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Haemocyte Classification and Differential Counts in the Freshwater Crab, *Potamon fluviatilis*

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Abstract: Three haemocyte groups were identified in the freshwater crab, *Potamon fluviatilis*: hyalinocytes, semigranulocytes and granulocytes. The proportion of hyalinocytes was found to be 15%, that of semigranulocytes 54.25% and that of 30.75% granulocytes.

Key Words: Freshwater crab, *Potamon fluviatilis*, haemocyte, hyalinocytes, semigranulocytes and granulocytes

Tatlısu Yengesinde, *Potamon fluviatilis* Hemosit Sınıflandırılması ve Diferansiyel Sayıları

Özet: Tatlısu yengeci, *Potamon fluviatilis* hemolymphinde üç hemosit grubu tanımlendi: hyalinosit, semigranulosit ve granulosit. Hyalinosit oranı% 15, semigranulosit oranı% 54.25 ve granulosit oranı% 30.75 olarak bulundu.

Anahtar Sözcükler: Tatlısu yengeci, *Potamon fluviatilis*, hemosit, hyalinosit, semigranulosit and granulosit

Introduction

Crustacean haemocytes play important roles in a host's immune response; however, there is no a uniform classification scheme for crustacean haemocytes (1).

Haemocyte classification in various crustaceans lacks consistency. Classification of the haemocyte types in decapod crustaceans is based mainly on the presence of cytoplasmic granules in hyaline cells, semigranular cells and granular cells (1-3). The same classification was given by Söderhall et al. (4) and Johansson and Söderhall (5) for crayfish. Similarly, Jussila et al. (6) identified haemocytes in western rock lobsters, *Panulirus cygnus*, as hyalinocytes, semigranulocytes and granulocytes.

In contrast, in the Indian spiny lobster, *Panulirus homarus*, haemocytes were classified into four types – prohyalocytes, hyalocytes, eosinophilic granulocytes and chromophilic granulocytes (7). In the circulation of the blue crab, *Callinectes sapidus*, three types of haemocytes—hyaline cells, small granule and large granule haemocytes— were identified by Clare and Lumb (8).

The circulating haemocyte number is a stress indicator (9) and haemocyte counts may be a valuable tool in monitoring the health status of crustacean species (6,10). 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 crab, *Potamon fluviatilis*.

The primary purposes of this research were to describe and classify the circulating haemocytes as well as establish a basic profile for total and differential counts of haemocytes in the freshwater crab, *P. fluviatilis*.

Materials and Methods

Crabs

Specimens of the freshwater crab (N=40), *Potamon fluviatilis*, in the intermoult stage of the moulting cycle, were collected from the Sakarya River, Central Anatolia. The carapace length was between 3.9 and 4.3 cm. All the freshwater crabs were female.

The crabs were kept in four 80-l aquaria for four days following collection from the river. The water temperature was $26 \pm 2^\circ\text{C}$. The dissolved oxygen varied between 5.4 and 5.7 mg/l. The aquaria were under continuous aeration. pH was 6.8 ± 0.5 . Freshwater crabs were not fed throughout the study.

Haemolymph collection

Haemolymph was collected by cardiac puncture using anticoagulant (2).

Haemocyte classification

P. fluviatilis haemocytes were classified using the simple criteria, the presence or absence of cytoplasmic granules, stated by Bachau (11), Mix and Sparks (10), and Johansson et al. (1).

Total and differential counts of haemocytes

Total and differential counts of haemocytes were performed according to Persson et al. (2,12).

Statistical analysis

The mean values of total haemocyte counts were calculated using a computer analysis program (Excel 7.0 for windows).

Results

The haemocytes in *P. fluviatilis* haemolymph were classified into hyalinocytes, semigranulocytes and granulocytes. Hyalinocytes were devoid of granules or sometimes contained only a small number of tiny intracellular inclusions (Fig. 1). The semigranular cells enclosed fewer smaller and generally less refractive granules (Fig. 2). The granular cells were filled with numerous large, highly refractive granules (Fig. 3).

The mean total haemocyte count was $10.53 \pm 11.10 \times 10^5$ /ml. The range of total haemocyte counts was between 3.3 and 13.9 ($\times 10^5$ /ml). The most abundant type was semigranulocytes, constituting 54.25% of total haemocytes. The percentages of hyalinocytes and granulocytes were 15% and 30.75% respectively.

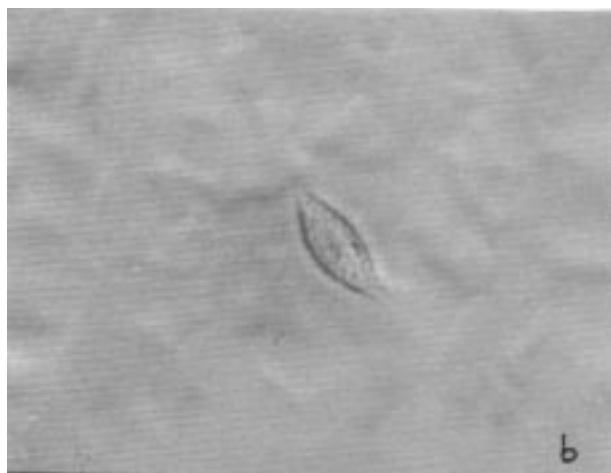
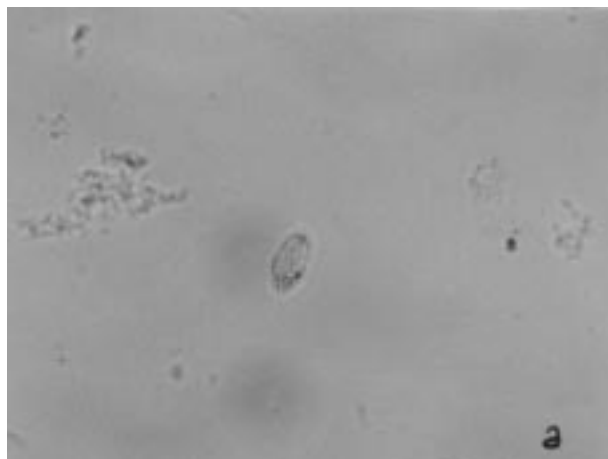
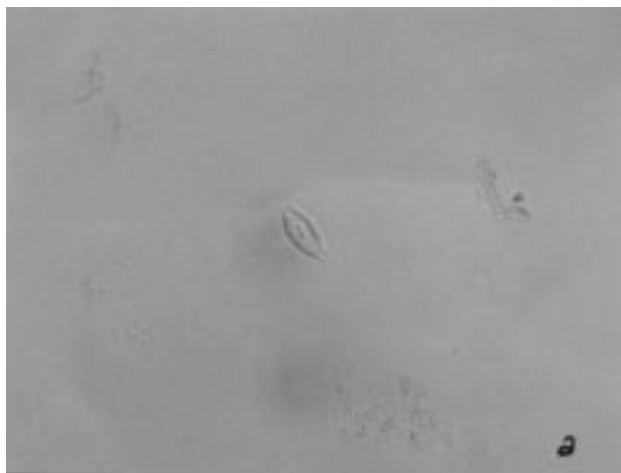


Fig. 1. Hyalinocyte in the haemolymph of *P. fluviatilis*.
a: x 400
b: 200% magnified by photocopying

Fig. 2. Semigranulocyte in the haemolymph of *P. fluviatilis*.
a: x 400
b: 200% magnified by photocopying

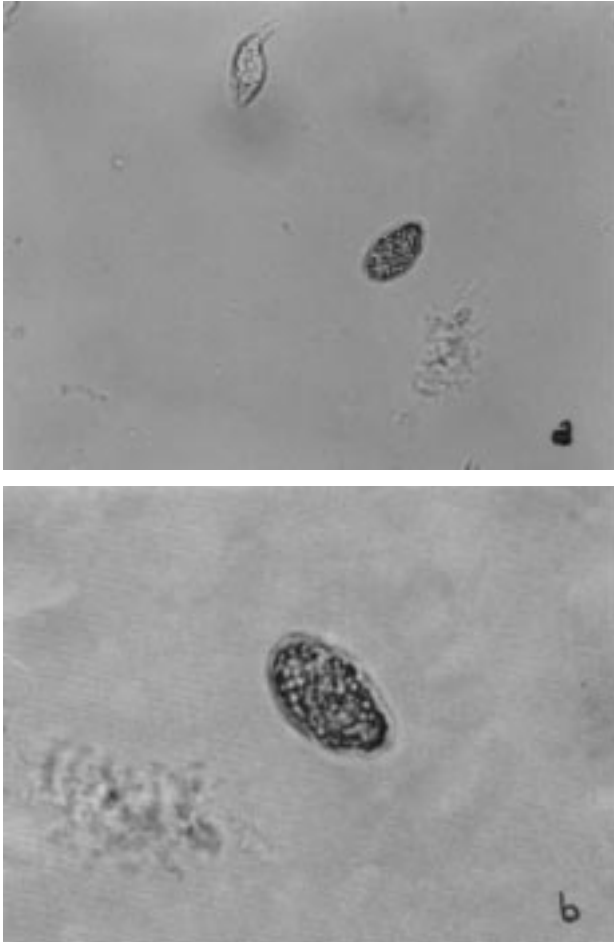


Fig. 3. Granulocyte in the haemolymph of *P. fluviatilis*.
a: x 400
b: 200% magnified by photocopying

Discussion

Three types of haemocytes (hyaline, semigranular and granular) were identified in the freshwater crab, *P. fluviatilis*, in this study. The types of freshwater crab haemocytes (hyalinocyte, semigranulocyte and granulocyte), classified according to cytoplasmic granules are in agreement with some previous reports. In crustaceans there are generally three morphologically distinct cell types –hyaline, semigranular and granular cells (3,5,11). Similarly, in the western rock lobster (6) and the shrimp (*Penaeus styliostris*) (13) haemolymph, three major haemocyte groups (hyalinocytes, semigranulocytes and granulocytes) were identified. However, this classification does not comply with the classification of Manjula et al. (7) for the Indian spiny lobster. In addition, Clare and Lumb (8) named hyaline cells, small granule and large granule haemocytes for the blue crab, *C. sapidus*.

There is no report concerning total and differential haemocyte counts of *P. fluviatilis*. Therefore, the values of total and differential haemocyte counts measured in this study may provide a useful source for further studies. Nevertheless, total and differential haemocyte counts could change depending on factors such as sex, water temperature, moulting cycle and starvation, as stated by Johansson et al. (1), Manjula et al. (7) and Le Moullac and Haffner (9).

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