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SENSITIVITY OF JUVENILE AND ADULT FRESHWATER CLAMS (MERCENARIA MERCENARIA) TO ACUTE AND SUBLETHAL TOXICITY OF CHLORINE CONCENTRATION

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ABSTRACT

This study evaluated the Sensitivity of Adult and Juvenile Freshwater Clam (*Mercenaria mercenaria)* to Chlorine toxicity. Six concentrations of Calcium Hypochloride were tested using 96-h static renewal toxicity tests and 8 weeks using the water renewal system respectively for acute and sub-lethal tests. Based on their respective mean 96-h lethal concentration to 50% (LC_{50}s), Juvenile Clams (52.78 mg/L Ca $(0H)_2$) were more sensitive than Adult Clams (53.56 mg/L $Ca(0H)_2$). For the 96 h bioassay at a significant difference of $p<0.05$, mortality ranged from 3.40±0.92 to 0.00±0.00 mg/L for the Adult Freshwater Clams and varied from 3.66±0.33 to 0.00±0.00 mg/L. The variation of water quality in the acute test showed the Total Dissolved Solids (mg/L) varying from 356.33±0.88 to 330.67±0.88 for adults and 3.67.50±2.53 and 347.00±0.00 for juvenile Clams, Dissolved Oxygen (mg/L) ranged from 5.66±0.06 to 4.49±0.15 for adult Clams and 6.75±0.05 to 3.51±0.15 for juvenile Clams, pH varied from 8.95±0.02 to 9.67±0.00 for adult *M.mercenaria* while the juveniles showed 9.68±0.00 and 8.96±0.03, Electrical Conductivity (µS/cm) ranged from 612.33±0.88 to 693.67±0.88 for adult Clams and 683.25±5.25 to 613.33±0.88 while the Temperature (ºC) ranged from 23.00±0.00 to 25.40±0.01. Water quality parameters were also varied in the sub-lethal test and they showed the highest Total dissolved solids of 358.67±4.12 at 0.87mg/L for adult Clams and 345.88±1.06 for juveniles while the lowest of 333.79±3.36 for adults and 329.79±2.39 for juveniles. The Dissolved Oxygen ranged from 7.00±0.00 to 3.84±0.07 for adult Clams and 6.27±0.03 to 4.82±0.02 for juvenile Clams. The pH ranged from 9.65±0.01 to 9.57±0.01 for adults and 9.61±0.01 to 9.50±0.01 for the juvenile Clams. The Electrical Conductivity varied from 6.62.96±7.38 to 714.96±9.34 for the adults and 662.00±3.66 to 689.79±3.58 for the juvenile *M. Mercenaria.* Temperature varied minimally from 24.80±0.14 to 24.89±0.13 for the adult Clams and 24.82±0.13 to 24.95±0.13 for the juvenile Clams. Mortality in the sub-lethal test for adult *M. Mercenaria* of 1.00±0.00 occurred at 1.78mg/L on week 6, concentration 3.57 mg/L on week 5 and 6 and concentration of 4.46mg/L on weeks 4, 5, 6 and 8 while for Juvenile Clams, mortality of 1.00±0.00 occurred at week 7 and 8. Comparison of LC_{50}s reported for other aquatic organisms to the 96-h LC_{50}s calculated for juvenile and adults of *Mercenaria mercenaria* shows this bivalve mollusc species to be among the most sensitive to Chlorine toxicity. Based on reported levels of discharge of chlorine effluent in the aquatic environment from anthropogenic sources, it may be an important limiting toxicological factor to freshwater Clam populations. The results indicate that the younger life stages of Clams generally were more sensitive to chlorine than the older life stages. The study also investigated the Histological alterations of the gills of *M. mercenaria* following a long-term exposure to sub-lethal levels of Chlorine toxicant. Clam

gills were extracted and taken to the laboratory to determine the Histological damage of this tissue. It was observed that most histological alterations were highly localised in the glandular cells of the gills; secretions of the glandular cells was found to be increased by increased exposure time. Histological changes in the gills used for this evaluation were the common lesions were epithelial lifting, hyperplasia and hypertrophy of the respiratory epithelium, lamellae fusion, and aneurysms in the gills. Histo-pathological results showed the activation of resistance mechanisms that allowed the Clams to survive under sub-lethal stresses. Thus, histological changes on localised tissues were sensitive and they had positive correlation to the time of exposure to the toxicant suggesting that they may serve as biomarkers for roundup exposure.
Keywords: Toxic, Histology, chlorine, Clams.

INTRODUCTION

Freshwater Clams are among the most imperilled groups globally (Lydeard *et al*., 2004). Their decline has been attributed to a number of factors including loss of habitat, predation, over-harvesting, invasive species, and exposure to environmental pollution (Williams *et al*., 2013). In fact, nearly 70% of all freshwater Clams in the United States are designated as either threatened, endangered, or in decline (U.S. Environmental Protection Agency, 2004; Williams *et al*., 2013). Aquatic ecosystems are exposed to contaminants that enter the waterway from various sources and contaminate different compartments of the ecosystems. Because of their aquatic distribution, contaminants affect a wide range of non-target organisms, like invertebrates and fishes, those inhabiting an aquatic environment (Otludil *et al.*, 2004). The likely release of chlorination by-products in the effluent stream in the process of chlorination and their possible damage to the aquatic life in the natural environment have raised concern in the use of technology. Another important disadvantage of chlorination is the species-dependent tolerance to hypochlorite; some species, even from the same phylum, are far more resistant to chlorination than others. Chlorination efficiency is further temperature-dependent: if the water temperature is low (<15 °C), the time required for effective chlorination will be prolonged (Jenner and Janssen- Mommen, 1993).

There have been suggestions that environmental contaminants may be responsible for failed reproduction or recruitment. These other factors potentially contributing to the continued low Clam abundance include: modification to the hard Clam food supply through changes in the phytoplankton assemblages and/or phytoplankton production resulting in a reduction in hard Clam growth rate, changes in predation rates and/or predator assemblages resulting from the modified conditions (Polyakov *et al.*, 2007), a decreased recruitment of Clams (Kraeuter *et al*., 2005). As with most organisms, the early life stages of freshwater Clams are the most sensitive to contaminant exposure but until recently, adults have been the focus of studies addressing the effects of waterborne contaminants on Clams (Kelly and Dram, 2012). There are a number of potential chemical threats in the watersheds where many endangered species of Clams are found. Furthermore, Valenti *et al*., (2005) stated that the data provided by laboratory toxicity tests with freshwater Clams are critical to their conservation since such controlled exposures are not possible in the field. Although there have been studies that have assessed Clam sensitivity to a range of environmental contaminants including pesticides (Bringolf *et al*., 2007), ammonia (Augspurger *et al*., 2003; Newton and Bartsch, 2007), chlorine (Valenti *et al*., 2006), there is as yet no clear consensus as to the contribution of water borne contaminants in the decline of freshwater Clams.

MATERIALS AND METHODS

Collection, Handling and Acclimatization of Clams

Juveniles and adults of *Mercenaria mercenaria* were collected from the Wadata axis of River Benue. Clams were collected with shovel and separated from the sandy – gravel substrate using a screen. Before any experiments, Clams were acclimatized in concrete tanks for at least 2 weeks to rescue Clam behavior such as burrowing and siphon retraction. Clams were removed from the sediment and sent in moistened plastic bags to the laboratory. Previous research and experience with Clams (unpublished data) demonstrated that *Mercenaria mercenaria* can survive forced valve closure in open air for seven to ten days, after which accumulation of metabolic end products or starvation (or

both) resulted in death or heightened sensitivity to toxicant. However, in all tests described here, less than 30 hours elapsed from the time of collection to placement in the laboratory. Clams were sorted into various size classes and acclimated to the eventual test temperature. The Clams were fed a concentrated algal mixture and zooplanktons as well as minced fish and shrimps once daily during all testing and acclimatization periods.

Plate 1. Shells of live Freshwater Clams showing Chlorine infestation

Behavioral Toxicity Assays

Valve movement behavioral toxicity assays were performed from July to September 2018. About 10 Clams of a specific size class i.e., mean shell length = juveniles were those whose lengths spanned from 4-10 mm shell length and adults 14-20 mm shell length (in line with Jasper, 2007) were randomly selected and transferred into each test media containing water to obtain the does-response profiles with various exposure chlorine concentrations under different integrated response times. The behavioral endpoint is valve closing response. The size segregated Clams were placed in groups of ten (10) in plastic bowls for identification. Test durations were 96 hours for acute and 8 weeks for the sublethal toxicity test which correspond with generally accepted *Mercenaria mercenaria* control practices (Yaldiz, 2008). From the results of the 96 hours LC_{50} by Auta (2001), the sublethal concentrations were chosen by taking fractions; one sixtieth (1/60), one thirtieth (1/30), one twentieth (1/20), one fifteenth (1/15) and one twelfth (1/12) of the concentrations from below the lowest limits which were taken as sub-lethal using the method according to Abubakar (2013) to arrive at each concentration. All tests were triplicated.

Various collection locations and dates were used so that minimal differences existed between ambient collection, acclimation and test temperatures. Exposure concentrations were chosen based on evidence on the literature for toxicity at various levels. Five concentrations (including the control) were used in each experiment. In all tests, juveniles and adults were simultaneously exposed. Adults and juveniles in these tests were exposed in discrete size classes to evaluate size dependent (length and weight) effects. Shell length was determined using Vernier callipers to ± 0.025 mm. Toxicants were applied to each treatment using pipette for all the concentrated solutions. Chlorine exposures were administered with a very slow dilution water inflow. Treatment temperature and toxicant concentrations were checked once daily during the chlorine tests. The Clams were considered dead and then removed when their valves parted easily upon probing with a small spatula or if the Clam was found open and not able to respond to gentle prodding.

The acute toxicity of biocides to Clams was evaluated from July to September, 2018 thus; acute (0, 20, 40, 60, 80 and 100 mg TRC/ L for both adults and juveniles) and sublethal (Adults; 0, 0.89, 1.78, 2.67, 3.57 and 4.46 mg TRC/L then Juveniles; 0, 0.87, 1.75, 2.63, 3.51 and 4.39 mg TRC/L) was tested for biocidal effectiveness.

Histo-Pathology of Clam Gills

The gills of the Clams were extracted after the sub-lethal test that lasted for eight (8) weeks and then fixed in Davidson's fixative (alcohol: formalin: acetic acid mixture in the ratio 70: 10: 5 for a

minimum of 24 hours. After fixation, a transverse sectional slice of fixed Clam tissue approximately 5 mm thick was taken from the middle part of the body for histology, ensuring that the gill tissues would be represented in sections. Sections were made at 4 mm using a rotary microtome and were stained in hematoxylin and eosin, and mounted in Permount synthetic mounting medium. Slides were examined microscopically for sub-lethal toxicological effects of chlorine on this tissue of Clams as well as pathological alterations. Histological sections were examined under a light microscope and all images were captured using a spot rising tech camera (Diagnostic Instruments, Inc.).

The samples were randomly selected from the triplicates for each concentration for both the adult and juvenile Clams. Consequent upon preservation of tissues in formalin in specimen bottles, the test tubes were duely labelled per concentration of toxicant and name of tissue. Thereafter, the entire labelled specimen was moved to the Department of Anatomy, College of Veterinary Medicine, Federal University of Agriculture, Makurdi for analysis. The laboratory analysis involved the following stages; Collection of samples, Fixation of collected samples, Dehydration of collected samples, Embedding, Sectioning, Floatation, Staining and Microscopy.

Water Quality Analysis

Toxicant concentrations were measured in exposure systems to validate actual exposure levels. Water temperatures were recorded daily for all tests. The pH was measured daily by a pH meter while the Dissolved oxygen was measured by a dissolved oxygen meter.

Statistical Analysis

Since biocidal effectiveness may not be reflected by lethal concentration (LC) statistics (e.g. a 4 day LC_{50} would indicate that the biocide was not effective and only half of the clams were killed), data was analysed for time to lethality (LT_{50} and LT_{100}; LT- lethal time). In this way, comparism of time to 50 percent and total (100%) effectiveness was compared on a per-concentration basis. Information was processed using the Fisher LSD and one way ANOVA for differences between levels of treatments.

Collection of Planktons

Clams under test conditions were fed a concentrated plankton mixture once daily during all testing and acclimatization periods. This plankton mixture was collected from the ponds in University of Agriculture Makurdi fish farm and the effluent site at the Makurdi abattoir. These were collected with the use of plankton net. Collection for zooplankton was done using a 243-mm mesh plankton net with a 12.5 cm opening. This was done by pulling the fine mesh net through the water, either vertically or horizontally, and then collecting the organisms that were retained by the net. Collections for phytoplankton was made using a surface grab technique. Prior to each use, there was a careful cleaning and thorough rinsing of the interior of the plankton nets and buckets.

Exposure System

During the acute toxicity test for both adults and juveniles, the exposure system used was the static exposure system where the test exposes the organism in still water. The toxicant is added to the water in order to obtain the correct concentration to be tested. The control and test organism were placed in the test solutions and the water was not changed for the entirety of the test. In the sub-lethal toxicity test for both juveniles and adult Clams, the renewal exposure system was used. The renewal test exposed the organism to the toxicant in a similar manner as the static test because it is in still water. However, in the renewal test, the test solution was renewed periodically at constant intervals of one week by transferring the organism to a fresh test chamber with the same concentration of toxicant. Therefore, static test condition was maintained in the acute test according to a protocol previously established (APHA, 1995). Renewal system was used because half-life of Calcium hypo-chloride in water at pH 8 is less than nine days at 25^{o}C and three days at 35^{o}C. It is hydrolyzed more rapidly at higher pH (Wang *et al*., 1994).

RESULTS

Acute Mortality in Freshwater Clams

Generally, the mortality of Clams increased as the concentration of chlorine increased (Table 2). The concentrations of chlorine toxicant ranged from 0 mg/l (control) to 100 mg/l. It was observed

that there was an increased rate of mortality recorded as the period of exposure increased from 24 hours to 96 hours. At 24 and 48 hours, mortality was not significantly different at different concentrations of chlorine, but at 72 and 96 hours, mortality showed significant difference at the different concentrations of 0 to 100 mg/l. Mortality increased steadily from 24 hours to 96 hours for adults Clams. During this period, the total mortality showed a significant difference ($p<0.05$) from 24 hours to 96 hours. It was observed that mortality of Clams increased as the Chlorine concentration increased (Table 2). There was an increased rate of mortality recorded as the period of exposure increased from 24 hours to 96 hours. In other words, as the period of exposure of Clams to toxicant increased, more mortality was recorded.

At 24, 48 and 72 hours, mortality was not significantly different at different concentrations of chlorine, but at 96 hours, mortality showed significant difference at the different concentrations of 0 to 100 mg/l. Mortality increased steadily from 24 hours to 96 hours for juvenile Clams. During this period, the total mortality showed a significant difference ($p<0.05$) from 24 hours to 96 hours as the concentrations of toxicant also increased.

Table 2. Mean Mortality Records of Adult Clams under Acute Exposure to Chlorine.

Concentration of Toxicant (mg/L)	Period of Exposure to Toxicant (Hrs)				Mean Mortality
	24	48	72	96	
0	0.00 ± 0.00^{c}	0.20 ± 0.20^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.20 ± 0.20^{f}
20	0.60 ± 0.40^{bc}	0.40 ± 0.24^{bc}	0.40 ± 0.24^{cd}	0.80 ± 0.20^{cd}	2.20 ± 0.20^{e}
40	0.80 ± 0.20^{abc}	1.40 ± 0.24^{a}	0.60 ± 0.24^{cd}	1.00 ± 0.31^{cd}	3.80 ± 0.20^{d}
60	1.20 ± 0.37^{ab}	1.00 ± 0.31^{ab}	1.20 ± 0.37^{bc}	2.00 ± 0.00^{bc}	5.40 ± 0.24^{c}
80	1.00 ± 0.31^{ab}	1.60 ± 0.24^{a}	2.00 ± 0.54^{ab}	3.20 ± 0.58^{ab}	7.80 ± 0.20^{b}
100	1.60 ± 0.51^{a}	1.60 ± 0.24^{a}	2.80 ± 0.37^{a}	3.40 ± 0.92^{a}	9.40 ± 0.24^{a}
CV	*99.27*	*74.02*	*103.36*	*92.08*	*67.32*
P-Value	*0.05*	*<0.01*	*<0.01*	*<0.01*	*<0.01*

Means on the same column with different superscript are statistically significant ($p<0.05$)

Variation of Water Quality Parameters in Acute Test

In the course of the 96 hours of the acute test on the adult Clams, a static exposure system were the test exposes the organism in still water, the toxicant is added to the water in order to obtain the correct concentration to be tested. The control and test organisms where placed in the test solutions and the water was not changed for the entirety of the test. During this period, water quality parameters were measured and recorded at the start and end of the experiment accordingly. The total dissolved solids (TDS) showed no significant difference (0.05%) at the concentration of 0 to 60 mg/l but showed some level of significance at 80 and 100 mg/l (Table 3). Dissolved oxygen (DO) showed no significant difference at 20 mg/L while at 0, 40, 60, 80 and 100 mg/L there was observable significant difference at the confidence limit of 0.05%. The pH also showed its significant difference at 20 mg/l while no level of significance was observed at 0, 40, 60, 80 and 100 mg/l. The Electrical conductivity and Temperature showed no statistical significance from 0 to 100 mg/l in the experiment.

In the acute test, the acute exposure system was used; therefore, the water was not renewed throughout the period of exposure. The total dissolved solids (TDS) showed significant difference (0.05%) at concentrations of 0, 40, 60, 80 and 100 mg/l while there was no significant difference at 20 mg/l. Statistical significant difference for pH at 0.05% was observed at 60 and 80 mg/l while there was no significant difference for 0, 20, 40 and 100 mg/l. Electrical conductivity showed no significant difference at 0.05%. Dissolved oxygen (DO) showed significant difference at 80 and 100 mg/L while at 0, 20, 40, 60 mg/L.

Temperature showed significant difference at 40, 60, 80 and 100 mg/l at 0.05% while showing no statistical difference at 0 and 20 mg/l.

Table 3. Variation in Water Quality Parameters in Acute Test for Adult Clams

Concentration of Toxicant (mg/L)	Water Quality Parameters									
	TDS (mg/L)		DO (mg/L)		pH		EC (µS/cm)		Temperature (°C)	
	Start	End	Start	End	Start	End	Start	End	Start	End
0	335.67±3.38	330.67±0.88	5.66±0.06	5.63±0.10	8.99±0.00	8.95±0.02	613.67±0.88	612.33±0.88	23.00±0.00	25.30±0.00
20	342.33±4.26	344.33±1.20	5.65±0.15	5.66±0.08	9.30±0.04	9.00±0.00	651.33±8.33	665.00±2.00	23.00±0.00	25.35±0.00
40	346.33±4.70	344.33±0.33	5.21±0.01	5.19±0.05	9.47±0.05	9.42±0.06	660.67±9.13	688.33±3.18	23.00±0.00	25.20±0.10
60	350.33±1.20	346.00±0.57	5.14±0.02	5.12±0.07	9.52±0.06	9.47±0.04	666.67±7.22	690.00±1.53	23.20±0.01	25.40±0.00
80	352.00±1.15	346.00±1.00	4.99±0.10	4.90±0.12	9.61±0.02	9.55±0.05	676.33±8.41	692.00±0.57	23.00±0.00	25.36±0.03
100	356.33±0.88	346.33±0.88	4.50±0.25	4.49±0.15	9.67±0.00	8.57±0.97	688.67±5.81	693.67±0.88	23.20±0.02	25.40±0.01
Df	*2*	*2*	*3*	*2*	*2*	*2*	*4*	*2*	*2*	*2*
T-Value	*1.43*	*1.39*	*0.42*	*0.45*	*1.39*	*1.25*	*1.07*	*1.59*	*0.62*	*0.53*
P-Value	*0.28*	*0.69*	*0.29*	*0.37*	*0.67*	*0.65*	*0.34*	*0.25*	*0.81*	*0.57*

* indicates statistical significance at 0.05%

Table 4. Variation of Water Quality Paramreters in Acute Test for Juvenile Clams

Concentration of Toxicant (mg/L)	Water Quality Parameters									
	TDS (mg/L)		DO (mg/L)		pH		EC (µS/cm)		Temperature (°C)	
	Start	End	Start	End	Start	End	Start	End	Start	End
0	350.00±0.40	347.00±0.00	6.75±0.05	6.69±0.00	8.96±0.03	8.95±0.03	613.33±0.88	616.00±2.52	23.50±0.00	24.10±0.00
20	356.25±1.55	346.75±0.47	6.55±0.05	6.52±0.25	9.14±0.04	9.04±0.03	623.75±1.89	643.00±10.10	23.50±0.00	24.10±0.00
40	359.50±3.20	348.75±0.47	5.10±0.20	5.00±0.15	9.35±0.03	9.17±0.06	636.00±2.38	656.00±8.81	21.50±0.00	24.10±0.00
60	367.50±2.53	349.50±0.64	4.45±0.25	4.41±0.06	9.54±0.02	9.26±0.05	648.25±2.29	663.75±8.44	23.50±0.04	24.12±0.02
80	359.50±3.20	348.75±0.47	4.42±0.20	4.40±0.25	9.61±0.01	9.36±0.05	661.50±2.25	668.00±7.78	23.47±0.02	24.15±0.06
100	367.50±2.53	349.50±0.64	3.50±0.05	3.51±0.15	9.68±0.00	9.49±0.06	682.00±5.02	683.25±5.25	23.42±0.04	24.15±0.06
Df	*3*	*3*	*2*	*2*	*2*	*2*	*4*	*2*	*2*	*4*
T-Value	*1.73*	*1.43*	*0.21*	*0.37*	*1.47*	*1.20*	*1.25*	*1.29*	*0.50*	*0.27*
P-Value	*0.31*	*0.71*	*0.31*	*0.20*	*0.53*	*0.70*	*0.24*	*0.30*	*0.82*	*0.50*

* indicates statistical significance at 0.05%

Variation in Water Quality Parameters in Sub-Lethal Test

In the sub-lethal test, the exposure system was the water renewal system where the organism is exposed to the toxicant in a similar manner like the static system; because it is in still water. However, the water was renewed periodically (at constant intervals of one week. i.e. at the end of each week) by transferring the organism to a fresh test chamber with the same concentration of toxicant. All temperatures measured and recorded during the test period showed no significance difference statistically. At the concentration of 0 and 0.87 mg/l, it was observed that TDS and EC showed some level of statistical difference ($p < 0.05$) but from 2.63, 3.51 and 4.39 mg/l, no significance difference was observed at that limit. The dissolved oxygen (DO) at the concentration of 0, 0.87, 1.75 and 4.39 mg/L showed significance difference at $p < 0.05$ while no significance difference was observed with the same confidence limit at the concentration of 2.63 and 3.51 mg/L. The pH clearly showed statistical difference at 0, 0.87 and 1.75 mg/l while no statistical difference was observed at $p < 0.05$ for 2.63, 3.51, 4.39 mg/l.

While the water quality parameters were varied for juveniles in sub-lethal test, it was observed that temperature showed no significant difference statistically. Electrical conductivity showed significant difference ($p < 0.05$) at the highest concentration of toxicant (4.39 mg/l) but there was no significant difference for the other concentration son that limit. The dissolved oxygen (DO) at the concentration of 4.39 and 3.51 mg/L as well as 1.75 and 2.63 mg/L showed no significant difference at the confidence limit of $p < 0.05$. At the control and 0.87 mg/L, there was an observed significant difference. While the pH was varied, significant difference was observed at 0, 0.87 and 4.39 mg/l at ($p < 0.05$) but showed no significant difference between 1.75 and 3.51mg/l. Total dissolved solids (TDS) showed no significant difference ($p < 0.05$) at 0.87, 1.75 and 3.51 mg/l while significant difference was observed at 0, 2.63 and 4.39 mg/l respectively.

Table 5. Variation of Water Quality Parameters for Adult Clams in Sub-lethal Test

Concentration of Toxicant (mg/L)	Water Quality Parameters				
	TDS (mg/L)	DO (mg/L)	pH	EC (µS/cm)	Temperature (°C)
0	333.79±3.36^{c}	7.00±0.00^{a}	9.65±0.01^{a}	662.96±7.38^{c}	24.89±0.13
0.87	358.67±4.12^{a}	6.15±0.05^{b}	9.62±0.00^{ab}	714.96±9.34^{a}	24.87±0.13
1.75	345.54±4.18^{b}	5.66±0.06^{c}	9.59±0.01^{bc}	691.79±7.54^{b}	24.81±0.13
2.63	346.17±2.32^{b}	4.65±0.05^{d}	9.58±0.00^{c}	694.17±3.75^{b}	24.80±0.13
3.51	346.88±2.45^{b}	4.48±0.08^{d}	9.57±0.01^{c}	687.96±4.63^{b}	24.80±0.14
4.39	344.50±2.52^{b}	3.84±0.07^{e}	9.58±0.01^{c}	688.25±5.75^{b}	24.80±0.14
CV	*4.99*	*0.77*	*0.62*	*5.15*	*2.65*
P-Value	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*0.99^{ns}*

Means on the same column with different superscript are statistically significant ($p<0.05$);
ns = not significant

Sub-Lethal Mortality of Clams in the Experiment

The test showed about 80% survival in the control media in all the triplicates (Table 7). Negligible mortality was recorded in the control unit. At the concentration of 0.00, 0.89 and 2.67mg/l, a significant difference at $p < 0.05$ was observed at week six of the experiment while weeks 4, 5 and 8 showed no significant difference at that same confidence limit. Weeks 1, 2, 3 and 7 showed to be non significant. At the concentration of 3.57 mg/l, a significant difference ($p < 0.05$) was observed at week 5 while weeks 4, 6 and 8 showed no significant difference at that same confidence limit. Weeks 1, 2, 3 and 7 showed no significance at all. It can be observed that at the concentration of 4.46 and 1.78 mg/l on week six of the test, a significant difference was observed while at weeks 4, 5 and 8; no significant difference was recorded at ($p < 0.05$). Weeks 1, 2, 3 and 7 showed no significant difference at all.
The mortalities in the sub-lethal test for the juveniles showed that at the concentration of 1.78 mg/l, there was no significant difference in the mortality of clams at this concentration at the confidence limit of $p < 0.05$ for weeks 3, 7 and 8 while weeks 1, 2, 4, 5 and 6 showed no significant difference in all.

At 0.89 mg/l, a significant difference was observed at week 7 while weeks 8 and 3 showed no significant difference both at the confidence limit of $p < 0.05$. Weeks 1, 2, 4, 5 and 6 showed to be non significant. At the concentration of 2.67, there was no observed significant difference in mortality of clams on weeks 3, 7 and 8 at $p< 0.05$ while the entirety of weeks 1, 4, 5 and 6 recorded no significant difference in all. At the concentration of 3.57 mg/l, the significant difference in mortality

at p<0.05 was observed on week 8 while weeks 3 and 7 showed no significance difference at the same confidence limit. Weeks 1, 2, 4, 5, and 6 showed no significant difference on a whole. At the concentration of 4.46 mg/l, it was observed that a statistical difference (p<0.05) in mortality of Clams was seen on week 3 while weeks 7 and 8 showed no significance difference at the same confidence level. Weeks 1, 2, 4, 5 and 6 showed no statistical difference in all.

Mortality Analysis in the Freshwater Clams

The concentration of calcium hypo-chloride that caused 50% mortality (LC_{50}) after exposure for 96hrs was determined to be 53.56 mg/l for the adults and 52.78 mg/l for the juveniles respectively. The toxicity was directly proportional to the concentration of the toxicant in the exposure for all exposures in both the adult and juveniles. From the Figure one (1) below, as the concentration of the toxicant (Chlorine) increases, the rate of mortality also increased. Therefore, we can deduce that the mortality of Clams is directly proportional to the concentration of chlorine toxicant in this experiment. Results from the probit curve shows that mortality increased with increasing concentration. From the analysis of the result, the upper and lower confident limits were determined to be 61.21 and 46.11 mg/L for adults respectively.

Table 6. Mean Sub-lethal Mortalities of Adult Freshwater Clams

Period of exposure to toxicant	Concentration of Toxicant (mg/L)						P-Value
	0.00	0.89	1.78	2.67	3.57	4.46	
Wk 1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-
Wk 2	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-
Wk 3	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-
Wk 4	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	1.00 ± 0.00^{a}	0.02
Wk 5	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.50 ± 0.02^{a}	0.50 ± 0.02^{a}	0.03
Wk 6	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	1.00 ± 0.00^{a}	0.00 ± 0.00^{c}	0.50 ± 0.02^{b}	0.50 ± 0.02^{b}	0.03
Wk 7	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-
Wk 8	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	1.00 ± 0.50^{a}	0.02
Total	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	1.00 ± 0.00^{b}	0.00 ± 0.00^{c}	1.00 ± 0.00^{b}	3.00 ± 0.05^{a}	0.01

Means on the same row with different superscript are statistically significant (p<0.05)

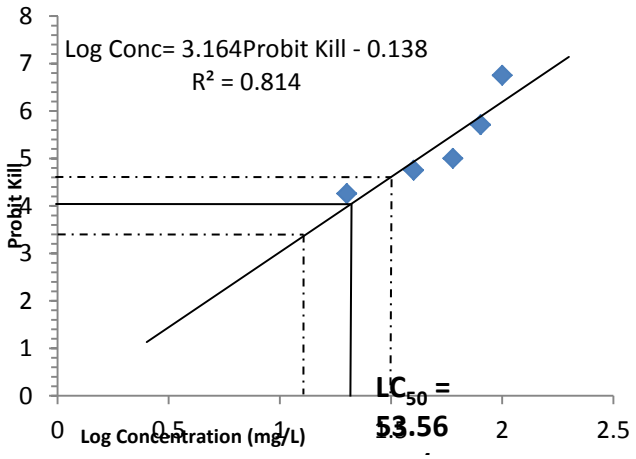


Fig. 1: Probit Curve for Mortality in Adult Freshwater Clams

From the figure two (2) above, as the concentration of the toxicant (Chlorine) increases, the rate of mortality also increased. Therefore, we can deduce that the mortality of Clams is directly proportional to the concentration of chlorine toxicant in this experiment. Results from the probit curve shows that mortality increased with increasing concentration. From the analysis of the result, the

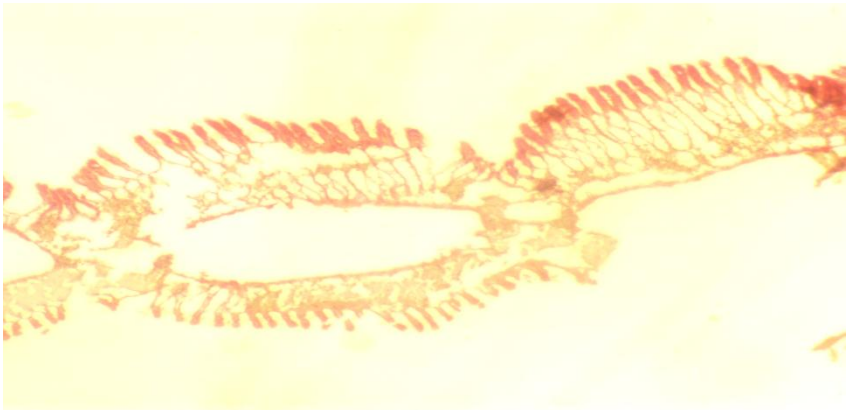
upper and lower confident limits were determined to be 60.07 and 45.64 mg/L for juveniles respectively. Figure 3 above shows the mortality of clams observed per concentration of toxicant on the adult clams. It can be seen that with an increasing concentration, the clams experienced an increase in mortality. At a concentration of 20 mg/L, mortality stood at two clams while at 100 mg/L, a total of 10 clams were recorded. Figure 4 below shows the mortality of clams observed per concentration of toxicant on the adult clams. It can be seen that with an increasing concentration, the clams experienced an increase in mortality. At a concentration of 20 mg/L, mortality stood at two clams while at 100 mg/L, a total of 10 clams were recorded. Therefore, we can deduce that the mortality of Clams is directly proportional to the concentration of chlorine toxicant in this experiment.

Pathology of Clam Gills (Ctenidium)

Plate 13 below shows the normal histology of the ctenidium of the adult clam at 0 mg/L concentration of chlorine. The gills can be shown to possess normal primary and secondary lamellae with the presence of mucous and epithelial cells surrounding them. In the control sample, there is no observed histo-pathological change or alteration in the tissues of the gills. It shows clusters of basophilic cells (bc) on parts of the adjacent filaments and numerous bundles of muscle fibers (mf). The water channels (wc) and haemal sinuses (hs) are evident in the centre of the gills. The gills of the control clam showed uniform arrangement of the lamellae (l) with uniform interlamellae space (ils). The surface of each lamellae or filament is covered by a thick cuticle underlined by a monolayer epithelium.

Plate 14 below shows disintegrated basophilic cells (bc) on parts of the adjacent filaments and few bundles of muscle fibers (mf). The water channels (wc) and haemal sinuses (hs) are evident in the centre of the gills but gradually fading away. There is an observed vacoulation of the gill filaments and mild inflammation of gill filaments with lesions. Chlorine exposed gills featured interlamellae epithelial hyperplasia, vacuolation and hypertrophy of cells. Plate 15 shows the basophilic cells of the gills completely disintegrated from their base. The basophilic cells are no more occurring in clusters. The water channels are also ruptured and diffuse. These anomalies found are said to be at stage 1 in severity; these include dilation of the marginal channel, hyperplasia of the epithelial cells, and lifting of the lamellar epithelium. There is visibly mild swelling and bio-accumulation of contaminant. The gill filaments are also fused.

Plate 14: Photomicrograph showing the normal histology of adult *M. mercenaria* gills in the control system (0 mg/L) magnification ×40



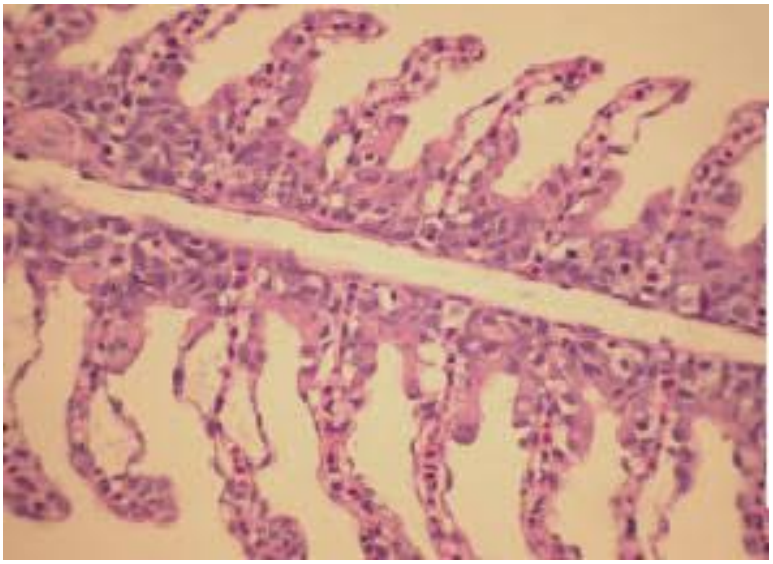


Plate 15: Histological alterations on the gills of an adult *M. mercnaria* (0.89 mg/L) showing disintegration of gill filaments

Plate 16 below shows disintegrated basophilic cells (bc) on parts of the adjacent filaments and few bundles of muscle fibers (mf). The water channels (wc) and haemal sinuses (hs) are evident in the centre of the gills but gradually fading away.

There is clear distortion of the gill filaments. The ciliary interfilamenter junctions are replaced by metaphilic cellular junctions.

The structure in plate 17 below shows histology of partially damaged gill filaments. The epithelium is completely hyperplastic. The hyperplasia was severely damaged, resulting in the fusion of some secondary lamellae.

Plate 18 below shows the totally damaged gill filaments indicating heavy bio-accumulation of chlorine toxicant granules. The cells have become necrotic as a result of the chronic inflammatory reaction in the gills. Frequently, alterations such as blood congestion, hypertrophy of epithelial cells and lamellar disorganisation were also observed.

Plate 19 below shows the normal histology of the ctenidium of the juvenile clam at 0 mg/L concentration of chlorine. The gills can be shown to possess normal primary and secondary lamellae with the presence of mucous and epithelial cells surrounding them. In the control sample, there is no observed histo-pathological change or alteration in the tissues of the gills.

It shows clusters of basophilic cells (bc) on parts of the adjacent filaments and numerous bundles of muscle fibers (mf). The water channels (wc) and haemal sinuses (hs) are evident in the centre of the gills.

Plate 20 below shows disintegrated basophilic cells (bc) on parts of the adjacent filaments and few bundles of muscle fibers (mf). The water channels (wc) and haemal sinuses (hs) are evident in the centre of the gills but gradually fading away.

There is an observed vacoulation of the gill filaments and mild inflammation of gill filaments with lesions.

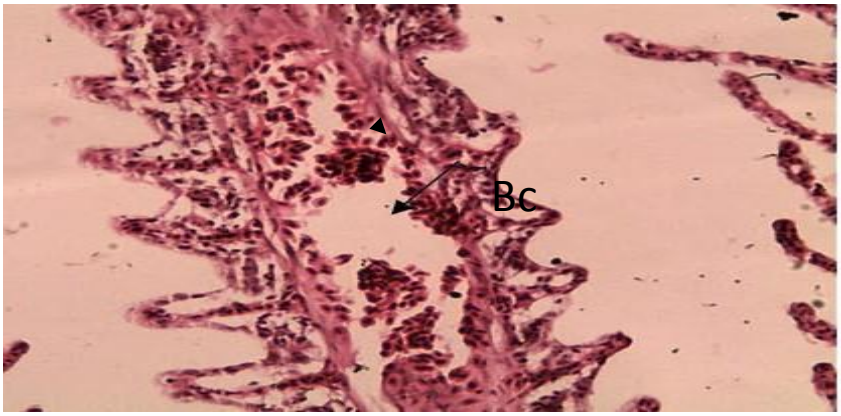


Plate 16: Photomicrograph showing the histology of an adult *M. mercnaria* gills showing disintegrated basophilic cells and ruptured water channels (1.78 mg/L)
Bc = basophilic cells
Wc= water channels

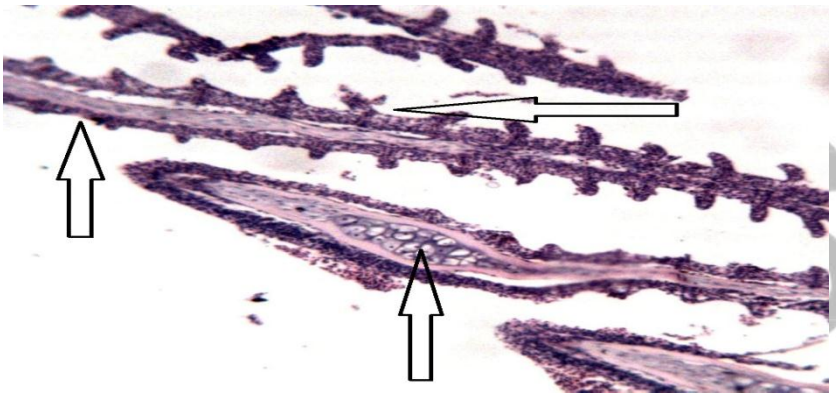


Plate 17: Photomicrograph of *M.mercenaria* gills snapped at x250 magnification showing degeneration of gill filament.

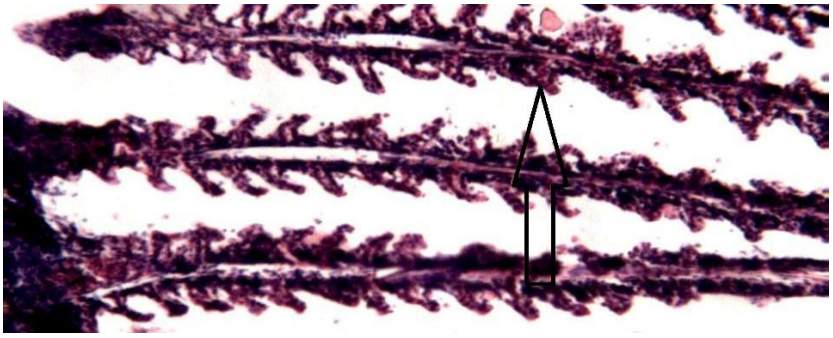


Plate 18: Histology of the partially damaged gill filaments of an adult *M. mercenaria* gills at 3.57 mg/L concentration of chlorine toxicant.

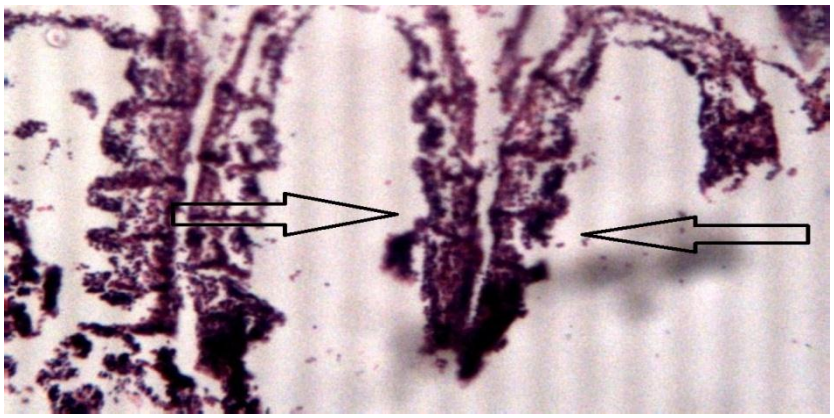


Plate 19: Photomicrograph of the histology of adult *M. Mercenaria* gills at 4.46 mg/L concentration of chlorine toxicant showing necrosis and degeneration of the filaments

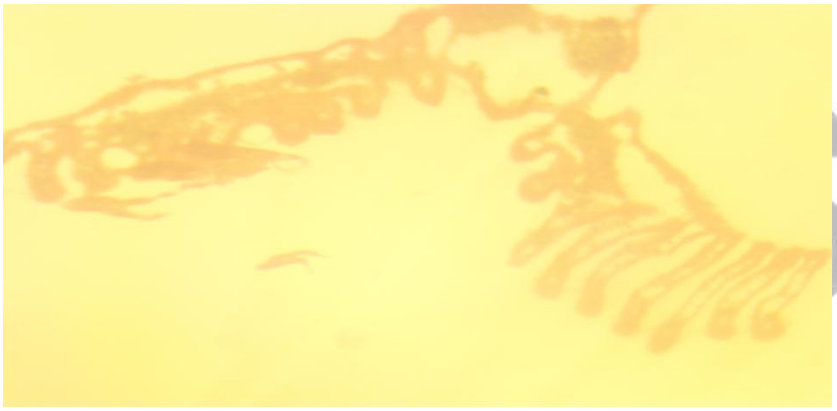


Plate 20: Photomicrograph showing the normal histology of a juvenile *M. mercenaria* gills in the control system at 0 mg/L.

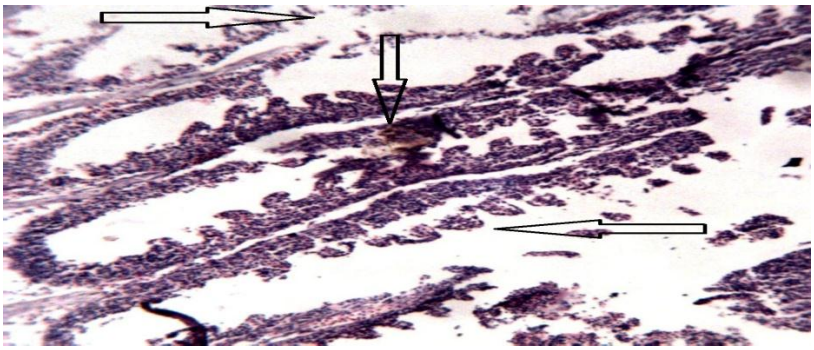


Plate 21: Histological Alterations on the Gills of a Juvenile *M. mercenaria* (0.87 mg/L) Showing Inflamationof the Gill Filaments with Lessions

Plate 21 below shows the basophilic cells of the gills completely disintegrated from their base. The basophilic cells are no more occurring in clusters. The water channels are also ruptured and diffuse.

There is visibly mild swelling and bio-accumulation of contaminant. The gill filaments are also fused.

Plate 22 below shows disintegrated basophilic cells (bc) on parts of the adjacent filaments and few bundles of muscle fibers (mf). The water channels (wc) and haemal sinuses (hs) are evident in the centre of the gills but gradually fading away.

There is clear distortion of the gill filaments. The ciliary interfilamenter junctions are replaced by metaphilic cellular junctions.

The structure in plate 23 below shows histology of partially damaged gill filaments. The epithelium is completely hyperplastic

Plate 24 below shows the totally damaged gill filaments indicating heavy bio-accumulation of chlorine toxicant granules. The cells have become necrotic as a result of the chronic inflammatory reaction in the gills.

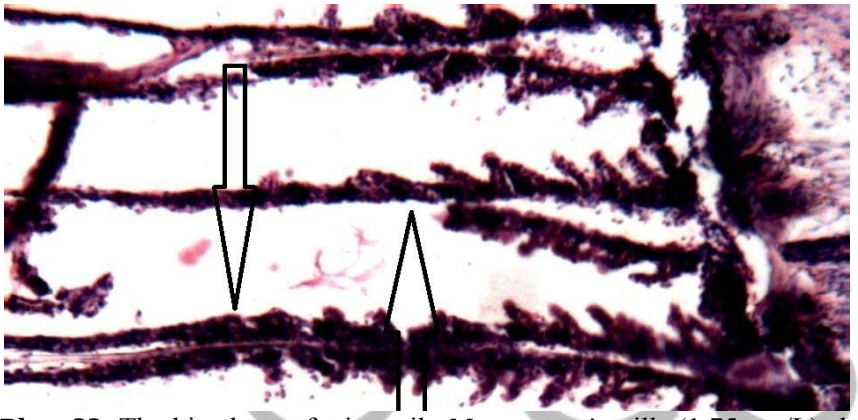


Plate 22: The histology of a juvenile *M. mercnaria* gills (1.75 mg/L) showing distorted gill filaments and fused gill filaments

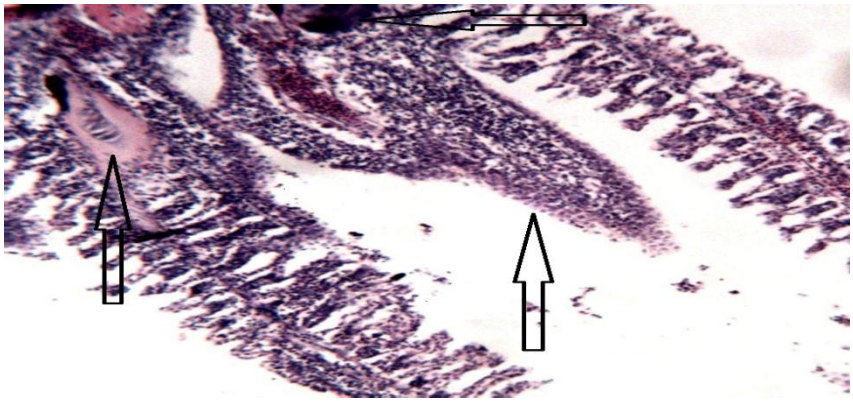


Plate 23: Distorted histology of a juvenile *M. mercenaria* gills at 2.63 mg/L concentration of toxicant.

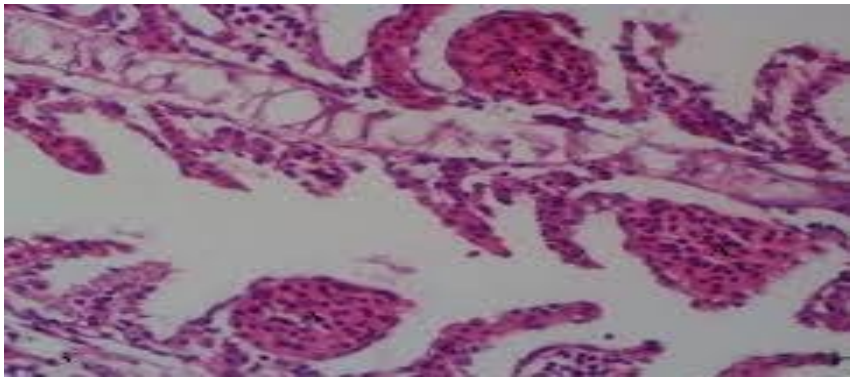


Plate 24: Histology of the partially damaged gill filaments of a juvenile *M. mercenaria* gills at 3.51 mg/L concentration of chlorine toxicant.

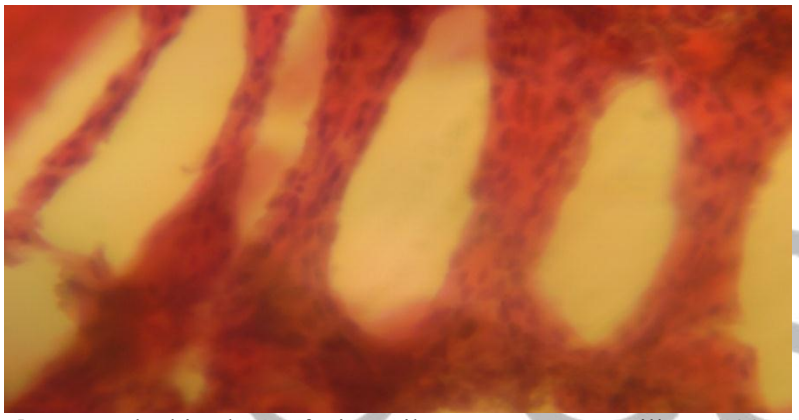


Plate 25: The histology of a juvenile *M. Mercenaria* gills at 4.39 mg/L concentration of chlorine toxicant showing damaged gill filaments

DISCUSSION

Acute mortality in freshwater Clams

As expected, increased concentrations of chlorine produced-oxidants led to increased mortalities of both stages (adult and juveniles) of *Mercenaria mercenaria.* A similar situation prevailed for the soft Clam *Mya arenaria* tested under comparable conditions (Roosenburg *et al*., 2008). It can be seen that the juveniles exhibited a greater sensitivity to chlorine compared to the adult Clams. The generally greater sensitivity of the juvenile clams to chlorine compared to the adults shows the greater sensitivity of younger life stages of bivalves to environmental factors (Pelseneer, 2000; Loosanoff and Davis, 2002; Kennedy *et al*., 2003a, b). There was an increased rate of mortality as the concentration of the chlorine toxicant increased from 24 hours to 96 hours. This difference in sensitivity in a previous study was also found for *Mya arenaria* juveniles exposed to chlorine concentrations (Roosenburg *et al*., 2008). Further, although the techniques and experimental designs differed from those of Roberts and Gleeson (1999), their study of chlorine toxicity in relation to bivalve molluscs supports this generalization. The lethal concentration value for 50 % of this population after 96 hrs of exposure was 53.56mg/L and 52.78mg/L for the adults and juveniles respectively. Thus, these even younger juvenile stages demonstrated greater sensitivity to chlorine addition than did the older adult stages. Another study that has examined the toxicity of Chlorine to *Mercenaria mercenaria* is Goudreau *et al*. (2000). The LC50 value reported in their study was 34 mg/L for *Mya arenaria*, which is substantially lower than the comparable value calculated in this study (52.75 mg/L for juveniles and 53.56 mg/L for adults). Despite the large difference in endpoints, data generated in each study may reflect toxicological endpoints that are similar.

Variation of water quality parameters in acute test

Water quality conditions in the test chambers were not problematic for both the adult and juveniles. Dissolved oxygen concentrations remained well above the 5-mg/L threshold recommended for freshwater bivalves (Havlik and Markin, 2004). The maximum recorded conductivity of 693 µS/cm for adults and 683 µS/cm for juveniles respectively was below the level (1,500 µS/cm) found to be safe by the water quality criteria for *M. mercenaria.* Conductivities above 1,500 µS/cm (0.15 S/m) has been associated with impaired water bodies (CATESB, 2001) and may reflect a decrease in water quality (Olsen *et al*., 2001)

It is possible that variations in water chemistry parameters between the tests with *Mercenaria mercenaria* adults and juveniles may have contributed to the observed differences in sensitivity. Hardness of the dilution water was substantially different for the two tests, ranging from 330 to 360 mg/L for the adults tests and from 320 to 350 mg/L for the juvenile tests. Either hardness range is suitable for juvenile survival, although higher hardness levels (450 mg/L) are recommended for long-term culture in order to provide adequate calcium for shell growth (Steg, 2008). The pH ranges for the adults were from 8.99 to 9.67 while the juveniles ranged from 8.96 to 968. The differing pH levels for the two tests may also have had an effect on the two stages of sensitivity. As with hardness and alkalinity, current data on whether pH affects the toxicity of chlorine are inconclusive. Some researchers report lower toxicity of chlorine toxicity with increasing pH, while in other cases no relationship is observed (Adams and Bealing 2004, Hickey and Martin 2009).

In our study, juveniles of *Mercenaria mercenaria* were significantly more tolerant of chlorine than the adults; however, if higher hardness, alkalinity, or pH levels actually buffer the toxic action of chlorine, the difference in sensitivity between the two life stages may be attributable to the differences in these water quality characteristics. Additional studies have also reported that other species of freshwater organisms have substantially lower acute tolerances to chlorine than those reported for *Mercenaria mercenaria* in this study.

Variation in water quality parameters in the sub-lethal test

Water quality conditions in the test chambers were not uniform since the exposure system was water renewal system. As such, it had effects on the test water chemistry and the test organisms. Fisher *et al*. (2003) reported similar trends pertaining to the tolerances of freshwater organisms during experiments comparing continuous versus intermittent exposures. The maximum recorded conductivity of 694 µS/cm for adults and 689 µS/cm for juveniles respectively was below the level (1,500 mmhos/cm) found to be safe for long term exposures of *Mercenaria mercenaria* based on tests conducted in the laboratory. Hardness of the dilution water was substantially different for the two tests, ranging from 333 to 346 mg/L for the adults tests and from 329 to 345 mg/L for the juvenile tests. Either hardness range is suitable for juvenile survival, although higher hardness levels (450 mg/L) are recommended for long-term culture in order to provide adequate calcium for shell growth and strength (Steg, 2008). The pH ranges for the adults were from 9.57 to 9.65 while the juveniles ranged from 9.50 to 9.61. The differing pH levels for the two tests may also have had an effect on the two stages of sensitivity. As with hardness and alkalinity, current data on whether pH affects the toxicity of chlorine are inconclusive. Some researchers report lower chlorine toxicity with increasing pH, while in other cases no relationship is observed (Adams and Bealing 2004, Hickey and Martin 2009).

In our study, juveniles of *Mercenaria mercenaria* were significantly more tolerant of chlorine than the adults; however, if higher hardness, alkalinity, or pH levels actually buffer the toxic action of chlorine, the difference in sensitivity between the two developmental stages may be attributable to the differences in these water quality characteristics.

Sub-lethal mortalities of clams in the experiment

A comparison of sensitivities for the adult and juveniles tested in this study revealed that younger clams were more sensitive to chlorine exposure than older adults (the difference in sensitivities for the respective age-groups was more apparent when contrasting survivorship results). This observation is consistent with a trend often apparent for other freshwater species because early

life stages of organisms are generally more sensitive to toxicant exposure than older, more developed individuals. Taylor (2000) observed that chlorine was substantially more toxic to early life stages of *Mercenaria mercenaria* than the adult stages. Although chronic studies examining the toxicity of Chlorine to Clams are scarcely conducted, studies examining effects of Chlorine exposure to other species of bivalves are extensive because chlorination is often used as a biofouling control agent (Rajagopal *et al.,* 2002, Rajagopal *et al.,* 2003). These studies also suggest that younger age classes of bivalves are more susceptible to Chlorine exposure than older classes. Researchers have commented that freshwater Clams may be useful as surrogate test species for assessing environmental risk for bivalves since they have similar physiological and ecological traits (Hull *et al.,* 2002). These similarities are useful for Chlorine toxicity to freshwater clams interpreting toxicological impacts of exposure because most bivalves reside in the benthos, rely on suspension or deposit feeding, and have the affinity to accumulate trace elements and metals from the water column, sediment, and interstitial water (Hull *et al.,* 2002, Vaughn and Hakenkamp 2001).

Of greater importance to this present study are the behavioral similarities shared by bivalve clams, the most obvious one being their ability to temporally avoid toxicants by closing their valves for prolonged periods. When they were exposed to high concentrations of Chlorine, *Mercenaria mercenaria* demonstrated avoiding the uptake of toxicants by sealing their valves, reducing filtration, and relying on an-aerobiosis. This is in conformity to other findings which has it that in some species, the valve closure is for extended periods (Rajagopal *et al.,* 2002, Rajagopal *et al.,* 2003). Consequently, because of this behavioral response, researchers often describe a time lag between the initiation of exposure and first observation of substantial mortality during laboratory studies. This time lag occurs even when exposure concentrations are extremely high and is typically 14 d or more for the bivalve species *Mercenaria mercenaria* (Rajagopal *et al.,* 2003). It was observed that the onset of mortality did not occur until energy resources were depleted or metabolic wastes reached toxic levels. This is in agreement with the findings of Rajagopal *et al.,* 2003. The greater susceptibility of earlier age classes of *Mercenaria mercenaria* to chlorine may be attributed to the fact that younger clams have less energy reserves and are unable to store as much wastes. The ability of the younger age class to avoid toxicant exposure may also be less because of thinner more permeable shells. Exposure to Chlorine likely reduced the filtration rate of juveniles, thereby leading to less food being ingested. This impaired their filter feeding activity since juveniles would have less energy for assimilation into new body tissue. Currently, the U.S. EPA defines chronic toxicity tests as exposures to individuals of a species that are equivalent to approximately one-tenth of their life span. Although bioassays this long in duration are not practical for bivalve Clams given their long life spans, which may exceed decades, it is important to note for conservation purposes. Impairment at lower concentrations become more apparent over long exposure times and suggests that young juveniles may be at risk to Chlorine exposure at concentrations below current water quality criteria.

Mortality analysis in the freshwater clams

During adult and juvenile Clam bioassays, we were able to maintain Chlorine concentrations close to target levels by intermittently or continuously dosing test chambers with solutions of calcium hypochlorite. However, since no recognized uniform pattern was observed in the physicochemical interaction of Chlorine and water, it is difficult to accurately infer toxicity based solely on chlorine concentrations (Stewart *et al.,* 2006). Additional studies also have reported substantial interspecific variability in the tolerances of *Mercenaria mercenaria* from various species to other contaminants, such as malathion and ammonia (Augspurger *et al.,* 2003, Keller and Ruessler 1997). It is unclear why some species have lower survivorship after being exposed to contaminants; however, it may be due to physiological differences.

Pathology of the Clam gills (ctenidium)

The gills or the ctenidium of freshwater Clams are attached on each side of the visceral mass. They extend from the attachment site of the labial palps to the anterior edge of the siphon septum and divide the mantle cavity into two regions, the suprabranchial chamber and the infrabranchial chamber. The apparent double set of gills on each side of the body actually arises by the folding of a single gill. In addition to the function of gas exchange during respiration, the gills also trap and transport food particles to the labial palps. A section of the inner folds of the gills extends towards the mouth between each pair of palp. Clusters of basophilic epithelial cells are also observed in the gill filaments

and are mainly confined to the most proximal and most distal filaments of the gill. Clams from the entire study had an inflammatory reaction in varying degrees. Most of the clams had gill lesions (a localised abnormal structural change in a bodily part), where the gill filaments were fused, ciliary interfilamenter junctions were replaced by metaplastic cellular junctions, epithelium was hyperplastic, and there was a chronic inflammatory reaction in the gills. The normal histology of clam gills as can be seen in the control (0 mg/L) for both adult and juveniles is made of two gill-plates at each side of freshwater clam. The gill-plate is composed of a number of gill filaments.

The wall of the gill filament is lined with ciliated columnar epithelial cells with ovoid nuclei; between them there are a number of mucous secreting cells with circular based located nuclei. The core of the gill-plates as well as the gill filament is made of a loose connective tissue with interlamellar tissue. The histopathological changes of gills are represented in the arrangement or regularity of gill lamellae or the cells and bioaccumulation of toxicant residues. At 0.89 mg/L and 0.87 mg/L concentration in both the adult and juvenile test for chlorine exposure, mild inflammation of the gill filaments with lesions and vacuolation occurred while at 1.78 mg/L and 1.75 mg/L, mild swelling and bioaccumulation of contaminant was observed. There was also fusing of the gill filaments. Swelling in the glandular cell of the gill has been observed also in Asian clam *Potamocorbula amurensis* from San Francisco bay (Clark *et al*., 2000). These observations could be confirmed by increasing the size of glandular cells in chlorine toxicant-treated clams compared with the respective controls (El-Shenawy *et al*., 2007). At this point, Clams tend to show a very common reaction to stress, which is also demonstrated by bivalves, by secreting mucous. This is in tandem with the findings of Janssen *et al*., 2002.

The histopathological changes observed in the study confirmed that Chlorine affected gill glandular cells. Molluscs increased mucus secretion in general as their first line of defense against entry of undesirable substances and many kinds of the stressors (El-Shenawy *et al*., 2007). Distortion of gill filaments occurred at the concentration of 2.67 mg/L and 2.63 mg/L of chlorine treatment leading to the replacement of ciliary inter-filamenter junctions by meta-plastic cellular junctions. At the concentration of 3.57 mg/L and 3.51 mg/L, there was observed partial damage of gill filaments which lead to the epithelium being hyper-plastic while at 4.46 mg/L and 4.39 mg/L, there was total and complete damage of the gill filaments indicating heavy bioaccumulation of toxicant granules and the cells became necrotic (that is the localised death of living cells as from infection or the interruption of blood supply as it is with this case) as a result of the chronic inflammatory reaction in the gills. This agrees with the findings of Choi *et al*., 2003 who opined that the epithelial cells of the gill play a crucial role in the physiological functions of bivalves. Therefore, the damage in the epithelium may result in serious dysfunction of the tissues, consequently leading to deleterious effects at the higher biological organization levels. As feeding and respiration of bivalves is essentially dependent on the gill system, the animal possesses different measures of defense against environmental hazards, e.g. particle rejection and formation of pseudofaeces, reduced filtration rate and valve closure (El-Shenawy *et al*., 2001, 2007).

Feeding efficiency in freshwater clams is directly related to mucin production from mucocytes (scattered in the gill filament) between the ctenidia of the clam which can be affected by toxins (El- Shenawy *et al*., 2007). The gills, which participate in many important functions in fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water, are considered the primary target of the contaminants (Mazon *et al*., 2002; Fernandes & Mazon, 2003). Alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defense mechanisms, since, in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (Fernandes & Mazon, 2003). However, freshwater clams have the capacity to increase their ventilation rate, to compensate low oxygen uptake (Fernandes & Mazon, 2003). Most part of the gill lesions caused by sublethal exposures affects lamellar epithelium (Hinton & Laurén, 2000); however, some alterations in blood vessels may also occur, when clams suffer a more severe type of stress. In this case, damaged pillar cells can result in an increased blood flow inside the lamellae, causing dilation of the marginal channel, blood congestion or even an aneurysm (Rosety-Rodríguez *et al*., 2002). The formation of an aneurysm is related to the rupture of the pillar cells (Martinez *et al*., 2004) due to a bigger flow of blood or even because of the direct effects of

contaminants on these cells. This is a severe type of lesion, recovery from which is possible, but more difficult than the epithelial changes (Poleksic & Mitrovic-Tutundzic, 2004). Winkaler *et al.* (2001) found anomalies such as hyperplasia, hypertrophy and dilation of the soft clam, *Mya arenia,* which corroborates with the hypothesis that the water really showed high level of chlorine contaminant and that exposure to this water caused structural damage to the clam gill.

CONCLUSION

Results of this bioassay suggest that *Mercenaria mercenaria* is more tolerant of Chlorine concentrations and dosages than many aquatic species. In particular, researchers have reported toxicological endpoints for other freshwater bivalves that are substantially lower than those recorded in this study for clams. Relating the results of this study to the current water quality criteria suggests that the environmental risk of chlorine exposure to *Mercenaria mercenaria* is fairly minimal. Although juvenile clams may be able to survive high dose acute exposures, the impact of long-term exposure to low doses may result in sub-lethal impairment that could lower their chances of surviving the multi-year, juvenile stage and being recruited to the reproducing population. Therefore, freshwater organisms as *M. mercenaria* may be more appropriate as test organisms for assessing Chlorine pollution, especially since no recent studies have reported species with tolerances below current water quality criteria. The analysis of the variation in the histological parameters leads to the conclusion that histopathological alterations are good biomarkers for field assessment and environmental contamination. It must be emphasized that histopathology is able to evaluate the early effects and the responses to acute exposure to chemical stressors. In conclusion, given the demonstrated susceptibility of *Mercenaria mercenaria* to chlorine addition to their environment, it appears important that industries and utilities which release chlorinated effluent into an area in which clams might be growing should carefully monitor their effluent to prevent excessive amounts of these biocides from killing these freshwater organisms.

RECOMMENDATIONS

Based on the results obtained from this study, it is recommended that water treatment plants as well as other chemical industries whose major effluents discharge points are the water bodies in which our aquatic organisms grow in (including the freshwater clams) should make sure the effluents are converted into benign forms before they are finally disposed off into this water bodies. This will make for an effective survival and growth of aquatic organisms and it will promote the enhancement of the modern aquaculture practice. However, the following areas could be focused to improve on this practice: Stringent effluent discharge laws should be put in place for violators of the modern/ acceptable practice. Bivalve molluscs which are filter feeders can be used as bio assay organisms to set water quality criteria. Additional research can be conducted in this area to harness the potentials that lie waste in these un-exploited benthic organisms.

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