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Effect of salinity on the hemocyte profile and phagocytosis in the Indian edible oyster, *Crassostrea madrasensis* (Preston)

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Abstract

Groups of Indian edible oysters (*Crassostrea madrasensis*) of average size 6.6 ± 1.5 cm x 4.2 ± 0.2 cm, were maintained at different salinities of 6, 12, 25 and 35 ppt for a period of 1 month. On termination of the experiment, hemolymph samples were withdrawn from adductor muscle sinuses and analyzed for total hemocyte counts, differential hemocyte counts and phagocytic function of the hemocytes. The results revealed that the total hemocyte counts were significantly high at 35 ppt and it was mainly contributed by semigranulocytes. In animals maintained at all other salinity levels, hemocyte counts did not show significant variations. The phagocytic index was significantly low ($p < 0.05$) in oysters maintained at 6 and 35 ppt salinities compared to other treatment groups.

Key words: *Crassostrea madrasensis*, salinity, hemocyte count, phagocytosis

Introduction

Molluscs are osmoconformers and therefore environmental factors such as salinity and temperature definitely influence their defense mechanisms (Chu, 2000). The defense system of molluscs includes cellular and humoral factors. Cellular factors comprise of hemocytes and these are of prime importance in the defenses. The hemocytes of the mollusc are generally classified as granulocytes and hyalinocytes based on the presence or absence of granules in the cytoplasm (Cheng and Foley, 1975; Rodrich and Ulrich, 1984). The granulocytes are further classified into semigranulocytes and granulocytes (Moore and Lowe, 1977; Rasmussen *et al.*, 1985). The hyalinocytes are the immature cells which mature into the other types of cells (Balquet and Poder, 1985). Phagocytosis is the most important cellular defense mechanism and granulocytes are more actively phagocytic compared to other cell types (Anderson and Anderson, 2000).

Since the hemolymph of the marine molluscs follows the osmotic strength and ionic composition of the ambient water, the hemocytes are directly exposed to salinity variations (Gilles, 1979). The present study was intended to understand the influence of different levels of salinities on the hemocyte pattern as well as on the function of hemocytes *i.e.* phagocytosis.

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Material and methods

Maintenance of C. madrasensis

The edible oysters, *C. madrasensis* (mean size 6.6 ± 1.5 cm x 4.2 ± 0.2 cm) were collected from the backwaters of Cochin around Vypeen Islands, Kerala. The salinity recorded from the sample collection sites ranged from 10-15ppt. The experiments were carried out in fiberglass tanks of 50 liters capacity holding 30 liters of filtered and aerated seawater. Through out the course of experiment, the animals were fed on *Chaetoceros* sp. *ad libitum*. Four groups of animals were exposed to different salinities viz. 6, 12, 24 and 35 ppt. Each group had three replicates with 15 animals each. Every day the tanks were cleaned after siphoning out the dirt and feacal matter and 50% of water was exchanged. Water quality parameters were checked frequently and were maintained at optimum levels. The animals were exposed to respective salinities for a period of 1 month, before hemolymph sampling was done.

Hemolymph collection

The hemolymph samples were collected from the adductor muscle sinuses following the method of Chen (1996). A notch was filed on the dorsal aspect of the shell valve, adjacent to adductor muscle and about 0.5ml - 2ml of hemolymph was collected from each animal using a 27-gauge needle attached to a 5-ml sterile syringe. The hemolymph samples were immediately stored at 4°C until analysis.

Total and differential hemocyte count

The total and differential hemocyte counts were estimated using hemocytometer according to the method of Nakayama *et al.* (1997) after staining with 0.3% of May-Gruenwald's eosin-methylene blue solution. The total count was expressed as mean number of cells in each of the four squares x 10^4 cells/ml of hemolymph.

Based on the staining characteristics using May-Gruenwald's stain, the hemocytes were classified as granulocytes, semigranulocytes and hyalinocytes (Gijo, 2004). For differential count, a total of 200 cells were counted and the percentage of each type of cells such as granulocytes, semigranulocytes, and hyalinocytes was calculated.

Phagocytosis

Powdered yeast was used for phagocytic studies as per the method of Bayne *et al.* (1979) with slight modification. Formalin killed yeast cells were washed 3 times with sterile filtered 2% seawater and was dispersed in appropriate quantities of 2% sterile seawater to have a density of 4 x 10^6 cells per ml.

Hemocyte monolayers were prepared

on glass slides, rinsed with 2% seawater and incubated with yeast suspension in 2% seawater for 60 minutes at 25° C. Subsequently, the slides were rinsed three times with 2% seawater, fixed in 10% methanol for 15 minutes, air dried and stained with dilute Giemsa (diluted 10 times with distilled water) for 20 minutes, differentiated in acetone and mounted in DPX. The cells were observed under light microscope and about 200 cells were counted on each slide. The phagocytic index was calculated as per the following formula.

Phagocytic index =

$$\frac{\text{Number of hemocytes with phagocytosed yeast cells}}{\text{Total number of hemocytes counted}} \times 100$$

Results

Total and differential hemocyte count

The mean hemocyte counts of *C. madrasensis* exposed to different salinities are depicted in Fig. 1. The total hemocyte counts at 6, 12 and 25ppt did not show any significant difference, whereas, at 35ppt salinity it was significantly higher ($p<0.05$). The percentage of granulocytes, semigranulocytes and hyalinocytes showed no significant difference ($P<0.05$) between various treatments (Fig. 2). However, the percentage of semigranulocytes was high at 25 ppt and 35 ppt, the maximum increase was seen at 25 ppt. The maximum reduction of hyalinocyte was seen at 35 ppt. The percentage of granulocytes was more or less same in all the treatments.

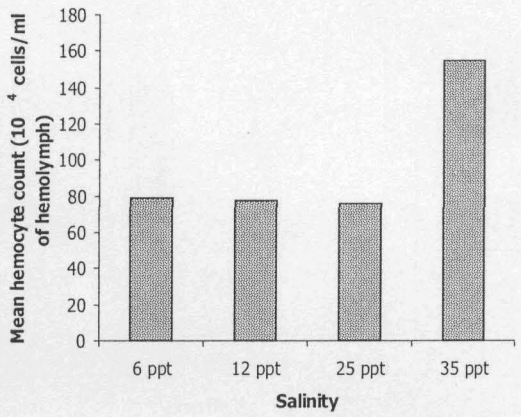

Fig. 1. *Mean hemocyte count (x 10^4 cells per ml) of the hemolymph of* C. madrasensis *at different salinities*

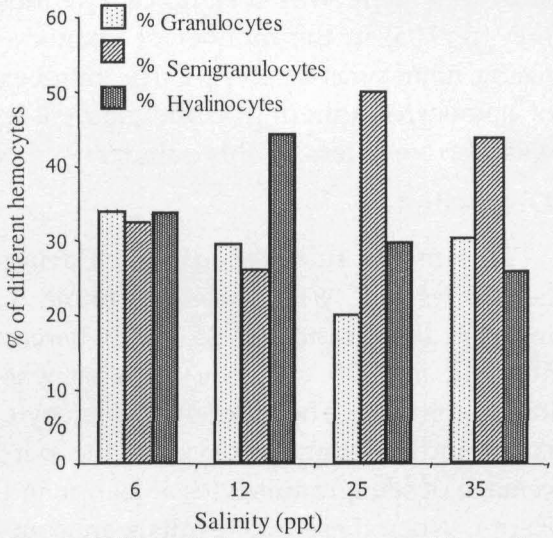

Fig. 2. *Differential hemocyte counts of* C. madrasensis *exposed to different salinities*

Phagocytosis

The phagocytic indices of hemocytes of *C. madrasensis* at different salinities are shown in Fig.3. Compared to the animals exposed to 6 ppt salinity, the indices recorded in animals at 12 and 25 ppt

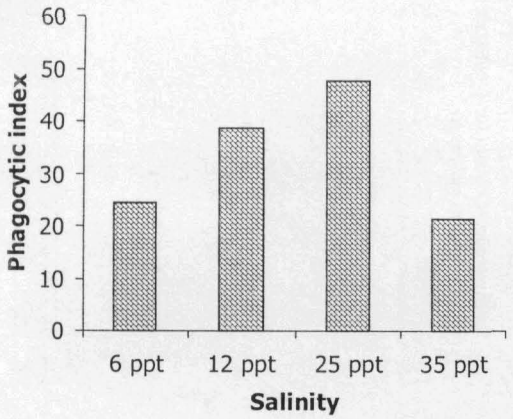

Fig. 3. *Mean Phagocytic index of the hemocytes of* C. madrasensis *at different salinities*

salinity were significantly ($p<0.05$) higher. However, there was a significant reduction ($p<0.05$) in the number of phagocytosing hemocytes at 35 ppt. The number of hemocytes adhering to the glass slide was also very less at this salinity.

Discussion

The mean number of circulating hemocytes in *C. madrasensis* was found to increase significantly at 35 ppt, whereas at 6, 12 and 25 ppt, the number was almost the same. The differential hemocyte count indicates an increase in the percentage of semigranulocytes at 25 ppt and 35 ppt. When these two results were compared, it was clear that the increase in the total hemocyte count is contributed by the increase in number of the semigranulocytes. As the total count increased considerably, it has to be assumed that hemopoiesis is occurring at a higher rate at these salinities and the first formed cells namely hyalinocytes are maturing into semigranulocytes. An increase in the agranular hemocytes at high salinity has been reported in *Crassostrea virginica* (Fisher and Newell, 1986). However, further maturation of semigranulocytes into granulocyte is not occurring, which may be the reason for the increase in percentage composition of semigranulocytes in the hemolymph.

The mean phagocytic indices of *C. madrasensis* at different salinities indicate that the salinities at which the functional property of hemocytes is not affected are 12 and 25 ppt. At 6 and 35 ppt, the phagocytic indices recorded were low. At 35 ppt adherence to the glass was also affected. Although the number of hemocytes was very high at 35 ppt and the increased number was mainly contributed by semigranulocytes and granulocytes, which are highly phagocytic, the phagocytic index recorded was less. The result indicates that the functional property of the hemocytes was highly affected at this salinity. A reduction in hemocyte spreading and rate of locomotion have been reported in *C. virginica* at high salinity (Fisher and Newell, 1986). Proper functioning of the granular hemocytes is highly essential for correct defense against infection by pathogens and parasites. An increase in the infestation by parasites in *C. virginica* has been reported in high salinity regions compared to low salinity areas (Haskin and Ford, 1982).

C. madrasensis is a euryhaline species which are found mainly in the backwaters and estuaries. The result of the present study indicates that the species can be

cultured at salinities ranging from 12 to 25 ppt, without compromising the defense system. At 6 ppt, even though the total hemocyte count was normal, the phagocytic index was significantly low compared to 12 and 25 ppt. At higher salinities hemocyte profile and function are affected, indicating a stressful condition for the species as the immune system is compromised.

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