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ENERGY DISPERSIVE X-RAY MICROANALYSIS AND ULTRASTRUCTURE OF SCALE OF *BARILIUS BARNA* USING SCANNING ELECTRON MICROSCOPE

R.K. Negi*, Tarana Negi and Tanuj

Department of Zoology & Environmental Sciences, Gurukula Kangri University, Haridwar 249404.
*Department of Zoology, Govt. College, Panchkula (HR)

Corresponding Author

R.K. Negi
Email: negi_gkv@rediffmail.com

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ABSTRACT

Present study was undertaken to analyze the ultrastructure and mineral content of the scale of fish species *Barilius barna* using SEM and EDX from the samples collected from the selected site of Kaushlya River adjoining Panchkula. The scales of *Barilius barna* were cycloid scales with an indistinct focus in the anterior region. The inter-circular space was maximum in the posterior region and minimum in the anterior part. Only two types of radii these were reported in the scale of species under report viz., primary radii and secondary radii with one distinct annulus, which indicated that this fish belonged to one year of age class. Six minerals were reported from the scale of the fish under report after the scale was subjected to energy-dispersive X-ray microanalysis (EDX) viz., calcium, carbon, oxygen, sodium, potassium and phosphate. It can be concluded that the circuli and the anterior part of the scale contains maximum percentage of minerals as compared to the other regions of the scales.

Keywords: Scale, *Barilius*, EDX, SEM.

INTRODUCTION

Agassiz [1] was the first to use the fish scales for the classification of fishes. As more than 80% of the fishes are covered by scales, hence, the knowledge of their detailed structure employing some advance techniques can be used for various purposes e.g. identification, classification, growth and pollution studies. Tandon and Sharma [2] described lateral line scales of some marine fishes of India and tried to show their utility of scales for the identification of fishes by giving a lepidological key, structure of fish scale can be used in the identification of fishes upto major groups [3,4], species levels [5], age determination [6].

The importance of scale morphology used in the classification was strengthened with the introduction and development of SEM [7], hence present study was undertaken to analyse the ultrastructure and mineral content of the scale using SEM and EDX from the samples collected from the selected sites viz. Bitna, Kanguwala and Datyar on the Kaushlya river adjoining Panchkula,

MATERIAL AND METHOD

During the course of the study fish samples belonging to the genus *Barilius barna* were collected using cast and hand nets brought to the laboratory for further analysis. For the study of ultra-structure of the scales, the scales were gently removed with tweezers and fine forceps from the left side of the body between the dorsal fin and lateral line in such a way that while removing the scales no damage is being done to the scale and cleaned by gently rubbing them with the help of thumb and finger tips. Immediately after removal they were cleaned mechanically using a fine brush and rinsed

with distilled water. The dried scales were fixed on the metallic stub with the help of double adhesive tape. They were coated with layer of gold having the thickness of 100 Å in a gold coating unit and were viewed under vacuum in SEM/EDAX Quanta 200 scanning electron microscope at accelerating voltage of 20kv and photographed. For the EDX studies, the cleaned and dried scales were mounted on the gold stub and coated with 100Å thick gold layer. The desired part of the scale was magnified on the SEM screen for the analysis and mineral content of different regions of the scales was analyzed by SEM/EDAX Quanta 200 from IIT Roorkee.

RESULT AND DISCUSSION

The scales of *Barilius barna* were cycloid scales which were almost circular in shape. The anterior face was smooth, embedded in the skin and was overlapped by the posterior part of the preceding scale. As the scales were transparent, they were suitable for study by using transmitted light. The typical scale when viewed under the SEM revealed that the scale can be divided into anterior, posterior and lateral regions (Fig.1a). The anterior scale was overlapped by the preceding scale whereas the posterior part was exposed. In this species, the focus was indistinct and lied in the anterior part of the scale. The shape of focus was oval or round having mesh like network (Fig.1d). From the focus lines of growth started appearing which were named as circuli (Fig.1b). The space between the circuli is called intercircular space. The intercircular space was maximum in the posterior region and minimum in the anterior part (Fig.1c). It was due to the anterior location of focus on the scale. Each circuli was wedge shape, having broad base and sharp end. In the anterior and lateral parts, the circuli were partitioned by deep and narrow grooves that run radially towards the focus. They were called as radii. Depending upon the point of origin of radii these were classified into two types viz., primary radii and secondary radii (Fig. 1a). Primary radii, originating from focus, reaching the margin of the scale and secondary radii, originating midway between focus and margin. There was no significant relationship between the number of radii and scale size, as the number of radii depends upon the location of the scale on the fish body. In the anterior region only primary radii were reported whereas, posterior region contains maximum number of primary radii followed by one or two secondary radii. The radii are open channels cut across the surface of the scale and provides flexiility. Whenever an annulus was formed, the circuli show broken pattern. In the present study one distinct annulus was reported, which indicated that this fish belonged to one year of age class. This pattern was very evident in between the two radii on anterior side the formation of annulus.

Energy dispersive X-ray Microanalysis (EDX) of scales of *Barilius barna*

The peak values of different elements recorded from the scale of *Barilius barna* have been documented in the Figs. 2. For the element detection, different parts of the scale viz., whole scale, focus, left circuli, anterior region and posterior region were subjected to energy-dispersive X-ray microanalysis (EDX). Six minerals were reported from the scale of the fish under report viz., Calcium, Carbon, Oxygen, Sodium, Potassium and Phosphate (Table 1). The region wise analysis (Table 2) of the scale indicated that posterior region of the scale contains maximum percentage of carbon (67.27%) followed by the focal region (66.74%).

Maximum concentration of Oxygen (25.07 %) was reported from anterior region followed by left circuli (24.47%) and whole scale (23.65%). Maximum concentration of Calcium was found in the circuli (18.75 %) and minimum in posterior region (4.98%). Potassium was reported with very low concentration in all the regions of the scale. Maximum percentage of Phosphorus was reported in the left circuli (9.33 %) followed by whole scale (3.82%) and minimum in focus (3.07%). Maximum sodium was reported in the focus (1.60%), followed by anterior region (1.18%) and posterior region (1.10%). Hence, it can be concluded that the circuli and the anterior part of the scale contains maximum percentage of minerals as compared to the other regions of the scales.

Table 1. Percentile values of different elements recorded from different region of the fish scales of *Barilius barna* using energy dispersive X-ray microanalysis.

Element	Whole scale	Focus	Left Circuli	Anterior region	Posterior region
CK	65.01	66.74	46.51	65.04	67.27
OK	23.65	22.86	24.47	25.07	22.36
NaK	00.98	01.60	00.51	01.18	01.10
PK	3.82	03.03	09.33	3.07	3.46
KK	00.58	00.42	00.24	00.50	00.43
CaK	5.95	05.36	18.75	05.13	04.98
Standard Deviation	24.83	20.71	23.15	25.07	30.29

Figure 1. SEM microphotograph of *Barilius barna (a)* normal scale (b) enlarged view of circuli (c) enlarged view of Radii (d) enlarged view of Focal region (F), C: Circuli; PR: Primary radii; SR: Secondary radii; F: Focus; AR: Anterior region; PR: Posterior region, C: Circuli; ICS: Intercircular space.

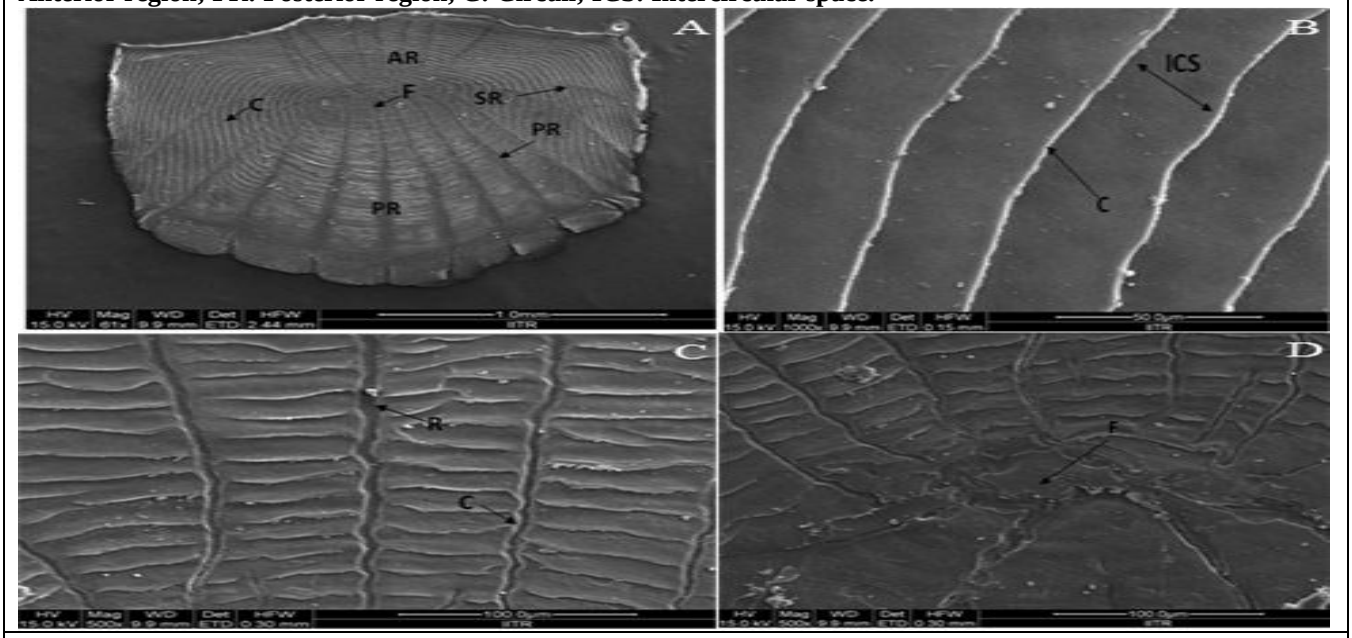
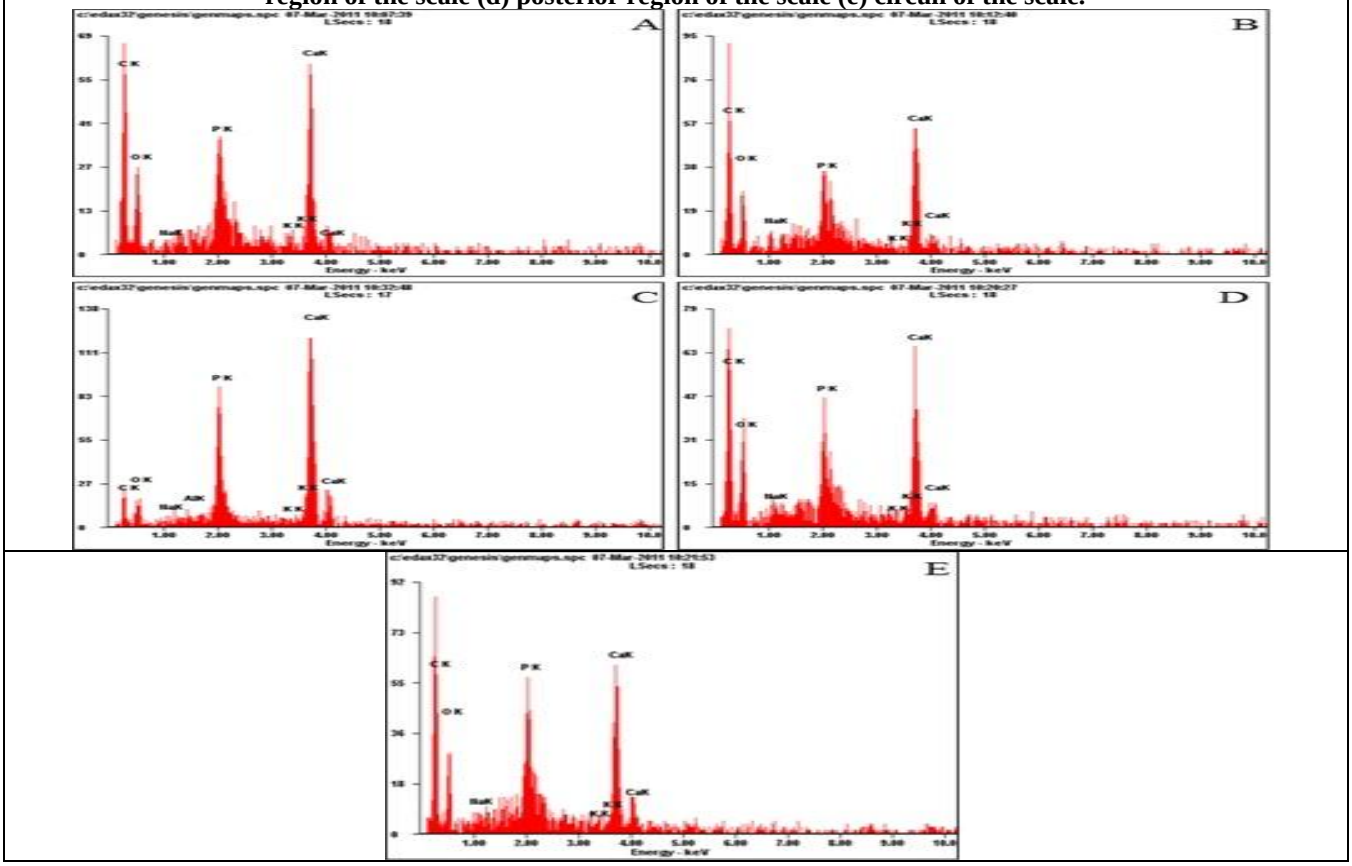


Figure 2. Peak values of different elements of *Barilius barna* (a) whole scale (b) focal region of scale (c) anterior region of the scale (d) posterior region of the scale (e) circuli of the scale.



DISCUSSION AND CONCLUSION

According to Van Oosten [8] circuli arises whenever bone forming materials occurs in a quantity greater than that can be utilized at the growing edges. They probably help in anchoring the sale in its pockets. The shape of circuli differs in different group of fishes. In carps, they are in the form of concentric rings. Radii on the scale increase the flexibility of circuli. De Lamater *et al*. [9] have given a good account of scales and ctenii. The elaborate hinged joints in the ctenoid scales of the fished appear in to the related to the flexibility of the body allowing extensive bending of the scales without fracture. Yang [10] studied the general morphological characters of scales of the body surface in cyprinoid fishes in his view lentic and lotic habitats have no relation to the phase of scale. Tandon and Sharma [2] described the lateral line scale of some marine fishes of India and tried to show the utility if scales in the identification of fishes giving lepidological key. However, plenty of work has been done on annulus formation in the different groups of fishes. Jhingran [11] recorded the annulus formation on the scales of *Cirrhinus mrigala* from the river Ganga at Buxar, on the scale of *Catla catla* from river Yamuna. In *Tor putitora* from Gobind Sagar [12] in *Cyprinus carpio* fron river Gagger, Nangal lake, Sukhna lake and Gobind Sagar Reservoir. During the investigation no annulus formation was recorded, this may be because of smaller size of fish. The causative factors for the annulus formation on the hard part of Indian fishes have been fully described [11-15]. It is very difficult to assign key role to any one factor responsible for the formation of annulus, logically, it appears that temperature and low electrolyte level of water are the most important factors. It can thus be concluded that the lateral line scale which represent the sixth sense in fishes is extremely important and helps the animal in either orientation or in location of prey or escaping from the enemy. In any case the role of the lateral line system in life of the fish cannot be underscored or is very dominant. Thus cycloid scales are the characteristics of the soft rayed fishes e.g. Carps Etc. whereas ctenoid scales are found in the spiny-rayed fishes. These two types of scales are found in almost all the bony fishes except cat fishes which are devoid of scales.

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