

On haematological characteristics of blood clam, *Tegillarca rhombea* (Born, 1778)

Abstract

The blood clam *Tegillarca (Anadara) rhombea* is one of the important bivalves that show the presence of haemoglobin in its blood as against haemocyanin that is present in other bivalves. The haematological characteristics of this clam found along the south west coast of Maharashtra, India were studied from February 2014 to March 2015, excluding June-July 2015 due to heavy monsoon, in randomly selected specimens. The blood showed the presence of red blood corpuscles with round nucleus, white blood corpuscles with kidney bean shaped nucleus and non-nucleated platelets. The average count of RBCs was 1.76×10^9 per ml, WBCs 4.87×10^6 per ml. and platelets were 66×10^8 per μl . Haemoglobin (Hb) was found in the range of 2.03 to 8.87 g dL^{-1} and seen to be increasing with size. The general trend in the relationship between blood parameters and body size is that the bigger the animal, the higher the values of its haematological parameters.

Keywords: *Tegillarca (Anadara) rhombea*, Haemoglobin, Clams, RBCs, WBCs, Platelets, Haematological Characteristics, Blood Clam, Lymphocytes, Monocytes, Nucleus, Cytoplasmic fragments, Coagulation, Blood cells

Volume 3 Issue 2 - 2015

Meshram AM,² Swapnaja Mohite¹

¹Associate Professor, Department of Fisheries Biology, College of Fisheries Biology, India

²Research Scholar, Department of Fisheries Biology, College of Fisheries Biology, India

Correspondence: Swapnaja Mohite, Associate Professor, Department of Fisheries Biology, College of Fisheries Biology, Ratnagiri, Maharashtra, India, Tel +919545030642, Email sa_mohite@yahoo.co.in

Received: October 01, 2015 | **Published:** November 30, 2015

Abbreviations: Hb, Haemoglobin; WG, Wright-Giemsa; PCV, Packed Cell Volume; Rbcs, Red Blood Cells; WBC; White Blood Cells; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Haemoglobin; OCC, Oxygen Carrying Capacity

Introduction

Many species of clams occur abundantly along Indian coast, particularly in the estuaries and backwaters, forming sustenance fisheries. The clams are rich in protein, glycogen and minerals which are easily digestible. Being lower in food chain, they are admirably suited for 'on-bottom' farming. *Tegillarca (Anadara) rhombea* belongs to the family Arcidae and it is popularly known as blood clam owing to the red colour of its flesh, which is due to the presence of hemoglobin in the blood.^{1,2} Due to the realization about the high nutritive value of clams and their importance in the economy of the coastal fishing villages coupled with the development of an export market for the frozen clam meat, stimulated research which resulted in a wealth of information on this important group during the last decade.³⁻⁶ In India, species such as *A. granosa* and *T. (A.) rhombea* are fished along with other bivalves and gastropods.⁷ Rich beds of *T. rhombea* are found along the south west coast of Maharashtra, India. Current work was done to study the haematologica characteristics of this clam.

Materials and methods

The study was conducted from February 2014 to March 2015. Weekly random samples ranging between 21 to 68 mm were collected by hand dredge net as well as by hand picking. Monthly 100 blood clam specimens were collected during the study period. Standard haematological procedures described by Brown⁸ were employed in the assessment of the various blood parameters. Blood samples were collected from 10 clams per month. The blood counts included red blood cells, white blood cells, platelets, haemoglobin and packed cell volume (%). Smear blood samples of this clam were stained in Wright-Giemsa (WG) solution and studied under the light microscope. Haemoglobin (Hb) was determined by the cyano methaemoglobin method, Packed Cell Volume (PCV) to check the percentage of RBCs

in the whole blood by microhaematocrit method. The capillary tubes were filled (two third by volume) by the clam blood and one end of the tube was sealed. The tubes were placed in the microhaematocrit centrifuge and centrifuged at 10,000 to 12,000 rpm for 5 minutes. The readings were taken by placing the tubes in the microhaematocrit reader. Oxygen carrying capacity of the clam blood was calculated by multiplying the haemoglobin content by 1.25, oxygen combining power of Hb/g.⁹

Results

Only a few species of bivalve mollusc have been reported to contain haemoglobin in their blood cells. *T. (A.) rhombea* is one such clam which shows the presence of haemoglobin. Red blood cell of the *T. (A.) rhombea* was nucleated and ovoid (8.12 - 12.9 x 12.5 - 15 μm). The nucleus was round and basophilic (WG) with a few dark brown structures dispersed in the pink cytoplasm (Table 1) (Plate 1 & 2). The average count of red blood cells was 1.76×10^9 per ml. In White blood cells, granulocytes (Neutrophils 60% of the WBCs) and agranulocytes (Lymphocytes (36% of WBCs) and Monocytes (4% of WBCs) were observed. The average white blood cell count was 4.87×10^6 per ml. Non-nucleated platelets about 2 μm in diameter, were also seen as small cytoplasmic fragments within the blood. In a blood smear, the platelets were seen as individual units but more often they adhere to each other, forming small clusters. The mean values of haematological profiles recorded were, haemoglobin (Hb) $4.67 \pm 1.56 \text{ g dl}^{-1}$ and Packed Cell Volume (PCV) $12.34 \pm 6.79 \%$. Oxygen carrying capacity was calculated as $6.425 \pm 2.48 \text{ vol. } \%$.

The blood clams used for the haematological profile were classified into five size classes using their length as follows: group 1 (20-30 mm), group 2 (31 to 40 mm), group 3 (41 to 50 mm), group 4 (51 to 60 mm) and group 5 (61 mm and above). The haematological profiles of *T. (A.) rhombea* in the various size groups (Table 2) indicated that in most of the parameters the values tended to increase with size. The haemoglobin count was lowest in Group 1 ($2.03 - 2.10 \text{ g dL}^{-1}$) and the highest was noted in Group 5 ($6.65 - 8.87 \text{ g dL}^{-1}$). The red blood cells count was 1×10^9 for the Group 1 and it was seen to be the highest

(2.24×10^9) for the Group 5. Similar trends were also observed for white blood cells, platelets and the packed cell volume (%). During blood sampling the bigger size appeared to have more quantity of

blood than the smaller ones, based on the ease of collection with the syringe.

Table 1 Blood components of blood clam *T. (A.) rhombea*

SL. No	Blood parameters	Average number or percentage per ml
1	RBC	1.76×10^9
2	WBC	4.87×10^6
WBC Differential Count		
Granulocytes		
	i) Neutrophils	60% of total WBCs
	ii) Eosinophil	0
	iii) Basophil	0
Agranulocytes		
	i) Lymphocytes	36% of total WBCs
	ii) Monocytes	4% of total WBCs
	Platelets	66×10^8 (per μ l)

Table 2 Variations in hematological parameters in *T. (A.) rhombea* according to clam size

Parameters	Group 1 (20 – 30 mm)	Group 2 (31 to 40 mm)	Group 3 (41 to 50 mm)	Group 4 (51 to 60 mm)	Group 5 (61 mm and above)
Hb (gdL ⁻¹)	2.03 - 2.10	2.08-3.9	3.78 - 5.84	5.65 - 7.08	6.65 - 8.87
RBC (per ml)	1×10^9	1.24×10^9	1.56×10^9	1.78×10^9	2.24×10^9
WBC (per ml)	2.87×10^6	3.34×10^6	3.78×10^6	4.56×10^6	4.90×10^6
Platelets (per μ l)	27×10^8	36×10^8	43×10^8	52×10^8	60×10^8
PCV (%)	5.03 ± 5.79 %	7.05 ± 6.45	11.2 ± 6.87	17 ± 6.45	22 ± 6.89

Hb:Haemoglobin (gperdeciliter);RBC:RedBloodCells (Cells $\times 10^9$ ml⁻¹);WBC:WhiteBloodcells(cells $\times 10^6$ cellsml⁻¹);PLT:Platelets ($10^8\mu$ l⁻¹);PCV:PackedCellVolume (%)
*Blood samples studied per group - 10.

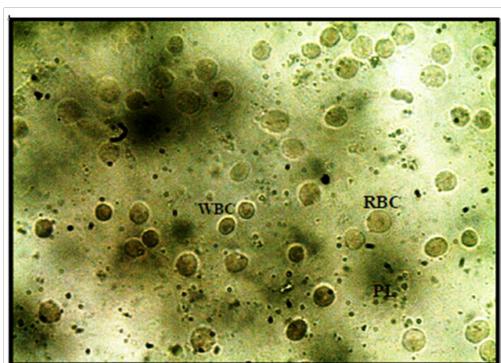


Plate 1 Blood cells in *T. (A.) rhombea* blood

RBC: Red Blood Corpuscles; WBC: White Blood Corpuscles; PL: Platelets (Magnification 40X)

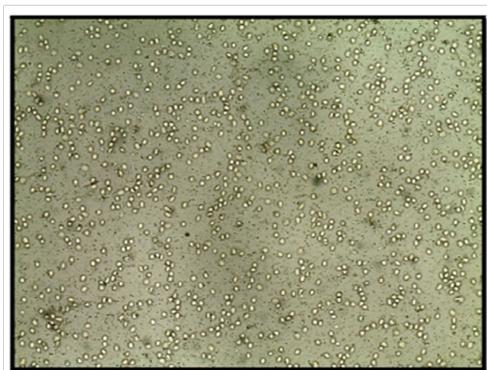


Plate 2 Platelets observed in *T. (A.) rhombea* blood (Magnification 40X).

Discussion

In the Arcidae, the presence of the hemoglobin pigment of vertebrates is most interesting. It is found simply dissolved (not in corpuscles) in both the blood and tissues and colors the muscle red.¹⁰ This is not typical of bivalves, which generally absorb oxygen from water directly into the tissues, without the aid of oxygen transport pigments like hemoglobin (or hemocyanin, which is found more typically in some gastropods). Where the water is very muddy, oxygen may be deficient, in which case any of these pigments facilitate oxygen transport into the tissue.

Nucleated and ovoid Red blood corpuscles or erythrocytes were observed in *T. (A.) rhombea* during the present study. Afati.¹¹ reported that the RBCs of *A. granosa* contained a nucleus but seemed to have no cytoplasmic granules which have postulated to provide oxygen during periods of desiccation. During the current study, it was seen that the RBCs of *T. (A.) rhombea* were nucleated. RBCs act as significant oxygen storage during periods of hypoxia.

The white blood corpuscles showed kidney bean shaped nucleus, which are much less common than the RBCs. Among granulocytes, phagocytic neutrophils that are capable of destroying damaged tissue and bacteria were also seen. Agranulocytes included lymphocytes, thesecond most common WBCs that are capable of producing antibodies were also found in *T. (A.) rhombea*. Monocytes, the third most common WBC type were also present in this clam. These cells phagocytose dead cells and bacteria. These are important in the inflammatory response of the body. These findings need further study as the information might be useful in using *T. (A.) rhombea* as a candidate species for blood related research for medical purposes. The average count of RBCs was 1.76×10^9 per ml, WBCs 4.87×10^6 per ml and platelets were 66×10^8 per μ l. Haemoglobin (Hb) was found

in the range of 2.03 to 8.87 gdl⁻¹ and seen to be increasing with size. Similar findings were reported by Gabriel et al.²

During the present study, non-nucleated platelets were found in the blood of *T. (A.) rhombea*. Platelets are small cytoplasmic fragments within the circulating blood and are reported as the main source in haemostasis.¹² These cytoplasm fragments do not contain nucleus.¹³ Platelets help in blood clotting. If platelet concentration is decreased, the risk of hemorrhage is increased.¹⁴ Blood elements are involved in the building of organisms and are necessary for its proper functioning. They play important role in metabolism, act as biocatalysts for enzymes, hormones, proteins, bone and blood formation etc.¹⁵

Kanchanapangka et al.¹ studied the blood smears of *A. granosa* stained in Wright-Giemsa (WG) solution and studied under the light microscope. They reported that the cockle red cell was nucleated and ovoid (8.8-12.5 x 12.5-15 µm). The nucleus was round and basophilic (WG) with a few dark brown structures disperse in the pink cytoplasm. These structures appeared as dense granular membrane bound inclusions which resembled cytolysosomes and lipofuscin granules under the transmission electronmicroscope.

Gabriel et al.² studied the haematological characteristics of *A. senilis*. The mean values of haematological profiles recorded were (mean ±S.D), haemoglobin (Hb) 4.08 ±1.88g dl⁻¹; Packed Cell Volume (PCV) 10.98±6.79 %; Red Blood Cells (RBC) 1.97 ±0.68 x 10¹² cells l⁻¹; White Blood Cells (WBC) 3.76 ±1.51 x 10⁹ cells L⁻¹ Platelets (PLT) 75.36 ± 88.36%; Mean Corpuscular Haemoglobin (MCH) 20.06 ± 3.98pg; Mean Corpuscular Volume (MCV) 50.64 ±19.30fl; Mean Corpuscular Haemoglobin (MCHC) 43.79 ±13.71 gdl⁻¹; Oxygen Carrying Capacity (OCC) 6.11 ± 2.83 vol. %. The highest range of the parameters was recorded in platelets, while the lowest was observed in RBC. During the current study, haemoglobin (Hb) was found to be 4.67 ± 1.56 g dl⁻¹ and Packed Cell Volume (PCV) 12.34 ± 6.79 %, which were higher than the parameters reported in *A. senilis*. This can be attributed to the larger size of *T. (A.) rhombea*. Average count of red blood cells was 1.76 X 10⁹ per ml, whereas the average white blood cell count was 4.87 X 10⁶ per ml. The oxygen carrying capacity can be correlated to the red blood cells. Neutrophils are phagocytic and help to destroy damaged tissue and bacteria. They self destruct after one burst of activity. Lymphocytes are the second most common white blood cells and they play a role in the immune system of the blood clam. They help in phagocytosis of dead cells and bacteria. The platelets were found in high number in *T. (A.) rhombea*. In vertebrate animals, they are produced in the bone marrow, the same as the red cells and most of the white blood cells. These light and smallest cells are the main defense against any injury to the epithelial layers of the body providing the initial seal to prevent bleeding by forming of a hemostatic plug. They can activate coagulation mechanisms through the exposure of an adequate phospholipidic surface, acting as a catalytic site for the development of coagulation and the consolidation of the hemostatic plug.¹⁶ The role of platelets in *T. (A.) rhombea* needs to be researched further. The general trend in the relationship between blood parameters and body size is that the bigger the animal, the higher the values of its haematological parameters. Gabriel et al.² reported that the higher Hb value was an indication that these bivalves required high concentration of oxygen as a result their burrowing activities.

They also reported that that the size of these bivalves had an influence on their haematological parameters. According to Babatunde et al.¹⁷ any changes in the constituent component of blood sample when compared to the blood profile could be used to interpret the metabolic and health status of the animal. Gabriel et

al.² suggested that the correlation coefficient among haematological parameters and size of *A. senilis*, indicated that size have influence on its haematological parameters. This was attributed to different rates of bivalve activity that demanded different levels of metabolic activity and these activities required several physiological adjustments which involved haematological parameters.

Conclusion

T. (A.) rhombea showed red blood corpuscles with round nucleus, white blood corpuscles with kidney bean shaped nucleus and non-nucleated platelets. The average count of RBCs was 1.76 X 10⁹ per ml, WBCs 4.87 X 10⁶ per ml. and platelets were 66 X 10⁸ per µl. Haemoglobin (Hb) was found in the range of 2.03 to 8.87 gdl⁻¹ and seen to be increasing with size. The general trend in the relationship between blood parameters and body size was that the bigger the animal, the higher the values of its haematological parameters. The haematological characteristics of *T. rhombea* need more investigations as these would help in further research on the use of antithrombotic agents and other anticoagulant anti platelet agents related to medical treatments.

Acknowledgments

None.

Conflicts of interest

None.

References

1. Kanchanapangka S, Sarikaputi M, Rattanaphani R, et al. Cockle (*Anadara granosa*) Red Blood Cell: Structure, histochemical and physical properties. *Jour Thai Vet Pract.* 2002;14(3):2545–2546.
2. Gabriel UU, Akinrotimi OA, Orlu EE. Haematological characteristics of the Bloody cockle *anadara senilis*(L.) From Anadoni Flats, Niger delta, Nigeria. *Science World Journal.* 2011;6(1):1–4.
3. Jones S. The molluscan fishery resources of India. *Proc Symp MolluseaMBAI.* 1970;12:906–918.
4. Alagarwami KK, Narasimham A. Clam, cockle and oyster resources of the Indian coasts. In: Proc Symp Living Res seas around India. *Central Marine Fisheries Research Institute Special Publication*, Cochin, India. 1973;pp.648–658.
5. Nayar KN, Mahadevan S. Edible Bivalves: Clams and others. In: Nair RV & Rao KS (Eds.), *The Commercial Mollusca of India Bull Cent Mar Fish Res Inst.* 1974;25:40–53.
6. Silas EG, Alagarwami K, Narasmham KA, et al. Bivalve Culture in Asia and the Pacific – India. *Proceedings of a workshop held in Singapore.* 1982;p.34–43.
7. Narasimham KA. Taxonomy of the blood clams *Anadara (Tegillarca) granosa*(Linnaeus, 1758) and *A. (T.) Rhombea*(Born, 1780). *J Mar Biol Asso India.* 1988;30(1&2):200–205.
8. Brown BA. Haematology. Principles and procedure. (3rd edn), *Lea and Fabiger*, Philadelphia, USA. 1980;pp. 356.
9. Johansen K. Air-breathing fishes. Fish physiology. In: *Hoar WS & Rendall DT* (Eds.), Acad Press, New York, USA. 1970;pp.361–411.
10. Morton JE. Molluscs—An introduction to their form and function. In: Harper & Brothers (Eds.), *Harper Torchbook*, (1st edn). 1960;pp. 232.
11. Afiati N. Cytoplasmic Granules in the Red Blood Cells and the Karyotype of Rounded Ecomorph of *Anadara granosa* (L.) (Bivalvia: Arcidae) from Central Java, Indonesia. *Indonesia Jour Mar Sci.* 1999;4(2):52–59.

12. Suganthi K, Bragadeeswaran S, Prabhu K, et al. In vitro Assessment of Haemocyte and Thrombocyte Count from the Blood Clam of *Anadara inequivalvis*. *Middle-East Journal of Scientific Research*. 2009;4(3):163–167.
13. Ross MH, Reith EJ. *Histology. A text and Atlas*. 1990.
14. Marcus AJ, Safier LB. Thromboregulation: multicellular modulation of platelet reactivity in hemostasis and thrombosis. *FASEB J*. 1993;7(6):516–522.
15. Rajesh KM, Mendon MR. Role of vitamins and minerals in fish and shellfish nutrition. *Aqua Intl*. 2001;18–21.
16. Jurk K, Kehrel BE. Platelets: physiology and biochemistry. *Semin Thromb Hemost*. 2005;31(4):381–392.
17. Babatunde GM, Fajimi AO, Oyejide AO. Rubber seed oil versus palm oil in broiler chicken diet. Effect on performance nutrient, digestibility, haematology and carcass characteristics. *Animal Feed Science Technology*. 1992;35:133–146.