Anais da Academia Brasileira de Ciências (2012) 84(4): 943-949
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
www.scielo.br/aabc

Hematological parameters of Iranian cichlid *Iranocichla hormuzensis* - Coad, 1982 (Perciformes) in Mehran River

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Manuscript received on January 1, 2011; accepted for publication on April 26, 2011

ABSTRACT

This study describes the hematological parameters in *Iranocichla hormuzensis*, an Iranian freshwater cichlid important as ornamental and food fish. Forty fish were captured with seine net at Mehran river Hormozgan province, Iran. Blood was used to determine the total counts of red blood cells (RBC) and white blood cells (WBC), hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and morphometric data of erythrocytes. The Iranian fish showed lower RBC and WBC values than the other cichlids (*Oreochromis niloticus*, *O. aureus*, *O. mossambicus*, *O.* hybrid, *Cichlasoma dimerus* and *Cichla monoculus*). Hematocrit did not vary among the species, but MCV, MCH and MCHC in *I. hormuzensis* were higher than those for *O. niloticus*, *O. aureus*, *O.* hybrid, *C. dimerus* and *C. monoculus*. These differences may be related to different life habit of fish. This study suggests that *I. hormuzensis* is well acclimated to the environment being the first report for its hematology. It is also suggested high efficiency in oxygen transportation, and an efficient inflow of oxygen by the gills, indicating the welfare of fish on this environment.

Key words: Cichlidae, Iran, hematology, erythrocytes.

INTRODUCTION

The family Cichlidae comprises about 150 genera and 1,300 species, making it the second largest perciform family. They are found in fresh and brackish waters of Central and South America, Africa, Madagascar, the Levant, Southern India, Sri Lanka and Southern Iran. These fish present colorful body and are mostly used as ornamental fish. *Iranocichla hormuzensis* Coad 1982 is the unique native species in Iran, which is recognized by darkened rays and lack of spots in the pectoral fin specially by the single nostril opening on each side of the head (Coad 1982). This Iranian cichlid is endemic to Fars and Hormuzgan provinces in Southern Iran. Its habit is benthopelagic, being found in waters with high temperature and mineral content draining into the Persian Gulf at the Strait of Hormuz. The fish mainly feeds itself by scraping the algae and detritus (Esmaeili et al. 2010). Iranocichla hormuzensis is locally called "Mahi-e-Karoo" and usually eaten by local people when it is available in large numbers during spring (Esmaeili et al. 2010). Although considered as an aquarium fish in Iran, this species has not been kept in aquarium and there is no information on its reproduction for aquaculture.

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Hematological parameters are considered an important indicator of fish health status, and provide valuable information to assess the fish welfare (Azevedo et al. 2006). Hematology is also used as an indicator of physiological and pathological changes in fish (Chekrabarty and Banerjee 1988, Martins et al. 2008). It can be affected by several factors including gonad maturation (Ranzani-Paiva and Godinho 1985), dissolved oxygen alterations (Ranzani-Paiva et al. 2000), gender (Lusková 1998), spawning and water temperature (Joshi 1982), lotic or lentic environment (Val et al. 1985), handling stress and transportation (Gbore et al. 2006), fish inflammation (Martins et al. 2006), size, feeding and stocking density (Rey Vázquez and Guerrero 2007), microbial infection and parasitism (Martins et al. 2004, Azevedo et al. 2006. Jamalzadeh et al. 2009).

Except for farmed fish, there is no information on the fish hematology in the Middle East fish species. Hematological assessment in wild freshwater fish was performed in *Barbus holudi*, *Cyprinus carpio*, *Labeo umbratus* and *L. capensis* from Dams of the Orange Free State, South Africa (van Vuren et al. 1978); in *Hypostomus regain* from Southeastern Brazil (Val et al. 1985); in 20 fish species from the floodplain of the upper Parana River - Brazil, including a cichlid *Cichla monoculus* (Ranzani-Paiva et al. 2000); in *Capoeta trutta* from Karakaya Dam Lake and Munzur River, Turkey (Danabas et al. 2010); in nine fish species from Tibagi River, Southern Brazil (Silva Souza et al. 2002); in *Salminus maxilosus* from Mogi-Guacu River, Southeastern Brazil (Ranzani-Paiva et al. 2003); in *Cichlasoma dimerus* from Corrientes, Argentina (Rey Vázquez and Guerrero 2007); in *Pimelodus maculatus* from the Itajaí-Açu River, Southern Brazil (Jerônimo et al. 2009); and in *Auchenipterus nuchalis*, *Psectrogaster amazonica* and *Squaliforma emarginata* from Tocantins River, Northern Brazil (Carvalho et al. 2009).

The aim of this study is to determine the hematological parameters in *I. hormuzensis* of the lower Mehran River, Iran. The collected data provide the first information on the blood composition for this species and could be used as an important tool for fish health diagnosis.

MATERIALS AND METHODS

Forty fish with mean weight 7.51 ± 1.91 g and mean length 74.2 ± 6.6 mm were captured with seine net from lower Mehran River (26°52′53″ N, 55°16′21″E) at 31 meters altitude during August 2009. The water temperature and salinity during the collection period were 33° - 34°C and 34 g/L, respectively.

Blood samples were taken from the caudal vein of each fish using a syringe rinsed with heparin (5000 unit mL^{-1}, Rotexmedica) and transferred to individual sterilized vials (at 4°C) containing 10 µL heparin, respectively. If a fish was too small to have enough blood for analysis, blood samples from two fish would be pooled to make a single sample according to Silva et al. (2009). After blood collection in the riverside, the samples were maintained on ice and sent to the laboratory of the Isfahan University of Technology. All procedures were carried out according to the international practices for animal use and care under the control of an internal committee of the Isfahan University of Technology. Hematocrit percentage was determined according to Goldenfarb et al. (1971) and hemoglobin concentration was obtained by the cyanomethemoglobin spectrophotometry method (JENWAY 6400 Spectrophotometer) (Blaxhall and Daisley 1973).

Total counts of red blood cells (RBC) and white blood cells (WBC) were determined in a hemocytometer after dilution with Race solution which containing 0.1 g brilliant cresyl blue, 3.8 g sodium citrate, and 0.2 mL formaldehyde 37% in 100 mL distilled water. The mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Houston (1990).

In the laboratory, blood smears were stained with Giemsa (10% in Sorenson buffer at a pH 6.8). Twenty to thirty cells from each slide of each fish

(n = 40) were selected for measurement of the major and the minor diameters of the erythrocytes and their nuclei with a Leica light microscope (Wetzlar, Germany) (Benfey et al. 1984). The cell surface, nuclear area and their volume were calculated using the formula suggested by Lemoine and Smith (1980):

$S = a \times b \times \pi/4$ and $V = [a/2] \times [b/2]\ 2 \times \pi \times 4/3$
Where *a* is the major and *b* is the minor axis of the cell or nucleus.

RESULTS

Iranocichla hormuzensis showed 0.96 ± 0.38 x 10^6/mm^3 and 10.05 ± 4.90 x 10^3/mm^3 in size, respectively for RBC and WBC (Table I). Hematocrit percentage was 26.9 ± 5.15% and hemoglobin concentration 6.67 ± 1.46 g/dL, while the mean corpuscular hemoglobin was 79.26 ± 31.63 µg/cell, mean corpuscular volume 307.24 ± 118.09 nm^3 and mean corpuscular hemoglobin concentration 0.26 ± 063 g/dL. The ratio WBC/RBC was 0.014. In terms of erythrocyte's size, the minor and major axis were 3.74 ± 0.45 and 5.51 ± 0.46 µm and of its nucleus 1.44 ± 0.24 and 2.24 ± 0.35 µm, respectively. The erythrocyte surface area was 16.22 ± 2.57 µm^2 showing a volume 41.08 ± 11.04 µm^3. Nuclear surface area was 2.56 ± 0.25 µm^2 with a volume of 3.42 ± 1.09 µm^3 (Table II).

DISCUSSION

Apart from some reports on the reproductive biology of *I. hormuzensis* from Mehran River by Esmaeili et al. (2010), nothing is known on its hematological parameters. If compared to cichlid fish, the mean RBC values of *I. hormuzensis* were lower than the reported by *Tilapia zilli* (Ezzat et al. 1974), *Sarotherodon melanotheron* (Lea Master et al. 1990), *O. niloticus* (Benli and Yildiz 2004, Silva et al. 2009, Kayode and Shamusideen 2010), *Cichla monoculus* (Ranzani-Paiva et al. 2000), Etroplus suratensis (Pathiratne and Rajapakshe 1998), *O. aureus* (Silveira-Coffigni et al. 2004), *Oreochromis* hybrid and *C. dimerus* (Rey Vázquez and Guerrero 2007), and *O. mossambicus* (Jaffar Ali and Rani 2009). Tavares-Dias et al. (2008) have suggested that species with high red blood cell counts have a greater adaptation to survive in environments with low levels of oxygen, as well as a higher metabolic activity. This could be explained for *I. hormuzensis* as a result of their lotic environment habit and consequently its low counts of RBC.

The main function of hemoglobin is to transport oxygen. Hemoglobin must be able to join O_2 strongly and release it when necessary (Perutz 1978). Erythrocytes are the dominant cell type in the blood of the majority of fish and they are important to keep the hemoglobin (respiratory pigment functionality). Studies on fish red blood cell, hematocrit and hemoglobin are important for health assessment and for investigation of oxygen carrying capacity (Tavares-Dias and Moraes 2004, Tavares-Dias et al. 2008). In this study, the hemoglobin concentration of Iranian cichlid *I. hormuzensis* was similar to other cichlids such as *Paralabidochromis beadlei* and *O. niloticus*, but was lower than those observed in *Pseudocrenilabrus multicolor* and *O. leucostictus*, as reported by Chapman et al. (2002). Hemoglobin concentration varies greatly in different animals and there is a correlation between hemoglobin concentration and the fish activity. As more active is the fish species, highest hemoglobin concentration can be observed (Tavares-Dias and Moraes 2004). Except for *O. mossambicus* (Jaffar Ali and Rani 2009) and *O. niloticus* (Kayode and Shamusideen 2010), *I. hormuzensis* showed higher values of MCV, MCH and MCHC when compared to other cichlids. Hematocrit percentage was similar to other cichlids. On the other hand, *I. hormuzensis* showed low number of erythrocytes, but high MCV and hemoglobin concentrations suggest high efficiency in oxygen transportation and efficient inflow of oxygen by the gills. According to Ranzani-Paiva et al. (2000) reduced hemoglobin concentration was reported in parasitized fish. Consequently, stressful conditions

TABLE I

Means (± standard deviation), minimum and maximum values of hematological parameters in Iranian cichlid *Iranocichla hormuzensis* in Mehran River and other cichlid fish.

Parameters	*I. hormuzensis* Present study	*S. melanotheron* Lea Master et al. 1990	*C. monoculus* Ranzani-Paiva et al. 2000	*O. niloticus* Benli and Yildis 2004	*C. dimerus* Rey Vázquez and Guerrero 2007	*O. niloticus* Silva et al. 2009	*O. mossambicus* Jaffar Ali and Rani 2009
RBC (10^6/mm^3)	0.96 ± 0.38 0.33-1.80 (20)*	1.4 ± 0.1	2.9 ± 0.8	2.2 ± 2.4	3.1 ± 1.0	2.1 ± 0.2	1.7 ± 0.2
Hematocrit (%)	26.90 ± 5.15 19.00-34.00 (10)	30.8 ± 2.7	58.0 ± 1.0	24.3 ± 0.9	31.3 ± 5.0	25.9 ± 15.0	30.0 ± 0.3
Hemoglobin (g/dL)	6.67 ± 1.46 4.83-11.11 (20)	7.3 ± 0.4	8.2 ± 0.5	7.7 ± 0.3	6.8 ± 1.0	-	8.1 ± 1.2
MCH (µg/cell)	79.26 ± 31.63 44.00-168.61 (20)	34.0 ± 6.6	27.9 ± 1.0	-	24.5 ± 8.3	-	41.8 ± 4.6
MCV (fL)	307.24 ± 118.09 122.22-463.41 (10)	228.0 ± 37.0	197.5 ± 1.8	-	110.3 ± 38.2	-	-
MCHC (g/dL)	26.00 ± 0.06 18.00-36.00 (20)	23.8 ± 2.0	14.1 ± 0.6	-	22.3 ± 3.8	-	29.2 ± 3.2
WBC (10^3/mm^3)	10.05 ± 4.90 3.00-21.00 (20)	6.2 ± 0.1	12.2 ± 3.8	12.0 ± 0.8	-	13.9 ± 0.8	4.2 ± 0.4
WBC/ RBC	0.014 ± 0.010 0.003-0.050 (20)	-	-	-	-	-	-

MCH - mean corpuscular hemoglobin, MCV - mean corpuscular volume, MCHC - mean corpuscular hemoglobin concentration, WBC - white blood cells, RBC - red blood cells. *Number of samples or measurements.

TABLE II

Means (± standard deviation), minimum and maximum values of erythrocyte size in Iranian cichlid *Iranocichla hormuzensis* in Mehran River. *Number of samples or measurements.

Parameters	Erythrocytes size	
Cell minor axis (µm)	3.74 ± 0.45	2.44-4.73 (173)*
Cell major axis (µm)	5.51 ± 0.46	4.4-6.6 (201)
Cell surface area (µm^2)	16.22 ± 2.57	8.95-23.75 (173)
Cell volume (µm^3)	41.08 ± 11.04	14.57-74.85 (173)
Nuclear minor axis (µm)	1.44 ± 0.24	0.88-2.16 (175)
Nuclear major axis (µm)	2.24 ± 0.35	1.56-3.53 (170)
Nuclear surface area (µm^2)	2.56 ± 0.25	1.21-4.6 (170)
Nuclear volume (µm^3)	3.42 ± 1.09	0.72-7.66 (170)

or parasitic infections could alter the hemoglobin concentration and fish blood profile, causing severe anemia. Nevertheless, the fish used on this work were apparently healthy with no signs of disease.

White blood cells play an important role in fish and are involved in the defense of the organism under several conditions, such as stress (Silveira-Coffigni et al. 2004), inflammation (Martins et al. 2006), and parasitism (Azevedo et al. 2006). The number of leukocytes is normally low in healthy fish and could be used as an indicator for infectious diseases (Jamalzadeh 2009). The average of WBC count was similar to the reported by *Morone* hybrid (Hrubec et al. 2001), *T. zilli* (Ezzat et al. 1974), *O. niloticus* (Azevedo et al. 2006) and *C. dimerus* (Rey Vázquez and Guerrero 2007). Nevertheless, the WBC count in *I. hormuzensis* was lower than that described for *Cyprinus auratus* and *C. carpio* (Groff and Zinkl 1999), *O. niloticus* (Benli and Yildis 2004, Silva et al. 2009), and higher than the related by *S. melanotheron* (Ezzat et al. 1974), *E. suratensis* (Pathiratne and Rajapakshe 1998) and *O mossambicus* (Jaffar Ali and Rani 2009). Low values of WBC in *I. hormuzensis* could be explained by its habit. This fish is found in a river environment, differently from those reported by other authors (Benli and Yildis 2004, Silva et al. 2009) or from fish farm (Azevedo et al. 2006).

Non-mammalian erythrocyte such as in fish, amphibians, reptiles and birds is nucleated, flattened and ellipsoidal (Rowley and Ratcliffe 1988). Studies on erythrocyte and its nucleus size are useful to determine the health status of the fish (Najiah et al. 2008). Nuclear size and shape can vary among the species (Jagoe and Welter 1995). In the present study, the cell surface and nuclear area in *I. hormuzensis* was clearly lower than the observed in cichlids *O. niloticus* (Najiah et al. 2008), *Oreochromis* hybrid (Rey Vázquez and Guerrero 2007), *Lepomis macrochirus* (Murray 1984), *Clarias* sp., *Pangasius sutchi* and *Mystus nemurus* (Najiah et al. 2008), *Dicentrarchus labrax* (Esteban et al. 2000) and *C. dimerus* (Rey Vázquez and Guerrero 2007). High MCV of *I. hormuzenis* compared to other cihlid fish could be related to lower surface/volume ratio of red blood cells (Tavares-Dias et al. 2008). In contrast, higher cell surface and nuclear area were reported in *I. hormuzensis* when compared to *A. nuchalis*, *P. amazonica* and *S. emarginata* (Carvalho et al. 2009).

There is a relationship between metabolic rate and cell size. The cells with the smaller surface have lower metabolic cost per unit of cell mass (Olmo et al. 1989). *Iranocichla hormuzensis* is found in waters with high temperature and mineral content, as well as slow flow of water. By the fact that *I. hormuzensis* presented high concentrations of hemoglobin and lower erythrocyte number and dimensions, this fish possibly could be well acclimated on this environment.

This work was the first contribution to the knowledge on red blood cells parameters, erythrocytes morphology and leukocytes count of a wild Iranian cichlid fish. These baseline values can be used for future studies on its physiology when exposed to an experimental condition. Further studies must be carried out to evaluate the reference ranges along the seasons to determine the hematological profile.

ACKNOWLEDGMENTS

The authors thank Professor Mahboobi Soofiani, dean of Department of Natural Resources, for providing laboratory facilities and testing site; M.S. Sohrabi, E. Behnam,V. Kiyani and G.H Sharifi for their help in the field work and fish sampling; N. Gilannejad, A. Zaerin, S. Kouhi and E. Paknahad for their help in laboratory analysis; and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-Brazil) for grant to M.L. Martins. This study was financially supported by Student Affairs Isfahan University of Technology. We thank Dr. Marcos Tavares-Dias (Embrapa Amapá, Brazil) and Dr. Dehai Xu (ARS, USDA, Aquatic Animal Health Research Laboratory, Auburn, Alabama, USA) for critical review of the manuscript.

RESUMO

Este estudo descreve os parâmetros hematológicos em *Iranocichla hormuzensis*, ciclídeo iraniano de água doce, importante como peixe ornamental e como de consumo. Quarenta peixes foram capturados com rede no rio Mehran, província de Hormozgan, Irã. O sangue foi usado para determinar as contagens totais de eritrócitos (RBC) e leucócitos (WBC), hematócrito, volume corpuscular médio (MCV), hemoglobina corpuscular média (MCH), concentração de hemoglobina corpuscular média (MCHC) e dados morfométricos de eritrócitos. O peixe iraniano mostrou valores menores de RBC e WBC do que outros ciclídeos (*Oreochromis niloticus*, *O. aureus*, *O. mossambicus*, *O.* hybrid, *Cichlasoma dimerus* e *Cichla monoculus*). O hematócrito não variou entre as espécies, mas MCV, MCH e MCHC em I. hormuzensis foram maiores do que em *O. niloticus*, *O. aureus*, *O.* hybrid, *C. dimerus* e *C. monoculus*. Estas diferenças podem estar relacionadas aos diferentes hábitos de vida dos peixes. Este estudo sugere que o *I. hormuzensis* está bem aclimatado ao ambiente, sendo o primeiro relato de sua hematologia. Sugere-se também alta eficiência em transporte de oxigênio, e eficiente influxo de oxigênio pelas brânquias, indicando o bem-estar do peixe neste ambiente.

Palavras-chave: Cichlidae, Irã, hematologia, eritrócitos.

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