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Red blood cell Morphology of some fish species in Ha Tinh and Nha Trang, Vietnam

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ABSTRACT

The article presents the research results of red blood cell morphology of 6 fish species caught in the wild (*Mugilidae sp.*, *Decapterus sp.*, *Epinephelus fuscoguttatus*, *Scatophagus argus*, *Aluterus monoceros*, *Pampus argenteus)* and 2 cultured fish species (*Sciaenops ocellatus, Lates calcarifer*) in Ha Tinh and Nha Trang. The data obtained showed that the red blood cells of the wild and cultured fish groups both exhibited cytoplasm disorders but the degrees of manifestation were different, with the main abnormal forms being nucleus distribution and erythrocyte nucleus shape disorders, no disorder in the form of micronuclei in the cytoplasm were found. The group of fish caught from the natural environment and *Lates calcarifer* have a low rate of red blood cell morphological disorder, ranging from 0 to 2%, while for *Sciaenops ocellatus*, although the areas show different levels of disorders, all show serious morphological disorders, particularly it was up to 87% in some certain areas–which means that *Sciaenops ocellatus* is being negatively affected by external factors. Images and detailed analysis can be found in the article.

Key words*: Blood, Fish, Red blood cells, Pollution, sea.*

Introduction

Most of man-made waste follows water to flow into water bodies, rivers or lakes or runs into the sea, including various xenobiotics such as biphenyls, organic chlorine pesticides, toxic metals, polycyclic aromatic hydrocarbon compounds, dioxins and others (Van der Oost *et al.*, 2003). Heavy metals are produced from a variety of natural and man-made sources (Bauvais *et al.*, 2015). In aquatic environments, heavy metal pollution is the result of direct atmospheric deposition, geological weathering or through agricultural, municipal waste discharge, from residential areas or industrial plants, even from wastewater treatment plants (Demirak *et al.*, 2006; Maier *et al.*, 2015). Fish are considered an important biological indicator in aquatic system to estimate the degree of metal contamination (Benaduce *et al.*, 2008); they offer some advantages in describing the natural characteristics of aquatic systems as well as assess the changes in living environment (Has-Schön *et al.*, 2008). In addition, fish is located at the end of the aquatic food chain, being capable of accumulating metals and causing chronic, acute diseases in human through food (Linbo *et al.*, 2009).

Since they eat and live in aquatic environments, fish are particularly vulnerable and heavily polluted as they cannot escape from the adverse effects of pollutants (Mahboob *et al.*, 2014; Saleh and Marie, 2015). Fish, compared to invertebrates, are more sensitive to many toxins and a convenient test subject to indicate ecosystem health (Marshall Adams and Ryon, 1994; Authman, 2011; Saleh and Marie, 2015). Metal ions present in water enter the fish's body and accumulate in various organs such as liver and kidney (Al-Mohanna, 1994; Shukla *et al.*, 2007). Blood indices are used as a measure of the sensitivity to stress when fish are exposed to various pollutants and toxins, such as metals, biocides, pesticides, chemical industrial wastewater, etc (Singh *et al.*, 2008).

This study aims at evaluating the red blood cell morphology of some wild fish species and cultured fish in Cua Khau, Cua Sot - Ho Do Bridge (Ha Tinh) and Nha Trang Bay area (Khanh Hoa).

Materials and Methods

Wild fish are caught by using fishing rods and nets in the waters of Cua Sot, Cua Khau (Ha Tinh) and Cai estuary, Hon Tam (Nha Trang Bay, Khanh Hoa). Blood samples were collected from 48 fish individuals of 6 species, each with 2-3 individuals: *Mugilidae sp.*, *Decapterus sp.*, *Epinephelus fuscoguttatus*, *Scatophagus argus*, *Aluterus monoceros*, *Pampus argenteus*.

Cultured fish were collected from cages in Ho Do Bridge, Cua Khau (Ha Tinh) and cages in Hon Mieu (Nha Trang). Research on red blood cell morphology of 18 fish individuals of 2 cultured species was conducted. At each farming site, sampling was conducted with 3 individuals of each species: *Sciaenops ocellatus* and *Lates calcarifer*.

Blood was taken from the caudal vein of the fish, regardless of whether the individual was male or female. Heparin was used as an anticoagulant with a concentration of 15-20 µL per 1 mL of fish blood. Fish blood collection tubes were stored at 8-10^{o}C. Fish blood specimens were made in 1-4 hours after blood sample collection.

An amount of whole blood was diluted 2-5 times with Ringer solution (Hoar and Hickman, 1975) for bony sea fish. This diluted blood is used to make a whole blood specimen.

Another part of blood was centrifuged at a rate of 2000×g for 15 minutes to get the blood rich in white blood cells. Specimens are made on a sterile slide with the blood rich in white blood cell.

The specimens were fixed with pure methanol for 10 minutes and allowed to dry in the air. They were stained with Romanowsky-Giemsa 10% for 20 minutes and rinsed with water. Olympus CX43 microscope was used to capture images at × 100 magnitude with oil, using Adobe Photoshop CS6 to homogenize the received image. White blood cells were classified after centrifugation based on descriptions of authors (Ivanova, 1983; Campbell and Grant, 2010).

Results and Discussion

For wild caught fish, the blood composition of all 6 fish species include red blood cells, white blood cells and platelets –which are the basic blood components found in different fish species (Ivanova, 1983; Campbell and Grant, 2010).

The red blood cells of these individuals are oval-shaped, slightly rounded or slightly elongated (Figure 1). The cytoplasm of the red blood cells is neutral in color, light blue or reddish-purple. Most cells have intact and continuous membranes with balanced cell shape. The purple, purple-blue, or dark blue colors are characteristics of Giemsa stain. It was recognized the disorders of red blood cell morphology in different forms such as: unbalanced cell shape, wrinkled cell membrane, unbalanced nucleus shape or having a sub-nuclear in cytoplasm. However, the ratio of such red blood cells on the specimens was relatively low, only 0 - 0.3%, with the exception of *Scatophagus argus*.The morphology imbalance of erythrocyte nucleus structure was noted at 0.2-2% (Table 1).

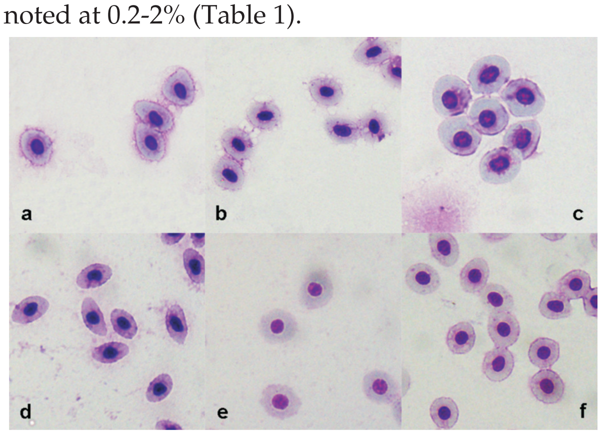

Fig. 1. Red blood cells of some fish species. a – *Mugilidae sp.*, b – *Decapterus sp.*, c – *Epinephelus fuscoguttatus*, d – *Scatophagus argus*, e – *Aluterus monoceros*, f – *Pampus argenteus*.

Table 1. Percentage of cell structure disorder in some wild fish species

Fish species	Cell morphological disorder expression		
	Cell shape	Membrane structure, wrinkles	Nucleus structure and shape
Mugilidae sp.	–	–	0 – 0.2%
Decapterus sp.	–	0 – 0.2%	–
E. fuscoguttatus	0 – 0.1%	–	–
S. argus	0 – 0.2%	–	0.2 – 2.0%
A. monoceros	–	0 – 0.3%	–
P. argenteus	–	0 – 1%	–

The results of this research indicated that the group of wild fish showed stress due to the effect of external factors on red blood cells but at a low level. Morphological disorder patterns and cellular disorder rate were not the same among fish species. The obtained study data were similar to other research results, under the same experimental conditions, affected by the same factor's exposure different fish species showed different hematologic disorders (Vosyliene, 1999; Monteiro *et al.*, 2011; Gill and Pant, 1987; Singh and Reddy, 1990).

For two fish species collected in cages, the blood cell compositions were similar, including red blood cells, white blood cells and platelets. Red blood cells were represented primarily as round or oval shapes. The nuclear was purple-blue in round or oval shapes.

For *Sciaenops ocellatus*, research on blood specimen by microscope showed that, besides normal red blood cells in oval shape, round shape with balanced nucleus, homogeneous-distribution nucleus, many red cells with unbalanced shape appeared, with deformed nucleus of unbalanced structure located in the cytoplasm, and the nucleus is not uniformly distributed. Abnormal red blood cell morphology in *S. ocellatus* blood was found in 3 cages in 3 study sites (Figure 2). The proportion of groups of cells with morphological disorders in *S. ocellatus* was quite high (Table 2).

Abnormal form of red blood cell nucleus distribution in *S. ocellatus* at L1 was from 63 to 83%, L2 from 72 to 82% while at L3, it was only recorded at less than 1%. With the nucleus shape disorder, fish in the L1 and L2 areas also showed high rates, at 72 - 87% and 65 - 78% respectively. At L3, the rate was lower, from 8-25%.

For *Lates calcarifer*, in the same farming conditions as *Sciaenops ocellatus* in two locations of Ho Do Bridge and Cua Khau, the number of abnormal red blood cells, the "deformed" nucleus is quite few, with the of only about 0, 2-0.5%. At the same time, the abnormal variation in the nucleus was at a lower level than that of *Sciaenops ocellatus*. On the *Lates calcarifer* blood specimen appeared red blood cells with "defective" nucleus (Figure 3).

The normal morphology of red blood cells in many fish species has also been well studied (Witeska, 2013; Fange, 1994; Gill and Pant, 1986; Witeska, 1998; Harr *et al.*, 2018). Fish red blood cells are very sensitive to various environmental effects, but Vosyliene (1999) believed that, basic quantitative hematological parameters (e.g. hematocrit, red

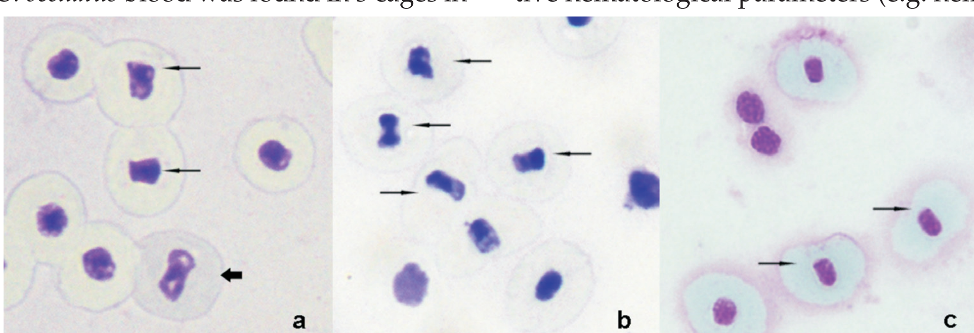

Fig. 2. Red blood cells of *Sciaenops ocellatus* in fish cages. *a – Fish blood in cages in Ho Do Bridge, b – Fish blood in cages in Cua Khau, c – Fish blood in cages in Hon Mieu. The thin arrows indicate the deformed erythrocyte nucleus / uneven nuclear distribution; the fat arrow indicate the mild alkaline erythrocytes.*

blood cell count or hemoglobin concentration) in fish tend to be stable due to a significant compensation capacity (Witeska, 2013). Furthermore, even though there is a quantitative change in the number of red blood cells in contaminated fish, they can show different directions of variation: possibly decrease or increase (Jezierska and Witeska, 2001), and they depend on the species of fish, the toxic agent and the level of toxicity (time and concentration) (Witeska, 2013). On the other hand, various authors have reported that toxic substances may cause morphological abnormalities in erythrocytes, including nuclear anomaly (Buckley, 1977; Gill and Pant, 1986; Witeska, 2004; Bagdonas and Vosyline, 2006; Witeska *et al*., 2006; Monteiro *et al.*, 2011), cell deformation (Vosyliene, 1996; Yang and Chen, 2003; Witeska, 2004; Witeska *et al*., 2006; Naskar *et al*., 2006), non-mitotic cell proliferation (Gill and Pant, 1987; Hofer *et al*., 1992; Witeska, 2004) or hemolysis (Fletcher and White, 1986; Shandilya and Banerjee, 1989; Singh and Reddy, 1990; Vosyliene, 1996; Bogé and Roche, 1996). Research results show that the abnormality can be caused by a range of organic and inorganic pollutants, damaging or disrupting both the nucleus and red blood cells. Nuclear abnormalities include changes in chromosome arrangement in the nucleus, nucleus deformation or fragmentation, and formation of sub-nuclear. Due to their sensitivity to xenobiotic, fish erythrocytes are often used to assess the potential for genetic toxicity of pollutants or other environmental adverse factors (Witeska, 2013). Aquatic contamination can also cause non-mitotic cell proliferation in erythrocytes. Cell abnormalities exhibit both internal and external damage to erythrocytes. Severe intoxication often creates "swelling" or dissolves red blood cells.

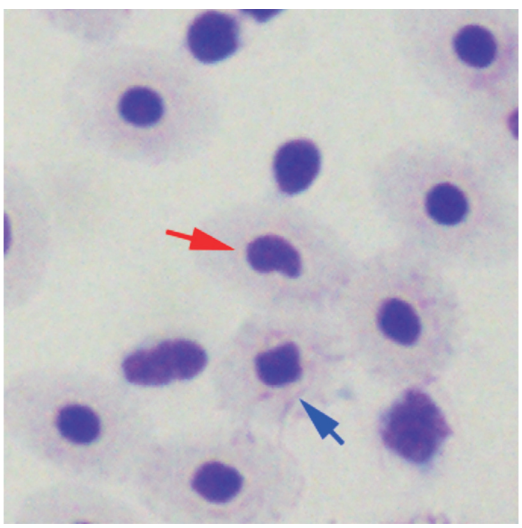

Fig. 3. *Lates calcarifer* blood cells in cage farming conditions. *The red arrow indicates missing-nucleus erythrocyte morphological disorder; the blue arrow indicates erythrocyte morphological disorder in nuclear shape.*

Erythrocyte damage caused by adverse environmental factors can be compensated for by the release of new cells from the spleen or / and the head of the kidney, indicated by an increase in the number of immature cells in peripheral blood. Consequently, the morphological characteristics of fish erythrocytes (cytoplasm anomalies, nucleus anomalies or the appearance of immature cells) appear to be more sensitive and reliable than baseline blood parameters when living in polluted environmental conditions (Witeska, 2013).

Conclusion

1. Six wild fish species collected in three locations of Cua Sot, Cua Khau (Ha Tinh), Nha Trang Bay (Khanh Hoa), including: *Mugilidae sp.*, *Decapterus sp.*, *Epinephelus fuscoguttatus*, *Scatophagus argus*, *Aluterus monoceros*, *Pampus argenteus* showed red blood cell morphological disorder at low level, at the rate of 0-2%. In other words, these fish species were affected by environmental pollution in the above-mentioned locations but not significantly.
2. *Sciaenops ocellatus* collected in cages at Ho Do bridge, Cua Khau (Ha Tinh), Hon Mieu (Nha Trang) showed high morphological disorder,

Table 2. The rate of red blood cell morphological disorder in *Sciaenops ocellatus*

Location	Forms of red blood cell morphological disorders			
	Cell shape	Membrane structure, wrinkled	Nuclein distribution	Nuclein shape
L1	1 – 2%	0 – 3%	63 – 83%	72 – 87%
L2	0 – 1%	1 – 5%	72 – 82%	65 – 78%
L3	0 – 1%	0 – 2%	0 – 1%	8 – 25%

especially the nucleus distribution nucleus shape forms. These fish individuals are under severe stress due to the influence of environmental factors.

3. In the same cage farming conditions, *Lates calcarifer* showed red blood cell morphological disorder at low level, only 0.2 - 0.5%. In other words, in the same farming conditions today, *Lates calcarifer* does not fall into a state of stress and is not significantly affected by the environment.

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