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Histopathological Changes in the Hepatopancreas of Freshwater Mussel, *Lamellidens marginalis* in Response to Seasonal Variations

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Abstract

Histological investigations on the digestive tissues of Lamellidens marginalis *reveal dynamic seasonal changes associated with feeding and environmental conditions. The digestive gland serves as the major site of intracellular digestion and is composed of blind-ending tubules connected to the stomach by ciliated ducts. Two main cell types, digestive and secretory (basophile) cells, constitute the tubule lining, while amoebocytes occur within the intertubular connective tissue. The gland exhibits cyclic phases of digestion—normal, absorptive, disintegrating, and reconstituting—corresponding to variations in food availability and environmental factors. During the monsoon, fragmentation of spherules within the digestive tubules indicates active digestion and intracellular breakdown of food. Post-monsoon and winter seasons are characterised by holding and absorption phases that reflect reduced feeding activity. The height and size of digestive cells increase during monsoon to post-monsoon periods (June–October), coinciding with enhanced food intake and preparation for gonadal maturation. Conversely, during summer, holding and absorption phases dominate due to fluctuating environmental conditions and lower food availability. The cyclic pattern of feeding and digestion in* Lamellidens marginalis *is closely correlated with freshwater current dynamics, turbidity, and seasonal nutrient fluctuations. These findings support earlier interpretations that digestion and waste elimination in bivalves occur simultaneously through continuous intracellular and extracellular processes within the digestive diverticula.*

Keywords: Lamellidens marginalis, *digestive gland, histopathology, intracellular digestion, seasonal variation, freshwater bivalve.*

Introduction

The freshwater pearl mussel *Lamellidens marginalis* (Lamarck, 1819) is one of the most common in freshwater habitats (rivers, ponds) mussels in South Asia and has been employed in aquaculture and biomonitoring applications. As natural filters that aid in water purification, nutrient cycling, and benthic–pelagic coupling, freshwater bivalves are essential to aquatic ecosystems (e.g. filter-feeding bivalves remove phytoplankton, particulate organic waste) [1].

Bivalves are extremely sensitive to changes in their surrounding environment, such as seasonal variations in temperature, food availability, hydrology, and pollution load (such as chemical, heavy-metal, and organic stressors), because of their sedentary filter-feeding lifestyle. These changes in the environment can affect physiological functions like tissue integrity, growth, metabolism and reproduction.

The Hepatopancreas involved in digestion, absorption, storage, and detoxification is the found in bivalves. It is especially susceptible to environmental stress and seasonal changes due to its high metabolic load and exposure to filtering materials. Thus, histopathological alterations in the hepatopancreas function as sensitive indicators for ecosystem health and sub-lethal stress. Research on other aquatic invertebrates, such as prawns and crabs, has shown that anthropogenic and seasonal stresses cause changes in the hepatopancreas, including lumen collapse, hemocyte infiltration, tubular necrosis, and thickening of the basement membrane. However, there are reported histological reactions to exposure to heavy metals (such as Cd and Hg) in the hepatopancreas in *Lamellidens marginalis* [2].

In the view of the general agreement that various cell types in the digestive tubule of bivalve molluscs do undergo a pattern of cytological and structural changes in accordance with a rhythm of environment [3]. It was felt essential to know as to how these changes can be correlated with day night cycle in different seasons and endogenous recurring factors.

The purpose of this study is to examine the histopathological alterations in the hepatopancreas of *Lamellidens marginalis* from freshwater lentic and lotic habitats in the Godavari River at Paithan during the three distinct seasons (monsoon, winter, and summer).

We aim to (i) establish baseline histological profiles for each season, (ii) identify specific tissue alterations that may indicate seasonal stress, and (iii) contribute to the use of *Lamellidens marginalis* as a sentinel organism for seasonal and anthropogenic change in freshwater ecosystems by connecting structural changes in the hepatopancreas with seasonal fluctuations in environmental parameters and the mussel's physiological status.

Materials and Methods

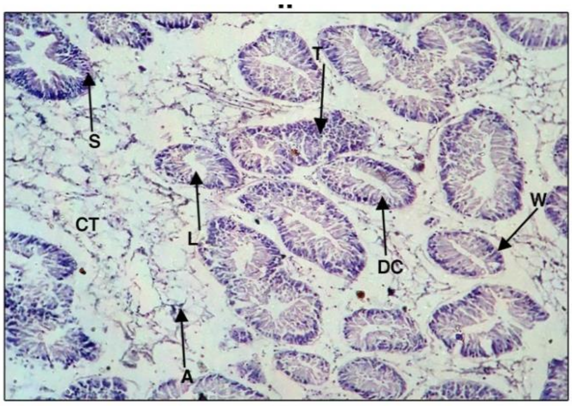
During the monsoon, winter, and summer seasons, freshwater pearl mussels, *Lamellidens marginalis*, were taken from the lentic and lotic environment of the Godavari River near Paithan (Latitude 190 29'8.7" N, Longitude 750 22'12" E). They were cleaned and brushed with water to get rid of mud and algae biomass after being brought to the lab. The animals were acclimated to laboratory conditions for a full day each season. Following that, measurements were taken of the shell's height, width, length, and total body weight. The weight of the soft bodily tissues was measured after the animals were dissected. For the investigation, five mussels were chosen. The hepatopancreas were removed and allowed to harden for a full day in Bouin's Hollande fixative. Tissues are removed from fixative and placed under tap water to eliminate any remaining fixative for subsequent processing. Water was removed from xylene and ethyl alcohol concentrations ranging from 30%, 50%, 70%, 90%, and 100% by the dehydration process. After the dehydration process, tissues were molded into L-shaped steel blocks by immersing them in xylene wax for 30 minutes. After two hours at room temperature, they were frozen for the duration of the night. After removing the ribbons from the freezer, portions were cut using a Spencer rotary microtome to a thickness of 6.0 to 7.0 µm. Wax is melted by gently running sections on a slide over a flame Sections were kept in xylene for two minutes after staining, and then the slides were hydrated in decreasing order of alcohol grade, from 100% to 30%. Mounting was done using DPX. The hepatopancreas and its cells were stained with hematoxylin and eosin [4]. Before microphotography, the entire section was examined under the research binocular microscope, and hepatic tubule measurements were taken as needed.

At mussel habitat sample sites, water quality indicators like temperature, dissolved oxygen, hardness, and pH were recorded on a monthly basis.

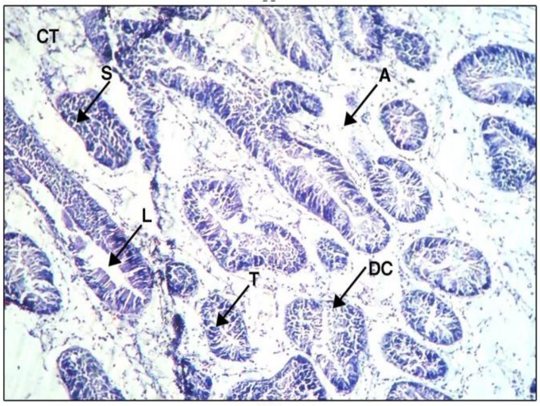
Results and Discussions

The water parameters studied the habitat of bivalve molluscs collected from lotic and lentic environments. In lentic water, summer temperatures range from 31.7°C to 33.0°C, pH 7.6 to 7.9, hardness 90 to 102 ppm, and dissolved oxygen content 5.9349 to 6.3243 ml/lit; monsoon temperatures range from 25.6°C to 27.7°C, pH 7.3 to 7.8, hardness 153.85 to 157.13 ppm, and dissolved oxygen content 5.1132 to 7.37535; winter temperatures range from 22.0°C- 23.4°C, pH 7.1- 7.6, hardness 107.52 - 114.13 ppm and dissolved oxygen content 7.1132- 7.375354 ml/lit. In lotic environment, Temperature 30.8°C–32.5°C, pH 7.3–7.7, hardness 94.71–103.11 ppm, and dissolved oxygen content 5.2130–5.3169 ml/lit during the summer; pH 7.5–8.2, hardness 133.81–144.95 ppm, and dissolved oxygen content 6.7469–7.0019 ml/lit during the monsoon; and temperature 22.2°C–23.1°C, pH 7.50–9, hardness 85.50–9, and dissolved oxygen content 6.50–7 during the winter.

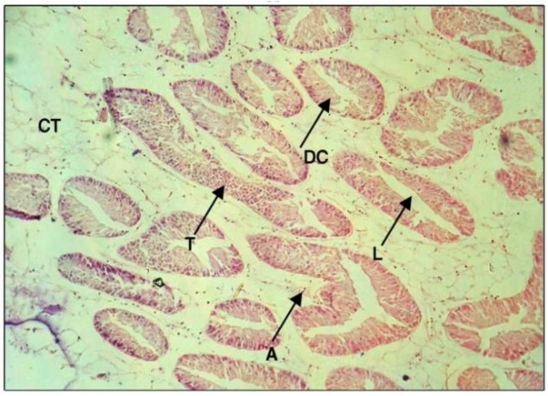
The digestive diverticulitis in bivalve molluscs is made up of a number of blind-ending tubules that connect to the stomach through a network of ducts. The digestive diverticulitis is generally acknowledged to serve as an organ of absorption and intracellular digestion in the majority of bivalves. There have also been suggestions for a secretary role; however, the purpose could just be the transfer of unneeded intracellular digestion cycle enzymes into the stomach. Hepatopancreatic cells may at certain times, be responsible for the production of new tubules. There is a general agreement that the digestive cells of the tubules are primarily organ of endocytic absorption and intracellular digestion[5].



(a) Monsoon Season



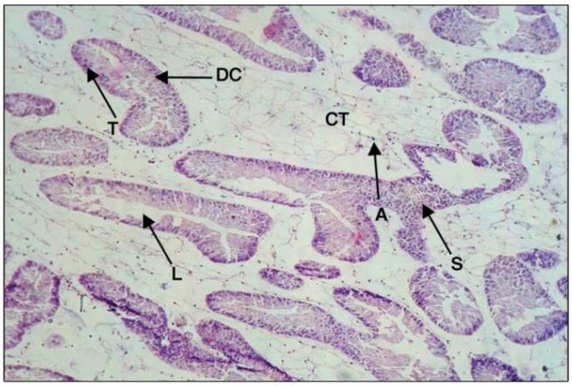
(b) Winter Season



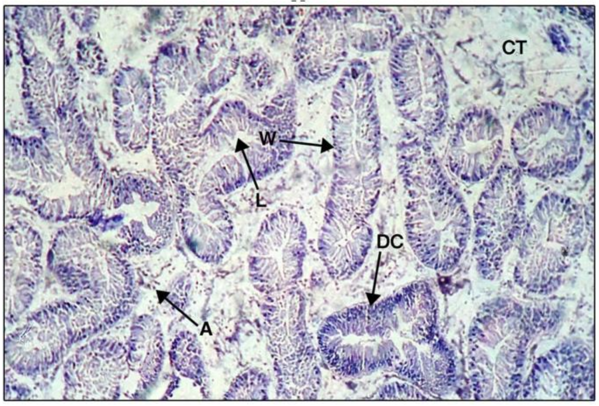
(c) **Summer Season**

Fig 1: Habitat specific histological changes in hepatopancreas from lotic water environment of *Lamellidens marginalis* during monsoon, winter and summer seasons.

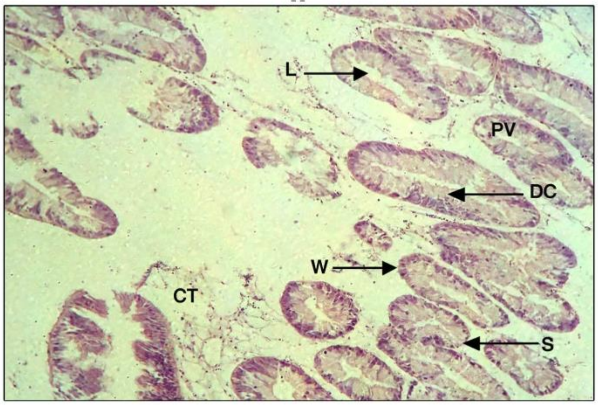
T	=	Tubule
W	=	Wall of the tubule
L	=	Lumen of the tubule
DC	=	Digestive cells
S	=	Secretory cells
CT	=	Connective tissues
A	=	Amoebocytes



(c) Monsoon Season



(b) **Winter Season**



(d) Summer Season

Fig 2: Habitat specific histological changes in hepatopancreas from lentic water environment of *Lamellidens marginalis* during monsoon, winter and summer seasons.

T	=	Tubule
W	=	Wall of the tubule
L	=	Lumen of the tubule
DC	=	Digestive cells
S	=	Secretory cells
CT	=	Connective tissues
A	=	Amoebocytes

In the present study, it is observed that fragmentation of spherules occurred with the onset of rainy season. The tubules were observed in this stage on pre monsoon and post monsoon in bivalves. The absorption phase was observed during summer. The events of digestion of food in the digestive tubules showed absorption of the digested food before the arrival for rainy season in July and August. In the rainy season tubules entire in the fragmentation stage, the absorption phase occurred during winter season i.e. from December to January in bivalves. The water level over the habitat was increased and the turbidity was slowly decreased. During this period tubules showed holding phase in summer with alternate absorption phase, holding and absorption phase are considered probably to occur as the food availability and external environment fluctuation. The size of digestive cells was increased in monsoon to post monsoon then in winter. In general, it is observe that the lumen of tubule had maximum diameter during holding phases, then followed by absorption and fragmentation phases. The height of the digestive cells including fragmentation spherules was also maximum during fragmentation than holding and observation. The diameters of the secretary cells were decreased during absorption phase. It was also observed that the height of the digestive cells increased during June, July and October because of much fund intake in Lamellidens *marginalis* and this period favorable to the animals for feeding and to build up the body reserves for gonad maturation. Amoebocytes were found scattered in the intertubular connective tissue much abundant during monsoon of fragmentation phase. Feeding and digestion in a cyclic pattern in *Lamellidens marginalis* is linked to freshwater flow across the animal bed during the monsoon, post-monsoon, summer, and periods of increased water pumping and prolonged feeding.

Numerous histologists have interpreted their findings from histological investigations of bivalves' digestive tissues to fit the idea that food is constantly being observed and trash is being expelled. In bivalves, cell breakdown and waste material stimulation may happen concurrently with food absorption. The primary site of intracellular digestion is the brown or black digestive gland, which is made up of blind-ending tubules connected to the stomach via a number of ciliated ducts. There is a constant two-way flow through these ducts: waste products depart on their way to the stomach and intestine, while materials enter the gland for intracellular digestion and absorption. The tubules are composed of two cell types, Digestive cells and basophile (secretory) cells [6]. Histological sections of digestive glands of *Mercenaria mercenaria* show that a number of digestive tubules are clustered around secondary ducts [7]. Food stuff would be given to each cluster tubule at the same time because of this structural arrangement. Within the same tubule cluster, correspondingly related tubule types are typically observed. In *Indonaia caeruleus*, tubules were mostly in absorption and fragmentation phase during monsoon season (August) [3]. In post monsoon and winter in tubules are small sized bivalves exhibited only holding and absorption phases. The author stated that holding and absorption phases occur due to food availability and external environment fluctuations. In the present study *Lamellidens marginalis*, revealed tubules and ducts arranged in the shape of tiny lobules that were loosely divided and joined by interlobular connective tissue made of collagenous fibers. Each tubule is bounded by muscle threads which determine the contraction of tubule. Two types of cells viz. digestive and secretary cells are present in each tubule. Amoebocytes are also found at several places, especially in the connective tissue. In *Lamellidens marginalis* are pumping water into and out of mantle cavity, supposedly continually in all the fortnights because of permanent submergence, except during late summer (May) and pre-monsoon (June). Digestion of ingested material in bivalves occurs in two phases comprising an extracellular process with the stomach and intracellular phase within the digestive diverticulitis correlation of fragmentation spherules from digestive tubules show that these spherules if reputed in the stomach can aid primary extracellular digestion of food [8].Extracellular digestion process occurring in the stomach followed by an absorption and dissolution of style and secretion of acid protease from the basophile cells, intracellular phase within the digestive diverticulitis. The greater part of protein digestion takes place interacellularly within the digestive cells of the tubules of diverticulitis [9]. In bivalve, food is typically subjected to an intracellular digestion cycle in the digestive diverticulitis. In show for a number of bivalves, that when they are fed, the height of digestive cells increased [10]. The rhythms of intracellular digestion have been described for numerous species of bivalve molluscs. Evidence of these rhythms is based primarily on differences in the morphological appearance of digestive gland tubule between animals, over a period of time [11]. The sequence of events in this dynamic possess can be divided into at least four phase, normal tubules (Type 1 tubules), absorptive (Type 2), disintegrating (Type 3) and reconstituting (Type 4) [12]. The neighboring cells of the same tubule were thought to be the sites of absorption and degradation. By identifying the tubule type that is most common throughout the digestive gland, one can evaluate the overall level of intracellular digestion inside a single bivalve. By examining the variations of each tubule type in several individuals over the desired environmental element, like the season, the digestion pattern within the population can be determined.

Conclusion

The digestive tubules of *Lamellidens marginalis* showed that ducts and tubules grouped in the small lobules, separated by interlobular connective tissues. Each tubule is bounded by muscle fibers. The tubule consists of two types of cells (1) digestive cells and (2) secretary cells. The amoebocytes are found scattered in the interlobular connective tissue. Three types of digestive phases namely holding, absorption and fragmentation was observed in majority of the tubules of both groups bivalves. Fragmentation, spherules occurred with the onset of rainy season i.e. during September, holding phase occurred in winter season i.e. in December in lentic bivalves. Absorption phase occurred from winter to early summer. The absorption of the food took place before arrival of the monsoon and the tubules entered in fragmentation phase, with the commencement of monsoon. This period also corresponded with the spawning activity of the animals. Holding phase was observed on December, when the animals showed subsequent gametogenesis. In winter, the absorption phase was dominant where the water level remained high and turbidity was less. During summer season the tubules were in holding phase.

Thus seasonal pattern of digestive tubules in holding, absorption and fragmentation was observed, which was depended on the food availability, favorable environmental conditions present over two different habitats.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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