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Study of Histomorphological Changes in the Digestive Tubules of Freshwater Bivalve, *Indonaia caeruleus* from Kukadi River at Yedgaon Dam During Different Seasons

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Abstract

The freshwater bivalve molluscs, *Indonaia caeruleus* (75-78 mm shell length) were collected from banks of Kukadi River at Yedgaon during summer, monsoon and winter seasons for the study of histological changes in the digestive tubules. The histological study showed that, ducts and tubules of digestive gland grouped in the form of small lobules, separated by inter lobular connective tissues. The tubule consists of digestive cells and secretary cells. The primary tubules in the *Indonaia caeruleus* showed synchronized pattern of different phases such as fragmentation of spherules, holding phase and absorption phase, according to changes in water level over the animal bed, rainfall, oxygen contents and probable changes in the detritus matter (i.e. food availability) on the habitat. Tubule consists of two types of cells (a) digestive cells and (b) secretary cells. Amoebocytes are found scattered in the interlobular connective tissues. The diameter of primary digestive tubules was found maximum during monsoon while it was found minimum during summer and winter seasons. During monsoon tubules were in fragmentation spherules phase. The tubules progressed in holding and absorption phases from late monsoon to winter season. The absorption phase in the tubules was found dominant during winter which probably indicated less availability of food. The results of the study are discussed in the light of effect of environmental parameters over the habitat.

***Key words*:** Digestive tubules, *Indonaia caeruleus*, Seasonal variations, Freshwater bivalve

Digestive diverticula comprise a series of blind ending tubules that communicates with the stomach via system of ducts in bivalve molluscs [1]. These tubules communicate with the stomach through a system of ducts. This anatomical arrangement is crucial for the digestive process in bivalve mollusks, as it allows for the transfer of food and digestive enzymes between the stomach and the diverticula, facilitating digestion and absorption of nutrients. The digestive tubules comprise two main types of cells i.e. digestive cells and absorptive cells [2]. The digestive cells are responsible for endocytic absorption and intracellular digestion [3-10]. The process of digestion occurs in two phases namely extracellular digestion in the stomach by dissolution of crystalline style and intracellular digestion in digestive diverticula of the stomach [11-13]. The digestive diverticula passes through phases of absorption, digestion, breakdown and reformation [14-17]. Alterations in digestive epithelial cells of muscles were observed by Lowen and Clarke [16]. Lysosomal changes of hepatopancreatic cells in response to extracellular signals, epinephrine, acetocholine etc. have been studied by Moore [17]. These alterations could include changes in cell morphology, function, or structure, potentially in response to environmental factors, diet, or physiological processes. The studies on the rhythmic changes in the digestive tubules in accordance with the environment of marine bivalve molluscs have been carried out by many workers [16], [18-19]. Similar studies on freshwater bivalves are comparatively less [15], [20]. Recently, Salve [10] studied the pattern of cytological and structural changes accordance with a rhythm of environmental fluctuation in the freshwater bivalve, *Indonaia caeruleus*. This species of freshwater bivalve is specifically mentioned, indicating that the study focuses on understanding how environmental fluctuations affect the cytological and structural characteristics of this particular species. Different species of bivalves may exhibit unique responses to environmental changes based on their physiological adaptations and ecological niche.

In the view of paucity of information, it was essential to know the, histological changes in the digestive tubules of *Indonaia caeruleus* from Godavari River, hence the present study was undertaken.

MATERIALS AND METHODS

The freshwater bivalve, *Indonaia caeruleus* (75-78) in shell length were collected (between 11:00 am-1.00 pm) from banks of Kukadi River at Yedgaon during summer (April and May), monsoon (July to September) and winter (December and January) over a period from June 2021 to April 2022.

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Immediately after bringing to the laboratory, cleaned animals were then defaecicated or depurated (not acclimatized) for 12-13 hours in laboratory s under constant aeration. During this period no food was given to the animals. Physicochemical characteristics of water (i.e. temperature, pH, hardness and dissolved oxygen) on the habitat were also measured.

During each season 10 individual animals appropriate same size, were dissected and after removing the shell valves, flesh of the individual animals were fixed into Bouins Hollande fixative for 24 hours. The fixative was renewed for next 24 hours to facilitate better fixation of the tissues. During all three seasons, hepatopancreas (digestive gland) from each individual bivalve was removed and processed for preparation for paraffin blocks. Dehydration of tissue was done through serial grades of ethyl alcohol and tertiary butanol, while xylene was replaced by toluene during the processing. The tissues were embedded in Ranchem paraffin wax at 58-60°C and sections were cutout at 6-7 µm thickness using Spence Rotary-Microtome and stained with haematoxylene and eosin stain. All the sections were observed under Labomed Trinocular Research Microscope. The measurements of the digestive tubules were also made before microphotography.

RESULTS AND DISCUSSION

The physicochemical characteristics of habitat water during different seasons were temperature (31 - 33.0°c), (27.3 - 28.8°c) and (22.1-23.5°c); pH (7.5 - 7.9), (7.8 - 8.5) and (7.1 - 7.7); hardness of water (85 - 102 ppm), (145 - 164 ppm) and (107-125 ppm) and dissolved oxygen content (5.7428 - 6.9918 mg//h), (6.2910 - 7.022 mg/l/h) and (7.5334 - 8.37 mg/l/h) during summer, monsoon and winter seasons respectively. These data points illustrate variations in temperature, pH, water hardness, and dissolved oxygen content across different seasons, namely summer, monsoon, and winter. These variations are crucial for understanding the ecological dynamics and health of the habitat water.

The histological changes in the digestive tubules during different seasons were given in (Table 1, Plate 1). The transverse section of digestive gland showed that, ducts and tubules grouped in the form of small lobules indistinctly separated and connected by interlobular connective tissue, consisting of the collagenous fibers. Each tubule is bounded by small muscle fibers, forming basement membrane due to which changes the volume of the tubules. The tubule consists of two types of cells, i.e. large digestive cells with basal nuclei and pyramid shaped secretary cells with oval nuclei. Both the types of cells are responsible for absorption and intracellular digestion. The assimilated food is passed out from the base of the digestive cells into the bathing haemocolelic blood, whereas the waste material of digestion is packed as fragmentations spherules which are budded off apically into the lumen of the tubule and finally discharged out via intestine. Amoebocytes are also found which surrounds the tubules. The structure of the digestive tubule can be classified into four different phases via holding, absorption, fragmentation of spherule and reorganization or reconstitution. The histological study of the digestive tubule, during different seasons revealed synchronous cycling in the phases of the digestive cycle according to changes in the external environment and availability of food materials during different seasons. The measurement of the diameter of the tubule and size of the lumen of the tubules during different seasons is given into the (Table 1) and photomicrographs of the tubules taken during different seasons are given in (Plate 1).

The amoebocytes are found abundant with the interlobular connective tissues. Comparatively, the number of amoebocytes increased during the fragmentation phase than holding and absorption phases of the tubules. The amoebocytes observed in more number in fragmentation spherule phase, holding phase on August during monsoon and they got the associated with the digestive cell during this period for assimilate more ingested food. In *Indonaia caeruleus* scattering of the amoebocytes in the digestive diverticula was found less abundant during winter, probably due to less availability of food (feeding activity was halted for most of this time [4]. The earlier review by Owen [5] commented upon the role of amoebocytes in the phagocytosis of food stuffs and digestion of fat and protein in bivalve intestine. Pal and Modak [21] have suggested for three intertidal bivalves, that amoebocytes make fragment which was characteristic of habitat- specific association with the digestive cells of the digestive diverticula that playing their role in digestion, assimilations and removal of waste is more important that is at present realized.

The study showed that, the tubules were in the fragmentation spherule stage from August to September during monsoon, holding phase occurred from January during winter to April and May during summer season. Absorption phase was found on December and January during winter season.

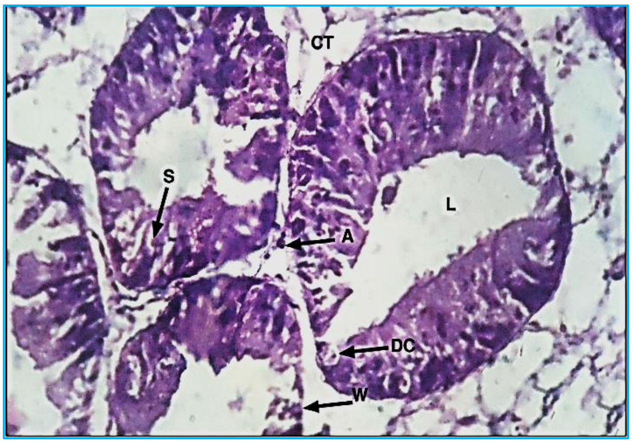
Table 1 Changes in the size of digestive tubules in *Indonaia caeruleus* during different seasons

Season	Months	Phases of digestive tubules	Diameter of the digestive tubules	Size of the digestive tubules
Summer	April	Holding	143.943 ± 6.128	58.438 ± 3.956
	May	Holding and absorbing	140.187 ± 5.365	62.841 ± 4.392
Monsoon	July	Absorption and holding	146.836 ± 4.874	64.311 ± 3.863
	August	Fragmentation	139.97± 6.387	52.784 ± 4.896
Winter	December	Absorption	142.883 ± 4.392	47.447 ± 5.869
	January	Holding absorption	137.688 ± 6.123	55.838 ± 3.126

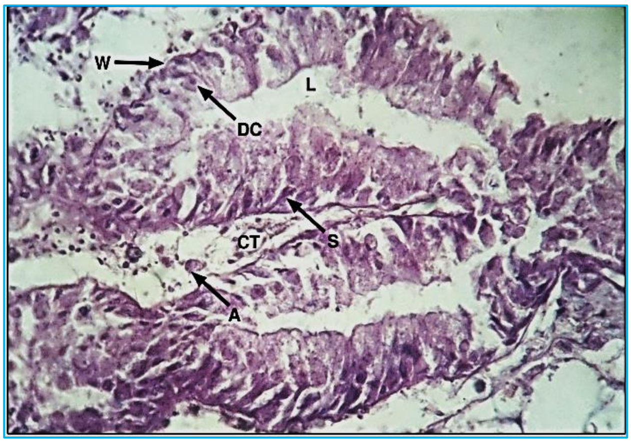
In the present study the diameter of the digestive tubules of the *Indonaia caeruleus* was decreased (142.883 ± 4.392 µm) on December and (137.688 ± 6.123 µm) on January during winter season. The diameter of the tubule was increased (146.836 ± 4.874 µm) on July during monsoon as well as (143.943 ± 6.128 µm) on April during early summer season. Similarly, size of the lumen of the digestive tubules was increased (62.841 ± 4.392 µm) on May and (58.438 ± 3.956 µm) on April during summer season. The diameter of the lumen showed more increase (64.311 ± 3.863 µm) on July during early monsoon.

In the present study, *Indonaia caeruleus* from Kukadi River received plenty of food from (August) monsoon to winter and tubules showed many fragmentation spherules. This is the period when rainfall occurred. This time many mature gametes were released. The holding phase of tubules was observed on July, January, April and May.

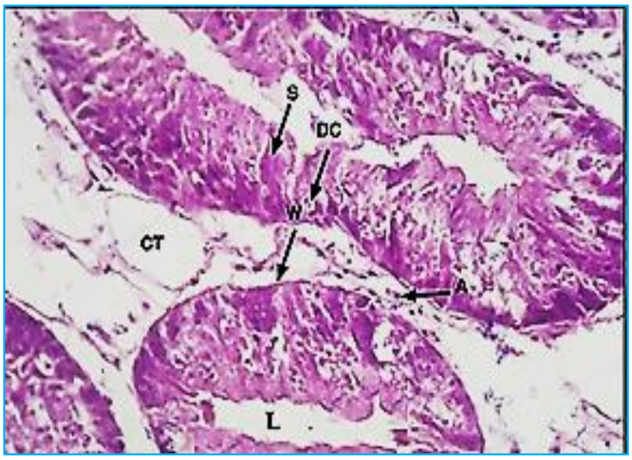
Thus, after rainy season, the tubules were progressed in the holding and absorption phases till early winter and from middle of the winter to summer tubules showed absorption phase. Thus, the present study revealed that, from winter onwards till first rainfall the availability of food from the environment was decreased. Both oxygen content and temperature during this period counteracted upon the food availability and the animals metabolic demand [22] and secondly the lysis of unspawned gametes and maturation and release of gametes seen during this period. Hence parallel to these reproductive events and decreasing food availability and low temperature during winter, animals showed absorption phase.



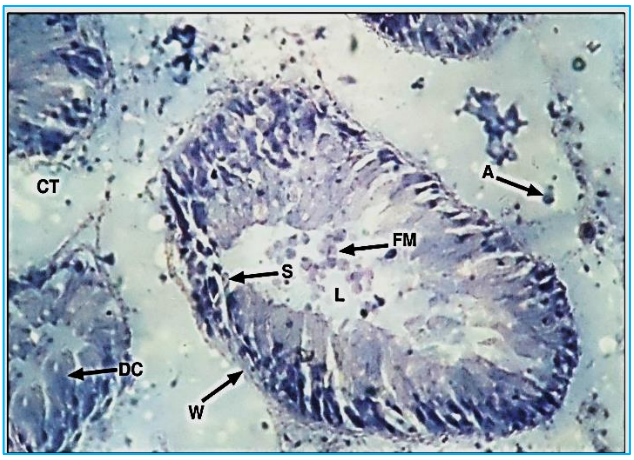
April (Holding phase)



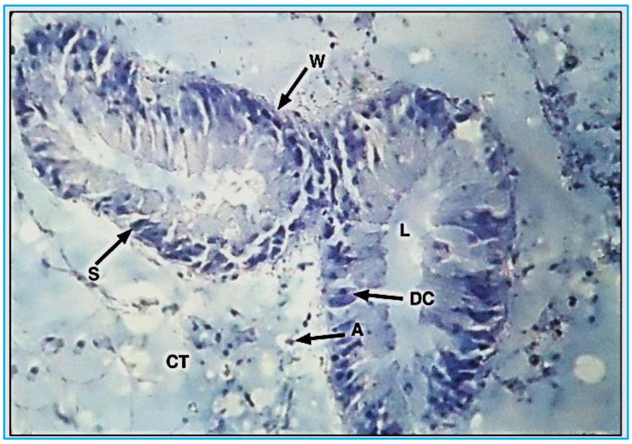
May (Holding and Absorption phase)



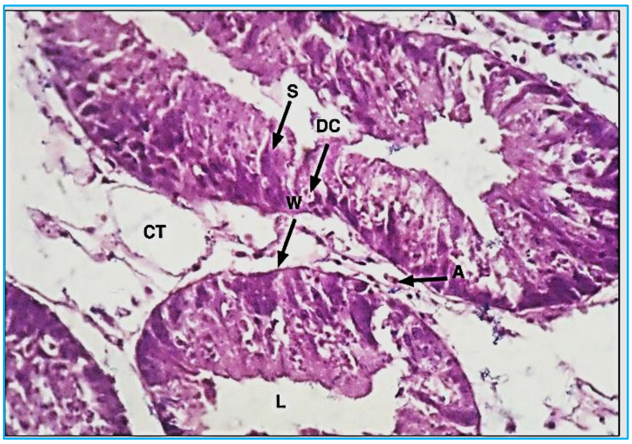
July (Absorption and Holding phase)



August (Fragmentation and Spherules phase)



December (Absorption phase)



January (Holding and Absorption phase)

T = Tubule, SC = Secretary cell, W = Wall of tubule, A = Amoebocyte
L = Lumen of the tubule, DC = Digestive cell, CT = Connective tissue

Plate 1 Changes in the digestive tubule of *Indonaia caeruleus* during different seasons

In addition to the above findings, it is further seen that there occurs period of short absorption holding phase on January during winter but longer period phase on April during summer season. On the other hand, there was occurrence of short holding and secretary phase from May to July. This period coincided with the long day length and high-water temperature. This period can be further correlated with low food level, increased metabolic demand and decrease in the rate of respiration [9].

The significance of local environmental variables is similar during different seasons may vary from lotic to lentic habitat [4]. It is possible that, other species either from lotic and lentic environments are similarly influenced by the changes in environmental parameter, especially, oxygen content, temperature, water level, water current and food availability. The seasonal cycle of digestion was presented in *Indonaia caeruleus* by Saokar [4]. The seasonal inadequacy of current of water over the habitat had a major effect on the expression of cycle in *Indonaia caeruleus*.

CONCLUSION

Thus, in the present study, on the *Indonaia caeruleus* from Kukadi River, in the flowing conditions digestive cells were partly filled with food and breakdown was not complete. During the speedy current of water over the animal habitat with heavy rainfall and cloudy days during monsoon, a continuous cycle of the digestive diverticula occurred and hence the phases of absorption and fragmentation spherules occurred in the tubules with rainy season. This period correlated with abundant food availability and the cells rapidly filled with materials. On the other hand, water temperature decreased with the onset of winter and no feeding occurred for a short period on December, when tubules showed reorganization phase.

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