Available online at: www.mbai.org.in doi: 10.6024/jmbai.2017.59.1.1911-07

# Effect of salinity on digestive gland of estuarine clam *Paphia laterisulca*

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Received: 14 Mar 2016, Accepted: 20 Jun 2017, Published: 30 June 2017

Original Article

## Abstract

Present investigation was aimed to study the seasonal variation in salinity induced histological alterations in digestive gland of estuarine clam *Paphia laterisulca*. In this study, clams (35-40 mm) were exposed to lower salinity ranges (100% to 10%) during all three seasons (summer, post-monsoon and winter). Salinity induced histological alterations in clam was prominently observed below 70% salinity range of exposure. On the basis of histological changes in digestive gland, it is clear that, overall lower salinity tolerance limit of the clam is up to 70% salinity i.e. 30% reduction in salinity as compared to the normal salinity of estuary in that season. Below their adaptive limit of the salinity range of 50% and 40% salinity, critical structural alterations in hepatopancreas were marked in the clam species.

***Keywords***: *Paphia laterisulca, salinity, digestive gland, histology, seasons*

## Introduction

In bivalves, the energy balance is directly associated with the quality of environment (Smaal and Widdows, 1994) and positive energy defined as the scope for utilization of energy for both, growth and/or reproduction (Resgalla *et al*., 2007). Gonad is the organ with the highest energy demand (Bayne, 1976), whereas the digestive gland plays a central role in the digestion and resorption of food (Morton, 1983) and both tissues affected by starvation. It is known that, such stresses as shortage of food supplies or pollution induce cytological changes in various bivalve tissues (Moor and Lowe, 1985). As target organs, the digestive glands and gonads were chosen to study the effect of starvation (Bielefeld, 1991). In marine animal physiological processes such as metabolism, respiration and excretion were altered with sudden change in salinity (Kim *et al*., 1998).

Marine benthic communities are subject to changing environmental conditions, therefore for evaluation of the functioning of these communities, better understanding of the physiological mechanisms that link environmental conditions to ecological responses is necessary (Menge *et al*., 2002; Niemi and McDonald, 2004). In the present investigation, structural alterations in hepatopancreas under lower salinity exposure was studied to represent the response of clam *P. laterisulca* to salinity stress and its adaptive limit.

## Material and methods

### *Clam collection and maintenance*

The estuarine clam, *Paphia laterisulca* was collected from Bhatye estuary (Ratnagiri, Maharashtra) during low tide by hand picking and digging with knife. The clams were cleaned and washed with the estuarine water. After cleaning, clams (35-40 mm) were acclimatized for 48 hours under laboratory conditions at 38, 29 and 36 ‰ during summer, post-monsoon and winter season, respectively. In all selected seasons *viz*. summer (March-May), post-monsoon (August-October) and winter (November-January), the same procedure was followed for animal collection and their maintenance in the laboratory. For experimental work only healthy clams were selected and tested.

### *Experimental design*

During experiment, clams were exposed to ten lower salinity ranges (100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%) for 08 days, here 100% saline water is 38‰ during summer, 29‰ during post-monsoon and 36‰ during winter season which was normal water of estuary collected during high tide, therefore it was considered as control range in all the seasons. These salinity ranges were maintained throughout experiment by adding fresh water. Daily changes in double filtered estuarine water of the respective salinity range were made with 6 hour interval.

### *Histological study*

During the different seasons i.e. summer, post-monsoon and winter, after completion of 08 days salinity exposure period, few clams were sacrificed and whole body of clam preserved in Bouin's Fixative for histological study. Hepatopancreas from clams preserved in solution was removed to study the histological alterations in the clams due to different salinity exposure. Routine technique of tissue processing for wax embedded tissue preparation and sectioning were carried out.

The prepared wax embedded blocks of hepatopaancreas were sectioned at 6 $\mu$m thickness on rotatory microtome (Erma, Japan). These sections were stained with Harris heamatoxylene and alcoholic eosin and mounted in D. P. X. During all the observations, the microphotography was made under the Olympus CH 20i (U.S.A.) microscope at 400X. All the changes in hepatopancreas of clams exposed to various salinity ranges were compared with hepatopancreas of normal clams for histological observations in respective seasons.

## Results

### *Histology*

Histological study gives clear picture of any physiological disturbance in the animal, which results from natural or artificial stress. In the present study, an attempt is made to understand the alteration in the basic cellular framework of hepatopancreas of estuarine clam *P. laterisulca* exposed to environmental stress like different salinity in different seasons.

### *Control*

During all the three selected seasons (summer, post-monsoon and winter), stained sections of hepatopancreas in clam *P. laterisulca* from control salinity range (Fig. 1 from Plate I, II and III) showed similar structural integrity.

### *Summer season*

During summer season estuarine clam, *P. laterisulca* exposed to lower salinity ranges for the period of 8 days showed significant histopathological alterations in hepatopancreas of clams exposed below 70% salinity range. In 70% salinity (Plate I, Fig. 2), only vacuolization of digestive cells appeared in few digestive tubules. While in 60% salinity (Plate I, Fig. 3), vacuolization progressively appeared with the disintegration of the cell membrane of digestive

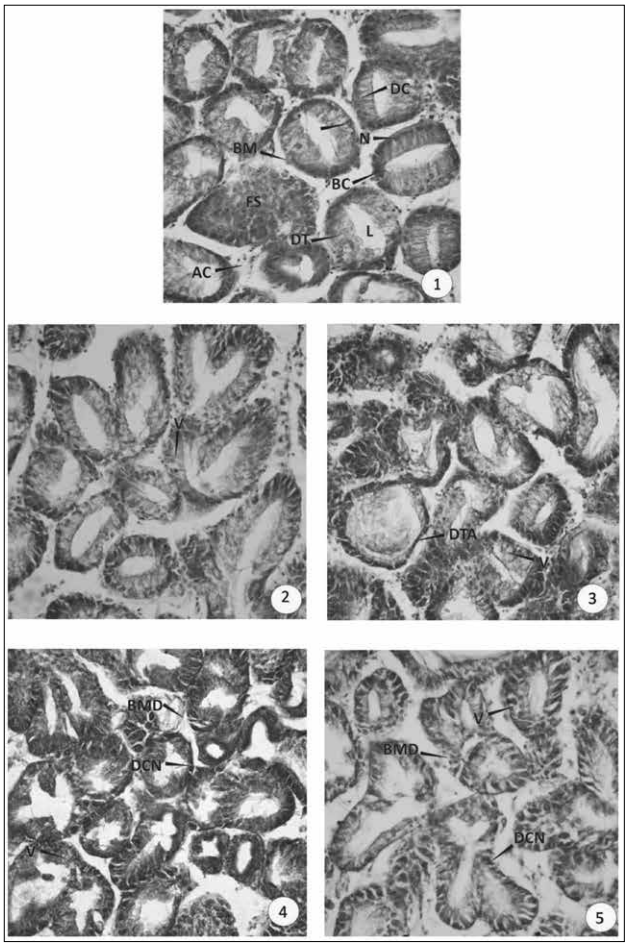


Plate 1. Microphotograph of section passing through hepatopancreas of clam *Paphia laterisulca* after 8 days period of exposure to lower salinity during summer season (Fig. 1 to 5; 1. Control group, 2. 70% salinity group, 3. 60% salinity group, 4. 50% salinity group, 5. 40% salinity group).

cells, which resulted in to leaking of cytoplasm into the lumen of digestive tubule. Comparatively, such alterations were prominently observed in clams exposed to 50% salinity (Plate I, Fig. 4) along with the degeneration of digestive cells and the basement membrane of few digestive tubules. Clams from 40% salinity after 8 days period of exposure, appeared with severe destruction in digestive tubules. Clams from 40% salinity showed heavily vacuolated digestive cells. Increment in lumen of tubules was due to digestive cell atrophy. In some tubules, both digestive and basophilic cells undergo necrosis (Plate I, Fig. 5).

### *Post-monsoon season*

Clams exposed to different lower salinity ranges for 8 days period of exposure, showed histopathological alterations in hepatopancreas below 70% salinity. Hepatopancreas of clams exposed to 70% salinity (Plate II, Fig. 2) showed slight alteration with reduction in inter-tubular connective tissues, increased size of lumen of digestive tubule and vacuole formation in few digestive cells of hepatic tubules. The clam from 60% salinity (Plate II, Fig. 3) showed more prominent alteration in hepatopancreas, which was marked by displacement of the basement membrane of tubular cells, loss of connective tissues, digestive cell necrosis as well as the formation of vacuoles in between digestive cell and basement membrane. Damage to the hepatopancreas was most prominently observed with further dilution in salinity. In 50% salinity, digestive cell atrophy resulted in to increase in lumen size. Digestive cell necrosis was increased as compared with previous salinity ranges. Tubular integrity was lost (Plate II, Fig. 4). In 40% salinity, in addition to these alterations in the hepatopancreas, digestive cell nuclear enlargement or swelling was evident (Plate II, Fig. 5).

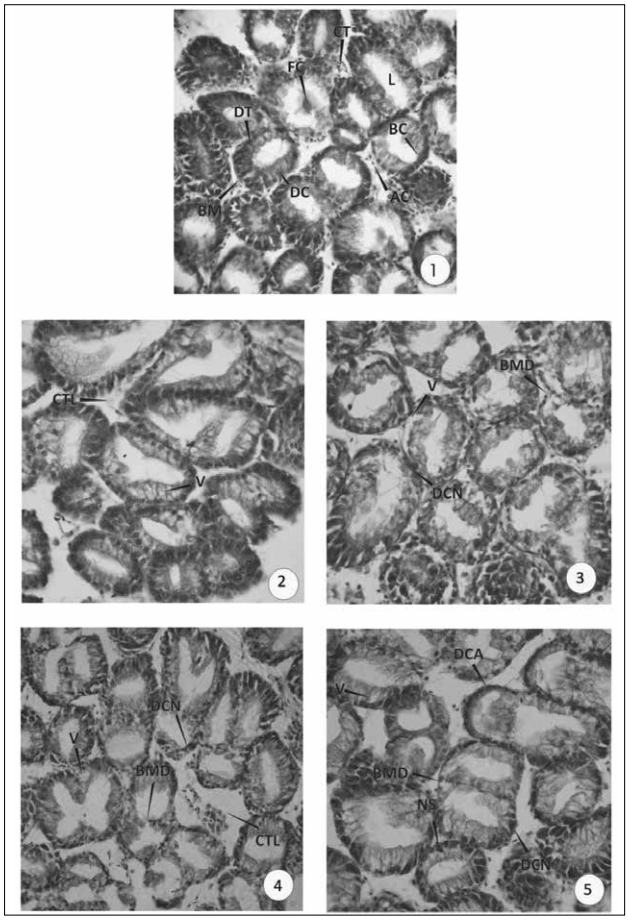


Plate II. Microphotograph of section passing through hepatopancreas of clam *Paphia laterisulca* after 8 days period of exposure to lower salinity during post-monsoon season (Fig. 1 to 5; 1. Control group, 2. 70% salinity group, 3. 60% salinity group, 4. 50% salinity group, 5. 40% salinity group).

### *Winter season*

Clams exposed to lower saline water for the period of 8 days during the winter season showed significant alteration in hepatopancreas below the exposure range of 60% salinity. In 60% salinity (Plate III, Fig. 2), hepatopancreas of clams showed loss of connective tissues which increases the inter-tubular space, while in 50% salinity (Plate III, Fig. 3), more prominent alteration in hepatopancreas was marked by loss of basement membrane, heavy vacuolization of digestive cells as well as its rupture and atrophy of basophilic cells. Comparatively, histological sections of the hepatopancreas of clams from 40% salinity (Plate III, Fig. 4), showed a total loss of structural integrity as a result of loss of the basement membrane and breaking of digestive tubules.

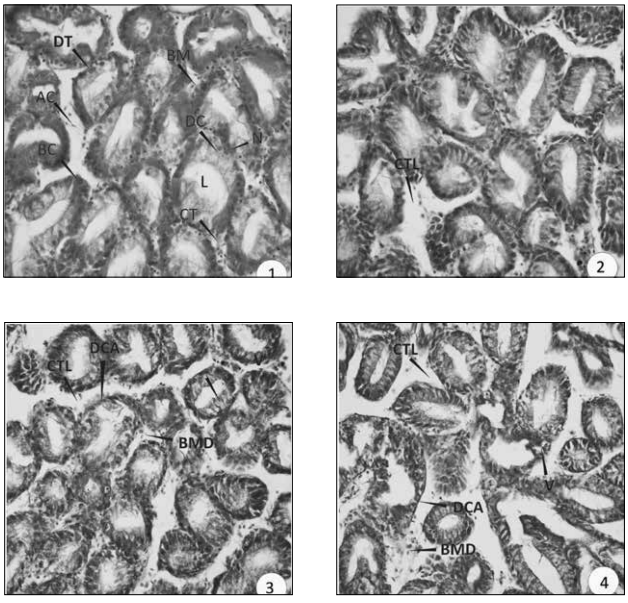


Plate III. Microphotograph of section passing through hepatopancreas of clam *Paphia laterisulca* after 8 days period of exposure to lower salinity during winter season (Fig. 1 to 4; 1. Control group, 2. 60% salinity group, 3. 50% salinity group, 4. 40% salinity group).

AC - Amoebocyte cell, BC - Basophilic cell, BM - Basement membrane, BMD - Degeneration of basement membrane, CT - Connective tissues, CTL - Connective tissue loss, DC - Digestive cell, DCN - Digestive Cell Necrosis. DT - Digestive tubule, DTA - Digestive tubule atrophy, FS - Fragmentary spherules, L - Lumen, N - Nucleus, V - Vacuole

## Discussion

Different phases of digestive tubules have been documented in the digestive gland of a normal specimen (Morton, 1983). Percentage of tubules in particular phase was taken as indicative of the activity of the gland (Bielefeld, 1991). But, due to difficulties in differentiation of phases of tubules, he could distinguish only disintegrating phase, which is distinct from other phases. Remaining all phases is considered as integer phases, because all the cells of the tubules are in the resorbing and digesting process. In present study, during all three selected seasons (summer, post-monsoon and winter), hepatopancreas of the clam *P. laterisulca* were shown similar structural integrity as described by Morton (1983). Even after 8 days period, structural integrity of hepatopancreas from control salinity clam remain unchanged with variation in the size of digestive tubules as well as variation in fragmentary spherules and size of lumen.

According to Shumway *et al.* (1977) bivalves found in intertidal and estuarine habitat are generally tolerant to abrupt and large changes in salinity. Poor nutrition during low salinity event caused digestive tubule atrophy in oysters from Apalachicola Bay (Winstead, 1995). Thus, reduced salinity may be indicative of various other stressors that, in combination, result in mortality or impact on clam health.

Some reports stated that, the breakdown of the digestive epithelium to be the generalized stress response resulting not only after exposure of clams to various pollutants but also to physiological extremes like increased salinity and starvation (Gold-Bouchat *et al*., 1995; Weinstein, 1997). The dominance of calcium cells in the digestive tubules resulted from a combination of hyperplasia and hypertrophy of calcium cells and loss of digestive cells. Both hypertrophy of basophilic cells and loss of digestive cell have been already described as a response of molluscs towards environmental stress (Zaldibar *et al*., 2007a, b).

Elston *et al*., 2003 observed swelling of absorptive cells of the digestive glands of clams exposed to a salinity of 10 ‰, possibly due to the absorption of hypoosmotic seawater, followed by the sloughing of these cells into the lumen of the digestive gland. At the same salinity range, yellow clam *Mesodesma mactroides* showed alterations in hepatic tubules at 96 h of exposure (Carvalho *et al*., 2015). In the present seasonal investigation, the estuarine clam, *P. laterisulca* was shown significant effect of lowered salinity on histological alterations of hepatopancreas. Results reveals that, hepatopancreas from the clams up to 70% salinity range was unable to display any significant structural alteration. Therefore, clam exposed to lower salinity range tolerate up to 20% reduction in salinity from that of normal salinity of their habitat. This range of salinity tolerance was observed in summer and post-monsoon season for *P. laterisulca.* As histological alterations were evident below 60% salinity range, it could be considered that, tolerance capacity increased in winter season. Such seasonal difference in tolerance limit may be resultant of degree of variation in salinity experienced by clam in that particular season.

From the results of histological alterations in hepatopancreas of estuarine clam *P. laterisulca*, it is clear that, low salinity altered hepatic tubule structure from 70% salinity range, which was marked by appearance of formation of vacuoles in the digestive cells. This vacuolization progressively increased with further decline in salinity exposure along with damage to the tubular integrity by digestive cell atrophy, disintegration of basement membrane and loss of inter-tubular connective tissues. More prominent and damaging alterations were seen in lowermost salinity range (40% salinity range) of exposure defined by shrinking of digestive tubules with breaking of basement membrane, loosing structural architecture, and both digestive and basophilic cell degeneration/necrosis. In all three seasons (summer, post-monsoon and winter) identical results were obtained.

Under reduced salinity, Ark Shell (*Scapharca subcrenata*) showed epithelial layer necrosis in the gills as well as increased numbers of hemocytes, nuclear condensation, and cytoplasmic enlargement in the digestive glands (Shin *et al*., 2009). Wang *et al.* (2008) found that, salinity events results in highly vacuolated hepatocytes in liver and even caused severe kidney damage of *Chalcalburnus chalcoides aralensis*.

Physiological stress is often reflected by important cytological changes in hepatopancreas as this organ is the central site of metabolism. The deterioration of the tubular epithelium is evidenced by the loss of contact between cells and with the basal lamina (Cuartas *et al*., 2003). This process was progressive in *Palaemonetes argentinus* as a consequence of the increasing salinity, suggesting a gradual reduction of the metabolic function. Hepatopancreas of *P. argentinus* showed severe histological alterations in individuals from all the salinity ranges, except the control (100%) (Diaz *et al*., 2010). They also observed epithelial desquamation, necrosis and folded basal lamina at tested salinities. Folded basal lamina has also been observed in individuals under nutritional stress (Storch and Anger, 1983; Diaz *et al*., 2002).

It is clear that, hepatopancreas of the clam *P. laterisulca* from the control salinity range showed structural integrity with digestive tubules in different phases of digestion after 8 days period of exposure. In the clams, histological alterations in hepatopancreas occured below 70% salinity range. Such alterations are prominent in the lowermost salinity range (40% salinity). As compared to other seasons, tolerance of clam increases during winter

season, as histological alterations in hepatopancreas marked from 60% salinity range in this season. Beside salinity effect, there is possibility of other factors influencing the tolerance and sensitivity of clams.

In the Bhatye estuary, degree of salinity varied from season to season. During summer season, salinity of estuarine water fluctuates from 38‰ to 35‰ with respect to tidal cycle i.e. 10% reduction as per experimental set carried out in laboratory conditions. Similarly, clams experience salinity reduction of 30 - 40% during post-monsoon and 20 % reduction during winter season. Therefore, the clam experience reduction in salinity from 10% to 40% depending on season.

## Acknowledgements

Authors are thankful to Prof. U. H. Mane, Centre for Coastal and Marine Biodiversity for providing valuable suggestion and facilities for this work. Authors are also thankful to UGC non-SAP for financial support in the form of fellowship during this work.

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