

Morphological Characterization of Hemocytes in *Himantura walga* (Dwarf Whipray) in Persian Gulf and Oman Sea

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Abstract: Much of our knowledge regarding vertebrate blood and blood cells is based on mammalian references. The critical study of fish blood is relatively new and comparatively few investigations have been published that focus on marine animals. The morphology of hemocytes (blood cells) of *Himantura walga* (Dwarf whipray) was characterized via light microscopy. Based on this characterization, we classified hemocytes into 7 distinct cells: RBC, thrombocyte, lymphocyte, monocyte, heterophil, eosinophil and basophil.

Key words: Blood morphology % Light microscopy % *Himantura walga* (Dwarf whipray)

INTRODUCTION

Himantura walga (Müller & Henle, 1841) (Dwarf whipray) live in Marine environment and are demersal. Their distribution is in tropical, subtropical and temperate environments of Western Pacific. This fish is one of the most common elasmobranchs in Persian Gulf and Oman Sea. They are from elasmobranchs and have cartilaginous bones. These fishes often lie themselves in the bottom of coastal waters. They are harmless for people. This species is assessed as Near Threatened (IUCN Red List Status (Ref. 84930) [1].

There are few diagnostic tools available to veterinarians and fish health professionals to evaluate disease and abnormalities in this fish. Besides, data regarding blood cell characteristics and blood cell size are limited. Basic knowledge from this study is important for hematological research, conservation and clinical diagnosis.

MATERIALS AND METHODS

25 Dwarf whipray were obtained from Persian Gulf and Oman Sea. The blood collected in heparinized

syringes by cardiac puncture. Smears were made, fixed with methanol and stained with Gimsa [2]. Blood cell morphology was studied and their measurements were taken by means of a Dino-Capture Occular Micrometer.

RESULTS

Peripheral Blood Cells Were Classified into Following 7 Types: Erythrocytes, thrombocytes, lymphocytes, monocytes, neutrophils, eosinophils and basophils (Fig. 1).

Erythrocytes: These cells are nucleated. They are oval in shape with an oval to rounded nucleus (Fig. 1A). The immature form of erythrocyte-reticulocyte- was seen in the blood smear (Fig. 1B). The mean total length (L) and width (W) of the erythrocytes and their nucleus length (NL) and width (NW) are given in Table 1.

Lymphocytes: They are the most common circulating leukocyte. They are classified as small (the predominant form) and large. They have a spherical shape with centrally located, large, round nucleus. The large nucleus covers the majority of the cells area. The cytoplasm is

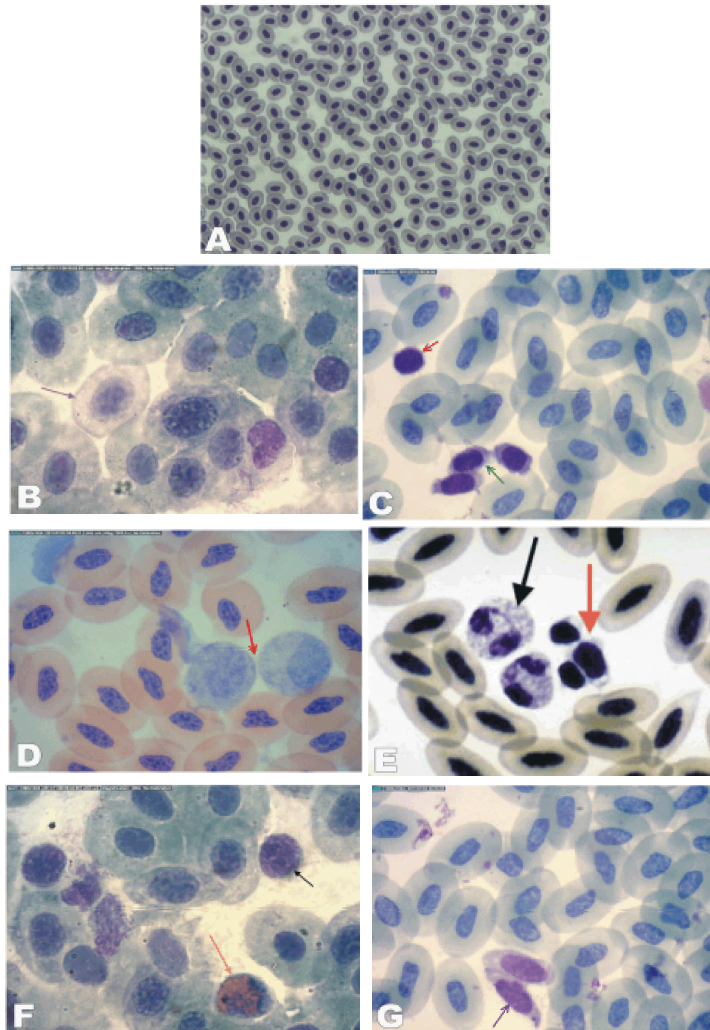


Fig. 1: The blood cells of *Himantura walga*: A- Erythrocytes, B- Reticulocyte, C- Lymphocyte (Red arrow) and Thrombocytes (Green arrow), D- Monocytes, E- Heterophil (black arrow), Thrombocytes (red arrow), F- Eosinophil (Red arrow), Basophil (Black arrow) and G- Thrombocytes

Table 1: Sizes of blood cells in *Himantura walga*.

Blood Cells	RBC				Thrombocyte		Lymphocyte Diameter		Monocyte Diameter	Heterophil Diameter	Eosinophil Diameter	Basophil Diameter
	CL	CW	NL	NW	CL	CW	Small	Large				
	84.44±2.15	66.78±3.12	53.21±0.77	38.47±1.12	10.22±1.61	4.87±1.13	14.09±3.45	17.87±2.34	18.25±5.21	19.65±4.333	18.34±3.53	16.05±3.76

stained pale blue and the nucleus is purplish blue using the Gimsa stain. No granule formation was observed in the cytoplasm (Fig. 1C).

Monocytes: They are large leukocytes and consisted of an oval to reniform nucleus and a weak basophilic cytoplasm, rich in cytoplasmic organelles, which were scattered in the cytoplasm. Sometimes a few large phagosomes were observed in the cells (Fig. 1D).

Neutrophils: Their morphology is variable and the nucleus may range in shape between kidney-like to having two or three lobes. The cytoplasm contains very thin, very pale eosinophilic granules (Fig. 1E).

Eosinophils: The nucleus is bilobulated and eccentrically located. The cytoplasm was filled with deep acidophilic granules of various sizes. These cells take the fourth place in the preparation of smears after lymphocytes, neutrophils and monocytes (Fig. 1F).

Basophils: These cells are oval-shaped with dark bluish purple granules within a light blue cytoplasm, which covers the nucleus. They are rarely seen in the preparation (Fig. 1F).

Thrombocytes: They are spindle-shaped cells with the oval nucleus. These cells are dark-stained. They may make clusters (Fig. 1C, 1E, 1G).

DISCUSSION

As stated in the result section leukocytes were identified on basis of their cell size and shape. These include: monocytes, neutrophil, eosinophil, basophil, lymphocyte and thrombocyte.

Almost all the fish have nucleated erythrocytes. Remarkable exceptions are *Maurolicus muelleri* a teleost with small non-nucleated erythrocyte [3]. In Dwarf whiplay the RBCs are oval in shape with round nucleus. Lymphocytes are the most common circulating leukocyte found in fish. They are generally classified as small or large [4]. Small lymphocytes were found about $14.09 \pm 3.45 \mu\text{m}$ in diameter. These cells had a dark purple large nuclei, clumped chromatin and pale blue cytoplasm. Large lymphocytes were found about $17.87 \pm 2.34 \mu\text{m}$ in diameter. Cytoplasm ratio was higher in large lymphocytes comparing to small lymphocytes. Ponson *et al.* studied characteristics of leukocytes of captured and cultural Asian eel and found that eels had small cell I: 6.43 ± 0.89 and $6.5 \pm 0.84 \mu\text{m}$ in diameter and small cell II: 5.338 ± 0.972 and $5.400 \pm 0.853 \mu\text{m}$ in diameter [5].

Monocytes were the largest and most variably shaped of the peripheral blood leukocytes. Monocytes of Dwarf whiplay were found about $18.25 \pm 5.21 \mu\text{m}$ in diameter in blood film. The large spherical or reni-form nucleus of this rarely encountered leukocyte type, occupied about half the cell, occasionally exhibiting the classical horseshoe shape. The relatively abundant basophilic cytoplasm was often vacuolated. Ponson *et al.* reported the size of monocytes of Asian eel about 10.96 ± 2.225 and $10.08 \pm 1.580 \mu\text{m}$ in diameter with large spherical or indented nucleus occupied about half the cell [5].

In fishes, the heterophils has been variably called neutrophils depending on the size of cytoplasmic granules. They were found $19.65 \pm 4.333 \mu\text{m}$ in diameter and their cytoplasm contains very tiny and pale eosinophilic granules and the nucleus was variable in shape from kidney-like to having 2 to 3 lobes. According to Ponson

et al. studies on leukocytes of Asian eel, neutrophils had striped gray to basophilic with irregular border cytoplasm and occasionally contains vacuoles with basophilic intracytoplasmic inclusion bodies [5].

Eosinophils are described for some fish but are poorly understood. Granules in fish eosinophils may have bar-shaped crystalloids similar to those described in human. There is a lack of knowledge regarding the full function of fish eosinophils, but they seem to function in a similar manner to mammalian mucosal mast cell [6]. The eosinophil of Dwarf whiplay had eccentrically bi-lobed nucleus and cytoplasm with acidophilic granules. The cell was $18.34 \pm 3.53 \mu\text{m}$ in diameter in blood film. These cells take the 4th place in blood smears after lymphocytes, neutrophils and monocytes. Studies performed on Asian eel showed that eosinophils were 10.357 ± 1.598 and $10.411 \pm 2.265 \mu\text{m}$ in diameter [5]. These cells were round, light purple with eccentric nucleus and euchromatin. The cytoplasm was lightly basophilic and contained numerous eosinophilic granules, which occasionally could obscure the nucleus.

Basophils are the most variable cell type in fish. They are apparently absent in certain species, such as zebra fish [7] and sea bass [8], but present in others, like the sea bream [9] and carp [10]. When present, basophils occur in very low numbers. Fish that do not have basophils also appear to lack mast cells, immunoglobulin E and serotonin [11]. In Dwarf whiplay, basophils were oval shaped cells with dark bluish purple granules within a light blue cytoplasm, which covers the nucleus. They are rarely seen in blood smears and were $16.05 \pm 3.76 \mu\text{m}$. Studies performed on blood leukocytes of Asian eel by Ponson *et al.* didn't show or discuss basophils among peripheral blood leukocytes [5].

Fish thrombocytes are variable in shape and may be rounded to oval or spindle shaped. Activation is frequent in blood smears obtained from the caudal vein and some of the changes noted in the cells on a smear may be caused by the activation of the clotting cascade [12]. The thrombocytes of dwarf whiplay were 10.22 ± 1.61 and $4.87 \pm 1.13 \mu\text{m}$ in diameter, spindle shaped cells with an oval nucleus. These cells are dark stained and can make clusters. In previous investigations on Asian eel these cells were measured about 9.790 ± 1.710 and $9.06 \pm 2.28 \mu\text{m}$ in diameter and had clear cytoplasm, purple nuclei and occasionally vacuolated. Thrombocytes showed some similarities to lymphocytes in Asian eel but thrombocytes were larger and can radiate cytoplasmic pseudopodia [5].

According to Kusuda and Ikeda [13], which studied characteristic of leukocytes of Japanese eel (*Anguilla japonica*); eels composed thrombocytes, neutrophils and monocytes. The principal characteristics of these leukocytes were similar to our findings on peripheral leukocytes of Dwarf whiptail except that we could recognize typical eosinophils and basophils in the blood smears. As with other vertebrates, fish thrombocytes are involved in blood clotting, phagocytosis and other possible immunologic function.

CONCLUSION

Dwarf whiptail (*Himantura walga*) composed of 5 distinguished leukocytes in peripheral blood. These are lymphocytes, the most abundant cells in the blood film, monocytes, eosinophils, basophils and thrombocytes.

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