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Short Communication
Does Circadian Rhythm Disorder Always Harm Organisms? Positive Effects of Circadian Rhythm Disorder in *Lates calcarifer*

[1,2]Jiawei Hong, [1,2]Dachuan Cheng, [1,2]Shengjie Zhou and [1,2]Zhenhua Ma

[1]Tropical Aquaculture Research and Development Center, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, 572018 Sanya, China
[2]Key Laboratory of South China Sea Fishery Resources Exploitation and Utilization, Ministry of Agriculture, 510300 Guangzhou, People's Republic of China

Abstract

Background and Objective: Circadian rhythm disorder was proved that may make a result of a wide variety of adverse health consequences, however, does circadian rhythm disorder always harm organisms in their whole life process? The aim of the present study was to evaluate the influence of circadian rhythm disorder on barramundi *Lates calcarifer* at different life stages. **Materials and Methods:** The growth and immune responses of larval and juvenile barramundi *Lates calcarifer* under various circadian rhythm disorder situation (13, 16, 19 and 24 h light time) were investigated in this experiment. **Results:** The results showed that total length of larval and juvenile significantly increased comparing to the control group when the light time was extended to 16 h, but the length of fish in 24 h light time group showed significantly lower than the control. The cortisol level of larvae in 16 and 19 h light time groups significantly differed from other 2 groups. To juvenile, circadian rhythm disorder didn't make any influence on both T-SOD and ACP activity, the same result also appeared in cortisol concentration. The activity of three immune enzymes of larval from 16 and 19 h light time groups were all stimulated to increase by the homologous circadian rhythm disorder compared to the control. **Conclusion:** In brief, lightly circadian rhythm disorder (prolonged 3 h light times) can benefit in fish growth. Although it brought some immune response to larvae which may cause adverse effects, all these negative effects would be eliminated when they grow up to juvenile.

Key words: *Lates calcarifer*, growth stage, cortisol, immune enzyme, circadian rhythm

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Corresponding Author: Zhenhua Ma, Tropical Aquaculture Research and Development Center, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, 572018 Sanya, China

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The earth rotates once every 24 h. To adapt to the rotation of our planet, plants, animals and humans adjust their biological rhythm to synchronize with the Earth's revolution and circadian rhythm was formed as a result[1]. Circadian rhythm is common as a nature law but we always forget its existence. It plays an important role in maintaining the various physiological functions of the body and regulating the adaptability of the body to the internal and external environment. However, circadian rhythm is not immutable and can be affected by external environmental factors (zeitgebers) and light is the most important zeitgeber of biological clock system[2]. When the external environment and internal biological clock do not match (such as different light time), corresponding disorders will occur to circadian rhythm. Circadian rhythm disorder was proved that may make a result of a wide variety of adverse health consequences, such as metabolic syndrome, cardiovascular dysfunction, immune dysregulation and reproductive problems[3]. However, does circadian rhythm disorder always harm organisms? This question should be further discussed.

Fish, as an aquatic organism, live in a more complex and varied environment compared to the terrestrial organism. It is more easily influenced by the surrounding environment changes. Furthermore, Larvae and juvenile stages of fish postembryonic development are of great importance, during which changes of major functional, structure and morphological happen[4,5]. Environment factors have a greater impact on fish at these two stages and of which the effects of the circadian rhythm disorder characterized by different photoperiods can't be ignored. Barramundi (*Lates calcarifer*), also named Asian sea bass, is an important marine food sh species of the world[6]. At present, little research has been done on the effects of photoperiod on the growth and development of larvae and juveniles of fish, preliminary studies have been carried out on the effects of the growth rate and feeding capacity of Barramundi. The results showed that prolonging the light time properly is beneficial to the growth and the increase of food intake[7,8]. However, the action mode and the mechanism inherent need to be further studied, especially to physiology.

Therefore, in order to evaluate the influence level and find out the action regularity to fish, we used barramundi in 2 growth stages as research object, investigated the effects of the circadian rhythm disorder caused by changing light time on growth and immune responses of fish.

MATERIAL AND METHODS

Animals source and experimental design: Barramundi fertilized eggs were produced by Tropical Aquaculture Research and Development Center, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Science sand hatched in 500 L incubators at 29.5°C. On 2 DPH (days post hatch), larvae were transported to 80 L rearing tanks and kept at a density of 20 individuals L^{-1}. Rotifers *Brachionus rotundiformis* at a density of 15 rotifers mL^{-1} were used to feed the larvae from 2-16 DPH. The rotifers fed with baker yeast were enriched with the *S. presso* (INVE Aquaculture, Salt Lake City, UT, USA) for12 h before the rotifers were added into the larval rearing tanks. On 9 DPH, *Artemia nauplii* were first introduced at 1 nauplii mL^{-1} and then added with a daily increment of 110%. *Artemia nauplii* were enriched with DHA protein selco (INVE Aquaculture) following the manufacturer's instruction before adding them to each tank. Starting from 20 DPH, *Artemia nauplii* were gradually phased out at a daily reduction until entirely replaced by commercial micro-diet. The commercial micro-diet (Otohime A1 Marubeni Nisshin Feed Co., Ltd. Tokyo, Japan) was delivered by hand at a 4 h interval from 08:30-19:00 h daily and the amount of feed was adjusted to reach the level of apparent satiation. Each pond bottom was siphoned daily to remove dead fish, uneaten food and faeces. About 1.5 times of the water volume of the pond was exchanged daily in the way of flowing water.

Four circadian rhythm treatments were tested in this study including light time: 13, 16, 19 and 24 h (means the photoperiods: Light/dark 13/11, 16/8, 19/5 and 24/0 h), in which 13 h light time group was the nature circadian rhythm group (control). Every treatment was in triplicates. Two growth stages of fish (larval and juvenile) were used as the research object: (1) Larval stage trial started from 3 DPH (initial feeding) and finished when entered in juvenile stage on 16 DPH, (2) Juvenile stage trial started from 17 DPH and ended on 24 DPH. The density of fish was kept at 20 individuals L^{-1} all the time no matter which fish stage trial. During the trial, light intensity was adjusted among the range of 600-1000 lx. Other ambient conditions, was measured as follow: Salinity 31±0.8, temperature 29.5±1.0°C, pH 7.7±0.2, dissolved oxygen 7.5±0.7 mg L^{-1}. At the end of trial, 5 fishes were randomly collected from each tank and stored at -80°C for further usage.

Sample handling and determination of physiological and biochemical indexes: Whole fish samples were homogenized on ice after adding 9 fold volume of 0.86% saline solution. The homogenate was centrifuged for 10 min at 15000 r min^{-1} and the supernatant was collected and kept at -80°C for the further analysis. The total protein of homogenate was quantified by the bicinchoninic acid (BCA) method using the assay kit (Nanjing Jiancheng Bioengineering Research Institute, Nanjing, China). Cortisol Content (COR) was determined using the cortisol assay kit (Jiangsu Kurt Biotechnology Co., Ltd., Yancheng, China) following the manufacturer protocols. Total superoxide dismutase (T-SOD), peroxidase (POD) and acid phosphatase (ACP) activities were determined using commercial assay kits (Nanjing Jiancheng Bioengineering Research Institute, Nanjing, China) according to the manufacturer's instruction. All enzymes' activity was expressed as units per milligram of protein (U mg^{-1} protein).

Statistical analysis: The results were analyzed using the SPSS 19.0 statistical software packages. All data were expressed as the mean±standard deviation (Mean±SD). Comparisons between different groups were conducted by one-way ANOVA and Tukey's multiple comparisons. Significance was set at $p<0.05$.

RESULTS

Total length of larval and juvenile barramundi was measured at the end of each trial. Significance was shown between groups at both two growth stages (Fig. 1a). Higher total length was observed when the light time was extended to 16 h. When light time continually increase to 24 h, the total length of larvae and juveniles significantly lower than the control ($p<0.05$).

Cortisol concentration of larval and juvenile was shown in Fig. 1b. Circadian rhythm disorder situation did not significantly affect the cortisol level in juvenile ($p>0.05$). However, the cortisol level in larvae, by contrast, presented a significant difference between different groups. Fish in 16 h and 19 h light time groups significantly differed from other two groups. Prolong light time for 3 and 6 h could result in inhibiting the secretion of cortisol.

Roughly equal phenomenon was found at 3 immune enzymes' activity (T-SOD (Fig. 2a), POD (Fig. 2b) and ACP (Fig. 2c) under various circadian rhythm disorder treatments. The activity of three immune enzymes of larval from 16 and 19 h light time groups were all stimulated to increase by the homologous circadian rhythm disorder, but their differences weren't significant except T-SOD compared to the control. Moreover, to juvenile, circadian rhythm disorder didn't make any influence on both T-SOD and ACP activity, only showed a significant effect on POD activity of 24 h light time group.

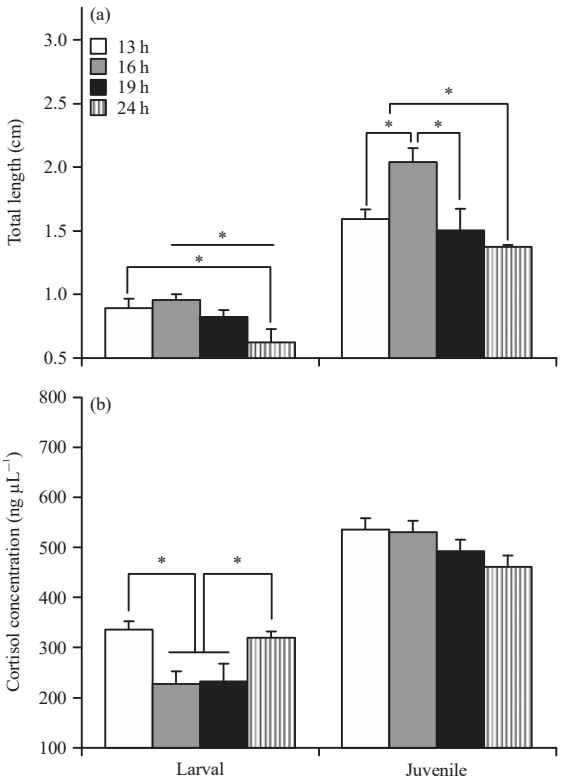

Fig. 1(a-b): (a) Total length and (b) Cortisol concentration of larval and juvenile barramundi under various circadian rhythm disorder situation (13, 16, 19 and 24 h light time)
*Indicates a significant difference ($p<0.05$) between different photoperiod groups

DISCUSSION

The 2017 Nobel Prize in Physiology or Medicine was awarded jointly to Jeffrey C. Hall, Michael Rosbash and Michael W. Young for their discoveries of molecular mechanisms controlling the circadian rhythm. They found out some genes that control the biological rhythm clock from fruit flies and the protein these genes encode. The coded proteins work together and regulate gene transcription rhythmically in cells using a negative feedback model. It was proved by them that the adjustment of circadian rhythm is endogenous and at a molecular level[9-11]. However, it is coordinated with external factors such as light[12-15]. The extent of the change of light time can decide how much the pressure given to internal control system. Pressure and regulation feedback are uncertain to be a simple linear relationship, it needs experimental verification.

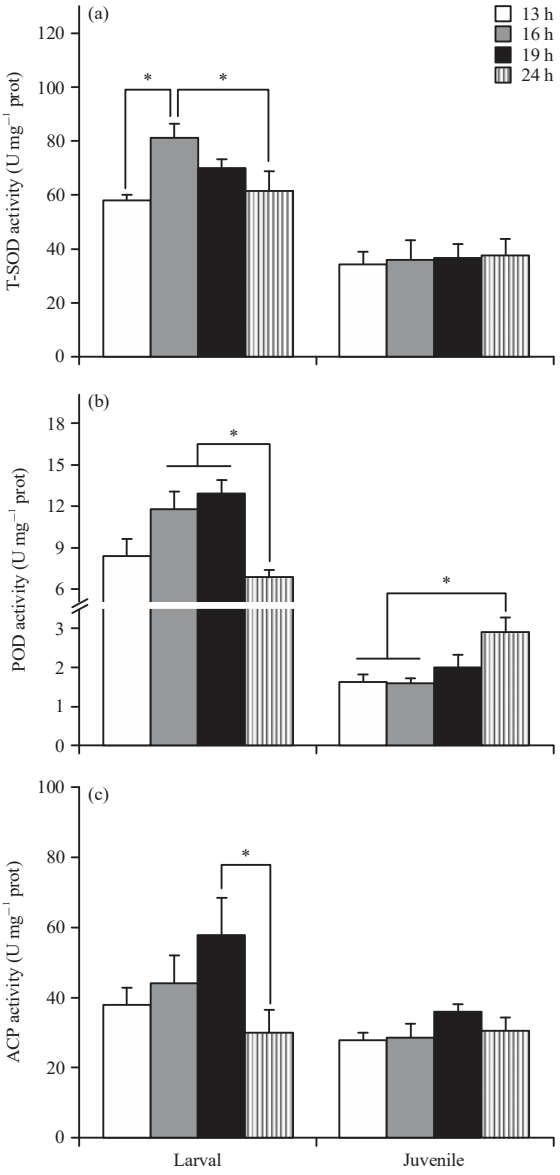

Fig. 2(a-c): (a) T-SOD, (b) POD and (c) ACP activity of larval and juvenile barramundi under various circadian rhythm disorder situation (13, 16, 19, 24 h light time)

*Indicates a significant difference ($p<0.05$) between different photoperiod groups

In our study, total length of larval and juvenile barramundi got an increase compared to the control when the light time was extended to 16 h, but 24 h light time group showed significantly lower than the control. The results indicated that properly extending light time can promote the growth of larval and juvenile to some extent. This is similar with previous studies, such as *Atractoscion nobilis*[16], *Thunnus albacares*[17] and *Epinephelus septemfasciatus*[18], they all showed an increase on growth rate and survival rate with extending of light time.

Cortisol can inhibit the extraction of amino acids from muscle tissue, accelerate the oxidation process of fat, promote the generation of a large amount of energy in a short time to cope with environment stress, but it can cause growth slow down and dysplasia, so the content of cortisol in blood is usually used as an important indicator of fish stress response[19]. We could find that there was no significant effect on the cortisol level of juvenile under various circadian rhythm disorder situation. However, the cortisol level in larvae from 16 and 19 h light time groups significantly differed from other 2 groups. The results demonstrated that prolonging light time for 3 and 6 h could result in inhibiting the secretion of cortisol. These degrees of circadian rhythm disorder, on the contrary, increased food intake of fish and put metabolism at a high level, fish obtained adequate energy and don't need cortisol to get more supply through other paths.

Environmental stress will simulate the production of excessive reactive oxygen species (ROS) which are toxic to cells[20]. At that time, non-specific immune system plays a leading role in coping with adverse environmental stress in which SOD and POD are of great importance in eliminating free radicals on immune regulation process[21]. In present study, the activity of three immune enzymes of larval from 16 and 19 h light time groups were all stimulated to increase by the homologous circadian rhythm disorder compared to the control. It suggested that these two light time treatments have stimulated responses of immune enzyme, they already did harm for the health of fish.

In addition, to juvenile, circadian rhythm disorder didn't make any influence on both T-SOD and ACP activity, the same result also appeared in cortisol concentration. These means that the larval had a better adaptability to circadian rhythm disorder, were stronger enough to face these challenges. Animals' ability of circadian rhythm adjustment and adaptability to disorder were not fixed all the time, making changes with growth instead.

CONCLUSION

Interestingly, lightly circadian rhythm disorder (extend 3 h light time) can benefit for growth of fish. Although it brought some immune response to larval which may cause adverse effects, all these negative effects will be eliminated when they grow up to juvenile. The fact of larval and juvenile barramundi making different performance when suffering various circadian rhythm disorder situation illustrated that circadian rhythm disorder don't always do harm to organisms.

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