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Sexual Dimorphism in *Barilius bendelisis* (Hamilton, 1822) Based on Scale Structure as Revealed by SEM and EDX Studies

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Abstract— *Fishes abode almost every type of water conditions, which vary to an greater extent. As there is a great variation in aquatic ecosystem, hence, fishes show maximum diversity in form and structure amongst the vertebrates. In the monitoring of aquatic environment fishes play a very important role. Amongst carps, minnows are the inhabitants of clean, highly oxygenated and flowing waters. Their presence or absence reflects the water quality and overall health of the aquatic ecosystem. Minnows are considered to be excellent aquarium fishes because they show different colour patterns. Amongst minnows, the fishes belonging to the genus Barilius (Ham.) are typically tropical in distribution. There are 25 species belonging to the genus Barilius (Ham.). Barilirus bendelisis (Ham.) is distributed throughout India except in Kerala and is also present in Bangladesh, Nepal, Pakistan and Sri Lanka. In Himachal Pradesh its abundance has been observed in all hillstreams. As most of them inhabits the hill streams and have little economic importance from food and culture point of view, their biology and systematic have received little attention in the past. The present work is undertaken with clear objective to understand the sexual dimorphism of Barilius bendelisis which is clearly demarcated on the basis of scale structure. Very little attention have been paid on structure of lateral line canals especially using SEM technique. Therefore, an attempt has been made using SEM. technique lo study the lateral line scale of male and female Barilius bendelisis in order to study whether there is any sexual dimorphism pinpointed on the basis of structure of lateral line canal. For the quantitative analysis of mineral elements in the fish scales, the Energy Dispersive X-ray (EDX) microanalysis was undertaken.*

Keywords— *Barilirus bendelisis ,sexual dimorphism, lateral line scale, lateral line canal, (EDX) microanalysis*.

I. INTRODUCTION

Aquatic resources constitute an important factor in prosperity of a nation. Amongst the aquatic fauna, fishes constitute the most important group of study. Aquaculture has made an enormous contribution to the world food production. Among the aquatic fauna fishes constitute the most important group for study. The fish fauna of India consists of 2500 fish species out of which 930 species belonging to 64 families,16 orders arid 233 genera occurs in various types of freshwater.[1]

For better adaptability in water fishes are provided with a thick armor of scales which constitutes an exoskeleton rendering the skin thin and delicate. Scales are the activities of the skin and owe their existence to the presence of lime salts in the body[2]. Scales are present in all teleost fishes except the catfishes. The absence of scales is considered as an advanced character and can be correlated to active mode of life. Scales are dermal in origin. Scales consists of the calcified bony layer and no calcified fibrillar plate. The scales are arranged differently in different regions on the body of a fish. The scales along the lateral line are perforated by a canal through which opens the neuromast organs of lateral line system. The scales along the bases of unpaired fins are arranged in forms of tiles, thus giving an additional strength to fins. The scales in the axillae of the paired fins, especially of pelvics, are modified to form dragger-shaped structure.

Antony Van Leeuwenhoek (1696) made the first attempt not only to describe the general terms and the mode of growth of scales but also suggested that they might afford an index of age. He based his preliminary observations on sectioned preperation of Eel's'scales and concluded that each layer represents a year in the life of the fish.[3] supported him and observed that the markings on the scales afford some evidence of their rate of growth. The close relationship of growth of scale with the growth of individual was concluded by[4], while working on Norwegian herring scales. Fraser (1916) was the first to point out that the scale appears on the body of fish after it has attained certain length. [5] pointed that the number of radii in the scale is variable within the same species and also even in scales from the different parts of the body of same fish. [6]stated that the rings formed on the scale are annual in nature.

Channa punctatus scales can be used as pollution indicators is confirmed by [7]end they also observed drastic alteration in mineral composition of the scale when fish is exposed to sub lethal doses of an insecticide, endosulfan. [8]collected three exotic carps from Gobindsagar, Himachal Pradesh, India and they observed the presence of four important minerals Aluminum, phosphorus, calcium and iron in various regions of scale.[9]studied the mineral composition of different regions of scale of an endangered fish *Tor putitora* using Energy Dispersive X-ray microanalysis technique. [10]while reviewing the scale in Indian fresh water fishes opined the scales is the best tool to study the fish biology. The present work is undertaken with clear objective to understand the sexual dimorphism of *Barilius bendelisis* which is clearly demarcated on the basis of scale structure.

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II. Materials and Methods

For lepidological studies scales of *Barilius bendelensis* (Hamilton,1822) were removed from left side of the body of the fish between lateral line and the dorsal fin preferably from the third or fourth row from the dorsal fin. Lateral line scales were removed from the lateral line of both sexes preferably from .13th -15th lateral line scale .Each scale was removed with help of tweezers and washed thoroughly in tap water, cleaned by gently rubbing them with the help of thumb and fingertip. The scales were subjected to sonication thrice with acetone, for 5 minutes each time to remove the mucous, dust and other extraneous matter from scales .The scales were thoroughly dried between folds of Whatman filter paper No.1. For Scanning Electron Microscopic (SEM) studies, the cleaned and dried scales were mounted on the aluminum stubs with the help of double adhesive tape with dorsal surface upward and ventral surface sticking to the double adhesive tape; coated with a layer of gold-palladium al loy in' JEOL, FINE COAT ION SPUJTER JFC-1100'sputter coater. The scale samples were then viewed under'JEOL, TSM-1600 SCANNING MI CROSCOPE,' at an accelerating voltage of 20 KV. Clear view of scale was visible on th fluorescent screen of Scanning Electron Microscope. The images were photographed at different magnifications.

For the quantitative analysis of mineral elements in the fish scales, the Energy Dispersive X-ray (EDX) microanalysis was undertaken. For EDx same mounted stubs were used. The specimen stub was handled only with forceps because the skin oils may contaminate the specimen stub and microscope column. The elemental composition was determined by Leica EDX analyser attached to Leica Stereoscan 360 Scanning Electron Microscope. The image was observed on the screen of Scanning Electron Microscope at an accelerating voltage of 20 KV and the microprobe was set on desired part of the scale and the elemental composition was recorded at different sites. The data was analysed by Hewlett Packard Pentium 120 MHz computer with cache memory 256 Mb and RAM memory 16 Mb loaded with link ISIS software of Oxford Instrument Corporation for X-ray microanalysis .The analysed data was then displayed on the screen & printed.

III. Observations and Discussion

The scales of *Barilius bendelensis* (Ham) is cycloid and more broad than long (Fig.1). like normal scale it has anterior (A), Posterior (P) and Lateral (L) field.The most distinct feature of both male and female scales of *Barilius bendelensis* is the anterior ward location of focus (F) from which arises the primary radii. Secondary radii are present between the primary ones. The anterior part of the scale is overlapped by the posterior side of preceding scale, therefore it is soft and translucent. Whereas the exposed posterior part is hard and semi-opaque. Ventral part of scale attached to skin is shiny and smooth as compared to dorsal part which is rough.

Normal Body Scale of Barilius bendelensi (Male)

The scale is divided into Anterior(A), posterior(P) and Lateral (L)regions (Fig. 1). There is a distinct focus (F) which lies in the anterior half of the scale. The circuli are clear in the anterior and lateral regions, whereas indistinct in posterior region due to the presence of a covering of epithelial layer. The length –breadth ratio of 1:1.31.The radii are present anterior and posterior to focus and absent in the lateral fields. Posterior region has a triangular shape. Numerous elevations are present in the posterior region in the form of chromatophores (Ch) (Fig. 1). The circuli do not run in circular fashion. In the anterior region they assume semi-circular fashion and in lateral region they arrange themselves in a vertical fashion (Fig. 1). The circuli in the marginal area of anterior region are ill organized and in the rest of the anterior region they are cut of by radii (Fig 1). The posterior region of the scale of male fish has protuberances which imparts roughness to the body surface. They are of varying sizes and show different configurations (Fig. 2). Each protuberance is an aggregation of irregular cells on the margin and show clear cut finger like projections which are visible in the central part (Fig. 3). The projections show the presence of microvilli like structures, which are the modifications of the epithelial layer (Fig. 4). There is clear cut demarcation between the anterior and posterior regions of scale on the dorsal part of scale. The margin between anterior and posterior part of scale has large number of pustules filled with mucous. The pores present on the epithelial layer may help in the transfer of mucous from skin to outside and these pustules may act as reservoirs of mucous (Fig. 5). Long canals are observed in the posterior region of scale at higher magnification. It is assumed that these canals – like structures provide passage for movement of mucous when it is secreted (Fig. 6). The circuli in the marginal area of anterior region are ill organized and in rest of the anterior region they are cut off by radii (Fig. 7). At low magnification, the exact shape of circuli in the anterior region could not be interpreted. Actually, the circuli in this region don't show clear straight line and are broken. The anterior most circuli are without protuberances (Fig. 7) but the circuli located-away from the anterior margin shows presence of protuberances which are of irregular shape. The radii show clear canal like structures (Fig. 7).

Fig. 1. SEM microphotograph of complete scale of *Barilius bendelisis* (male) showing posterior side (P)with anterior (A), lateral (L), posterior (P) , radii(R), circuli(C), focus (F), chromatophores (Ch)

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Fig. 2. SEM microphotograph of posterior field (P)of scale of *Barilius bendelisis* (male) showing chromatophore

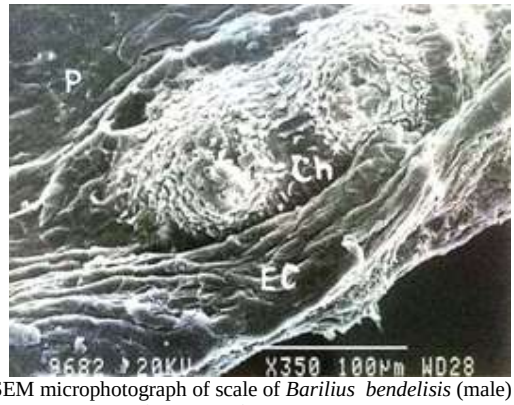

Fig. 3. SEM microphotograph of scale of *Barilius bendelisis* (male) showing magnified view of chromatophores (Ch)

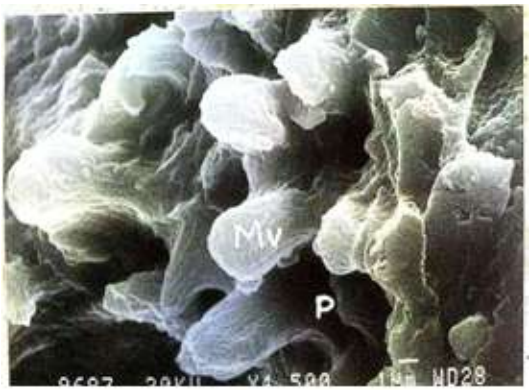

Fig. 4. SEM microphotograph of scale of *Barilius bendelisis* (male) showing posterior field (P) with microvilli (Mv) like structures in chromatophore

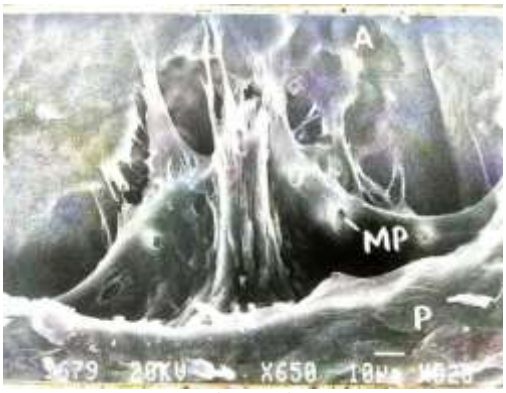

Fig. 5. SEM microphotograph of scale of with *Barilius bendelisis* (male) showing opening of mucouspore (MP) in margin between anterior(A) and posterior (P) regions of scale

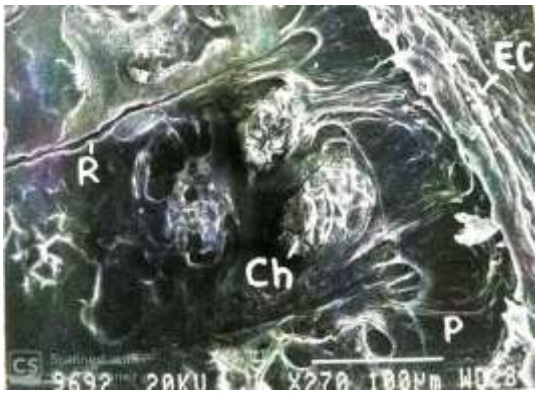

Fig. 6. SEM microphotograph of scale of *Barilius bendelisis* (male) showing magnified view in posterior region (P) with epitheli

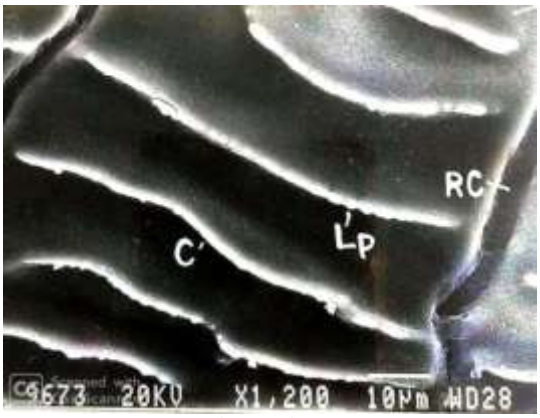

Fig. 7. SEM microphotograph of scale of *Barilius bendelisis* (male) showing magnified view of circuli(C) with poorly developed, Lepidonts (Lp) and radial canal (RC)

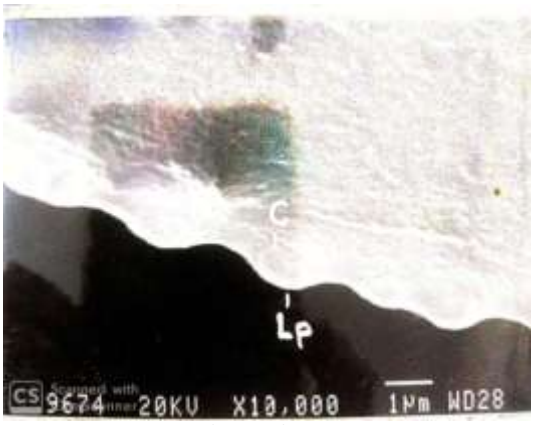

Fig. 8. SEM microphotograph of scale of *Barilius bendelisis* (male) showing circuli (C) with lepidont (Lp)

Secondary radii arise close to the anterior margin but away from focus. Circuli in the anterior marginal region are in organized and broken (Fig. 8). The base of each circulus is not well marked and on its outer surface it has clear distinct protuberances of varying sizes (Fig. 8).

Normal Body Scale of Barilius bendelensi (Female)

The outline of scale is like that of male, but differs from it in having length –breadth ratio of 1:1.12. The circuli are clear in the anterior and lateral region and indistinct in posterior region. The posterior region of scale is semi -oval and free

from protuberances (Fig.9). The radii appear from focus which is not pinpointed. In anterior region there is no distinct circuli approximately upto 80µm and then regular formation of circuli takes place. In region posterior to focus irregular circuli formation has been observed. The radii cut circuli in vertical canal –like structures. The circuli in the anterior region are without protuberances (Fig. 10) whereas inn malespecimens protuberances of irregular shape observed (Fig. 8.). In posterior region of scale pores of various size are seen but chromatophore are absent (Fig. 11). The area between the anterior and posterior region of scale is clearly demarcated by absence and presence of epithelial covering (Fig. 12).

Fig. 9. SEM microphotograph of complete scale of *Barilius bendelisis* (Female)

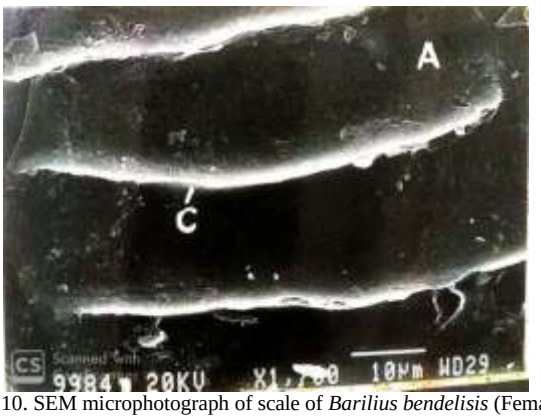

Fig. 10. SEM microphotograph of scale of *Barilius bendelisis* (Female) anterior sides showing circuli (C) with no lepidont

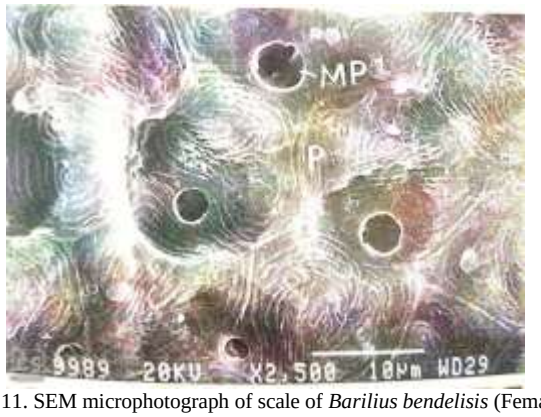

Fig. 11. SEM microphotograph of scale of *Barilius bendelisis* (Female) posterior side with Mucous pore(MP)

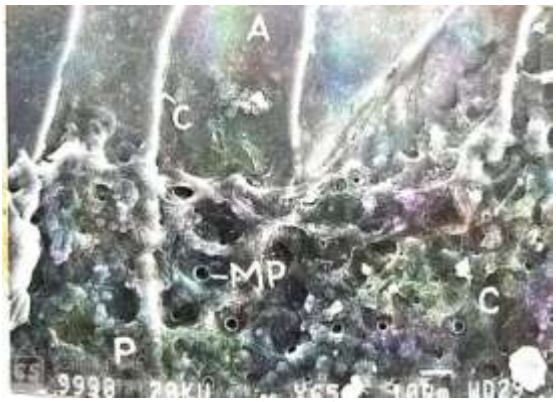

Fig. 12. SEM microphotograph of scale of *Barilius bendelisis* (Female) showing margin between anterior (A) and posterior side of scale with opening of mucous pore(MP)

Fig. 13. SEM microphotograph of Lateral line scale of *Barilius bendelisis* (male) showing anterior (A) ,posterior (P),lateral fields (L), Lateral line canal(LC) ,anterior opening of line canal(AOLC), posterior opening of line

Lateral Line Scale of Barilius bendelensi (Male)

The lateral line scale is divided into Anterior(A), posterior(P) and Lateral (L) regions. The focus is obliterated because of the presence of lateral line canal in the centre. The anterior margin is neither is neither rounded nor circular but somewhat flat (Fig. 13). Circuli are absent in the region anterior to lateral line canal,which is occupied by its opening. Circuli more prominent in lateral and antero-lateral portion. Posterior region is more or less triangular in shape. The pore of lateral line canal opens in the anterior face of scale and is guarded by eve-like projections. Numerous elevations are present in the posterior region in form of protuberances but absent in female lateral line scale. The scale is more broad than long and has the ratio of 1:1.37.The canal has a cup like cover at its tip whose eve-like projections are rounded .The posterior region of lateral line canal merges with posterior region of scale .The eve like projection look like a spatula (Fig. 14). Circuli are ill organized around the lateral line canal.

Lateral Line Scale of Barilius bendelensi (Female)

The outline of lateral line anal is more or less like that of male but differs in possessing distinct circuli anterior to the opening of the canal(Fig. 15)Thus distinct circuli can be marked on the lateral and antero-lateral regions. A few circuli are present on the posterior region which are absent in case of male lateral line scale. The circuli start appearing close to

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lateral line canal whereas in case of male they appear at a distance from lateral line canal in the lateral region of scale. There is not much difference between length and breadth of scale and has a ratio of 1 : 1. 23. The protuberances so distinct in male are absent on the posterior side of scale in case of female (Fig. 15). The pore of lateral line canal opens on the anterior region. On the left side the eve-like projection is produced into a spine. There is no such projection on the right side which is at a lower level than the left side (Fig. 16). A few radii are present on the lateral sides of the scale which belong to the class of primary radii. Anterior part of lateral line canal is not spatula shaped as in case of male lateral line canal (Fig. 14) and (Fig. 16). Posterior part of lateral line canal which merges with epithelial layer in the posterior side of scale has ill organized circuli and radii arising from the side of lateral line canal (Fig. 16) .In the middle portion of lateral line canal the width is narrow as compared male lateral line canal which is much broader (Fig. 14) and (Fig. 16). Posterior region scale has epithelial layer which secretes mucous inside and around it through rounded pore-like openings (Fig. 15).

Fig. 14. SEM microphotograph of Lateral line scale of *Barilius bendelisis* (male) showing Anterior part of Lateral line canal (ALC), Middle part of Lateral line canal(MLC), Posterior part of Lateral line canal(PLC)

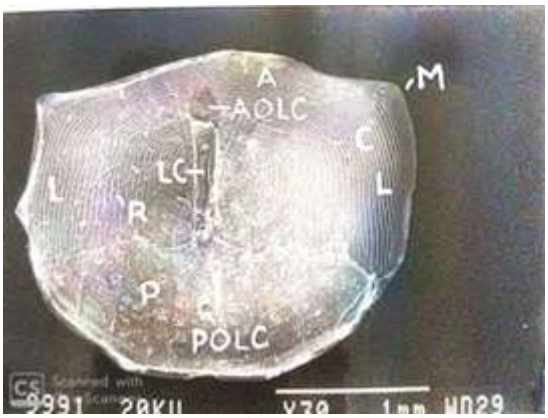

Fig. 15. SEM microphotograph of Lateral line scale of Barilius bendelisis (Female) showing Anterior opening of Lateral line canal(AOLC), Posterior opening of Lateral line canal(POLC)

Fig. 16. SEM microphotograph of Lateral line scale of Barilius bendelisis (Female) showing Anterior opening of Lateral line canal(AOLC), with eve like projections (EP)

The perusal of literature has indicated that the fish scales have been successfully employed for the identification of fishes. From time to time (as early as 1696 by Leeuwenhoek), the structure of fish scale was related to its growth. In the later year some workers like Williamson (1849), Hoffbauer (1898) and Thomson (1902) did mention that scales can be used as growth indicators.

In the second decade of 20th Centuryy various workers e.g.[6], [11],[12], [2], [13],[14], [15,16], [17], [18] worked extensively the structure of scale in depth to make it suitable for age determination and growth studies. The attempt of most of the workers has been rewarding and contributed for the management of commercial fishery and for the conservation of either threatened or endangered fish species. The use of various parts of scale for, the confirmation of sexual dimorphism is the first attempt not described earlier. On the basis of present studies, it is suggested that in other fishes where sexual dimorphism exists, scales both normal and lateral line should be subjected to SEM investigations. In the past the number of lateral line scales on the lateral line has been used in the identification of fish species. Very few attempts have been made to use the structure of lateral line to show the sexual dimorphism, which is clearly evident in *Barilius bendelensis*. [19]has mentioned the possible important implication of the morphological variation in structure of lateral line canal in taxonomy, in scale growth and development, and in lateral line mechanoreception. [15]pointed out that the structure of lateral line canal, coupled with other structures, can contribute for determining and classifying fishes. Thus there is strong evidence that not only general morphology of the scalebut lateral line scales of male and female *Barilius bendelensis* showed marked sexual dimorphism

B. Mineral Analysis

EDX microanalysis is the analysis of very small samples and is one of the dependable methods of analyzing microscopic substances. It is ideally suited to elemental concentration with minimum detectable limits ranging between 0.1% to 1%. It is based on the principle that when electrons of appropriate energy strike a sample, they cause the

emission of x-rays whose energies and relative abundance depend upon the composition of the sample.

The use of EDX microanalysis for the study of mineral composition of fish scale is a more reliable. Chemical analysis of a fish scale by ordinary methods is possible [2]but not reliable. The mineral composition of different parts of the scale is helpful in separating the populations living under different ecological conditions, in determining the past of a fish in question, sexual dimorphism etc. [9]studied the mineral composition of different regions of the scale of an endangered fish, golden mahseer, Tor putitora. In following years more attempts have been made on scales of other fishes [78].

For the present study different regions of the scale were subjected to Energy Dispersive X-ray microanalysis technique to assess the elemental composition of different regions of the male and female scale. It has been observed that the scales of *Barilius bendelensis* indicated the presence of carbon, oxygen, sodium, magnesium, phosphorus, chlorine, calcium, iron and sulphur. From the data presented in Table 1 it is evident that the scale has maximum percentage of oxygen followed by calcium, carbon, phosphorus, sodium, magnesium, iron, chlorine and sulphur respectively. The percentage composition clearly indicates that oxygen, calcium, carbon and phosphorus constitute the major elemental part of the scale whereas percentage of elements like sodium, magnesium, iron, chlorine and sulphur varies between 0.051-0. 739% and hence they are termed as trace elements.

TABLE 1. Weight % of different elements present in different regions of normal body scale of male and female of *Barilius bendelisis* (Ham)

Elements		Elemental Composition(%)				Range%	Range difference%	Mean %
		Normal Scale						
		Entire scale	Anterior Field	Circuli	Focus			
Carbon	♂	26.715	*	*	*	26.715	26.715	26.715
	♀	16.050	31.217	5.170	8.786	31.217-5.170	26.047	15.306
Oxygen	♂	41.117	60.212	49.022	30.687	60.212-30.687	29.525	45.260
	♀	44.951	33.615	45.361	47.263	47.263-33.615	13.648	42.798
Sodium	♂	*	0.605	0.449	0.316	0.605-0.316	0.289	0.457
	♀	0.332	0.313	0.499	0.445	0.499-0.313	0.186	0.397
Magnesium	♂	0.214	*	0.353	0.271	0.353-0.214	0.139	0.261
	♀	0.270	0.188	0.322	0.300	0.322-0.188	0.134	0.27
Phosphorous	♂	11.102	14.229	18.204	23.334	23.334-11.102	12.232	16.717
	♀	13.702	11.038	17.599	15.792	17.599-11.038	6.561	14.533
Chlorine	♂	*	0.010	0.032	0.136	0.136-0.010	0.126	0.059
	♀	0.0415	*	0.010	0.103	0.103-0.010	0.093	0.051
Calcium	♂	20.114	24.759	31.844	45.098	45.098-20.114	24.984	30.454
	♀	24.652	23.629	30.850	27.310	30.850-23.629	7.221	26.610
Iron	♂	*	0.184	0.096	*	0.184-0.096	0.088	0.14
	♀	*	*	0.187	*	0.187	0.187	0.187
Sulphur	♂	0.739	*	*	*	0.739	0.739	0.739
	♀	*	*	*	*			

* Below detectable limits

The percentage composition of oxygen and calcium is 45.260% and 30. 454% respectively in case of scale of male and 42.798% and 26. 610% respectively in case of scale of female and are dominant in all regions of the scale. The percentage of carbon is 26.715% in the entire scale and insignificant in anterior field, circuli and focus in case of male whereas in female percentage of carbon is 15. 306% and shows fluctuations in different parts of scale i.e 5.170% in circuli and is present in significant amount in 3l.217% in the anterior field. The percentage of sulphur in the entire scale of male is 0.739% and elsewhere below detectable limits but its percentage is below detectable limits in the scale of female. In the anterior field of scale oxygen is present in high amount in male 60.212% as compared to female 33. 615%. Thus it can be concluded that oxygen element is most important component in scale of *Barilius bendelensis*. The percentage of calcium is 30.454% in case of male and 26.610% in case of female scale. Thus roughness of scale of male can be attributed to the maximum occurrence of calcium in all the parts of scale.

[9]studied the mineral profile of Indian major carps, Amblypharyngodon microlepis, Danio devarro and Tor putitora respectively. [2]studied the scales of *Cyprinus carpio* and *Labeo* spp. And reported the occurrence of calcium, phosphorus and magnesium. The occurrence of other elements in minor quantities I. e. F, Li, cu, Na, Ba, Cr depends upon their occurrence in surrounding waters, where the fish abodes .

t[9] have reportecl the maximum porcentage of calcium (4l.97%) followed by phosphorus (27.32%), iron (15.08) and alluminium (14.9T%) in scales of *Tor putitora* from Gobindsagar. The occurrence of sodium and silicon in different regions of the scale is attributed to the varying types of habitat, where the fish lives during the different phases of life. The EDX of the scales of other fishes like *Cyprinus*, *Labeo* showed the presence of less number of elements as their habitat is very much restricted.

The presence of oxygen and chlorine is helpful in the formation of the salts of the various minerals present in the scale. The percentage composition of the minerals varies in the different regions of the scale and in sexes. The presence of sulphur in the entire scale in male, though in lesser amount is of great interest and its absence in female clearly indicate that the mechanism of its uptake from the surrounding water is more efficient in male than female. The scale of *Barilius*

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bendelisis follows the general characteristic pattern of cyprinoid scale that is characterized by the presence of maximum percentage of calcium whereas other elements e. g. sodium, iron, present in trace quantities. Surprisingly the phosphorus showed its presence in fairly good amount.

IV. Summary

The present Work deals with sexual dimorphism in *Barilius bendelensis* (Ham.) based on scale structure as revealed by SEM. For SEM studies, the scales were washed in sonicator in acetone and Mounted on the aluminium stubs with double adhesive tape. The gold- palladium alloy coating was done to make the specimen conductive. The stubs were then mounted in'JEOL, TSM-1600 SCANNING MICROSCOPE', processed in vacuum at a voltage of 20 KV. The various images were observed on the screen of SEM.

The general structure of the scale of *Barilius bendelisis* resembles a typical cyprinoid scale, but shows sexual dimorphism. The normal body scale of male has tubercle-like structures in the posterior field whereas in the female posterior field has only epithelial layer containing openings of mucous pores. In male the tubercles have well developed villi which are the derivatives of the epidermal covering on the posterior side of the scale. The length-breadth ratio of the scale is 1:1. 31 and 1:1.12 in males and females respectively showing once again sexual dimorphism.

Lateral line scale of male of *Barilius bendelensis* has eve-like projection on the anterior side of lateral line canal produced into a spatula shaped structure whereas in case of female eve-like projection is produced into a spine on the left side of lateral line canal .The lateral line scale of female differs from male in possessing distinct circuli anterior to the opening of the lateral line canal. In the middle portion of lateral line canal, the width is narrow in case of female as compared to male lateral line canal, which is much broader. Posterior side of lateral line scale of male has protuberances whereas it is absent in case of female.

Energy Dispersive X-ray (EDX) microanalysis technique shows the occurrence of different minerals viz., carbon, oxygen, sodium, magnesium, phosphorus, chlorine, calcium, iron and sulphur. Of all these minerals oxygen, calcium, carbon and phosphorus constitute the major elemental part of scale whereas percentage of elements like sodium, magnesium, iron, chlorine and sulphur varies between 0.051-0. 739% contributing to the trace elements. The percentage of carbon is 26. 715% in the entire scale and insignificant in anterior field, circuli and focus in case of male whereas in female percentage of carbon is 15. 306% and. shows fluctuations in different parts of scale i.e. 5. 170% in circuli and 31. 217% in the anterior field. The percentage of sulphur in the entire scale of male is o. 739% but is below detectable limits in the scale of female. The percentage of calcium is 30. 45% in case of male and 26. 610% in case of female scale. Thus roughness of scale of male can be attributed to the maximum occurrence of calcium in all the parts of scale again confirming sexual dimorphism. It can, therefore be concluded that scales of *Barilius bendelisis* can be used to study sexual dimorphism.

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