

Note

Precision of age estimates from different ageing structures of *Labeo bata* (Hamilton) collected from river Ganga, India

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ABSTRACT

Scales, opercular bones, vertebrae and otoliths collected from *Labeo bata*, inhabiting river Ganga were used to identify the most suitable structure for age estimation. Age estimates were evaluated between readers and between pairs of ageing structures by calculating the percent agreement, average percentage of error and coefficient of variation. Percent agreement between readers was highest for scales (87%), followed by opercular bones (81.2%), vertebrae (78.9%) and otoliths (76%). Due to highest values of percent agreement, lowest average percent error and coefficient of variation values between two readers, scales were considered to be the most suitable structure for age estimation of *L. bata*. When age of scales were compared with other alternative structures viz., opercular bones, vertebrae and otoliths, the highest percent agreement (72.7%), lowest average percent error (7.61%) and coefficient of variation (10.77%) values were found between scales and opercular bones. Also, mean values of age estimates from scales were comparable ($p > 0.05$) to those obtained from opercular bones, and were significantly ($p < 0.05$) different from age estimates using otoliths and vertebrae.

Keywords: Age estimation, Ganga River, *Labeo bata*, Opercular bones, Otoliths, Scales, Vertebrae

Labeo bata (Hamilton), belonging to the family Cyprinidae, has been reported from rivers and lakes of India, Bangladesh and Pakistan (IUCN, 2012). This species has also been introduced into reservoirs where the species is cultivated. Conservation status of this species has been assessed as lower risk near threatened (Molur and Walker, 1998) and least concern (IUCN, 2012). Loss of habitat, overexploitation and siltation may be some of the threats to wild populations of the species (Molur and Walker, 1998; IUCN, 2012).

The assessment of age within and among populations is a critical component of many fisheries investigations. Data on fish age are necessary to examine growth, mortality and reproductive characteristics of a population (Hoxmeier *et al.*, 2001). Age of fishes can be estimated using a variety of hard structures such as scales (Johal and Tandon, 1985; Gursoy *et al.*, 2005), otoliths (Abecasis *et al.*, 2006; Stewart and Hughes, 2007; Khan *et al.*, 2011a; Khan *et al.*, 2013a), fin rays and spines (Paragamian, 2003; Metcalf and Swearer, 2005; Al-Zibdah and Odat, 2007), vertebrae (Polat *et al.*, 2001; MacNeil and Campana, 2002; Khan *et al.*, 2013b) and opercular bones (Qasim and Bhatt, 1964; Blake and Blake, 1978; Nargis, 2006). The most reliable method may vary among species and it is necessary to evaluate precision and accuracy of bony structures used for age estimation (Polat *et al.*, 2001). Age estimation is often

accompanied by several sources of error that can have significant effects on many population parameter estimates (Campana, 2001). Although age estimates that are precise may not necessarily be accurate (Maccina *et al.*, 2007), understanding the precision of structures used for age estimation can reduce variation and increase the utility of age and growth information (DeVries and Frie, 1996). Comparison of age estimates between structures is an alternative technique for validation that may provide useful information on the accuracy and bias of age estimating structures (Sylvester and Berry, 2006). Several studies have focused on comparing ages enumerated from different bony structures in an attempt to quantify the precision and to identify possible bias associated with each structure. To date, no published information is available on the precision of age estimates from different ageing structures in *L. bata*. Therefore, the present study was undertaken with the objectives to evaluate and compare different ageing structures (*i.e.*, scales, opercular bones, otoliths and vertebrae) in order to identify the most suitable ageing structure and quantify the differences in precision and bias between readers and among the pairs of ageing structures.

L. bata is cultivated along with Indian major carps in bheries and estuarine waters in the Sundarbans (West Bengal) and extensively used for stocking in tanks in several parts of India (Froese and Pauly, 2011). This

species along with the Indian major carps comprise significant proportion of the inland capture fisheries of the country. The information generated may be useful for the formulation of scientifically sound fishery management policies for *L. bata* inhabiting the river Ganga.

A total of 265 *Labeo bata* specimens were collected from river Ganga from September 2010 to December 2011. The total length (TL) of each fish was measured to the nearest 0.1 cm and the total weight (TW) was recorded to the nearest 0.1g. Scales, otoliths, vertebrae and opercular bones were removed from the fish and prepared for ageing. Scales were removed using forceps 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 mucous by washing them in tap water and rubbing in between the finger tips. To visualise scale structures more clearly large scales were dipped in a weak solution of KOH (1%) for about 5–10 min, and then washed in tap water and air dried (Tandon and Johal, 1996). Opercular bones were immersed in boiling water and the attached tissue was removed using a brush. Cleaned opercular bones were dried at room temperature and stored separately until used for examining under transmitted light with naked eye. Sagittal otoliths were removed from the fish, rinsed in distilled water to remove any adhering tissue, dried and then immersed in alcohol to examine under microscope. Otoliths with unclear annual rings were ground with sand paper to make the annuli more distinct (Tandon and Johal, 1996). Vertebrae (4th to 10th) were removed and placed in boiling water for 10–15 min to clear the attached muscles. All processed vertebrae were then dried for 2 weeks to count the annual rings under a dissecting microscope (Phelps *et al.*, 2007). All the scales, opercular bones, vertebrae and otoliths, were aged independently by two readers who did not have knowledge of fish length, weight, or date of collection. Such data were utilised to calculate the precision of age estimates between the two readers. In order to produce information on measures of precision, the age estimates were subjected to appropriate calculations and statistical treatments (Khan and Khan, 2009). Precision was measured by calculating the percent agreement (PA), coefficient of variation (CV) and average percentage of error (APE) between the readers and between the pairs of ageing structures. Percent agreement is expressed as percentage of the number of observations showing similar age estimates to the total number of observations on age estimates. Percent agreement was calculated using the templates for calculating ageing precision (Sutherland, 2006). APE was calculated using the formula presented by Beamish and Fournier (1981):

$$APE = \frac{1}{R} \sum_{i=1}^R \frac{|x_i - \bar{x}|}{\bar{x}} \times 100$$

where x_{ij} is the i^{th} age determination of the j^{th} fish, \bar{x}_j is the average age calculated for the j^{th} fish and R is the number of times each fish was aged.

The coefficient of variation (Campana, 2001) was calculated as the ratio of standard deviation over the mean, and can be written as:

$$CV_j = 100\% \times \frac{\sqrt{\frac{\sum_{i=1}^R (X_{ij} - \bar{X}_j)^2}{R-1}}}{\bar{X}_j}$$

where CV_j is the age precision estimate for the j^{th} fish.

Age bias graphs (Campana *et al.*, 1995) were constructed to examine potential bias between readers and between pairs of ageing structures. Mean age readings 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 ($p > 0.05$) differences among themselves (Khan and Khan, 2009). All statistical analyses were done using MS-Excel and SPSS (version 16.0).

The age composition of the sampled fish specimens based on different bony structures exhibited variation in their age estimates (Fig. 1). Percent agreement (PA) of ages between the age readings of two independent readers was highest for scales followed by opercular bones, vertebrae and otoliths (Table 1). When scale age estimates were compared with other alternative structures, highest PA was found between scales-opercular bones, followed by scales-vertebrae and scales-otoliths (Table 1). Age bias graphs between two readers for scales, opercular bones, vertebrae and otoliths are presented in Fig. 2. In case of scales, no age bias was found between readers, while small error was present in opercular bones. Disagreement

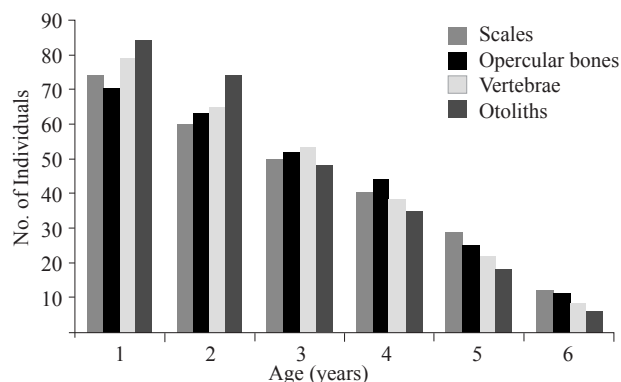
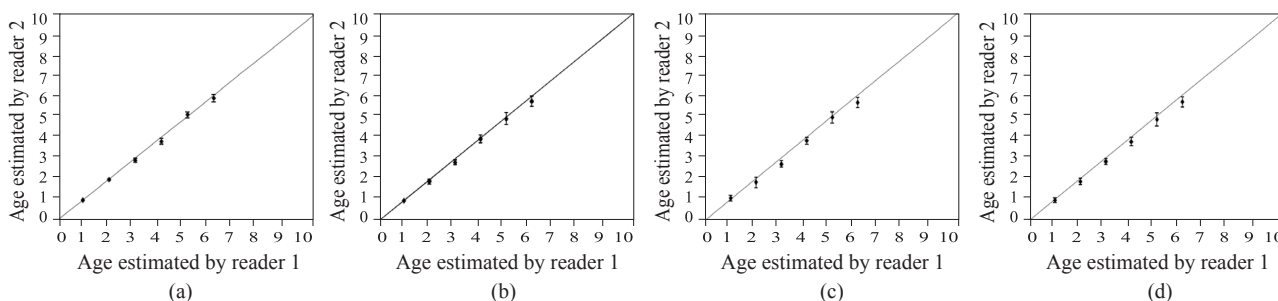


Fig. 1. Age estimates derived from the readings of different ageing structures in *Labeo bata*.

Table 1. Comparison of mean values of age estimates from ageing structures in *Labeo bata* collected from river Ganga

Group	PA	APE	CV
Between Readers			
Scales	87	2.38	3.37
Opercular bones	81.2	3.22	4.81
Otoliths	76	6.01	8.50
Vertebrae	78.9	5.28	7.48
Between Structures			
Scales vs. Opercular bones	72.7	7.61	10.77
Scales vs. Vertebrae	68.8	9.95	14.07
Scales vs. Otoliths	62.3	11.72	16.5

Fig. 2. Age bias graphs between two readers for the age estimates derived from (a) scales, (b) opercular bones, (c) vertebrae and (d) otoliths of *Labeo bata* collected from river Ganga. Points below the line indicate ages that were underestimated.

between readers increased with age for otoliths and vertebrae after age 2, and these increased with fish age as indicated by large standard error bars. Mean values of age estimates from different structures, when compared using ANOVA followed by DMRT, showed that mean age estimates from scales were significantly ($p < 0.05$) higher from otoliths, but comparable ($p > 0.05$) to the values obtained from opercular bones (Table 2). The values of age estimates from vertebrae and otoliths did not differ significantly ($p > 0.05$).

Table 2. Comparison of mean values of age estimates (years) from ageing structures in *Labeo bata* collected from river Ganga

Bony parts	Mean Values of age estimates
Scales	2.8361 ^a
Opercular bones	2.5902 ^{ab}
Otoliths	2.0492 ^c
Vertebrae	2.2459 ^{bc}

Note: Values bearing different superscripts are significantly different ($p > 0.05$) from each other.

Scales had the highest values within and between reader agreement and lower ageing error, and they were also easier to read and non-destructive to fish as compared to other structures (*viz.*, opercular bones, vertebrae and otoliths). Chatterji *et al.* (1979) studied age and growth of *L. bata*, by analysis of annuli found on the scale and by length-frequency distribution. Scales are one of the most frequently used fish ageing structures due to their ease of collection and also the fish can be released alive (DeVries and Frie, 1996). Researchers have reported scales as the precise ageing structure in *Micropterus salmoides* (Prentice and Whiteside, 1974), *Pomoxis nigromaculatus*

(Kruse *et al.*, 1993), striped bass (Welch *et al.* 1993), *Labeo rohita* and *Channa marulius* (Khan and Khan, 2009) and *Cirrhinus mrigala* (Khan *et al.*, 2011b). Scales with their growth marks were reported to be better structure than opercular bones used for ageing in *Prochilodus nigricans* (Louberis and Panfili, 1992). The annuli on scales of *Capoeta trutta* were reported better than those on otoliths and operculum (Ozdemir and Sen, 1983). Scales were easy to collect and process, but the growth marks in old specimens were very close to the edge and almost

indistinguishable, as reported for *Boops boops* from the Gulf of Lion (Girardin and Quignard, 1986). Scales were reported to underestimate the age in old specimens of *Helicolenus percooides* (Withell and Wankowski, 1988) and *Etheostoma caeruleum* (Beckman, 2002).

Opercular bones showed the second highest agreement between readers, and the highest agreement with scale age estimates. In several studies of cyprinid age, small differences were found between opercular and scale age estimates (L'Abée-Lund, 1985; Sinis *et al.*, 1999). Opercular bones were found to be superior to other ageing structure in *Cyprinus carpio* (McConnell, 1952) and *Catla catla* (Khan and Khan, 2009).

Vertebrae have rarely been used for age estimation in fishes. In the present study, rings on vertebral centra were not very clear and showed numerous minute marks unrelated to cyclic events. Since we used whole vertebral centra for age estimation in *L. bata*, the degree of clarity and sharpness of the growth rings were low. In contrary, Polat *et al.* (2001) reported vertebrae as the most reliable structure with minimal ageing error, for age determination in *Pleuronectes flesus luscus*.

Otoliths showed lowest percent agreement and highest ageing error values between two readers and with scale derived age estimates. Annual rings on otoliths were often difficult to interpret in *L. bata*. As a result, percent agreement between readers and agreement with scale ages were low in otoliths. Otoliths were reported to be inferior to scales and vertebrae for age estimation in *Leuciscus*

cephalus orientalis (Ozdemir and Sen, 1986). Sometimes, the interpretation of the otoliths is complicated due to presence of false rings (Morales-Nin, 1992) which are often deposited subsequently to the crucial moments of the life cycle such as sexual maturity as suggested by Colloca *et al.* (2003). In the present study, the first and second annuli were usually easily identified in younger fish. However, in older fish the otoliths were thicker which made the identification of annual rings more difficult. It seems plausible that the use of whole otoliths could have led to underestimation of age which corroborates the findings in *Helicolenus dactylopterus* (Abecasis *et al.*, 2006).

Results of the present study indicated that scales are the most suitable hard part for age estimation in *L. bata* up to 6 years of age. However, when size class is not taken in to account, the difference between age estimates derived using scales and opercular bones are not significant.

Acknowledgements

Authors are thankful to the Chairman, Department of Zoology, Aligarh Muslim University, Aligarh, India for providing necessary facilities for the study. Financial assistance (UGC-MAN Fellowship) provided to the first author by the University Grants Commission, New Delhi, is gratefully acknowledged.

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Date of Receipt : 22.11.2012

Date of Acceptance : 18.11.2014