Acute Effect of NH₃ on Mangrove Crab *Neopisesarma medri* (Crustacea-decapoda)

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Abstract: Objective: The influence of ammonia on histopathology and some of the metabolic effects of the *N. medrii* crabs were studied. Disruption of pilaster cells and subsequent collapse of gill lamellae were the main effects observed. Epithelial necrosis and hyperplasia were also detected. **Material and method:** Histopathology was processed by standard histological techniques with light microscopy. The important tissular alterations in the connective tissue, epithelial retraction in some tubules and folded basal lamina were observed in treated hepatopancreas. **Results:** Numerous tubules presented an enlarged and irregular lumen with the epithelium atrophied or completely absent. In general, the lesions were particularly located in the medullar region of the hepatopancreas. Exposure of ammonia had decreased the weight gain and increased the food consumption of the treated crabs. Similarly ammonia treatment had also decreased the weight of mandibular organs and had altered the various behaviours of these crabs. The feed intake, water intake and number of burrows made between controls and treated differed significantly ($p < 0.05$). **Conclusion:** These changes suggest that the observed histopathological damage and the associated metabolic disorders affected the neuroendocrine system of the animal, possibly leading to death.

Key words: Metabolic effects, pilaster cells, epithelial necrosis, hyperplasia, irregular lumen, mandibular organs

INTRODUCTION

Environmental contamination and exposure to pollutants is a serious growing problem throughout the world as these are causing some metabolic disorders and are also found in increasingly hazardous concentrations in air, food and water. Because of the growing evidence of a cause-effect relationship between environmental contaminants and the occurrence of toxicopathic effects in both shell and fin fish (Stehr *et al.*, 1998; Stentiford *et al.*, 2003; Feist *et al.*, 2004), studies on histopathology have increasingly been incorporated in national marine biological effects monitoring programmes (Lang, 2002; Feist *et al.*, 2004). Environmental ammonia concentrations may be raised by industrial pollution, domestic sewage and drastic changes of environmental conditions such as water temperature and salinity. In some areas of the mangroves, ammonia concentrations in the pore water, where crabs make their burrows, can reach values as high as 1.2 mM (total ammonia) (Baumgarten and Niencheski, 1990). Despite the high tolerance to sublethal concentrations, crabs exhibit a small resistance time to ammonia concentrations close to the corresponding 96 h LC₅₀ value (Rebelo *et al.*, 1998).

On an international level, toxicological studies are in the process of being implemented in integrated environmental monitoring and assessment programmes. Studies on fish toxicology are studied in details from all over the world but such studies on crabs are few and are not carried out in detail and hence the present

investigation in this experiment was aimed at better understanding of histopathology and some of the metabolic disorders of medri crabs due to the exposure of ammonia.

MATERIALS AND METHODS

Animals: Forty animals were used for histological examination and detection of their metabolic malfunctions were obtained from Prangipattai near Center of Advanced Study in Marine Biology, Annamalai University, Tamil Nadu India (Lat; 11° 29' N and Long; 79° 46' E). The experimental duration was 30 days. Immediately after collection, the crabs were brought to the laboratory and acclimatized to its conditions (Salinity 10-34 ppt; Dissolved oxygen 4.2 -5.8 ml L⁻¹; Temperature 22- 30°C and pH 8.0-8.5). The animals were stocked at the density of 10 crabs per trough and were divided into two groups, control and test animals. Animals were exposed to ammonia (as ammonium sulphate) for 96 h at the following concentrations: 0 (control) and 27.5 mM. Ammonia free seawater was obtained and used to prepare test solutions and the solutions were renewed every 24 h.

Histological examination of crab tissue: To determine whether ammonia results in structural changes in internal tissues such as the gills and hepatopancreas, both control (normal) and treated crabs were examined histologically. Selected crabs were killed by injection of ca 50 mL of

Bouin's sea-water fixative through the unsclerotised membranes at the base of the fifth pereiopod. Crabs were rapidly dissected and gills and hepatopancreas were placed in an excess volume (ca 25 mL to <1 g tissue) of fixative for storage in the dark at room temperature until processing. Picric acid was removed from the fixed tissue samples by several washes in 70% propan-2-ol that had been previously saturated with lithium carbonate. Tissues were then dehydrated through a graded series of alcohols (70, 90 and 100% propan-2-ol, each for 30 min) and cleared in Histo-clear (National Diagnostics, Hull, UK) for 3 h 5 µm sections were prepared with a Zeiss HM 350 microtome and stained with hematoxylin and eosin.

Weight measurements: Weights of the crabs were taken using electronic balance after blotting the animal on a filter paper. Initial and final weights of live individuals in each experimental group were measured and recorded.

Feeding: The crabs were fed with decayed mangrove leaves twice per day. For feeding, food, approximately

equivalent to 20% of the body weight of the animal was used. The unused food was removed after 6 to 8 h. The troughs were cleaned daily.

Behavior: Each behavioural activity was observed for four days. Observations were made daily for three hours (morning, midday and afternoon) throughout the duration of the experiment.

Somatic index measurement of mandibular glands. Index was determined using the standard formula:

$$\text{Index} = \frac{\text{Wet weight of the organ}}{\text{Wet weight of the crab}} \times 100$$

RESULTS

Histopathology: The results of the histopathological effects of ammonia on the hepatopancreas and gills after 30 days of exposure are shown in Fig. 1. As shown in Fig. 1a-c, control gills, exhibit a very thin cuticle, a single layer of epithelial cells and the characteristic pilaster cells.

Fig. 1(a-f): Low power micrographs of the gills and hepatopancreas of *N. medrui* crabs. (a and c) Normal gills, (b and d) Treated gills, (e) Normal hepatopancreas and (f) Ammonia treated hepatopancreas

In contrast, exposed gills exhibit collapsed lamellae, due to disruption of pilaster cells (Fig. 1b and d). A detail of this pathology can be seen in Fig. 1b and d. Epithelial necrosis and hyperplasia were also observed (Fig. 1b). These pathologies together with the absence of pilaster cells, led to collapse of the entire lamellae (Fig. 1d). Histologically, each tubule of the hepatopancreas was surrounded by haemal spaces that contained haemocytes and apparent blood vessels (Fig. 1e). The hepatopancreatic tubules were enclosed by a basal lamina and contained a central lumen (Fig. 1e, f), the main histopathological alterations present were epithelial retraction, folded basal lamina and numerous cells were completely detached from the basal lamina showing signs of necrosis (Fig. 1f). The lesions did not comprise the entire organ but they were located in the medullar region of the organ.

Growth: The results of 30 days growth trial are presented in Table 1. Exposure of ammonia decreased the food consumption of these crabs. Maximum weight gain was noticed in normal crabs. To investigate the growth rate of

Fig. 2: Showing mandibular gland index of control and treated of *N. medrui* crabs, $p < 0.05$

Table 1: Effect of ammonia on growth of mangrove crab (Weight in grams)			
Parameters	Control	Treated	p-value
Initial weight	7.10±0.05	6.98±0.02	>0.05
Final weight	8.40±0.32	7.2±0.1	<0.05
Percentage growth	15.52±0.52	3.22±0.26	<0.05
Average daily growth	0.50±0.05	0.09±0.005	<0.05

Table 2: Effect of ammonia treatment on some of the behavioural changes in *N. medrui* crab

Parameters	Control				Treated				p-value
	1 day	2 day	3 day	4 day	1 day	2 day	3 day	4 day	
Feed intake (g)	1.3±0.33	1.6±0.51	1.6±0.001	1.4±0.42	1.5±0.05	2±0.32	1.9±0.05	1.3±0.06	$p < 0.05$
Water intake (mL)	3±0.06	3.2±0.64	3.5±0.09	3.6±0.69	3.5±0.52	3.52±0.54	4.1±0.03	3.1±0.02	$p < 0.05$
Number of burrows made	3±0.65	3±0.5	4±0.06	6±0.60	2±0.02	3±0.005	3±0.087	1±0.002	$p < 0.05$

crabs after the treatment of ammonia, a comparison between control and treated animals was made. Student t-test done showed that growth rates between control and treated differed significantly ($p < 0.05$).

Mandibular gland index: The weight of the mandibular glands decreased with increasing body weight of crabs and decreased when the animal is exposed to ammonia (Fig. 2). The results also showed that there is positive correlation between weight gain and mandibular indices of normal crabs (Fig. 3), however no such correlation had been found in ammonia treated crabs (Fig. 4). Student t-test done showed that mandibular gland index between control and treated differed significantly ($p < 0.05$).

Behaviour changes due to the exposure of ammonia: The various behavioural changes of medrui crabs are presented in Table 2. Exposure of ammonia had altered the various behaviours of this crab. Student t-test done

Fig. 3: Correlation of mandibular gland indices and weight gain of normal *N. medrui* crabs ($R^2 = 0.603$)

Fig. 4: Correlation of mandibular gland indices and weight gain of treated *N. medrui* crabs ($R^2 = 0.000$)

showed that feed intake, water intake, number of burrows made, between control and ammonia treated crabs differed significantly ($p < 0.05$).

DISCUSSION

There are several reports in the literature on the histopathological effects of pollutants in the gills of crustaceans and fish (Bigi, 1996; Randi *et al.*, 1996; Medesani *et al.*, 2000). Nevertheless, just a few of them are related to ammonia concentrations (Paul and Banerjee, 1997; Winkaler and Martinez, 1998). Cardoso *et al.* (1996) detected changes in cells and branchial tissue of larvae and juveniles of *Lophiosilurus alexandri*, exposed to concentrations of unionized ammonia close to the LC_{50} values. Paul and Banerjee (1997) reported for the epithelial linings of the operculum of a catfish massive and quick damage due to necrosis and sloughing of the outer surface epithelial cells with degeneration and disappearance of club cells, after acute exposure to ammonium sulfate. Winkaler and Martinez (1998) also found gill lamellae fusion to be an effect of ammonia in fish exposed to 0.41 mM of gaseous ammonia. They also reported severe aneurysm as a specific effect of such exposure. In *N. medrii*, we detected extensive structural alterations, involving cellular hyperplasia, necrosis and disruption of pillar cells after acute exposure to ammonia. The histopathological effect of ammonia on gill epithelium showed gill to be independent, since we found similar effects in gill lamellae. This led to considerable thickening of the gill epithelium and reduction of haemolymph spaces resulting in restriction of respiratory gas exchange as shown by hypoxia and hypercapnia, as reported by Rebelo *et al.* (1998). In the hepatopancreas of *N. medrii*, several alterations were observed, indicating that the organ reacts to environmental changes. Miglioranza *et al.* (2002) reported that the biota accumulates significant amounts of organochlorine pesticides *in situ*, particularly DDT. Nimmo and Blackman (1972) found that penaeid shrimps accumulate more DDT in the hepatopancreas than in other organs. The important degenerative desquamation, tubular dilation, loss of cellular limits and epithelial atrophy described in the present study were also mentioned for other decapods exposed to contaminants (Vogt, 1987; Sousa and Petriella, 2007). Alterations like atrophy, reduction in the height of tubular epithelium, necrosis and degenerative desquamation have been noted in hepatopancreas of mollusks and decapods crustaceans after exposures to organochlorines like Aroclor and DDT (Lowe *et al.*, 1971, 1972), petroleum compounds (Fries and Tripp, 1976), organophosphates like methyl parathion

(Lowe *et al.*, 1971) and heavy metal salts (Establier *et al.*, 1978). Most of the alterations in the hepatopancreas of *N. medrii* crabs were focused on the medullar region of the organ, where medial and proximal zones of the tubules are located. Coincidentally, other researchers studying the effect of heavy metals on decapod crustaceans found the highest amount of metal granules in R-cells located at the proximal zone of the tubules (Hopkin and Nott, 1979). The increased desquamation is related to the epithelial renovation and the elimination of damaged cells, probably containing the toxicants. The important haemocytic infiltration observed in the present study is involved in the phagocytosis of necrotic cells. Similarly, Doughtie and Rao (1983) and Sousa and Petriella (2007) found a pronounced activity of phagocytes in the hepatopancreas of *Palaemonetes pugio* exposed to pollutants; the authors also observed ultrastructural alterations similar to those observed in the present study. Thus the present results are in agreement with the above mentioned studies.

The present study demonstrated that ammonia treatment decreased the growth of *N. medrii* crabs and also increased their feed utilization efficiency. Decrease in growth was also reported in *Physocypria kraepelini* (Yu *et al.*, 2009). Dagmar *et al.* (2005) also reported a decrease in growth in *Rhodococcus erythropolis* and *Candida maltose*. Similar results were also reported in different crustaceans (David *et al.*, 1979; Miranda-Filho *et al.*, 2009).

The weight loss of ammonia exposed crabs are in agreement with the findings of the above mentioned works. In the present study, ammonia treated animals consumed more food than normal animals. The high food requirements of the ammonia treated crabs might be due to increase demand of energy after exposure.

In this study, ammonia had a negative effect on growth of mandibular organs of these crabs. Sanjeevraj *et al.* (1996) in his study showed that moulting and growth rates in eyestalk-ablated prawns were higher. Hinsch (1977) and Le Roux (1980, 1983) also observed hypertrophy of the MO in response to eyestalk ablation in *Libinia emarginata*, *Palaemonetes varians* and *Pisidia logicornis*. Eyestalk removal significantly increased the levels of MF in the hemolymph of spider crab, *L. emarginata* (Laufer *et al.*, 1987), American lobster *Homarus americanus* (Tsukimura and Borst, 1992) and *Orconectes virilis* (Tsukimura *et al.*, 1989). A mandibular organ inhibiting hormone (MOIH) has been isolated from the sinus gland (Liu and Laufer, 1996). In the crab *O. senex senex* also, the weight of the MO increased as the crab approached ecdysis. Similar results were observed for crayfish *P. clarkia* (Landau *et al.*, 1989) and

fresh water prawn *M. rosenbergii* (Sagi *et al.*, 1991). Thus the findings of the present study corroborate the above works in the sense that exposure of ammonia might have interfered with the X-organ-sinus gland. This is also evident from decreased size of mandibular glands in this experiment.

Further, observation recorded in the present study clearly demonstrated that ammonia treatment induces behavioural changes in *N. medrii*. One of these changes is hyperactive state of the crab after exposure to ammonia and in hyperstate, crab needs more oxygen and food, hence the crab takes more water and food to meet extra demand of oxygen and energy. Other parameters also show that these behavioural changes probably may occur due to the intoxication of ammonia on central nervous system which in turn leads disfunction of other body system.

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