Quantitative Changes in the Hepatopancreas Cells of the Bivalve Mollusk *Batissa Violacea* Exposed to Anthropogenic Activities in Catubig River, Northern Samar, Philippines

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**Abstract.** This study aims to determine the quantitative changes in the hepatopancreas cells of bivalve mollusk as a bioindicator of pollution on aquatic species. Clams *Batissa violacea* inhabiting in Catubig River, Northern Samar was used as the bioindicator of pollution. Clams were collected during high flow and low flow periods at two different sites along the Catubig River. Pooled hepatopancreas were used for quantitative analyses; from chronically exposed group, depurated laboratory control group and those clams that were brought back to the river and immersed for 72 hrs. Histological sections of hepatopancreas were examined by Ken-a-vision microprojector microscope. Results have shown that acute exposures in the number of digestive tubules were significantly different from chronically exposed group of clams. The hepatopancreas epithelium heights also showed significant differences during high and low flow regimes. Overall, quantitative changes found in the hepatopancreas cells after acute and chronic exposures, could serve as a biomarker of exposure to pollutants.

1. Introduction

Rapid population growth, increased industrial activities and agricultural practices, among others that caused water pollution became environmental problem in the world [1]. Most bodies of water are vulnerable to anthropogenic activities due to human routinary movements. These includes the use of agricultural chemicals such as organophosphates, industrial chemicals like metals and surfactants [1,2]. Previously, Snyman et al. [3] reported the percentage area on the oocyte number and the effects of accumulated copper on the hepatopancreas epithelium height on the snail *Helix aspersa*. Results showed a significant lower mean digestive epithelium height for those collected two months after fungicide application compared to snails collected after one-week fungicide application. Similarly, seasonal histological changes in the digestive glands of male and female *Nephrops norvegicus* at the Pagasitikos Gulf were observed. Female lobsters sampled in May had more lipid droplets per tubule and a smaller F-cell tubule ratio than males [4].

Bivalve mollusk are widely used as bioindicator of environmental pollution [2,5,6]. Hepatopancreas of mollusk are good accumulators of toxic substances [7]. The hepatopancreas is a tubulo-acinar gland which occupies the greater part of the shell spiral cavity of mollusk. The digestive tubules are lined with simple epithelium which consists of two cell types namely: digestive and secretory cells [8]. Qualitative techniques in determining effects of pollutants on the molluscan digestive cell structure has been the subject of investigation by various researchers [9–11]. On the other hand, quantitative techniques can also be good indicator of toxicity with greater sensitivity and precision other than qualitative descriptions alone [12].
This study aims to determine the usefulness of quantitative changes as a biomarker of pollution and to validate the histopathological qualitative findings reported in Salinas [11]. Specifically, this will also confirm the effectiveness of the hepatopancreas as pollutant accumulator compared to other body organs of mollusk.

2. Materials and methods

2.1 Experimental design

The experimental design followed were similar to those described by Salinas [11]. Portion of the total soft tissue with hepatopancreas of each animal was rapidly excised and were fixed in 10% formalin, dehydrated in ethanol, and embedded in paraffin. Immediately these were cut 5 um thick, mounted on glass slides and stained with Harris hematoxylin eosin. Slides were examined using Ken-a-vision microprojector microscope with the sections projected either onto blank white paper or millimetre graph paper. For the number of digestive tubules, three randomly squares (A=3mm²) were chosen per section and the total number of tubules was counted. Subsequently, randomly selected intact tubules were traced on millimetre graph paper and evaluated for epithelium height. Five tubules representing different regions of each section were analyzed.

2.2 Statistical Analysis

Data from the two flow regimes, between sites, and treatments were tested for normality and homogeneity of variance before they were subjected to ANOVA (Analysis of Variance). Significance levels were set at P<0.05. ANOVA by post hoc multiple comparisons were made using least significant difference test (LSD) by means of SPSS version 23, to determine which values differed significantly.

3. Results and discussion

The mean number of digestive tubules following chronic and acute exposures of *Batissa violaceae* was shown in Fig. 1. Acute exposure was significantly different from those chronically exposed clams (P=0.000). Significant difference between sites was also noted except within the resident chronically exposed and depurated group of clams during high flow period. P value for this difference ranged from 0.000-0.028.

![Figure 1](image)

Figure 1. The mean number of digestive tubules (Means±SEM) of *Batissa violaceae* at two different sites in the Catubig River following chronic and acute exposures during high flow and low flow conditions of the river.

Further in Site 1, during high flow period, the number of digestive tubules was significantly higher in depurated group of clams (169.5±17.31) compared to that of resident clams (114.66±1.48).
Fig. 2 shows the mean epithelial height of the hepatopancreas tubules in the two sites during the flow regimes. There was significant difference between the two flow regimes except in the in the depurated group of clams in site 2. During high flow, the mean epithelial height was significantly higher, ranging from (34.81±0.85-15.80±1.31). In low flow period site 1, epithelial height of tubules was significantly greater in the depurated group of clams 14.07±0.43 as compared to that of the resident clams, 2.22±0.032. Between sites, no significant difference was observed during high flow period.

The results of this study confirmed the histopathological qualitative findings of Salinas [11] that the hepatopancreas of *Batissa violaceae* is a good accumulator of various substances. Earlier study, [7] considered the same known organ as a target for environmental pollution assessment. Previously, the disintegration in the digestive tubules during low flow period in Site 1 were observed [11]. Quantifiable changes with reduced height of epithelium were noted in the present study. It was concluded that the responses could be attributed to the environmental stressors like municipal sewage and pesticides that were present in Site 1 during low flow period. The results of this study concur with the data reported by other researchers. Rocha et. al [13] noted the decrease in epithelium height of the mussel *Mytilus galloprovincialis* after exposure to cadmium quantum dots (QD) for 14,15 and 17 days. Likewise, the reduction in mean epithelial thickness (MET) of the mussel *Mytilus galloprovincialis* after long term exposure of the hepatopancreas to the Prestige oil spill in Galicia and Bay of Biscay [14] was in agreement with the current research.

In this study, the number of digestive tubules was higher in depurated group of clams, during high flow period. Also, the results of histopathological qualitative analysis have shown amoebocyte infiltration in the same group of clams. This is indicative of clam’s natural protective defense against environmental stressors. This probably explains the large number of digestive tubules and amoebocytes in the depurated clams during high flow period. The self-cleansing mechanism (depuration) for eliminating contaminants was also documented in various researches [15–17]. In the qualitative study, pathological changes in the resident chronically group of clams showed reduced epithelium height and tubular atrophy. These results corroborated with the reduced number of digestive tubules and epithelial thinning in chronically exposed clams. The reduced number of digestive tubules in chronically exposed group of clams as typical response to stressors were in agreement with the results from earlier studies [2,13,14,18]. The thinning of epithelial cells could be due to autophagy and the role of calcium cells during the detoxification process [12].

For those clams that were depurated and immediately returned to the river, pathological changes were observed in the hepatopancreas epithelium but their damage were not as prominent compared to resident group of clams [11]. The previous results can be correlated with the present study wherein clams returned to the river showed reduced epithelial thickness but not at the same level with the resident chronically group of clams.
Since this study was conducted in natural field setting, it is possible that other factors could induced the observed pathological changes. Stressors that include metals, pesticides, oil, and municipal sewage, among others are known anthropogenic activities that cannot be avoided being part of human daily routine [19].

The results of the quantitative analysis in conjunction with previous qualitative histopathological findings, lead to conclusion that the changes in the number of digestive tubule and the hepatopancreas epithelium height are measurable indicators of environmental pollutants and can be considered as biomarker of pollution. Although, the main contributing factor that caused cellular changes was not identified, they can be a subject of future studies.

4. References

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**Acknowledgment**

I thank Dr. Glorina Pocsidio of University of the Philippines, Diliman for her valuable suggestion. Likewise, Reinerio E. Irinco from National Irrigation Administration for all the assistance specially in the preparation of the manuscript.