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Comparison of cleithra, urohyal and hyomandibular bones for age estimation in Indian major carp *Labeo rohita* (Hamilton)

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Abstract
Labeo rohita specimens were collected from local fish market of Aligarh to observe the hard structures providing the most suitable age estimate. Total length (T_L) of each fish was measured from tip of snout to the longest fin ray of the caudal fin. Each fish was weighed separately on a weighing balance, and the age of each fish was determined by using cleithra, hyomandibular bone and urohyal bone. Age estimates from different hard anatomical structures were used to calculate the correlation between the age readings from different pairs of ageing structure. Data generated on age estimates from the selected ageing structures were also subjected to student t- test .Standard deviation and standard error was also calculated for the age reading from a different pair of ageing structures. The statistical analysis was carried out by SPSS v.12. Comparison of different structures revealed cleithra to be the best ageing structure for estimating age. So, in case of fish with unreadable scales use of cleithra for age estimation of *L. rohita* was recommended.

Keywords: Age estimate, cleithra, urohyal bone, hyomandibular bone, *Labeo rohita*

1. Introduction
Fish age determination is an important tool in fishery biology, especially for the assessment of life history, growth rate, age at the first maturity and population dynamics. Knowledge on these aspects has an important and useful application in fisheries resource management. Different methods have been employed for the determination of fish age. Among all the methods, the mark-release capture method is considered as the most accurate but its application is limited in fisheries due to a number of constraints such as time and money. Fisheries are marked or tagged, and released after recording initial measurements of lengths and weight; the fish are recaptured after an interval of a few months and measured again. Various types of tag bearing particulars about the fish and date of tagging etc. are attached to the body, so that they remain in position and are not lost. A tag may be a disc made of plastic, aluminium, silver; nickel etc. Tag may be attached to the body by means of thin wire without actually piercing it. The wire may be tied by encircling the jaw or caudal peduncle. In some cases a thin wire is used to actually pierce the tissue of a body. The marked or tagged fishes provide more realistic data regarding the growth and movements of the fish, but are more difficult to recapture, hence a very large number of fish must be used in this method.
Another method of fish age determination is based on analysis of length frequency data. This method is based on the expectation that length- frequency analysis of the individuals of a species of any one age group, collection on a same day will show variation around the mean length. It is further based on the assumption that when data in a sample of the entire population are plotted, successive ages at successive given lengths will be clumped together, making possible a separation of various age groups. For a fairly reliable result of the above method, it is necessary that the sample consists of a large number of individuals collected preferably on a single day, and should include representatives of all sizes and age group in a population.
The hard parts that can be studied for age determination are scales, otoliths vertebral centra, dorsal and pectoral spines or rays, opercular bone, urohyal bone, coracoids, hyomandibular etc. Of these the most commonly used are the scales and otoliths.
Generally, scales are used for age determination of fish. This is the simplest and most accurate

method, and with some experiences, annuli on the scales can be easily counted. The focus of the scale is the first part to develop and is usually located in the centre. An annulus is usually marked by a clear, narrow streak, encircling the focus. One annulus represents one year, and the addition of successive annuli indicates the number of years the fish has attained. The age of a fish is determined by scales on the basis that successive rings are formed as the fish grows in age. During summer and autumn, plenty of food is available and the fish grows at a faster rate so that the rings formed in this period are widely spaced. During winter, the food supply is limited and growth is restricted so that the rings are tightly packed, giving an appearance of alternate light and dark bands. These two bands are together considered to have been formed in one year and are called the annuli. These numbers of annuli on the scale gives the age of a fish. Sometimes the false annuli also appear in some fishes and are sometimes difficult to distinguish from true annuli and make an age determination less accurate. Van Oosten (1957) [42] attributes these accessory annuli to growth cessation due to fluctuation in temperature, food, disease, spawning, injury or starvation etc. Generally a true annulus runs all along the surface of a scale, and is formed at the same time each year. But a false annulus does not run all along the scale and can be formed at any time due to various factors. It was formerly thought that availability of food was responsible for the formation of rings, and so this method is valid for fish living in temperate zones, where the amount of food varies with the season, while in tropical areas where the food is available throughout the year, this method is not reliable. However, it is now believed that food alone is not responsible for the formation of annuli and it can be due to cessation of feeding during the process of maturation and spawning.

Scales have been successfully used for the age and growth study of different fish species such as *Labeo rohita* (Khan and Siddiqui, 1973) [23]; *Catla catla* (Johal and Tandon, 1992) [16]; *Cirrhinus mrigala* (Kamal, 1969) [20] *Channa marulius* (Dua and Kumar, 2006) [7]; *Capoeta trutta* (Aydin *et al*; 2003); *Scardinius erythrophthalmus* (Gursoy *et al* 2005) [11] etc.

Otoliths are formed in embryo as soon the inner ear is formed, and are located on either side of skull. Of the three otoliths (Lapillus, asteriscus, and Sagitta), Sagitta is the largest and commonly used in age determination of fishes. Otoliths have been used for age estimation in *Tor putitora* (Pathani, 1979) [30]; *Mystus keletius* (Santhakumar *et al*; 1983) [38]; *Sillago indicia* (David and Pancharatna, 2003) [3]. Vertebral centra have been used for age determination in a wide range of marine and freshwater species such as *Pleuronectes flesus luscus* (Pallas) (Polat *et al*; 2001) and *Cyprinus carpio* (Linnaeus) (Phelps *et al*; 2007) [32].

Opercular bones have been used for age determination in different fish species by many workers in different fish species viz. Perch (Bardach, 1955; Le Cren, 1947) [24]; *Cyprinus carpio* (Mc Connell, 1952) [26]; *Ophiocephalus punctatus* (Qasim and Bhatt, 1964) [36]; *Labeo senegalensis* (Blake and Blake, 1978); *Catla catla* (Nargis, 2006) [27] etc.

Dorsal fin rays were reported to be more suitable for ageing different fish species such as *Salmo trutta* (Burnet, 1969) [20]; *Cyprinus carpio* (Wichers 1976; Kamilov 1984) [43, 21]; Age studies of various fish species have been undertaken by several researchers using cleithra in different fishes such as esocid (Casselman, 1977; Harrison and Hardely 1979) [2, 13]; *Esox lucius*, (Euchner 1988) [8]; *Yellow perch* (Schmitt and Hubert, 1982) [39]. The urohyal bone is a single median triradiate solid bone with anterior tip generally connected to the ventral hypohyals, and the anteriodorsal part connected to the *first* basibranchial and the posterior part connected to the pectoral girdle by means of muscles. It has horizontal and vertical components, which are flat. Urohyal bone has been used by different researchers for age estimation such as, *Hypophthalmichthys molitrix* (Johal *et al*; 2000, 2002); *Lutjanus vittus* (Davis and West, 1992) [4] etc. Limited information is available on age determination using cleithra and urohyal bone on Indian fresh water fishes. To best of our knowledge, there is no previous information available for age determination of fish by using hyomandibular bone.

Some workers used different hard anatomical structures in the same fish for age estimation. Comparison of age estimates between structures is an alternative to the validation that may provide useful information on the accuracy and bias of age estimating structures (Sylvester and Berry, 2006).Various bony structures of *Liza ramada* population from Mersin bay were compared for age determination (Gocer and Ekingen 2005) [9]. Scales, pectoral fin rays and opercular bones were compared for age determination of Ontario red horse, *Moxostoma* species i.e. *Moxostoma anisurum, M. Carinatum M. macrolepidotum* and *M. Valenciennesi*) (Reid, 2007) [37]. Scales, opercular bones, vertebrae, dorsal fin rays and otoliths were used for ageing common carp [32]. Age determination is invariably accompanied by various sources of error. A variety of methods exist through which age interpretation can be validated [1]. Cleithra and scales were compared for age and growth analysis of yellow perch (Schmitt and Hubert, 1982) [39]. Comparison of cleithra and scales were done for age and growth studies of esocids [13]. Khan and Khan (2009) studied comparison of age estimates from scales, opercular bone, otoliths, vertebrae and dorsal fin rays in *Labeo rohita, Catla catla and Channa marulius*. Results indicated scales to be the most suitable structure for ageing *L. rohita* and *C. marulius* and opercular bone for *C. Catla*. The objective of present study was to evaluate and compare age estimates between different ageing structures (cleithra, hyomandibular, and urohyal bones) for the age estimation of Indian major carp, *Labeo rohita* (Hamilton)

2. Materials and Methods

2.1. Sample Collection

The study material consisted of 13 specimens of *Labeo rohita*. Fish samples were collected from the local fish market of Aligarh. Fishes were caught using gill nets of varying mesh sizes.

2.2. Body Measurement

Total length (TL) of each fish was measured from the tip of the snout to the longest fin ray of the caudal fin. All fish samples were measured to the nearest 1 mm. Each fish was weighed separately on a weighing balance.

3. Age reading techniques

3.1 Collection and preparation of cleithra

Cleithra was removed from fresh specimens and cleaned by soaking them in warm water for several minutes and then rubbing with a finger and soft brush to remove any adhering tissue. Cleaned and dried bones were stored in envelopes and observed under microscope for age reading (Johal *et al*; 2001) [19].

3.2 Collection and preparation of hyomandibular bone

The hyomandibular bone was removed and cleaned in hot water. The cleaned hyomandibular bone was then dried and stored. Age was determined by counting the number of annuli under a binocular microscope.

3.3 Collection and preparation of urohyal bone

Urohyal bones were removed from fresh specimens and muscles were separated by dipping them in water at 60-70 degree centigrade for 5 min. The cleaned and dried Urohyal bones were stored in ordinary envelopes. Urohyal bone was examined dry on a black surface under incident light using a dissecting microscope.

3.4 Data analysis

Age estimates from different hard anatomical structures (viz. cleithra, hyomandibular bone and urohyal bone) were used to calculate the correlation between the age readings from different pairs of ageing structures such as cleithra-hyomandibular bone; hyomandibular bone-Urohyal bone and cleithra –Urohyal bone. Data generated on age estimates from the selected ageing structures were also subjected to student t-test. Standard deviation and standard error was also calculated for the age reading from different pair of ageing structures. The statistical analysis was carried out by SPSS v.12.

Table 1: Morphometric measurements and number of annuli on different hard parts of *Labeo rohita*

S.no	Total length	Standard Length (cm)	Total Weight (gm)	Number of rings		
				Cleithra	Hyomandibular bone	Urohyal bone
1	42.5	35	1150	3	2	2
2	47.5	40	1450	3	2	3
3	41	34.5	1300	3	3	3
4	50	42.5	1670	3	3	2
5	45	38.5	1250	2	3	2
6	50	42.5	1750	2	3	3
7	52	45.2	2000	4	4	4
8	47.5	40	1400	3	2	2
9	50	42.5	1700	3	3	3
10	51.5	44.5	1850	3	2	4
11	55	48.5	2100	4	4	3
12	52.5	48	2200	3	3	3

Table 2: Paired samples correlation of age estimates from different bony parts of *Labeo rohita*

Ageing structures	Paired sample correlations	
	N	Correlation
Cleithra-Hyomandibular bone	13	0.527
Hyomandibular-Urohyal bone	13	0.422
Cleithra-Urohyal bone	13	0.527

Table 3: Mean of age estimates for ageing structures along with results of pair-wise comparisons of the age estimates among ageing structures using paired t-test

Paired ageing structures	Paired differences			
	Mean	Standard deviation	Standard Error mean	t-value
Cleithra Hyomandibular bone	.1538	.6887	.1910	.805
Hyomandibular bone- Urohyal bone	.0000	.8165	.2264	.000

4. Results and Discussion

Labeo rohita specimens ranged from 41 to 57.5 cm in total length and 1150 gm to 2200 gm total weight (Table 1). Overall, cleithra, hyomandibular bone and urohyal bone showed a regular growth pattern in the form of alternating opaque and translucent bands. In *L. rohita*, cleithra showed clearer and sharper annual growth rings. After cleithra, urohyal bone showed clear rings while, rings present on hyomandibular bone were less distinct. The age readings from different anatomical structures were subjected to one way analysis of variance (ANOVA), correlation coefficient, and student t-test in order to establish the relationship among readings of different structures. Mean values of age estimates from different structures, when compared using (ANOVA), showed insignificant ($p<0.05$) differences. The correlation coefficient values between age reading of paired structures was same for cleithra – hyomandibular bone (0.527) and cleithra- urohyal bone (0.527) while low value of correlation coefficient was found between hyomandibular bone - urohyal bone (0.422). Results of student t-test between paired structures showed insignificant differences between cleithra – hyomandibular bone, hyomandibular bone urohyal bone and cleithra –urohyal bone (Table 3). Euchner (1988) studied the collection, preparation and use of northern pike cleithra for age determination. They reported that cleithra is a more reliable structure for assessing the age structure of pike stocks. Harrison and Hardely (1979) [13] studied the comparison of the cleithra and scales for age and growth studies of esocids. They found cleithra as a suitable ageing structure. Johal *et al* (2001) [19] studied the comparison of back –calculated lengths of silver carp by using scales, cleithra and urohyal bone. They reported linear relationship were found between body length-scale radius, body length-cleitrum length and body length – urohyal length (r=0.949, 0.948 and 0.974 respectively). Laine *et al*. (1991) studied the accuracy of using scales and cleithra for ageing northern pike from oligotropic Ontario lake. They reported both scales and cleithra are equally suitable for assigning age structure to squeers lake northern pike

In the published literature available on freshwater fishes, scales have been exclusively used for age studies in *L. rohita*

[23] *Catla catla* (Johal and Tandon, 1987; 1992) [18, 16] and *Cirrhinus mrigala* (Jhingran, 1957, Johal and Tandon 1983) [15, 17]. In most of cyprinids, age has been estimated from scales (Kamilov, 1984) [21]. Scales of *L. rohita* are elongated and broad having focus which is sharp in the beginning but becomes prominent as scales grow in size and the circuli (circuli concentric lines) are formed periodically with the growth of scales showing closely and widely spaced arrangement representing slow and fast period of growth [41]. Horpilla and Nyberg (1999) [14] studied the validity of different methods (scale proportional hypothesis; body proportional hypothesis and Fraser-lee method) in the back calculation of lengths of roach by using cleithra and scales. They found cleithra very useful especially in determining in the ages of old and slow-growing roach and other cyprinids, the scales of which are often impossible to read. The detection of the first annuli from cleithra of older individuals is often difficult due to the thickness of the structure, a phenomenon found also in opercula of roach [25, 12, 31].

5. Conclusion
Comparison of different structures (i.e. cleithra hyomandibular bone and urohyal bone) revealed cleithra to be the best ageing structure for estimating age. So, in case of fish with unreadable scales, use of cleithra for age estimation of *Labeo rohita* is recommended.

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