Effects of lighting mode on the growth performance, cortisol level and oxidative stress of juvenile Chinese three-keeled pond turtle (*Chinemys Reevesii*)

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Abstract: To improve the light environment and welfare of the turtle cultured indoors, the effects of lighting mode on growth performance, cortisol level, and oxidative stress of juvenile Chinese three-keeled pond turtle, *Chinemys reevesii*, were investigated in this study. The experimental turtles with an initial weight of 5.61±0.09 g were reared in tanks under four different lighting modes: three groups with light (lighting the basking area and water area, LBW; lighting the water area only, LW; lighting the basking area only, LB) and control group (no light, NL). The experiment was conducted for more than six months, with each group having three replicates. After 203 d of the experiment, the turtle in the LW group exhibited higher weight gain rate (WGR) and a specific growth rate (SGR, %/d) compared to other treatments. Also, results showed that the final body weight of the turtle exposed to LW was higher than that exposed to other treatments. On the physiological level, serum cortisol level in turtles exposed to LW was significantly lower than that in other treatments. Regarding oxidative stress, the level of catalase (CAT) in turtles exposed to LW and LB was significantly lower than that exposed to LBW and NL. The malonaldehyde (MDA) activity in turtles exposed to LW was significantly lower than other treatments. Based on the growth performance and health status, it is suggested that lighting the water area only is the optimal lighting mode for the juvenile three-keeled pond turtle cultured indoors.

Keywords: Chinese three-keeled pond turtle, lighting mode, growth, stress response, oxidative stress
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1 Introduction

According to the recent literature, it can be found that light plays an important role in the growth[1-3], behavior[4-6], and physiology[7-9] of aquatic animals. Numerous studies have investigated the significant impact of light on aquatic animals, such as the effect of light intensity[2,10-12], the light spectrum[9,13,14], and photoperiod[3,15,16]. However, few studies about the effect of lighting mode on aquatic animals have been conducted.

As a special aquatic animal, the Chinese three-keeled pond turtle, *Chinemys reevesii*, has been used as food, pet, and traditional medicine in many regions of the world[17-19]. In nature, turtle goes into hibernation when the temperature is low[20]. Besides, one of the biggest problems in culturing turtles is the high mortality rate of juvenile turtles during hibernation[21]. Greenhouse can solve the problem above and help to enhance the survival rate and reduce mortality during the cold weather since it can maintain the water and air temperature stable. However, the environment of a traditional greenhouse is dark. While in nature, a turtle can sometimes be observed basking on the rocks, branches, and shore. Therefore, it is necessary to study the effect of light on *Chinemys reevesii* cultured in the greenhouse.

Recently, the effects of light on turtles have been reported in several studies. For instance, the effect of light pollution on the nesting and the population of hatching turtles[22-25], the effect of ultraviolet radiation on the 25-hydroxyvitamin D3 of turtles[26,27] and the effect of lighting mode on Chinese soft-shelled turtle[28]. Although the optimal lighting mode for the Chinese soft-shelled turtle has been studied, the impact of lighting mode on *Chinemys reevesii* cultured indoors is still little known. According to the previous studies, it can be learned that the effects of light intensity, photoperiod, and light spectrum on aquatic animals are species-specific. Thus, regarding lighting mode, whether it has a species-specific impact on aquatic animal needs further research.

As an important environmental factor, light may result in a stress response, which was indicated by plasma cortisol, glucose

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levels[12,29]. In addition, light can also have significant effects on antioxidant enzymes[30]. Previous studies have shown that when animals are in oxidative stress, the antioxidant system plays a critical role in protecting the organism from oxidative damage, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-PX)[31-33].

Therefore, this study aimed to investigate the influence of different lighting modes (no light, NL; lighting the basking area and water simultaneously, LBW; lighting the water only, LW; and lighting the basking area only, LB) on the growth, cortisol level and oxidative stress of *Chinemys reevesii*, to examine whether the effect of lighting mode on aquatic animals is species-specific, and to find the optimal lighting mode for the culture of *Chinemys reevesii*.

2 Materials and methods

2.1 Animals and experimental conditions

All experimental protocols in this study were under the approval of the Care and Use of Animals of Zhejiang University committee. The approval number of the relevant animal welfare document was ZJU20190082.

The experiment was conducted in an aquatic farm (Hangzhou Weikang Agricultural Development Co.) sited in Hangzhou, China, from September 2018 to March 2019. The juvenile turtles, with an initial body weight of (5.61±0.09) g (mean±SD) were purchased from a turtle hatchery in Jiangxi Province, China. All turtles selected were healthy and had been acclimated for two weeks before the experiment. The stocking density was 60 individuals per square meter, according to the research of Zhang et al.[34]. During the experiment, the water temperature ranged from 28°C to 30°C, the pH 7.2-7.5, dissolved oxygen (DO) concentration was maintained above 2 mg/L, and the total ammonia nitrogen and nitrite were within a safe range. The animals were fed 3% of their body weight twice daily at 08:00 and 16:00. The commercial feed used in this study came from Zhejiang Jinjia aquatic feed Co., Ltd. (Huzhou, Zhejiang, China).

Twelve tanks for rearing the turtles were randomly divided into four groups. The size of the tank was 200 cm×100 cm×30 cm. Each tank had a water area and basking table (60 cm×40 cm). The basking table had a slope of 3%. All tanks were placed in a concrete pond, and an air-source heat pump heated the water in the pond to maintain the water at about 30°C. Turtles were randomly divided into four groups and cultured under different lighting modes: no light (NL), lighting the basking area and water simultaneously (LBW), lighting the water only (LW), and lighting the basking area only (LB). The sunlamps, which consist of UV and visible light, were produced by Changzhou Xinghuo Lighting Co., Ltd. (Changzhou, China)[28]. The design of the culture tank, the detail of the arrangement of the sunlamp in different treatments, the light intensity, and the light spectrum were the same as research of Li et al.[28]. Sunlamps were switched on from 06:00 to 18:00.

2.2 Calculation of growth performance and survival rate

At the end of the experiment, the turtles from each tank were counted to calculate the survival rate. Growth parameters, including weight gain rate (WGR), feed conversion ratio (FCR), specific growth rate (SGR, %/d), and the survival rate (SR, %), were calculated according to Equations (1) to (4):

$$WGR = \frac{(W_f - W_i)}{W_i} \tag{1}$$

$$SGR = \frac{\ln W_f - \ln W_i}{d} \times 100\% \tag{2}$$

$$FCR = \frac{F_c}{W_g} \tag{3}$$

$$SR = \frac{T_{fn}}{T_{in}} \times 100\% \tag{4}$$

where, W_i is initial body weight, g; W_f is final body weight, g; F_c is feed consumption; W_g is weight gain, g; T_{fn} is total final number and T_{in} is total initial number.

2.3 Sampling

At the end of the experiment, the turtles were deprived of food for 24 h before sampling. Six turtles were randomly selected from each tank. A 120 mg/L MS-222 (*tricaine mesylate*) was used to anesthetize the turtle. When the turtle was completely anesthetized, blood was quickly drawn from the turtle's head using a 5 mL centrifugal tube. After two hours, the blood sample was centrifuged at 2500 r/min for 10 min. All serum samples were frozen and stored at -80°C for subsequent analysis of cortisol levels. After blood collection, the liver was also sampled. The liver was flushed with a normal saline solution and then stored at -80°C for the analysis of antioxidant enzyme activity.

2.4 Analysis of physiological indexes

Serum cortisol level was detected with enzyme-linked immune sorbent assays (ELISA) using commercial kits (reference codes H094). The total antioxidant capacity (T-AOC), superoxide dismutase (SOD) activity, catalase (CAT) levels, and malonaldehyde (MDA) activity [reference codes A015-1-2 (total antioxidant capacity assay kit), A001-1-2 (total superoxide dismutase assay kit), A007-1-1 (catalase assay kit) and A003-1-2 (malondialdehyde assay kit)] in turtle liver were determined using commercial kits. All commercial kits were purchased from Nanjing Jiancheng Institute, Nanjing, China.

2.5 Statistical analysis

This study analyzed experimental data from each treatment using SPSS Statistics (ver. 20.0, IBM). Data were expressed as the means ± SD of three replicates and were compared using one-way ANOVA. Before analyses, the data were analyzed for normality and homogeneity of variances. If significant (p<0.05) differences were found, Turkey's HSD multiple range test was used to compare different treatments.

3 Results

3.1 Growth performance and survival rate

The growth performance and survival rate of juvenile turtles at the end of the experimental period subjected to different lighting modes are presented in Table 1. The final body weights of the turtles showed variations between (87.32±1.37) g and (96.11±3.64) g for the different lighting modes, and the highest values were observed in turtles exposed to LW, followed by NL, LB, and LBW. Similar trends were also observed in WGR and SGR. The final body weight, WGR, and SGR of turtles in LW were significantly higher than that in LBW (p<0.05), but no significant differences were observed between LW, LB, and NL (p>0.05). Further, the survival rate is highest in NL among the treatments, while no statistical differences were shown among animals under four lighting modes (p>0.05).

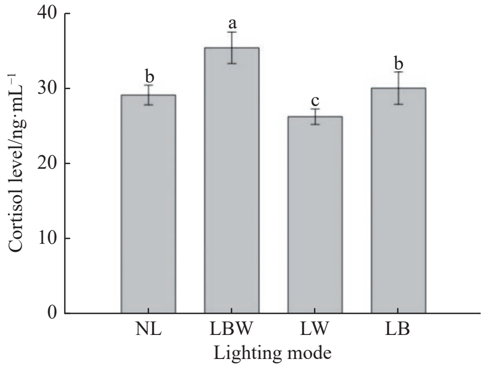
Data were presented as means ± SD from three replicates. Values with different superscripts in the same column are significantly different (p<0.05). The treatments were no light (NL), lighting the basking area and water simultaneously (LBW), lighting the water only (LW), and lighting the basking area only (LB).

Table 1 Influence of lighting mode on the growth performance and survival rate of juvenile three-keeled pond turtles at the end of the experiment (203 d)

Lighting mode	W_i/g	W_f/g	WGR	SGR/(%·d^{-1})	FCR	SR/%
NL	5.65±0.06	91.63±4.43ab	14.94±0.77ab	1.37±0.02ab	1.15±0.06ab	94.45±1.47
LBW	5.67±0.02	87.32±1.37b	14.19±0.24b	1.35±0.01b	1.19±0.07a	91.95±1.21
LW	5.50±0.02	96.11±3.64a	15.72±0.63a	1.41±0.02a	1.14±0.03b	92.08±1.25
LB	5.57±0.01	91.21±3.49ab	14.86±0.61ab	1.38±0.02ab	1.15±0.04ab	90.42±2.09

3.2 Cortisol level

Figure 1 shows the influences of different lighting modes on the serum cortisol level of the turtle. Serum cortisol level was significantly affected by the lighting mode. The serum cortisol level of the turtle in LW was significantly lower than that in other treatments (p<0.05). No significant differences were observed between NL and LB (p>0.05).



Note NL: no lighting; LBW: lighting the basking area and water simultaneously; LB: lighting the basking area only; LW: lighting the water only. Values are presented as means±SD (n=6 individuals per replicate). Different letters on the bar chart indicate significant differences (p<0.05).

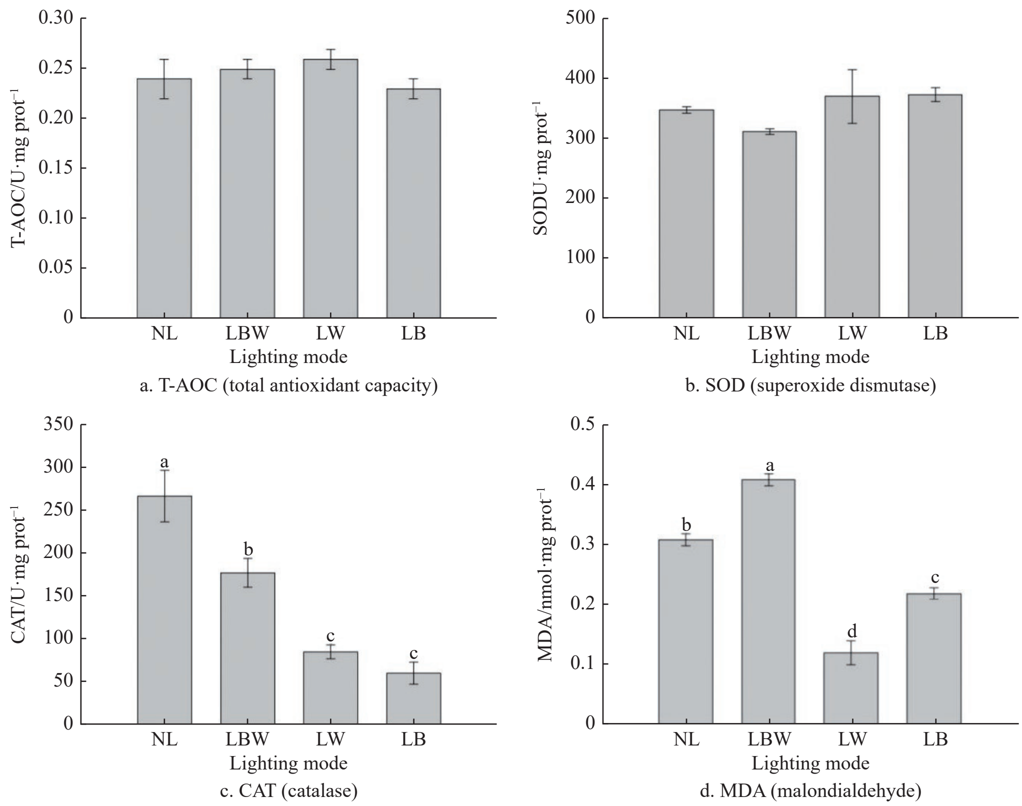
Figure 1 Influences of different lighting modes on the serum cortisol level of Chinese three-keeled pond turtles at the end of the experiment

3.3 Oxidative stress

The antioxidant enzyme activities of turtles subjected to different lighting modes are shown in Figure 2. There were no significant differences (p>0.05) in the activities of T-AOC and SOD of turtles among the treatments (Figures 2a and 2b). For the activities of CAT, the turtle exposed to LW and LB had a significantly lower value (p<0.05) than that exposed to LBW and NL, but no significant differences (p>0.05) were observed between LW and LB (Figure 2c). Concerning the activities of MDA, significant differences were observed among the treatments, and the lowest values were observed in turtles exposed to LW (p<0.05), followed by LB, NL, and LBW (Figure 2d).

4 Discussion

In the natural environment, light is a complex factor for aquatic animals. It is affected by water depth and turbidity, resulting in different light intensities and spectrums. The impact of varying light intensities and spectrums on aquatic animals was discussed previously[7,10,12,35]. However, to our knowledge, little was reported



Note: Data were presented as means±SD from three replicates (n=6 individuals per replicate). Significant differences (p<0.05) among treatments were indicated by different letters. The treatments were no light (NL), lighting the basking area and water simultaneously (LBW), lighting the water only (LW), and lighting the basking area only (LB).

Figure 2 Effects of lighting mode on the antioxidant enzyme activities in three-keeled pond turtles at the end of the experiment about the effects of lighting mode on *Chinemys reevesii*. The present study demonstrated that the growth performance of turtles exposed to LW was the best among the four treatments. The results also showed that the lighting mode of LW caused less stress in the turtle since the levels of serum cortisol, CAT, and MDA were significantly lower compared to other treatments.

4.1 Growth performance

In the current study, the results suggested lighting mode had significant effects on the growth performance among the treatments. The growth performance of the turtles exposed to LW was the highest among the treatments, followed by the turtle exposed to NL, and the lowest group was the turtles exposed to LBW. This might be the feed conversation ratio (FCR) of the turtle exposed to LW was improved. In addition, the growth performance of turtles exposed to LW was better than NL and LB. It could be explained that the lighting mode made the turtle exposed to LW have a higher feeding incidence compared to the turtle exposed to the treatments with no light above the water (NL and LB). The results were in accordance with the previous study that juvenile *E. coioides* has more active feeding at the light intensity of 320-1150 lx than at the light intensity of 0 lx, which were ascribed to the high feeding incidence at the light intensity of 320-1150 lx[12]. However, the results were contrary to another work on the Chinese soft-shelled turtle[36], which indicated that the Chinese soft-shelled turtle grew better under darkness. This may be caused by the composition of light. In the study of Zhou et al.[36], the fluorescent lamp was used as a light source containing visible light, whereas in the current study, the light contains visible light, UVA light, and UVB light, which is similar to the component of sunlight. Additionally, regarding the treatments with light, the growth of turtles exposed to LW was significantly higher than that exposed to LBW. It was possible that the lamp lighting the full tank made the turtle exposed to LBW more stressed than that exposed to LW, which can be evidenced by the cortisol level in the turtle shown in Figure 2. Moreover, the finding that lighting mode had a significant effect on the growth performance of Chinese three-keeled pond turtle was contrary to another research where the lighting mode had no significant impacts on the growth performance of Chinese soft-shelled turtle[28]. It can be explained that Chinese three-keeled pond turtle and Chinese soft-shelled turtle are two species and the effects of lighting mode on the growth performance are species specific.

4.2 Stress response

Cortisol is a vital stress hormone[37] and plays a significant role in numerous processes in fish, such as growth, behavior, reproduction, and stress response[38]. In the current study, the cortisol level of the turtle exposed to LBW was significantly higher than that in other groups, which indicated that the turtle might be stressed when exposed to LBW. This finding was similar to the research of Li et al.[28]. This outcome may be because both the Chinese three-keeled pond turtle and Chinese soft-shelled turtle could not adapt to the lighting mode of LBW. In this lighting mode, light acted as a stressor to stimulate cortisol secretion to adjust the physiological state. Additionally, the cortisol level of the turtle exposed to LW was significantly lower than that in other groups, which indicated that the turtle exposed to this lighting mode suffered less stress. Combining the final body weight and cortisol level, it can be found that high cortisol levels reduced the growth of the turtle. In contrast, the turtle with a lower cortisol level obtained better growth. This finding coincided with previous research that chronic stress may reduce fish growth since the energy was allocated to restore homeostasis, such as tissue repair, locomotion, and respiration[39-41]. However, this result was contrary to the effect of lighting mode on the cortisol level of the Chinese soft-shelled turtle[28]. The possible reason is that three-keeled pond turtle and Chinese soft-shelled turtle are two different species and their habits are also different. Therefore, it can be concluded that the effects of lighting mode on aquatic animals are species-specific.

4.3 Oxidative stress

The antioxidant system of aquatic animals is mainly composed of antioxidant enzymes, which can counteract the negative effects of reactive oxygen species (ROS) and play a significant role in protecting cells from oxidative stress. In general, organisms protect themselves from the toxic effects of ROS by increasing the level of antioxidant enzymes[1]. In the present study, the activity of CAT in turtles exposed to NL was significantly higher than in those exposed to other treatments. The increase of CAT may help to eliminate the ROS induced by a dark environment, thereby protecting the cells from oxidative damage. The result indicated that the turtle exposed to NL suffered the highest oxidative stress among the treatments, which was following the study by Wei et al.[6], who found that a dark environment caused hepatopancreas oxidative stress of juvenile gibel carp. Regarding the treatments with light, the activities of CAT in turtles exposed to LW and LB was significantly lower compared to the turtles exposed to LBW, which indicated that the lighting mode of LBW induced liver oxidative stress.

The oxidative stress can further be proved by the concentration of MDA. MDA can reflect the lipid peroxidation levels of cells and is usually used to appraise the animal's health status[42]. In this study, the concentration of MDA in turtles exposed to LW was significantly lower than in those exposed to other treatments. This result indicated that the turtle exposed to LW had a low level of lipid peroxidation, which further suggested that the turtle in LW suffered less oxidative stress than that exposed to LBW, LB, and NL. High antioxidant-related enzymes indicated that the turtle exposed to LBW, LB, and NL suffered oxidative stress, which might be the reason for turtle's low growth performance in LBW, LB, and NL.

5 Conclusions

In conclusion, it is evidenced that the growth, stress response, and oxidative stress of juvenile three-keeled pond turtles were significantly affected by the lighting mode. The turtles in LW had higher WGR and SGR than other groups. In addition, LW could have caused less oxidative stress for the turtles. Therefore, based on the turtle's growth performance and health status in this experiment, it is believed that lighting only the water area is the best lighting for the juvenile three-keeled pond turtle cultured indoors.

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