

A Technique to Evaluate the Erythropoietic Efficiency in Fish

S. HOMECHAUDHURI* and A. JHA

Fisheries and Ecology Laboratory, Aquatic Biosystems Research Unit
Department of Zoology, University of Calcutta
35 Ballygunge Circular Road, Calcutta 700019
India

Abstract

This experiment describes a technique for quantifying erythropoietic efficiency in the pronephros and the spleen of *Cyprinus carpio* and *Oreochromis niloticus*. The methodology is reliably accurate to produce reproducible results suggestive of its utility in evaluating normal erythropoiesis and stress response in fish .

Discussion

The spleen and pronephric kidney are the main erythropoietic organs in teleosts. In some fishes both organs function equally whereas in others any of the two remains more active than the other (Catton 1951). However, in some fishes only the spleen (e.g. in perch) or the head kidney (e.g. in mrigal) shows activity. There are several reports on ontogeny of erythrocytes (Mahajan and Dheer 1980, Weining 1990), erythropoietic response against various stressors (Peters and Schwarzer 1985), histology and ultrastructure of erythropoietic tissues (Khadre et al. 1989, Bodammer et al. 1990) and also histopathology of such organs (Wlason and Dabrowska 1989 and Noyes et al. 1991). Except for a single work (Sordyl and Osterland 1991) the erythropoietic efficiency of the spleen and head kidney has not been quantified by any useful method although such quantification is absolutely necessary to assess normal activity as well as response against stressors. Hence, a methodology has been tried in order to establish the relative efficiency of erythropoietic organs quantitatively.

For this experiment, two cultivated exotic fish *Oreochromis niloticus* and *Cyprinus carpio* were chosen because they possess both the spleen and the head kidney functioning as erythropoietic organs. Fishes in this study were all females either in developing (for *O. niloticus*) or gravid (for *C. carpio*) stages of reproductive maturity.

Live specimens were obtained from a local fish farm. Apparently healthy and disease free fish were maintained in well aerated water in a large aquarium (4' x 1.5' x 1.5') containing 5 to 6 specimens each for 7 days of

physical acclimation. The range of values for water parameters recorded in the fish holding aquaria were: temperature (24 to 26°C), pH (6.9-7.2), total alkalinity (70 to 80 ppm), dissolved oxygen (5 to 7 ppm) and dissolved free CO₂ (2 to 3 ppm). For the study, the whole spleen and the head kidney were dissected from the live fish and excess peripheral blood was removed by blotting using Whatman filter paper. Both organs were weighed, placed in two separate watch glasses containing 1 ml of red blood cell diluting fluid and cut into pieces (4 pieces of head kidney and 7 pieces of spleen). Cells were dispersed by gently holding pieces of tissue with fine forcep in the diluting fluid for exactly six minutes. The blast cells along with the other cells were freed from the tissue and precipitated in the watch glass. The duration of dispersion of cells was estimated by repeated trials to obtain sufficient cells in the suspension. After a thorough mixing of the cells in the diluting fluid, 100 µl of the mixture was micropipetted into a dry and clean centrifuge tube and centrifuged for 1 minute at 1000 rpm. The blast cells remained in the supernatant and other debris were pelleted. Ten microliters of the supernatant was carefully transferred to a Naubaer's double haemocytometer for total counting of different blast cells. Special care was taken at this stage to avoid mixing the debris in the testing sample. The temperature during tissue handling process and centrifugation was maintained at 18°C. Simultaneous spleen and kidney tissue imprints were prepared following Ashley and Smith (1963) and stained with Graham knoll's benzidine method followed by counterstaining with Giemsa (Mahajan and Dheer 1979). Differentiation of blast cells, mature reticulocytes, erythrocytes and their morphometry were made following descriptions of Mahajan and Dheer (1980). Presence of different blast cells *viz.* small lymphoid haemoblast, basophilic erythroblast, polychromato-philic erythroblast, acidophilic erythroblast and other lineage cells *viz.* young reticulocyte, mature reticulocyte and erythrocyte in the haemocytometer were confirmed by comparing their morphometry with that of the various cell types identified from tissue imprint preparation. The following process was used to estimate the level of erythropoietic efficiency of the two tissues. The blast cells in the four corners of the haemocytometer, each having 1 mm² in area, and 1/10 mm in depth, with a cubic content of 1/10 mm³ were counted. Thus, the total number of blast cells counted from four chambers represent their density per 0.4 mm³ of suspension. The number of blast cells/1 mm³ of suspension was then extrapolated by multiplying the density value by 2.5. This quantification method enabled accurate distinction between the erythropoietic activity of spleen and head kidney at a given time. Following this method, the female *O. niloticus* at developing maturity stage showed a value of 1430.8 ± 413.0 blast cells/mm³ in the spleen and 1297.2 ± 294.37 blast cells/mm³ in the head kidney, indicating a marginally higher potential erythropoiesis in the spleen. Gravid *C. carpio* female showed 5141.83 ± 371.08 blast cells/mm³ in the spleen and 5312.33 ± 524.27 blast cells/mm³ in the head kidney (Table I). Individual variations were pronounced despite similar maintenance protocols, because other factors *viz.* ambient O₂ content, nutritional status and individual metabolic functions might lead to variations in the erythropoietic activity in fishes. Greater erythropoietic activity observed in this experiment in gravid *C. carpio* might be related to greater de-

Table 1. Erythropoietic activity in *C. carpio* and *O. niloticus* measured in pronephros and spleen.

Species	Sex and maturity status	Length range of the fish (cm)	Weight range of the fish (g)	Weight range of pronephric kidney (g)	No. of blast cells/mm ³ in pronephric kidney Mean \pm S.D.	Weight range of spleen (g)	No. of blast cells/mm ³ in spleen Mean \pm S.D.
<i>Oreochromis niloticus</i> (# 5)	O ₊ Developing	11 - 12.5	21 - 28	0.012 - 0.066	1297.2 \pm 294.37	0.020 - 0.037	1430.80 \pm 413.0
<i>Cyprinus carpio</i> (# 6)	O ₊ Gravid	12 - 18.5	93 - 108	0.080 - 0.087	5312.33 \pm 524.27	0.039 - 0.098	5141.83 \pm 371.08

mand of red blood cells in the gonads following maturation of eggs and greater bulk of the tissues. It is also evident that both the pronephric kidney and spleen play almost equal roles in erythropoiesis in *C. carpio* and *O. niloticus*. In conclusion, the usefulness of this method is suggested in evaluating erythropoietic activity of the spleen and pronephros in different fishes. Such quantification can also help in the assessment of the relative efficiency of these tissues whenever necessary.

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