

Identification of Different Types of Haemocytes in Freshwater Crab *Paratelphusa masoniana* (Henderson).

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Abstract

For the first time haematological studies on freshwater crab *P. masoniana* (Henderson) has been carried out in Jammu, India. The aim of studies were to determine various type of hemocytes, their total and differential count as well. Based on morphological studies of hemocytes under microscope three types of haemocytes were identified viz., Hyalinocytes (H), Semigranulocytes (SG) and Granulocytes (G). These cells were varied with respect to their proportion, Semigranulocytes being the heighest (54%) follow by Granulocytes (30%) while Hyalinocytes were least (16%) among three cells.

Key words: *Paratelphusa*, haemocytes, morphological features

Introduction:

Freshwater crustacean like other aquatic organism remain continuously exposed to several types of pollutants as well as pathogenic organism inhabiting water bodies and hence need an effective immune system. Immunity in crustacean is provided by haemocytes circulating freely in the body because they have an open vascular system (Matozzo and Marin, 2010b). Moreover invertebrates compared to vertebrates possess only cellular mediated immunity because they lack antibody mediated immunity and hence their immune system is not adaptive but rather happens to be of innate type (Galloway and Depledge, 2001).

Haemocytes provide immunity via phagocytosis, encapsulation and lysis of foreign cells and opsonation (Soderhall and Smith, 1983; Matozzo and Marin, 2010c). Crustacean being completely dependent on cellular mediated immunity so their formed elements i.e. haemocytes present in haemolyph appear to play

very significant role in providing them immunity besides acting as chief indicator of their physiological status (Galloway and Depledge, 2001).

To best of our knowledge no study concerning identification of circulating haemocytes from the crab *P. masoniana* (Henderson) has previously been performed. Presently therefore an endeavour has been made to study and identify different types of haemocytes in crab *P. masoniana* under light microscope and classify on the basis of their morphological features. Additionally attempt has also been made to determine total and differential hemocyte count.

Material and methods:

Animal: Adult crabs (3.5 cm mean carapace width) were collected from lower region of river Chenab, kept in the laboratory in disinfected plastic tubs then fed with mussels, algae & mud rich in organic matter. The crabs were acclimatised in the laboratory for 3 days.

Haemolymph sampling

Crabs were anaesthetised on ice for 15 minutes and the haemolymph was collected by puncturing heart directly by 1ml insulin syringe, thin smear was prepared immediately and subjected to staining. Staining was done by using 10% Giemsa stain (Matozzo and Marin, 2010).

Photography

Slides of blood smear were scanned and photographed using Olympus Microscope CH20 i.

Results:

Type of hemocytes

Three types of haemocytes have been identified viz., Hyalinocytes (H), Semigranulocytes (SG) and Granulocytes (G). The different morphological characteristics features taken into account for the identification of haemocytes are (i) Size (ii) shape (iii) presence or absence of cytoplasmic granules (iv) position of nucleus and (v) nucleo: cytoplasmic ratio. Chief morphological characteristics of these hemocytes are given in table (1) and are described as under:

Hyalinocytes (fig. 1):

Hyalinocytes have been observed to be ellipsoidal in shape and small in size. Cytoplasm of these cells show purple colour and have a centrally placed dark blue coloured nucleus. Size of these cells was found to be $8.3 \pm 1.2 \times 7.6 \pm 0.32 \mu\text{m}$ with nucleus having size $5.84 \pm 1.14 \times 4.2 \pm 0.20 \mu\text{m}$. Based on this their nucleus to cytoplasmic (n:c) ratio was found to be $0.6255 \pm 0.02 \mu\text{m}$. These cells did not reveal any type of granules in their cytoplasm.

Semigranulocytes (fig. 2):

Semigranulocytes have been observed to be circular/ovoid and their cytoplasm exhibited purple colour. These hemocytes too had a centrally placed dark blue nucleus. Size of these cells has been found to be $12\pm 2.24 \times 10.2\pm 0.52\mu\text{m}$ and that of nucleus $7.2 \times 5.2\mu\text{m}$. Their nucleus to cytoplasmic ratio has been calculated to be $0.44\pm 0.06\mu\text{m}$. These cells were observed to contain sparsely placed blue coloured cytoplasmic granules.

Granulocytes (fig. 3):

Granulocytes are the hemocytes which have comparatively large size, circular in shape with purple stained cytoplasm. These characteristically had an eccentrically placed nucleus. Size of these cells has been recorded to be $14.3\pm 1.34 \times 12.6\pm 0.24\mu\text{m}$ and that of nucleus to be $8.50\pm 14 \times 7.20\pm 36\mu\text{m}$. Nucleus to cytoplasmic ratio has been found to be $0.51\pm 0.02\mu\text{m}$. These cells were densely packed with blue coloured cytoplasmic granules.

Relative proportion of different haemocytes:

All the 3 hemocytes were varied in their proportion (table 1) Semigranulocytes being the highest (54%) followed by Granulocytes (30%) while Hyalinocytes (16%) were least among three. This simply indicates that semigranulocytes are the dominant hemocytes in hemolymph of crab *P. masoniana*.



Fig.1 Hyalinocytes

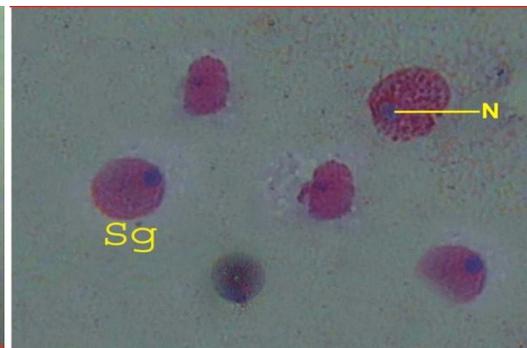


Fig. 2 Semigranulocytes

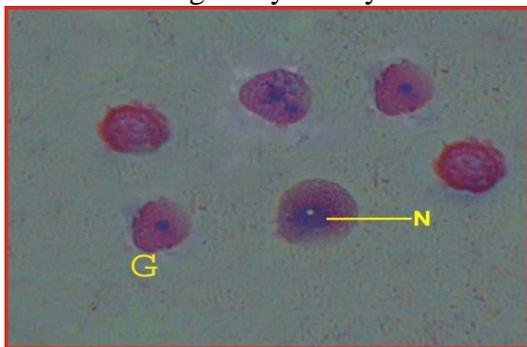


Fig. 3 Granulocytes

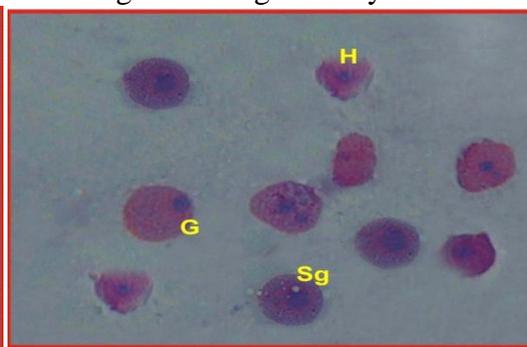


Fig. 4 Different types of cells

N-Nucleus, G-Granulocytes, Sg-Semigranulocytes, H-Hyalinocytes

Discussion:

Types of haemocytes: Review of literature (Hose *et al.*, 1990; Heng and Lei, 1998; Johanson *et al.*, 2000; Laxmilatha and Laxminaryana, 2004; Gelibolu *et al.*, 2009; Kakoolaki *et al.*, 2010 and Matozzo and Marin, 2010b) reveals that classification of hemocytes has remained a controversial aspect. Controversy usually appears to be because of lack of uniformity of classificatory criterions that have been employed to distinguish these different type of hemocytes.

Different schemes have been proposed for classification of hemocytes based on either morphology (Heng and Lei, op. cit.) or morphological and chemical features (Kakoolaki *et al.*, op. cit. and Matozzo and Marin, op. cit.) or functional properties (Matozzo and Marin, op. cit.). Presently in tune to Heng and Lei (op. cit.) hemocytes too have been classified based on their morphological characteristics of size, shape, presence or absence of granules, position of nucleus and nucleus to cytoplasmic ratio as Hyalinocytes, Semigranulocytes and Granulocytes..

Proportion of hemocytes:

Present results are in tune with those of Yildiz (2002) who while working on freshwater crab (*Potamon fluvitalis*) also reported almost similar relative proportion of hemocytes as 15% Hyalinocytes, 54.2% Semigranulocytes and 30.75% Granulocytes. Similar results were also reported by Geilbolu *et al.* (2009) for marine crab, *Callinectes sapidus*, who found different hemocytes to be in the proportion of 55% (semigranulocytes), 31% (Granulocytes) and 14% (Hyalinocytes). Contrarily, however, Matozzo and Marin (2010b) found Hyalinocytes (44%) to outnumber Granulocytes (28%) and Semigranulocytes (27%).

On the basis of above discussion and present observation on hemocytes it can aptly be concluded that *P. masoniana* like other decapods crabs also have three types of hemocytes in their hemolymph viz. Hyalinocytes, Semigranulocytes and Granulocytes and these hemocytes differ from each other not only in their morphological characteristics but also in respect of their relative proportion.

Table: 1. Shows the classification, characteristics features and relative proportion of different hemocytes of crab *P. masoniana*.

S. No.	Types of Hemocytes	Shape	Size (μm)	Granules	Position of nucleus	Nucleus to cytoplasmic ratio (μm)	Relative proportion (in %)
1.	Agranulocytes						
1.a.	Hyalinocytes	Ellipsoidal	$8.3\pm\times 7.6\pm 0.32$	Absent	Centrally located	0.6255 ± 0.02	16
2	Granulocytes						
2.a.	Semi granulocytes	Circular/oval	$12\pm 2.24\times 10.2\pm 0.52$	Sparsely present	Centrally located	0.44 ± 0.06	54

2.b.	Granulocytes	Circular/oval	14.3±1.34×12.6±0.24	Densely packed	Eccentrically located	0.51±0.02	30
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