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Research Article

A QUANTITATIVE AND QUALITATIVE EVALUATION OF HAEMATOPOIESIS IN THREE HABITAT SPECIALIST FISHES, *ACANTHOCOBITIS BOTIA*, *DEVARIO AEQUIPINNATUS* AND *BARILIUS BARNA* FROM A HILL STREAM ENVIRONMENT

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ABSTRACT

The aim of the present study was to execute quantitative and qualitative assessments of developing blood cells in the head kidney of three habitat specialist fish species of River Murti *Acanthocobitis botia*, *Devario aequipinnatus* and *Barilius barna*. The variance in activity pattern and preference of specific habitat of these three fishes were correlated with their haematopoietic profile. Eleven types of hematopoietic precursors and mature cells in erythroid and lymphoid lineage were recognized in head kidneys of the three fish species. The erythroid lineage was found to be the most abundant as well as diverse one with a special focus on the size and quantity of the small lymphoid haematoblasts, young and mature erythrocytes. Significant difference regarding the rate of maturation of developing blood cells and erythropoietic efficiency was found between these three species through cytological descriptions. These findings were corroborated through cell cycle study by flow cytometry analysis, which showed high, medium and low values of S-phase cells in *A. botia*, *B. barna* and *D. aequipinnatus* respectively, which corresponded to the turnover number that are associated with their respective habitat. The differential pattern of erythropoiesis might indicate its functional significance in the employment of different survival strategies that dictate varied activity and specialisation of habitat in fishes.

Keywords: *Acanthocobitis botia*, *Barilius barna*, *Devario aequipinnatus*, Haematopoiesis, Head kidney, Survival strategy.

INTRODUCTION

Freshwater environments throughout the world are experiencing serious threats to their biodiversity resulting in ecosystem instability (Suski and Cooke, 2006). Freshwater fish being one of the most threatened taxonomic groups (Darwall and Vie, 2005) due to their high sensitivity to the quantitative and qualitative alteration of aquatic habitats (Laffaille *et al.,* 2005; Kang *et al.,* 2009) are viewed to be good biological indicators of the status of aquatic communities and river environments (Schneiders *et al.,* 1993) and they often play a key role in environmental planning schemes (Schiemer, 2000; Sarkar and Bain, 2007). Over the last century the riverine ecosystems have suffered from intense human intervention. This coupled with slow and inadequate conservation measures to mitigate the impact of the pressures (Sarkar *et al.,* 2010), have largely resulted in habitat loss and degradation and as a consequence, many fish species have become highly endangered.

India is endowed with a rich fish biodiversity (2,200 fish species) and ranks ninth in term of freshwater mega biodiversity (Mittermeier and Mittermeier, 1997). The freshwater resources of India are currently experiencing an alarming decline, particularly in the fish biodiversity due to several factors and as a result, a sizeable portion of freshwater fishes have been categorized as threatened (Sarkar *et al.,* 2008). River conservation and management activities in most countries, including India suffer from inadequate knowledge of the constituent biota. This emphasizes an immediate need for initiating global research to develop alternative conservation planning schemes to protect the biodiversity of these freshwater aquatic systems (Margules and Pressey, 2000; Lipsey and Child, 2007; Pusey *et al.,* 2010; Sarkar *et al.,* 2012).

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The ichthyofaunal diversity of the Eastern Himalayas and its foothills is unique in terms of the lotic water ecosystems (Acharjee and Barat, 2011). Correlation between fish species richness and hydrological attributes shows good relationship where water depth, water velocity, dissolved oxygen and pH generally act as the most important variables in shaping fish assemblage with the fishes acclimatizing along the altitudinal gradient (Lakra *et al.*, 2010), thereby demonstrating the significance of altitude as an important physical factor. Water velocity and the amount of dissolved oxygen increase with increasing altitude, and the fish may respond to both of these factors in a variety of ways. It has been supposed that increase in haemoglobin concentration or number of red blood cells is an essential feature of acclimatization in such conditions (Hall *et al.,* 1936; Smeda and Houston, 1979). Although variations in haemoglobin, triggered by temperature change is a known fact (Graham, 1985), there was no information regarding effects of environmental conditions on formation of blood cells.

Haematopoiesis in the vertebrate is characterized by the induction of ventral mesoderm to form haematopoietic stem cells and the eventual differentiation of these progenitors to form the peripheral blood lineages (Bahary and Zon, 1998). Since haemopoietic tissue is known to be sensitive to various environmental cues due to its high cell turnover rate (Kondera and Witeska, 2013), the process of haematopoiesis might be considered as one of the adaptive strategies in such fishes to survive in their specialised habitats.

Acanthocobitis botia, *Devario aequipinnatus* and *Barilius barna*, despite being residents of the same hill stream environment vary in their activity pattern and preference of specific habitat. The present study is thus designed to examine the haematopoietic profile in these three habitat specialist fish species as well as to generate baseline information on the same.

MATERIALS AND METHODS

Study Area and Fish Sampling

Sampling was conducted at Murti river which rises from the Mo forest (close to the Neora Valley National Park) in the Darjeeling Himalayas (2211 m above sea level or asl) flowing its way along the foothills before it meets the Jaldhaka River (102 m asl). The study site was selected at North Dhupjhora (26°50.631′ N 88°49.704′ E). Monthly sampling was carried out for 3 years (from July 2013 to June 2016) in the river at the sampling site (for a stretch of 5 km) using cast net and gill net. *Acanthocobitis botia* (Hamilton, 1822), *Devario aequipinnatus* (McClelland, 1839) and *Barilius barna* (Hamilton, 1822) with a body mass of 4.1 ± 2.1 g, 5.4 ± 1.6 g and 5.2 ±1.7 g respectively and length of 7.2 ± 1.5 cm, 9.3 ± 1.3 cm and 8.9 ± 1.6 cm respectively were obtained by sampling. The fish species were identified to the lowest taxonomic level according to Talwar and Jhingran (1991) and Day (1889).

Haemopoietic study and Morphometric analysis

Twelve individuals belonging to each of the three fish species were taken for further experiments and subsequent haemopoietic studies were conducted upon them, using the imprint or impression technique (Mahajan and Dheer, 1979, 1980). Imprint of the head kidney tissue was made on multiple glass slides which were air dried and subsequently stained with benzidine (Forteza Bover, 1964) followed by counterstaining with Giemsa. Morphometry of haemopoietic cells were studied by ocular micrometer (LM magnification 10 × 100) in light microscope (Leica DM1000). Haemopoietic efficiency of the three fish species was evaluated following the method of Homechaudhuri and Jha (2001).

Calculation of means, standard deviations (SD) of the means, standard errors (SE) of the means from whole range of data were done using Descriptive Statistics tool of Microsoft Excel 2007. One way ANOVA and Tukey's post hoc analysis was carried out with SPSS Statistics version 17.0. At $p < 0.05$ level, the differences were considered significant.

Flowcytometric analysis

Distribution of different phases of cell cycle from nuclear DNA of head kidney cells was determined using FACS calibur (Becton Dickinson) by BD Accuri C6 software. Cells were fixed with 3% para-formaldehyde followed by permeabilization with 0.5% Triton X-100. Subsequent labelling of nuclear DNA with Propidium Iodide (PI) was done after RNase treatment. Total 10,000 events were acquired for analysis. DNA contents were analysed by histogram plotting of PI-fluorescence at X-axis versus counts at Y-axis (Loken, 1990).

RESULTS

Acanthocobitis botia, *Devario aequipinnatus* and *Barilius barna* were selected for the study based on the variation of their activity pattern and preferred habitat type as listed in Table 1.

The pronephric kidney indicated its role as the main haemopoietic tissue in all the three fish species, *Acanthocobitis botia* (Hamilton, 1822), *Devario aequipinnatus* (McClelland, 1839) and *Barilius barna* (Hamilton, 1822). 11 types of haematopoietic precursors and mature cells were identified by light microscopy observation in the same. The present study documented cytological description and morphometry of the different developmental stages of erythrocytic and leukocytic lineage in the head kidney of *Acanthocobitis botia*, *Devario aequipinnatus* and *Barilius barna* (Table 2). Haematopoietic efficiency was also evaluated to understand the adaptive physiology of them to survive in the specialised habitat of the swift flowing river.

Table 1. Activity pattern and preferred habitat type of some prioritized fish species of the river Murti.

Fish species	Activity pattern	Preferred Habitat type				
		River Depth (m)	Velocity of water (ms-1)	Dissolved Oxygen (mgl^{-1})	pH	Substratum
Acanthocobitis botia	Active	1.1±0.7	0.9±0.12	8.2±0.3	7.4±0.06	Sand, gravel, small boulders and bedrocks
Devario aequipinnatus	Sedentary	1.2±0.7	0.2±0.09	7.1±0.2	7.9±0.09	Sand and gravel
Barilius barna	Migratory	1.4±1.1	0.8±0.31	7.9±0.7	7.3±0.11	Sand, gravel, and bedrocks

Table 2. Characteristics of haematopoietic cells, namely Small Lymphoid Haematoblast (SLH), Basophilic Erythroblast (BE), Polychromatophilic Erythroblast (PE), Acidophilic Erythroblast (AE), Young Reticulocyte (YR), Mature Reticulocyte (MR), Young Erythrocyte (YE), Mature Erythrocyte (ME), Lymphocyte (LYM), Neutrophil (NEU) and Macrophage in *A. botia, D. aequipinnatus and B. barna* (n=12).

Cell type	Area (μm^2)						Percentage distribution		
	Cell			Nucleus					
	A. botia	*D. aequipinnatus*	*B. barna*	*A. botia*	*D. aequipinnatus*	*B. barna*	*A. botia*	*D. aequipinnatus*	*B. barna*
SLH	23.12 ±1.81	25.41 ±1.92	23.35 ±0.70	9.42 ±1.15	11.50 ±0.72	9.55 ±0.98	5.68 ±0.72	11.15 ±0.59	7.51 ±0.28
BE	73.14 ±1.28	75.38 ±2.29	75.15 ±2.25	45.51 ±1.22	45.98 ±1.75	46.35 ±1.49	1.48 ±0.14	2.8 ±0.21	2.72 ±0.42
PE	133.18 ±2.06	134.54 ±3.14	128.35 ±3.17	53.14 ±1.21	54.55 ±1.95	51.39 ±2.59	5.19 ±0.25	6.24 ±0.98	5.85 ±0.48
AE	107.29 ±2.19	109.25 ±2.58	85.46 ±2.51	43.50 ±1.51	45.66 ±1.38	39.42 ±1.85	1.65 ±0.09	2.47 ±0.41	2.05 ±0.35
YR	72.80 ±2.98	76.95 ±1.56	75.50 ±1.66	15.14 ±1.43	18.96 ±0.95	15.84 ±1.24	16.75 ±1.12	19.43 ±1.34	18.48 ±2.09
MR	61.31 ±2.17	65.39 ±2.12	61.15 ±1.52	13.12 ±1.49	13.74 ±0.56	13.54 ±1.15	11.88 ±0.76	14.82 ±1.15	13.26 ±1.56
YE	53.45 ±1.45	58.98 ±1.39	54.90 ±1.85	14.42 ±0.48	15.94 ±0.95	14.35 ±1.04	30.69 ±1.41	21.54 ±1.08	27.15 ±1.68
ME	41.53 ±1.52	48.55 ±1.31	45.90 ±1.93	10.19 ±0.54	12.73 ±0.58	11.84 ±0.63	19.28 ±1.39	15.38 ±1.18	17.69 ±1.84
LYM	25.84 ±2.69	23.71 ±2.04	20.76 ±0.82	6.45 ±0.85	7.42 ±1.05	5.66 ±0.68	5.85 ±0.37	4.89 ±0.56	3.87 ±0.69
NEU	24.65 ±1.13	23.84 ±1.08	28.19 ±1.06	9.56 ±0.80	9..67 ±0.64	9.89 ±1.13	1.15 ±0.08	0.72 ±0.09	0.67 ±0.15
MAC	177.79 ±3.51	156.88 ±2.91	166.55 ±3.11	38.29 ±1.72	35.14 ±2.58	35.56 ±3.69	0.4 ±0.02	0.56 ±0.06	0.75 ±0.08

The erythrocytic lineage consisted of eight stages namely small lymphoid haematoblast (SLH), basophilic erythroblast (BE), polychromatophilic erythroblast (PE), acidophilic erythroblast (AE), young reticulocyte (YR), mature reticulocyte (MR), young erythrocyte (YE) and mature erythrocyte (ME). Lymphocyte, neutrophil and macrophage were identified in the lymphocytic lineage (Figure 1, 2, 3) in all the three species. The percentage distribution of SLH (which is a storage form of developing erythrocytes) was found to differ significantly in an increasing order in *Acanthocobitis botia, Barilius barna* and *Devario aequipinnatus.* In contrast to this finding, the ME cells were found to be most abundant in *A. botia* and least abundant in *D. aequipinnatus* (Figure 4, Table 2). Size of ME varied significantly among the three species, with the largest and the smallest being found in *D. aequipinnatus* and *A. botia* respectively. *B. barna* maintained consistency in both size and number of haematopoietic cells (Table 2). However no significant structural variation between the developing pronephric cell populations of the three fish species was observed.

The measurement of haematopoietic efficiency in the three species revealed highest erythropoietic efficiency in *A. botia* and lowest in *D. aequipinnatus* reflecting different adaptive strategy of them inhabiting diverse macro habitats in the hill streams (Figure 5). This finding was further supported by cell cycle study of the head kidney tissue by flow cytometry analysis. The results showed 73.2% cells in the G0-G1 (undivided and first growth) phase (M1), 9.3% cells in the S (synthetic) phase (M2) and 3.5% cells in the G2-M (second growth and mitotic) phase (M3) of the cell cycle in *A. botia*, whereas 93.5% cells in the G0-G1 phase (M1), 2.7% cells in the S phase (M2) and 0.9% cells in G2-M phase (M3) in *D. aequipinnatus.* Maintaining uniformity in its cell turnover rate, *B. barna* was shown to have 82.9% cells in the G0-G1 phase (M1), 5.8% cells in the S phase (M2) and 2.3% cells in G2-M phase (M3) (Figure 6).

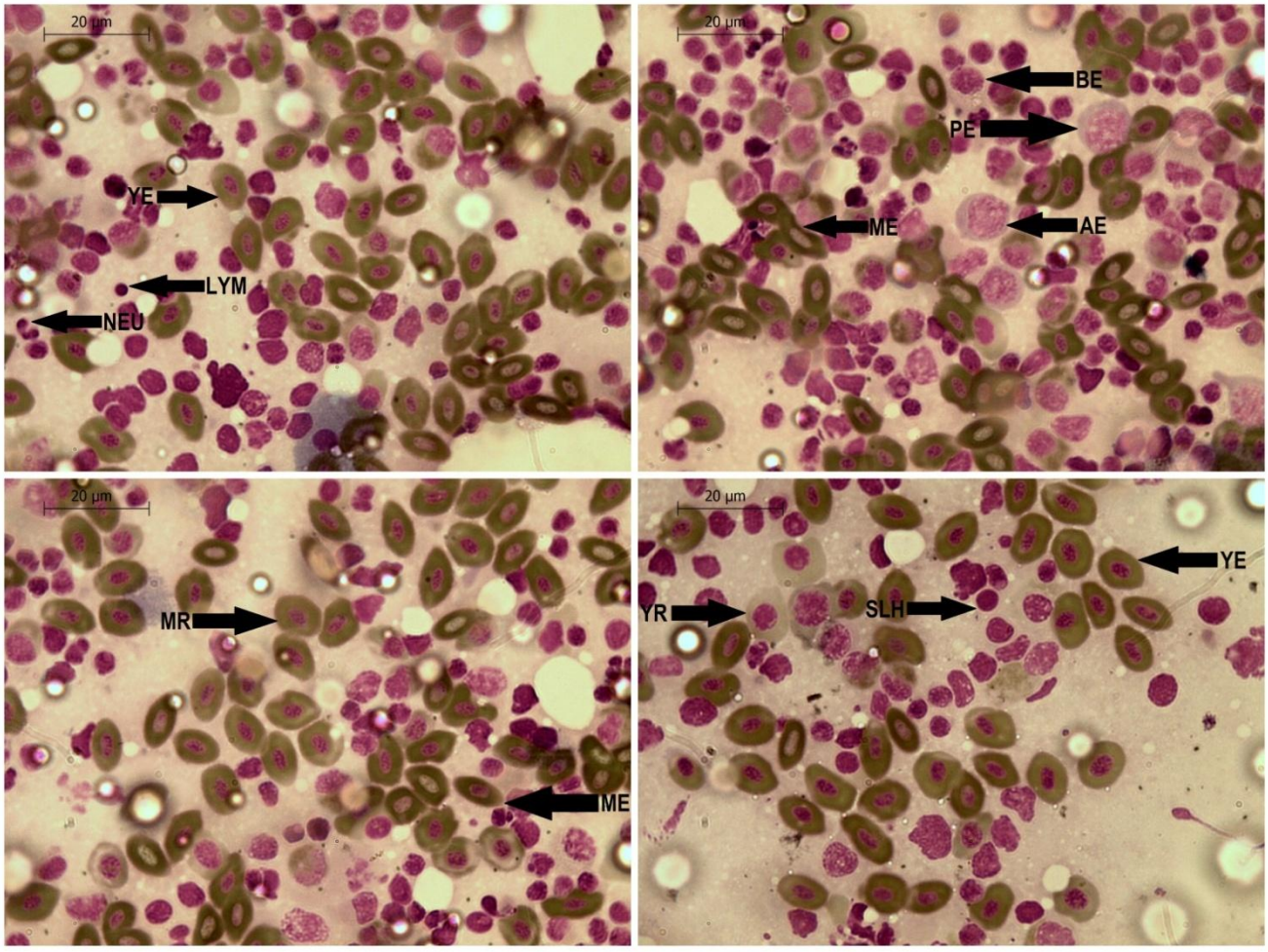

Figure 1. An imprint from the head kidney of *Acanthocobitis botia* showing Small Lymphoid Haematoblast (SLH), Basophilic Erythroblast (BE), Polychromatophilic Erythroblast (PE), Acidophilic Erythroblast (AE), Young Reticulocyte (YR), Mature Reticulocyte (MR), Young Erythrocyte (YE), Mature Erythrocyte (ME), Lymphocyte (LYM), Neutrophil (NEU) and Macrophage.

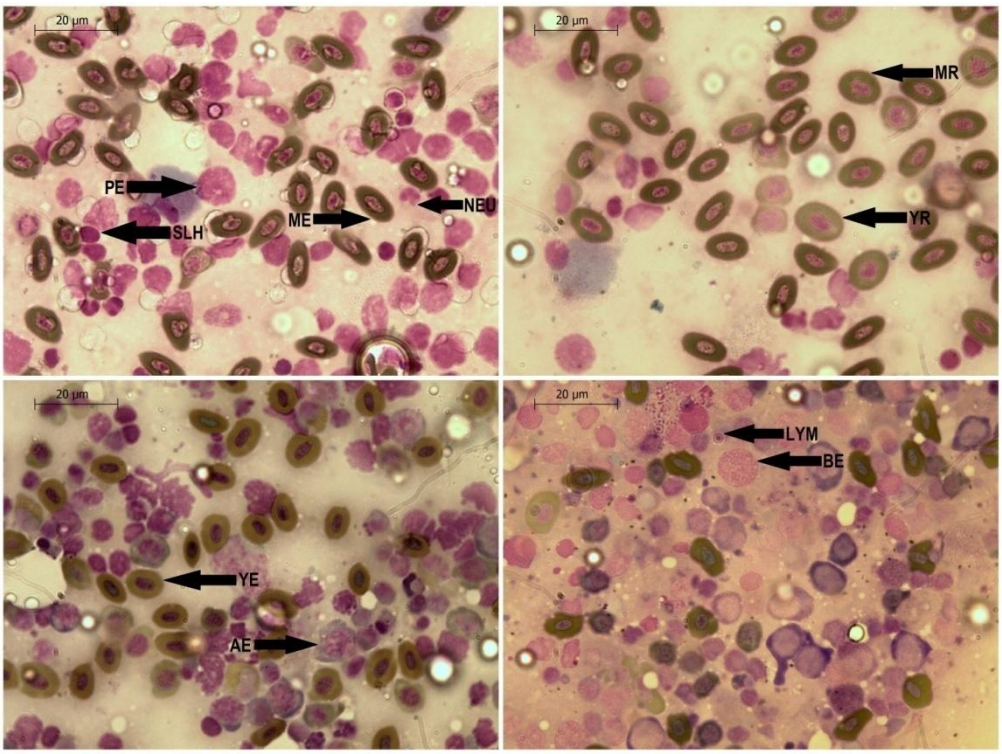

Figure 2. An imprint from the head kidney of *Devario aequipinnatus* showing Small Lymphoid Haematoblast (SLH), Basophilic Erythroblast (BE), Polychromatophilic Erythroblast (PE), Acidophilic Erythroblast (AE), Young Reticulocyte (YR), Mature Reticulocyte (MR), Young Erythrocyte (YE), Mature Erythrocyte (ME), Lymphocyte (LYM), Neutrophil (NEU) and Macrophage (MAC).

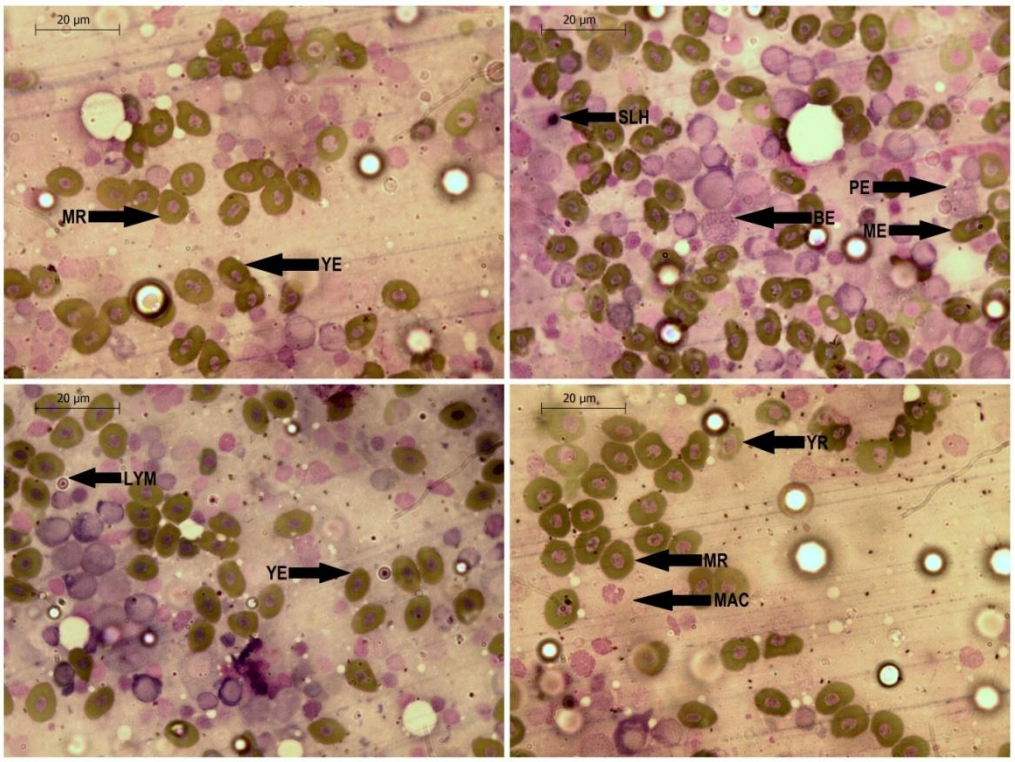

Figure 3. An imprint from the head kidney of *Barilius barna* showing Small Lymphoid Haematoblast (SLH), Basophilic Erythroblast (BE), Polychromatophilic Erythroblast (PE), Acidophilic Erythroblast (AE), Young Reticulocyte (YR), Mature Reticulocyte (MR), Young Erythrocyte (YE), Mature Erythrocyte (ME), Lymphocyte (LYM), Neutrophil (NEU) and Macrophage (MAC).

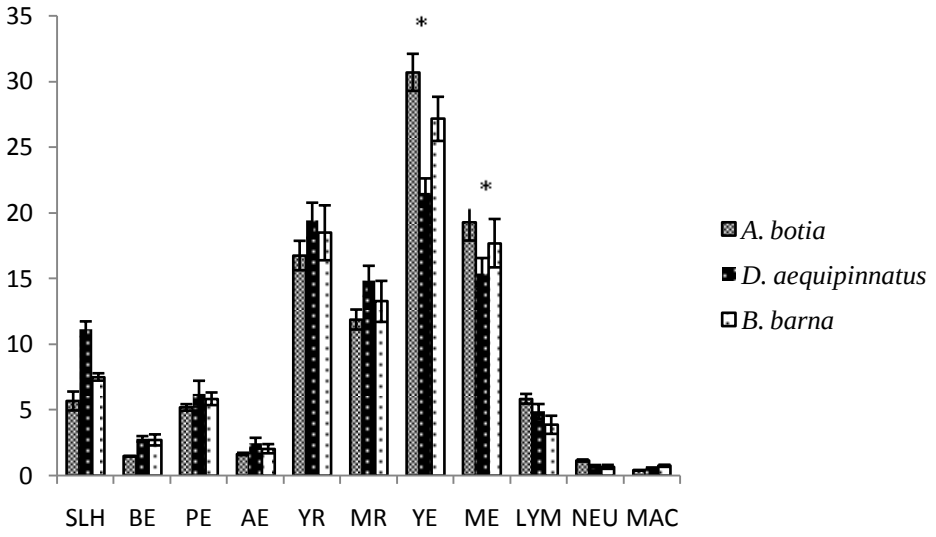

Figure 4. Comparative study on percentage distribution of haematopoietic cells in *A. botia*, *D. aequipinnatus* and *B. barna* *significant (P<0.05).

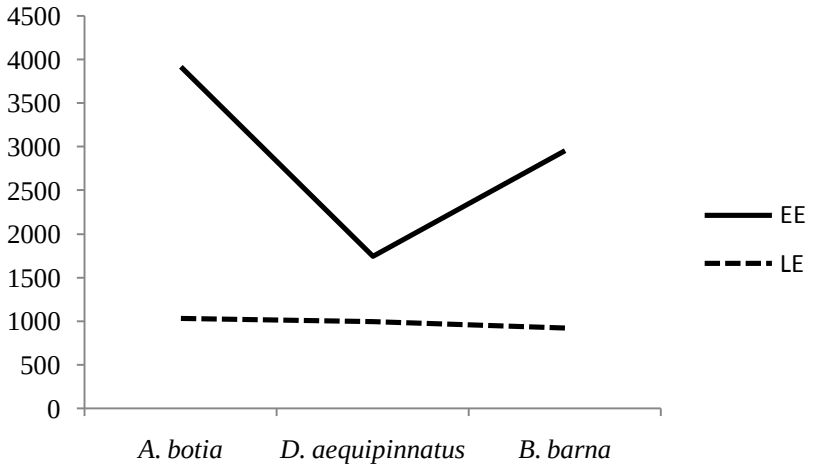

Figure 5. Comparative study of Haematopoietic efficiency in head kidney of *A. botia, D. aequipinnatus and B. barna* (EE: Erythropoietic Efficiency and LE: Leukopoietic Efficiency).

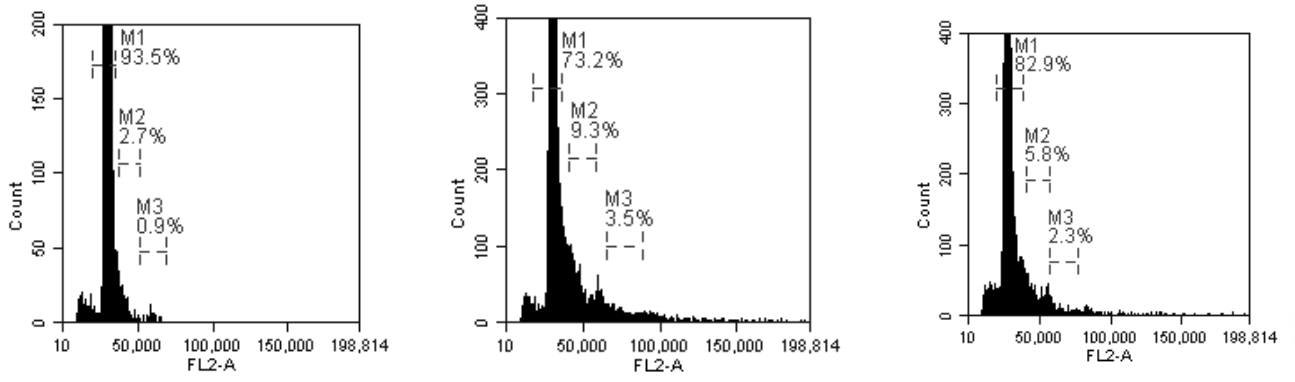

Figure 6. Flow cytometric study of head kidney in *A. botia* (a) and *D. aequipinnatus* (b) and *B. barna* (c) showing phases of cell cycle. (M1: G0-G1 (undivided and first growth) phase, M2: S (synthetic) phase and M3: G2-M (second growth and mitotic phase).

DISCUSSION

The teleost head kidney has been considered a haematopoietic organ similar to the bone marrow of higher vertebrates and has also been described as a primitive system (Tomonaga *et al.,* 1973, Abdel-Aziz *et al.,* 2010). The present findings reinforce the data of Grizzle and Rogers (1976) and Fijan (2002a) about the main role of kidneys in haematopoiesis.

In the present study, haematopoiesis was produced continuously in the head kidney of *A. botia*, *D. aequipinnatus* and *B. barna* and the erythropoietic series was most frequent among all identified hematopoietic tissue cells, similar to *D. labrax* (Esteban *et al.,* 1989), *S. aurata* (Zuasti and Ferrer, 1989), *C. carpio* and *O. niloticus* (Homechaudhuri and Jha 2001), *I. punctatus* (Fijan, 2002a, 2002b), *O. niloticus* (Abdel-Aziz *et al.,* 2010), *C. parallelus* (Santos *et al.,* 2011) and *S. cephalus* (Kondera, 2014). The developmental stages of Erythroid lineage, however, were more numerous than any other cell type similar to the results of Fijan (2002), suggesting erythropoiesis as a potent parameter to study the adaptive physiology in fishes.

The percentage of SLH in all the species was found to be considerably lower than other erythroid developmental stages contradictory to the results obtained by Gangopadhyay and Homechaudhuri (2011) in *C. batrachus*. Erythrocytes were more numerous than erythroblasts, unlike the findings of Wlasow and Dabrowska (1989) in *C. carpio*, Fijan (2002b) in *I. punctatus*, Som *et al.* (2009) in *L. rohita*, Gangopadhyay and Homechaudhuri (2011) in *C. batrachus*, Kondera (2011) in *C. carpio* and Kondera (2014) in *S. cephalus*. Erythropoietic efficiency of the three species under study was found to be much higher than that of *O. niloticus* as reported by Homechaudhuri and Jha (2001). Thus the present study might signify the efficiency of erythropoiesis to maintain the required oxygen demand and survive in different habitat conditions of the hill stream environment.

As per the data attained by various authors underlining the percentage of lymphoid cells in the head kidney, it has been found that these cells often differ dramatically despite belonging to the same fish species (Kondera, 2011). The percentage of these head kidney lymphoid cells reported in the present study were comparable to the already reported values by Wlasow and Dabrowska (1989) in *C. carpio*, but were found to be much lower than that reported by Fijan (1961) for common carp, Fijan (2002b) for channel catfish and Kondera (2011) for common carp with the lymphocyte being the most frequently occurring cell of the lymphoid lineage in the kidney of *A. botia, D. aequipinnatus* and *B. barna*. These results were concomitant with the work done by Wlasow and Dabrowska (1989), Quentel and Obcach (1992), Fijan (2002b), Liu *et al.* (2004) and Kondera (2011). Moreover the data corresponding to the leukopoiectic efficiency also revealed the fact that fewer lymphoid cells were required in each of the three species under study.

Following Abdel-Aziz *et al.* (2010), quantitative and morphological methods were jointly applied for categorical stratification and consequent identification of the various fish haematopoietic cells for progressive classification from their present temporary conditional state. Cell cycle study of the head kidney by flow cytometry found the percentage of cells at different phases. However, significantly high values of the S phase cells were recorded in *A. botia*, similar to the findings of Chaudhuri *et al.* (2017) in *G. gotyla gotyla*, which might denote very high rate of cell division suggesting great efficiency to survive in the swift flowing river. In contrast, a significantly less number of S-phase cells in *D. aequipinnatus* might indicate the slow turnover rate of the developing blood cells.

Previous studies have mentioned that the structure of the developing blood cells and their cell populations found in the haemopoietic organs of different fish species differ only in the relative numbers of the various types of cells and this may be due to the physiological condition of the fish, their immediate environment and the season of the year (Ezzat *et al.,* 1974; Smith *et al.,* 1976; Safer and El-Sayed, 1986; Abdel-Aziz *et al.,* 2010). In the present study, significantly high percentage of YE and ME, erythropoietic efficiency and S phase cells and significantly low percentage of SLH in *A. botia* might thus be suggestive of higher turnover rate of the storage form (SLH) into mature erythrocyte (ME) responsible for satisfying high oxygen demand for active swimming and clinging on to the pebbles in the substratum against the flow of the river. Again, significantly high percentage of SLH in *D. aequipinnatus*is was noteworthy since blood oxygen carrying capacity can be increased by release of stored cells (Murad *et al.,* 1990). Significantly large size of YE and ME, having greater haemoglobin content might also be an adaptive strategy for sudden hypoxic conditions in shallow habitats (Gangopadhyay and Homechaudhuri, 2011). Conversely, significantly low percentage of YE and ME, erythropoietic efficiency and S-phase cells in the same is indicative of its sedentary habit and less requirement of dissolved oxygen in its preferred riverine habitat. *B. barna*, maintaining a consistent pool of SLH as well as YE and ME (also supported by the erythropoietic efficiency and number of S-phase cells) justified its migratory habit and adaptation for active swimming and suitability for a long range of habitat.

CONCLUSION

In conclusion, the cellular composition of the head kidney, designated as primary hematopoietic tissue, in various fish species is similar, but there are quantitative differences. The proportion of various blood cells in fish is a very labile parameter that depends on many factors, including season, physicochemical water parameters, water contamination, stressors, etc. and this dictates the adaptation of organisms to variable environmental conditions (Kondera, 2014). A quantitative and qualitative approach on the haematopoiesis of these three fish species might thus help in understanding their survival strategy in specific macro habitats. Since freshwater fish are under immediate threat of environmental degradation and habitat destruction mainly

due to natural and anthropogenic stresses, the knowledge of fish physiology finds its relevance to develop appropriate planning for biodiversity conservation strategies for wild fish population of the lotic ecosystem.

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REFERENCES

Abdel Aziz, E.S.H., Abdu, S.B.S., Ali, T.E.S. and Fouad, H.F., 2010. Haemopoiesis in the head kidney of tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae): a morphological (optical and ultrastructural) study. *Fish Physiol. Biochem.,* 36, 323-336. doi: 10.1007/s10695-008-9297-z.

Acharjee, M.L. and Barat, S., 2011. Spatio-temporal Dynamics of Physico-Chemical Factors of River Relli in Darjeeling Himalaya, West Bengal, India. NBU *J. Anim. Sci.*, 5, 24-33.

Bahary, N. and Zon, L.I., 1998. Use of the Zebrafish (*Danio rerio*) to define hematopoiesis. *Stem Cells,* 16, 89-98. doi: 10.1002/stem.160089.

Chaudhuri, A., Gangopadhyay, K. and Homechaudhuri, S. 2017. Understanding Haematopoiesis as survival strategy in two characteristic habitat specialist fishes inhabiting a hill stream environment. *J. Env. Biol.*, 38, 477-482. doi: 10.22438/jeb/38/3/MS-245.

Darwall, W.R.T. and Vie, J.C. 2005. Identifying important sites for conservation of freshwater biodiversity: extending the species based approach. *Fish Manag. Ecol.,* 12. 287-293. doi: 10.1111/j.1365-2400.2005. 00449.x.

Day, F. 1889. The fauna of British India: Fishes. Taylor and Francis, London.

Esteban, M.A., Meseguer, J., Garcia, A.A. and Agulleiro, B. 1989. Erythropoiesis and thrombopoiesis in the head-kidney of the sea bass (*Dicentrarchus labrax* L.): an ultrastructural study. *Arch Histol. Cytol.,* 52,407-419. doi: 10.1679/aohc.52.407.

Ezzat, A.A., Shabana, M.B., Farghaly, A.M., 1974. Studies on the blood characteristics of *Tilapia zillii* (Gervais) I. Blood cells. *J. Fish. Biol*., 6, 1-12. doi:10.1111/j.1095-8649.1974. tb04516.x.

Fijan, N., 1961. Haemopoietic function of kidneys in some species of freshwater fish. Bioloski Glasnik, 14,167-208.

Fijan, N., 2002a. Morphogenesis of blood cell lineages in channel catfish. *J. Fish. Bio.,* l60, 999-1014. doi: 10. 1111/j.1095-8649.2002.tb02424.x.

Fijan, N., 2002b. Composition of main haematopoietic compartments in normal and bled channel catfish. *J. Fish Biol.,* 60, 1142-1154. doi: 10.1111/j.1095-8649. 2002.tb01711.x.

Forteza Bover, G., 1964. Atlas of Blood Cytology, Grune and Stratton, New York and London.

Gangopadhyay, K. and Homechaudhuri, S. 2011. Descriptive characteristics of haemopoietic cell lineages in a facultative air breathing fish *Clarias batrachus* (L.). *Turk. J. Zool.,* 35, 737-746. doi:10. 3906/zoo-0905-37.

Graham. J.B., 1985. Seasonal and environmental effects on the blood hemoglobin concentrations of some Panamanian air-breathing fishes. *Env. Biol. Fish*., 12(4), 291-301. doi: 10.1007/BF00005459.

Grizzle, J.M. and Rogers, W.A., 1976. Anatomy and Histology of the Channel Catfish. Auburn, Agricultural Experiment Station, Auburn University.

Hall, FG., Dill, D.B, and Guzman, E.S., 1936. Comparative Physiology. In: High Altitudes Barron. J. *Cell Comp. Physiol.,* 8, 301-313. doi: 10.1002/jcp. 1030080302.

Homechaudhuri, S. and Jha, A., 2001. A technique to evaluate the erythropoietic efficiency in fish. *Asian Fish. Sci.,* 14, 453-455.

Kang, B., He, D., Perrett, L., Wang, H., Hu, W., Deng, W., Wu, Y., 2009. Fish and fisheries in the Upper Mekong: current assessment of the fish community, threats and conservation. *Rev. Fish. Biol. Fish.,* 19, 465-480. doi: 10.1007/s11160-009-9114-5.

Kondera, E., 2011. Haematopoiesis in the head kidney of common carp (*Cyprinus carpio* L.): a morphological study. *Fish Physiol. Biochem.*, 37, 355-362. doi: 10.1007/s10695-010-9432-5.

Kondera, E., 2014. Cell composition of the head kidney of European chub (*Squalius cephalus* L.). *Arch. Pol. Fish.,* 22, 271-280. doi: 10.2478/aopf-2014-0029.

Kondera, E. and Witeska, M., 2013. Cadmium and copper reduce hematopoietic potential in common carp (*Cyprinus carpio* L.) head kidney. *Fish. Physiol. Biochem., 39:* 755-764. doi: 10.1007/s10695-012-9738-6.

Laffaille, P., Acou, A., Guillouet, J. and Legult, A., 2005. Temporal change in European eel, *Anguilla anguilla*, stock in a small catchment after installation of fish passes. *Fish Manag. Ecol.,* 12,123-129. doi: 10.1111/j. 1365-2400.2004.00433.x.

Lakra, W.S., Sarkar, U.K., Kumar, R.S., Pandey, A.K., Dubey, V.K., Gusain, O.P., 2010. Environmentalist Fish diversity, habitat ecology and their conservation and management issues of a tropical River in Ganga basin, India. *Environmentalist* 30(4), 306-319. doi: 10.1007/s10669-010-9277-6.

Lipsey, M.K. and Child, M.F., 2007. Combining the fields of reintroduction biology and restoration ecology.

Conserv. Biol., 21, 1387-1388. doi: 10.1111/j.1523-1739.2007.00806.x

Liu, Y., Zhang S., Jiang, G., Yang, D., Lian, J. and Yang, Y., 2004. The development of the lymphoid organs of flounder, *Paralichthys olivaceus*, from hatching to 13 months. *Fish Shellfish Immun.*, l16, 621-632. doi: 10.1016/j.fsi.2003.10.001.

Loken, M.R., 1990. Immunofluorescent technique in flow cytometry and sorting. Willey, 2nd Edn.

Mahajan, C.L. and Dheer, J.M.S., 1979. Autoradiography and differential haemoglobin staining as aids to the study of fish haematology. *Experientia*, 35, 834-835. doi: 10.1007/BF01968282.

Mahajan, C.L. and Dheer, J.M.S., 1980. Origin and development of neutrophils in an air breathing fish *Channa punctatus Bloch. Acta Zool.*, 61, 251-254. doi: 10.1111/j.1463-6395.1980.tb01310.x.

Margules, R.C. and Pressey, L.R., 2000. Systematic conservation planning. *Nature* (London), 405, 243-253. doi:10.1038/35012251.

Mittermeier, R.A. and Mittemeier, C.G., 1997. Megadiversity: Earth's Biologically Wealthiest Nation. In: McAllister, D. E., Hamilton, A. L. and Harvery B. (Eds.). *Global Freshwater Biodiv*. Sea Wind, Cemex, Mexico City, 11, 1-140.

Murad, A., Houston, A.H. and Samson, L., 1990. Haematological responses to reduced oxygen carrying capacity, increased temperature and hypoxia in goldfish (*Carassius auratus* L.). *J. Fish Biol.*, 36, 289-305. doi: 10.1111/j.1095-8649.1990.tb05610.x.

Pusey, B.J., Arthington, A.H., Stewart-Koster, B., Kennard, M.J. and Read, M.G., 2010. Wide spread omnivory and low temporal and spatial variation in the diet of fishes in a hydrologically variable northern Australian river. *J Fish Biol*., 77, 731-753. doi: 10.1111/j.1095-8649.2010.02712.x.

Quentel, C. and Obcach, A. 1992. The cellular composition of the blood and haematopoietic organs of turbot *Scophthalmus maximus* L. *J. Fish Biol.*, l41: 709-716.doi: 10.1111/j.1095-8649.1992.tb02700.x.

Safer, A. and El-Sayed, N., 1986. The fine structure of the nephronic tubule of the mudskipper *Periophthalmus koelreuteri* Pallas. *J. Morphol.*, 187, 109-121. doi:10.1002/jmor.10518 70109.

Santos, A.A., Gutierre, R.C., Antoniazzi, M.M., Ranzani-Paiva, M.J.T., Silva, M.R.R., Oshima, C.T.F. and Egami, M.I., 2011. Morphocytochemical, immunohistochemical and ultrastructural characterization of the head kidney of fat snook *Centropomus parallelus. J. Fish. Biol.*, 79. 1685-1707. doi: 10.1111/j.1095-8649.2011.02718.x.

Sarkar, U.K. and Bain, M.B., 2007. Priority habitats for the conservation of large river fish in the Ganges river basin. Aquat. Conserv., 17, 349-359. doi: 10.1002/aqc. 782.

Sarkar, U.K., Gupta, B.K. and Lakra, W.S., 2010. Biodiversity, ecohydrology, threat status and conservation priority of the freshwater fishes of river Gomti, a tributary of river Ganga (India). *Environmentalist*, 30, 3-17. doi: 10.1007/s10669-009-9237-1.

Sarkar, U.K., Pathak, A.K. and Lakram W.S., 2008. Conservation of freshwater Fish resources of India: new approaches, assessment and challenges. *Biodivers. Conserv.*, 17, 2495-2511. doi: 10.1007/s10531-008-9396-2.

Sarkar, U.K., Pathak, A.K., Sinha, R.K., Sivakumar, K., Pandian. A.K., Pandey. A., Dubey, V.K. and Lakra, W.S., 2012. Freshwater fish biodiversity in the River Ganga (India): changing pattern, threats and conservation perspectives. *Rev. Fish Biol. Fisheries*, 22, 251-272. doi: 10.1007/s11160-011-9218-6.

Schiemer, F., 2000. Fish as indicators for the assessment of the ecological integrity of large rivers. *Hydrobiologia*, 422/423, 271-278. doi: 10.1023/A:1017086703551.

Schneiders, A., Verhaert, E., De Blust, G., Wils, C., Bervoets, L. and Verheyen, R.F., 1993. Towards an ecological assessment of watercourses. J. Aquat. Ecosyst. Heal., 2(1), 29-38. doi: 10.1007/BF00043332.

Smeda, J.S., Houston, A. 1979. Evidence of weight-dependent differential hematological response to increased environmental temperature by carp, *Cyprinus carpio*. *Env. Biol. Fish*., 4(1), 89-92. doi: 10.1007/BF00005933.

Smith, A.M., Potter, M. and Merchant, E.B., 1967. Antibody-forming cells in the pronephros of the teleost *Lepomis macrochirus*. *J. Immunol.*, 99(5), 876-882.

Som, M., Kundu, N., Bhattacharyya, S. and Homechaudhuri, S., 2009. Evaluation of hemopoietic responses in *Labeo rohita* Hamilton following acute copper toxicity. *Toxicol. Environ. Chem.*, 91, 87-98. doi:10.1080/02772240801990700.

Suski, C.D, and Cooke, S.J., 2006. Conservation of aquatic resources through the use of freshwater protected areas: opportunities and challenges. Biodivers, Conserv., 16(7), 2015-2029. doi:10.1007/s10531-006-9060-7

Talwar, P.K. and Jhingran, A.G., 1991. Inland Fishes of India and Adjacent Countries. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, Vol. 1 and 2.

Tomonaga. S., Hirokane, T. and Awaka, K., 1973. Lymphoid cells in the hagfish. *Zool. Mag.*, 82. 133-135.

Wlasow, T. and Dabrowska, H., 1989. Cellular changes in the blood and haemopoietic tissues of common carp exposed to sublethal concentration of ammonia. *Aquat. Living Resour*., 2, 169-174. doi: 10.1051/alr:1989020.

Zuasti, A. and Ferrer, C., 1989. Haemopoiesis in the head kidney of *Sparus auratus*. *Arch Histol. Cytol*., 52, 249-255. doi: 10.1679/aohc.52.249.