HAYATI
Journal of Biosciences

Vol. 30 No. 4, July 2023 711-715
DOI:10.4308/hjb.30.4.711-715
ISSN: 1978-3019
EISSN: 2086-4094

Morphological Alterations of Fish Erythrocytes as Their Response to Environmental Conditions

D.H. Quyet*, **P.T. Dung**

Institute of Tropical Ecology, Vietnam-Russia Tropical Center, 63 Nguyen Van Huyen Str., Nghia Do Ward, Cau Giay District, Hanoi, Vietnam

ARTICLE INFO

Article history:
Received September 15, 2022
Received in revised form January 27, 2023
Accepted February 16, 2023

KEYWORDS:
erythrocyte,
marine fish,
red blood cells,
disorder,
environment

ABSTRACT

Fish bodies respond to changes in environmental conditions in various ways and degrees. Seven fish species were collected from three study locations, including two nearshore sites (Ha Tinh province and Nha Trang city, Khanh Hoa province) and one offshore site (Toc Tan island, Khanh Hoa province) to evaluate alterations in erythrocyte morphology under certain environmental conditions. Heavy metal concentrations at all study sites were analyzed and met the acceptable technical criteria for each site. Fish blood samples were taken from the tail veins, anticoagulated with heparin, and stained with Giemsa stain. The blood cell morphological study was performed using an optical microscope. The results revealed two types of morphological abnormalities of fish erythrocytes, including nuclear deformation and nuclear-matter distribution.

1. Introduction

Environmental and human factors impact both freshwater and marine fish. Each type of factor may greatly impact the physiology and biochemistry of fish from the cellular to the organ system level. Anthropogenically induced physical factors, such as noise and vehicles, cause stress and behavioral changes in fish (Leduc *et al.* 2021; Mickle and Higgs 2018). Pathogenic agents like bacteria, viruses, and parasites may quantitatively alter the composition of the blood (Martins *et al.* 2008). For instance, microorganisms in the blood, toxic chemicals, and heavy metals cause morphological changes in red blood cells and cell division disorders (Novotny *et al.* 2004; Quyet 2021; Witeska 2013).

The erythrocytes of fish are susceptible to changes in environmental conditions (Witeska 2013). However, basic quantitative measurements of fish blood cells (such as hematocrit, red blood cell count, or hemoglobin concentration) tend to be stable due to their significant potential offset (Vosylien 1999). Depending on the species and contributing variables, the number of red blood cells in fish can increase or decrease (Jezierska and Witeska 2001). Several investigations have confirmed that harmful chemical substances typically induce morphological alterations in fish erythrocytes, which are expressed in various degrees. In addition, the abnormal expression of fish erythrocytes depends on the concentration of chemical substances, exposure period, and species features (Witeska 2013). As a result, fish erythrocytes are considered biological indicators representing the quality of the aquatic environment and the nutrition of organisms.

The objective of this study was to evaluate how different fish species in Ha Tinh province and Khanh Hoa province (Nha Trang city and Toc Tan island) responded to the environmental conditions based on the expression of morphological abnormalities in their red blood cells.

2. Materials and Methods

2.1. The Study Areas and Sampling Strategy

The study was conducted in two nearshore sites in August and September 2019 (Thach Kim commune, Loc ha district, Ha Tinh province (18°27'8.12"N, 105°55'5.67"E) and Nha Trang city, Khanh Khoa province (12°11'6.17"N, 109°13'13.29"E), and one offshore site in October 2020 (Alison Reef (Toc Tan island, 8°48'27.52"N, 113°58'46.92"E), Spratly Islands (Truong Sa archipelago), Khanh Hoa province).

The temperature and salinity of the water were measured at each sampling site during the time of

* Corresponding Author
E-mail Address: dhquyet@gmail.com

sampling using a thermometer and a refractometer to understand how environmental parameters vary in the study areas, respectively. The surface water samples were collected and preserved for the determination of heavy metal content (Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Hg, Pb) by Inductively coupled plasma mass spectrometry (ICP–MS) using the method described in the SMEWW 3125B:2017 (APHA *et al.* 2017).

2.2. Fish Sampling and Blood Preparation

2.2.1. Fish Sampling

At the two nearshore sites, marine fish were either captured in the coastal area using fishing rods or purchased directly from cage fish farms. At the offshore study site, fish were only caught with fishing rods.

2.2.2. Ringer's Physiological Saline Solution Preparation

Physiological saline solution for marine fish was prepared according to the recipe of Hoar and Hickman (1975). In one liter of distilled water, dissolve the following salts: 7.8 g NaCl, 0.18 g KCl, 0.166 g $CaCl_2$, 0.095 g $MgSO_4$, 0.084 g $NaHCO_3$, and 0.06 g NaH_2PO_4.

2.2.3. Fish Blood Preparation

Blood was drawn from the tail veins of the fish with a 5 ml syringe containing 1-2 drops of Ringer solution with heparin (500 IU/ml). The tube was shaken at least 8 times inversely for the optimum anticoagulant action. When drawing blood, either fish male or female was considered. 4-8 individuals represented each species.

Fish blood specimens were prepared using the standard method of distributing blood on slides and staining with 5% Giemsa dye. The blood cells were examined and photographed at 40× and 100× magnifications using an Olympus CX43 microscope and ImageJ software (v.152r). The images were homogenized colors and removed stains by Photoshop CS6 software. ImageJ software (v.152r) was applied to measure the blood cells.

3. Results

The water parameters at the three study sites, including temperature, salinity, and concentration of heavy metals, are listed in Table 1.

The study was carried out on erythrocytes from seven fish species, including *Epinephelus fuscoguttatus, Lates calcarifer, Scatophagus argus, Aluterus monoceros, Sciaenops ocellatus, Balistoides viridescens, Elagatis bipinnulata.* The findings revealed that the erythrocytes of fish undergo morphological alterations that are mostly expressed in four features, including cellular deformation, nuclear deformation, membrane wrinkling, and nuclear-matter distribution (Figure 1).

Furthermore, one of the seven mackerel species showed a moderate disorder, and two expressed disordered morphological traits. The remaining four displayed either an ambiguous or a slow rate of change (Table 2).

Compared to other species in the study, the red drum Sciaenops ocellatus and the titan triggerfish *Balistoides viridescens* showed the most apparent erythrocyte morphological disorders in comparison with other species in the study. The highest rates of nuclear deformation (34.98%) and nuclear-matter dispersion (96.45%) were found in *Balistoides viridescens.* The nuclear deformation rate in Sciaenops ocellatus

Table 1. Water parameters at the study sites

Targets	Unit	HT#	NT#	QC10	Alison reef (TS)	QC44	MPC^
Temperature	°C	27.00	27.00	-	27.00		-
Salinity	%	24.00	35.00	-	33.70		-
Chromium	µg/l	0.76	0.92	100	-	50.0	-
Manganese	µg/l	1.56	128.07	500	-		-
Iron	µg/l	54.78	176.23	500	-		($Fe^{2+/3+}$): <100–200*
Nickel	µg/l	0.42	-	-	-		30–75**
Copper	µg/l	0.74	1.18	200	5.29	10.0	1–10*
Zinc	µg/l	4.01	7.24	500	11.05	20.0	100–1,000**
Arsenic	µg/l	1.39	2.34	20	1.36	5.0	3,000–30,000**
Cadmium	µg/l	0.04	0.14	5	0.14	1.0	0.2–1.0* 2,000–20,000**
Mercury	µg/l	ND	0.10	1	-	0.2	0.3–2.0* 200–4,000**
Lead	µg/l	2.03	1.87	50	2.03	5.0	4–70* 1,000–1,000,000**

ND - Not Detected; QC10, QC44 – National technical regulation on marine water quality (Ministry of Natural Resources and Environment); MPC –maximum permissible concentration; # – Quyet (2021); ^ – Svobodova *et al.* (1993); * – acceptable limitations in aquaculture; ** – lethal concentration of the parameter for fish is determined by various variables, including organic and inorganic compounds, water hardness, fish species, and others

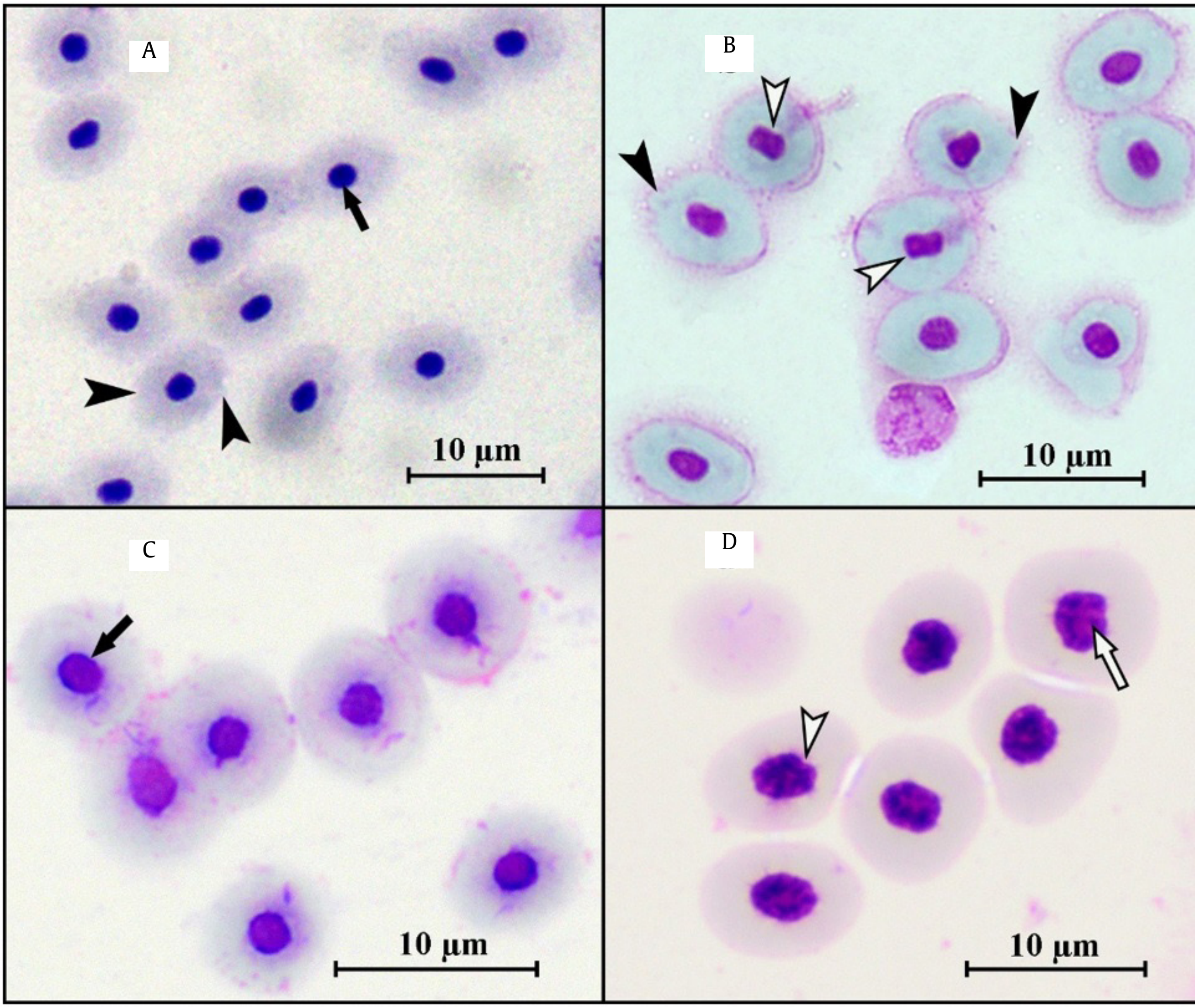

Figure 1. Dysmorphic condition of erythrocytes in fish (100×). (A) *Lates calcarifer*, (B) *Scienops ocellatus*, (C) *Elagatis bipinnulata*, (D) *Balistoides viridescens*. Black arrowhead-membrane wrinkling; white arrowhead-nuclear deformation; black arrow-normal cell nucleus; white arrow-nuclear-matter distribution

Table 2. The rate of fish erythrocyte morphological disorders

Name of the species (cell count)	Location	The rate of fish erythrocyte morphological disorders			
		RLc (%)	RLn (%)	RLm (%)	RLcn (%)
Epinephelus fuscoguttatus (503)	HT	–	–	–	–
Lates calcarifer (718)*	HT	–	0.42	0.28	–
Scatophagus argus (509)	NT	0.20	–	–	–
Aluterus monoceros (891)	NT	–	1.12	–	–
Sciaenops ocellatus (1113) *	NT	0.54	16.44	1.08	0.54
Balistoides viridescens (1015)	TS	–	34.98	–	96.45
Elagatis bipinnulata (734)	TS	0.27	3.95	2.18	1.09

* - cage-raised fish, HT – Ha Tinh, NT – Nha Trang, TS – Truong Sa, RLc – cellular deformation, RLn – nuclear deformation, RLm – membrane wrinkling, RLcn – nuclear-matter distribution

was lower, at 16.44%. *Elagatis bipinnulata* expressed all four types of erythrocyte morphological disorders at low rates (less than 4%). The remaining four examined species under study environmental conditions showed no erythrocyte disorder or low abnormality rate (less than 4%).

4. Discussion

Fish respond differently to various combinations of water parameters. Abiotic factors that affect the fish body are often divided into physical and chemical. Temperature, light, or human activity like freight transportation, tourism, wharf activities, and noise are examples of physical factors. Wastes of daily life, tourism, fishing explosives, and the exploitation of marine resources, which are discharged into the ocean (either fully or partially treated), as well as heavy metals, pH, or salinity, are among the chemical factors. Biotic factors include aquatic animals, vegetation, microbes, and all species' interaction and competition.

Fish have been reported to have erythrocyte morphological disorders when the environment is contaminated by a variety of factors, particularly organic substances such as phenol compounds (Sharma and Chadha 2021), detergents (Zeni *et al.* 2002), tributyltin chloride (TBTC) (Tiano *et al.* 2003), thiamethoxam (Ghaffar *et al.* 2020), tetrabromodiphenyl ether (Bolognesi *et al.* 2006), as well as heavy metals like Cr (Suchana *et al.* 2021), Hg (Gwoździński 1992), Pb (Ahmed *et al.* 2022; Hofer *et al.* 1992; Monteiro *et al.* 2011; Witeska 2004), Cd (Hofer *et al.* 1992; Witeska 2004; Witeska *et al.* 2006), Cu (Bagdonas and Vosylienė 2006; Gwoździński 1992; Witeska 2004), Zn (Bagdonas and Vosylienė 2006). Fish also exhibit a wide range of erythrocyte morphological disorders, which vary between species. They include broken red blood cells, nuclear deformation, nucleolus appearance, concave cell nucleus, segmented cell nucleus, the appearance of Heinz body, cell deformation, membrane defects, wrinkled membranes, and mitosis (Witeska 2013). Under the same lead exposure conditions, erythrocytes in *Prochilodus lineatus* species demonstrated cell deformation and nucleus fragmentation (Monteiro *et al.* 2011), but in *Cyprinus carpio*-anomalies included chromatin condensation at the nucleus border, nuclear malformations, cytoplasm vacuolization, swelling and deformation of entire cells (Witeska 2004). Alternatively, Gwoździński (1992) found anomalies in the red blood cell membranes of *Cyprinus carpio* that had been exposed to Cu and Hg.

The study indicates a variety of erythrocyte morphological disorders under certain environmental conditions. However, our results also show that the concentrations of heavy metals both nearshore and offshore are within acceptable limits according to the National technical regulation on marine water quality (QC44 2012; QC10 2015). Furthermore, offshore seawater's acceptable heavy metal limits are relatively low compared to nearshore seawater. From the perspective of resource and environmental management, these criteria assure the general safety of humans and marine life. Therefore, our findings demonstrate that, despite the low concentrations of heavy metals in seawater, their simultaneous presence has specific impacts on the fish body at the cellular level. This result is also in line with previous observations of Vosylienė (1999) and Zeitoun and El-Sayed (2014 on erythrocyte morphology disorder under several environmental factors. The erythrocyte morphological disorders in fish observed in this study do not necessarily indicate contaminated seawater; rather, some fish species exhibit these abnormalities in response to unfavorable environmental conditions. The major evidence is that some fish species have high rates of erythrocyte morphological disorders while others have much lower rates.

Commonly, commercial and nearshore fish species are typically more impacted by human activities than offshore ones. However, the study's results showed that the erythrocytes of three nearshore fish species (*Epinephelus fuscoguttatus, Scatophagus argus*, and *Aluterus monoceros*) exhibited a lower rate of disorders than the offshore fish *Balistoides viridescens*. Despite being wild fish, the nearshore and offshore fish species displayed erythrocyte morphological disorders at very different rates. That is why we believe that heavy metal content and other chemical substances in water environments influence erythrocyte morphological disorders in fish more than physical factors like aquaculture and human activities.

Acknowledgements

The author would like to express his heartfelt gratitude to the officers of the 6th Navy Group's ship 632, two colleagues, Tran Van Dat and Nguyen Tai Tu, for their assistance collecting samples during the survey. I sincerely thank Dr. Astakhov DA for his assistance in identifying the fish species based on the images. The was mainly supported by the Grassroots-level project "Evaluation of erythrocyte morphological characteristics and hematological parameters of common fish species in the central coastal area (Ha Tinh) at risk of pollution due to discharge," funded by the Vietnamese State's funding for scientific research activities, and partly by the Ministry of National Defense project KCB-TS01 (project coordinator Dr. Hoang Thi Thuy Duong).

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