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Hematological studies of the freshwater Zooplankton *Macrocyclops edax*

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ABSTRACT

According to the literature review, this is one of the pioneer works in the observation of hemocytes in the cylopoid copepods. The homogenization and centrifugation methods were used to isolate the hemolymph of the zooplanktons. Using eosin stain, hemocytes were stained and identified under a trinocular light microscope. The presence and absence of cytoplasmic granules in the hemocyte cells were used to classify the hemocytes identified. Granulocytes, semi-granulocytes, and hyalionocytes were identified as three forms hemocytes. In a Neubauer counting apparatus, total and differential counts of hemocytes were performed. The results were recorded as 80,000 cells per µl of hemolymph and 45% hyalinocytes, 39% Granulocytes and 16% semi granulocytes.

Key words: Zooplankton, Copepods, hemocytes, Granulocytes, semi-granulocytes, hyalionocytes, *Macrocyclops edax*

INTRODUCTION

Aquatic animals are surrounded by microorganisms in the aquatic environment that are capable of initiating infection in these animals. Unlike fish that possess both innate and adaptive immunity, the lower aquatic animals especially invertebrates lack true adaptive immune systems and they depend upon their innate

immune system for the defence mechanism against the invading pathogen. In normal conditions, crustaceans maintain a healthy state by mounting a defence reaction against potential pathogens.

Crustacean hemocytes (blood cells) play a central role in their immune reactions and are capable of phagocytosis, encapsulation, nodule formation, and mediation of cytotoxicity. When the host defence encounters a pathogen, a series of pathways get activated to protect the crustacean against infection by a variety of microorganisms. The entry of a pathogen into the hemocoel of the host triggers a complex system of innate defence mechanisms involving cellular and humoral immune components.

Three types of circulating hemocytes have been reported in various crustaceans, they are hyaline (H) cells, semigranular (SG) cells and granular (G) cells (Rowley, 2016) Among microcrustaceans a handful of studies are available for the hemocyte mediated immune mechanisms in Daphnia (Shan Liu, 2020). Praveena and Venkatalakshmi (2019) have reported isolation and identification of hemocytes in the cyclopoid copepod. A premilinary method of isolation of hemolymph and hemocytes have been reported. The scarcity in hematological studies in copepods leads to the present investigation.

Copepods have a vital role in ecosystems because of their position as major and secondary consumers in food webs, as well as their ability to be exploited by humans in a variety of ways (Dussart and Defaye, 2001). Microcyclops, Megacyclops, and Mesocyclops can be employed as biological agents in mosquito control (Marten and Reid, 2007), Copepods are used in monitoring research in the fields of functional genetics and transcriptomics, as well as in the lab. In limnological investigations, the calanoid/cyclopoid-cladoceran ratio is utilized as a water quality indicator. The present work has been undertaken to identify the dominant copepod of the Pillaiyarkoil pond, vanniyadi, Papanasam and to study the hematology of the same.

MATERIALS AND METHODS

Plankton collection;

Zooplankton species were collected by Towing method using Henson's standard plankton net (150 µm) in zigzag, horizontally at depth of 50 to 100 cm 10 minutes with a uniform speed, in the freshwater pond (Pillaiyar pond) located in vanniyadi, Thanjavur disdrict, Tamil nadu. From the Zooplankton collected, cyclopoid copepods were isolated and cultured separately.

Isolation of Hemolymph, plasma, and Hemocytes

The methodology was followed as per Praveena and Venkatalakshmi (2019) with few modifications. 50 mature adults were separated under a hand lens. lens. Using a mortor and pestle the planktons were gently crushed using a physiological saline solution. The homogenate was then centrifuged at 3000 rpm for 10min. the tissue debris was discarded. The supernatant was supposed to be the hemolymph. To centrifuge 6000 rpm for 10 min this resulted in the separation of plasma and packed cells out of the hemolymph. The plasma was separated in an Eppendorf tube using a micropipette. The packed hemocytes were re-suspended in phosphate buffer saline for further identification.

Microscopic Examination of Hemocytes

Freshly packed hemocytes were smeared and stained with eosin and examined under light microscopy (LM) to determine the cell size, cell shape, and presence of cytoplasmic granules under a magnification of 100X. total and differential count of hemocytes were carried out in Neubauer counting chamber.

RESULTS

i. Plankton Identification

Plate 1 shows the microscopic photograph of the dominant copepod which was identified as *Macrocyclops edax.*

These cyclopoids have 5th pair of legs, which are much smaller than legs 1-4 and have a different shape. The *Macrocyclops*, 1st antenna of 17 articles; body large to 3 mm and distal segment of leg 5 bearing 3 processes. The *edex*, first antenna usually much shorter than body; anterior part of body much broader than posterior part; females with two lateral egg sacs.

ii. **Total count of hemocytes**

The total hemocyte count of *Macrocyclops edax* has been recorded as 80,000/µl. of hemolymph

iii. Differential count of hemocytes

Three types of hemocytes have been identified viz., Hyalinocytes (H), Semigranulocytes (SG), and Granulocytes (G). The different morphological characteristics feature taken into account for the identification of hemocytes are (i) Size (ii) Shape (iii) presence or absence of cytoplasmic granules and (iv) position of the nucleus.

Hyalinocytes:

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. These cells did not reveal any type of granules in their cytoplasm.

Semigranulocytes:

Semigranulocytes have been observed to be circular/ovoid and their cytoplasm exhibited purple colour. These hemocytes to had a centrally placed dark blue nucleus. These cells were observed to contain sparsely placed blue coloured cytoplasmic granules.

Granulocytes:

Granulocytes are the hemocytes that have comparatively large size, circular in shape with purple stained cytoplasm. These characteristically had an eccentrically placed nucleus. These cells were densely packed with blue-colored cytoplasmic granules. Fig 1 shows the differential count of the hemocytes observes and recorded.

DISCUSSION

The most important requirement of the aquaculture practice is the production of appropriate, nutritional, balanced, unpolluting, economically viable, and readily acceptable feed in order to realize optimum growth and survival of the cultivable stock. Live food organisms are preferred by most cultured fish larvae compared to artificial supplementary feed. The zooplanktonic population forms the important major live feed organism in the natural environment. Plankton is a highly valuable food and plays an important role in the purification of polluted waters. Zooplankton plays a pivotal role in sustaining, especially, young fishes, it also forms much of the food for planktivorous fishes and also supplements the food of other fishes. Its importance to practical aquaculture cannot be overstated (Alekseev, 2002).

Copepods, a micro crustacean group, and hosts of several species of parasites; the most important being the microsporidian, nematodes, and cestodes. But surprisingly little is known about infection processes and potential anti-parasite defense of copepods. copepods are the intermediate hosts for *Dracunculus medinesis*, a human parasite; *Auguillicola crassus* and *Camallanus sp*., the fish-parasitic nemetodes (Livensen and Jokabcen, 2002) and also they act as 'transport hosts' from nemetodes to nematotes Joachin Kurtz., 2007 experimentally showed that there is specificity and memory in the immune mechanism of copeode*Macrocyphinus albidus* against nematode parasite in terms of reduction in re-infection for sibling parasites compared to prior exposure to unrelated species. Little et al., 2003 also showed that there might be the maternal transfer of specific protection. It was suggested that through copepods slowly relying on innate immunity, there should be an alternative mechanism that might establish specific recognition within the innate immune system.

Franz and Kurfz (2002) suggested that tapeworms directly manipulate copepod behaviour. Hoffmann and Reichhart (2002) studied Drosophila's innate immunity. Pasternate et al., (1995) explained the changes in metabolism and behaviour of the freshwater copepod Cyclops, *Strenusalys souram* infected with *Diphyllo bothrium spp*. However, the knowledge of the immune system in these microcrustaceans is rare. Change-Bum Jeong et al., (2015) showed the increase in transcriptional levels of dorsal and dorsal-like genes upon exposure to immune modulators in the cyclopoid copepod *Paracyclo pinaniana*. However, the observation of hemocytes in the freshwater copepod *Mesocyclops leuckarti* was first reported by Praveena and Venkatalakshmi (2019). Following the works of the same, the present investigation was carried out in *Macrocyclops edax* similar results were obtained. In addition, the differential counts of hemocytes were also analyzed.

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Plate 1. Light microscopic picture of *Macrocyclops edax*

Plate 2. Cyclopoid copepoid haemocytes stained with Eosin (1drop) observed in g; granulocytes; sg; semigranulocytes; h; hyalionocytes.

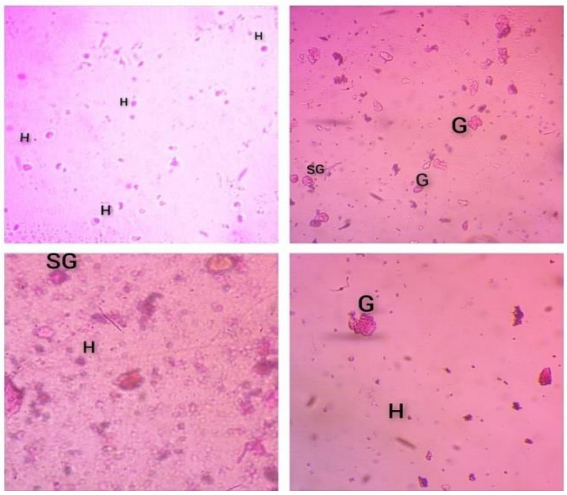

Fig 1. Differential count of Hemocytes