



Comparison of age estimates from scale, opercular bone, otolith, vertebrae and dorsal fin ray in *Labeo rohita* (Hamilton), *Catla catla* (Hamilton) and *Channa marulius* (Hamilton)

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ABSTRACT

The present study was undertaken with a view to compare the precision and reliability of the age readings obtained from different bony structures of some important freshwater teleosts viz., *Labeo rohita* (Hamilton), *Catla catla* (Hamilton) and *Channa marulius* (Hamilton). Standard procedures were followed to prepare and study the age structures. In *L. rohita* and *C. marulius* percent agreement between reader's age estimates was highest for scales, i.e. 96.3% and 90.5%, respectively and in *C. catla* percent agreement was highest (93.3%) for opercular bone. When scale ages were compared with other alternative structures viz., otoliths, opercular bone, vertebral centra and dorsal fin rays, percent agreement was found highest between scale and opercular bone age estimates (77.8%) in *L. rohita* and between scale and otoliths (94.8%) in *C. marulius*. In case of *C. catla* highest percent agreement was found between opercular bone and scale age estimates. In *L. rohita* each of the ageing structure showed significant ($P < 0.05$) underestimation of age in comparison to scales. In *C. catla* mean age estimates from opercular bone were comparable ($P > 0.05$) to the values obtained from all other structures except dorsal fin rays. In *C. marulius* mean age estimates from scales were comparable ($P > 0.05$) to those from all other structures except from dorsal fin rays. Results indicated scales to be the most suitable structure for ageing *L. rohita* and *C. marulius* and opercular bone for *C. catla*. However, in *C. catla* also scales may be used as a non-destructive method of age estimation with satisfactory results.

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1. Introduction

Estimation of accurate fish age is considered as an essential step for age-based assessment of fish population and successful resource management. Comparison of age estimates from various ageing structures have been reported to be undertaken in a number of fishes with a view to identify the most suitable structure for a fish population (Reid, 2007; Phelps et al., 2007; Maceina and Sammons, 2006).

In the published literature available on Indian freshwater fishes, the ageing structures were used not to test the precision for age estimates but to correlate age with growth of the fishes (Johal et al., 1983; Johal and Tandon, 1985, 1992; Dua and Kumar, 2006). There are no published reports available on comparison of different structures for ageing the fishes selected for the present study (rohu, *Labeo rohita* Hamilton; catla, *Catla catla* Hamilton and giant snakehead, *Channa marulius* Hamilton). These fishes constitute major catch amongst the riverine and the reservoir fisheries

of India. Indian major carps (*L. rohita*, *C. catla* and mrigal, *Cirrhinus mrigala* Hamilton) are considered as most important freshwater food fishes in Indian subcontinent and comprise bulk of the total freshwater fish production in the region. *C. marulius*, the largest of the snakeheads, enjoys strong consumer preference and usually fetches higher market price than carps and catfishes in many parts of the country. But unfortunately its culture is not popular and also the fish appear in very low numbers in wild catch. Riverine fisheries, in general, are considered to generate yields below subsistence level. Catch statistics over many years indicate a declining trend for riverine catches, both in quantitative and qualitative terms. The average yield of major carps in river Ganga declined from 26.62 to 2.55 kg/ha per year during the last four decades. Biologically and economically desirable fish species have started to be replaced by low-value species as their populations are rapidly declining (Upare, 2007). However, conservation and proper utilization of germplasm is a prerequisite for the sustainable management of and increased production from aquatic resources (Gupta et al., 2005).

Since various methods for age determination in fishes sometimes show variation in readings, therefore, the present study has been undertaken with a view to compare and select the suitable skeletal structure for reliable age estimation in some of the

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commercially important freshwater fishes viz., *L. rohita*, *C. catla* and *C. marulius*.

2. Materials and methods

2.1. Sampling

The study material consisted of 108 *L. rohita* specimens, 104 specimens of *C. catla* and 116 specimens of *C. marulius*. Samples were collected monthly from January 2007 to April 2008 from the river Ganga at Narora (27°30'N, 78°25'60E), U.P., India.

Total weight, total length and standard length of each sample were recorded. Otoliths, vertebrae, opercular bone, scales and dorsal fin rays were removed from the fish and prepared for ageing.

2.2. Scale preparation and reading techniques

Scales were removed from above the lateral line near the tip of the pectoral fin. Scales were washed, cleaned and studied as dry mounts, after removing the extraneous matter and mucus by washing them in tap water and rubbing in between the finger tips. To make scales more clear and soft (in case of large scales), dipped in weak solution (1%) of potassium hydroxide (KOH) for about 5–10 min, then washed in tap water and dried in air. Small sized scales were mounted between two glass slides and studied with the help of compound microscope (Tandon and Johal, 1996).

2.3. Opercular bone preparation and reading techniques

The opercular bone were detached with the help of scalpel and dipped in boiling water for few minutes to remove extraneous tissue. A bristled brush was used to remove tissue that boiling water did not loosen. Cleaned opercular bones were dried at room temperature and examined under transmitted fluorescent light with naked eye (Phelps et al., 2007).

2.4. Otolith preparation and reading techniques

Sagittal otoliths were removed from otic capsules by opening the otic bulla. In *L. rohita* and *C. catla*, otoliths were washed, cleaned and read whole by immersion in alcohol while in *C. marulius*, otoliths were studied by placing them in glycerol and examined under microscope using reflected light. Otoliths with unclear annual rings were ground with sand paper to make the annuli more distinct for age reading (Tandon and Johal, 1996).

2.5. Vertebrae preparation and reading techniques

Vertebrae (4th to 10th) were removed and placed in boiling water for 10–15 min to clear the attached muscles. Vertebrae were then dried for 2 weeks to count annual rings. Vertebrae were examined by shining a fiber-optic light near the bottom of the structure to illuminate annuli under dissecting microscope (Phelps et al., 2007).

2.6. Dorsal fin ray preparation and reading techniques

Dorsal fin rays were sectioned with jeweller's saw. Sections were placed on a microscopic slide and viewed under dissecting microscope (Phelps et al., 2007).

All the structures (otoliths, scales, vertebrae, opercular bone and dorsal fin ray sections) were aged independently by two readers without the knowledge of fish length. In case of disagreement between readers the ageing structures were reexamined together by the two readers until a consensus was reached.

2.7. Calculations and statistical analyses

Age estimates were compared by calculating the coefficient of variation, percent agreement (PA) and average percent error (APE) between the readers and between the pairs of ageing structures. APE was derived using the formula presented by Beamish and Fournier (1981):

$$APE = \frac{1}{R} \sum_{i=1}^R \frac{|x_{ij} - x_j|}{x_j} \times 100,$$

where x_{ij} is the i th age determination of the j th fish, x_j the average age calculated for the j th fish and R the number of times each fish is aged.

Beamish and Fournier (1981) described APE to be sensitive not only to age disagreement but also to the magnitude in the difference in age assignment between or among readers.

In *L. rohita* and *C. marulius* each alternative structure (otoliths, opercular bone, vertebrae and dorsal fin rays) was paired with the scale (which showed more clear and sharp rings) and in case of *C. catla* each alternative structure was paired with opercular bone. Scales in the *L. rohita* and *C. marulius* and opercular bone in *C. catla* were considered to be accurate ageing structures on the basis of clarity and sharpness of the annual rings and also prior validation of these structures in the respective fishes (Khan and Khan, unpublished data).

For each fish species, mean age readings (consensus data) obtained from various hard anatomical parts were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) (Gomez and Gomez, 1984) in order to explain whether the readings from different hard anatomical parts of the same species showed significant differences among themselves. Although mean age estimate is not an indicator for the reliability of ageing structure but it may provide useful information regarding over or under estimation of age by a structure irrespective of fish size-class. This may prove useful in selecting the structures which may give statistically indifferent readings when size-class is not taken into account.

3. Results

3.1. *L. rohita*

In *L. rohita* specimens ($N=108$; total length = 18–64 cm) annual growth rings were clearer and sharper in scales thereby producing lesser errors in age estimation. Percent agreement between the two independent readers was highest for the scales (96.3%) followed by opercular bones (89.8%), vertebral centra (75.0%), otoliths (63.9%) and dorsal fin rays (63.0%). When scale ages were compared with other alternative structures, highest percent agreement was found between scale and opercular bone (77.8%), followed by vertebrae (69.4%), otoliths (61.1%) and dorsal fin rays (39.8%). In *L. rohita* scale and opercular bone showed lowest values of APE and CV as compared to other structures (Table 1). Mean values of age estimates from different structures showed significantly ($P<0.05$) underestimation of age by opercular bone, vertebrae, otoliths and dorsal fin rays when compared to the values obtained from scales (Table 2).

3.2. *C. catla*

In *C. catla* ($N=104$; total length = 19–61 cm) percent agreement of ages between independent readers was higher for opercular bone (93.3%) followed by otoliths (84.6%), scales (79.8%), vertebrae (71.2%) and dorsal fin rays (65.4%). When opercular bone

Table 1

Comparison of percent agreement (PA), average percent error (APE) and coefficient of variation (CV) between the hard anatomical structures in *Labeo rohita*, *Catla catla* and *Channa marulius*.

Between structure	PA	APE	CV
<i>L. rohita</i> (N = 108)			
Scale-otolith	61.1	10.40	14.72
Scale-opercular bone	77.8	5.66	8.01
Scale-vertebrae	69.4	7.55	10.69
Scale-dorsal fin rays	39.8	16.14	22.83
<i>C. catla</i> (N = 104)			
Opercular bone-scales	95.2	1.13	1.60
Opercular bone otolith	97.1	0.70	1.00
Opercular bone-vertebrae	74.0	6.26	8.86
Opercular bone-dorsal fin rays	65.4	8.05	11.85
<i>C. marulius</i> (N = 116)			
Scale-otolith	94.8	1.16	1.65
Scale-opercular bone	87.9	3.07	4.34
Scale-vertebrae	69.8	7.60	11.0
Scale-dorsal fin rays	68.1	7.95	11.24

N: number of observations.

ages were compared with other alternative structures, highest percent agreement was found between opercular bone and otoliths (97.1%) followed by scale (95.2%), vertebrae (74%) and dorsal fin rays (65.4%). In *C. catla* opercular bone and otoliths showed lowest values of APE and CV as compared to other structures (Table 1). Mean values of age estimates from different structures (except dorsal fin rays) exhibited comparable ($P > 0.05$) estimation of age when compared to the values obtained for opercular bones (Table 2).

3.3. *C. marulius*

In *C. marulius* (N = 116; total length = 11–88 cm) percent agreement of ages between readers was higher for scales (90.5%) followed by otoliths (83.6%), opercular bones (81.0%), vertebrae (70.7%) and dorsal fin rays (66.4%). When scale ages were compared with other alternative structures, highest percent agreement was found between scale and otoliths (94.8%) followed by opercular bones (87.9%), vertebrae (69.8%) and dorsal fin rays (68.1%) (Table 1). Scale and otoliths showed lowest values of APE and CV as compared to other structures (Table 1). Mean values of age estimates from different structures (except dorsal fin rays) showed comparable ($P > 0.05$) estimation of age when compared to the values obtained for scales (Table 2). However, mean age estimates from dorsal fin rays were insignificantly ($P > 0.05$) different from the values obtained for all other structures except scales.

4. Discussion

4.1. Scales

In most of the cyprinids, age has been estimated from scales (Kamilov, 1984). In common carp, *Cyprinus carpio* Linnaeus, com-

Table 2

Comparison of mean values of age estimates from different bony parts in *Labeo rohita*, *Catla catla* and *Channa marulius*.

Bony parts	Mean values of age estimates ¹		
	<i>Labeo rohita</i> (N = 108)	<i>Catla catla</i> (N = 104)	<i>Channa marulius</i> (N = 116)
Scales	2.3056 ^a	2.6346 ^a	2.1983 ^a
Otolith	1.9167 ^b	2.6442 ^a	2.1466 ^{ab}
Opercular bone	2.0833 ^b	2.6635 ^a	2.0948 ^{ab}
Vertebrae	2.0000 ^b	2.4231 ^{ab}	1.9138 ^{ab}
Dorsal fin ray	1.6944 ^c	2.2981 ^b	1.8793 ^b

¹ Values having similar superscripts in each column are insignificantly different ($P > 0.05$) from each other.

parison of different structures (scales, opercular bone, dorsal spines and sagittal otoliths) revealed scales to be the best structure for estimating age but in case of fish with unreadable scales use of fin sections was recommended (Lubinski et al., 1984). Scales with their growth marks were reported to be better structure than the opercular bone used for ageing black prochilodus, *Prochilodus nigricans* (Spix and Agassiz) (Loubéris and Panfili, 1992). Amongst all age structures, scales were found to be the most suitable ageing structure in *L. rohita* and *C. marulius* in the present study. In addition to having clear and sharp annuli, scales also have the advantages such as easy collection, preparation and being non-destructive to the fish. This is in corroboration with the findings of other studies. In shir mahi, *Capoeta trutta* (Heckel), Ozturk et al. (1997) found rather clear annuli on scales though best annuli were reported in dorsal fin ray. The annuli on scales of *C. trutta* were reported better than those on the otoliths and operculum (Ozdemir and Sen, 1983). Sharper and clearer annuli on *L. rohita* scales, observed in the present study, may be due to the fact that the maximum age observed in the fish group was 6 years and the clarity and sharpness of annuli on the scales of 6 years age fish was slightly less than those of the younger fish. However, there are reports where *L. rohita* and other *Labeo* species had been aged upto 7+ and 8+ years using scales only (Johal and Tandon, 1985; Tandon et al., 1989). These authors did not use any other structure for age reading in the fishes.

Several researchers have reported that scales can provide unreliable estimates of fish age (Boxrucker, 1986; Hammers and Miranda, 1991). The imprecise and inaccurate age enumeration from scales have been attributed to reabsorption and deposition of false annuli due to stress and food limitation, and annuli becoming obscure because scale growth tends to cease as fish grow older (Beamish and McFarlane, 1987; Maceina and Sammons, 2006). Scale ages were on an average 9 years less than the ages estimated from sectioned otoliths in striped bass, *Morone saxatilis* (Walbaum) older than 20 years, but scales were reported to estimate age adequately up to the age of 12 years (Secor et al., 1995). In some scientific reports, the use of scales was criticized mainly because of the frequent underestimation of the ages of older fish (Beamish and McFarlane, 1987).

4.2. Otoliths

Otoliths exhibited clear growth rings in *L. rohita*, *C. catla* and *C. marulius*. Age agreement for otoliths between independent readers was 63.9%, 84.6%, 83.6% for *L. rohita*, *C. catla* and *C. marulius*, respectively. Accurate age estimation using otoliths is also supported by the fact that otoliths do not show reabsorption and their growth is acellular rather than by calcification (Secor et al., 1995) and also because otoliths are reported to be metabolically inert and thus do not reflect physiological changes that may occur throughout the life of fish (Phelps et al., 2007). Sometimes, the interpretation of the otoliths is complicated due to presence of false rings (Morales-Nin, 1992) which are often deposited corresponding to the crucial moments of the fish life cycle such as sexual maturity. There are reports that the use of whole otoliths, as used in the present study, can lead to underestimation of the ages when compared with sliced otoliths (Abecasis et al., 2006). Otoliths were reported to be the most reliable ageing structure in transcaucasian barb, *Capoeta capoeta umbla* (Heckel) (Ekingen and Polat, 1987) and Atlantic horse mackerel, *Trachurus trachurus* (Linnaeus) (Polat and Kukul, 1990). Mosegaard et al. (1989) observed that reader precision was high for both scales and otoliths obtained from roach, *Rutilus rutilus* (Linnaeus) in Sweden, but ages discerned from scales were much lower than those observed from fish that were >10–11 years old estimated from otolith examination.

Isermann et al. (2003) while comparing the scales, sagittal otoliths and dorsal spines reported that otoliths provide the most

time efficient and precise approach for age estimation in walleyes, *Stizostedion vitreum* (Mitchill). Brown et al. (2004) validated the use of asteriscus otolith annuli for the age determination in *C. carpio*. Morioka et al. (2002) in their study on otoliths of African carp, *Labeo mesops* (Gunther) juveniles reported lapillus otoliths to be suitable for age determination. Age assignment from spines and scales was less precise than from otoliths for fish collected from upper Hudson river and for the older fish scale ages were progressively lower than otolith age (Maceina and Sammons, 2006). Kruse et al. (1993) observed that ages determined using scales and otoliths were reported to be similar in black crappies, *Pomoxis nigromaculatus* (Lesueur). Erman (1959) found that annual rings on otoliths were more difficult to identify. Ozdemir and Sen (1986) found that scales and vertebrae were better as compared to otoliths in chub, *Leuciscus cephalus* (Linnaeus) for age reading.

4.3. Opercular bone

Opercular bones were reported to be superior to scales for age estimation of *C. carpio* (McConnell, 1952). Nargis (2006) used opercular bones of *C. catla* for age reading. The determination of age and growth of fish from opercular bone is well established in fishes of temperate waters and has been found to be more satisfactory than other methods such as scales, vertebrae, spines or other hard parts in common pike, *Esox lucius* (Linnaeus) (Frost and Kipling, 1959) and European perch, *Perca fluviatilis* (Linnaeus) (Shafi and Maitland, 1971).

Rings on opercular bone of younger age group fishes were clearer and more easily identifiable than in the older age groups. Similar observations have also been reported by other researchers (Frost and Kipling, 1959; Nargis, 2006; Shafi and Maitland, 1971). Khemiri et al. (2005) found opercular bone unsuitable for age estimation in bogue, *Boops boops* (Linnaeus) from Tunisian waters. In four redhorse species, *M. anisurum*, *M. carinatum*, *M. macrolepidotum* and *M. valenciennesi*, age estimates from scales were significantly lower than those obtained from fin rays and opercles (Reid, 2007). Jimenez-Badillo (2006) mentioned that the extraction, preparation and reading of opercular bones in comparison with scales were relatively easier and cited that the rings on scales and opercular bones were due to periods of fast or slow growth imposed by reproductive energetic demands in blue tilapia, *Oreochromis aureus* (Steindachner). Blake and Blake (1978) used opercular bones for studying the age and growth of African carp, *Labeo senegalensis* (Valenciennes) from Lake Kainji, Nigeria and mentioned that growth rings were formed as a result of minimum water temperature and for onset of the rains associated with a limited food supply.

4.4. Vertebral centra

Vertebrae were reported to be the most reliable part of the skeleton for age determination in Nile perch, *Lates niloticus* (Linnaeus) (Mishrigi, 1967). Polat et al. (2001) compared different bony parts of north Atlantic flounder, *Pleuronectes flesus luscus* (Pallas) for age determination and reported vertebrae as the most reliable structure having minimal ageing error. Guinn and Hallberg (1990) reported that vertebrae and otoliths gave similar age estimates in burbot, *Lota lota* (Linnaeus). In golden snapper, *Lutjanus johnii* (Bloch), sectioning of otoliths and vertebrae enhanced the ability to differentiate opaque zones in otoliths and interpret growth checks in vertebrae and produced higher age estimate than those obtained from whole vertebrae and otoliths (Marriott and Cappel, 2000). Clark (1987) compared vertebrae, otoliths and scales for ageing fall chum salmon, *Oncorhynchus keta* (Walbaum). The author concluded that time required to process and read vertebrae (20 times as long as scales) made them less practical to use but the precision and accuracy involved with vertebrae made them best of

the three structures researched. Vertebrae have rarely been used to study age estimation in fishes which show clear growth marks in other ageing structures that cause minimal or no damage to the fish. In the present study, the rings on vertebral centra were not very clear and showed numerous minute marks unrelated to cyclic events which corroborates with the observations made by Hill et al. (1989) in blue marlin, *Makaira nigricans* (Lacepede) and Khemiri et al. (2005) in *B. boops*.

4.5. Dorsal fin rays

Dorsal fin rays were found to be more suitable than the otoliths for ageing brown trout, *Salmo trutta* (Linnaeus) (Burnet, 1969) and to other structures for ageing *Barbus rajanorum mystaceus* (Heckel) and *C. trutta* (Polat, 1987a, 1987b). In thin-lipped grey mullet, *Liza ramada* (Risso) population from Mersin bay, dorsal fin rays were found to be the most reliable bony structure for age determination followed by scales, vertebrae, otoliths and operculum (Gocer and Ekingen, 2005). Red gurnard, *Chelidonichthys kumu* (Lesson and Garnot) was reported to be aged satisfactorily by using fin rays for live specimens and otoliths for dead specimens (Staples, 1971). If non-lethal procedure is needed for age determination of *C. carpio* population, then pectoral fin ray sections have been recommended for fish up to the age of 13 years (Phelps et al., 2007). The authors also explained that most ages in *C. carpio* were overestimated by scales, vertebrae and opercles through age 6 but underestimated beyond age 10. Use of fin rays does not require sacrificing the fish, and rays can be removed without any apparent harm to the fish (Mills and Beamish, 1980). Fin ray annuli are reported to remain prominent for older fish when scale annuli are not identifiable (Beamish and Chilton, 1977). If first annulus not identified correctly then the fish age will be underestimated leading to over estimation of growth and naturally mortality coefficients with drastic implications in fish stock management (Leaman and Nagtegaal, 1987).

5. Conclusions

Scales were found to be the reliable bony structure for ageing *L. rohita* and *C. marulius*, and opercular bones in *C. catla* up to 6 years of age. In *C. catla*, since values of different parameters for scales were very close to otoliths and opercular bones and also because of several advantages discussed elsewhere (Section 4.1), scales may be recommended as ageing structure, if non-destructive method is to be used.

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