E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2020; 8(3): 574-576
© 2020 IJFAS
www.fisheriesjournal.com
Received: 24-03-2020
Accepted: 28-04-2020

Suresh M Kumbar
Department of Zoology, Arts, Commerce & Science College, Palus, District Sangli, Maharashtra, India

Swapnali B Lad
Department of Zoology, Arts, Commerce & Science College, Palus, District Sangli, Maharashtra, India

Jyoti A Kumbar
Department of Zoology, Arts, Commerce & Science College, Palus, District Sangli, Maharashtra, India

Corresponding Author:
Suresh M Kumbar
Department of Zoology, Arts, Commerce & Science College, Palus, District Sangli, Maharashtra, India

Formation of annual growth layers in the Southern Indian freshwater fish *Labeo rohita* (Hamilton, 1822); (Teleostei: Cyprinidae)

Suresh M Kumbar, Swapnali B Lad and Jyoti A Kumbar

Abstract
Fingerlings of the freshwater fish rohu *Labeo rohita* were maintained in the farm pond (size 33 x 33 x 3 m) at Gavhan village, district Sangli, Maharashtra, India to know the periodicity of formation of growth marks in the calcified materials. Approximately 1.0 – 1.2 cm body sized fingerlings were released on May 2015 and maintained for a year in the fish farm pond under natural conditions. All fish were collected back in July 2016 by using cast net with the help of local fisherman. Among the collection, 100 fish with different body size (range 18 – 39 cm) were selected for age determination study. There was a significant increase in body mass and body size in each fish. All these fish showed one line of arrested growth each in scales, otoliths and vertebrae sections, suggesting that the formation of growth marks is annual in this fish.

Keywords: Cypriniformes, age determination, year ring, tropical

1. Introduction
Studies on growth, age and attainment of sexual maturity of commercially important fishes provide baseline information for successful stock identification [1, 3]. The age and growth studies of India major carps namely, *Labeo catla* (Hamilton, 1822); *Cirrhina mrigala* (Bloch, 1795) and *Labeo rohita* (Hamilton, 1822) have been investigated in detail [4, 5]. The applicability and the accuracy of age determination studies depend upon the periodicity of formation of growth marks [4, 6]. However, very few experimental studies have established that the formation of growth marks in Indian freshwater fishes is annual; therefore, they can be regarded as 'year rings' for the estimation of age [7, 8]. Previous studies are limited to north-east region of India, where the variation in annual mean temperature does exceed more than 25°C. Similar type of work is totally lacking in southern region of India, where annual mean temperature variation does not exceed 10 °C. It is not much clear that whether the formation of growth mark is annual or not and which factors are responsible for the formation of growth marks in the southern part of India. In order to bridge this lacuna and to know the periodicity in the formation of growth marks in the freshwater fish *L. rohita* inhabiting Sangli, Southern India.

2. Materials and Methods
Fish farm pond (size 33 x 33 x 3 m) was constructed at Gavhan village (20´ 68º N and 76´ 70º E), district Sangli, Maharashtra, India. Gavhan is a small village located 45 km east to the Sangli city. Study area has an average elevation of 560 m ASL and it receives an average annual rainfall of 450 mm from South West monsoon. About 1000 fingerlings of *L. rohita* (approximately SVL = 1-1.2 cm) were released into the farm pond in May 2015 and maintained for a year under the natural conditions. Fish were fed on natural and artificial foods (Ground nut cake, Grobest Fish feed - 9200 and Grower - Growfin, Tamboli Fish Farm, Pune) *ad libitum* regularly. Fish were collected back in the month of July 2016 by using cast net with the help of local fisherman. From the collection, different body size (range 18 – 39 cm) fish (n = 100) were selected for the age determination study. They were brought to the laboratory where body mass (to the nearest 0.01g) and body size (measured to the nearest cm using a thread) of each fish were recorded. Simultaneously lateral line scales, otoliths and central vertebrae were collected and fixed in 10% formalin solution for further studies. Formalin fixed

scales of each specimen was cleaned in water by rubbing gently with fingers. Cleaned scale was placed in between two clean slides. The slides were tied with rubber band on either side and observed under binocular microscope (Magnus MSZ-BI) for enumerating the ring counts present on the scales and photographed by using a digital camera (ABBOT DEC- 2000). The otoliths were washed in water and cleaned from all extraneous tissue and weighed to the nearest 0.001 mg. The otoliths were then immersed in 50% glycerol and observed under a binocular microscope. The growth rings were clearly visible as alternate opaque and translucent zones were enumerated. The central vertebrae (5th and 10th) of each fish were cleaned, washed in water for 1 h and decalcified with 5% nitric acid. Decalcified vertebrae were washed under running tap water for 24 h to remove the traces of formaldehyde and nitric acid and preserved in 70% alcohol. These vertebrae were embedded in paraffin wax and sectioned (10 µm thickness) by using a rotary microtome (Model GE-70). Mid-diaphyseal sections were stained with Harris haematoxyline and observed under compound microscope for enumeration of growth marks. The relationship if any, between body size and body mass, body size and otolith weight was determined by calculating the correlation coefficient '*r*' by Karl Pearson's method [9].

3. Results and Discussion

At the beginning of the experiment, small sized (approximately 1-1.5 g weight; 1-1.2 cm length) fingerlings were released and maintained up to one year in the natural agriculture pond under regular feeding (Fig. 1A). Hundred different body sized (range 18 – 39 cm) fish were selected from the stock in July 2016 (Figure 1B). The body mass, size and the otolith mass of each fish were increased significantly compared to the corresponding values at the beginning of the experiment (Table 1). All these fish showed one LAG (Lines of Arrested Growth) in scales, otoliths and vertebrae sections (Table 1; Fig. 1C-E). There was a high degree of positive correlation between body mass and body size (r = 0.87), body size and otolith mass (0.82).

The freshwater fish *L. rohita* is widely distributed in peninsular India, and is one of the highly consuming commercial food fish [10]. Generally physiological processes of cold blooded animals are regulated by the temperature of the water in which they live. Annual cyclicity in calcified materials leaves growth marks that indicate the number of osteogenic cycles experienced, and indirectly the age of the individual. Although the age and growth of Indian major carps inhabiting north-east region of India have been investigated in detail [5, 6, 11, 12] such type of work is scanty in northern fishes [13]. In the present study, 100 different body sized (fish of representative sizes from the stock) fishes were selected after one year for age determination study. All these fishes showed one LAG each in their scale, otolith and vertebrae sections which may be formed during the lapsed period, assuming that one LAG is laid down per year. The present results reveal that the formation of LAG is annual in southern Indian freshwater fishes. The fact that many of the southern Indian freshwater fishes exhibit marked seasonality in the body growth and reproductive activity [14-15] suggests that scales, otolith and vertebrae growth is a cyclical phenomenon leading to the formation of LAGs. Although, gonado-somatic-index (GSI) values increase from May - September, which coincides with the onset of monsoon rains and breeding activity of the fish; they attain their maximal values in August and from October onwards there is a decrease in GSI [15, 18]. Therefore, in this fish, the LAG(s) may be laid down between May - September when the body growth is almost ceased coinciding with the wet season of the year. From January onwards when GSI masses begin to restore, the next growth cycle may set in. Similarly, annual rings found in the scales of *Puntius sarana* from River Ghaggar in Rajasthan and in Sukhna lake in Punjab, formed during March-May owing to spawning stress [18]. The work of *Catla catla* from Harike and Gobindsagar showed the formation of the annuli in June-July coinciding with the commencement of spawning and the beginning of the southwest monsoon [8]. Therefore, enumerating the presence of growth marks in the cross sections of vertebrae sections may be used to estimate the age and longevity of *L. rohita* and perhaps other tropical freshwater fishes. Furthermore, high degree of correlation between body mass and size and body size and otolith weight indicates that body size analysis is also one of the reliable techniques for assessment of age in this fish. In conclusion, present study suggests that LAGs are formed annually in the scales, otoliths and vertebrae sections of freshwater fish *L. rohita* inhabiting southern region of India and therefore could be regarded as annual rings for estimating the age of freshwater fishes.

Table 1: Table showing the changes in body mass and size, otolith mass, and number of LAG in the freshwater fish *Labeo rohita* collected from the agriculture pond after one year. (Values in mean ± SD).

Sl. No.	No. of fish	Body Mass (g)	Body Size (cm)	Otolith Mass (mg)	Growth Layer
Initial (May 2015)	1000	1.0 - 1.5	1.0 - 1.20	--	0 LAG
Final (June 2016)	100	267.167 ± 163.36	25.052 ± 7.169	16.02 ± 7.058	1 LAG

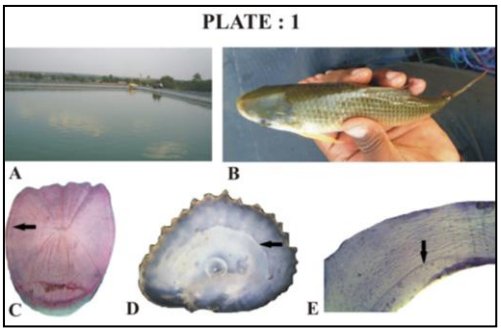

Fig. 1A - E: A: Fish farm pond. **B**: One year old fish *Labeo rohita*, **C***:* One LAG in the scale, **D**: otolith; **E:** vertebral cross section of the same fish; Scale line = 100 µm.

Abbreviation: Arrow = Lines of Arrested Growth (LAG).

4. Acknowledgements

Authors are thankful to Management and Principal for providing necessary laboratory facilities. The study was financially supported by the University Grants Commission, New Delhi for Major Research Project File No. 42-619/2013 (SR).

5. References

1. Klevezal GA. Recording structures of mammals, Determination of age and reconstruction of life history. Rotterdam/Brookfield: A. A. Balkema. 1996, 274.

2. Pawson MK, Jennings S. A critique of methods for stock identification in marine capture fisheries. Fishery Research. 1996; 25(3-4):203-217.
3. Griffiths MH. Age and growth of South African silver Kob *Argyrosomus inodorus* (Sciaenid), with evidence for separate stocks. African Journal of Marine Science. 1997; 17:37-48.
4. Jhingran AG. Age determination of Indian major carp, Cirrhina mrigala (Ham.) by means of scales. Nature, London. 1957; 179:468-469.
5. Khan RA, Siddiqui Q. Studies on age and growth of rohu, *Labeo rohita* (Ham.) from a pond (Moat) and Rivers Ganga and Yamuna. Zoological survey of India, Calcutta. 1973; 39(5):537-597.
6. Mir JI, Sarkar U, Gusain OP, Dwivedi AK, Jena J. Age and growth in the Indian major carp *Labeo rohita* (Cypriniformes: Cyprinidae) from tropical rivers of Ganga basin, India. International Journal of Tropical Biology. 2013; 61(4):1955-1966.
7. Menon NG. Age and growth of the marine catfish *Tachysurus thalassinus* (Ruppell) from Mandapam waters. Indian Journal of Fishries. 1986; 33(4):413-425.
8. Johal MS, Tandon KK. Age and growth of the carp *Catla catla* (Hamilton 1922) from north India. Fisheries Research. 1992; 14:83-90.
9. Zar JH. Biostatistical Analysis, 3rd Edition Englewood Cliffs, NJ: Prentice-Hall, 1984, 662.
10. Jayaram KC. The Fresh water Fishes of the Indian Region. Second Edition. Narendra Publishing House, Delhi, 2010, 616.
11. Natarajan AV, Jhingran AG. On the biology of *Catla catla* from the river Jamuna. Proceeding of Indian National Science Academy. 1963; B29:326-355.
12. Bhatt BJ, Jahan N. Determination of Age and Growth Rate of Fresh Water Fish *Labeo rohita* (Ham. 1822) by Using Cycloid Scales. International Journal of Pure & Applied Biosciences. 2015; 3(3):189-200.
13. Seshappa G. Recent studies on age determination of Indian fishes using scales, otoliths and other hard parts. Indian Journal of Fisheries. 1999; 46(1):1-11.
14. 14. Patil HS, Saidapur SK. Reproductive cycles of reptiles, In: S. K. Saidapur (ed.), Reproductive Cycles of Indian Vertebrates, Allied Publ. Ltd., New Delhi, 1989, 275.
15. Sharma AP, Naskar M, Joshi KD, Bhattacharjya BK, Sahu SK, Das S *et al.* Impact of climate variation on breeding of major fish species in inland waters. Bulletin, 2014, 35.
16. Sarkar SK, Saha A, Dasgupta S, Nandi S, Verma DK, Routray P *et al.* Photothermal manipulation of reproduction in Indian major carp: a step forward for off-season breeding and seed production. Current Science. 2010; 99:960-964.
17. Chakrabarti P, Chatterjee N. Seasonal changes in the architecture of hepatocytes in relation to ovarian activities during growth, maturation, spawning and post-spawning phases in *Mystus vittatus* (Bloch, 1790). Journal of Entomology and Zoology Studies. 2014; 2(4):212-217
18. Roy K, Mandal D. Maturity stages of ovary of a minor carp, *Labeo bata* (Hamilton-Buchanon, 1822). International Journal of Fisheries and Aquatic Studies. 2015; 2(6):19-24.
19. Tandon KK, Johal MS. Age and growth of the minor carp *Puntius sarana* (Ham.). Zoologica Poloniae. 1983(a); 30(1-4):47-55.