

# 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  $1.76 \times 10^9$  per ml, WBCs  $4.87 \times 10^6$  per ml. and platelets were  $66 \times 10^8$  per  $\mu\text{l}$ . Haemoglobin (Hb) was found in the range of 2.03 to 8.87 g/dL<sup>1</sup> 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

## Research Article

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**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 Brown [8] 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  $\mu\text{m}$ ). 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  $1.76 \times 10^9$  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  $4.87 \times 10^6$  per ml. Non-nucleated platelets about 2 $\mu\text{m}$  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)  $4.67 \pm 1.56$  g dl<sup>-1</sup> and Packed Cell Volume (PCV)  $12.34 \pm 6.79$  %. Oxygen carrying capacity was calculated as  $6.425 \pm 2.48$  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 g dL<sup>-1</sup>) and the highest was noted in Group 5 (6.65 - 8.87 g dL<sup>-1</sup>). The red blood cells count was  $1 \times 10^9$  for the Group 1 and it was seen to be the highest ( $2.24 \times 10^9$ ) 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.

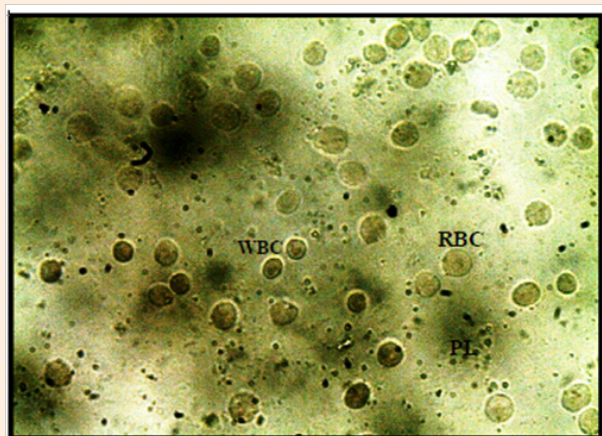
**Table 1:** Blood components of blood clam *T. (A.) rhombea*.

SL. No	Blood Parameters	Average Number or Percentage Per Ml
1	RBC	$1.76 \times 10^9$
2	WBC	$4.87 \times 10^6$
<b>WBC Differential Count</b>		
<b>Granulocytes</b>		
	i) Neutrophills	60% of total WBCs
	ii) Eosinophil	0
	iii) Basophil	0
<b>Agranulocytes</b>		
	i) Lymphocytes	36% of total WBCs
	ii) Monocytes	4% of total WBCs
	<b>Platelets</b>	$66 \times 10^8$ (per $\mu$ l)

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

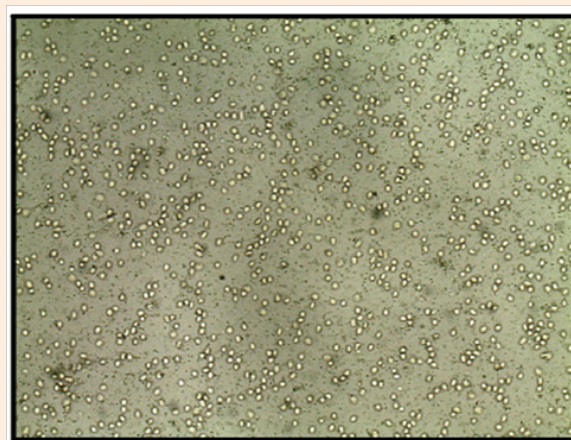
Parameters	Group 1 (20 - 30 mm)	Group 2 (31 to 40 mm)	Group 3 (41 to 50 mm)	Group 4 (51 to 60 mm)	Group 5 (61 mm and above)
Hb (gdL <sup>-1</sup> )	2.03 - 2.10	2.08-3.9	3.78 - 5.84	5.65 - 7.08	6.65 - 8.87
RBC (per ml)	$1 \times 10^9$	$1.24 \times 10^9$	$1.56 \times 10^9$	$1.78 \times 10^9$	$2.24 \times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

Hb: Haemoglobin (g per deciliter); RBC: Red Blood Cells (Cells  $\times 10^9$  ml<sup>-1</sup>); WBC: White Blood cells (cells  $\times 10^6$  cells ml<sup>-1</sup>); PLT: Platelets ( $10^9 \mu$ l<sup>-1</sup>); PCV: Packed Cell Volume (%) \*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 1:** 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, these second 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  $1.76 \times 10^9$  per ml, WBCs  $4.87 \times 10^6$  per ml and platelets were  $66 \times 10^8$  per  $\mu\text{l}$ . Haemoglobin (Hb) was found in the range of 2.03 to 8.87  $\text{g dL}^{-1}$  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 cytoplasmic 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  $\mu\text{m}$ ). 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 electron microscope.

Gabriel et al. [2] studied the haematological characteristics of *A. senilis*. The mean values of haematological profiles recorded were (mean  $\pm$  S.D), haemoglobin (Hb)  $4.08 \pm 1.88 \text{ g dl}^{-1}$ ; Packed Cell Volume (PCV)  $10.98 \pm 6.79 \%$ ; Red Blood Cells (RBC)  $1.97 \pm 0.68$

$\times 10^{12}$  cells  $\text{l}^{-1}$ ; White Blood Cells (WBC)  $3.76 \pm 1.51 \times 10^9$  cells  $\text{L}^{-1}$  Platelets (PLT)  $75.36 \pm 88.36\%$ ; Mean Corpuscular Haemoglobin (MCH)  $20.06 \pm 3.98 \text{ pg}$ ; Mean Corpuscular Volume (MCV)  $50.64 \pm 19.30 \text{ fL}$ ; Mean Corpuscular Haemoglobin (MCHC)  $43.79 \pm 13.71 \text{ g dl}^{-1}$ ; Oxygen Carrying Capacity (OCC)  $6.11 \pm 2.83 \text{ vol. } \%$ . 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  $4.67 \pm 1.56 \text{ g dl}^{-1}$  and Packed Cell Volume (PCV)  $12.34 \pm 6.79 \%$ , 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  $1.76 \times 10^9$  per ml, whereas the average white blood cell count was  $4.87 \times 10^6$  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 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  $1.76 \times 10^9$  per ml, WBCs  $4.87 \times 10^6$  per ml. and platelets were  $66 \times 10^8$  per  $\mu\text{l}$ . Haemoglobin (Hb) was found in the range of 2.03 to 8.87  $\text{g dL}^{-1}$  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.

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