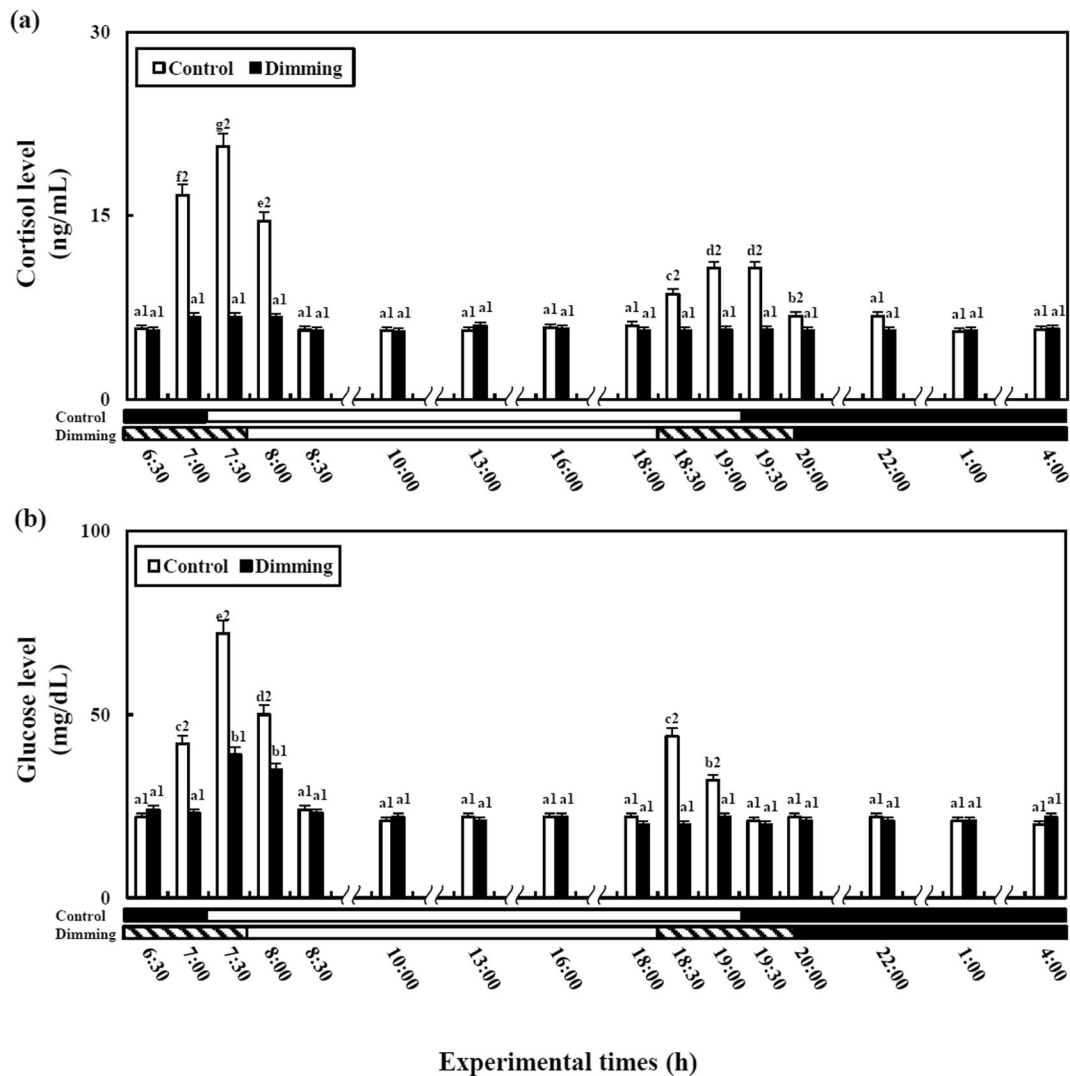


Fig. 4 Changes in the expression of cortisol plasma level (a) and glucose plasma level (b) in goldfish exposed to two different light conditions (control and dimming). White bar represents the light period, black bar represents the dark period, and hatched bar represents the dimming period. Values with different numbers

indicate significant difference among different experimental periods ($P < 0.05$). The lowercase letters indicate significant differences between different light conditions ($P < 0.05$). All values are shown as the means \pm SE ($n = 5$)

Discussion

In the present study, by measuring various stress indicators in goldfish, we compared the endocrinological differences under stress conditions when light intensity was rapidly changed every 12 h with those observed under gradual light dimming.

We analyzed CRH and POMC mRNA expressions and activities in the goldfish diencephalon of the control and dimming experimental groups. We found that CRH and POMC mRNA expressions and activities were

temporarily significantly increased in the control group, in which light intensity was rapidly changed at 07:00 and 19:00. However, CRH and POMC mRNA expressions and activities showed no or slight differences in the dimming experimental group in which light intensity was gradually changed (increasing light from 7:30 to 8:30 and decreasing light from 18:30 to 19:30), similar to the natural sunrise and sunset times.

Additionally, plasma cortisol and glucose levels in goldfish in the control and dimming experimental groups were analyzed. As a result, plasma cortisol and

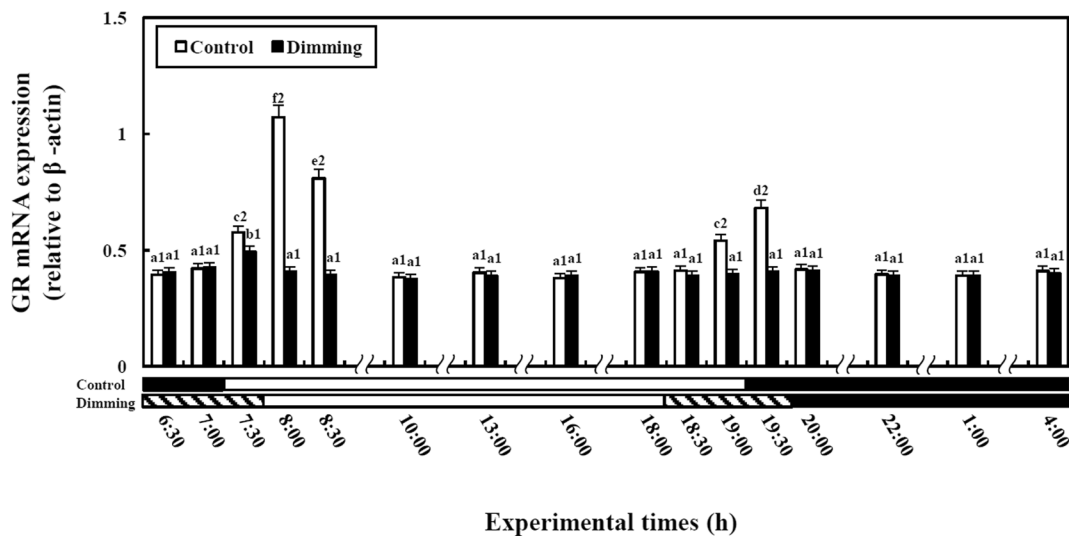


Fig. 5 Changes in the expression of glucocorticoid receptor (GR) mRNA in the diencephalon of goldfish exposed to two different light conditions (control and dimming). White bar represents the light period, black bar represents the dark period, and hatched bar represents the dimming period. Values with different numbers

indicate significant difference among different experimental periods ($P < 0.05$). The lowercase letters indicate significant differences between different light conditions ($P < 0.05$). All values are shown as the means \pm SE ($n = 5$)

glucose levels in the control group were temporarily increased. However, the cortisol level in the dimming group did not change, and glucose, which was temporarily increased during the gradual brightness changes (from 7:30 to 8:30 and from 18:30 to 19:30), was significantly lower than in the control group. Similarly, GR mRNA expression in the goldfish diencephalon of the control group was significantly increased compared to that in the dimming experimental group. Additionally, CRH and POMC mRNA expressions and activities, plasma cortisol and glucose levels, and GR mRNA expression in the control and dimming groups when changing from a dark to bright state (6:30 to 7:30) were significantly higher than when changing from a light to dark state (18:30 to 19:30).

Tian et al. (2015) exposed the blunt snout bream *Megalobrama amblycephala* to 800 and 1600 lux for 2 weeks to investigate the stress caused by light. As a result, the stress indicator cortisol was significantly increased compared to that in the control group exposed to light of 100 lux, indicating that strong light induced stress. Additionally, Tian et al. (2019) analyzed cortisol levels after exposing the blunt snout bream to different light conditions and evaluated stress levels in the fish body. They discovered that the cortisol level was significantly increased during the light changes in all

experimental groups, and cortisol level at light changing time was the highest during 24 h.

Sudden changes in light can cause behavioral and endocrine changes in fish, which may adversely affect homeostasis (Manci et al. 1992; Pupalla et al. 2004). Particularly, rapid light changes from dark to light can induce light-shock stress by causing sleep disorder in fish (Fuller, 1998). Therefore, in the current study, sudden light changes appeared to act as stress factors, and based on the results obtained in the study conducted by Fuller (1998), changing from dark to light states induced stronger light-shock stress.

Of the previous studies showing that cortisol was induced by light stress, only a few studies have examined how other indicators related to the HPI axis, such as CRH, POMC, GR, and glucose, are affected by light stress. However, cortisol is a major index of the HPI axis, which is a hormone defense mechanism that operates under stress conditions. Many previous studies have reported that various types of physical and chemical factors increase cortisol by increasing CRH and POMC, which induces an increase in glucose levels (Winberg and Lepage 1998; Ghisleni et al. 2012; Zhang et al. 2015). Additionally, Barton and Iwama (1991) reported that rapid environmental changes act as stressors to the fish body and increase cortisol levels

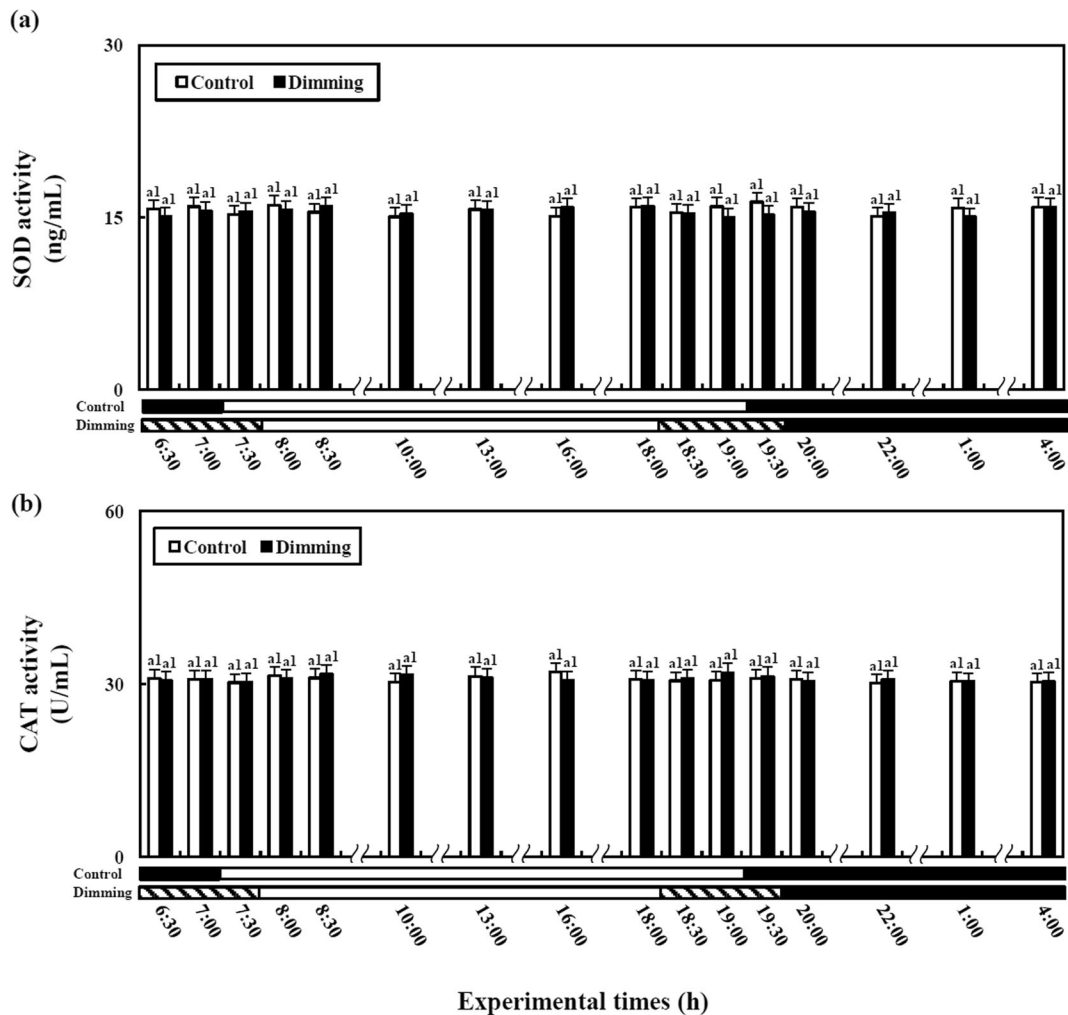


Fig. 6 Changes in the plasma superoxide dismutase (SOD) activity (a) and plasma catalase (CAT) activity (b) in goldfish exposed to two different light conditions (control and dimming). White bar represents the light period, black bar represents the dark period, and hatched bar represents the dimming period. Values with

different numbers indicate significant differences among different experimental periods ($P < 0.05$). The lowercase letters indicate significant differences between different light conditions ($P < 0.05$). All values are shown as the means \pm SE ($n = 5$)

through the HPI axis, thereby increasing not only GR expression but also glucose levels.

Therefore, in this study, it was considered that the rapid light changes were a stress factor resulting in the generation of CRH and POMC, which are important indicators of the HPI axis. The light changes also increased cortisol levels and glucose production as a result of increased levels of the cortisol receptor GR.

The results for the activity of the antioxidant enzymes, SOD and CAT, were in contrast to the abovementioned results. There was no significant difference in SOD and CAT activity in the control or dimming group, whereas HPI axis-related indicators

were significantly increased when light was rapidly changed (07:00 and 19:00) in the control group. Similarly, a previous study showed that the antioxidant index was increased by stress while antioxidant enzymes were not. Jia et al. (2015) investigated the effect of nitrite, a stress inducer in the fish body, on turbot *Scophthalmus maximus*, and reported that plasma cortisol and glucose levels were increased in turbot but did not lead to increased SOD and CAT activities. In the present study, sudden changes in light resulted in transient stress on the fish but did not induce significant changes in SOD and CAT activity, indicating that sudden and transient light changes do not induce oxidative stress.

The results of the present study indicate that the control group subjected to the sudden light change had higher levels of stress factors than the dimming group in which light changes corresponded to natural sunrise and sunset. Additionally, a sudden light change is a stress factor that increases indicators related to the HPI axis, but does not act as a continuous stressor that induces oxidative stress; the light change from the dark to the light state was a stronger stress factor than that from light to dark. Therefore, application of a dimming system can minimize stress to fish and positively affect aquaculture.

In this study, sudden light changes did not act as a stressor to induce oxidative stress during the short period of the experiment. However, it is necessary to investigate the intensity of light and efficient dimming conditions that reduce physiological stress on various fish species.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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