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Comparative Hematological Values, Morphometric and Morphological Observation of the Blood Cell in Capture and Culture Asian Eel, *Monopterus albus* (Zuiew)

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Abstract: Problem statement: *Monopterus albus* (Zuiew), Asian Eel, is one of the common fish found mainly in Asia. At present, the habitats of Asian Eels have been on the decrease. Contrarily, consumption of Asian Eel has increased. Whereas, data regarding blood cell characteristics, blood cell sizes and hematological values of Asian Eel are limited. As such, the objective of this study was to establish the blood cell characteristics, blood cell dimension and hematological values of capture and culture Asian Eel. Basic knowledge from this study is important for hematological research, conservation, clinical diagnosis and in-depth study of this Eel. **Approach:** Blood samples of capture (n = 13) and culture (n = 19) Asian Eel, *Monopterus albus* (Zuiew) were collected in northeastern Thailand. Hematological values, morphometric and morphology of the blood cells were determined using standard techniques. Hematological values and morphometric between captive and cultural Eel were compared. **Results:** Hematological values and morphometrics of the capture and cultural Eel were not significantly different ($p>0.05$), but the hemoglobin and neutrophil of the capture eel were significantly higher than those of the culture eel ($p<0.05$). Neutrophil, monocyte, eosinophil and thrombocyte characteristics of Asian Eel were not different from other Eels. Nucleus characteristics, cytoplasmic shape and nucleus: Cytoplasm ratio of small cell I and small cell II were different. Lymphocyte of Asian Eel was different form the previous report. **Conclusion:** This study indicated that sources of eel influenced to some hematological values.

Key words: Hematological values, morphometric, morphology, capture, culture, Asian eel *Monopterus albus* (Zuiew)

INTRODUCTION

Hematological parameters that are used in veterinary and clinical medicines have been established as health indicators[1]. Quantity and quality of leucocyte cells, which are hematologic parameters, are generally used to determine immune reactions and disease. Moreover, changes in leucocytes also occur when fish are stressed and environmental quality is altered[2]. Sahan *et al*.[3] used some hematological values as parameters to determine the effect of water pollution induced stress in European eel. Blood cell characteristics of fish show distinct morphological similarities to those of birds and mammals and comprise lymphocyte, monocyte, granulocyte and thrombocyte. The most notable differences in fish relate to thrombocytes, which are nucleated and much larger than their mammalian counterparts and granulocytes which vary in the appearance of their granules[4].

Monopterus albus (Zuiew), Asian eel, is one of the common fish found mainly in Asia, from India, Southern China to Malaysia and Indonesia[5]. This fish is an important protein source for people in the northeastern part of Thailand. Eels are classified in kingdom Animalia, phylum Chrodata, class Actinopterygii, order Synbranchiformes, family Synbranchidae, genus *Monopterus*, species *Monopterus albus* and binomial name *Monopterus albus* (Zuiew). During the rainy season, or summer, people catch them by trap or hook for making food. At present, the habitats of Asian eels have been on the decrease. Contrarily, consumption of Asian eel has increased. Aquaculture of Asian eel is adversely affected by production related disorders and infectious diseases.

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Unfortunately, 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, blood cell sizes and hematological values of Asian eel are limited. As such, the objective of this study was to establish the blood cell characteristics, blood cell dimension and hematological values of capture and culture Asian eel. Basic knowledge from this study is important for hematological research, conservation, clinical diagnosis and in-depth study of this eel.

MATERIALS AND METHODS

Animals: Thirty two Asian eel (13 capture and 19 culture eels) captured from a pond and a rice field, were purchased from a market in Maha Sarakham province, in the northeastern part of Thailand during March-May 2008 They were taken to the laboratory at the Faculty of Veterinary Medicine and Animal Science, Mahasarakham University.

Hematological techniques: Blood samples of 1 mL were collected from the vein under the vertebrae using a 3 mL syringe, a 23-gauge needle 1.5 inches in length and placed in a micro-tube with EDTA for determining hematological values. The samples were cooled to approximately 4°C, using icepacks. Differential white blood cell counts were performed on blood films prepared, fixed in 95% methanol for 5 min., stained with Wright-Giemsa and then photos were taken with a camera under light microscopy. Blood cell dimensions were determined by using a stage and ocular micrometer. The PCV was determined after the blood had been transferred to microcapillary tubes and centrifuged at 2500 g for 5 min. Total white blood cell counts were determined manually with the improved Neubauer counting chamber after the blood was diluted with Natt and Herrick's solution. Total red blood cell count was performed by diluting the eel blood with Grower's solution then counting the red blood cells in 5 red blood cell squares of the improved Neubauer counting chamber. The hemoglobin concentration was determined by the cyanmethemoglobin method. MCV, MCH and MCHC were calculated.

SEM technique: The blood sample was dropped in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 overnight at 4°C then washed in the same buffer. Samples were postfixed with 1% Osmium tetroxide for 2 h then rinsed with distilled water, dehydrated in 20, 40, 60, 80, 100, 100% acetone and left to air dry. Gold coated blood films were examined with a SEM (JSM 6460 LV).

Statistical analysis: The results were given as mean ± SD, hematological values between males and females were compared by t-test and a level of significance set at $p<0.05$.

RESULTS AND DISCUSSION

Analyses: Summaries and comparison of hematological values and morphometrics of blood cell in capture and culture Asian eel are shown in Table 1. Some hematological values of Asian eel in this study, i.e., PCV, total red blood cell, MCV, MCH, MCHC and total white blood cells were different from the report of Siang *et al*.[5] that studied the effect of pesticides on some hematological values of Asian eel in Malaysia. Sahan *et al*.[3] studied the effect of different stations in the Ceyhan river (Adana, Turkey) on hematological values of European eel (*Anguilla anguilla* L., 1758) and found that water quality, i.e., temperature, NO_3-N, NO_2-N, NH_3-N, SRP and COD had an effect on the total white blood cell and neutrophil. Moreover, the packed cell volume of the Asian eel in this study, both capture and cultural, were higher than the packed cell volume of the European eel *Anguilla anguilla* L., 1758, as reported by Yavuzcan *et al*.[6] and Van Ginneken *et al*.[7].

Table 1: Comparison of hematological values and cell sizes (Mean ± SD) between capture and culture Asian eel, *Monopterus albus* (Zuiew)

Hematological values	Capture eel (n = 13)	Culture eel (n = 19)
PCV (%)	55.769±12.262	52.684±9.882
HB (g dL^{-1})	17.469±1.541	15.889±1.668*
RBC (x10^6 cell μL^{-1})	2.920±1.150	2.413±0.754
MCV (fL)	214.725±90.593	238.040±81.459
MCH (pg)	69.224±27.574	72.540±24.883
MCHC (g dL^{-1})	36.065±24.557	31.371±7.582
WBC(x10^4 cell/µl)	1.280±0.688	1.687±0.781
Small cell I (%)	50.000±5.490	60.000±14.126
Small cell II (%)	9.400±7.07	6.800±5.586
Monocyte (%)	6.100±7.489	10.500±5.421
Neutrophil (%)	34.200±11.858	22.200±12.795*
Eosinophil (%)	0.300±0.480	0.500±0.707
Erythrocyte: Length (µm)	11.640±1.007	11.420±0.912
Erythrocyte: Width (µm)	9.285±1.101	8.335±0.870
Erythrocyte's nucleus: Length (µm)	3.905±0.380	3.785±0.503
Erythrocyte's nucleus: Width (µm)	3.295±0.455	3.425±0.483
Thrombrocyte (µm)	9.790±1.710	9.060±2.281
Small cell I (µm)	6.430±0.890	6.500±0.846
Small cell II (µm)	5.338±0.972	5.400±0.853
Monocyte (µm)	10.960±2.255	10.080±1.580
Neutrophil (µm)	11.690±1.690	11.850±10.254
Eosinophil (µm)	10.357±1.598	10.411±2.265

PCV: Packed cell volume: HB: Hemoglobin concentration: RBC: Red blood cell: WBC: White blood cell: MCV: Mean corpuscular volume: MCH: Mean corpuscular hemoglobin: MCHC: Mean corpuscular hemoglobin concentration, *: $p<0.05$

These documents indicated that the environment and species of eel had an effect on hematological values. Besides, the neutrophil's percentage of capture Asian eel was higher than culture eel. This result was in accordance with the report of Sahan *et al*.[3]. They found that different environments had an effect on the neutrophil of eel and that an increase of neutrophil meant environmental stress. Generally, fish show a large variation in the number of hemoglobin components which relates to their ability to adapt to widely different environmental condition[8,9]. In this study, the hemoglobin of the capture was higher than culture eel. Therefore, all above documents showed that the different sources effect to neutrophil and hemoglobin of Asian eel.

Cell morphology and morphometric: Leucocytes were identified on basis of number, their cell size, shape, structure and ultrastructure. These were: Small cell I, Small cell II, monocyte, neutrophil, eosinophil and thrombocytes. Summaries of the blood cell morphology of capture and cultural Asian eel were as follow:

Small cell I was found about 50-60% in blood film. Small cell I of capture and cultural Asian eel were 6.430±0.890 and 6.500±0.846 µm in diameter. Small cell I had a dark purple, segmented nuclei, clumped chromatin with clear cytoplasm. Frequently, small cell I had cytoplasmic pseudopods. Small cell II was found about 6-9% in blood film. Small cell II of capture and cultural Asian eel were 5.338±0.972 and 5.400±0.853 µm in diameter. Small cells II were characterized by a large nucleus which usually spherical. Nucleus of Small cell II was round and dark purple in color. Nucleus: cytoplasm ratio was higher than small cell I. Monocytes were the largest and most variably shaped of the peripheral blood leucocytes. Monocyte of Asian eel was found about 6-10% in blood film. Monocyte of capture and cultural Asian eel were 10.960±2.255 and 10.080±1.580 µm in diameter. The large spherical or indented nucleus of this rarely encountered leucocyte type occupied about half the cell, occasionally exhibiting the classical 'horse shoe' shape. The relatively abundant basophilic cytoplasm was often vacuolated. Neutrophil was found about 20-30% in blood film. Neutrophil of capture and cultural Asian eel were 11.690±1.690 and 11.850±10.254 µm in diameter. Neutrophil cytoplasm was striped, gray to slightly basophilic and had occasional vacuoles and basophilic, intracytoplasmic inclusion bodies. Cytoplasmic borders were irregular. Eosinophil was found about 0-1% in blood film. Eosinophil of capture and cultural Asian eel were 10.357±1.598 and 10.411±2.265 µm in diameter. Eosinophil of Asian eel had round, light purple, often eccentric nuclei with open chromatin. Eosinophil cytoplasm was lightly basophilic and contained numerous eosinophilic granules. The eosinophil granules occasionally obscured the nucleus. Thrombocyte of capture and cultural Asian eel were 9.790±1.710 and 9.060±2.281 µm in diameter. Thrombocyte had clear cytoplasm, purple nuclei and occasionally vacuolated. The thrombocyte showed some similarities to the lymphocyte, but the thrombocyte was larger and able to radiate cytoplasmic pseudopodia.

Kusuda and Ikeda[10] studied characteristics of leucocyte of eel, *Anguilla japonica* and found that eel composed of four leucocytes, i.e., lymphocyte, thrombocyte, neutrophil and monocyte. They reported the principal characteristic of the leucocytes isolated from the eel was as follows: Lymphocyte-like cells were round and ranged from 5-10 µm in diameter, with round nuclei. Cytoplasmic volume was relatively small and cytoplasm was stained dark gray. Neutrophil-like cells were round or oval and ranged from10-15 µm in diameter and with eccentrically located, oval or bilobed nuclei. Cytoplasm was stained whitish or whitish-gray. Monocyte-like cells were round and ranged from 10-15 µm in diameter; round or oval nuclei occupied more than half of the cytoplasm. Cytoplasm was stained light to dark gray. Thrombocyte-like cells were oval to spindle shape and ranged from 8-10 µm in length, 3-5 µm in width, with an oval to spindle nuclei. The cytoplasm was stained light pink. Moreover, McArthur[11] reported the morphology of erythrocytes, neutrophils, lymphocytes, thrombocytes of the New Zealand freshwater eels, *Anguilla australis Schmidtii* (Phillips) and *A. dieffenbachii* (Gray). Neutrophil of the New Zealand freshwater eels was oval to round fine granules comparable to the mammalian neutrophil and this leucocyte found rarest in blood smear. The largest mature cell, neutrophils vary tremendously in size among eels (diameter 7-13 µm, mean 10 µm). The nucleus of mature neutrophil is usually eccentric and moderately clumped and stains deep purple. The cytoplasm of neutrophil of the New Zealand freshwater eels stain light grey and has varying number of colorless or basophilic threadlike inclusions which give it a reticulate or web-like appearance. Lymphocytes of the New Zealand freshwater eels are divided into small (4-6 µm in diameter, mean 5.3 µm) and large (7 µm) lymphocytes. Mature large lymphocytes have a bean-shaped nucleus less dense than that of mature small lymphocytes. The small lymphocyte was round to oval with a large, densely chromatic nucleus. Thrombocyte of the New Zealand freshwater eels was 8.5×4.7 µm in diameter.

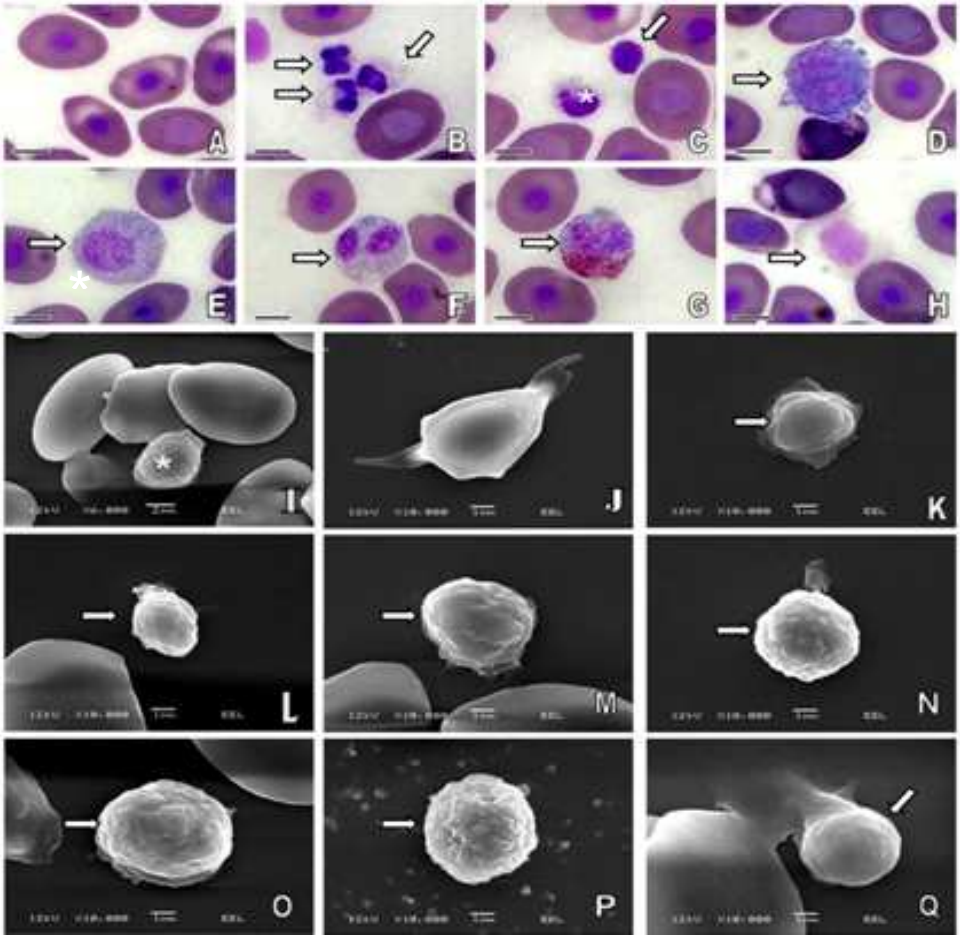

Fig. 1: A-H micrograph of Asian eel blood cells with Geimsa-Wright stained. I-Q SEM micrograph of Asian eel blood cells. (a): 5 Erythrocytes; (b): 3 small cell I (arrows); (c): A small cell II (arrow) compared to small cell I (*); (d): A monocyte (arrows); (e): Neutrophil with round nucleus (arrow), (f): Neutrophil with bilobe nucleus (arrow); (g): Eosinophil (arrow); (h): Thrombocytes; (I) 4 erythrocytes with a small cell I (*); (j): Abnormal erythrocyte; (k): Small cell I (arrow); (l): Small cell II; (m): Monocyte (arrows); (N-O): Neutrophil (arrows); (p): Eosinophil (arrows); (q): Thrombocyte (arrows) (from A-H, bar = 10 µm I, bar = 2 µm J-Q, bar = 1µm)

The blood cell characteristics of the eel, *Anguilla japonica* that was reported by Kusuda and Ikeda[10] was different than some findings of this study. In this study, we found 2 types of cell-like lymphocyte, small cell I was different from the lymphocyte of *Anguilla japonica*. However, small cell II was similar to the lymphocyte of eel, *Anguilla japonica*. The differences between leucocytes were the nuclei shape and diameter. Furthermore, the differences between the Asian eel and the New Zealand freshwater eels were classification of lymphocyte: The New Zealand freshwater eels are divided into small and large lymphocytes, but the Asian eel's 2 types of lymphocytes were Small cell I and small cell II. The results from this study showed that leucocytes of eels were different in classification, especially the characteristics of lymphocyte.

Under SEM examination, the mature erythrocytes of the Asian eels were flat with a smooth surface. The small cell I was round and irregular membrane surface. The small II was round with smooth or fine irregular membrane surface. Monocytes were larger than lymphocytes. They were round cells with a rough membrane. The neutrophil were round with irregular membrane surface. Eosinophils were round cells with many spherical spines protruded from their membrane surface. The thrombocyte was round and presence of a spread monolayer. SEM micrograph of blood cells in Asian eels that reported in this study was the first report (Fig. 1).

CONCLUSION

Environment and location have influenced some hematological values of Asian eels. Lymphocyte characteristics of Asian eel was divided into two types', i.e., small cell I and small cell II and lymphocytes of Asian eel were different from the previous report on the Japanese and the New Zealand eel.

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