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Pervaiz Ahmed Pervaiz1
Department of Zoology,
School of Biological Science,
Dr. Harisingh Gour University,
Sagar -470003 (M.P). India.

Madhu Sudan2
Department of Zoology,
University of Jammu, Jammu-180006 (J&K). India.

Malabika Sikdar1
Department of Zoology,
School of Biological Science,
Dr. Harisingh Gour University,
Sagar -470003 (M.P). India.

Correspondence
Malabika Sikdar1
Department of Zoology,
School of Biological Science,
Dr. Harisingh Gour University,
Sagar -470003 (M.P). India.

Effect of photoperiod and temperature on development and growth of hepatopancreas of freshwater prawn *Macrobrachium dayanum*

Pervaiz Ahmed Pervaiz, Madhu Sudan, Malabika Sikdar

Abstract
The effect of photoperiod and temperature on the development of Hepatopancreas was studied in laboratory for 30 days and divided into three different light regimes. 5 types of cells E-(embryonic) cells, F-(fibrillar) cells, B-(blister-like) cells, R-(resorptive/absorptive) cells and M-(myoepithelial/midgut) were found in Hepatopancreas of *Macrobrachium dayanum*. From the studies of histology and hepatosomatic index, (24L:00D) group showed an increase of the lumen of the tubules with a consequent compression of the epithelial cells, which formed a thin layer. In 24D:00L group illustrated cellular disruption, highest degree of abnormality with heamocytic infiltration and disrupted cells, disappearance of lateral cell membranes and lyses of cytoplasm in many areas. In control (12L:12D) prawns exhibited the well-organized glandular tubular shape normally seen in prawn species.

Keywords: Hepatopancreas, *Macrobrachium dayanum*, Lumen, Cellular disruption, Lyses, Glandular tubular.

1. Introduction
The crustacean hepatopancreas is a multilobate diverticulum of the midgut, which consists of a multitude of blind tubules lined with a single layer of epithelial cells [1]. The hepatopancreas, being analogous to the liver and combining many of the functions of the liver, pancreas, and intestine of vertebrates, plays an important role in several metabolic processes in crustaceans [2, 3]. Studies on the hepatopancreas at different biological levels such as the structure, development, physiology, metabolism, and biochemistry concluded that this digestive organ possesses several functions, including absorption, digestion, storage, and secretion [2]. Therefore, the hepatopancreas, or so-called mid-gut gland, is a very important organ for crustaceans.
The Decapod hepatopancreas is the primary location of energetic lipid storage, and fatty acid composition is determined by a combination of diet, biosynthesis [4] and temperature. [5] found that environmental temperatures and diet influenced fatty acid composition in caprellidean amphipods. As a result of temperature-induced changes, lipid membranes and fatty acid stores undergo homeoviscous adaptation, ideal viscosity for immediate environmental conditions [6]. This may occur by fatty acid synthesis or by the oxidation/reduction of existing fatty acids [7].
The hepatopancreas due to its critical role in the digestion of food is richly supplied with hemolymph through main blood vessels that branch out into small capillaries to nourish each individual hepatopancreatic tubule [8]. Proper functioning of the hepatopancreas is therefore important to the health, growth and survival of cultured prawns [9]. As for nutrient utilization, it is an excellent model for food digestion and cell secretion because of its primary role in the synthesis and secretion of digestive enzymes, final digestion of the ingested food and subsequent uptake of nutrients [10]. The other important role of hepatopancreas is detoxification such as toxin in feed and pollutants in environment [11, 12].
Typically, the organs with the highest content of lipid are the hepatopancreas and the ovary. Changes in the relative size of hepatopancreas (hepatosomatic indices) and its moisture content are used to evaluate nutritional status, as well as crayfish condition and exposition to environmental stress [13]. The annual cycle of freshwater crayfish can be characterized by changes in hepatopancreas energy content, which serves as the main source for the ovarian development, growth and moulting of the crayfish [14, 13]. [15] studied the relationship between gonad indices and hepatopancreas indices in males and females of *Sesarma intermedia*,

while [16] investigated changes of hepatopancreas and testis indices during the reproductive period of male fiddler crabs, *Uca lactea*.

The principal aim of this research was to elucidate the effects of photoperiodism and temperature mechanism that regulate the key physiological processes of maturation and growth of hepatopancreas in *Macrobrachium dayanum* which helps overall development of an animal.

2. Materials and Methods

2.1 Procurement, Maintenance and Acclimatization of Test Animals

Freshwater prawn *Macrobrachium dayanum* (length 3.5-7.0 cm, weight 700-25, 00 mg) collected from local Lake of Sagar. The animals were brought to the laboratory under oxygen packing in live conditions and kept in fiber aquaria of the size of 36x12x12" contained non-chlorinated fresh water under controlled conditions well aerated and continuously recycled through aerator. Before experimentation, prawns were treated with 0.1 $KMnO_4$ solutions to obviate any dermal infection and acclimatized for two weeks to laboratory conditions. The experiment was done for one month from 1st May to 30th May 2011 and then was terminated.

2.2. Experimental Setup

2.2.1 Effects of Photoperiod on Hepatopancreas of *M. dayanum*

60 animals of *M. dayanum* of both male and female of equal weight were selected for experiment. The prawns were divided into three groups viz. group (I) control (12L:12D), group (II) continuous darkness (24D:00L) and group (III) continuous light (24L:00D).

2.2.2. Histology of Hepatopancreas

The effects of photoperiod and temperature on hepatopancreatic functions were assessed by histological techniques. At the completion of the experiment the prawns were dissected out quickly for the hepatopancreas and body weight and hepatopancreatic weight were recorded immediately after sacrifice. Gravimetric data were expressed in terms of hepatosomatic index (hepatopancreas weight/body weight) x 100. After weighing, hepatopancreas were fixed and processed. Paraffin blocks were prepared and sections of the hepatopancreas 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. Hepatopancreatic sections were examined under Zeiss binocular phase contrast microscope for maturation and differentation of different types of cells.

2.2.3. Statistical analysis

All the series of experiments were done for hepatopancreas in triplets. 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 (±SD) values for the experimental data.

3. Results

3.2. Histopathological Observation of Hepatopancreas

Histological sections of hepatopancreatic epithelium in this study suggests that tubules were composed of five main cell types in *M. dayanum* (Fig. 1, 2 and 3) viz. E (embryonic) cells, F (fibrillar), R (resorptive), B (blister-like) cells and M (myoepithelial/midget) cells, distributed throughout the whole tubule.

3.3. Group I. Control

Phase contrast microscope preparations of control group (12L:12D) (Fig. 1) demonstrated that the hepatopancreas consisted of numerous blind ending tubules loosely bounded together by connective tissue and branching in a racemose manner. The hepatopancreas of control shrimp exhibited the well-organized glandular tubular structure. A longitudinal section of the apical region of a hepatopancreatic tubule showed that the cell surface facing the lumen was covered with a microvillus brush border and the tubule apex contained undifferentiated embryonic cells (E-cells). Moving away from the apex, the cells began to differentiate into developing absorptive, storage (resorptive) cells (R-cells). Middle proximal region of the tubules showed that the tubules were empty of food material and appeared in a hexagonal arrangement or ''star shape" in the lumen, and the basal lamina outlined each tubule.

Control specimens showed five kinds of cells that were easily differentiated. The E-cells (embryonic cells) were the undifferentiated cells. The embryonic cells predominated at the distal ends of the tubules and were known to give rise to the remaining cell types. The light cells (resorptive, R or absorptive cells) predominated over the dark cells (fibrillar or F-cells) and B-cells. F-cell contained a single vacuole of variable dimensions lying distal to the nucleus. The B-cells were known as the secretory cells, blister like cells. They were characterized by a large vacuole which often projects into the lumen of the tubule. B-cells occur in large number in association with R and F-cells. The cells were loaded with secretory granules inside and at the peripheral regions of the vacuoles. The M-cells (myoepithelial/ midget cells) were scattered throughout most of the length of the tubule. They were the smallest and least numerous of all the cell types and always occur individually at the basal part of the epithelium. The hepatosomatic index observed in this group was 3.688 ± 0.722 (Table 1).

3.4. Group II. Continuous dark (24D:00L) Exposed

Sections of continuous darkness treated group illustrated cellular disruption and disappearance of lateral cell membranes. The basal lamina appeared folded in some places. Moreover, the cells were shrinked leaving the basal lamina behind. On the other hand, necrosis of the microvilli border was noted in some places and distorted appearance was observed in others. The study suggests lyses of cytoplasm in many areas. Many vacuoles containing cytoplasmic debris could be observed with highly increased frequency than normal. Hepatopancreas in this group also showed heamocytic infiltration in the interstitial sinuses. Mitochondria and lipid droplets could not be easily detected in the cytoplasm. Most of them contained abundant heterochromatin distributed both centrally and peripherally.

Specimens exposed to 24 hour dark showed an increased proliferation of F-cells in the middle region of tubules. The proliferation of vacuolated B-cells almost up to the distal end of the tubules and reduction in the number of E-cells were also noticed. The tubules appeared shrunk and disintegrated in a majority of cases. Collapse of structural integrity of the tubules was evident with the heavily vacuolated cells and cell debris

piled up in the lumen. Shrinkage and breakage of tubules and enlargement of tubule lumen resulted in structurally void areas in the hepatopancreas. The tubules appeared shortened and the middle region of the hepatopancreas almost devoid of any distinct and defined morphological features (Fig. 2). The hepatosomatic index observed in this group was 3.096 ± 0.935 (Table 1).

3.5. Group III. Continuous light (24L:00D) Exposed

Treatment with 24 hour continuous light showed an enlargement of the lumen of the tubules with a consequent compression of the epithelial cells, which formed a thin layer. The lumen of the tubules was occupied by an abundant secretion. Histologically, there were slight differences in the size of hepatopancreatic tubules when compared with the control group, this was confirmed from the hepatosomatic index also. Growing R-cells were those in which the cytoplasm characteristically contained numerous vacuoles and lipid droplets, while (fibrous) cells (F-cells) were more basophilic than R-cells. This region also contained the large distinctive secretory (blister) cells (B-cells), each of which contained one large apical secretory vacuole.

M. dayanum showed single row of E-cells, indicating the formation of fewer new cells. Response to a 24 hour continuous light group showed an increase in the number of B-cell vacuoles and their presence right up to the distal region of the tubules accompanied by an extremely reduced number of F- cells. A number of cells were found extruded into the lumen in the proximal region. However, no shortening or distortions of tubules were noticed (Fig. 3). The hepatosomatic index observed in this group was 3.971 ± 0.971 (Table 1).

Table 1: Showing the effect of different photoperiods on hepatopancreas of *M. dayanum*.

S. No.	Treatment	HSI = (Wt. of hepatopancreas/ wt. of body) x 100. (Mean ± S.D)
Group I	Control (12L:12D)	3.688 ± 0.072
Group II	24 h. dark (24D:00L)	3.096 ± 0.093^{ns}
Group III	24 h. light (24L:00D)	3.971 ± 0.097^{ns}

Data has been represented as mean ± standard deviation (N=3)
ns, not significant.

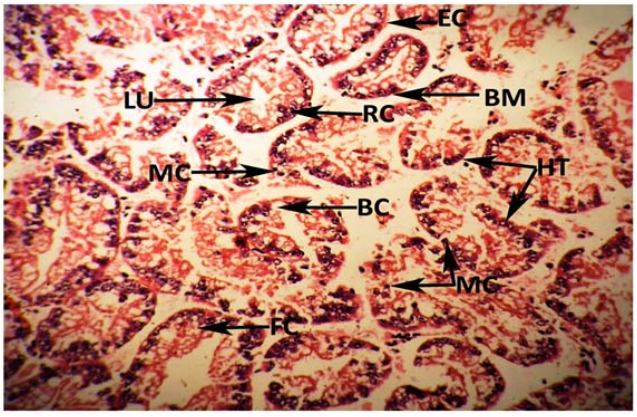

Fig. 1: Photomicrograph of longitudinal section of hepatopancreas of control group (12L:12D) showing normal cells (X 100). H& E Stain.

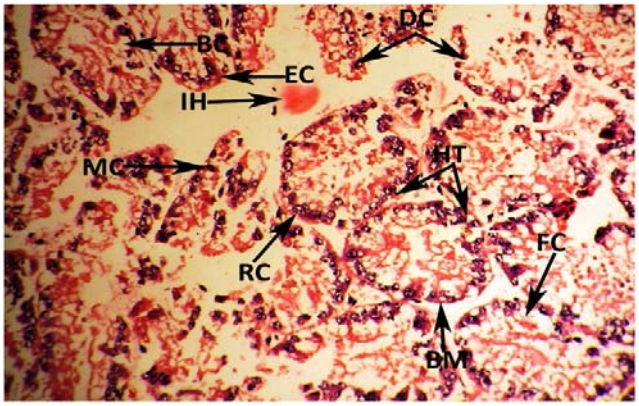

Fig 2: Photomicrograph of longitudinal section of hepatopancreas of continuous dark group (24D:00L) showing disrupted cells (X 100). H& E Stain.

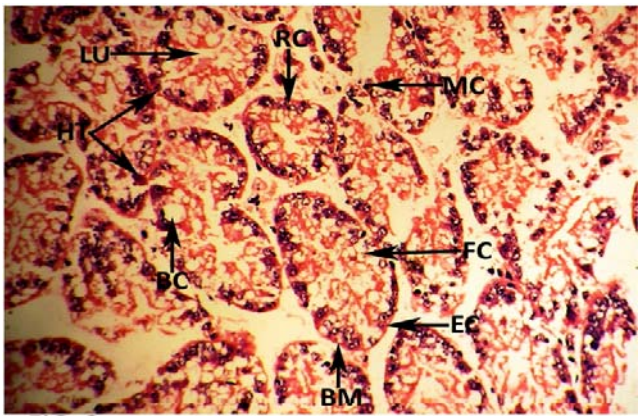

Fig 3: Photomicrograph of longitudinal section of hepatopancreas of continuous light group (24L:00D) showing enlarging tubules (growing cells) (X 100). H& E Stain.

3.5. The Effect of Photoperiod on the Hepatopancreatic Growth of *M. dayanum*

There was statistically no significant increase or decrease observed in the hepatopancreas of both groups (III and II) continuous light and continuous dark in comparison to the control group I ($P>0.05$), but according to values obtained in table 1, there was slight increase of growth in group III as compared to group I. On the other hand in group II, growth was less as compared to group I.

4. Discussion

Results of the photoperiodic study on hepatopancreas of *M. dayanum* demonstrated increasing degrees of cell abnormality in prawns kept in 24 hour continuous dark. The highest degree of dystrophy of tubular epithelial and nodule formation in hepatopancreatic tissues of prawns of 24 hour continuous dark group supported the poorest growth responses as was also evident from hepatosomatic index. The observed damage to hepatopancreas disturbs its normal functions like secretion as well as absorption and storage of nutrient materials and obviously growth get too retarded. The tubule appeared shrunk and disintegrated in a majority of cases. Whereas in (24L:00D) group the myoepithelial cells remain largely intact though in several areas the tubule did not remain as tightly packed as in comparison to control animals. Increased vacuolation, secretions in the lumens, breakage of tubule connective tissue lining and sloughing off of the tubule epithelium have to be set in (24D:00L) treated prawns.

Coinciding with present findings, [17] and [18] also observed five types of cells in the hepatopancreatic tubule epithelium that takes part in the digestion process. In the present study, undifferentiated E-cells were found at the distal ends of each tubule with proximal nuclei and conspicuous nuclear bodies. The multi-vacuolated R-cells occur throughout the hepatopancreas surrounded by a network of myoepithelial cells. B-cells had smaller nuclei and nucleoli which were displaced to the periphery by a single large vacuole that were formed by the aggregation of smaller vacuoles as digestion progresses and were excretory in nature. The M-cells were smallest and least numerous of all the cell types at the basal part of the epithelium. F-cells were basophilic and intersperse between B- and R-cells in the middle region of tubules. [19] Observed holocrine mode of B-cells, rejecting any secretory role for the B-cells, which were recognized to be excretory in nature. However, [19] have proposed that only F-cells can perform this function. In this case B-cells were thought to be the main site for nutrient absorption and digestion (but not for their storage) and for the accumulation of waste products. In addition, the hepatopancreas fulfils a key role in the temporary storage of exoskeleton calcium, phosphate, glycogen and lipids during the different phases of the moulting cycle [20, 21]. In contrast to present findings, hepatopancreas are composed of blind tubules, which were internally lined with a single epithelial layer delimiting the lumen and consisting of at least four cell types, E (embryonic) cells, confined to the blind distal ends, and F (fibrilar), R (resorptive) and B (blister-like) cells, distributed throughout the whole tubule [22].

The *M. dayanum* hepatopancreas were the primary location of energetic lipid storage, and fatty acid composition and biosynthesis. [5] Observed that environmental temperatures and diet influenced fatty acid composition in caprellidean amphipods and as a result of temperature induced changes, lipid membranes and fatty acid stores undergo homeoviscous adaptation, ideal viscosity for immediate environmental conditions [6]. Desaturase enzymes control homeoviscous adaptation, and are affected by both temperature and ingestion of saturated lipids regardless of temperature. This may occur by fatty acid synthesis or by the oxidation/reduction of existing fatty acids [7].

Crustacean hepatopancreas was the most important organ in the general economy because it serves as the main energy reserve for growth and moulting [23]. Physiological stress was often reflected by important cytological changes in this sensitive organ hepatopancreas, as the central site of metabolism, presented histological alterations in individual's cells from continuous darkness. Photoperiodism increases temperature of water. This process was progressive in *M. dayanum* as a consequence of the increasing photoperiodism, suggesting a gradual increase of the metabolic function in light treated group. So the main organ affected by this was fatty acids found in hepatopancreas. These fatty acid changes may also help to reveal details of energy utilization.

Enhanced weight of the hepatopancreas of continuous light group reflects the provision of energy utilization and was favorable for growth and metabolism as is clear from hepatosomatic index table 1 of control and continued darkness. Particularly, the hepatopancreas are also considered the major storage organ in Decapods crustaceans, mainly accumulating lipids and to a lesser degree, glycogen according to [24] who support that carbohydrates have low storage capacity and low capability of enzymatic processing. The hepatopancreas was involved in diverse metabolic activities, such as accumulation and cyclic mobilization of reserves, contribution of nutrients to the ovary during vitellogenesis, digestion and absorption [25, 26, 27].

On the other hand, levels of the digestive enzymes in Decapod crustaceans do not remain constant during the developmental cycles. The most common causes of the alterations of enzymatic values were seasons, circadian rhythm and amount and quality of food [28, 29]. Among the digestive enzymes detected in crustaceans, trypsin was considered the most important in the digestion of proteins [29].

The present observations get confirmation from the studies of [30] and [31] who observed an ultrastructural alteration in F, and B cells indicated the sensibility of these cells. The main alterations were detected in F and R cells. F cells were implicated in the synthesis and secretion of digestive enzymes and R cells store the lipids and glycogen, and were also implicated in the detoxification processes. The profound damages observed in these cells, particularly in R cells, such as disrupted cells, hemocytic infiltration, nuclear retraction, cytoplasmic vacuolization, and cytoplasmolysis largely in (24D:00L) group. [30] Suggested that R cells are the most sensitive to metabolic and environmental changes such as starvation, salinity and pollutants.

Hepatopancreatic cells play a considerable role in massive calcium movements between the exoskeleton and the hepatopancreatic and gastrolith storage sites during premoult and postmoult stages of the moulting cycle in crustaceans [32]. Recently, [33] demonstrated that in *Homarus americanus* both R and E-cells show a significant increase in calcium content during premoult, whereas F and B-cells do not. Within the hepatopancreatic cells, possible mechanisms of calcium sequestration include attachment to calcium-binding proteins (calmodulin, calreticulin), formation of $CaPO_4$/$CaSO_4$ granules, and storage in many intracellular organelles such as mitochondria, endoplasmic reticulum, lysosomes, nucleus, Golgi complex, and endosomes [32, 34]. A possible role of crustacean epithelial endoplasmic reticulum and mitochondria in transcellular calcium transport and storage has already been demonstrated [35, 33].

The effect of the average monthly temperatures may also partially explain the difference in fatty acid saturation categories. Accumulation of lipids occurs in algae when there was high illumination and little available nitrogen, which together permits photosynthesis and inhibits protein synthesis and growth. In addition, [36] showed differences in the fatty acid profile of various algae. Therefore, since algal species and abundances in the intertidal zone fluctuate with environmental temperatures and other seasonal factors [37], the hepatopancreatic fatty acid (HFA) composition of *P. crassipes* may fluctuate seasonally as a result of algal consumption.

In the present study, we observed that an activity rate of *M. dayanum* was slower during cooler temperatures than in warmer temperature and photoperiod, most likely resulting in a lower foraging rate and less food consumption in continuous darkness. In (24L:00D) *M. dayanum* treated group, metabolism was shown to increase, beginning approximately two weeks before undergoing a moult, and was maintained at that rate throughout the moulting process. Since *M. dayanum* fasts while moulting, it was necessary to build up energy stores prior to the energetically demanding moult cycle. Because a higher moulting rate was found in continuous light (24L:00D) than (24D:00L), there was likely to be a lower abundance of fatty acids during the continuous light (24L:00D) that may be anticipated from the high activity and

foraging rate of *M. dayanum*. Coinciding with present results, [38] also observed in lobster, *Jasus edwardsii* that temperature has broad reaching effects on the physiology and behavior of animals, such as seen in growth rates of this species.

A lot of research work has been carried out on toxicological effects on hepatopancreas such as [19, 39]. Since the above studies, were made on toxicological effects on crustaceans, the present findings appeared to provide first evidence on the role of combined temperature and photoperiodic effects in the hepatopancreatic growth of *M. dayanum*.

From the studies of histology and hepatosomatic index it was found, that in (24L:00D) group showed an increase of the lumen of the tubules with a consequent compression of the epithelial cells, which formed a thin layer. The lumen of the tubules was occupied by abundant secretions. In 24D:00L group illustrated cellular disruption and disappearance of lateral cell membranes. Moreover, the cells were shrunken leaving the basal lamina behind. On the other hand, necrosis of the microvilli border was noted in some places and distorted appearance was observed in continuous dark group. The present study also revealed lyses of cytoplasm in many areas. Many vacuoles containing cytoplasmic debris could be observed with highly increased frequency in (24D:00L) group than the normal.

The difference in growth of hepatopancreatic tubules was not significant but some change occurs in weight of hepatopancreatic tubules as was clear from hepatosomatic index. From histological point of view, there were alteration found in hepatopancreatic cells between these two groups whereas in control (12L:12D) group the hepatopancreas consisted of numerous blind ending tubules loosely bound together by connective tissue and branching in a racemose manner. The hepatopancreas of control (12L:12D) prawns exhibited the well-organized glandular tubular shape normally seen in prawn species. The lumen of the tubules was narrow. Longitudinal sections of the hepatopancreas of *M. dayanum* maintained under control (12L:12D) conditions did not showed any differences in the number or structure of various cell types or tubular shape and arrangement.

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