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Impact of Calcium Carbonate on the Juveniles of the Brackish River Prawn, *Macrobrachium macrobrachion* (Herklot, 1851) Under Laboratory Conditions

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ABSTRACT

The acute toxicity and the effect of sub-lethal concentrations of calcium carbonate on the morphology of *M. macrobrachion* were investigated under laboratory conditions. The prawns were exposed at 0, 160, 320, 640, 1280, 2560 and 5120 mg L^{-1} of $CaCO_3$ using static renewal bioassay for 96 h for the acute toxicity test. The prawns were also exposed for the chronic test at 0, 20.0, 40.0 and 80.0 mg L^{-1} for a period of two weeks. Mortality occurred in the bioassay tanks at random and the bioassays were monitored throughout the exposure period. Results showed that exposed prawns were not significantly impacted when compared with the control group and correlations were significant at $p<0.05$. There were no mortalities in the tanks with the highest concentrations while one death was recorded in the control tank and in the tanks with the 160 and 320 mgL^{-1} of $CaCO_3$, respectively. The gills of exposed prawns were analyzed using photomicrography and the results showed that the organs had their normal morphological status after the exposure time, implying that $CaCO_3$ is non-toxic to *M. macrobrachion*.

Key words: Calcium carbonate, prawn, toxicity, photomicrography

INTRODUCTION

The input of inorganic chemicals into the environment at undetectable rates could pose risks to the inhabiting organisms. A lot of agro-chemicals have been in use for so long for the sole aim of boosting food production as well as enhancing growth and survival in farm animals including fish. Although this has been an age long practice which has enhanced water quality management for suitable and productive fisheries, uncontrolled use and adoption into several other sectors could lead to misappropriation, hence, it is expedient that the toxicity of this valued substance for best practices and optimal usage is duly assessed for proper nutrient budgeting and use (Weston and Seelig, 1994). Furthermore, uncontrolled applications of these substances result in deleterious impacts on our ecosystems. Some of these inorganic compounds are adopted in so many other environmental activities today such as remediation of polluted environments. Calcium carbonate has been employed in oil explorative activities as a sealing agent to increase the density of the

drilling fluid and seal off heavy pores in the system while the use of calcium hydroxide in combination with sodium sulphide is in use today for remediating metal polluted soils (Zeng *et al.*, 2005) and as an electron acceptor.

Toxicants cause changes in the normal metabolic activities in fish which could lead to other defects such as biochemical, histological, growth, reproductive defects and others. It can also impact the environment causing negative shifts in juvenile recruitment patterns and also destroy species diversity or completely destroy a fishery. More so, there is the need to build data bank for pooling information, this will serve as baseline reference data for environmentalists and for policy formulation as regarding the use of calcium carbonate and its application for non-agricultural activities.

MATERIALS AND METHODS

Juveniles of *M. macrobrachion* weighing between 1.83-4.22 g with total body length of 5.0-6.6 cm were collected from the canal located in the African Regional Aquaculture Centre (ARAC), Port Harcourt, Rivers State. The prawns were collected using non-return valve traps. The test prawns were kept in cages submerged in the ponds for one week to acclimatize and afterwards transferred to ARAC laboratory for the bioassays. The acute toxicity test was carried out using lethal concentrations of 160, 320, 640, 1280, 2560, 5120 and 0 mg L^{-1} (as control). The sublethal test was also carried out using concentrations of 20l, 40 and 80 mg L^{-1} plus the control (0 mg L^{-1}) for chronic examinations. They were stocked at one prawn per 2 L of water and six prawns were tested for each concentration. Calcium carbonate (agricultural lime) was used in this experiment as the toxicant.

The daily water quality status of the test media was determined by testing the following parameters: Temperature (°C) using mercury in glass thermometer, pH using the aquaculture test kit model AQ-Z, Code 3633-03 LaMotte. Dissolved oxygen (mg L^{-1}), total alkalinity (mg L^{-1}) and total hardness (mg L^{-1}) were all analyzed using titration methods with the aquaculture test kit. They were all monitored throughout the duration of the experiment following the methods of APHA (1985) for both the acute and sublethal tests.

One gram of $CaCO_3$ was dissolved in distilled water and made up to 1000 mL and made a gram per liter (1 g L^{-1}) solution. From 1 g L^{-1} solution which is equivalent to 1000 mg L^{-1}, 1 mL of stock solution was made up to 1 L to give 1 mg L^{-1} (Reish and Oshida, 1987; EIFAC, 1983). The analysis of variance (ANOVA) and correlations were used in the statistical analysis at $p \leq 0.05$ for testing the significant differences of the means. The mortality analysis was carried out using probit method and regression for the dose-response curves to determine the LC_{50} and LT_{50}.

RESULTS AND DISCUSSION

The water quality parameters determined for the toxicity test media fell within the tolerable ranges for tropical fish species (Alabaster and Lloyd, 1980). Temperature range was between 27.98±0.17 and 28.55±0.38 as the lowest and highest values recorded in the acute test media. While pH ranged from 6.25±0.29 to 7.00±0.00, dissolved oxygen ranged from 3.45±0.55 to 5.0±0.00, Total Alkalinity (TA) ranged from 71.20±14.53 to 120.15±8.90 and Total Hardness (TH) from 75.65±0.00 to 137.95±8.90 (all in mg L^{-1}) throughout the test period as the lowest and highest values, respectively. The analysis of variance (ANOVA) showed that there were significant differences at $p<0.05$ among the means.

The response of *M. macrobrachion* to calcium carbonate is illustrated graphically in Fig. 1. The water quality parameters determined for the sublethal toxicity test media where temperature (27.80±0.20 to 27.93±0.20), pH (6.40±0.00 to 7.00±0.00), dissolved oxygen (3.60±1.00 to 4.40±0.80),

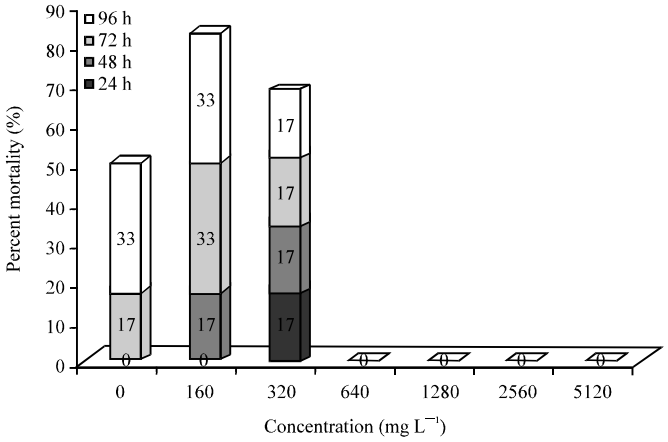

Fig. 1: Histogram of percentage mortality against concentration for *M. macrobrachion* exposed to calcium carbonate after 96 h

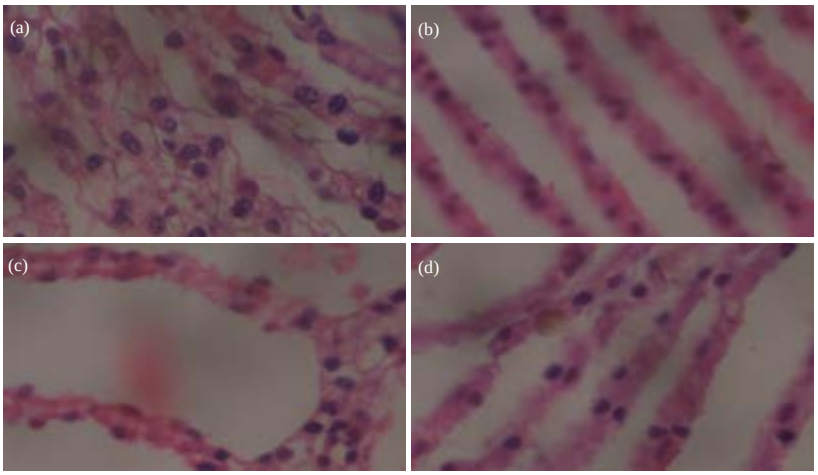

Fig. 2(a-d): Gill morphology of *Macrobrachium macrobrachion* after 14 days exposure to different concentrations of $CaCO_3$ (a) Control, (b) 20, (c) 40 and (d) 80 mg L^{-1}, Normal histology of gill without necrosis and lesions at exposed concentrations

TA (57.85±0.00 to 75.65±35.60) and TH (84.55±55.00 to 120.15±0.00). They were monitored throughout the test period and the result showed, they fell within recommended ranges (Alabaster and Lloyd, 1980). The histological studies of the gills showed no significant differences from the control (Fig. 2).

The toxicity testing using calcium carbonate on the juveniles of *M. macrobrachion* revealed that calcium carbonate is non-toxic to the prawns at the concentrations exposed. This supports the inferences made by some researchers that calcium carbonate and hydroxide is more or less of low toxicity after exposing some mammals to the calcium compounds. They reported median lethal doses of 6,450 mg kg^{-1} (Lewis, 1996), 7,340 mg kg^{-1} (Stewart, 2012) and 1,400 mg kg^{-1} (ITII, 1988) for rats exposed to calcium carbonate, calcium hydroxide and calcium chloride, respectively. The high

LD_{50} indicates low toxicity of calcium compounds which makes them good candidates in wide range of domestic, food, health and environmental applications (Singh *et al*., 2000).

The water quality parameters measured were within the tolerable ranges for the species. The desirable levels of total hardness and alkalinity for fish culture generally fall within 20-300 mg L^{-1} (Boyd and Lichtkoppler, 1979). In this experiment, the total alkalinity and hardness occurred at rates suitable and also favourable to *M. macrobrachion* which was also enhanced by the liming effect of $CaCO_3$. In addition, as pH drops, more of carbonate ion in the test media is converted to bicarbonate ion making calcium more soluble and effecting a buffer. This could be the simple explanation why the test media was conducive for the prawns even as molting occurred in some tanks with high calcium carbonate concentration.

The mortality report revealed that LC_{50} was not deducible from the acute toxicity bioassay experiment. Mortality was not high enough to extrapolate the concentration at 50% death of prawns. There was no significant impact of calcium carbonate on the exposed organs buttressing the inference that this inorganic compound is non-toxic to the brackish river prawn, *Macrobrachium macrobrachion*.

CONCLUSION

In conclusion, calcium compound is considerable for water quality maintenance in prawn culture in order to enhance growth and survival and therefore boost production on the long run. This has also opened an avenue for further research to establish best application rates.

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