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ORIGINAL ARTICLE

# HAEMOCYTES CLASSIFICATION AND DIFFERENTIAL COUNTS IN THE FRESHWATER CRAB, PARATELPHUSA HYDRODROMOUS

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**Abstract:**

*The morphological study of haemocytes from the freshwater crab Paratelphusa hydrodromous was carried out by light microscopy. Total haemocytes count and differential count of crab haemocytes were made. Total count of haemocytes was noted as 140+3.288 cells/mm3. Three types of haemocytes were identified in fresh water crab Paratelphusa hydrodromous: hyalinocytes, semigranulocytes and granulocytes. The proportion of hyalinocytes was found to be 35%, whereas semigranulocytes were found as 62% and granulocytes 43%.*

**KEYWORDS-**

Freshwater crab, Paratelphusa hydrodromous, Haemocytes, Hyalinocytes, Semigranulocytes, Granulocytes.

## INTRODUCTION

Like other invertebrates, crustacean immune defense is mediated by circulating haemocytes and include phagocytosis, encapsulation, nodule formation, clotting, agglutination, melanisation and microbicidal activity (Smith 1991; Smith et al., 1986; Soderhall 1992; Le Moullac et al., 2000). Crustacean haemocytes play important role in host immune responses; however, there is no uniform classification scheme for crustacean haemocytes (Johansson et al., 2000). Although one of the most debated point is the morphological classification of crustacean haemocytes, three types of circulating haemocytes are generally recognized in most species of crustacea. They are hyalinocytes (the smallest cells without evident granules) semigranulocytes (which contain small granules) and granulocytes (with abundant cytoplasmic granules) (Bauchau et al., 1981). In addition to the differing morphological features, each one of the cell type was suggested to have a main function. Hyalinocytes were considered as phagocytes (Soderhall et al., 1986), semigranulocytes (also called small granule haemocytes), which display some phagocytic capacities, would be specialized in particle encapsulation (Persson et al., 1987) and granulocytes (also called large granule haemocytes).

The circulating haemocyte number is a stress indicator (Le Moullac et al., 2000) and haemocyte counts may be a valuable tool in monitoring the health status of crustacean species (Jussila et al., 1997 and Mix et al., 1980). The haemolymph and haemocyte components of the arthropod circulation are responsible for various protective mechanisms ranging from coagulation and would participate in cell-mediated immunity (Theopold et al., 2004; Iwanaga et al., 2005; Jiravanchpaisal et al., 2006). Much of our current understanding of the invertebrate immune system has arisen from the investigation of common research species of those of commercial importance.

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Studies on the haemocytes of crustaceans contribute to the accumulation of the basic knowledge on haemocytes especially with regard to the physiological condition of the animal. However, there is no information on total and differential haemocyte counts of the freshwater crabs. Hence the present attempt was made to study the total and differential count of haemocytes of the freshwater crab Paratelphusa hydrodromous.

## MATERIALS AND METHODS

### Animal collection

The intermoult adult males of Paratelphusa hydrodromous (4 cm mean carapace length) were collected from different sites of paddy fields in Pethankuppam Village, Cuddalore District, Tamil Nadu, India and brought to the laboratory for the collection of haemolymph.

### Haemolymph collection

Crabs were anaesthetized on ice for 10 min and the haemolymph (atleast 250 µl/crab) was collected from the unsclerotised membrane of the walking legs using a 1-ml plastic syringe, placed in Eppendorf tubes and diluted with (1:2) an anticoagulant solution of sodium citrate buffer pH 4.6 (2.1 v/v / EDTA- NaCl 0.45 w/v) and stored at 4°C until use.

### Smear preparation

Collected haemolymph was spread out upon a clean slide into thin films and air dried. The air dried smears were fixed with methanol and stained with Giemsa stain. The slide was dipped in 70% ethanol until the desired amount of dye was removed from the smear. Then the smear was permanently mounted in balsam under a cover slip.

### Total haemocyte counts (THCs)

The counting of free haemocytes was done by using a haemocytometer with improved double Neubauer ruling. The haemolymph from the crab was collected on a glass slide and quickly drawn into a pipette used for the counting of WBC of mammals. The pipette was filled with the haemolymph upto the 0.1 mark in each case and then the diluting fluid (Tuerk's fluid) was added and shaken for three minutes. Three drops of the mixture were dropped into counting chambers (Jones et al., 1962). The THC / mm3 were determined by the method of Kolmer et al. (1969).

The THC / mm3 was determined by the formula x/4 x 10 x Y where x = total no. of haemocytes counted in 4 chambers in the corner.

| | | |
|---|---|---|
| 4 | = | No. of chambers |
| 10mm | = | Depth of the chamber |
| Y | = | Dilution |

### Differential haemocyte counts (DHCs)

The method employed for counting of different types of haemocytes was especially suited to the picture of crabs as suggested by Vinson (1971). DHC were made (number of different haemocytes per 100 haemocytes) by observing stained blood films under microscope.

### Measurement of cell size

The diameter of the haemocytes was calculated using a compound microscope fitted with micrometers.

### Results

The haemocytes in the haemolymph of Paratelphusa hydrodromous were classified into hyalinocytes, semigranulocytes and granulocytes. Hyalinocytes were devoid of granules or only a small number of tiny intracellular inclusions (Fig.1).

Semigranulocytes were intermediate cell type between hyalinocytes and granulocytes; they showed an acentric, spherical nucleus and had less granules than granulocytes (Fig.2). The granulocytes were filled with numerous large, highly refractile granules (Fig.3).

The total haemocyte count was 140 cells / mm3. The range of total haemocytes count was between 90 and 140 cells / mm3. The most abundant type was semigranulocytes, constituting 60.78 + 1.735 of total haemocytes. The number of hyalinocytes was found to be 35.486+3.142 whereas granulocytes were found 44.278+2.790 respectively.

Haemocyte size frequency distribution was determined by using stage micrometer and ocular micrometer. In in vivo observation, cells were classified on the basis of the presence/absence of cytoplasmic granules as granulocytes, which are 0.056±0.008 m in diameter with more number of refractile granules, semigranulocytes are 0.072±0.011 m in diameter containing a variable number of refractile granules and hyalinocytes are 0.010±0.002 m in diasmeter without evident cytoplasmic granules.

| Types of haemocytes | THCs No. of cells / mm$^3$ $\overline{X}$ ± S.D | Diameter of cells µm $\overline{X}$ ± S.D | % of individual haemocytes |
|---|---|---|---|
| Hyalinocytes | 35.486 ± 3.142 | 0.010 ± 0.002 | 0.35% |
| Semi-granulocytes | 60.78 ± 1.735 | 0.072 ± 0.011 | 0.62% |
| Granulocytes | 44.278 ± 2.790 | 0.056 ± 0.008 | 0.43% |
| Total | 140±3.288 | | |

$\overline{X}$ **Mean value obtained from six observations.**

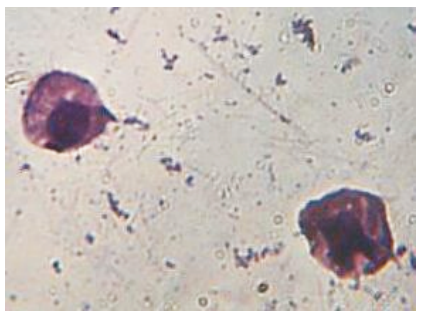

Fig.1 Hyalinocytes
X600 Magnification

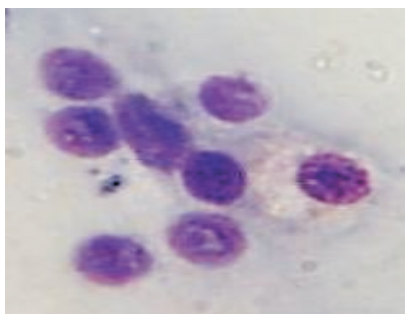

Fig.2 Semigranulocytes
X400 Magnification

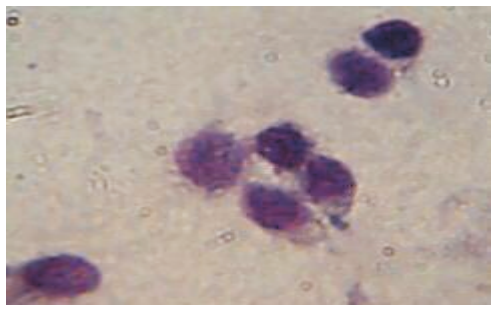

Fig.3 Granulocytes
X400 Magnification

## DISCUSSION

In the present investigation, under light microscope the, circulating haemocytes from the freshwater crab Paratelphusa hydrodromous were evaluated. They were classified on the basis of morphological (presence of cytoplasmic granules) features of cells.

In the present study, three types of haemocytes (hyaline, semigranular and granular) were identified in the freshwater crab, Paratelphusa hydrodromous. The haemocytes of freshwater crab were classified as hyalinocyte, semigranulocyte and granulocyte according to cytoplasmic granules are in agreement with previous findings. (Bauchau et a., 1981; Persson et al.,1986; Johansson et al.,1989).

Following the morphological criterion proposed by Bauchau et al., (1981) and Hose et al.,(1990) the presence or absence of refractile granules and three basic cell types were identified in our study also.

Haemocytes were classified from both penaeid, Penaeus paulensis and two Palaemonids, Macrobrachium acanthurus and Macrobrachium rosenbergii, as hyaline haemocytes, small granule haemocytes (semigranulocytes) and large granule haemocytes (granulocytes) (Jusila, 1997; Yauzcan, 2002; Hammond, 2002; Gargioni and Barracco, 1998). Although the significance of this marked variation in THC remains unclear, influences on moult cycle (Tsing et al., 1989), diet (Want et al., 2005), harvesting (Jusila et al., 1997), diseases (Smith et al., 1980; Eddy et al., 2007) and environmental contaminants (Smith et al., 1995) cannot be excluded.

In addition, a recent study Li and Chen (2008) demonstrated that both high (10.1) and low (6.5) pH values significantly decreases THC in Litopenaeus vannamei. In most crustacea, THC and DHC vary markedly among individuals (Sung et al., 2002; Johansson et al., 2000). The present study also showed of Paratelphusa hydrodromous a great inter-individual variability in the haemocytes. This important aspect will be more fully investigated in future studies. Nevertheless, differences in the values among Crustacean species may be due to the differing experimental procedures used to determine circulating haemocyte mean number.

## SUMMARY AND CONCLUSIONS

Results of this study indicated that Paratelphusa hydrodromous, has three types of haemocytes namely hyalinocytes, semigranulocytes and granulocytes. The values of total hyalinocytes counts measured in this study may provide useful tool for further studies. Nevertheless total and differential haemocytes counts could change depending on factors such as sex, water temperature, molting cycle and starvation.

In the present study, light microscopy analyses allowed us to identify three main haemocyte types in haemolymph from the fresh water crab, Paratelphusa hydrodromous. Preliminary morphological investigation of haemocyte under light microscope is considered necessary by investigators.

Future studies are to be performed in the ultrastructural and functional aspects of haemocytes to distinguish freshwater crab cell types and clarify their role in immune responses and the role of haemocytes on wound healing in the crab.

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