e-ISSN:2321-6190
p-ISSN:2347-2294

Research & Reviews: Journal of Zoological Sciences

Scanning Electron Microscopy and Edax Study of Scales of Genus Puntius

Roushan K. Thakur*, Negi RK and Raushani Raj
Department of Zoology and Environmental Sciences, Gurukula Kangri Vishwavidyalaya Haridwar (Uttarakhand), India

Research Article

Received date: 14/10/2015
Accepted date: 16/12/2015
Published date: 21/12/2015

***For Correspondence**

Department of Zoology and Environmental Sciences, Gurukula Kangri Vishwavidyalaya Haridwar (Uttarakhand), India

E-mail: roushanthakur6@yahoo.in

Keywords: SAM, EDAX, Scales, Puntius conchonius, Puntius sophore.

ABSTRACT

Present study was designed and an attempt has been made to study the Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray spectroscopy (EDAX) of fish of genus Puntius from Uttarakhand. Puntius conchonius and Puntius sophore (Spotted barbs) in the fast flowing water of Song River. The body of the fish in laterally compressed and is covered over by numerous small scales. The number of lateral line scale is approximately ninety. The fish adheres to the bottom by well-developed reflected lower lips. The typical scale when viewed under SEM reveals that the scale can be divided into anterior, posterior and focus regions. The scales of Puntius conchonius and Puntius sophore are cycloid scales which are roughly oval in shape. The scales from focus region of the black spot have 75% of degenerated scales as compare to normal body scale of genus Puntius. In the case of Puntius sophore black spot scale a total seven elements were found from the different region of the scales viz., Ca, P, O, C, Mg, Na and K. In the case of Puntius sophore (middle part) eight elements were found from the different region of the scales. These minerals were Ca, P, O, C, Mg, Na, K and Zn. Lateral line canal of lateral line scale of this species covered 80% of entire length of scale. It is tube like structure having two opening the anterior and posterior opening, anterior opening is circular in shape and open near the tip of the posterior end of the scale.

INTRODUCTION

Among the aquatic fauna, fishes constitute the most important group for study. Fish is an aquatic vertebrate, which breathe by means of gill and swim with help of fin supported by fin rays. Each fish species has a particular habitat which provides a condition of life most appropriate to its survival, such as mountainous streams for trouts. For the better adaptability in water, some fishes possess much scale which constitutes an exoskeleton rendering the skin thin and delicate. The presence of scale in fish body is the characteristic feature of class pieces of phylum Chordata. Scales are the result of the activities of the skin and on the presence of lime salts in the body. Amongst the hard parts of a fish, scales, if present, can be removed from the body of the fish without causing any visible injury. Fishes are known for indeterminate growth and this makes them an excellent material for study of age and growth. The overall growth of the fish is directly or indirectly influenced by factors like food, inter and intra-specific or intrageneric competition, sex, temperature, density, and productivity of water, the latter depends upon the prevailing physic-chemical conditions. It has recently been highlighted that, due to loss of fish habitat as a result of water management practices, the release of effluents into natural water bodies and several anthropogenic activities, most of the fish species of developing and under developing countries are under different types of the threats as is evidences from the squeezing in the stock of most of the fish species [1]. In some case the fish community structure is completely disrupted due to the reduction in fish stock. Fish biologist is unable to get large specimens for studied related to fish bionomic. Under these circumstances, a lepidological study of the best alternative, as fish scale is considered to be the best tool in fish biology[2]. Keeping in mind the above facts an attempts has been made to study the SEM and EDAX of fish of genus Puntius from Uttarakhand.

e-ISSN:2321-6190
p-ISSN:2347-2294

MATERIAL AND METHODS

To study the ultra-structure of the scale of Puntius conchonius and Puntius sophore, the fishes were collected from Song river using cart net and hand net during the period of Jan to April 2014.

Scales were gently removed with tweezers and fine forceps from the middle part above the lateral line and some scales from lateral line in such a way that while removing the scales no damage is being done to the scale. Cleaned by gently rubbing them with the help of thumb and fingertips to remove the mucous and extraneous matter and sonicated in a sonicator for 10 min to get rid of fine dust particles and other extraneous matter. Sonicated scales were dried on Whatman filter paper. Immediately after removal they were cleaned mechanically using a fine brush and rinsed with distilled water. For scanning electron microscope studies the cleaned and dry scales were mounted on the metallic stubs by double adhesive tape, with dorsal surface upward and the ventral surface sticking to the tape and coated with a 100A thick layer of Gold in Gold coating unit. The Gold coating overcomes the problem of charging and beam damage. Slight adjustments were made to increase sharpness and contrast of images of scale. The scales were viewed under vacuum at an accelerating voltage of 20 Kv in SEM/EDAX quanta 200 FEG scanning electron microscope (FEI). Various images of the scales were recorded on photographic plate.

After SEM scale is used for EXAD study, different region of the scale is selected separately for the EDAX study and then chemical composition has been evaluated.

RESULT AND DISCUSSION

Puntius conchonius and Puntius sophore (Spotted barbs) were collected from the fast flowing water of Song River which is spring fed stream. The body of the fish in laterally compressed and is covered over by numerous small scales. The number of lateral line scale is approximately ninety. The fish adheres to the bottom by well-developed reflected lower lips.

The scales of Puntius conchonius and Puntius sophore are cycloid scales which are roughly oval in shape **(Figure 1.1).** The posterior face is rough and bears chromatophores. As the scales are transparent, they are suitable for study by using transmitted light.

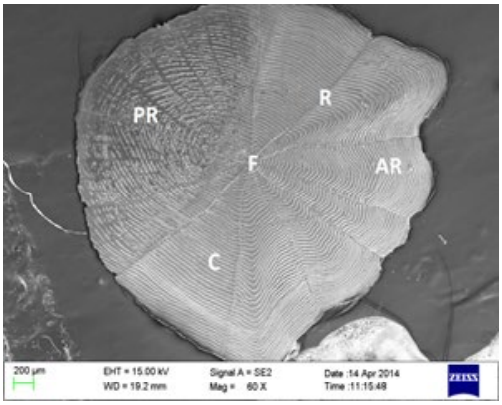

Figure 1.1. SEM microphotograph of *Puntius sophore* showing the complete normal body scale. Focus (F), Radi (R), Circuli (c), Anterior region (AR) and Posterior region (PR).

The typical scale when viewed under the SEM reveals that scale can be divided into anterior, posterior and lateral field. The anterior and lateral parts of scales are over lapping by preceding scales whereas the posterior part is exposed. Focus is present middle of the scale **(Figure 1.1)**; there are present circular lines of growth called circuli. The circuli are distinct in the all the sides with regular space pattern. Depending upon the point of origin the radii are primary, secondary and tertiary type **(Figure 1.2).** Primary radii originating from the focus and Reaches the margin of the scales, secondary radii originating midway between focus and margin, tertiary radii originating between mid-way and margin **(Figure 1.3).**

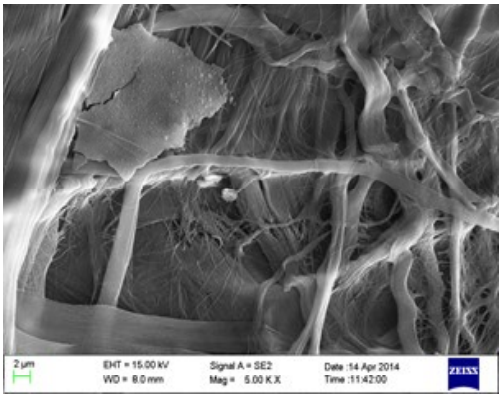

Figure 1.2. SEM image of a fractured section of a fish (*Puntius conchonius*) scale showing sliding of the collagen structure and pulling out and breakage of individual collagen fibers.

e-ISSN:2321-6190
p-ISSN:2347-2294

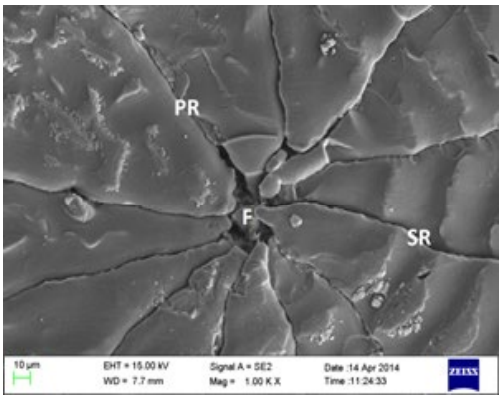

Figure 1.3. Focus (F) region of *Puntius sophore* showing primary radii (PR), Secondary radii (SR).

There was no significant relationship in 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 present study primary radii was reported maximum as compared to secondary radii and tertiary radii **(Figure 1.4)**.

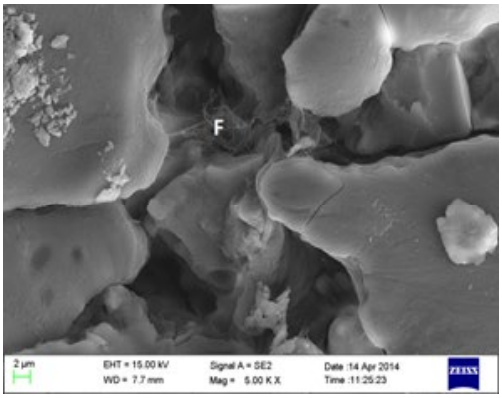

Figure 1.4. Enlarged view of focus region of scale of *Puntius sophore.*

There was uniform pattern of intercellular space. The radii have open channel **(Figure 1.5)** cut across the surface of the scale and provide flexibility. When selection of scale was subjected to magnification of 5000x it shows the collagen structure and pulling out and breakage of individual collagen fibres **(Figure 1.6).** During the course of investigation scale present on the black spot were also removed and were subjected for the SEM study **(Figure 1.7).** The cycloid scales of genus Puntius view under SEM revealed that these have not clear focus region. The scales present on the black spot have 75% of degenerated scales as compare to normal body scale of genus Puntius. Radii were uniformly distributed through the scales **(Figure 1.8).** Circuli and radii are distinct away from the focus region. There was no structural differentiation in the regenerated part of the scale.

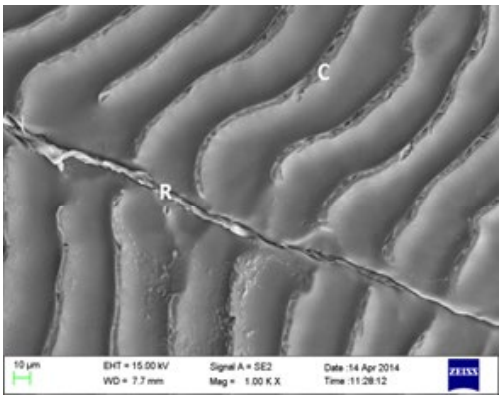

Figure 1.5. SEM microphotograph of scale of *Puntius sophore* showing circuli (C), radii (R) and inter circuli space (ICS) on anterior region.

Lateral line scale

Lateral line scale is roughly rectangular in shape. Focus is indistinct and present towards the anterior side. Circuli are well marked **(Figure 1.9).** Primary and secondary radii are very distinct. Lateral line canal covered 80% of entire length of scale. It is tube like structure having two opening the anterior and posterior opening, anterior opening is circular in shape and open near the tip of the posterior end of the scale **(Figure 1.10).** When lateral line scale was exposed to high magnification, a clear cut distinct focus was observed. From the anterior region of lateral line scale there is wide lateral line canal which narrow towards the

posterior region of the scale **(Figure 2.0).** There are fingers like projection of primary radii originating from the margin of focus is very much clear with distinct appearance of radii **(Figure 2.1).** Thus it can be conducted that all the characters such as shape of the scale, type of circuli, number of radii present and absence can be used for the taxonomic purpose. At this moment the data on the scale of Puntius conchonius and Puntius sophore is available here it is supported that the scale structure of other species of genus Puntius should be worked out to construct authentic key for the taxonomic purpose, hence the observation presented here from the base line data for understanding the structure of the scale in the future studies.

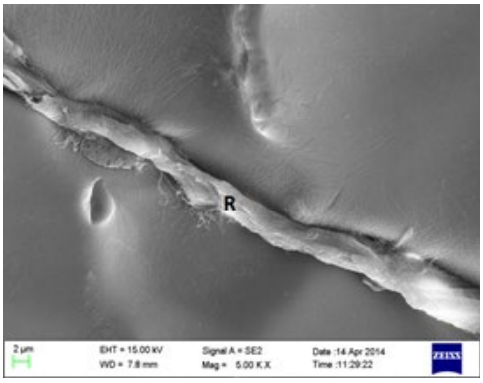

Figure 1.6. SEM microphotograph of scale of *Puntius sophore* showing radii on posterior region.

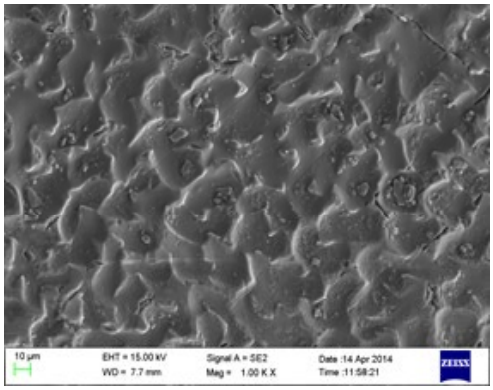

Figure 1.7. SEM images of the surface morphology of the osscous layer of scale showing porous granular texture of calcium phosphate crystals on the scale of *Puntius sophore*.

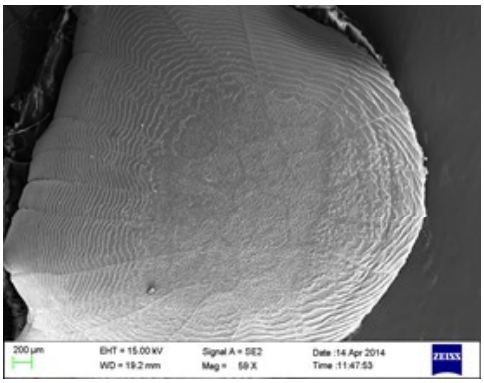

Figure 1.8. Regenerated scale of *Puntius sophore*.

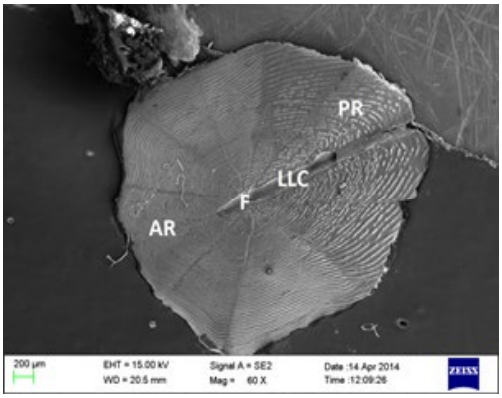

Figure 1.9. SEM microphotograph of complete lateral line scale of *Puntius conchonius* showing lateral line canal (LLC).

e-ISSN:2321-6190
p-ISSN:2347-2294

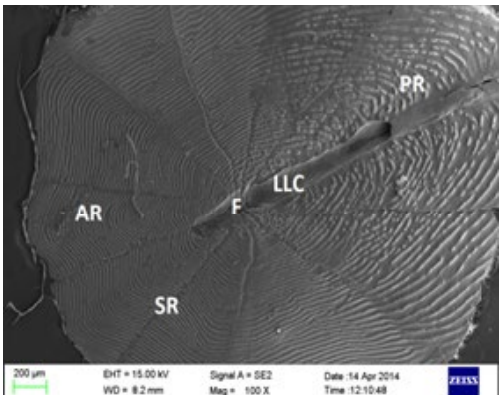

Figure 1.10. SEM microphotograph of complete lateral line scale of *Puntius conchonius* showing the posterior part of lateral line canal (LLC) along with primary radii (PR) and secondary tadii (SR).

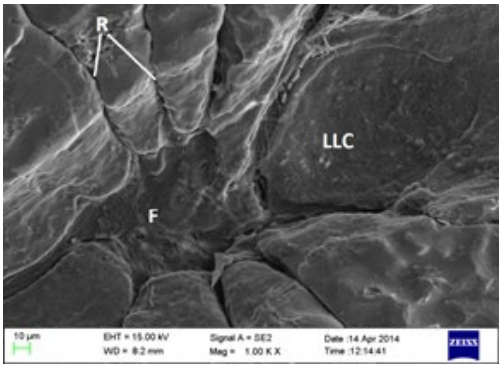

Figure 2.1. SEM microphotograph of lateral line scale of *Puntius conchonius* showing focus and a point from lateral line canal (LLC) starts, Radii (R) and Focus (F).

Energy Dispersive X-ray micro-analysis (EDXA) of scales of Puntius conchonius and Puntius sophore were carried out. The peak values of different elements recorded from the scale of Puntius conchonius and Puntius sophore . For the element detection different regions of scales viz, whole scale, focus region, anterior and posterior regions were selected to EDAX studies. The scale of Puntius Conchonius contains five minerals viz calcium, phosphorus, oxygen, carbon, magnesium in both types of scales (black spot & middle part) but only zinc is present in focus region of middle part of scale.

The region wise analysis of (black spot) scale of Puntius conchonius indicated that anterior region contains maximum (12.7%) calcium followed by focus (4.46%) and minimum amount of calcium was recorded (3.0%) at posterior region. Maximum concentration of oxygen (36.59%) was recorded from posterior region and minimum (30.23%) in anterior region. Percentage composition of phosphorus was reported low at all regions of the scales. Maximum percentage of carbon (60.07%) was reported from whole scales and minimum (49.99%) from anterior region. However magnesium was recorded from focus and anterior region of the scales **(Table 1.1).**

In the case of Puntius sophore black spot scale a total seven elements were found from the different region of the scales. These minerals were Ca, P, O, C, Mg, Na and K. During the present investigation maximum percentage of oxygen (46.36%) has recorded from the anterior region followed by (42.75%) in focus region. Whereas Ca was recorded (22.97%) in focus region and minimum from posterior region of the scale (17.61%). Phosphorus was record (14.46%) in focus region and minimum (9.86%) from posterior region. Maximum percentage of carbon (40.57%) was recorded from posterior region and minimum from focus region. Percentage concentration of magnesium was recorded very low in all regions of the scales. Sodium (Na) was recorded from whole scale, focus and anterior region only, whereas Potassium (K) has reported from focus, anterior and posterior region only **(Table 1.1).**

During the course of investigations comparative study of scales from middle part of fish body were also considered for the element composition. The region wise analysis of the scale Puntius conchonius (middle part) indicated that anterior region contain maximum (29.09%) calcium followed by focus (19.97%) and minimum amount of calcium was recorded (17.01%) at posterior region. Maximum concentration of oxygen (40.38%) was recorded from posterior region and minimum (36.77%) in focus region. Percentage composition of magnesium was reported low at all regions of the scales. Maximum percentage of carbon (31.45%) was reported from posterior region and minimum (16.11%) from anterior region. However zinc was recorded from focus region of the scales only **(Table 1.2).**

In the case of Puntius sophore (middle part) eight elements were found from the different region of the scales. These minerals were Ca, P, O, C, Mg, Na, K and Zn. During the present investigation maximum percentage of oxygen (48.47%) has recorded from the posterior region followed by (45.39%) in focus region. Whereas Ca was recorded (18.77%) in focus region and

Table 1.1. Showing chemical percentage in Puntius conchonius and Puntius sophore (Black spot).

ELEMENT NAME IN %	Puntius conchonius(Black Spot)								Puntius sophore(Black Spot)							
	REGION															
	Whole Scale		Focus		Anterior		Posterior		Whole Scale		Focus		Anterior		Posterior	
	Weight%	Atomic%	Weight%	Atomic%	Weight%	Atomic%	Weight%	Atomic%	Weight%	Atomic%	Weight%	Atomic%	Weight%	Atomic%	Weight%	Atomic%
Calcium	3.91	1.34	4.46	1.55	12.17	4.60	3.00	1.02	21.64	9.83	22.97	10.88	19.93	9.02	17.61	7.20
Phosphorus	2.01	0.89	2.47	1.11	7.07	3.45	1.43	0.63	12.97	7.62	14.46	8.86	11.99	7.02	9.86	5.21
Oxygen	34.01	29.16	34.82	30.21	30.23	28.60	36.59	31.25	41.18	46.86	42.75	50.75	46.36	52.58	30.91	31.65
Carbon	60.07	68.61	57.91	66.94	49.99	63.01	58.98	67.10	22.87	34.67	17.76	28.07	19.90	30.06	40.57	55.34
Magnesium			0.34	0.19	0.55	0.34			0.84	0.63	0.86	0.67	0.73	0.54	0.62	0.42
Sodium									0.49	0.39	0.54	0.44	0.81	0.64		
Potassium											0.67	0.32	0.29	0.14	0.43	0.18
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Table 1.2. Showing chemical percentage in *Puntius conchonius* and *Puntius sophore* (Middle part).

ELEMENT NAME IN %	**Puntius conchonius(Middle Part)**								**Puntius sophore(Middle Part)**							
	REGION															
	Whole Scale		**Focus**		**Anterior**		**Posterior**		**Whole Scale**		**Focus**		**Anterior**		**Posterior**	
	Weight%	**Atomic%**	**Weight%**	**Atomic%**	**Weight%**	**Atomic%**	**Weight%**	**Atomic%**	**Weight%**	**Atomic%**	**Weight%**	**Atomic%**	**Weight%**	**Atomic%**	**Weight%**	**Atomic%**
Calcium	21.66	9.75	19.97	8.74	29.09	14.42	17.01	7.15	19.35	8.66	18.77	8.29	22.82	10.68	18.81	8.45
Phosphorus	13.06	7.61	11.78	6.67	14.81	9.50	10.11	5.50	11.85	6.86	11.56	6.61	13.76	8.33	11.75	6.83
Oxygen	39.33	44.37	36.77	40.29	39.47	49.01	40.38	42.51	46.78	52.47	45.39	50.24	44.62	52.29	48.47	54.56
Carbon	25.01	37.57	29.85	43.57	16.11	26.65	31.45	44.11	20.81	31.09	23.17	34.16	17.98	28.07	19.38	29.06
Magnesium	0.94	0.70	0.66	0.47	0.52	0.42	1.05	0.73	0.75	0.56	0.75	0.54	0.82	0.63	0.73	0.54
Zinc			0.97	0.26												
Sodium									0.45	0.35					0.50	0.39
Potassium											0.36	0.16			0.37	0.17
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

e-ISSN:2321-6190
p-ISSN:2347-2294

maximum from anterior region of the scale (22.82%). Phosphorus was record (11.56%) in focus region and maximum (13.76%) from anterior region. Maximum percentage of carbon (18.82%) was recorded from anterior region and minimum from focus region. Percentage concentration of magnesium was recorded very low in all regions of the scales. Sodium (Na) was recorded from whole scale, focus and anterior region only, whereas potassium has reported from focus, and posterior region only and sodium has reported from whole scale and posterior region only **(Table 1.2).**

DISCUSSION

Almost in the centre of scale, there is focus, around the focus there are growth lines running in circular fashion, which are called circuli[3]. Johal and Chadde[4] studied scale structure of Barilus barila (Ham.) and reported that there is clear cut demarcation between the anterior and posterior region and also state that shape of the scale, presence or absence of letridents and tubercle can be employed for taxonomic purposes. SEM observation of scale of C. argus showed that there are two different kinds of scale denticle distributed along the inter radial circuli in the anterior field and circuli in the lateral fields respectively [5]. Mulligan et al., [6] studied the element composition of striped bass otoliths and reported Si, Al, Cl, S, Na, K, Mn, Tc, Cr, Cu, Re, V, and the lanthanide series elements. During the present investigation five and eight elements Ca, C, P, O and Mg from Puntius conchonius and Ca, P, O, C, Mg, Na, K and Zn from Puntius sophore respectively. Lapi and Mulligan[7] investigated Salmon stock identification using microanalytic technique to measure elements present in the freshwater growth region of the scale. Energy dispersive X- Ray analysis has been used to identify elements in such diverse structure and cnidarian statolith polychaetae jaw & scale of Barilus[8]. EDAX analysis gave Ca:P molar ratio of 1-85 that was calcium rich compare with that of 1.67 recorded to the scales of P. major[9]. Scale morphology provides new and useful information for systematic. Esmaeili and Johal[10] studied ultrastructure of the egg envelop of silver carp, Hypophthalmichthys molitrix.

From the above findings it may be inferred that elements composition in the fish scale may be used as pollution indicator as well as it also helpful in the identification on the basis of their element composition from different water bodies.

SUMMARY

Puntius conchonius and Puntius sophore (Spotted barbs) inhabits different habitats in the fast flowing water of Song River. The body of the fish is laterally compressed and is covered over by numerous small scales. The number of lateral line scale is approximately ninety. The fish adheres to the bottom by well-developed reflected lower lips. The typical scale when viewed under SEM reveals that the scale can be divided into anterior, posterior and focus regions.

The scales of Puntius conchonius and Puntius sophore are cycloid scales which are roughly oval in shape. In the present study primary radii was reported maximum as compared to secondary radii and tertiary radii. The circuli are distinct in the all the sides with regular space pattern. Corresponding SEM images of the fracture surface indicated that sliding of the collagen lamellae and pulling out of individual collagen fibers. The scales from focus region of the black spot have 75% of degenerated scales as compare to normal body scale of genus Puntius. Maximum concentration of oxygen (36.59%) was recorded from posterior region and minimum (30.23%) in anterior region in the scale of P. conchonius. In the case of Puntius sophore black spot scale a total seven elements were found from the different region of the scales viz., Ca, P, O, C, Mg, Na and K.

Maximum percentage of oxygen (46.36%) was recorded from the anterior region followed by (42.75%) in focus region. Whereas Ca was recorded (22.97%) in focus region and minimum from posterior region of the scale (17.61%). Middle part scale of Puntius sophore contains eight elements. These minerals were Ca, P, O, C, Mg, Na, K and Zink. In the case of Puntius sophore (middle part) eight elements were found from the different region of the scales. These minerals were Ca, P, O, C, Mg, Na, K and Zn. During the present investigation maximum percentage of oxygen (48.47%) has recorded from the posterior region followed by (45.39%) in focus region. The region wise analysis of the scale Puntius conchonius (middle part) indicated that anterior region contain maximum (29.09%) calcium followed by focus (19.97%) and minimum amount of calcium was recorded (17.01%) at posterior region.

Maximum concentration of oxygen (40.38%) was recorded from posterior region and minimum (36.77%) in focus region. Zinc was recorded from focus region of the scales only.Black spot scale of Puntius conchonius indicated that anterior region contains maximum (12.7%) calcium followed by focus (4.46%) and minimum amount of calcium was recorded (3.0%) at posterior region. Lateral line canal of lateral line scale of this species covered 80% of entire length of scale. It is tube like structure having two opening the anterior and posterior opening, anterior opening is circular in shape and open near the tip of the posterior end of the scale.

ACKNOWLEDGEMENTS

We thank Director, Indian Institute of Technology, Roorkee, Uttarkhand and Head, Dept. of Zoology and Environmental Science, G.K.V. Haridwar, Uttarakhand as well as Fishery Department of Uttarakhand for providing necessary facilities to carry out the study.

REFERENCES

1. Molour S and Walker SC. (ed) Report of the workshop conservation assessment and management programme (CAMP) for

freshwater fishes of India. Coimbatore, India. Zoo. Outreach organisation breeding spread group India. 1998.

2. Tandon KK and Johal MS. In advances in fish biology (Ed). Singh Hindustan Publishing Corporation India Delhi. 1994; pp 1-11.
3. Chugunova NI. Hand book for the study of age and growth of Fishes. National science foundation, Washington. 1963; 132 pp.
4. Johal MS and Chadha N. Scale structure of Barilius barila (Ham.) as reveled by SEM. Proc. The national seminar New Trends in fishery development in India. Organised by Dept. of Zoology, Punjab University, Chandigarh Feb. 2005; 16 –18, 29–34pp.
5. Liu WT, et al. Structure & composition to teleost scales from snakehead Channa argus (cantor) (Pesciformes; channidae). J.fish Biol. 2008; 72: 1055–1067.
6. Mulligan TJ, et al. A method of stock identification based on the element composition of striped bass, Morone saxatilies (Walbaum) otolith. J. Mar. Priol. Ecol. 1987; 114: 241-248.
7. Lapi LA and Mulligan TJ. Salmon stock identification using a Microanalytical technique to measure elements present in the freshwater growth region of scales. Can. J. Fish. Aquat. Sci. 2013; 38: 744-751.
8. Negi RK, et al. Scale ultra-structure & EDAX analysis of Barilius bendelisis using electron microscope. Novus natural science research. 2013; 2: 9–14.
9. Ikoma T, et al. Micro structure mechanical, and biometric properties of fish scale from Pagrus major. J structural Biol. 2003; 142: 327-333.
10. Esmaeili HR and Johal MS. Ultrastructural features of the Egg envelope of silver carp Hypophthalmichthys molitrix. Env Bio. Fish. 2005; 72: 373-377.