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Quantitative Comparisons of Erythrocyte Morphology in Healthy Freshwater Fish Species from Malaysia

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Abstract: Erythrocyte morphology of four freshwater fish species namely tilapia *Oreochromis niloticus*, *Pangasius sutchi*, catfish *Clarias* sp. and river catfish *Mystus nemurus* were studied. Variations of erythrocyte and nucleus size were observed among the fish species studied. The largest size of erythrocyte was observed in tilapia *O.reochromis niloticus* (60.79±3.00 μm^2) and the smallest size of erythrocyte was observed in catfish *Clarias* sp., (53.24±2.57 μm^2) while the largest size of nucleus was also observed in *O.reochromis niloticus* (12.18±0.70 μm^2) yet smallest in *P, sutchi*, (9.37±0.97μm^2).

Key words: Erythrocyte, Morphology, *Mystus nemurus*, *Pangasius sutchi*, *Oreochromis niloticus*, *Clarias* sp.

INTRODUCTION

In human and most mammals, erythrocytes are anucleated with the shape of a biconcave len. Non mammalian vertebrates particularly fish however, possess oval to ellipsoidal erythrocyte shape with its nucleus retained. Fish erythrocytes have a wide range of sizes among different species[1,2].

Jagoe and Welter [2] reported about the morphology of erythrocyte from 7 freshwater fish species namely largemouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), chain pickerel (*Esox niger*), yellow perch (*Perca flavescens*), mosquito fish (*Gambusia holbrooki*), red eye bass (*Micropterus coosae*) and rainbow trout (*Oncorhynchus mykiss*). They found that nuclear size and shape varied significantly among the species. Isolated nuclei were found to have conspicuous apertures or holes and the numbers as well as the sizes of these holes also varied significantly among species. However, variations in nuclear size and structure within species were smaller compared to interspecies differences. In Malaysia, up till now, there have been no studies of on erythrocyte and its nucleus size in healthy freshwater fish. This information would be valuable to determine the health status of the fish. Therefore, the study reported herein aims to determine the size of erythrocytes and its nucleus in four different species of healthy freshwater fish in Malaysia namely tilapia *Oreochromis niloticus*, catfish *Clarias* sp., *Pangasius sutchi* and river catfish *Mystus nemurus*.

MATERIALS AND METHODS

Ten male of each freshwater fish species without pathological lesions and equally size-age were used in this study. These species were tilapia *Oreochromis niloticus*, river catfish *Mytus nemurus*, *Pangasius sutchi* and catfish were kept in continuously aerated water. The fish were put under sedation using 250 mg/L of MS222 (Tricane methanesulfonate) and blood was obtained by caudal vein puncture using 25G syringe. Thin blood films were made on glass slides air dried and subjected to Giemsa staining. Twenty slides were examined from each fish species.

The slides were observed using a Digital Biological Microscope (Motic DMB3-223A and Motic B3-223ASC, Japan). On each slide, 50 erythrocytes and their nuclei were measured under objective lense (x40). Erythrocyte and nuclear sizes were calculated according to the formula [3] respectively:

$$ES = [(EL \times EW \times \pi)/ 4]$$

$$NS = [(NL \times NW \times \pi)/ 4]$$

ES = erythrocyte size; EL = erythrocyte length; EW = erythrocyte width; NS = nucleus size; NL = nucleus length; NW= nucleus width

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Table 1: Mean (±standard deviation) of erythrocyte and nuclei length, width and size in freshwater fish species studied

Species	EL, µm	EW µm	EL/EW	ES, µm^2	NL,µm	NW,µm	NL/NW	NS, µm^2	NS/ES
M. nemurus	9.92 (±0.30)	7.90 (+0.07)	1.26 (±0.04)	57.99 (±2.10)	4.25 (±0.15)	3.38 (±0.12)	1.26 (±0.05)	10.54 (±0.68)	0.18 (±0.32)
P. sutchi	9.02 (±0.23)	8.10 (±0.34)	1.11 (±0.04)	57.77 (±3.57)	3.71 (±0.22)	3.00 (±0.15)	1.24 (±0.06)	9.37 (±0.97)	0.16 (±0.27)
O.niloticus	10.38 (±0.18)	7.87 (±0.49)	1.32 (±0.09)	60.79 (±3.00)	4.88 (±0.12)	3.69 (±0.16)	1.38 (±0.08)	12.18 (±0.70)	0.20 (±0.23)
Clarias sp.	9.50 (±0.26)	7.50 (±0.20)	1.25 (±0.04)	53.24 (±2.57)	3.92 (±0.32)	3.22 (±0.29)	1.22 (±0.04)	9.70 (±1.65)	0.18 (±0.64)

*EL: erythrocyte length; EW: erythrocyte width; ES: erythrocyte size; NL: nucleus length; NW: nucleus width; NS: nucleus size

RESULTS AND DISCUSSION

The most important feature observed in erythrocyte of all freshwater fish species studied was centrally located prominent nucleus (Figure 1 to 4). Similar to the other previous studies, the erythrocyte of the fish are oval-shaped. However, there were some variations based on erythrocyte morphometric measurements as given in Table 1. *Oreochromis niloticus* had the largest erythrocyte mean length, EL (10.38±0.18 µm) while *P. sutchi* have the smallest mean EL (9.02±0.23 µm). However, it was observed that erythrocytes mean width, EW of *P. sutchi* was the largest (8.10±0.34 µm) and the mean EW was found in keli *Clarias sp.* (7.50±0.20 µm).

Meanwhile, *O. niloticus* had the largest size of nucleus, NL with mean length of 4.88 µm (±0.12) and mean width, NW of 3.69 µm (±0.16). The smallest nucleus was observed in Patin *Pangasius sutchi* with mean length of 3.71 µm (±0.22) and mean width of 3.00 µm (±0.15). *Oreochromis niloticus* has the largest erythrocyte size, ES and nucleus size, NS respectively (60.79 µm^2±3.00; 12.18 µm^2±0.70) as compared to other fishes.

The blood cells present in the peripheral blood of fishes are consisted of erythrocyte, thrombocytes, lymphocytes, monocytes, neutrophils, heterophils, eosinophil and basophils in which they serve similar functions as they do in mammals [4]. Fish erythrocytes unlike mammalian erythrocytes are nucleated and represent the most numerous cells in the blood[5]. Among four freshwater fish species, erythrocyte of both *O. niloticus* (Figure 3) and *Clarias* sp. (Figure 4) are elongated. However, *Clarias* sp. has smaller erythrocyte as compared to *O. niloticus*. The erythrocyte of all non-mammalian vertebrate (i.e. fish, amphibians, reptiles and birds) is nucleated, flattened and ellipsoidal[6]. In the present study, erythrocyte and nucleus of *P. sutchi* (Figure 2).and *M. nemurus* (Figure 1) are rounded to oval. In channel catfish *Ictalurus punctatus* immature erythrocyte is reported to be rounder while the nucleus is more centrally located as compared to mature erythrocyte [7]. According to Canfield [8], non-mammals erythrocytes are oval and ellipsoidal cells. In comparison to erythrocyte of birds, the erythrocyte of reptiles, amphibians and fish have rounder ends and rounder nucleus[8].

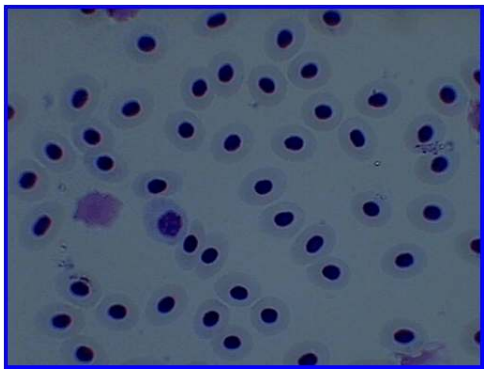

Fig. 1: Erythrocyte and nucleus of baung *Mytus nemerus* (x40)

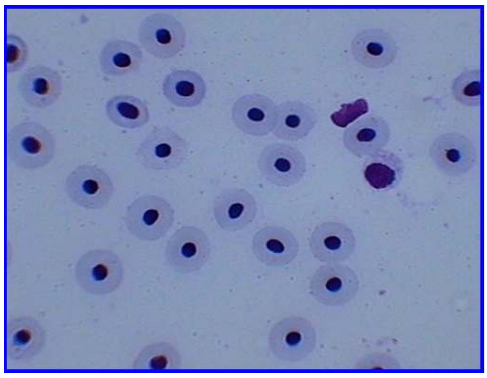

Fig. 2: Erythrocyte and nucleus of patin *Pangasius sutchi* (x40)

The differences in erythrocyte morphometric may be caused by dissolved gases. Erythrocytes are highly specialized for transporting oxygen O_2 [9]. Oxygen is important to the fish because fish obtains this vital gas directly from water via the gill. As an erythrocyte develops,

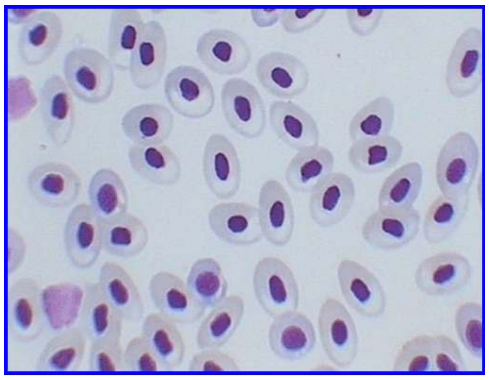

Fig. 3: Erythrocyte of tilapia *Oreochromis niloticus* (x40)

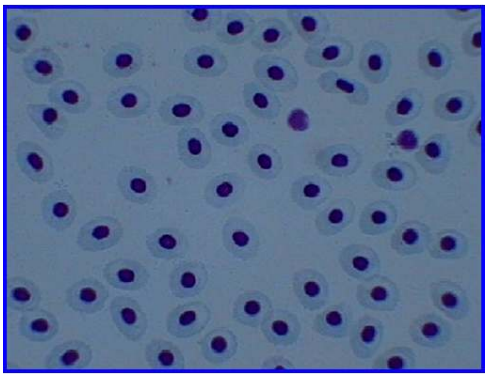

Fig. 4: Erythrocyte and nucleus of *Clarias* sp. (x40)

it produces great quantities of hemoglobin, the O_2 transporting pigment that gives vertebrate blood, red in colour [4].When the fish is in low oxygen level, it affects the circulatory system and causes respiratory stress that may alter the morphology of fish erythrocyte as well as decrease the erythrocyte number[10]. Erythrocyte has previously been studied in the mammalian microcirculation, where it has been showed to play an important role in oxygen uptake and release to tissues by diminishing the diffusion boundary layer fluid[11].

The erythrocyte became more elongated when squeezed between pillar cells of gills[11]. These were also observed in rainbow trout *Oncorhynchus mykiss* and roach *Rutilus rutilus* where the erythrocyte of both fish species were oval in the arterioles and arteries. During the erythrocyte passage through the gill lamella, the mean erythrocyte length was increased to 18.4±2.1 µm for trout *Oncorhynchus mykiss* and to 18.1±2.7 µm for roach *Rutilus rutilus*. Similar observation was also noted in the previous histological observation of fish secondary lamella [12].

In general, erythrocytes are longer in size in the lower orders, for example reptiles have larger erythrocyte than birds and birds have larger ones than mammals. However, erythrocyte size a lso varies greatly within orders for example in mammals the smallest erythrocyte occurs in deer, sheep and goats while the largest erythrocyte occurs in elephant and some large rodent [8].

Cartilaginous fishes *Elasmobranchii* such as shark *Selachii* and stingrays *Botoidea* typically have larger erythrocyte size although fewer in number, than erythrocyte of teleost. Even within the teleost, fishes which have more erythrocyte per milliliter of blood generally have smaller erythrocyte[13]. Furthermore, the energetic fish species tend to have more erythrocyte than sedentary fish group. Perhaps the smaller cells sizes in these active forms present a shorter mean diffusion path length for essential respiratory gases such as O_2 requirement [14].

From this study, tilapia *O. niloticus* had the largest erythrocyte size. The smallest mean size of erythrocytes was found in *Clarias* sp. In other study, in Order Cypriniformes, erythrocyte and nucleus size of goldfish *Carassius auratus* were 83.45 µm^2 and 17.28 µm^2 [15] and Japanese carp *Cyprinus carpio* 68.99 µm^2 while 9.90 µm^2 [16]. Based on these previous studies, both erythrocyte and nucleus size of *C. auratus* and Japanese carp *C. carpio* were larger than the present study. Compared with other study in the same class, *Anguila rostrata* erythrocyte size was 68.64 µm^2 and their nucleus size was 9.55 µm^2. In another species *O. cruentifer*, the erythrocyte and nucleus size were 65.92 µm^2 and 10.37 µm^2, respectively[17]. Both previous studies showed fish erythrocyte size was larger than the present study. However, nucleus size of tilapia *Oreochromis niloticus* in the present study was the largest compared to the previous study.

The smallest size of erythrocyte was found in *Clarias* sp. The size was smaller as compared to other study conducted by Stoskopf [5]. Although the body length of *Clarias* sp. is small it is not too possible to correlate these erythrocyte sizes with the body length. This is because various environmental factors play important role in the determining erythrocyte size [18]. Mature erythrocyte of channel catfish was found to be elliptical in shape with centrally located oval nucleus. As such, there are changes in erythrocyte size during the life cycle of Atlantic salmon, *Salmo salar*[19]. The erythrocyte size in the fingerling of Atlantic salmon, *Salmo salar* was 101.13 um^2 and for the smolts or adults' fish, the erythrocyte was slightly larger, 103.12 um^2.

Among these four freshwater fish species the ratio of erythrocyte size: nucleus size were *M. nemurus* 11:2, patin, *P. sutchi* 6:1, *O. niloticus* 5:1 and *Clarias* sp. 5:1. *O.niloticus* and keli, *Clarias sp.* have the same ratio of erythrocyte and nucleus size because the shape of both erythrocyte and nucleus are similar although their size is smaller in *Clarias* sp.

REFERENCES

1. Jagoe, C.H. and D. Welter, 1995. Quantitative comparisons of the morphology and ulrastructure of erythrocyte nuclei from seven freshwater species. Can. J. Zool., 73(2): 1951-1959.
2. Satchell, G.H., 1971. Circulation of Fishes. Cambridge University Press. London. England. pp: 145.
3. Ismail, H., S. Murat and S.Y. Hikmet, 2003. Erythrocyte size and morphology of tortoises and turtle from Turkey. Zool. Stud., 42(1): 173-177.
4. Campbell, T.W., 1990. An introduction to fish haematology Compend Contin education Practical Veterinary. 4(12): 528-533.
5. Stoskopf, M.K., 1992. Fish Medicine. College of Veterinary Medicine. North Carolina State University, Raleigh, North Carolina, pp: 882.
6. Rowley, A.F. and Ratcliffe, 1998. Vertebrate blood cells. Cambridge. University Press Cambridge, England. Available from: http://www.mbl.edu/BiologyBulletin/MMEK/CohBody.Html.[Accessed 21 July 2004]
7. Grizzle, J.M. and W.A. Rogers, 1976. Anatomy and Histology of the channel catfish. Opelika, AL: Craftmaster Printers, pp: 112.
8. Canfield, P.T., 1998. Comparative cell morphology in peripheral blood film from exotic and native animals. Aust. Vet. J., 12(76): 880.
9. Kisch, B., 1951. Observation on the haematology of fishes and bird. Exp. Med. Surg. 7: 326.
10. Brown, L., 1993.A aquaculture for veterinarians: fish husbandry and medicine. North Chicago. USA. pp: 440.
11. Nilsson, E.G., O.C. Lofman and M. Block, 1995. Extensive erythrocyte deformation in fish gills observed by in vivo microscopy: Apparents for enhacing oxygen uptake. J. Exp. Biol., 12(198): 1150-1156.
12. Laurent, P., 1984. Gill internal morphology. In: Fish Physiology. Orlando. pp: 183.
13. Ragan, H.A., 1999. Comparative hematology. In: Wintrobe's Clinical Haematology, pp: 2763.
14. Aust, V.J., 1998. Practical Laboratory Medicine, Comparative cell morphology in the peripheral blood from film exotic and native animals. The University of Sydney. Available from: p://www.Veterinary Anatomy and Pathology/RBC.html.[Accessed 5 July 2004]
15. Hardie, D.C. and P.D.N. Hebert, 2003. The nucleotypic effects of cellular DNA content cartilaginous and ray-finned fishes. pp: 706.
16. Conroy, D.A. and J.L. Rodriguez, 1966. Erythrocyte measurement of some Argentine fishes. Prog. Fish Cult., 28: 46.
17. Hartmann, F.A. and M.A. Lessler, 1964. Erythrocyte measurement in fishes, amphibians and Reptiles. Biol. Bull., 126: 83-85.
18. Altman, P.L. and D. Dittmer, 1961. Blood other body fluids. Federal America Society Biological. Washington. D.C. pp: 123.
19. Wilkins, N.P., G.I. Sangters and D.A. Conroy, 1970. Disease of Fish. Study on the haematology of the Atlantic salmon *Salmo salar*. Symposia of the Zoological Society of London. 30: 127.