Experimental observation

Various workers have reported morphological, functional and ontogenetic variants among decapod hemocyte types. Integrating all the above criteria, the hemocytes of *P. hydrodromous* could be conveniently categorised into four general types namely prohemocytes, hyalinocytes, intermediate granulocytes and eosinophilic granulocytes.

Hemopoietic organs of *P. hydrodromous* were defined histologically, which suggested their presence in the wall of the foregut as epigastric hemopoietic nodules. Total hemocyte count ranged from 4050 to 7100 cells/mm$^3$ and DHC showed prohemocytes (embryonic hemocytes) $1.5 \pm 0.69\%$, hyalinocytes $12.4 \pm 6.56\%$, intermediate granulocytes $48.85 \pm 6.21\%$ and eosinophilic granulocytes $37.75 \pm 5.13\%$. Hence, it was observed that granulocytes were the most dominant group among hemocytes.

Hemolymph biochemistry revealed a composition of organic and inorganic constituents. The range of total free sugars, proteins and total lipids were 32.4 to 46.4 mg%, 4.0 to 6.4 g% and 14.4 to 44.2 mg% respectively. Hemolymph showed a higher calcium index and smaller magnesium index. Na$^+$ ions in the hemolymph were very high $(245.37 \pm 15.37 \text{ m mol / l})$ and K$^+$ ions were low $(7.69 \pm 0.11 \text{ m mol / l})$. Copper in ionic state was very low $(2.17 \pm 0.8 \text{ m mol/l})$.

Densitometric analysis of electrophoretic pattern of the hemolymph proteins revealed a female limited protein. Much difference in the protein fractions could not be derived based on the size of the animal. However, densitometric analysis indicated quantitative changes in the protein fractions.

Hemogram numerations indicated that hemocytes were immuno-reactive and fluctuated based on the physiological state of the animal. Changes due to endogenous and exogenenous factors predisposed the functional integrity of the hemocytes.
Hemogram assay developed for urea and endosulfan toxicity manifested high counts in the circulating hemocyte population. Significant reduction in the percentage of hyalinocytes and intermediate granulocytes was observed. Remarkable increase in the percentage of eosinophilic granulocytes was also encountered.

Hemolymph composition assay indicated an increase in sugar level and decrease in the protein level.

Hemocyte lethality assay included gross morphological and cytological changes such as change in size of the hemocytes, presence of phagosomes in the cytoplasm, degranulation, coagulative necrosis and formation of pseudopods.

Immunotoxic response in various tissues included hemocytic infiltration, atypical amoeboïd motion, extracellular hemocyte clumping, melanocytic nodule formation and hemocytic neoplasia.

The cellular internal defence mechanism was clearly manifested by the granulocytes, which responded remarkably during experimental bacterial challenge. Gram negative bacteria V. alginolyticus and A. hydrophila employed in the challenge imparted damage to hemocytes and tissues. Both hyalinocytes and granulocytes overcame bacterial challenge by their immunomodulatory behaviour. Phagocytosis, encapsulation and formation of extra cellular clumps were common functions observed. However in severe infection the cellular defence mechanism was under threat, leading to extensive degranulation, phagosome formation and hemocytolysis.

**Conclusion**

Thus this work provides a potential model to understand and enrich information regarding crustacean hematology. Further this study lays the groundwork for better monitoring of the physiological state of the animal through the hemogram assay, hemocyte lethality assay and hemocyte behavioural assay. Moreover, the immunomodulatory behaviour of the hemocytes in the internal defense of the
organism and the mechanism underlying in the maintenance of hematological equilibrium (hemostasis) is understood.

Evidence from the present work substantiates the immunocompetant nature of granulocytes, which includes features such as their immunological responsiveness, great potential for phagocytic activity, infiltrate injured tissues early in an inflammatory response, localised increase in the number of granulocytes in an inflammatory site and prominence of granulocytes in encapsulation and healing responses.

The incidence of necrotic gill disease (NGD) with crabs exposed to endosulfan and gill rot disease (GRD) in the crabs challenged with *A. hydrophila* are promising bio-indicators in the health monitoring of decapods in a degrading environment.

**Recommendations**

1. Further studies by investigators utilising other decapods are necessary to test the usefulness of this classification scheme and to offer improvements by developing more specific criteria.

2. Cytochemical tests of decapod hemocytes are inadequate for proper differentiation of the various kinds of granular inclusions occurring in the cytoplasm.

3. More information is needed on the cellular immune reactions of crustaceans against viral and fungal disease organisms.

4. We are only in the early stages of knowing how different hemolymph cells communicate amongst themselves during defense reactions. More detailed studies are needed to unravel the molecular and biochemical processes involved in cell-to-cell communication and humoral mediated immunity.