2012 International Conference on Environment, Chemistry and Biology
IPCBEE vol.49 (2012) © (2012) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2012. V49. 5

Histopathology in the Digestive Gland of *Batissa violaceae* Lamark as a Biomarker of Pollution in the Catubig River, Northern Samar, Philippines

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Abstract. Clams (*Batissa violaceae* L.) collected from two different sites in the Catubig River in Northern Samar Philippines, were analyzed using histopathology as a biomarker in the digestive gland during high flow and low flow periods. Results indicate that clams during the low flow period have shown more disintegrated digestive tubules than high flow period. Field experiments with laboratory controls were also conducted. Resident clams previously depurated in the laboratory for 48 hrs were subsequently returned to their respective sites and immersed in cages for 72 hrs. Similarly depurated control clams were kept in the laboratory while immersed in clean aged tap water. Histopathological analysis, on the depurated group of clams revealed that the digestive tubules were closely packed and infiltrated with amoebocytes. The resident chronically exposed clams showed many damaged digestive tubules. In clams, depurated in aged tap water and subsequently immersed in river water, many digestive gland tubules were damaged; however, the intertubular spaces were more or less similar in extent. These histological changes suggest that histopathology can be indicative of pollution which is the inherent characteristic of Site 1 especially during the low flow period.

Keywords: histopathology, biomarker, digestive gland

1. Introduction

Environmental pollution is a worldwide problem, especially in the aquatic ecosystem which is increasingly affected by anthropogenic inputs (Zorita 2006; Bilbao et. al. 2006). Histopathology, as a tool or technique of analysis, can provide an idea of the status of the environment. Similar to assay techniques, histopathology provides biomarkers that are sensitive and responsive since tissues and organs are being altered even at lower concentrations of contaminants (Abdallah n.d.; Au 2004).

Bivalves are widely used as sentinel organisms since they are sessile in nature and filter feeders that possess a high capacity to take up chemical pollutants even at a considerable distance from the source of pollution (Bilbao et. al 2006).The selected organ is the lipid rich digestive gland since it plays a central role in accumulation and detoxification of various substances and considered to be a target organ in environmental pollution assessment (Klobucar et al. 1997).Generally, the digestive gland is composed of tubules that are bound by connective tissue and muscle fibers. The digestive epithelium consist of two cell types, the digestive cell which is involved in absorption and intracellular digestion and the basophilic cells which appear to be involved in synthesis of proteins and extracellular digestion (Zorita 2006).

The present study had the following objectives (1) to characterize and compare the histological changes in resident clams as well as acutely exposed clams under the two flow regimes (2) to compare the histological changes in clams from two sites of the river.

2. Materials and Method

Clams from two sites of the Catubig River in Northern Samar, Philippines were analyzed and compared. Site 1 is a commercial district and a relatively densely populated area. Site 2 is nearby agricultural farms, is

less populated, and where agricultural practices of farmers are traditional, i.e., without use of pesticides and chemical fertilizers and there is dependence on rainfall rather than irrigation from river dams.

The test organisms used were the freshwater clam *Batissa Violacea* Lamarck with approximate shell length of 4-6 cm. collected by professional divers at depths of 8 m at two distinct sites during high flow and low flow periods. Immediately, after collection the clams were dissected and subjected to histopathological analysis.

For laboratory control group of clams, from each study site in the river, 18 individual clams were collected. Immediately after collection, the clams were brought to the laboratory where all the clams were depurated by immersion in aged tap water for forty-eight hours. Afterwards, the clams were removed from the water and divided into two major groups: one group was immersed in aged tap water and served as laboratory control and the other group was placed inside nylon netted cages and immersed back in their respective source site in the river. The cages were securely tied to the irrigation pumps (in Site 1) and to fallen coconut tree trunks (in Site 2).

From each study site, during high flow and low flow periods in the river, a total of nine clams were used for histopathological analysis-three from the chronically exposed group; three from depurated laboratory control group which were exposed to tap water for 72 hours; three from the depurated group which was brought back to the river and immersed there for 72 hours. Portion of the total soft tissue with digestive gland were fixed in 10% formalin, dehydrated in ethanol, and embedded in paraffin. Afterwards these were cut 5 um thick, mounted on glass slides and stained with Harris hematoxylin eosin. The tissues were examined and photographed using Carl Zeiss optical microscope.

3. Results

Microscopic examination of the digestive gland of depurated *B. violacea* taken from site 1 during high flow period (Fig 1a-1b) revealed that the digestive tubules were closely packed and heavily infiltrated with amoebocytes. At low flow periods, the digestive tubules were widely separated from each other. The epithelial lining cells in some tubules became detached from its basement membrane, others were thinner and damaged (Fig. 1c-1d).

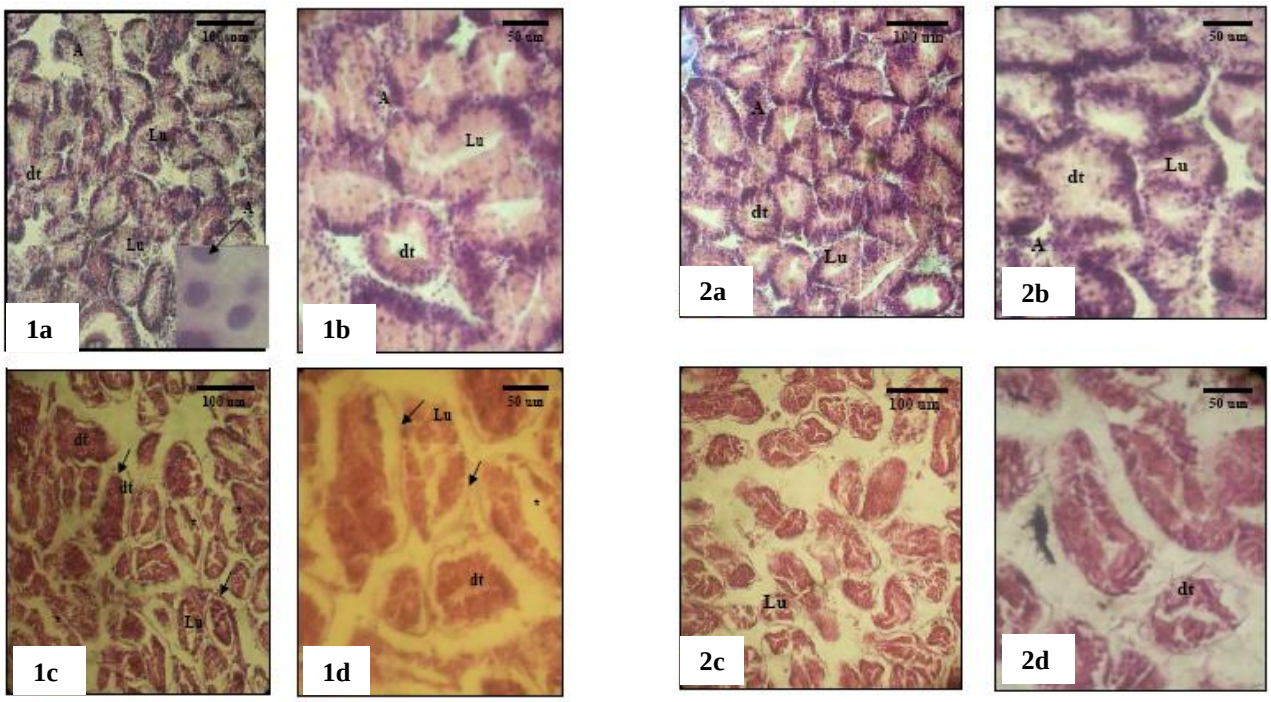
The resident chronically exposed clams sampled from site 1 during high flow period (Fig. 3a-3b) showed many damaged digestive tubules. Some of the cellular components of the tubules were not discernible. The damage was greater in those clams taken from the same site during low flow period (Fig. 3c-3d) since most of the cellular components and the normal architecture of the tubules was lost.

In clams taken from site 1 depurated in aged tap water and subsequently immersed in river water during high flow (Fig 5a-5b), the digestive gland tubules were more intact than those taken during low flow (Fig. 5c-5d). In the latter, it was further observed that many tubules were damaged.

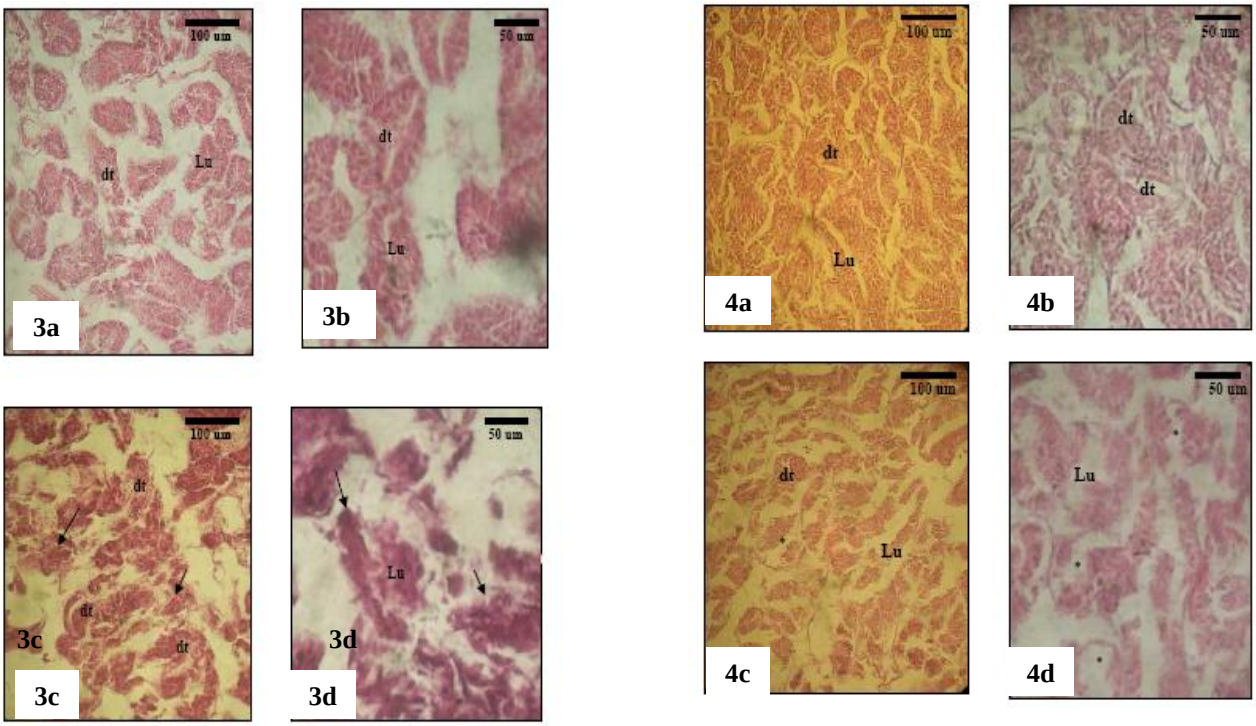
The digestive tubules of those clams taken from site 2 during high flow (Fig. 2a-2b) and depurated in aged tap water appeared similar to those clams taken from site 1 during the same period. In clams sampled during the low flow period (Fig. 2c-2d), the two cell types, digestive and basophilic cells were not distinguishable from each other. Also, the intertubular spaces became wider.

In chronically exposed clams from site 2, the digestive tubules during high flow (Fig. 4a-4b) appeared closely packed. However, their cellular components were not clearly distinct and became detached from its basement membrane. On the other hand, during low flow period (Fig. 4c-4d), most of the tubules were disintegrated.

Those clams taken from site 2 and depurated for 72 hours and subsequently immersed in river water for the same duration showed many shrunken digestive tubules (Fig. 6a-6b). A few have lost their cellular details. In contrast, the extent of damage was much greater in those clams taken during the low flow (Fig. 6c-6d). In addition, there was evident epithelial lining that was consequently increased the size of the tubular lumen.



Figs. 1 to 2.*Batissa violaceae* depurated in aged tap water for 72 hrs Fig 1. Sections of the digestive gland taken during high flow (Fig. 1a-1b) and low flow periods (Figure 1c-1d) in site 1.Fig 2. Digestive gland of clams in site 2 during high flow (2a-2b) and low flow (2c-2d) periods. Note the presence of amoebocytes (A) during high flow period in both sites.

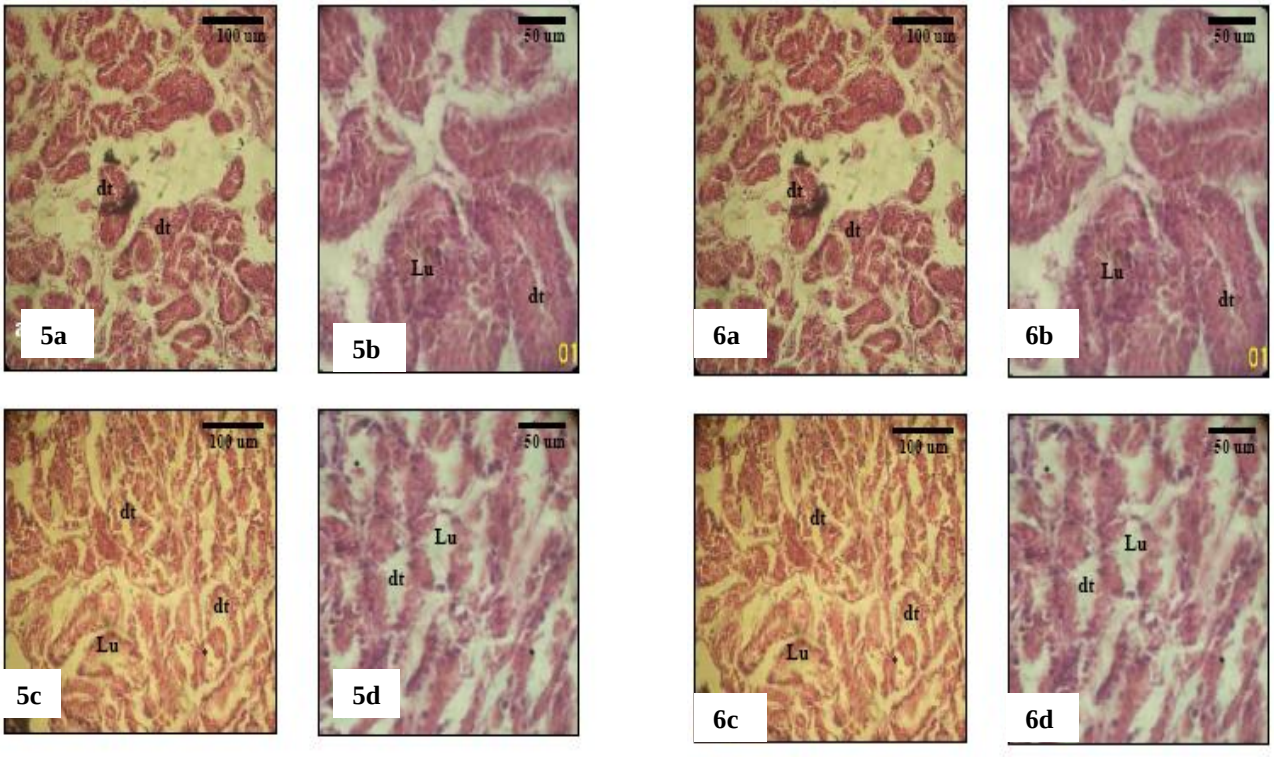


Figs. 3 to 4. Resident chronically exposed group of clams. Fig 3. Digestive gland during high flow (Fig. 3a-3b) and low flow periods (Fig. 3c-3d) in site 1. Fig.4. Digestive gland of clams in site 2 during high flow (4a-4b) and low flow (4c-2d). Notice the digestive gland tubules (dt) taken during low flow shows more disintegrated tubules than those taken during high flow period (*) in both site.

4. Discussion

In molluscs, the digestive gland is tubulo-alveolar and is composed of several digestive tubules separated from each other by a small amount of connective tissue. The digestive tubule is lined by two kinds of cells,the digestive and basophilic cells. The digestive gland cells are specialized in intracellular digestion

whereas the pyramidal shaped basophilic cells are for protein synthesis (Najle 2000,Otludil et al. 2004 and Zorita et al. 2006). In some instances, the digestive gland tubules contain a large number of amoebocytes, which were all considered to originate below the basement membrane.Amoebocyte infiltration is one of the natural protective responses of the clam to fight various environmental pollutants (Lee et. al. 2001). This could probably explain the present result wherein the depurated clams during high flow period show large number of amoebocytes.



Figs. 5 to 6. *Batissa violaceae* depurated in aged tap water and subsequently immersed in river water for 72 hrs. Fig. 5. Sections of digestive gland taken from site 1 during high flow (Fig. 5a-5b) and low flow periods (Fig.5c-5d).Fig. 6. Digestive gland of clams in site 2 during high flow (Fig.6a-6b) and low flow periods (Fig. 6c-6d). Notice that the digestive gland tubules (dt) taken during high flow were more intact than those taken during low flow periods in both sites

During low flow period, particularly in site 1, alterations in the digestive tubules were observed. The changes could be attributed to the environmental stressors that were more prevalent during low flow period in site 1. This caused lipid peroxidation and subsequent enlargement of tubular lumen due to reduced epithelial thickness. In an earlier work, Odendaal and Reinecke (2003) had also related the reduction in tubule epithelial thickness of woodlouse *Porcellio laevis* after exposure of the hepatopancreas to cadmium sulfate. Similarly, Snyman et. al (2005) showed the dose related decrease in glandular epithelium after six week exposure period of *H. aspersa* in copper.

The self-cleansing mechanism (depuration) was used to reduce potential health hazards. Earlier, Metcalf (1979) had utilized long depuration times for the elimination of greater number of contaminants although not all clams function with equal effectiveness in eliminating contaminants. Also, Cusson et. al (2005) showed that the time required to eliminate 100% of the coliform *E.coli* in bivalves is less than 48 hrs. However, according to Chen and Chou (2001) purple clams *Hiatula rostratais* detoxify PSP toxins for 3-4 weeks and the major toxin found in the digestive gland during the depuration period is GTX3 and GTX2 whereas the non-visceral tissues did not accumulate significant toxin levels, which suggest that the different toxin composition in the different portions of the whole soft body tissue might be due to its capacity to retain toxins in the tissue organs.

The effect of depuration was observed in the present study particularly in the chronically exposed group of clams. Numerous and most intact digestive tubules were seen in depurated group. The absence of apparent lesions seen in the depurated group of clams could perhaps be attributed to autophagy induced by nutrient

deprivation, which occurs during depuration. Autophagy provides a second tier of defense against oxidative stress (Moore et al. 2006).

In the present study, the resident chronically exposed groups of clams showed histological effects on the digestive gland that differs from depurated ones. Pathological changes showed disruption of integrity of digestive tubules as a result of greater areas of intertubular spaces between tubules, separation of digestive cells from the basement membrane, reduced epithelium height, and tubular atrophy.

In the case of those clams that were depurated but subsequently returned to the river during the low flow period, there was detachment of tubular component but only moderate changes were observed in the epithelium of the digestive tubule. These clams also exhibited pathological changes in the tubular structure but did not attain the same change observed in resident clams. Likewise, Wallner et. al. (2000) also observed that the oysters *C. rhizophorae* transplanted from unpolluted site to a contaminated site did not reach the same level of metal content found in resident oysters. The results indicate that the histopathological parameters have been adequate to signify differences between the low flowing and high flowing water.

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