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Histological organization of hepatopancreas in relation to the starvation in commercially important edible freshwater crab, *Barytelphusa cunicularis*, Westwood, 1836 (Decapoda: Crustacea)

Sherkhane U D
Department of Zoology, New Arts, Commerce and Science College, Shevgaon, Ahmednagar, Maharashtra, India

Abstract
The freshwater crab *B. cunicularis* is a commercially important species and is widely distributed in different regions of Maharashtra, Southern parts of India and Sri Lanka. In freshwater crabs, and many other crustaceans the hepatopancreas is the main digestive gland located in the cephalothorax region of the body. The hepatopancreas is usually considered the most important organ in crustaceans for the storage and mobilization of carbohydrates and lipids, much like the liver of vertebrates and the fat body of insects. The present research work gives a brief account of the histological organization of hepatopancreas and the effect of starvation on the histology of the hepatopancreas. Result obtained shows that the hepatopancreas consists of innumerable, blindly-ending hepatopancreatic tubules held together by means of connective tissues. The hepatopancreatic tubules are surrounded by haemal spaces that contain haemocytes with apparent blood vessels. The hepatopancreatic tubules are enclosed by a basal lamina and each tubule has a central lumen. The study of hepatopancreatic tubule cells shows that these cells can be categorized into three types: R-cells, F- cells and B-cells. Similar observations were also reported earlier by some workers. The R-cells (Reserve cells) are the most common cell type in hepatopancreatic tubules and are characterized by the presence of a large number of cytoplasmic vacuoles. It was observed that starvation results in a lower number of B cells and F-cells in the hepatopancreas.

Keywords: starvation, crab, hepatopancreas, histology

Introduction
The freshwater crab *B. cunicularis* is a commonly distributed crab in many regions of Maharashtra, Southern parts of India and Sri Lanka (Diwan, 1971; Ng and Tay, 2001; Sherkhane, 2007; Srivastava, 2005; Sutar, 2002) [6, 12, 17, 18, 20]. The hepatopancreas is usually considered the most important organ in crustaceans for the storage and mobilization of carbohydrates and lipids, much like the liver of vertebrates and the fat body of insects. Food is an important ecological factor that controls the growth, development and physiology of animals. If animals are starved, they show structural and physiological adaptations to withstand short periods of starvation. The hepatopancreas is a vital organ for the metabolism and storage of nutrients (Byard, 1975; Ramadevi *et al*., 1990; Vogt, 1994; Vogt *et al*., 1989) [4, 15, 22, 23]. Starvation induces ultrastructural changes in the hepatopancreas and changes in the blood proteins of crabs and other crustaceans (Al-Mohanna and Nott, 1989; Djangmah, 1970, Papathanassiou and King, 1984; Storch *et al*., 1982; Vinagre and Chung JS, 2016) [2, 7, 13, 19, 21]. Al-Mohanna and Nott (1989) [2] observed the lower number of secretory B cells in the hepatopancreatic tubules of a starved specimen of *Penacus semisulcatus*. In crab, *B. cunicularis*, it was found that there is an increased amylase activity in the hepatopancreas of the crabs fed normally (Sherkhane, 2007) [17]. There are numerous reports on the histological organization of marine crabs and other crustaceans (Diaz *et al*., 2010; Franceschini-Vicentini *et al*., 2009; Longo and Diaz, 2015) [5, 8, 10]. However, the perusal of the literature shows that the studies on the histological organization of hepatopancreas of freshwater crabs are scanty, outdated and insufficient (Diwan, 1971; Sakhare and Kamble, 2014; Sherkhane, 2007) [6, 16, 17]. In light of this background, the present work was undertaken to put a light on the histological structure of hepatopancreas and its relation to the starvation in crab *B. cunicularis*.

Material and methods
Collection and maintenance of Experimental animal
The live specimens of crab *B. cunicularis* were collected from the Kham river, Aurangabad and Nathsagar dam (District: Aurangabad, Maharashtra, India) with assistance from local fishermen. Animals measuring 1-9 cm in carapace length were used for the experimental purpose. The study was conducted for a period of two years from January 2003 to December 2005. The animals were kept in the trough in aerated condition with sufficient water at about 25-27◦C. Crabs were allowed to acclimatize to the laboratory conditions for a period of 48 hrs. The control crabs were regularly fed with earthworms while starved crabs were not fed for a period of 24 hrs.

Histological details of hepatopancreas

The histological changes in the hepatopancreas were studied in the *B. cunicularis*. This method gives an idea of the different types of the hepatopancreas in crabs. For this purpose, the hepatopancreas normally fed (control crabs) and hepatopancreas of 24 hr starved crabs were dissected out and fixed in Bouins fluid. After 24 hours of fixation, the tissues were dehydrated in alcohol grade series and were paraffin-embedded (58-60°C). The sections were cut at 8-10µ and stained with haematoxylin-eosin.

B. cunicularis of various sizes were selected for the study. The organs were fixed in 10% formalin, dehydrated in different grades of alcohol; paraffin-embedded, sectioned 8-10 mm thick and stained in the haematoxylin-eosin stain. Finally, slides were observed under the light microscope. For this purpose, the hepatopancreas was fixed in Bouin's fluid. After 24 hours of fixation, the tissues were dehydrated in alcohol grade series and were paraffin-embedded (58-60°C). The sections were cut at 8-10µ and stained with haematoxylin-eosin. The histological structures of all the tissues were observed under the light microscope.

Results and discussion

Histological structure of Hepatopancreas

In crabs and other crustaceans, the hepatopancreas is a metabolically important organ that is vital for the absorption and storage of nutrients, and can synthesize digestive enzymes for food digestion (Ramadevi *et al*., 1990; Vogt, 1994; Vogt *et al*., 1989) [15, 22, 23]. In freshwater crab *B. cunicularis*, the hepatopancreas consists of a right lobe and left lobe. These are located in the cephalothorax and occupy much of its part. It surrounds the stomach from its lateral, ventral and posterior sides. The colour of the hepatopancreas is usually yellowish, or yellowish-green or sometimes yellowish-brown. Histologically, the hepatopancreas consists of innumerable, blindly-ending hepatopancreatic tubules held together by means of connective tissues. The hepatopancreatic tubules are surrounded by haemal spaces that contain haemocytes with apparent blood vessels. The hepatopancreatic tubules are enclosed by a basal lamina and each tubule has a central lumen. The study of hepatopancreatic tubule cells shows that these cells can be categorized into three types: R-cells, F- cells and B-cells. Similar observations were also reported earlier (Ramadevi *et al*., 1990; Sakhare and Kamble, 2014) [15, 16]. The wall of the tubules is made of a single layer of columnar epithelium whose component cells can also be categorized into four types of cells: granular cells, ferment cells, hepatic cells with fat globules, and basal or replacing cells (Sherkhane, 2007) [17]. Ramadevi *et al* (1990) [15] described five types of cells in *Ocypoda platytarsis:* E-, B-, R-, M- and F-cells. Four cell types, E-, F-, B- and R-cells are reported in *Scylla serrata* by Monin & Rangnekar (1974) [11]. The R-cells (Reserve cells) are the most common cell type in hepatopancreatic tubules and are characterized by the presence of a large number of cytoplasmic vacuoles. These cells are also known as the absorptive or resorptive cells and their main function is to absorb food materials and minerals from the digestive tract.

Effect of starvation

The effect of starvation on the hepatopancreas of crab *B. cunicularis* was shown in terms of the effect on the size of the hepatopancreatic cells. The cells of the hepatopancreas synthesize and secrete amylase and other digestive enzymes (Asaro *et al*., 2017) [3]. The hepatopancreatic cells thus show profound secretory activity in fed crabs which is directly proportional to the size of the hepatopancreatic cells (Sherkhane, 2007) [17]. The size of the hepatopancreatic cells increases in fed crabs showing an increase in amylase secretion and thus the secretory activity of the cells. The size of the hepatopancreatic cells decreases in starved crabs showing a decrease in amylase secretion and thereby indicating a reduction in the secretory activity of the cells (Table-1). The number of granular cells also decreases in starved crabs.

Table 1: Secretory activity of hepatopancreatic cells in fed and starved crabs

Sr. No.	Day	Crab group	Hepatopancreatic cell diameter (µm)
1.	Day-1	Fed	33.85±8.10
		Starved	29.90±6.30
2.	Day-2	Fed	41.70±8.17
		Starved	30.99±=5.08
3.	Day-3	Fed	41.70±8.17
		Starved	30.99±5.08
4.	Day-4	Fed	33.85±8.10
		Starved	29.90±6.30
5.	Day-5	Fed	44.28±6.30
		Starved	26.65±7.04

In fed crabs, the digestive enzymes are released due to which the granular cells are more secretory compared to the non-fed as shown in Plate- I: Fig. 5 a & b. The circadian rhythmicity in the release of the digestive enzymes was observed (Sherkhane, 2007) [17]. The two groups, one starved and the second fed at a specific time in a day,

were maintained. It was observed that the secretory activity of the cells increased after two hours of feeding. The cells were seen more active in fed crabs and less active in the starved crabs. The secretory activity of the cells suggests that the amylase activity was increased in the hepatopancreas of fed crabs as compared to the non-fed crabs. In starved crabs, the amylase activity decreased due to the less activity of the digestive secretory cells.

Some studies indicate that water pollutants and pesticides affect the histological organization of hepatopancreas in *B. cunicularis* and other crustaceans (Kharat *et al*., 2014; Patil and Yadav, 2011; Sakhare and Kamble, 2014) [9,14, 16]. The hepatopancreas is usually considered the most important organ in crustaceans for the storage and mobilization of carbohydrates and lipids, much like the liver of vertebrates and the fat body of insects (Ramadevi *et al*., 1990; Vogt, 1994; Vogt *et al*., 1989) [15, 22, 23]. Papathanassiou and King (1984) [13] evaluated ultrastructural changes in hepatopancreatic cells of common prawn *Palaemon serratus* induced by 56 hrs of starvation. The authors registered the lower number of secretory B cells in the tubules of the starved specimen. It was observed that starvation-induced by moulting also results in the lower number of B cells in the hepatopancreas of *Penacus semisulcatus* (Al-Mohanna and Nott, 1985, 1989) [1, 2]. B cells are involved in intracellular digestion, whereas F cells produce digestive enzymes for

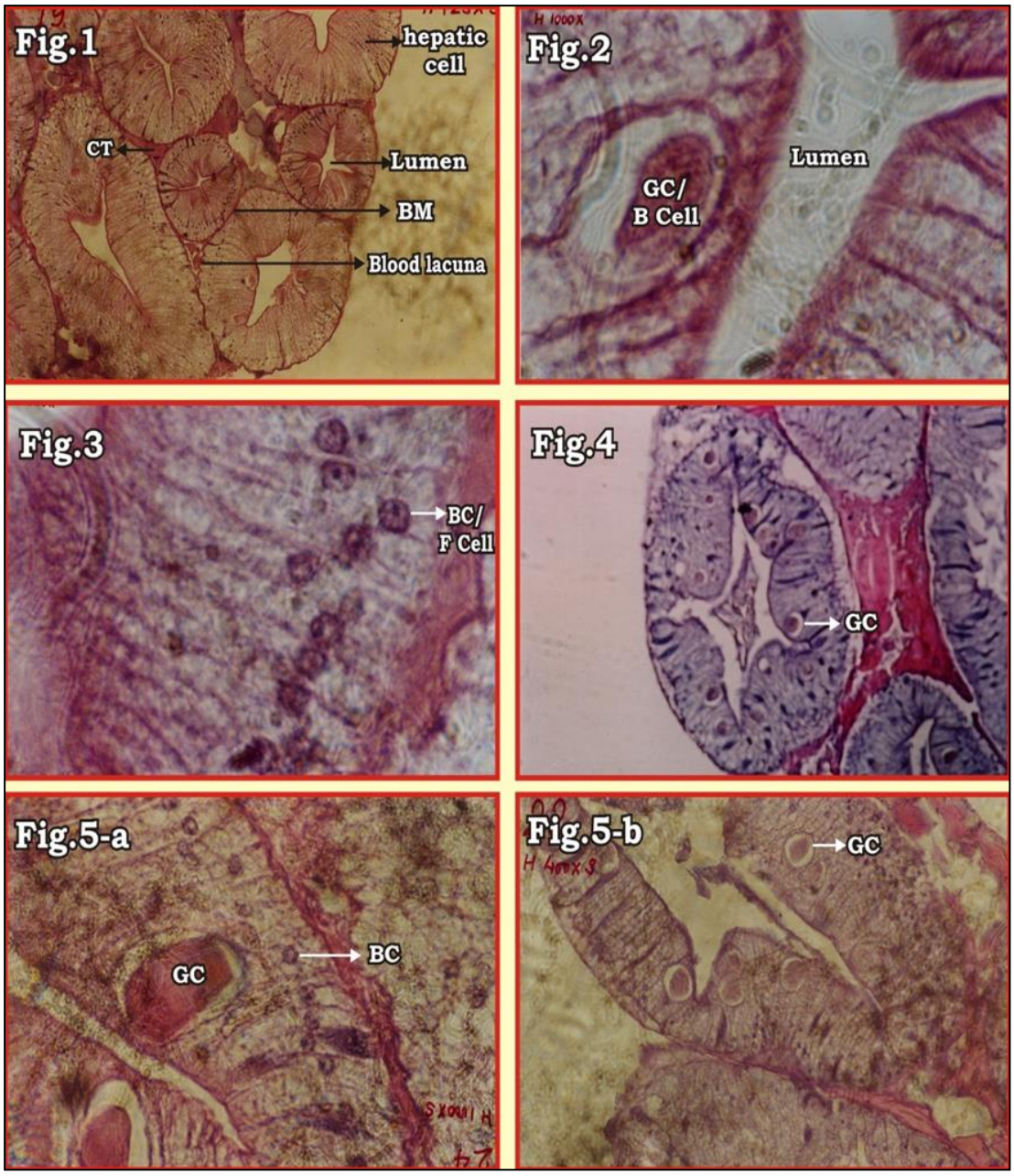

Plate 1: T.S. of Hepatopancreas showing its structure in fed crabs (Fig.1 -3) and starved crabs (Fig.4, 5a &b). Fig 1: T.S. of hepatopancreas (Magnification:100x). Fig 2: A part of hepatopancreas showing B-cell/Granular cell (Magnification:100x). Fig 3: T.S. of hepatopancreas showing ferment cells/basal cells (Magnification:1000x). Fig 4: T.S. of hepatopancreas in starved crab (Magnification:400x). Fig 5 a&b: T.S. of hepatopancreas in starved crab (Magnification:400x for 5a and 325x for 5b). Abbreviations: BC: Basal cell, GC: Granular cell, BM: Basal membrane, CT: Connective tissue.

extracellular digestion, although there is some controversy, it is considered that B cells are derived from F cells (Al-Mohanna and Nott, 1989; Papathanassiou and King, 1984) [2, 13]. In crab, *B. cunicularis*, it was found that there is increased secretory activity in the hepatopancreas of the crabs fed normally. The secretory cells (B cells) were lowered in the tubules in starved specimens compared to the fed. The results obtained will help formulate feeding strategies for commercial crab cultures.

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