Ultrastructure of the focus region of the regenerated cycloid scale of an exotic fish, *Cyprinus carpio communis* L. as a possible key to comprehensive understanding of populations

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Fish scales (except placoid) have been employed for age determination, growth studies and various growth parameters, which are useful in the formulation of various fishery management practices and the optimum exploitation of fishery stocks of commercial fish species. Fish-scale morphology and its morphometrics can be employed for taxonomic purposes and for phylogenetic relationships. The evaluation of populations/stocks of the fish species having wide zoogeographical distribution and inhibiting distinctly different ecological conditions has been done with the help of molecular markers. An alternate technique, i.e. to study the ultrastructural details of the focus region of the regenerated scale has been found to be more reliable and economical. This technique has been tested positively on the regenerated cycloid scale of an exotic fish, *Cyprinus carpio communis* L. inhabiting five distinctly ecologically different water bodies of northern India.

Keywords: Exotic fish, focus region, regenerated cycloid scale, ultrastructural details.

VARIOUS hard parts of fishes such as scales, otoliths, vertebrae, opercular bones, sections of the fin ray spines, eye lens, urohyal bone, cleithral and sub-cleithral bones have been employed for age determination, growth studies, fishery management and toxicological aspects, especially of commercial fishes both marine and freshwaters[1,2]. The growth data which include both length and weight ascertained from these hard parts have been used for the determination of 'old age', and for the calculation of harvestable size as legal fishable size in the fast-growing fish species for optimum exploitation of commercial fish species in natural waters[1,3]. Out of all these hard parts, fish scale, which is a mesodermal derivative, has also been employed for species identification, especially in those genera having a large number of fish species, where the morphometric and meristic characters are either ambiguous or overlapping[4,5]. Sometimes fish species identification is based on ill-defined characters. Under such situations, ultrastructure of fish scale has also been used for the exact species identification[3–5]. This fish taxonomic tool is a new innovation.

There are more than 30 exotic fish species that have been introduced in Indian freshwaters for several purposes, mainly to enhance the fish production[6]. Most of them have slipped into natural waters from the confined waters due to negligence and almost all the exotic fish species are now considered to be part of Indian native fish fauna, though their presence in natural aquatic environments is not desirable[6–8]. Out of 30 exotic fish species, the *Cyprinus carpio* having 3 subspecies occurs throughout India both in confined and natural aquatic ecosystems. Out of the three subspecies, *Cyprinus carpio communis* L. commonly known as common carp (Figure 1) is the most widely distributed species in India. As this breeds two times in natural waters; does not undertake even short distance spawning migration and breeds within the localized stocks, thus, there is high possibility of the occurrence of distinct populations of this fish inhabiting the different ecological conditions. The present study has been carried out with the concept that morphometric and meristic characters of this exotic fish species do not indicate the occurrence of different stocks in different ecological conditions; so there must be some other parameters which can help us discriminate the populations of this exotic fish species in different natural water bodies of North India.

One of us (M.S.J.) has worked on the age and growth studies of this fish species employing various hard parts such as scales, vertebrae and opercular bones from different natural water bodies of India and Europe, and found that even the growth rates differ significantly under different ecological conditions. This is a clear indication that the species has the tendency for the formation of distinct populations[9,10].

In the present study, samples of cycloid scale were collected from five different water bodies of North India having distinctly different ecological conditions, viz. Harike wetland, Ropar wetland, Nangal Lake, Gobindsagar reservoir and Katli Fish Farm, Ropar. Fish scales

Figure 1. *Cyprinus carpio communis* L. from Harike wetland.

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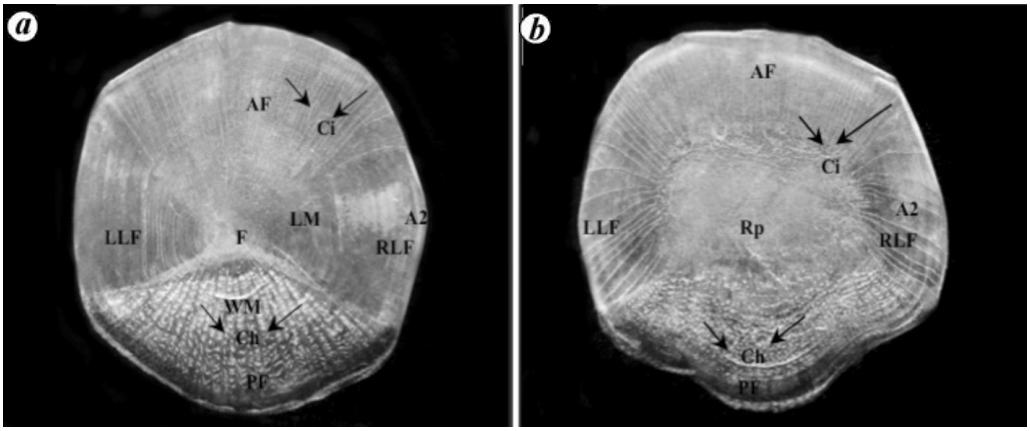

Figure 2. ***a***, Normal scale of *Cyprinus carpio* L. (approximately 38.7 cm length) collected from River Sutlej, Ropar. ***b***, Regenerated scale of *C. carpio* L. collected from river Sutlej, Ropar. The original scale was removed approximately at age of one and a half years. Second annulus (A2) is very clear and first annulus (A1) is missing. Focus (F), winter mark (WM) and larval mark (LM) are also absent. AF, Anterior field; Ch, Chromatophores; Ci, Circuli; LLF, Left lateral field; PF: Posterior field; RLF, Right lateral field; Rp, Regenerated part.

were removed from the left side of the fish, below the dorsal fin and above the lateral line, preferably from the second or third row and stored in envelopes with supporting data such as total fish length, weight, sex (if possible) and date of collection[1]. For the collection of fishes cast-net having mesh size 1 cm was used. The fish samples were collected during October 2012 from all the water bodies; they belonged to the age class 2+ and the total fish length varied between 32.27 and 38.69 cm (Figure 2).

In the laboratory, the scales were washed with tap water, gently rubbed between the 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 No. 1 filter paper for overnight in desiccator to remove the last trace of moisture. The dried scales were then mounted on aluminum stubs with the help of double stick tape in such a way that the ventral part of the scale touches the stub. The mounted scales were sputtered with a layer of gold (100 Å) in a gold sputtering unit. The casting of gold made the non-conductive scale conductive. The different images of the scales were viewed under vacuum at an accelerating voltage of 20 kV in JEOL SEM Model JSM 6100.

The cycloid scale of *C. carpio communis* L. is almost as long as broad (Figure 2). The part which touches the body of the fish is called the ventral part, which is smooth and shining; the opposite part is dorsal, rough to touch and non-shiny. For ultrastructural details of the scale, only the dorsal part has been considered. The general shape of the scale is semi-circular. The thickness of the scale decreases from the central to peripheral part. At the centre, there is a distinct focus (Figure 2), the point from where the scale makes its beginning. It is very distinct in the scale of this fish. The focus divides the scale into four distinct regions, namely anterior, posterior, left lateral and right lateral (Figure 2). The scales arrange themselves on the body of the fish in an overlapping manner with anterior part of the scale being covered by the posterior part of the preceding scale. Posterior part is comparatively thicker than the anterior part and is covered by a fold of skin epidermis having chromatophores arranged in the longitudinal rows (Figure 2). The focus does not occupy the central position, but it lies a little towards the posterior side of the scale. The scale of this fish has recorded a larval mark (Figure 2 *a*), which indicated the length when the larva of this fish came out of the vitelline membrane and started feeding on natural food. Inside the vitelline membrane, the larva feeds on the yolk of the egg. The larval mark is clearly visible in case of fast-growing fishes, whereas in slow-growing fishes it is absent or indistinct[11]. Around the focus there are growth lines called circuli (Figure 2 *a*). The circuli are arranged in a circular fashion and correspond to the overall shape of the scale. They are more distinct in the anterior and lateral parts compared to the posterior part. The intracircular distance depicts the growth rate. This distance is more during summers when the growth rate is high and less during winters, indicating the slow growth rate during the period. The shape of the circuli is species-specific[4]. The radii cut the circuli at right angles. The number of radii is more in the anterior part compared to lateral and posterior parts. During southwest monsoon, due to the high concentration of salts in the ambient water, there is imbalance in the osmoregulatory system; thus several circuli show breaking mechanism. A space is created, which permits the passage of light. This area is called annulus[1]. The number of annuli indicates the age of the fish. All the fishes selected for the present study belonged to age class 2+, which indicates they have completed two years of life and have entered in the third year. This fish starts breeding in the first year in the wild.

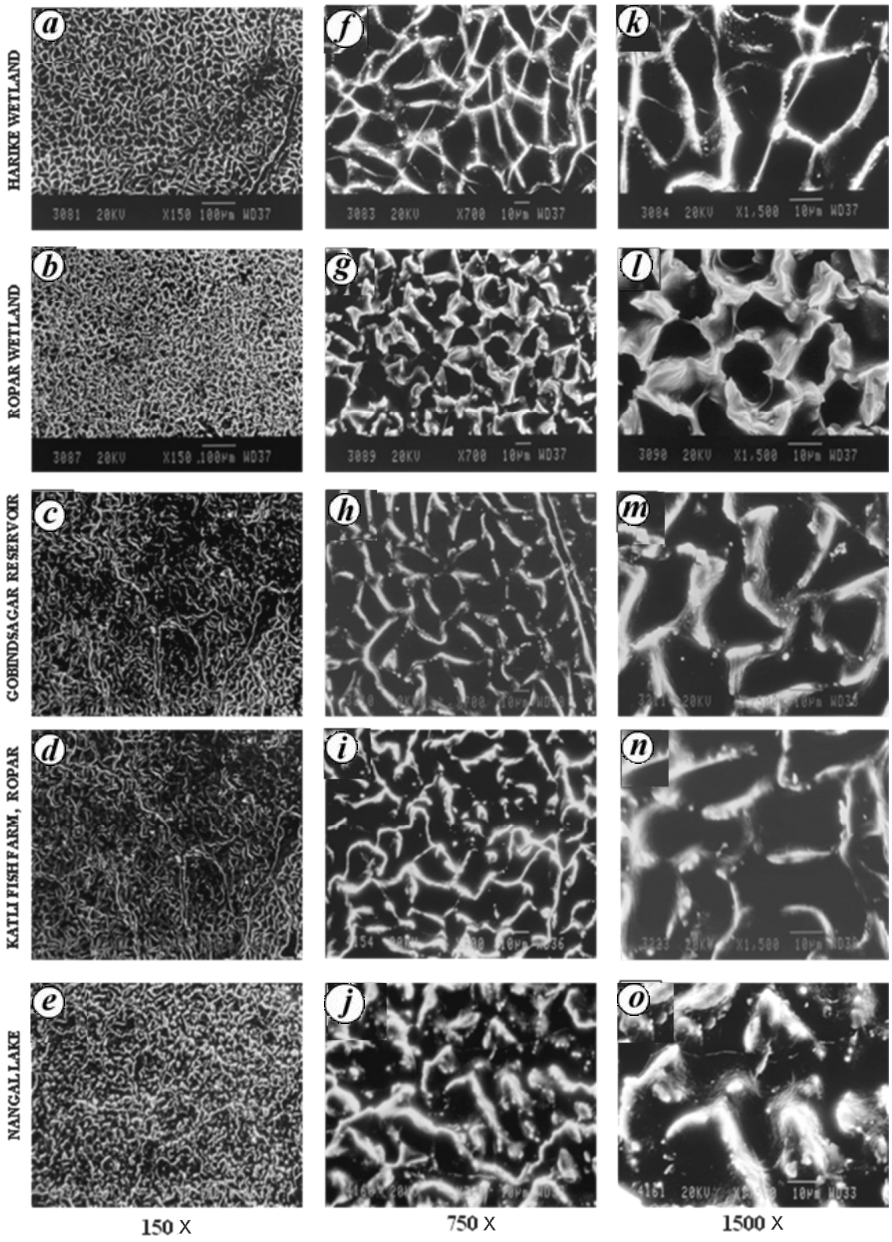

Figure 3 *a–o*. Regeneration pattern in central part of the scales of *C. carpio* L. collected from different localities in Punjab and Himachal Pradesh.

Therefore, the formation of annual mark appears from the first year. The formation of annulus and structure of the regenerated scale have no relevance with the age of the fish. Therefore, the regenerated scale from any age class of this fish can be employed for population analysis with great accuracy.

During overcrowding, while passing through the macrophytes and by rubbing against each other during spawning period, some scales are removed or shed. As the scale acts as an organ of protection, as well as defensive mechanism, a new scale appears on the area from where the original scale has been removed/shed. This scale is called the regenerated scale (Figure 2 *b*). The process of formation of a new scale is very fast. Hence, circuli are not formed till the stage the regenerated scale assumes the same size of the surrounding scales. Once the regenerated scale attains a size similar to those of the surrounding scales, the circuli formation begins (Figure 2 *b*). Almost all the fishes are covered by some percentage of regenerated scales. The maximum percentage of regenerated scales is present in the middle part of the body of the fish.

For the present study, ultrastructure of the focus region of the regenerated scale at magnifications 150×, 700× and

1500× of *C. carpio communis* L. inhibiting five ecological different localities has been employed for separation of stocks of this exotic fish species (Figure 3).

The ultrastructural details of the focus region of the regenerated scales from five different localities at the magnification of 100× indicates that, in this region the upper layer of the scale is mesh-like, which is in the form of villi having different shapes and configurations. The structure of the mesh in the scale of fish inhabiting Harike, Ropar and Nangal wetlands has almost similar type of mesh work, but the thickness is more in the case of Harike and Ropar populations compared to Nangal population. Similarly, the populations of this fish inhabiting Gobindsagar reservoir and the Katli Fish Farm, Ropar have focus regions of the scales with entirely different structures. Again, the grouping pattern is different in these two populations. The focus region of the normal scale of Indian major carps has poorly defined circuli, arranged in semi-circular fashion[11]. Similarly, the focus region of the scales of other cyprinids have similar structures[5], but clear-cut differences are observed. On the basis of the present and earlier studies, it can be concluded that the villi arrangement in the focus region as revealed by SEM studies can be employed for species identification[5,11]. As only one species of the genus *Cyprinus* occurs in this region, the ultrastructural details of the same region can be employed for discriminating the populations inhabiting the varied ecological conditions[12,13].

The regenerated scale is termed as the deformation of the central area of the replaced scale due to the lack of nutrients, especially calcium salts and the availability of less time, which is required for the formation of normal circuli. In this region the ridges assume different shapes with gaps of various sizes. This arrangement appears in the form of a network. The various types of network found in the regenerated part of the scale of *C. carpio communis* L. inhabiting the five water bodies are given in Figure 3. For the sake of comparison and uniformity, all the SEM photomicrographs are at the same magnification (150×, 700× and 1500×). From these figures it appears that the regenerated part of the scale of the population inhibiting Ropar wetland has more compact ridges with less space in between, firmly attached to each other (Figure 3 *b*, *g* and *l*). In other populations, calcium salts are diffusely deposited in the ridges; the ridges are loosely connected with each other and have wide variation in the gaps. Calcium particles are present between the villi from the populations from Gobindsagar (Figure 3 *c*, *h* and *m*), Katli Fish Farm (Figure 3 *d*, *i* and *n*) and Nangal Lake (Figure 3 *e*, *j* and *o*). The maximum intensity of these calcium particles is in Nangal Lake population followed by Katli Fish Farm and Gobindsagar populations. In addition to these characters, the configuration of the villi and the inter-villous space can also be employed for the separation of stocks of this exotic fish species. Needless to say that the differences are apparent and they are population-specific[14–16].

Normal shape and ultrastructural details of the fish scale of various fish species have been described here so that these can be employed for species identification and to determine the phylogenetic relationships between different groups[17,18]. The regenerated scales of pike-perch from different parts of North America were employed for the analysis of population with great accuracy[17]. It may be added here that the use of otolith morphology and otolith elemental fingerprinting is in vogue for population analysis and migratory routes, especially in marine fishes having wide zoogeographical distribution[19–22].

It can be concluded that the ultrastructural details of the cycloid scales can be employed for species identification. This can replace the conventional tool, i.e. identification on the basis of morphometric and meristic characters. Further, ultrastructural details of the focus region of the regenerated scales can be employed for population analysis for those fish species having wide zoogeographical distribution and which inhibit distinctly different ecological conditions. As this technique involves removal of scales directly from the fish without killing it and requires no chemical processing, the chances of artefacts are minimal. As the scales can be studied directly without any processing using SEM technique, only the processing and viewing charges need to be paid, whereas molecular studies involve laborious steps of DNA extraction, PCR using specific primers, etc. which is more expensive than SEM. Moreover, one can also save time when SEM studies are employed. Considering these facts, we are of the view that this tool can be used as a good alternate to molecular markers for analysis of fish populations.

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