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. 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. niloticus (12.18±0.70 µm^2) yet smallest in P. sutchi, (9.37±0.97 µm^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 disc. 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 Welter3 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), redeye 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 Mystus 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 lens (x40). Erythrocyte and nuclear sizes were calculated according to the formulae respectively:

\[ \text{ES} = \frac{[(EL \times EW \times \pi)]}{4} \]
\[ \text{NS} = \frac{[(NL \times NW \times \pi)]}{4} \]

\[ \text{ES} = \text{erythrocyte size; } \text{EL} = \text{erythrocyte length; } \text{EW} = \text{erythrocyte width; } \text{NS} = \text{nucleus size; } \text{NL} = \text{nucleus length; } \text{NW} = \text{nucleus width} \]
Table 1: Mean (±standard deviation) of erythrocyte and nuclei length, width and size in freshwater fish species studied

<table>
<thead>
<tr>
<th>Species</th>
<th>EL, µm</th>
<th>EW, µm</th>
<th>EL/EW</th>
<th>ES, µm+</th>
<th>NL, µm</th>
<th>NW, µm</th>
<th>NL/NW</th>
<th>NS, µm+</th>
<th>NS/ES</th>
</tr>
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<tbody>
<tr>
<td>M. nemurus</td>
<td>9.92</td>
<td>7.90</td>
<td>1.26</td>
<td>57.99</td>
<td>4.25</td>
<td>3.38</td>
<td>1.26</td>
<td>10.54</td>
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<td></td>
<td>(+0.30)</td>
<td>(+0.07)</td>
<td>(+0.04)</td>
<td>(+2.10)</td>
<td>(+0.15)</td>
<td>(+0.05)</td>
<td>(+0.68)</td>
<td>(+0.32)</td>
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<tr>
<td>P. sutchi</td>
<td>9.02</td>
<td>8.10</td>
<td>1.11</td>
<td>57.77</td>
<td>3.71</td>
<td>3.00</td>
<td>1.24</td>
<td>9.37</td>
<td>0.16</td>
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<tr>
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<td>(+0.34)</td>
<td>(+0.04)</td>
<td>(+3.57)</td>
<td>(+0.22)</td>
<td>(+0.15)</td>
<td>(+0.06)</td>
<td>(+0.97)</td>
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</tr>
<tr>
<td>O. niloticus</td>
<td>10.38</td>
<td>7.87</td>
<td>1.32</td>
<td>60.79</td>
<td>4.88</td>
<td>3.69</td>
<td>1.38</td>
<td>12.18</td>
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<tr>
<td></td>
<td>(+0.18)</td>
<td>(+0.49)</td>
<td>(+0.09)</td>
<td>(+3.00)</td>
<td>(+0.12)</td>
<td>(+0.16)</td>
<td>(+0.08)</td>
<td>(+0.70)</td>
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<tr>
<td>Clarias sp.</td>
<td>9.50</td>
<td>7.50</td>
<td>1.25</td>
<td>53.24</td>
<td>3.92</td>
<td>3.22</td>
<td>1.22</td>
<td>9.70</td>
<td>0.18</td>
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<tr>
<td></td>
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<td>(+2.57)</td>
<td>(+0.32)</td>
<td>(+0.39)</td>
<td>(+0.04)</td>
<td>(+1.65)</td>
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</tr>
</tbody>
</table>

*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 erythrocytes 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 µm²±3.00; 12.18 µm²±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 basophil in which they serve similar functions as they do in mammals[6]. Fish erythrocytes unlike mammalian erythrocytes are nucleated and represent the most numerous cells in the blood[7]. 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[8]. 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[9]. 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].

The differences in erythrocyte morphometric may be caused by dissolved gases. Erythrocytes are highly specialized for transporting oxygen O₂[9]. Oxygen is important to the fish because fish obtains this vital gas directly from water via the gill. As an erythrocyte develops,
erythrocyte size also varies greatly within orders for birds and birds have larger ones than mammals. However, order reptiles have larger erythrocytes than teleosts. Rutillus rutillus and Oncorhynchus mykiss erythrocyte length was increased to 18.4±2.1 µm for trout 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 Rutillus rutillus. Similar observation was also noted in the previous histological observation of fish secondary lamella.

In general, erythrocytes are longer in size in the lower orders, for example reptiles have larger erythrocytes than birds and birds have larger ones than mammals. However, erythrocyte size also 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.

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. 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 O2 requirement.

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 µm² and 17.28 µm² and Japanese carp Cyprinus carpio 68.99 µm² while 9.90 µm². 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 µm² and their nucleus size was 9.55 µm². In another species O. cruentifer, the erythrocyte and nucleus size were 65.92 µm² and 10.37 µm², respectively.[2] 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.[3] 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. 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.[4] The erythrocyte size in the fingerling of Atlantic salmon, Salmo salar was 101.13 um² and for the smolts or adults’ fish, the erythrocyte was slightly larger, 103.12 um².

Among these four freshwater fish species the ratio of erythrocyte size: nucleus size were M. nemurus 11:2, patin, P. puthi 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