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Studies on the Effect of Photoperiodism and Temperature on Moulting of a Freshwater Prawn *Macrobrachium dayanum*

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Abstract

The effect of Photoperiodism and temperature on moulting of a freshwater prawn *Macrobrachium dayanum* was investigated in the laboratory for 91 days. 60 healthy specimens of *M. dayanum*, were taken with uniform weight. The experimental prawns were divided into three groups viz, control, continuous 24hrs. dark and continuous 24hrs. light condition. Moulting frequency increased with corresponding increase in photoperiod and temperature 24L: 00D (17.50 °C) as compared to control (12L: 12D, 16.49 °C). While lowest moulting rate was recorded in (24D: 00L, 13.15 °C). The highest moulting observed in 24hrs. continuous light indicated longer photoperiodism and rise in temperature gave positive effect on the growth of *Macrobrachium dayanum*, consumed good feed, and grew faster, due to better assimilation efficiency.

Keywords: *Macrobrachium dayanum*, Moulting frequency, lowest, highest, growth, Assimilation.

Introduction

Moulting in crustaceans is an important phenomenon for growth, reproduction and metamorphosis. It involves the shedding of the hard exoskeleton to expose a soft new shell. The uptakes of water from the animal's immediate surroundings cause the new exoskeleton to expand, and finally get hardened. Moulting is a complex process that is affected by a range of external factors such as temperature, photoperiod, and nutrition^[1]. However despite extensive research, the moulting process in Crustaceans still remains poorly understood.

Survival and development of Decapods can be influenced by a variety of environmental factors including temperature, oxygen concentration, salinity and light^[2, 3, 4]. Intensity variations, spectral composition, light polarization and photophase duration also affect the growth and reproduction in Crustaceans. The daily cycle of light and dark and the seasonal changes in the proportions of light and dark are most likely to be of importance in the crustaceans^[5, 6] studied the effect of light intensity (750 and 75 lx) and photoperiod 12L/12D and 7L/5D on *P. merguensis* and found that light intensity and photoperiod significantly influence the percentage weight gain, but do not influence moult frequency.

Artificial light is used as a tool in species adapted to diverse environments and at different latitudes, to investigate the physiological dynamics, which could be involved in determining distribution and adaptation patterns of these animals^[7]. Seasonal effects on development, moulting, metabolic rate, induction of moulting, fully developed gonads and sex determination may be under photoperiodic control in some species^[2, 8] reported the atrophy of somatic muscles, the resorption of the old exoskeleton, and the formation of the new exoskeleton in preparation for the onset of ecdysis.

In view of meager knowledge, related to the photoperiodism and its affects in freshwater prawns, the present investigation has been undertaken to find out whether there exists any effect of photoperiod and temperature on moulting in *Macrobrachium dayanum*.

Materials and methods

Collection, Inuring and Maintenance of test animals

Freshwater prawn *Macrobrachium dayanum* (length 3.5-7.0 cm, weight 700-1000 mg) collected from local lake of Sagar and brought to the laboratory. Before experimentation, prawns were treated with 0.1 KMnO₄ solution to obviate any dermal infection and

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acclimatized for two weeks to laboratory conditions. The total duration of experiment was from 1st November 2009 to 30th January 2010 and then experiment was terminated. Uniform weights of animals were selected for experiment. Prawns were kept in glass aquarium (60 l) and fed with commercially available food. Prior to feeding fecal matter was collected with a net, presence of any dead prawn was recorded and any excess of food removed to preserve water quality, and along with this the moults were collected, counted daily and temperature was recorded with thermometer. The experimental prawns were divided into the following three equal groups each having 60 animals.

Group 1

This group was subjected to natural day and night light with normal duration and intensity.

Group 2

Animals were placed in an aquarium with thick card-board box and further covered with a black cloth, in order to maintain animals in constant darkness throughout the experimental work.

Group 3

Animals receive 24hrs of light per day. Lighting was provided by 40 watt unfrosted incandescent bulbs suspended from a frame approximately 15 inches above the water level.

Estimation of Growth

Arthropods do not possess any hard parts with periodic marking as were found in fishes on the other hand, most of the dimensional increase occurred at ecdysis which was a process of periodic moulting or shedding of exoskeleton. Therefore, estimation of growth in crustacean had always been a problem. The easiest method to understand growth in prawns was to count the number of moults undergone. Hence in the present study, the number of moults found in each group was counted to know the effect of temperature and photoperiodism on the growth pattern of *M. dayanum*.

Statistical analysis

Experiments were done for moulting in quintuplets. The significance was calculated using analysis of variance (ANOVA) followed by Tukey's multiple comparison test of columns of Graph pad instat 3 Demo statistical software for windows. A value of $P < 0.05$ was taken as statistically significant. And the results were calculated as mean with standard deviation (\pm SD) values for the experimental data.

Results

Effect of Photoperiod and Temperature on Moulting of *M. dayanum*

The total duration of treatment was 3 months. Moulting in prawns was monitored daily during morning hours when fecal matter was collected during the whole experimental period. Significant differences had been observed in the animals of the three groups with respect to moulting.

Group I

This group served as control, in which prawns showed an intermediate rate of moulting process as compared to the groups exposed to continuous darkness and continuous light. In this group moulting was recorded to be 65.600 ± 3.025 . The average temperature in this group was observed 16.49°C during the whole experiment.

Group II

This group was exposed to 24 hrs complete darkness under the conditions described. It showed the lowest number of prawns moulting during the whole experimental period. This was observed due to lowering of water temperature as food was not assimilated properly and also less intake of food as compared to other groups. Lowest moulting was recorded in complete darkness group (II) 56.600 ± 2.013 . The average temperature in this group was observed 13.15°C during the whole experiment.

Group III

This group was exposed to 24 hrs. Continuous light and showed the highest number of moults undergone during the whole experimental time. Maximum average temperature limit in the season of winter was recorded to be 17.50°C in 24hrs. Completely light exposed, while minimum temperature limit was recorded in continuously 24hrs. Darkness exposed group which was 13.15°C . Highest numbers of moults were recorded as 83.200 ± 2.132 in this group as compared to control and continuous dark group. Longer photoperiodism and higher temperatures were found to accelerate the moult cycle and growth of *M. dayanum*. At temperature of 17.50°C , moults occurred at a faster rate with a reduced moult cycle duration. Rate of feed intake was good at longer photoperiod and higher temperature 17.50°C and average at control 16.49°C . Less feed intake was observed in continuous dark group 24D: 00L at 13.15°C . This was found due to the heat generated from continuous illumination of bulb which increased the temperature of water and this process in turn had positive effect on the moulting of *Macrobrachium dayanum* can be seen clearly in (Table 1).

Mechanism of Moulting

The beginning of moulting was well characterized by the retraction of the epidermal matrix from the cuticle. This process was first visible at the bases of the terminal setae of the telson, proceeding only later through other body regions and appendages. Then epidermal infoldings or invaginations occurred. The internal enhancement of the tissue surface for morphological reconstruction processes including: the lengthening of already existing setae, the formation of new setae and the appearance of other completely new organs was observed. During these sub-surface reconstruction processes in the epidermal tissues, the cuticle not changed. When morphological reconstruction processes completed, a very thin new cuticle was secreted on the surface of new epidermal structures such as setae and appendages, while the gap between the old and new cuticle increased.

The moulting process took normally, at most, a few minutes and this process was very painful for the animals. It was initiated by the body flexing movements, followed by a dorsal rupture of the cuticle between the cephalothorax and the pleon. First, the telson was retracted from the old exoskeleton. Subsequently, the animals also shed their anterior part of body and moulting was completed.

The Effect of Photoperiod and Temperature on the Moulting of *M. dayanum*

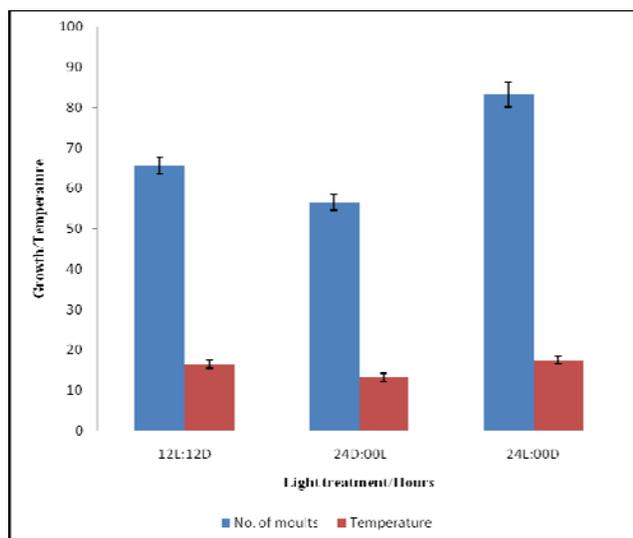
The table 1 and graph 1 shows the results obtained during experiments. Tukey's multiple comparison test of ANOVA shows in group II, moulting rate was significantly ($P < 0.05$) decreased as compared to group I, whereas in continuous light (group III) moulting rate was significantly ($P < 0.001$) increased

over those of control (group I) and continuous dark (group II). The graph also showed clear difference between the different groups. Highest growth was recorded in 24hrs. Continuous light exposed group compared to control, whereas, lowest growth was achieved in 24hrs. Continuous dark exposed group.

Table 1: Showing the effect of photoperiodism and temperature on the moulting (growth) of *M. dayanum*.

S. No.	Treatment	No. of animals	No. of moults Mean ± SD
Group I	Control (12L:12D, 16.49°C)	20	65.600 ± 3.025
Group II	Complete dark (24D:00L, 13.15°C)	20	56.600 ± 2.013*
Group III	Complete light (24L:00D, 17.50°C)	20	83.200 ± 2.132***

Data has been represented as mean ± standard deviation (N=5)



Graph 1: Showing the effect of photoperiod and temperature on moulting of *M. dayanum*.

Discussion

Results of the photoperiodic and temperature studies on moulting indicated that these were the two main important factors that directly affected the moult cycle and growth of *Macrobrachium dayanum*. Moulting frequency increased with corresponding increase in photoperiod and temperature 24L: 00D (17.50 °C). The effect of photoperiodism in crustaceans was generally contradictory. Observations of [9] conformed to the results of the present study, that lowest moulting rate occurred in complete darkness 24D: 00L (13.15 °C). However, [10] observed in *Panulirus argus* that photoperiod did not affect moulting rates significantly. In the present study, low moulting rate had been observed in complete darkness 24D: 00L (13.15 °C) while highest moulting occurred in complete light 24L: 00D (17.50 °C) which indicated that longer photoperiodism and rise in temperature caused due to the warmth generated from the bulb used above the aquarium and had a positive influence on the growth of *Macrobrachium dayanum*. Most of the studies on the effect of light in crustaceans indicated variable results. Increase in size has been observed in the larvae of *Homarus americanus* [2] when kept in continuous darkness. In the event of a sudden change in the start or at the end of lightening, moulting was regulated by an endogenous rhythm in *Panulirus japonicus phyllosoma* [11, 12] studied

influence of temperature and photoperiod in *Penaeus semisulcatus* and found that photoperiod and increased temperature induced successful maturation and even multiple spawning within the same moulting period. [13] also observed that highest moulting occurred at 32 °C as compared to 20 °C in Narrow-clawed crayfish *Astacus leptodactylus*.

During the present study, prawns being subjected to complete darkness (24D: 00L 18.55 °C) was similar to the eyestalk ablation, as in both the cases the main aim was to keep the optic ganglion from being exposed to light. Therefore the observation made in both the cases should have similar affects. [14] Observed that moult inhibiting hormone was produced in the eyestalk and stored in the sinus gland and moulting hormone is produced in Y-organ. Thus the removal of eyestalk causes an increase in ecdysteroid secretion from the Y-organ, which induces precocious moulting [13]. Ecdysteroid secretion from the Y-organ may be regulated not only by changes in the hemolymph MIH, but also by changes in the responsiveness of the Y-organ to MIH [15, 16, 17, 18, 19, 20] Reported that removal of the x-organ sinus gland complex (XO-SG) which secretes neurohormones that control the endocrine system regulating moult and reproduction, can induce both these processes. [16] Found that moult control in crustaceans is completely based on the presumption that (MIH) is released only during intermoult, in which its action is to repress ecdysteroid synthesis, leading to permissive entry to premoult and subsequent moulting. The findings of [21, 22] indicated that the processes of moulting, moult frequency and growth on noble crayfish *Astacus astacus* and Acorn barnacle *Austromegabalanus psittacus* are increased by warm water compared to cold water, which is in agreement with present findings. Photoperiod has been shown to affect food consumption, moulting frequency, the incidence of cannibalism and growth performance of crustaceans [2, 23]. Contrary to our findings, [24] reported that freshwater prawn juveniles grown for 110 days under total darkness (L0:D24) had higher weight gains than those grown under other light regimes at (L12:D12, L16:D8, or L20:D4).

It was interesting that prawns cultured at 24L: 00D (17.50 °C) consumed good feed, and grew faster, due to better assimilation efficiency. Observations at cleaning times suggest that prawns appeared less motile at continuous light group 24L: 00D (17.50 °C) than at control 12D:12L (16.49 °C). Less movement means more energy for somatic growth and may contribute to the growth improvement of prawns at 24L: 00D (17.50 °C). Although statistically significant results were obtained in this study, the tendency of more frequent moulting of prawns observed at 24L: 00D (17.50 °C) may be biologically important and deserves some attention.

The present study provides some preliminary evidence that moulting and growth of *M. dayanum* could be potentially stimulated, to a certain extent, by manipulating temperature and photoperiod in the culture system without any adverse effect on prawn survival. Although controlling light in outdoor culture ponds was likely to be unpractical, the findings of this current study may be applicable to indoor intensive systems only. Further research, however, was recommended to better understand the mechanism behind these findings, and to assess the potential of these findings for commercial prawn farming operations.

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