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Haemocytes classification, total and differential counts in the,Freshwater Crab, *Oziotelphusaravi*

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Abstract

For the first time haematological study has been performed on freshwater*Ozhiotelphusaravi*.In this present investigation the haemocytes have been examined using phase contrast microscope. The purpose of this study was to determine the different types of haemocytes, their total and differential counts as well. Haematological parameters such as THC and DHC were carried out in *O.ravi* used to understand and compare the haemograms with other related decapods crustaceans.Three types of haemocytes were distinguished based on the presence of cytoplasmic granules into Hyalinocytes (H), Semigranulocytes(Sg), Granulocytes(G). All the three haemocytes were varied in their proportion. Semi granulocytes being the highest (51%) in male followed by Granulocytes (41%) in female and Hyalinocytes (29%) in males were found. THC levels did not differ significantly between males and females, although the juveniles showed little variations.

INTRODUCTION

Crustaceans have an open circulatory system, similar to other arthropods, with much haemoglobin circulating freely in the hemolymph.Haemocytes from crustaceans provide vital tasks such wound healing and protection against bacteria, viruses, and parasites (Bauchau, 1981).The most contentious issue in the classification of crustacean haemoglobin has been and continues to be the absence of consistent classification standards that allow for the differentiation of different cell types (Johanssonet al., 2000). The majority of Crustacea species base their classification of haemoglobin on whether or not cytoplasmic granules are present in the cell. According to this standard, three categories of circulating haemoglobin are

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typically identified in Crustacea: granulocytes (which have a large number of cytoplasmic granules) and semigranulocytes (which have tiny granules) (Bauchau, 1981). Hyalinocytes are the tiniest cells without visible granules. Manjula et al., 1997 observed four different types of haemoglobin in the Indian spiny lobster, Panulirushomarus: pro-hyalocytes, hyalocytes, eosinophilic granulocytes, and chromophilic granulocytes. In contrast, Clare and Lumb, 1994 identified three different types of haemoglobin in the blue crab, Callinectes sapidus: hyaline cells, small and large granulocytes. Remarkably, morphological criteria were used to describe eleven different types of haemocytes in the American lobster, H americanus (Battison et al., 2003).Despite being essential to the immunological response of a host, crustacean haemoglobin is not standardised. There is a lack of uniformity in the classification of haemoglobin among various crustaceans. The primary classification of decapod crustacean haemocyte types is based on the presence of cytoplasmic granules in hyaline, semi-granular, or granular cells. Soderhall, Johansson, and Soderhall all gave the same classification to crayfish. Jussila also identified granulocytes, semigranulocytes, and hyalinocytes as the haemoglobin cells present in Panulirus cygnus, the western rock lobster. In contrast, prohyalocytes, hyalocytes, eosinophilic granulocytes, and chromophilic granulocytes were the four types of haemoglobin identified in the Indian spiny lobster, Panulirushomarus. In the blood of the blue crab, Callinectes sapidus, Clare and Lumb found three distinct types of haemoglobin: hyaline cells, tiny granule haemoglobin, and giant granule haemoglobin. Since haemoglobin levels in the blood indicate stress, haemoglobin counts can be an effective indicator of a species' general health. Research on the haemoglobin of crustaceans contributes to the basic understanding of haemoglobin, especially in relation to the animal's physiological state (YILDIZet al., 2002). The role of the cells in the haemolymph was also investigated; upon moulting, hyaline cells start the coagulation of the haemolymph and harden the exoskeleton (Vacca and Fingerman, 1983; Omori et al., 1989). According to Wood and Visentin (1967), Busselen (1970), Stang-Voss (1971), Wood et al. (1971), and Ravindranath (1980), granulocytes have a variety of functions, including phagocytosis, agglutination, coagulation, encapsulation, and storage of hemocyanin and glycoproteins.

The haemocyte categorization and differential counts in the freshwater crab Potamonfluviatilis were reported by Yavuzcan-Yildiz and Atar (2002). Nayan et al. (2010) carried out comparable investigations in Sartorianaspinigera. The freshwater crabs Paratelphus amasoniana and P. hydrodromous were found to have distinct forms of haemoglobin in Gupta et al. (2013) and Arulprakashet al. (2013), respectively. In O. ravi,

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there were no reports available. The current study's objective was to investigate haemoglobin using a phase contrast microscope. The findings of this study will shed light on O. ravi haemoglobin and encourage more research into it.

METHODS AND MATERIALS:

Animal collection

The crabs werecollected from different sites of banana fields in Pottalkulam Village, Kanyakumari District, TamilNadu,India and brought to the laboratory acclimatized and used for the collections of haemolymph.

Haemolymph collection

The haemolymphof *O.ravi*wascollected with syringe either through the arthrodial membrane of leg joint or by cutting the dactyl of the walking leg.The haemolymph was transferred immediately to glass tubes in an ice bath to minimize clotting. The haemolymph was diluted 1:1 (v/v) with saline.

Smear preparation

One ortwodrops of formalin (3.4%) and haemolymph were placed quickly on one end of a cleanslide and a thin film of haemolymph smear was made. The smear was air dried and subjected to staining using 10% Giemsa stain (Matozzo and Marin, 2010).

Total haemocyte counts (THC)

Using an enhanced double Neubauer ruling hemocytometer, the number of free haemoglobin was counted. The crab's haemolymph was gathered on a glass slide and swiftly inserted into an apipette, which is used to count the white blood cells (WBC) in mammals. Each pipette was filled with hemolymph up to the 0.1 mark, and after that, Tuerk's fluid a diluting agent was added and the mixture was agitated for three minutes.Jones et al. (1962)

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used three drops of the mixture to fill counting chambers. The THC / mm3 was calculated using the Kolmer *et al*., 1969 technique.

The formula x/4 x 10 x Y, where x = total no. of haemoglobin counted in 4 chambers in the corner, was used to calculate the THC/mm3.

4 = Number of chambers

10 mm is the chamber's depth.

Y is for Dilution.

Differential haemocyte counts (DHCs)

The technique used to count the various forms of haemoglobin was particularly well-suited to the image of crab haemoglobin, as recommended by Vinson (1971). DHC (number of distinct haemocytes per 100 haemocytes) was calculated by utilising a light microscope (phase contrast microscope) to take 100x magnification pictures of stained blood films.

RESULT:

Three different forms of hemolymph were identified in O. ravihaemolymph. Granular cells were the largest type of hemolymph cell. These cells' cytoplasm is made up of many granular features and tiny cytoplasmic vacuoles. The nucleus of this sort of cell was conflicting (Fig. A).

Semi granular haemocytes were intermediate between hyalinocytes and granulocytes. These are smaller than granular cells and consist of acentric nucleus and fewer granules. (Fig. B).

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The hyaline haemocytes were elliptical in shape with centrally embossed nuclei. The nucleus takes part in roughly three-quarters of the cell body and appeared to be a distinct nuclear membrane. (Fig. C).

This simply shows that semi granulocytes are the mastery haemocytes in haemolymph of *O.ravi.*

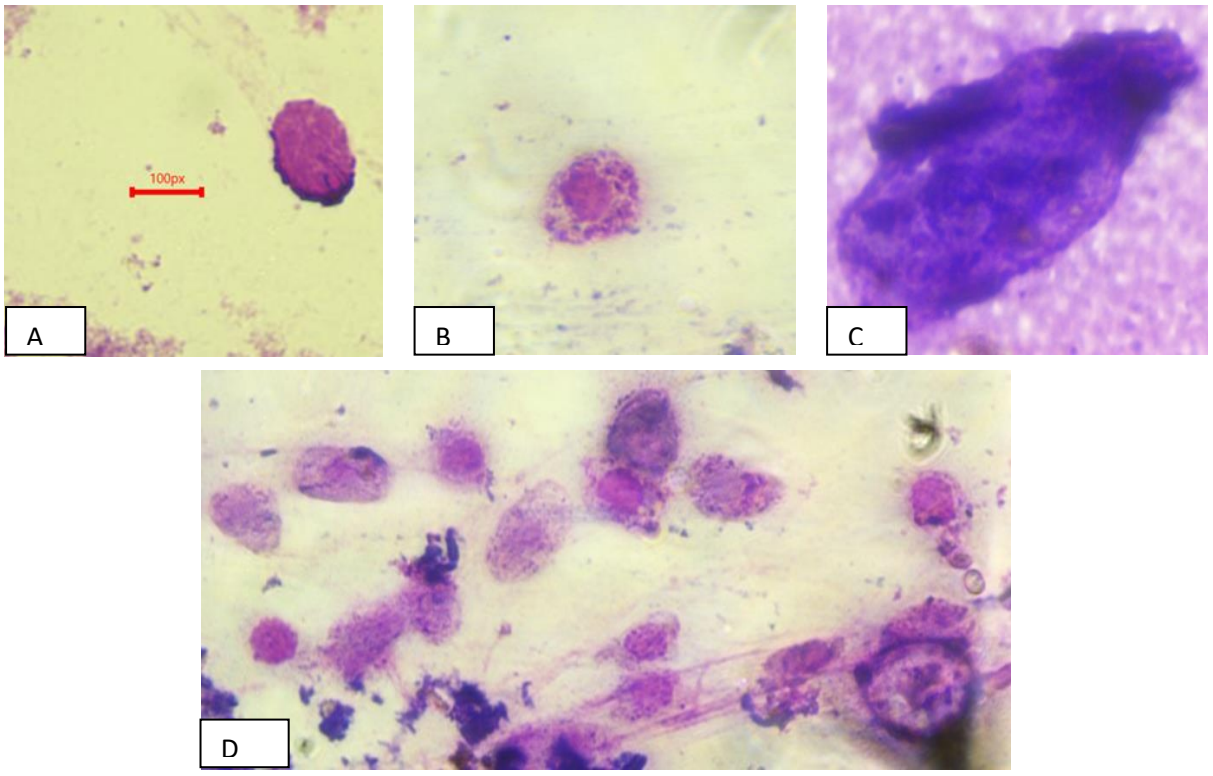

Fig: 1 A- Granulocytes (40x Magnification), B- Semi granulocytes (40x Magnification), C- Hyalinocytes (100X Magnification) D-Different types of cells (40x Magnification).

Table: 1Differential haemolytic counts of freshwater crab *O. ravi* in percentage

Types of cells	Males	Females	Juveniles
Hyalinocytes	29.207±1.85	28.107±1.62	24.07±0.98
Semi granulocytes	51.802±1.37	49.604±1.79	38.09±0.64
Granulocytes	34.621±0.67	41.05±0.29	30.07±0.71

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Among males the most abundant cell wassemi granulocytes as 51.802±1.37%, Hyalinocytes was found to be 29.207±1.85%, whereas granulocytes were found to be 34.621+0.67%. In females' 28.107±1.62% Hyalinocytes, 49.604±1.79%,Semi granulocytes and 41.05±0.29%granulocytes were found. Similarly in juveniles' 24.07±0.98%Hyalinocytes, 38.09±0.64%Semi granulocytes and 30.07±0.71% Granulocytes were found in percentage. (Table.1)

Table: 2Total Heaemocytic counts of *O. ravi*

Sex	THCs (cells/mm^3)
Male	5056±0.563
Female	5043±0.854
Juvinile	4712±0.458

The total haemocytic countsof*O.ravi*was found to be equal in male and female 5056±0.56 (cells/mm^3)and 5043±0.854 (cells/mm3) among females and among juvenile groupsit was found to beless compared to the adults (4718±0.458cells/mm^3).(Table.2).

DISCUSSION:

Based on shape and physiology, three forms of haemoglobin have been identified in O. ravi in this investigation: granulocytes, semigranulocytes, and hyalinocytes. These findings are consistent with earlier classification schemes (Arulprakashet al., 2013). Three forms of haemoglobin are detected for the first time in the haematological studies on the freshwater crab Paratelphus amasonian: granulocytes, semigranulocytes, and halinocytes (Gupta, 2013).According to morphological characteristics (Hen and Lei, 1998) or chemical aspects (Kakoolaki et al., 2010; Matozzo and Marin, 2010), haemoglobin is categorised.In this work,

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the haemocytes of the crab O. ravi were examined for morphological characteristics such as form, granule presence or absence, and nucleus position. According to this, three different forms of haemoglobin were seen: granular, semigranular, and halinocyte cells. This is the first report on O. ravi.

This study indicated that in men, the proportion of hyalinocytes was 29%, whereas granulocytes and assemi granulocytes were found to be 34% and 51%, respectively.In females, the measured values for hyalinocytes were 28°C, assemi granulocytes were 49°C, and granulocytes were 41°C. In females, the measured values for hyalinocytes were 28°C, assemi granulocytes were 49°C, and granulocytes were 41°C. Geilboluet al. (2009) also published similar results for the marine crab Callinectes sapidus, finding that the haemocyte proportions were 55% semi-granulocytes, 31% granulocytes, and 14% hyalinocytes. In contrast, Hyalinocytes (44%) were found to be more numerous than Granulocytes (28%) and Semi-granulocytes (27%), according to Matozzo and Marin (2010). Vargas-Albores et al. (2005) found that SGH was prevalent in penaeid shrimps (51%) and was followed by hyaline cells (29%) and LGH (19%). Tsing et al. (1989) and Gargioni and Barracco (1998) have documented the presence of different haemocyte proportions in penaeids and palaemonids. Semi-granulocytes, which made up 54.25% of all haemoglobin in P. fluviatilis, were the most prevalent cell type; the proportions of granulocytes and halinocytes were, respectively, 15 and 30.75% (Yavuzcan-Yildiz and Atar, 2002). Based on the preceding discourse and current observations regarding haemoglobin, it can be deduced that O. ravi, akin to other decapod crabs, possesses three distinct types of haemoglobin in their hemolymph: granulocytes, semi-granulocytes, and halinocytes. These haemoglobin subtypes vary from one another not only in terms of their relative proportion but also in their morphological attributes. The total haemoglobin counts determined in this investigation may yield valuable data for subsequent research.

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FINAL VERDICTAccording to haematological research done on O. ravi, the hemolymph contains three different forms of haemoglobin. Phase contrast microscopy morphological analyses allowed for the identification of granulocytes, semi-granulocytes, and hyalinocytes. The current investigation has made it possible to recognise and describe the distinct haemocyte types in O. ravi, which may also be typical of other decapod crustaceans. Thus, the current study has contributed to the development of a consistent classification system for haemoglobin. Additional hemogram research has shown that the THC and DHC values are consistent with those seen in other decapod crustaceans and can be a useful tool in determining the animal's overall health. Information on O. ravi haemoglobin will facilitate future research on the physiological and functional characteristics of freshwater crab haemoglobin.

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