Lipid Peroxidation as a Biomarker of Field Exposure in the Gills and Digestive Gland of the Freshwater Bivalve Batissa Violaceae Lamarck

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***Abstract*—This current research aims to determine the potential of lipid peroxidation as a biomarker for environmental water pollution. Post-hoc multiple comparisons between sites, flow regimes and tissues were made using least significant difference test (LSD) to determine which values differed significantly. Results of the TBARS assay on the acute exposures in the field and in the lab showed that after 24, 48 and 72 hrs. exposure, clams from both sites showed higher MDA levels in the digestive gland and gills during the low flow period than in the high flow period .The laboratory control clams showed progressive decline in MDA levels in low flow clams while the high flow clams showed relatively no change in the MDA levels. Most marked was the significant rise in the MDA levels of low flow clams of Site 1 after 72 hrs exposure in the field. Digestive glands were more sensitive to change in the levels of lipid peroxidation compared to gill tissues. The results suggest that lipid peroxidation levels can be a good bioindicator of pollution which in this study is inherent characteristic of Site 1 especially during the low period.**

***Index Terms*—lipid peroxidation, malondialdehyde, biomarker, gills, digestive gland.**

I. Introduction

The earth is affected by the presence of environmental contaminants as a result of increased industrial activities, anthropogenic inputs, irrigation systems, and non-traditional agricultural practices stimulated by rapid population growth and increased urbanization [1]. Biomarkers are important tools in the detection of various contaminants especially in aquatic ecosystem. Any biological response at cellular, biochemical and molecular levels that indicate the presence of pollutants are considered as biomarker [2].

Most water contaminants can convert oxygen to reactive oxygen species (ROS) that produce toxic effects by behaving as units called free radicals. Prime targets of ROS are the polyunsaturated fatty acid (PUFA) in the membrane lipids causing lipid peroxidation that eventually leads to a variety of human health disorders such as atherosclerosis, ischemia, reperfusion injury, UV induced carcinogenesis and even cell death [3].

In this study, the freshwater clam *Batissa violaceae* Lamarck (Family Corbiculidae) was tested as an indicator orgamism of water pollution in the Catubig River Northern Samar. The study on the mollusk as possible bioindicator of riverine water quality was premised on the fact that they have the ability to concentrate pollutants, since bivalves are sessile in nature and are filter feeders [4]. They have the ability to accumulate chemical or bacteriological contaminants and naturally occurring harmful substances in surrounding waters. It has been reported that a single clam may filter up to 300 times its weight in one hour [5].

The present study had the following objectives: (1) to determine the potential of lipid peroxidation as a biomarker of environmental water pollution (2) to determine the level of responsiveness of digestive gland and gills to a change in environment (3) to determine the extent of lipid peroxidation of resident clams as well as acutely exposed clams under the two flow regimes (4) to compare the extent of lipid peroxidation in clams from two sites in the river.

II. Materials and Method

A. Study Area

Clams collected from two different sites in the Catubig River were analyzed and compared during high and low flow periods. Observation sites in the Catuvig River were: Site 1- the riverbed within Barangay Poblacion which is nearest to the town proper of Catubig, and Site 2- the riverbed in Barangay Fidel Robis located between the towns of Las Navas and Catubig. Site 1 is relatively densely populated area, a commercial district. Site 2 is within a less populated area wherein the agricultural farm is largely dependent on rainfall and agricultural practices are traditional where pesticides and chemical fertilizers are not being applied.

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B. Test Organisms

Test organisms used were the freshwater clam *Batissa Violaceae* Lamarck. For the study, individual clams with approximate shell length of 4-6 cm were collected from the riverbed at depths of 8 m by professional divers at two distinct sites during the high and low flow periods.

C. Lipid Peroxidation (LPO) in Resident or Chronically Exposed Clams

Thirty bivalves were collected and subjected to TBARS assay for determination of LPO levels from each study site during the two flow regimes. The clams were dissected for digestive gland and gills, the tissues from three clams were pooled and subjected to the TBARS assay (n=5).

D. Experimental Depuration and Acute Exposures

For acute exposure, from each study site from the river, 180 individual clams were collected during high and low flow periods of the river. 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). Levels of tissue lipid peroxidation were determined after each exposure period (i.e. 24, 48, and 72hrs.) with n=5.

E. Thiobarbituric Acid Reactive Substances (TBARS) Assay

The extent of lipid peroxidation was determined by a modified TBARS method of measuring malondialdehyde (MDA) [6].The digestive glands were homogenized using a glass tissue homogenizer in a 0.05 M phosphate buffer (pH 7.4) at 1:2 proportion of tissue weight to buffer volume. Afterwards, 0.5 ml of homogenate was added to 2.5 ml 20% trichloroacetic acid and 1 ml 0.67% thiobarbituric acid and subsequently mixed with a vortex mixer. The tubes were heated at 100°C for 30 minutes on a water bath. After cooling to room temperature, 4 ml of butanol was added and was shaken vigorously on a vortex mixture. After centrifugation at 1000 g for 15 min. at 25° C, the amount of MDA formed was measured by the absorbance of the upper butanol layer at 535 nm. MDA (1, 1, 3, 3 tetraethoxy propane) was used as a standard for the assay and the level of lipid peroxidation was expressed as nanomoles MDA per mg protein. Protein concentration was determined by the Bradford method [7].

F. Statistics

Data gathered were subjected to analysis of variance (ANOVA). Significance levels were set at $P<0.05$. When ANOVA revealed significant differences, post hoc multiple comparisons between sites, tissues and flow regimes were made using least significant difference test (LSD) to determine which values differed significantly.

III. Results

Fig. 1 shows the lipid peroxidation (LPO) levels in digestive gland and gills of the chronically exposed bivalves or resident clams from the two sites in the two different flow periods of the river. In each site, MDA or LPO levels in the tissues of *B. violaceae* were significantly higher during low flow periods. DG1 (from site 1) during the low flow period showed the highest MDA (nM MDA/mg protein 276.92 ± 21.92 only approximating the levels of the gills (G1) with 137.04±18.70.

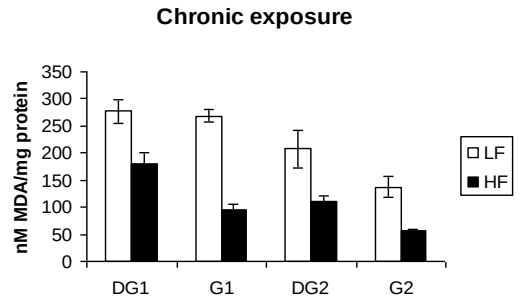

Figure 1. The MDA levels at two different sites in the Catubig River in the (DG=Digestive gland; G=gills) of Batissa violacea during high flow and low flow conditions of the river. All low flow values are significantly greater than their corresponding high flow values. All values for site 1 are significantly greater than for site 2 except for gills during high flow period.

Shortly after depuration, it was observed that LPO levels in DG and G dropped from the chronic levels (Fig. 1). P value for this difference ranged fom (0.000-0.030) except for the gills, in both sites during high flow conditions.

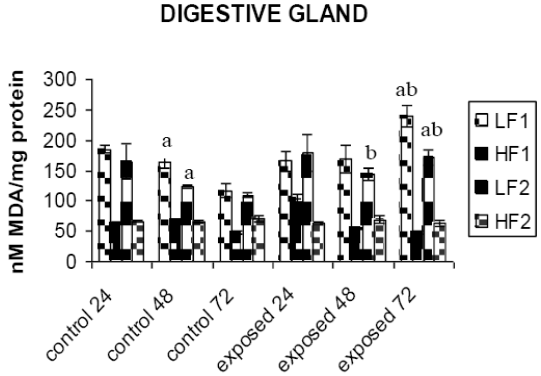

Figure 2. The MDA levels (Means ± SEM) of *Batissa violacea* at two different sites in the Catubig River following depuration and acute exposures during high and low flow conditions of the river. All low flow values are significantly higher than the corresponding high flow values. All values at low flow are not significantly different between sites except at a. Significant difference from corresponding depurated clams is indicated by b.

The effects of acute exposure in the digestive gland are shown in Fig. 2. There was the difference in LPO according to the flow regime (Low flow> High Flow). For the clams collected during the low flow period, marked reduction in the MDA level from the just depurated level was evident, especially in those that were immersed in clean tap water compared to those clams immersed in the river. MDA levels were reduced after 24

and 48 hours, but increased after 72 hours. Significant difference between sites was shown only just after depuration and after exposure of 72 hours during the low flow period.

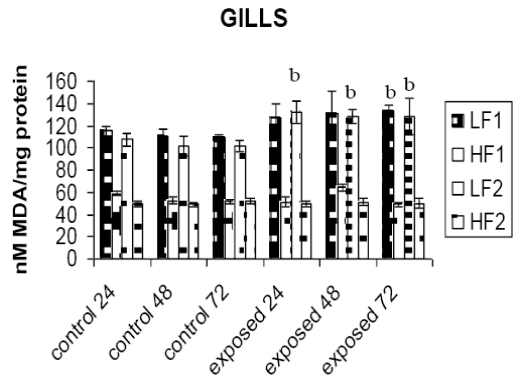

Figure 3. The MDA levels (Means ± SEM) of *Batissa violacea* at two different sites in the Catubig River following depuration and acute exposures during high and low flow conditions of the river. All low flow values are significantly higher than the corresponding high flow values. All values at low flow are not significantly different between sites. Significant difference from corresponding depurated clams is indicated by b.

Fig. 3 shows the responses of the gills. Again as described previously for the digestive gland, there is evident difference in the response of tissues during high flow and low flow periods. The levels of LPO in the gills were higher during the low flow period. There was no change in the LPO of gills from the just depurated clams and the gills of clams placed in tap water. However, while the clams were in the river, in both sites, during the low flow period, MDA levels increased, (P value range from 0.01-0.10). Gills from clams collected during the high low period did not show change in MDA levels from the depurated levels in both tap water and river water.

IV. Discussion

Biomarkers are useful tools in measuring the health status of the environment. Among the biomarker of stress, the primary key events in oxidative damage is lipid peroxidation [8].

The results of the assays for lipid peroxidation levels had obviously reflected the condition of the river. Since a consequence of low flow is a greater concentration of pollutants in the water, expectedly, high MDA levels were obtained from the clams during this time. Since the greater amount of undesirable environmental impacts due to anthropogenic inputs inevitably caused greater pollution in Site 1, expectedly, lipid peroxidation was significant in clams collected from this site. During low flow period less water is available to dilute whatever intrusions, inorganic or organic, harmful or otherwise, that maybe present in the water. During times of heavy rains or typhoons, volume flow as well as rate of flow of the water in the river is greatly increased.

It is expected that at this period of high flow, pollution would not be as much as that seen during low flow periods. The levels of LPO in digestive gland and gills were consistently higher during low flow periods in the river in both groups of clams that have been chronically exposed and acutely exposed after depuration. Moreover, expectedly, during the low flow period, levels of MDA were consistently higher in clams obtained from site 1, possibly due to higher level of pollution in this riverine areas. This observation closely paralleled on the clam *Melanoides tuberculata* in the Rietvlei wetland System in South Africa in which the levels of malondialdehyde in total soft tissue of the clam during low-flow periods of the river were also very much higher. In this particular study, the higher level of LPO had also been attributed to the increased concentration of different chemicals in the water [9].

Chronic and acute exposures in gills and digestive gland were done to establish the extent of adaptation of the organisms to oxidative stress, to determine their responsiveness to the level of environmental pollution and their applicability for biomonitoring. Clams that had been living in the river for some time showed marked oxidative stress as indicated by the marked difference in the MDA levels reached by the resident clams. There is the possibility that the chronically exposed clams are in the state of incomplete adjustment of their antioxidative defenses due to the continuous disturbance being brought about by activities in surrounding area. The prevailing interaction of prooxidant and antioxidant processes in the tissues must likely that lipid peroxidation still exceeds the activity of defensive antioxidant system. Cossu *et.al.* had reported the decreased antioxidants and increased lipid peroxidation in bivalves transferred to polluted sites [10]. Similarly, increased lipid peroxidation level and concomitant decreased of antioxidant level was observed in the bivalve *Unio tumidus* after exposure to copper and thiram [11]. On the other hand, however, higher levels of antioxidant enzymes (catalase,glucose 6-phosphate and 6-phosphogluconate) and decreased MDA content in clams *Scrobicularia plana* could be obtained from clams chronically exposed to metals and hydrocarbons which suggest the possibility of an adaptive mechanism against oxidative stress [4].

Furthermore, in the study regarding the uptake and clearance of PCB congeners, it was shown that the antioxidant superoxidase dismutase activity increased seven fold during the depuration process in the clam *Chamaelea gallina* [12]. This situation could explain the result of the present study of decreased lipid peroxidation levels in depurated clams after 24 and 48 hours of exposure as well as the result obtained from the depurated clams that were reimmersed in the river water. Possibly, there was an increased level of antioxidant enzymes during the depuration as well as the contribution of nutrient deprivation. According to Moore, nutrient deprivation could result in improved "housekeeping" through the efficient removal of cellular structures and components that had been damaged or no longer needed, as shown in the experiment involving depurated groups of clams [13].

Regarding the study on the depuration rates of American oyster *Crassostrea virginica*, wherein the period of time needed for depuration of accumulated

organic contaminants represented the degree of contamination of a site and could vary depending on the concentration of contaminants present in the tissues [14]. Pertinent to this report would be the obtained result of the present study on the continuing decline of MDA levels within a 72-hour period in the digestive gland from low flow period in clean water, which could then likely reflect the degree of pollution of the site. This trend was not seen in the Site 2 clams as well as in the high flow batches.

There is the possibility that the increase of MDA level for those clams that were returned to the river could be caused by stress due to handling. Camus *et al*. had considered transplantation to have a great effect on the antioxidant level of mussels. Possible sources of stress associated to transplantion such as handling, emersion and transport lead to subsequent depression of most antioxidant defenses [15]. However, according to Rodriguez-Ariza *et al.*, clams that are exposed to air can recover completely after re-immersion [12]. The present result wherein the clams that remained for 72 hours during the low flow period still showed enhanced lipid peroxidation could, therefore, suggest continued sensitivity and responsiveness to the pollution in the site.

Different biomarker responses have been correlated to different physiological functions and composition of tissues such as antioxidants, lipid content, metabolic rate and DNA repair activity [16]. In the present work, more enhanced lipid peroxidation was evident in the digestive gland since it contains high lipid reserves and high amounts of antioxidants [17]. Furthermore, in agreement with the result reported in 2002, there is a lower level of MDA in the gills than in digestive gland as in both the oyster (*Crassostrea gigas*) and the mussel (*Mytilus edulis)* that have been suggested to possess intensified antioxidant system [18].

In addition, in the study of brown mussel *Perna perna*, it was noted that there is a higher activity of glutathione peroxidase in gills than in digestive gland, which corresponds to decreased lipid peroxidation. Their results had been attributed to the fact that the gills are known to be the active site for excretion, providing the removal of unwanted contaminants from the organism [19].

V. Conclusion and Recommendation

Based on the findings of this study, lipid peroxidation level in the freshwater clam from two sites in the Catubig River which were mainly differing in the extent of anthropogenic inputs was greatly affected by the flow regime. Digestive gland had higher capacity to exhibit change in the levels of lipid peroxidation than the gills. In all clams, reduction of MDA levels was observed after their immersion in clean tap water; however upon return to the river water, increased in lipid peroxidation occurred. These results indicate that lipid peroxidation was a good biomarker of pollution. Yet, further studies are needed for the exploration of other organs or other components of the whole tissue to completely determine all the possible effects of the contaminants in a particular tissue. Other biomarkers such as the antioxidant system can be undertaken to fully understand the response of the organism to oxidative stress. Presence of chemicals before and after depuration should be determined. Proper handling and transport should be taken in consideration to avoid stress during transplantation. Furthermore, monthly biomonitoring should be evaluated to show or to give awareness to people of Northern Samar for the possible impacts of the environmental factors on the clams and the community.

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