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SCALE STRUCTURE OF A CYPRINID FISH, *CAPOETA DAMASCINA* (VALENCIENNES IN CUVIER AND VALENCIENNES, 1842) USING SCANNING ELECTRON MICROSCOPE (SEM)*

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Abstract – The normal and lateral line cycloid scales of a cyprinid fish *Capoeta damascina* (Valenciennes in Cuvier and Valenciennes, 1842) have been subjected to the scanning electron microscopy technique in order to study their detailed structure. The scales have the general morphological characteristics of the cycloid scales. In the normal scale located below the dorsal fin, the focus lies towards the anterior region and is covered by reticulate or honeycomb form structures with few mucous pores. There is a clear-cut demonstration between the anterior and posterior region. In the posterior section, the scale has several rows of pigmented granules (tubercles) with different shapes from round to oval, semi-oval and even oblong structure. On the dorsal side, the anterior circuli bear teeth-like structures called lepidonts which help the scale in firm attachment to the skin. The circuli may also have calcium projections. The lateral line scale has a canal which characteristically lies along the anterior-posterior axis, slightly towards the posterior part with two anterior and posterior openings. The anterior opening is wider than the posterior opening and is hidden by an evelike extension cantilevered over it. Based on the obtained results it could be concluded that the shape and size of lepidonts on the circuli crest and also the pattern of reticulate or honeycomb form structures in the focus region may provide reliable taxonomic tools.

Keywords – Scale, scanning electron microscopy, lepidont, *Capoeta damascina*

1. INTRODUCTION

Detailed structure of the fish scale can be helpful in the identification of fish up to major groups [1-3] and species levels [4-6], phylogeny [7-8], sexual dimorphism [9], age determination [10-13], past environment experienced by fish, discriminating between hatchery reared and wild populations, migration, the pathology of a fish scale due to water pollution of the water body [14-16], and for growth studies [17-26]. In the past numerous studies on the scale structure of commercial fish [17-21] have been conducted world wide and the information available from these studies has been successfully used in growth studies [22-26], the calculation of minimum harvestable size [27] so that legal fishable size can be prescribed, for the determination of old age in commercial fish [11], and the pollution status of the waterbody [14-16, 28]. Due to some subjective reasons, in the past most of the lepidological studies are on commercial fishes [11, 19-21, 23-24, 26-27].

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 underdeveloped countries are under different types of threats as is evidenced from the squeezing of geographical limits and the reduction in the stocks of most of the fish species [29]. In some cases the fish community structure is completely disrupted. Due to the reduction in

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fish stocks, fish biologists are unable to get large numbers of specimens for studies relating to fish bionomics. Under these circumstances, a lepidological study is the best alternative, as fish scale is considerd to be the best tool in fish biology [30-31].

During perusal of the literature it has been found that the age and growth studies on cyprinid native fishes of Iran are rarely chosen and some taxa are completely ignored. Cyprinid fishes form one of the most important links in the fish community structure in different water bodies, hence the standardization of the scale structure of these fishes can help in the understanding of their bionomics.

Keeping in mind the above facts, a first attempt has been made to study the scale ultrastructure of a native cyprinid fish, *Capoeta damascina*, employing scanning electron microscope. In Iran this fish is generally known as siah mahi, sardeh, twiny and gel cheragh.

2. MATERIAL AND METHODS

To study the ultrastructure of the scale of *C. damascina*, the fishes were collected from the Zanjiran spring-stream (52° 39´N 29° 04´E), in southwest Iran (Fig. 1) using a cast-net and an electro fishing device during the year 2005. The scales were gently removed with fine forceps from the left side of the body between the dorsal fin and lateral line and also from the lateral line (Fig. 2) in such a way that while removing the scales no damage is being done to the scale. Immediately after their removal they were cleaned mechanically using a fine brush and rinsed with triple distilled water. Cleaned scales were dehydrated in 30, 50, 70, and 90% ethanol and dried on filter paper [25]. The scales were not put in absolute alcohol as 100% ethanol caused the scale margins to curl. To avoid curling, after 70% ethanol, the scales were kept between the microslides for 2-3 days. The cleaned and dried scales were mounted on metallic stubbs by double adhesive tape with the dorsal surface upward and the ventral surface sticking to the tape and coated with a thick layer of gold in a gold coating unit (SC7640 SPUTTER COATER, Model: FISONS). The gold coating overcomes the problem of charging and beam damage. An additional advantage of gold coating is to improve the strength of secondary electron signals from the specimen's surface. The scales were viewed under vacuum in a Leica Cambridge scanning electron microscope at an accelerating voltage of 20 kv at low probe current. Various images of the scales were recorded on photographic film. When the gold coated scales were not being viewed, the stubbs were stored in a desiccator to avoid moisture.

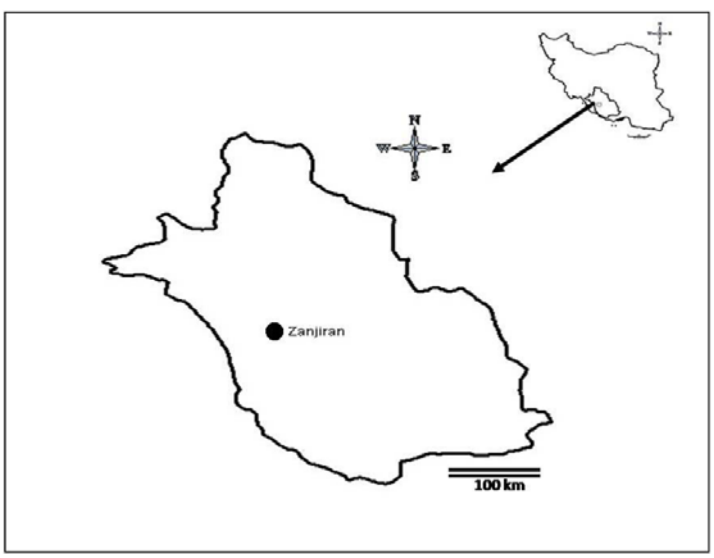

Fig. 1. Map of Iran showing fish collection site

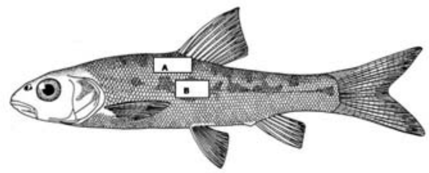

Fig. 2. Schematic drawing of *Capoeta damascina* (total length, 45cm) showing location of key scales using for scanning electron microscopy. a) scale below dorsal fin; b) lateral line scale

3. RESULTS AND DISCUSSION

The general structure of normal and lateral line scales of a hypothetical fish and that of *C. damascina* is given in Fig. 3. The length of the scale of *C. damascina* is greater than its breadth, so it does not have a circular shape (Fig. 3). The scales maintain the same morphological proportions located on the different parts of the body. The scales present below the dorsal fin, above the lateral line are the largest, while the scales on the other parts of the body are smaller in size. As the scale from this region depicts all the features, this scale has been designated as the ‘key scale’. The scale of this fish can be divided into anterior or rostral (A), posterior or caudal (P), and lateral fields (L). There is no cteni at the posterior part of the scale; hence, it is a cycloid scale. The anterior field is embedded in the skin and overlapped by the posterior side of the proceeding scale. The ventral part of a scale is shiny and smooth while the dorsal is rough, convex and has distinct structures, consisting of ridges, grooves and granules (tubercles).

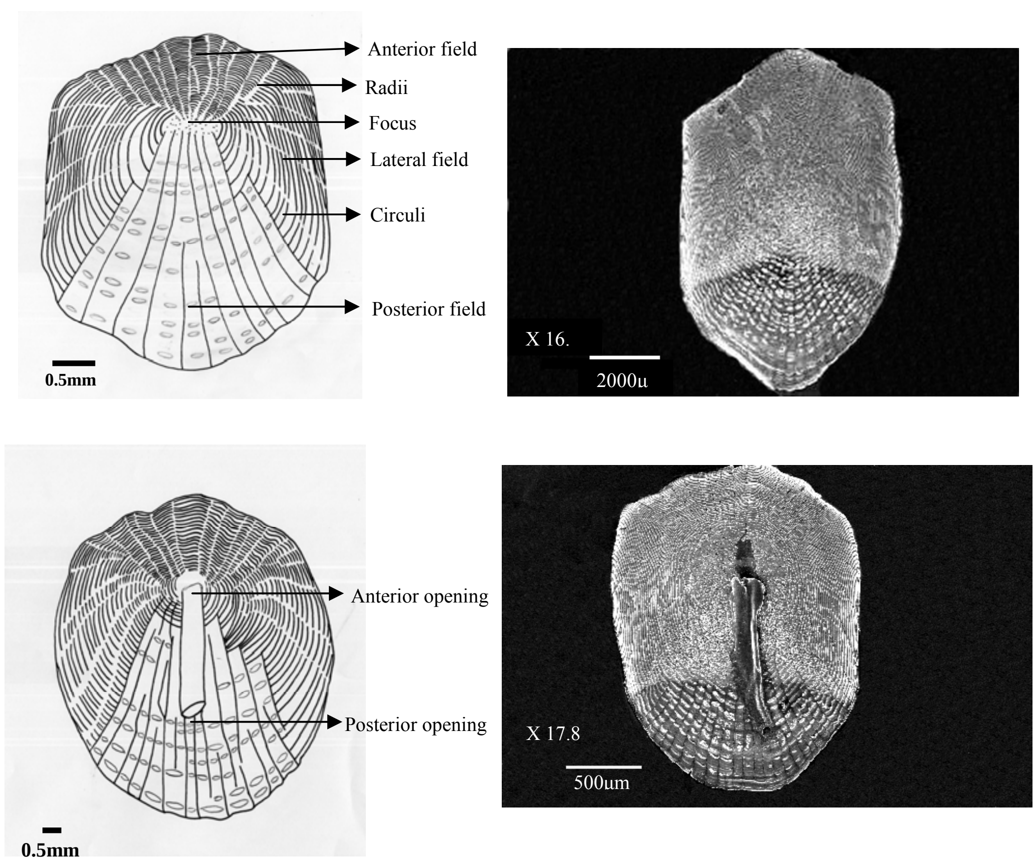

Fig. 3. a) Schematic drawing of a sectioned cyprinid scale. b), SEM microphotograph of a normal *C. damascina* scale. c) schematic drawing of a sectioned cyprinid lateral line scale. d) SEM microphotograph of *C. damascina* lateral line scale.Focus (F), posterior opening (PO), anterior opening (AO), posterior field (P), anterior field (A), lateral field (L)

Each scale has a focus, which is a part of the scale that developed first during ontogenesis (Fig. 3). The focus, which lies in the anterior part of the scale, divides the scale into anterior (cephalic to focus), posterior (caudal to focus) and lateral fields on the lateral sides of the scale (Figs. 3, 4B). The area enclosed by the focus is covered by reticulate or honeycomb form structures (Figs. 4B, C). Few mucous pores are found in the focus region. The same has been reported in the scale of some other cyprinid fishes such as *Catla catla* (Hamilton-Buchanan, 1822) and *Tor putitora* (Hamilton–Buchanan, 1822) [32]. From the focus lines of growth (the ridges) start appearing. These are named circuli (Figs. 3, 4). The space between circuli is called inter circular space. Circuli (the lines of growth) are distinct, overcrowded in the anterior part and widely separated in the lateral parts. This is because of the anterior location of the focus on the scale. The circuli are not found on the posterior part of the scale. The circuli formation is due to the excess calcium salts secreted by the skin and their subsequent deposition on the scale. So, the distance between circuli indicates a fast or slow growth period. Each circuli is wedge shaped, having a broad base and a pointed upper part (Figs. 4D, E, F). The arrangement of the circuli corresponds to the scale shape. The intercirculus space is minimum in the anterior part of the scale and maximum in the lateral parts. The circuli have calcium projections (Fig. 5 C). The interradial circuli are almost straight. According to Lippistsch [25] the shape of the first interradial circuli (convex, straight, or concave) is characteristic within species of cichlid fishes, but in some cases seems to be modified by external factors. In the interradial space the circuli bear small denticles or tooth-like structures that can be seen only under high magnification and are called lepidonts. These circuli are found only in the anterior part of the scale, therefore lateral interradial circuli have no lepidont. They are widely spaced, blunted and irregular teeth on the crest of circuli as seen in (Fig. 4F). Lepidnots of different size and shape have been reported in many fish species [25, 33, 34]. Delmater & Courtenay [33] illustrated these structures for several fish genera and suggested that they might characterize genera, and may even distinguish some taxa at a specific level. Lepidonts are not homologous to breeding tubercles and contact organs. The microscopic size and the covered position of lepidonts make the function as contact organs (to facilitate contact between males and females during reproduction highly improbable [33]. Javad & Al-Jufaili stated that there is no variation in the shape and size of the scale denticles (lepidonts) from different regions of the body of greater lizardfish [34].

In the anterior and lateral parts, the circuli are partitioned by deep and narrow grooves that run radialy towards the focus. They are called radii. These scales with well developed radii are here called "sectioned". On the scale of *C. damascina* the radii can be categorized into three types depending upon their point of origin on the scale including: Primary radii, originating from the focus, reaching the margin of the scale; Secondary radii, originating midway between focus and margin; and Tertiary radii originating between midway and margin (Fig. 4D). The relative number of primary and secondary radii is more as compared to the tertiary radii. There is no significant relationship between the number of radii and scale size, as the numbers of radii depend on the location of the scale on the fish body. However, in some other teleostes such as *Mullus surmuletus* L., 1758 and *M. barbatus* L., 1758, the number of radii is correlated to fish size [34]. Javad & Al-Jufaili [34] showed that in greater lizard fish, the number of radii is three and remains constant on a given scale during ontogeny. The consistency in the radii number makes it a good taxonomic criterion to identify this fish in a further comparative study [34]. The presence of primary and secondary radii is a growth phenomenon and obviously only weakly influenced by genetic factors [25]. The radii formation is considered to be related to the accommodation power of the large surface area of the anterior and lateral parts of the scale in the lesser space as these two parts of the scale are overlapped by the posterior part of the preceding scale. The higher number of radii correlate to the better nutritive conditions of the fish [11, 19]. Radii represent the line of scale flexibility.

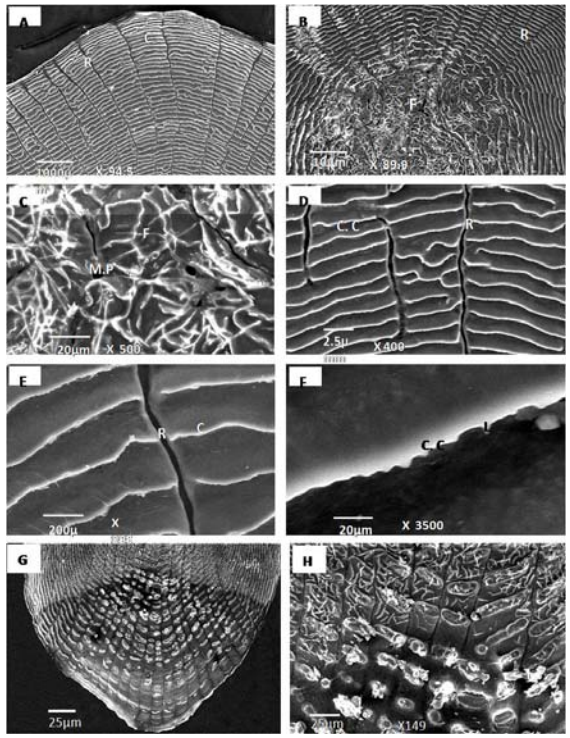

Fig. 4. SEM microphotographs of *C. damascina* scale. radii (R), circuli (C), lepidont (L), mucous pore (M.P), focus (F), crest of circuli (C.C), Primary radii (P.R), radii (S.R), tertiary radii (T. R) and tubercle (T)

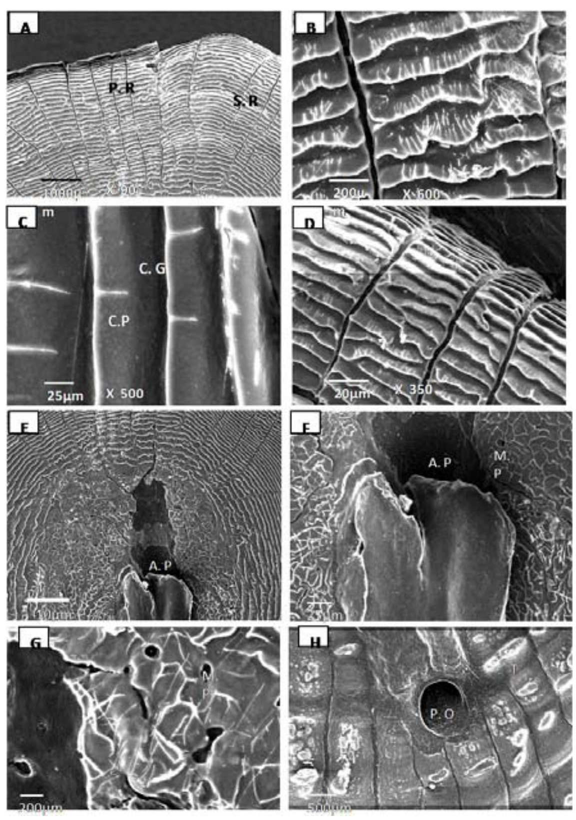

Fig. 5. SEM microphotographs of lateral line scale of *C. damascina.* radii (R), circuli (C), crest of circuli (C.C), circular groove (C. lateral circuli (l. C), primery radii (P. R), secondary radii (S. R), line of growth (L. G). Anterior pore (A. P), Posterior pore (P. O), Tubercle (T) and Mucus pore (M. P)

The scale of *C. damascina* records the annuli, hence, the age determination from this scale is easy and authentic (Fig. 4).

In the posterior part of the scale, which is exposed, the circuli lose their characteristic features. In this part the scale is covered with epidermis and has several rows of pigmented granules (tubercles) whose concentration depends on the location of the scale on the fish body (Fig. 4 G, H). The shape of tubercles varies from round to oval, semi-oval and even an oblong structure. The outer surface of a tubercle is not smooth; on the contrary, it has several warts and wrinkles. They vary considerably in their length and are located in the interradial space covering a large part of the caudal field. Tubercles are formed by the aggregation of the epithelial layer of the skin which covers the posterior part of the scale. They impart specific color to fish as they contain chromotophores in the outer surface. Granulations or the presence of chromatophores on the posterior part of a scale is a characteristic feature of the cycloid and ctenoid scales of carp and perch respectively [11, 19-21].

A schematic drawing of a lateral line scale is shown in (Fig. 3). A lateral line scale is also divided into anterior (rostral) and posterior (caudal) parts. The focus is absent in the lateral line scale. This scale has a canal which, characteristically, lies along the anterior-posterior axis, slightly towards the posterior part with two openings. The posterior opening lies towards the posterior margin and the anterior opening towards the anterior part of the scale. The anterior opening of the lateral line canal is wider than the posterior opening and is hidden by an evelike extension cantilevered over it (Fig. 5 E, F, H). The same structure has been reported in some other fish [35]. According to Delmater & Courtenay [35] scanning electron microscopy of lateral line scales of teleostean fish demonstrates a wide range of structural variation of the lateral line canal from a simple direct or slightly oblique perforation to an extended canal with or without simple to highly complex cantilevered extensions acting as covers for the anterior opening. The anterior part of the lateral line has several mucus pores (Fig. 5 G). In the anterior part, the circuli are densely spaced (Fig. A, B, D). The granulation on the posterior portion is the same as normal scales located below the dorsal fin, except that the canal is extended to the granulation area. The results presented in this lepidological study reveal that although the scale of *Capoeta damascina* shows the general structure of a cycloid cyprinid scale, the shape and size of lepidonts on the circuli crest, and the pattern of reticulate or honeycomb that form structures in the focus region may provide more reliable taxonomic tools.

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REFERENCES

1. Lagler, K. F. (1947). Lepidological studies. 1. Scale characters of the families of Great Lakes. *Transaction of the American Microscopical Society*, *66*(2), 149-171.
2. Van Oosten, J. (1957). The skin and scales. In: The Physiology of Fishes, *I*, (ed. E. B. Margaret), *Academic Press*, 207-243.
3. Norman, J. R. (1975). *A History of Fishes,* (Revised by P. H. Grenwood), London Ernest Benn Ltd.
4. Chu, Y. T. (1935). Comparative studies on the scales and on the pharyngeals and their teeth in Chinese Cyprinids, with particular reference to taxonomy and evolution. *Biol. Bull. St. John's Univ*., *2***,** 226.
5. Das, S. M. (1959). The scales of freshwater fishes of India and their importance in age determination and systematics. *Proc. Ist. All Indian Congr. Zool*., Part 2, 52.
6. Lanzing, W. J. R. & Higginbotham, D. R. (1974). Scanning microscopy of surface structures of *Tilapia mossambica* (Peters) scales. *J. Fish. Biol*, *6*, 307-310.

7. Kobayashi, H. (1951). On the value of the scale character as material for the study of affinity in fishes. *Jpn. J. Ichthyol*., *1*(4), 226-237.
8. Kobayashi, H. (1952). Comparative studies of the scales in Japanese freshwater fishes. With special reference to phylogeny and evolution.I. Introduction. *Japanese Journal of Ichthyology*, *2*, 183-191.
9. Johal, M. S. & Thomas, N. (2000). *EMSI Bull*., *1*(1), 16-19.
10. Jhingran, V. G. (1957). Age determination of the Indian major carp, *Cirrhinus mirgala* (Ham.) by means of scales. *Nature, London*, *179* (4557), 468-469.
11. Tandon, K. K. & Johal, M. S. (1996). Age and Growth in Indian Freshwater Fishes. New Delhi, *Narendra Publishing House*. 232.
12. Johal, M. S. & Tandon, K. K. (1989a). Age and growth of catla, *Catla catla* (Hamilton, 1822) from Rang Mahal (Rajisthan), India. *Bangladesh J. Agri*., *14*(2), 135-150.
13. Johal, M. S. & Tandon, K. K. (1992). Age and growth of carp *Catla catla* (Hamilton, 1822) from northern India. *Fish. Res*., *14*, 83-90.
14. Johal, M. S. & Dua, A. (1994). SEM study of the scales of freshwater snakehead, *Chana punctatus* (Bloch) upon exposure to endosulfan. Bull. Environ. *Contam. Toxicol*., *52*, 718-721.
15. Johal, M. S. & Dua, A. (1995). Elemental, lepidological and toxicological studies in *Channa punctatus* (Bloch) upon exposure to an organochlorine pesticide. *Bulletine of Environmental Eontamination and Taxicology*, ***55*,** 916-921.
16. Johal, M. S. & Sawhney, A. K. (1997). Lepidontal alterations of the circuli on the scales of freshwater snakehead, *Channa punctatus* (Bloch) upon exposure to malathion. *Curr. Sci., 72*(6), 367-369.
17. Cockrell, T. D. A. (1915). Scales of Panama fishes. *Proceeding of the Biological Society of Washington* (151-160).
18. Tandon, K. K. & Chaudhry, N. (1983-84). Variations in the scales of some freshwater Teleostean fishes of India. *Matsya*, *9-10*, 38-45.
19. Johal, M. S., Novak, J. & Oliva, O. (1984). Notes on the growth of the common carp Cyprinius carpio. In Nprthen India and middle Europe. *Vest. Cs. Spolec. zool*., ***48*,** 24-38.
20. Johal, M. S., Tandon, K. K. & Kaur, S. (1996). Scale structure, age and growth of *Labeo calbasu* (Hamilton, 1822) from northern India. *Acta. Hydriobiol*, *38*(1-2), 53-63.
21. Johal, M. S. & Agarwal, T. (1997). Scale structure of Oreochromis *mossambicus* (Peters). *Res Bull. Panjab Univ*., *47*(1-4), 41-49.
22. Chugunova, N. I. (1963). *Handbook for the study of age and growth studies in fishes* (English translation). Washington: National Science Foundation.
23. Qasim, S. Z. (1964). Occurrence of growth zones on the opercular bones of the freshwater murrel. *Ophicephalus punctatus Bloch. Curr. Sci*., *33*(1), 19-20.
24. Qasim, S. Z. & Bhatt, V. S. (1966). The growth of the freshwater murrel, Ophicephalus punctatus Bloch. *Hydrobiologia*,. *27*(3-4), 289-316.
25. Lippitsch, E. (1990). Scale morphology and squamation patterns in cichlids (Teleostei, Perciformes): A comparative study. *J. Fish. Biol*., 1990, *37*, 265-291.
26. Johal, M. S., Esmaeili, H. R. & Tandon, K. K. (2001). A comparison of back–calculated lengths of silver carp derived from bony structures. *J. Fish. Biol*., *59*, 1483-1493.
27. Johal, M. S. & Tandon, K. K. (1987). Harvestable size of two India major carps (Pisces: Cyprinidae). *Vest. cs. Spolec. zool*., *51*, 177-182.
28. Johal, M. S. & Sawhney, A. K. (1999). Mineral profile of focal and lepidontal regions of the scale of *Channa punctatus* as pollution indicator. *Pol. Res*., *18*(3), 297-299.

29. Molour, S. & Walker, S. (1998). (eds.), *Report of the workshop 'Conservation Assessmen And Management Plan (CAMP) for Freshwater Fishes of India*'. Coimbatore, India, Zoo Outreach Organisation Conservation Breeding Special Group India.
30. Tandon, K. K. & Johal, M. S. (1994). *Scales, a tool in fish biology*. Pages 1-11. In: Advances in Fish Biology (ed., Singh, H. R.). Delhi, India, Hindustan Publishing Corporation.
31. Johal, M. S. (2005). *Recent innovations in age determination using hard parts in Indian freshwater fishes*. Pages 91-98. In: New Horizons in Animal Sciences (eds. Sobti, R. C. and Sharma,V. L.). Jalandhar, Punjab, Visual Publishing Company.
32. Johal, M. S., Tandon, K. K. & Sandhu, G. S. (1999). *Age and growth of an endangered cold-water fish-golden mahseer Tor Putitora (Hamilton) from Gobindsagar, Himachal Pradesh, India*. Pages 59-73. In: Ichthyology, Recent Research Advances (ed., Saksena, D. N.). New Delhi, Calcutta, Oxford and IBH Publishing CO. PVT. LTD.
33. Delmater, E. D. & Courtenay, W. R. (1974). Fish scale as seen by scanning electron microscopey. *Biological Sciences*, *37*, 141-149.
34. Javad, L. A. & AL-Jufaili, S. M. (2007). Scale morphology of greter lizardfish *Saurida tumbil* (Bloch, 1795) (Pisces: Synodontidae). *Journal of Fish Biology*, *70*, 1185-1212.
35. Delmater, E. D. & Courtenay, W. R. (1973). Variations in structure of the lateral line canal on the scales of Teleostean fishes. *Z. Morph. Tiere*, *75*, 259-266.