Hematological characteristics of red drum *Sciaenops ocellatus* (Linnaeus, 1766) in freshwater experimental culture conditions

Do H. Quyet

Institute of Tropical Ecology, Joint Vietnam-Russian Tropical Science and Technology Research Center, 63 Nguyen Van Huyen, Nghia Do Ward, Cau Giay District, Hanoi, Vietnam. Corresponding author: D. H. Quyet, dhquyet@gmail.com

Abstract. This study aimed to examine the hematological characteristics of red drum (*Sciaenops ocellatus*) reared in freshwater experimental aquaria and fed a commercial diet. Red drum is a marine fish native from Central Mexico on the Gulf coast to Massachusetts in the Atlantic Ocean, farmed commercially and used as a valuable food source. This species is raised on farms in estuaries and faces many risks such as water pollution and temperature changes due to ocean currents. Fish blood profile data, which includes hematological indicators, blood cell morphological characteristics as well as biochemical indicators, are very useful in assessing the health status and living environment conditions of red drum. Blood samples were taken from red drum specimens on days 1, 5, 10, and 15 of the experiment. Standard hematological methods were used for the experiment. Research results show that red drum poorly adapts to the freshwater environment, with a mortality rate of up to 42% in a total of 20 days of experimentation. Hematological parameters, such as erythrocytes, leukocytes, hemoglobin, and blood biochemical indices, such as glucose, urea, triglyceride, aspartate aminotransferase, and alanine aminotransferase of red drum were stable during the experiment. According to the research, some red blood cells had morphological disorders with low rates, from 1 to 5%, such as loss of nucleus, bud nucleus, bi-nucleus, notched nucleus, and wrinkled cell membrane.
Key Words: erythrocytes, freshwater, hematology, heavy metal, red drum.

Introduction. The red drum *Sciaenops ocellatus* (Linnaeus, 1766) is one of the fish species commercially raised in many countries around the world, such as China, Ecuador, Guadeloupe, Israel, Martinique, Mayotte, Mauritius, and Mexico (FAO 2008). It was introduced into Vietnam in 1999 and is now grown in many coastal areas of Vietnam. This species has expanded in farming scale in many localities due to its nutritional value, meat quality, growth rate, adaptability to aquaculture conditions, and price and profits for investors (Gardes et al 2000). The red drum belongs to the Sciaenidae family and is a euryhaline organism, capable of adapting to water conditions with different salinities (Odell et al 2017). Fish farms are often found in estuaries, ponds, lagoons, or lakes near the sea. Red drum is also raised in coastal cages. However, estuaries are under pressure from economic development, shipping activities, and industries, leaving seawater at high risk of contamination (Burt et al 2019). This can significantly impact aquaculture activities in the area, and may even damage the farms.

Fish blood parameters, such as red blood cells (RBCs), white blood cells (WBCs), and component cells, such as lymphocytes, neutrophils or heterophils, basophils, eosinophils, monocytes or hemoglobin (Hb) content, and plasma biochemical indices are common biological indicators for evaluating fish health and physiological status (Docan et al 2018; Fazio 2019; Witeska 2013). The results of hematological analyses can also reflect the living environment conditions of fish, and the negative effects of various factors, both endogenous and exogenous, on the body of the fish.

This article presents the results of research on the hematological characteristics of red drum in freshwater experimental farming environments and the use of commercial feed. It also considers their ability to adapt to these experimental conditions.

Material and Method

Experimental design

Preparation of the aquarium. The study was carried out between February 17 and April 5, 2022, in Ky Anh Town, Ha Tinh Province. A square aquarium with the dimensions, length x width x height of 2.0 m x 2.0 m x 1.4 m, was used. The volume of water in the aquarium was about 4.6 m^3. The initial measured salinity was 26‰. Oxygen in the aquarium was enriched using the oxygen aerator Veratti (model ACO-012, China) with four spherical air stones (Figure 1).

Figure 1. Square aquarium for experiment.

Preparation of the fish. Fifty healthy red drum fish were purchased from a farm about 1 km from the experimental site. The health status of the fish was checked visually before being released into the aquarium. No individuals were injured or had parasites on their bodies. The fish's scales and fins were shiny, beautiful, and scratch-free. The average weight of the fish was 1.00±0.25 kg.

Release, care, and raising of the fish. The fish were not fed in the first two days following the release, and the water in the aquarium was changed every day. After five days, salinity was decreased to 1‰. At 8:45 a.m. and 5:15 p.m. every day, fish were fed commercial food, Nutrilis P3, produced by the company, Ocialis, of ADM Group. Feed ingredients according to information from the manufacturer include crude protein (min. 43%), crude fat (min. 7%), moisture (max. 12%), fiber (max. 3%), calcium (min. 1.5%), phosphorus (min. 0.5%), lysine (min. 2.6%), methionine and cystine (min. 1.4%), and crude ash (max. 16%).

Collection of fish blood and water samples. On days 1, 5, 10, and 15 from the time the water salinity in the aquarium reached 1‰, fish blood was taken from the caudal vein (Lawrence et al 2020) and heparin was used to prevent blood clotting. Fish were removed from the tank using a net and the time to take a fish's blood did not exceed two minutes after leaving the tank to minimize stress on the fish. A total of 4 mL was collected from each fish, of which 2 mL was for biochemical analysis and 2 mL was for cell count and blood smear.

Simultaneous with the four times blood samples were collected, water samples were also collected, preserved, and sent to analyze the composition of heavy metals, including chromium (Cr), iron (Fe), arsenic (As), manganese (Mn), mercury (Hg), nickel (Ni), cadmium (Cd), magnesium (Mg), zinc (Zn), copper (Cu), and lead (Pb) using Standard

Methods for the Examination of Water and Wastewater 3125B by inductively coupled plasma mass spectrometry (ICP-MS) system (APHA et al 2017).

Biochemical analysis. Tubes with blood samples used for biochemical analysis were placed in a blood transport cooler, stored at a temperature of 4-8°C, and transported to the laboratory within two hours following collection.

The Hb content was analyzed using the auto hematology analyzer, BC-2800 (Mindray, China). The remaining parameters - glucose, urea, creatinine, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and protein - were analyzed using the automated clinical chemistry analyzer, Olympus AU400 (Japan). The biochemical parameters were analyzed in the laboratory of Hoan Hao Clinic, Hai Thuong Lan Ong Street, Ha Tinh City, Ha Tinh Province, Vietnam.

Counting the components of blood. A portion of fish blood was diluted 250 times with a physiological saline solution for freshwater fish (Hoar & Hickman 1983). The number of RBCs was manually counted using a Neubaer cell counting chamber. Another part of whole blood was used for blood smear and stain Giemsa according to the classical method. These steps were carried out in the field laboratory (Figure 2). All blood samples from fish groups on different sampling days were marked, labeled, and preserved carefully. Other detailed research activities were carried out at the laboratory of the Institute of Tropical Ecology, Vietnam-Russia Tropical Center. Pictures of blood smears were taken using an Olympus CX33 optical microscope (Japan) and the relative ratio of WBCs and platelets to the number of RBCs was quantified directly through the slide images.

Figure 2. Red blood cell counting in the field laboratory.

Image processing and statistical analysis. Images were processed to increase contrast using Photoshop CS6 software and their resolution was increased using the free online image processing AI tool, Picwish.

The obtained data were statistically processed using IBM SPSS Statistics software (version 20) at the laboratory of the Institute of Tropical Ecology, Vietnam-Russia Tropical Center and the results were presented as mean±SD (standard deviation). The Kruskal-Wallis test and Dunn's test were conducted for biochemical test results and the analysis of variance (ANOVA) test was applied to RBC morphology data. Statistically significant differences were considered with p-value ≤ 0.05.

Results. The quantity of 11 heavy metals in the culture environment was analyzed and it was found that most were undetectable or at very low levels, except for Mg, less than 1 mg L^{-1} (Table 1).

Table 1

Quantity of 11 heavy metals in the culture environment

	Cr	*Fe*	*As*	*Mn*	*Hg*	*Ni*	*Cd*	*Mg*	*Zn*	*Cu*	*Pb*
D1	ND	0.011	ND	< 0.0045	ND	ND	ND	0.931	0.016	ND	ND
D5	ND	0.011	ND	< 0.0045	ND	ND	ND	0.877	0.017	ND	ND
D10	ND	0.011	ND	< 0.0045	ND	ND	ND	0.831	0.019	ND	ND
D15	ND	0.012	ND	< 0.0045	ND	ND	ND	0.096	0.017	ND	ND

Note: Unit of measure – mg L^{-1}; ND - not detected; D1, D5, D10, D15 - date of water sampling.

A total of 21/50 red drum individuals died during the experiment. Details of events and blood sampling dates are presented in Table 2.

Table 2

List of events that occurred during the experiment

No.	*Experiment day*	*Number of specimens from which blood samples were taken*	*Number of dead fish*	*Number of remaining fish*
1	1	10	0	40
2	5	8	0	32
3	8	-	2	30
4	9	-	2	28
5	10	8	1	19
6	12	-	6	13
7	13	-	2	11
8	14	-	5	6
9	15	3	3	0
Total		29	21	
Death rate		42%		

The results of a whole blood smear showed the components, RBCs, neutrophils, basophils, lymphocytes, platelets, and monocytes. In the platelet group, two morphological types were mainly recorded, including needle-shaped and spherical platelets (Figure 3).

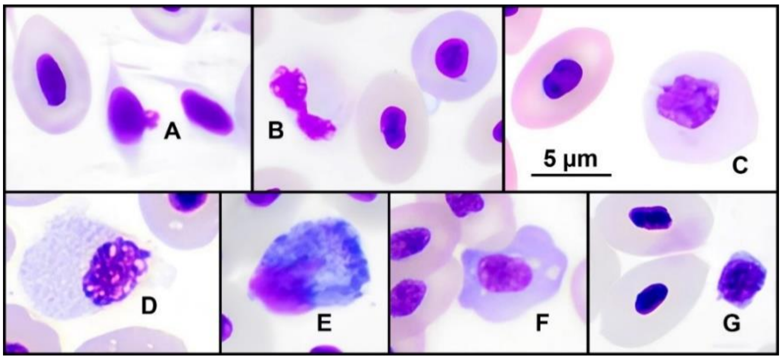

Figure 3. Red drum blood cell composition in freshwater culture conditions: A - thrombocytes, B - segmented neutrophil, C - monocyte, D – neutrophilic megagranulocyte, E - basophil, F - basophilic erythroblast, G - small lymphocyte. Ruler is 5 μm for all images.

The size of RBCs in fish groups on sampling days was measured and presented in Table 3.

Table 3

Morphological indices of the RBCs of red drum during the experiments

	Dc, μm	*dc, μm*	*Dn, μm*	*dn, μm*	*Sc, μm²*	*Sn, μm²*
D1	11.06±0.75ᵃ	7.01±0.68ᵃ	4.30±0.48ᵃ	2.86±0.46ᵃ	63.21±5.95ᵃ	9.80±2.47ᵃ
D5	10.41±0.94ᵇ	7.99±0.74ᵇ	3.72±0.39ᵇ	2.95±0.34ᵇ	66.31±9.77ᵇ	9.23±1.85ᵇ
D10	10.99±0.82ᶜ	7.29±0.58ᶜ	3.97±0.34ᶜ	2.72±0.27ᶜ	64.97±6.40ᵇ	8.18±0.82ᶜ
D15	10.04±1.63ᵇ	7.04±1.11ᵃ	4.03±0.59ᶜ	2.75±0.43ᶜ	65.55±8.59ᵇ	8.09±1.02ᶜ

Note: Dc, dc, Sc - cell length, width, and area; Dn, dn, Sn - nucleus length, width, and area; D1, D5, D10, D15 - date of blood sampling; [a, b, c,] – statistically significant difference between groups according to the column ($p < 0.05$).

At the same time, the results of the specimen under an optical microscope showed RBCs with morphological disorders related to the nucleus and nuclear membrane (Figure 4), although the rate is small at only 1-5%. Such forms include erythrocytes losing the nucleus (Figure 4A), bud-nucleus (Figure 4B), bi-nucleus (Figure 4C), notched nucleus (Figure 4D), and nucleus with wrinkled membrane (Figure 4E-G).

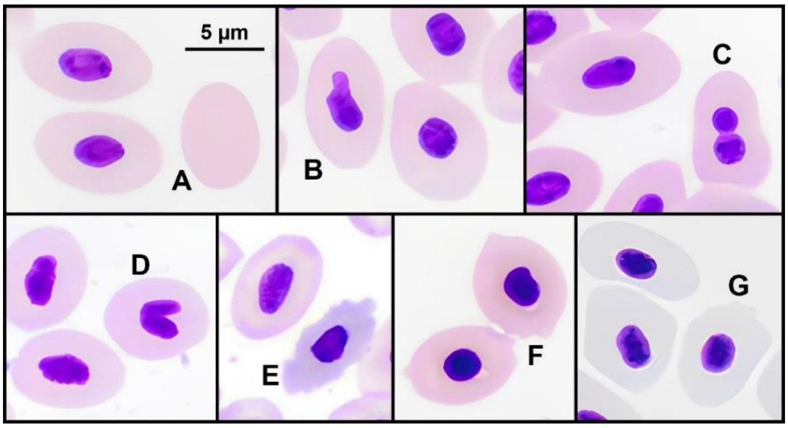

Figure 4. Red blood cell morphology disorder in red drum in the freshwater experimental environment using commercial feed: A (D1) - erythrocyte without a nucleus; B (D1) - erythrocyte with bud-nucleus; C (D1) - bi-nucleus; D (D1) - notched nucleus; E (D1), F (D5), G (D10) - wrinkled membrane. Ruler is 5μm for all images.

The results of measuring cell component index as well as biochemical indices are presented in Table 4.

Table 4

Blood cell composition and biochemical data

Hematological parameters	*D1*	*D5*	*D10*	*D15*
RBC ($x10^9$ mL^{-1})	2.67±0.48ᵃ	2.79±0.58ᵃ	3.33±0.94ᵇ	2.82±0.51ᵃ
WBC % (compared to 100 RBCs)	1.04±0.78ᵃ	1.65±0.79ᵃ	1.91±1.07ᵃ	1.85±1.01ᵃ
Thromb (compared to 100 RBCs)	1.13±1.03ᵃ	0.20±0.33ᵇ	0.80±0.82ᵃ	0.65±0.72ᵃᵇ
HgB (gL^{-1})	102.16±7.06ᵃ	103.33±6.47ᵃ	105.00±4.69ᵃ	104.11±5.24ᵃ
Glucose (mmol L^{-1})	5.62±1.65ᵃ	4.32±2.22ᵃ	5.70±2.00ᵃ	5.56±1.66ᵃ
Urea (mmol L^{-1})	1.76±0.63ᵃ	1.07±0.73ᵃ	0.55±0.51ᵃ	1.44±0.44ᵃ
Creatinine (μmol L^{-1})	28.66±5.19ᵃ	32.00±6.78ᵃ	17.75±4.92ᵇ	25.22±3.61ᵃᵇ
Triglyceride (mmol L^{-1})	3.07±1.12ᵃ	3.73±1.48ᵃ	3.20±0.27ᵃ	3.05±0.53ᵃ
AST (U L^{-1})	30.82±12.52ᵃ	33.92±26.51ᵃ	23.18±9.06ᵃ	22.10±4.59ᵃ
ALT (U L^{-1})	1.68±0.87ᵃ	1.72±1.49ᵃ	1.98±0.67ᵃ	1.48±0.59ᵃ
Protein (g L^{-1})	40.53±6.65ᵃᵇ	34.87±2.22ᵃ	42.15±5.11ᵇ	40.98±6.84ᵃᵇ

Note: RBC - red blood cell; WBC - white blood cell; Thromb – thrombocytes; HgB – hemoglobin; AST - aspartate aminotransferase; ATL - alanine aminotransferase; D1, D5, D10, D15 - date of blood sampling; [a, b, c,] – statistically significant difference between groups according to the row ($p < 0.05$).

During most of the experiments, RBCs, WBCs, HgB, glucose, urea, triglycerides, AST, and ALT were stable and the difference was not statistically significant. Platelet count and protein quantity decreased on day 5, while creatinine decreased on day 10 of the experiment.

Discussion. During the experiment, 21/50 individuals died, making up 42% of the total individuals, even though the rearing time was quite short. The total time was only about 20 days (including five days of switching from water with a salt concentration of 26‰ to fresh water with a salt concentration of 1‰). Research by Gullian-Klanian (2013) showed that the survival rate of red drum during an eight-month grow-out period in freshwater was 57.6%. It should also be noted that the stocking density in this study was very high, with 50 individuals with about 4.4 m^3 of water, even though the water was changed daily and abundant oxygen was provided. However, the mortality rate was high, this is believed to be related to stress because of the short period of adaptation to changes in salt concentration (five days) as well as the negative effects of waste products from the fish themselves. The low survival rate of red drum in freshwater conditions is thought to be related to fish plasma osmolality, characterized by the difference in the concentration of Ca^{2+}, K^+, and Mg^{2+} ions between the fish's body and the water environment (Wurts & Stickney 1993; Gullian-Klanian 2013). To be able to survive long-term in water with low salt concentrations, red drum must be able to maintain relatively stable plasma ion concentrations (Gullian-Klanian 2013) to ensure metabolic activities take place normally. However, the research results showed that 1‰ salt content was not a suitable environment for the ideal, long-term growth of red drum. This result is similar to the observation of Gullian-Klanian (2013) that red drum was not completely adapted to freshwater environments.

Hematological studies of fish play an important role, especially in health and disease monitoring. There have been studies related to the blood of red drum, however, most of the studies have provided data in marine environments. This study provides new data, including blood biochemical indices of this species under experimental conditions of freshwater culture and using commercial feed.

The composition of red drum blood cells in this study was similar to that of other bony fish species. However, eosinophils were not recorded in the granulocyte composition, which is different from the results of Harr et al (2018), in which the authors recorded the presence of a very small proportion of these cells. The absence or presence of a very small number in the blood composition of red drum may be due to the role of eosinophils that can be taken over by other cells (Ainsworth 1992).

The research result of Gullian-Klanian (2013) showed that the RBCs of red drum in marine water environment are from 1.54 to 1.75 x 10^9 mL^{-1}, in freshwater environment from 1.70 to 2.61 x 10^9 mL^{-1}. Research on red drum in some sea areas of Vietnam also reported the erythrocyte content from 1.46 to 3.43 x 10^9 mL^{-1} (Quyet 2022) and in this experiment it was from 2.19 to 4.27 x 10^9 mL^{-1}. The increase of erythrocytes in freshwater environment compared to saltwater environment is an adaptive mechanism that many species use to enhance the ability to transport dissolved oxygen in water better (Franklin et al 1991). The difference in the number of RBCs of red drum in both fresh and salt water once again confirms its dependence on other physiological characteristics, environmental characteristics, and living conditions.

The number of RBCs, the changes in their morphology such as size, surface area, volume and hemoglobin content in fish blood are closely related to the metabolic intensity and oxygen demand of fish (Maciak & Kostelecka-Myrcha 2011; Dal'Bó et al 2015). The results of the study showed that the RBC content increased on the day 10 compared to the first and fifth days, while the difference in hemoglobin content at different times was insignificant; at the same time, the size of red blood cells underwent complex changes. One of the important factors that directly affects the rate of oxygen and carbon dioxide exchange is the red blood cell surface area and here, this index on days 5, 10 and 15 were all higher than on the first day. We belive that, in addition to the increase in the number of RBCs, the increase in surface area is also one of the mechanisms that helps the hemoglobin oxidation and deoxygenation reactions to occur faster. This is also consistent with previous research results (Richardson & Swietach 2016; Jeamah et al 2023).

RBC morphology disorders have been reported in cases related to environmental pollution by pesticides (Saxena & Seth 2002) or heavy metals (Takashima & Hibiya 1995; Witeska 2013). Examination of RBC morphology through blood smears is one of the simple, easy-to-perform, and inexpensive methods that allows assessment of the quality of the fish's living environment (Carrasco et al 1990). The noteworthy point in the results of this experiment is that the RBC disorder was relatively low, in general, only about 1-5%. This is completely different from the results of blood research on red drum in 2018 in a saltwater environment (Quyet 2022) only about 1 km from the above-mentioned experiment location. At that time, the rate of morphological disorders in the RBCs was high in red drum with up to 82% for nuclear-matter distribution and 78% for nuclear deformation. Comparing the results of the current research with data from previous research (Quyet 2022) helps strengthen the hypothesis that the RBC disorder of red drum is related to the heavy metal content in the water environment even though they are all within the threshold of normal standards.

As is known, in lower animals, leukocytes play a major role in the immune system, fighting against foreign agents that invade the body (Tavares-Dias et al 2007), while platelets directly participate in blood clotting processes (Hill & Rowley 1996; Arasu et al 2016). However, the participation of RBCs and platelets in immune responses in lower animals such as fish has also been demonstrated (Nagasawa et al 2014; Stosik et al 2020; Ortiz & Esteban 2024). The ratio of leukocytes to red blood cells in red drum has been reported to range from 0 to 1% (Harr et al 2018) or from 2 to 4% (Quyet 2022). The number of leukocytes depends largely on the status of the immune system as well as the environment in which the fish lives. The white blood cell count in this study was quite variable, and the difference between experimental time points was not statistically significant. Meanwhile, platelets decreased significantly on day 5 and tended to stabilize on days 10 and 15 of the experiment. In most cases, acute negative effects usually increase the white blood cell count (Shaluei et al 2013), while in the long term, such effects weaken the immune system and the white blood cell count decreases (Witeska et al 2010). It should be noted that large fluctuations in white blood cell counts also indicate individual immune responses, or errors in the identification of small lymphocytes and platelets (Farrell 2011). The distinction between these two cell types is not always clear under light microscopy. However, we believe that red drum living under experimental conditions are exposed to many negative impacts from the aquatic environment, including their waste products and stress due to high stocking density.

The remaining hematological parameters, such as HgB, glucose, urea, creatinine, AST, ALT, triglycerides, and protein, can provide valuable information about the overall health status, metabolic capacity, and the influence of environmental factors on fish (Oner et al 2008). HgB and glucose reflect oxygen or energy needs, stress levels, and nutritional status (Jawad et al 2004; Tavares-Dias & Moraes 2004). Urea and creatinine indices indicate kidney function and whether there is a protein metabolism disorder (Kulkarni & Pruthviraj 2016). The two enzymes, AST and ALT, provide information about liver or muscle function (Kavadias et al 2004; Kulkarni & Pruthviraj 2016). The results of the study showed that most of the biochemical indices were quite stable, and the differences between time points were not statistically significant, except for the creatinine and protein indices. The response of fish to stress is considered extremely varied, and it is so diverse that it prevents the creation of a unified and general description of the effects of environmental stress factors on fish (Balasch & Tort 2019). This means it is difficult to predict or affirm anything specific based on the increase or decrease in creatinine or protein levels in the red drum mentioned above. It is clear that red drum in the above experimental conditions is less affected by external factors than fish living in the estuary environment where factors, such as changes in temperature, salinity, wastewater, and potentially contaminated components from residential areas can affect them adversely. As a result, fish blood biochemical values achieved relative stability with few major fluctuations during the experiment.

Conclusions. In a freshwater experimental farming environment using synthetic feed, the red drum showed poor adaptation with a mortality rate of up to 42%. Most hematological parameters (RBC, WBC, and HgB) and blood biochemical indices (glucose, urea, triglyceride, AST, and ALT) were stable during the experiment, except thrombocytes, creatinine, and protein. The author recorded a small number of RBC morphological disorders, such as loss of nucleus, bud-nucleus, bi-nucleus, notched nucleus, and wrinkled cell membrane. While red drum are often considered tolerant of higher stocking densities compared to their natural habitats, our study examined the combined effects of two potential stressors: high initial stocking density and very low salinity. This highlights the need for further research to elucidate the intricate interactions between these factors and their impact on erythrocyte morphological abnormalities in this species.

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Conflict of interest. The author declares that there is no conflict of interest.

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Author:
Do Huu Quyet, Institute of Tropical Ecology, Joint Vietnam-Russian Tropical Science and Technology Research Center, 63 Nguyen Van Huyen, Nghia Do Ward, Cau Giay District, Hanoi, Vietnam, e-mail: dhquyet@gmail.com

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