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EFFECT OF PHOTOPERIOD AND TEMPERATURE ON GONADAL DEVELOPMENT OF FRESHWATER PRAWN *MACROBRACHIUM DAYANUM*

PERVAIZ A.P.[1]*, SUDAN MADHU[2] AND SIKDAR MALABIKA[1]

[1]Department of Zoology, School of Biological Science, Dr. Harisingh Gour University, Sagar -470003 (M.P,) India.
[2]Department of Zoology, University of Jammu, Jammu-180006 (J&K). India.
*Corresponding Author: Email-pervaiznayak@gmail.com

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Abstract- The effect of photoperiodism and temperature on gonad of a freshwater *Macrobrachium dayanum* was investigated in the laboratory for 30 days. Animals with uniform testes and ovarian condition were selected for the experiment. The experimental prawns were divided into three groups i.e., control (12L: 12D, 24° C), continuous dark (24D: 00L, 27° C) and continuous light (24L: 00D, 30°C). On histological point of view, exposure to Continuous light (24L: 00D, 30° C) showed post-vitellogenic stage and mature spermatozoa predominant over those of corresponding groups. Under continuous darkness (24D: 00L, 27° C) gonadal maturation was regressed. On the other hand, Control group 12L: 12D (24°C) promote gonadal maturation more compared to treated group 24D:00L (27°C) in *M. dayanum*. Statistically also, there occurred a significant increase in the ovarian and testicular index in prawns subjected to complete light as compared to the prawns kept under control condition and the changes which occurred in complete darkness was not significant in comparison to control. Warm temperature alone cannot induce gonadal maturation; long photoperiod indeed was apparently an essential factor.

Keywords- *Macrobrachium dayanum*, post-vitellogenic, regressed, increase, gonadal maturation, apparently.

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Introduction

Seasonal changes in day length or photoperiod act as an external temporal clue to start a series of physiological processes. As a result, certain events like growth and spawning are restricted to specific times of the year. These photoperiodically controlled reactions suggests a capacity of the organisms to distinguish between short and long days and therefore to measure physical processes and phenomena. This measurement seems to be based, at least in some species, on originating rhythms [1].

The induction of ovarian maturation and spawning of female penaeid shrimps are mainly carried out by using the unilateral eyestalk ablation technique [2]. This technique is used worldwide in hatcheries, many difficulties, such as deteriorations in spawn, larval quality and quantity over time have been joined with it [3]. Other techniques to the eyestalk ablation method, such as temperature and/or photoperiod manipulations, hormone injections have been examined in different shrimp species. [4] with *Penaeus duorarum*, and [5], with *Penaeus semisulcatus* studied the effects of temperature changes on induced maturation and spawning with a high degree of success.

In general, long photoperiods and high temperatures were reported to be required for reproduction in *Penaeus duorarum* [4]. Low temperatures less than 25°C are known to discourage mating, gonad development and spawning in *Penaeus stylirostris* [6] and *Penaeus semisulcatus* [7]. Cycling temperature fluctuations between 20 and 28°C induce maturation and spawning in *Penaeus duorarum* [4] and *Penaeus semisulcatus* [5]. The cycling temperature fluctuation has been suggested to be an effective technique in obtaining off-season reproduction in the green tiger shrimp *P. semisulcatus* [5].

A few thermal manipulating experiments have also been conducted to induce spawning in *P. trituberculatus* at 21°C [8] and *Menippe mercenaria* at 25°C [9]. However, several experiments combining altered temperature and photoperiod conditions have been performed *Penaeus merguiensis* 22°C and 27°C, 10L:14D and 14L:10D [10,11] *Penaeus semisulcatus* 20-28°C, 10L:14D and 14L:10D [5] *Penaeus esculentus* 26°C, 14L:10D [12] *Jasus edwardsii* natural vs. compressed 9 month treatment [13] *Homarus americanus* 9.8-15°C, 8L:16D and 16L:8D [14] and 13-14°C, 8L:16D [15] *Panulirus japonicus* 13°C, 19°C, and 25°C, 10L:14D and 14L:10D [16]. All cited manipulated environmental conditions resulted in some degree of successful gonadal maturation of the respected species.

The principal aim of this research was to elucidate the effects of photoperiodism and temperature mechanism that regulate the key physiological processes of maturation of gonads in *Macrobrachium dayanum* with respect to understanding reproductive biology and growth. Moreover, such knowledge is necessary reliably to egg production for aquaculture of the crustaceans. To clarify the factors affecting initiation of gonadal development, further studies on the developmental processes of gonads, particularly connected to the function of reproductive hormones, were needed because the development was primarily inhibited by the endocrine system.

Materials and Methods

Location of collection

The specimens of *M. dayanum* were collected from local Lake of Sagar, and were brought to the laboratory of Department of Zoology, Dr. Harisingh Gour University Sagar (MP) under oxygen packing in live condition.

Acclimatization of test animals and Experimental setup

120 specimens of *M. dayanum*, both males and females looking apparently healthy in the size group of 35-70 mm in total length, were taken specimens with uniform testes and ovarian condition (immature) only were selected for the experiment, and were kept in aquariums. All aquaria were aerated with oxygen by supplying air continuously through air-stones from an aerator. They were 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. The water was changed after an interval of 2-3 days. The prawns were divided into the following three groups each having 20 specimens. Prawns were acclimatized to laboratory conditions for a period of two weeks no mortality was recorded during this period. After acclimatization, experiment was started from 1st May to 30th May 2010 for one month and then terminated. Temperature was maintained with automatic thermostat heater.

Group I

This group was subjected to natural day and night light duration and intensity and maximum temperature of water was found to be around 24˚C.

Group II

This group was kept under complete darkness for 24 hours throughout the experimental period. For the purpose, the aquarium was totally covered with thick cardboard box and further covered with a black cloth in order to maintain complete darkness and temperature was maintained at 27˚C.

Group III

This group was kept in continuous light for 24 hours. For this purpose a bulb of 40 Watt producing yellow light was lit continuously above the aquarium and temperature was maintained at 30˚C.

Histology of Gonads

The effects of different photoperiod and temperature on reproductive functions were assessed by gravimetric and histological techniques. The colour of the testes and ovaries were observed through carapace. At the completion of the experiment, the prawns were dissected out quickly for the gonads and body weight and gonadal weight was recorded immediately after sacrifice. Gravimetric data were expressed in terms of the gonadosomatic index (gonadal weight/body weight x 100) since gonadal size in this species depends on body weight. After weighing, ovary and testes were immersed in alcoholic Bouin's fluid for 24 hours. After proper fixation, the material was transferred to 70% alcohol. The alcohol was changed daily for 3 to 4 times till the Bouin's got completely removed. The material was then dehydrated in different grades of alcohol, cleared in xylene, passed through molten wax and finally the paraffin blocks were prepared. After embedding, the wax blocks were trimmed. Sections of the gonads were cut at 5µm in the thickness for histological preparations and sections were mounted serially on slides, which were further stained, by Harris-Hematoxylin and Eosin stain. All slides of gonadal sections were examined under Zeiss binocular phase contrast microscope for maturation and density of different types of cells.

Statistical analysis

All the series of experiments were done in hextuple. 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 determined as mean with standard deviation (±SD) values for the experimental data.

Results

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

Continuous light regime and higher temperature were found to accelerate the gonadal maturation to developed state in *Macrobrachium dayanum*. The shortest gonadal maturation was recorded in continuous dark group. The intermediate maturation was recorded in control group. All female and male prawns exposed to the long photoperiod and warm temperature advanced to the pre-spawning conditions.

Histological Observation of Ovaries

Group I. 12L:12D (24°C)

The ovaries of the control group 12L:12D and (24°C) revealed that it was mainly composed of oocytes in late stage of previtellogenic development. Oogonia were small, oval or rounded cells each with a spherical nucleus occupying almost the entire cell, and the cytoplasm was intensely basophilic. Oogonia were observed in the centre of the ovary near the previtellogenic oocytes (primary oocytes). Oogonia and previtellogenic oocytes were observed in abundance in this group. There were follicle cells aggregated in clusters in some places found around oocytes and vitellogenesis formation starts, which were clearly seen through vitellin enhancement in cytoplasm of oocytes. The colour of ovary in this group was greenish as observed. The average GSI value was about 2.469 ± 0.164 [Table-1] and [Fig-1].

Group II. 24D:00L (27°C)

In 24D:00L and (27°C) the oocytes appeared small in size compared to those in 12hL:12D and (24°C) group and some oocytes were observed in an abnormal architecture. The ovary in this group was mainly composed of oogonia and previtellogenic oocytes. The abnormal structure of these oocytes reflects the lower significance in gonadosomatic index than that of the prawns exposed for simulation of higher temperature conditions. Oogonia were observed near the periphery of the previtellogenic oocytes and the cytoplasm was intensely basophilic. Previtellogenic oocytes present in this group were mainly composed of previtellogenic oocyte I and II. There was a central proliferation zone oocytes I and II were prominent, oocyte III (previtellogenic) appeared, which was characteristic of this group. The colour of ovary in this group observed was translucent white. The average GSI value was about 1.978 ± 0.020 [Table-1] and [Fig-2].

Table-1 *Showing the effect of Photoperiodism and temperature on the ovarian development of M. dayanum.*

S. No.	Treatment	GSI = (wt. of gonad / wt. of body x 100). Ovarian index (Mean ± S.D)	Colour of ovary
Group I	Control (12L:12D,24° C)	2.469 ± 0.164	Greenish
Group II	Continuous dark (24D:00L, 27° C)	1.978 ± 0.020 ns	Translucent
Group III	Continuous light (24L:00D, 30° C)	5.483 ± 0.206***	Dark Green

Data has been represented as mean ± standard deviation (n=6)
ns, not significant; *** indicates values are highly significant P<0.001 compared to control respectively.

Table-1.1 *Relative abundance criteria employed for ovarian development in M. dayanum. Relative abundance of various oogonial cells in ovaries*

Treatment	Oogonia	Previtellogenic oocytes	Vitellogenic oocytes	Postvillogenic oocytes
(12L:12D,24° C)	+++	++++	+	-
(24D:00L, 27° C)	+++	++++	-	-
(24L:00D,30° C)	+	++	-	+++

+to+++++ indicates the degree of abundance; -, means not found.

Group III. 24L:00D (30°C)

The developed oocytes i.e. postvitellogenic oocytes were mainly observed in the ovaries of prawns maintained at 24L:00D and (30°C) and were in abundance over those of other oogonial cells. The cells that compose the ovary are of three main types i.e., oogonia, oocytes in different stages of development and follicle cells as observed. No degenerating oocytes were observed in this group. The follicle cells were found around the oocytes and were responsible for supplying nutrients. Cortical specializations, also called cortical rods or cortical bodies, were membrane-bound organelles that assembled during oocyte development and became associated with the cell membrane in mature eggs. Developed stage was

seen exactly before the spawning and can be considered as ultimate limit of the ovary maturity and gonads occupy anterior region of abdomen as well as carapace cavity. The mature oocytes have reached their maximum number. Histological examination in the gonads of *M. dayanum* revealed that the ovary was composed of oocytes in small and big sizes and devoid of yolk granules. The colour of ovary in this group was dark green as observed. The average GSI value was about 5.483 ± 0.206 [Table-1] and [Fig-3].

Histological Observation of Testes

Group I. 12L:12D (24°C)

Histological sections of testes revealed that each testicular lobe was composed of innumerable testicular acini held together by connective tissue. Testes of *M. dayanum* were made up of large number of seminiferous tubules of varying sizes held together by connective tissues. In histological sections of testicular lobes of *M. dayanum* a germinal zone containing spermatogonia cells and nurse cells were apparent. Each spermatogonium contains a thin rim of cytoplasm around a vesicular nucleus. This group was mainly composed of spermatocytes and spermatids in abundance.

The primary spermatocytes have eosinophilic cytoplasm. The primary and secondary spermatocytes did not show any marked difference in size. The secondary spermatocytes, followed by second maturation division, gave rise to spermatids and then spermatozoa. The colour of testes in this group observed was translucent. The average GSI value was about 1.745 ± 0.015 [Table-2] and [Fig- 4].

Table-2 *Showing the effect of different wavelengths and light intensities on Gonadosomatic index of testicular development of M. dayanum.*

S. No.	Treatment	GSI = (wt. of gonad / wt. of body x 100). Testicular index (Mean ± S.D)	Colour of Testes
Group I	Control (12L:12D,24° C)	1.745 ± 0.015	Translucent
Group II	Continuous dark (24D:00L, 27° C)	1.651 ± 0.074 ns	Translucent
Group III	Continuous light (24L:00D, 30° C)	2.168 ± 0.074***	Yellowish

Data has been represented as mean ± standard deviation (n=6)
ns, not significant; *** indicates values are highly significant P<0.001 compared to control respectively.

Table-2.1 *Relative abundance criteria employed for testicular development in M. dayanum. Relative abundance of various spermatogonial cells in testes.*

Treatment	Spermatogonia	Spermatocytes	Spermatids	Spermatozoa
(12L:12D,24° C)	+	+++	++++	++
(24D:00L, 27° C)	++	++++	+++	+
(24L:00D,30° C)	+	+	+	++++

+to+++++ indicates the degree of abundance; -, means not found.

Group II. 24D:00L (27°C)

The spermatogonia undergo meiotic division to give rise to smaller cells, the spermatocytes. In immature animals the testicular acini were completely empty. The aciner wall was found thicker in immature animals and contained only a small germinal zone with non-differentiated germ cells and spermatids. Spermatozoa were also seen but not so much developed as they were in early stage of development. Chromatin material found in nucleus was deeply stained with Hematoxylin. The colour of testes in this group observed was translucent. The average GSI value was about 1.651 ± 0.074 [Table-2] and [Fig.-5].

Group III. 24L:00D (30°C)

In histological sections, testes were thin, translucent and extremely delicate organs. This group was mainly composed of mature spermatozoa. In fully mature males, the germinal zone was very much restricted and acini were fully occupied with cells spermatids and spermatozoa. Nurse cells were found dispersed in between spermatogonia. By virtue of their close association with gonadal cells, they were assumed to have a nutritive and supportive role. The various entities of spermatogenesis *viz.* spermatogonia, spermatocytes, spermatids and spermatozoa were usually observed.

Spermatogonia were the first group of cells to appear during the process of spermatogenesis and hence were most populous near the germinative zone of maturing testes. These were circular and basophilic structures with a network of chromatin material and nucleoli but indistinct nuclear wall.

The spermatids were small rounded bodies. They had a little cytoplasm and most of their volume was occupied by a large nucleus. Chromatin material found in nucleus was deeply stained with Hematoxylin. Finally, the spermatids had undergone certain morphological changes to produce spermatozoa. The spermatozoa were crescent shaped structures bearing a short tail, midpiece and head. The colour of testes in this group observed was yellowish. The average GSI value was about 2.168 ± 0.074 [Table- 2] and [Fig- 6].

Gonadosomatic Index (GSI) Observations

(a) The Effect of Photoperiod and Temperature on the Ovarian Development of *M. dayanum*

The results obtained are summarized in [Table-1]. The examination of this table indicated clearly that there was a significant increase in the gonadosomatic index values varied at different combinations of photoperiod and temperature. The Tukey's multiple comparison test of ANOVA, however, indicated significant increase in the ovarian index in the prawns belonging to the group III as compared to group I ($P<0.001$). On the other hand, the ovarian index of group II decline non significantly as compared to group I ($P>0.05$). When we compared, group III and group II it was observed that ovarian index increased significantly in group III over those of group II ($P<0.001$). Postvitellogenic oocytes were observed in the ovaries of prawns of the group III [Fig-3]. On the other hand, the oogonia, previtellogenic oocytes and initiation of vitellogenin were observed in the ovaries of the prawns belonging to the groups I and II [Fig-1 and 2].

(b) The Effect of Photoperiod and Temperature on the Testicular Development of *M. dayanum*

[Table-2] depicts the results obtained in the experiment. The testes of the treated prawns belonging to the group III showed remarkable increase in their size when compared to those of the group I. Tukey's multiple comparison test of ANOVA shows significant increase in the average testicular index in the prawns of group III over those of the group I ($P<0.001$). On the other hand, the testicular index of group II was not significant as compared to group I ($P>0.05$). When we compared, group III and group II it was observed that testicular index increased significantly in group III than group II ($P<0.001$). In group III, testicular follicles were found to be full with spermatozoa [Fig-6], whereas in group I and II the testicular follicles were observed to contain the spermatogonia, spermatocytes, spermatids and sparsely distributed spermatozoa [Fig- 4 and 5].

Discussion

Photoperiod and temperature were necessary factors in regulating gonadal maturation in *M. dayanum*. An out of season long photoperiod-warm temperature stimulated testicular and ovarian development. Neither a long photoperiod nor a warm temperature alone could induce fully gonadal maturation to the spawning conditions. Similar findings had been given by [16] in *P. japonicus* that the effects of photoperiod and temperature on ovarian development were not independent and ovarian development depends on the combined conditions of these factors. Histological studies also revealed stimulatory effects of the long light regimes at high temperature on testicular maturation and stimulation of spermatogenic activity in *M. dayanum*, resulting in a longer spawning period. Similar photoperiod-induced changes have been reported by [17]. The testes were formed by

seminiferous tubules and like most Decapods, the spermatogonia were located in the most peripheral portion of these tubules [18].

Control group 12L:12D (24°C) promote gonadal maturation more compared to treated group 24D:00L (27°C) in *M. dayanum*. Therefore, warm temperature alone cannot induce gonadal maturation in this species. The present findings were however, in contrast to those observed in crayfish *A. leptodactylus* [19] exposed to three different light regimes natural day light regime control (10.04L:13.96D), (24D:00L) and (8L:16D) and found that the higher percentage of ovigerous females in the darkness group mated and spawned earlier than in the other groups. For other Decapods, higher temperature was the predominant cue while photoperiod supports the induction in *P. japonicus* [16], *P. merguiensis* [11], and *P. semisulcatus* [5]. [20] observed that temperature of the water was probably the most important environmental variable in prawn cultures, because it directly affected metabolism, oxygen consumption, growth, moulting and survival. Temperature was an important abiotic factor that modulates many aspects of *C. quadricarinatus* biology including growth and reproduction [21,22]. Not long past study by [23] on sexually undifferentiated juveniles showed that it was possible to manipulate the sexual differentiation of early juveniles towards a higher proportion of males by means of increasing temperature.

The present investigation gets confirmation from the studies of [24] that adult prawns were tolerant to wide range of water temperature (18-34^{o}C). However, in transport studies, [25] reported that lower temperatures (19-20^{o}C) lower metabolic rates, increase survival and reduce activity, oxygen consumption and nitrogenous excretion. However, in other species, temperature has a greater influence on spawning than photoperiod as seen in the mud crabs, *Scylla serrata* [26], *Scylla paramamosain* [27]. In these experiments, the animals were exposed to constant light conditions with different temperatures, which all resulted in higher production at higher temperature. However, findings by [28,29] does not lend much support to present observations as they found that the combined conditions of warm temperature (10-17°C) and darkness successfully accelerated ovarian maturation from mid-December to mid-January and induced egg laying 5 weeks earlier than in the control group. Long photoperiod exposure (16th of light per day) did not accelerate ovarian maturation to the same degree; it did not promote earlier egg lying.

Long photoperiods and temperatures above 25°C are known to be suitable for maturation of shrimp species such as *P. semisulcatus* [5]. In subtropical regions, temperatures less than 25°C, encountered during late-autumn, winter or early-spring, depress gonad development and spawning in shrimps (even in eyestalk ablated females) [5]. Despite providing optimal conditions, shrimp broodstock may not readily develop ovaries and spawn in off-reproductive season in captivity, but applying cyclic temperature fluctuations between optimal and sub-optimal levels (20 and 28°C) has proved to induce maturation and successful spawnings in *P. duorarum* by [4] and in *P. semisulcatus* by [5].

To observe effect of the photoperiod on growth and spawning efficiency of *Nile tilapia* broodstock in a recycling system, the fish were subjected to four photoperiod treatments (24L:00D) light dark, (18L:6D), (12L:12D), (6L:18D) by [30] who suggested that a (12L:12D) photoperiod regime should be adopted for maximum growth, seed making and spawning frequencies of *Nile tilapia* broodstock reared in intensive re-circulating systems. On the other hand, studies by [31] showed the effect of six photoperiod protocols on the spawning time of two rainbow trout, *Oncorhynchus mykiss*. They found that no significant difference was observed in the percentage of mature females between experimental and control group. However, [32] observed that photoperiod was not a significant predictor in the model in addition to temperature.

Conflicting results on reproductive processes upon a critical photoperiod near 12hr. L has been reported for several crustacean species [14]. Effects of environmental factors on reproduction had long been examined in many Decapods in order to understand and control the reproductive process. In contrast to present study, [33] observed that temperature was the prominent environmental factor controlling reproduction in Crustaceans. [34] observed that the particular combination of photoperiod and temperature inducing ovarian development varies with species or population because response to these factors depended on their natural environment and season of breeding. Concerning the factors that induce breeding in Decapods [35] reported that an increase in water temperature, worked as a trigger in the fresh water prawn *Macrobrachium nipponense*. However, in the present study it was found that in *M. dayanum*, prevention of ovarian development at higher temperatures under the short photoperiod obviously demonstrated that increase in water temperature alone does not induced ovarian development.

Decrease in the ovarian and testicular index in prawns subjected to complete darkness 24D:00L (27°C) and increase in ovarian and testicular index in prawns subjected to complete light 24L:00D (30°C) in comparison to the prawns kept under control condition 12L:12D (24°C) were observed in the present investigation. The reason for this variation was due to the change in photoperiodism and temperature.

Photoperiod and temperature, has a high potential not only for accelerating growth but also for producing broodstocks in a shorter time, although the hatching rate, the quality of eggs, and larvae should be further investigated. In the present investigation, development in size of ovary and testes and formation of advanced ova i.e. postvitellogenic oocytes and spermatozoa were observed in the prawns subjected to complete light in comparison to the control. This was predicted due to the decrease in the activity of the neurosecretory cells of the optic ganglion in the eyestalk in absence of light. At the same time the prawns subjected to complete light exhibited lesser development in the ovary and testes in comparison to control. This may be due to the increased activity of the neurosecretory cells in the eyestalk in presence of continuous light.

From the present investigation it could be concluded, that by keeping the prawns in complete darkness, one can reduce the secretion of gonadal inhibiting hormone (GIH) from the eyestalk. Therefore, eyestalk ablation, which was a painful surgical technique, could be avoided for early maturation. Moreover, a detailed study on this aspect could standardize the exact duration and intensity of photoperiodism required by different species of commercially important prawns. Combined results of these experiments indicated that, spermiogenesis and final oocyte maturation depend on a combination of long photoperiod and warm temperature. The long photoperiod and warm temperature regime resulted in stimulating spawning, whereas the short photoperiod and cold temperature regime resulted in gonadal regression. The effect of day length on gonadal activity thus seemed to be mediated via the hypothalamus.

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