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Evaluation of ageing precision from different structures of three threatened freshwater fish species, *Clarias batrachus*, *Heteropneustes fossilis* and *Wallago attu*

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Abstract. Ageing precision from otoliths, vertebrae and pectoral spines was studied in *Clarias batrachus*, *Heteropneustes fossilis* and *Wallago attu*. In *C. batrachus*, otoliths showed the highest (92.9 %) agreement between readers followed by vertebrae (87 %) and pectoral spines (83.8 %). The highest percentage of agreement (87 %) and lowest average percentage of error (1.89 %) and coefficient of variation (3.81 %) values were observed between otoliths and vertebrae age estimates. In *H. fossilis*, vertebrae showed the clearest annual rings and had highest (90.8 %) PA values between readers, followed by otoliths (80.3 %) and pectoral spines (73.7 %). The highest PA and lowest APE and CV values were found between vertebrae and otoliths estimates. In *W. attu*, otoliths showed the highest PA (86.7 %) values between readers followed by pectoral spines (70.3 %) and vertebrae (67.9 %). The highest PA and lowest APE and CV values were found between otoliths and pectoral spines. On account of the highest PA, lowest APE and CV values, the most suitable ageing structure was otolith in *C. batrachus* and *W. attu*, while vertebrae in *H. fossilis*. If mean age estimates are considered then in the absence of the most suitable ageing structure, alternatively, vertebrae can be used in *C. batrachus*, otoliths in *H. fossilis* and pectoral spines in *W. attu*.

Key words: age estimation, average percent error, otoliths, pectoral spines, percentage of agreement, vertebrae

Introduction

The fish species selected for the present study, the walking catfish, *Clarias batrachus* (Linnaeus), stinging catfish, *Heteropneustes fossilis* (Bloch) and Asian silurid catfish, *Wallago attu* (Bloch & Schneider) are highly popular food fishes in the Asian continent. The population of all these species is reportedly declining due to a number of factors such as high fishing pressure, habitat loss, water pollution, disease etc. (Molur & Walker 1998, Khan et al. 2012). Many of these factors are further aggravated by the fact that successful commercial culture of any of these species is not being undertaken particularly in terms of significant contribution to the total produce. Therefore, the conservation of aquatic germplasm resources is to be taken on priority basis in the present global scenario, where more fish species are being reported to be endangered and threatened (Lakra et al. 2010). For this purpose, we need to have accurate and updated basic biological information on the concerned fish species. But unfortunately, such information for many fish species is either incomplete or inaccurate.

Age structure of population provides information that appears essential for the understanding of several population parameters particularly the dynamics of the target fish species, recruitment, population growth rates, and mortality. In the fishes without scales, age can be estimated using a variety of hard structures, including otoliths, fin spines, vertebrae and opercular bones. Some hard structures provide more accurate and precise estimates of age than others. Selecting the proper structure for ageing is critical for providing useful information to managers (Quist et al. 2007). The choice among the bony structures for age determination varies from species to species. Generally, all the bony structures in the fish body do not exhibit the equal clarity and distinctness in their annual marks. Thus, it becomes necessary to find out the most suitable ageing structures in each fish species. For obtaining correct age data, the ages

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of fish are estimated by comparing readings from various bony structures and different readers. As the most reliable ageing method may vary among species, the precision of bony structures by readers should be studied for each species (Baker & Timmons 1991). Useful information on the accuracy and bias of age estimating structures may be obtained by the comparison of age estimates between structures which has been considered as alternative technique to validation of age estimates (Sylvester & Berry 2006). The present research investigation was undertaken in order to develop the necessary basic biological information required for the formulation and implementation of scientifically sound fishery management policies for *C. batrachus*, *H. fossilis* and *W. attu*. To date, no published studies have compared the age estimates from different ageing structures in *C. batrachus*, *H. fossilis* and *W. attu* in order to generate information on the ageing precision. Therefore, the present study was undertaken with the objective to evaluate and compare different ageing structures (i.e., otoliths, vertebrae and pectoral spines) so as to identify and quantify the differences in precision between readers and among the pairs of ageing structures of *C. batrachus*, *H. fossilis* and *W. attu* collected from the River Ganga.

Material and Methods

Study area and sample collections

The River Ganga rises in the Gangotri glacier (30°54' N, 78°54' E) in the Himalayas at an altitude of 7010 meter above mean sea level in the Uttarkashi district of Uttarakhand, India. It travels along the five Indian states of Uttarakhand, Uttar Pradesh, Bihar, Jharkhand and West Bengal. A total of 342 specimens of *C. batrachus*, 485 samples of *H. fossilis* and 320 *W. attu* specimens were collected monthly from January 2010 to October 2011 from the River Ganga at Narora (27°30' N, 78°25' E), U.P., India (Fig. 1). Total length (TL) was measured to the nearest 1 mm. Body weight was recorded to the nearest 0.1 mg as total weight (TW) including gut and gonads. Otoliths, vertebrae, and pectoral spines were removed. For each fish, annuli were counted on all these structures independently by two readers without prior knowledge of fish length and age estimates from other structures.

Ageing structures were prepared following the methods adopted by Khan & Khan (2009) and Khan et al. (2011b).

Sagittal otoliths were removed from otic capsules by opening the otic bulla. In *C. batrachus*, otoliths were washed, cleaned and read whole by immersion in 50

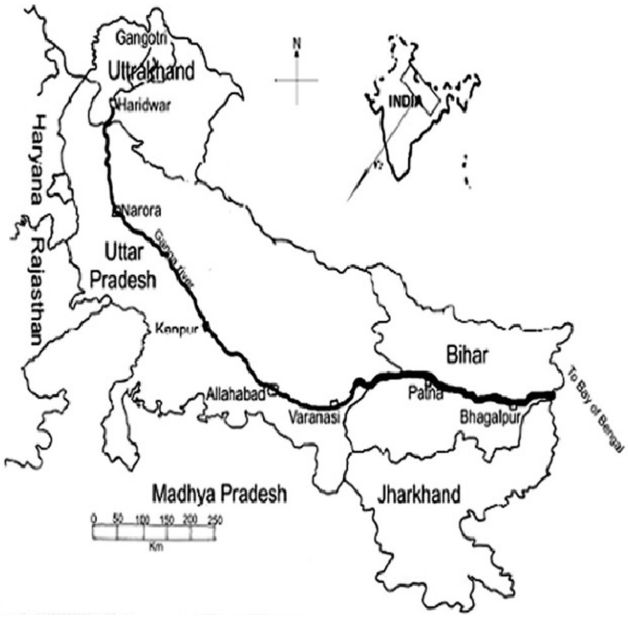

Fig. 1. Map showing the sampling location (Narora) of *Clarias batrachus*, *Heteropneustes fossilis* and *Wallago attu* inhabiting the River Ganga.

% glycerol and observed under microscope using reflected light. In *H. fossilis* and *W. attu* otoliths were read whole by immersion in ethanol and examined under microscope on a black background using reflected light. Otoliths with unclear annual rings were ground with sand paper to make the annuli more distinct for age reading (Tandon & Johal 1996).

Vertebrae (4^{th} to 10^{th}) were removed and placed in boiling water for 5-10 min to clear the attached muscles. All processed vertebrae were then dried and examined in xylol under the microscope (Yalcin et al. 2002).

Pectoral spines were sectioned using a jeweller's saw. Spine sections were mounted on microscope slides and viewed under dissecting microscope (Buckmeier et al. 2002).

Precision was measured by calculating the percent agreement (PA), coefficient of variation (CV) (Chang 1982), and average percent error (APE) (Beamish & Fournier 1981) between the readers and between the pairs of ageing structures for the selected fish species. APE was derived using the formula presented by Beamish & 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.

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

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

where CV_j is the age precision estimate for the j^{th} fish. Mean age readings (consensus data) obtained from various bony parts were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) (Gomez & Gomez 1984) in order to explain whether the readings from different bony parts of the fish showed significant differences among themselves. Although mean age estimate is not an indicator for the reliability of ageing structure, it may provide useful information regarding over or under estimation of age by a structure irrespective of fish size-class (Khan & Khan 2009). This may prove useful in selecting the structures which may give statistically

Table 1. Precision in age estimates between two independent readers and between pairs of ageing structures in *Clarias batrachus*. Measures of precision include percentage of agreement (PA), average percentage of error (APE) and coefficient of variation (CV).

	PA	APE	CV
Ageing structures	Between Readers		
Otoliths	92.9 %	1.36 %	1.93 %
Vertebrae	87 %	2.53 %	3.58 %
Pectoral spines	83.8 %	4.52 %	5.30 %
	Between ageing structures		
Otoliths-Vertebrae	87 %	1.89 %	3.58 %
Otoliths-Pectoral spines	63 %	7.49 %	10.60 %

Table 2. Comparison of mean values of age estimates from different ageing structures in *Clarias batrachus*, *Heteropneustes fossilis* and *Wallago attu*.

Bony parts	Mean values of age estimates[1]		
	Clarias batrachus	*Heteropneustes fossilis*	*Wallago attu*
Otoliths	2.50^{a}	1.92^{ab}	2.64^{a}
Vertebrae	2.26^{ab}	2.07^{a}	2.10^{b}
Pectoral spines	2.17^{b}	1.76^{b}	2.34^{ab}

[1] Values within a column having similar superscripts are insignificantly different ($P > 0.05$) from each other.

Table 3. Precision in age estimates between two independent readers and between pairs of ageing structures in *Heteropneustes fossilis*. Measures of precision include percentage of agreement (PA), average percentage of error (APE) and coefficient of variation (CV).

	PA	APE	CV
Ageing structures	Between Readers		
Vertebrae	90.8 %	5.52 %	7.82 %
Otoliths	80.3 %	10.62 %	16.89 %
Pectoral spines	73.7 %	17.81 %	25.20 %
	Between ageing structures		
Vertebrae-Otoliths	82.9 %	4.92 %	6.96 %
Vertebrae-Pectoral spines	77.6 %	6.42 %	9.09 %

Table 4. Precision in age estimates between two independent readers and between pairs of ageing structures in *Wallago attu*. Measures of precision include percentage of agreement (PA), average percentage of error (APE) and coefficient of variation (CV).

	PA	APE	CV
Ageing structures	Between Readers		
Otoliths	86.7 %	2.21 %	3.14 %
Vertebrae	67.9 %	5.66 %	8.01 %
Pectoral spines	70.3 %	6.38 %	7.77 %
	Between ageing structures		
Otoliths-Vertebrae	68.5 %	7.67 %	8.67 %
Otoliths-Pectoral spines	83 %	2.67 %	3.95 %

indifferent readings when size-class is not taken into account. All calculations and statistical analyses were done using MS-Excel and SPSS (version 17.0).

Results

C. batrachus

Of all the ageing structures examined, otoliths were the most reliable structure for ageing *C. batrachus* based on PA, APE and CV values. PA of ages between the two readers was the highest for otoliths (92.9 %) followed by vertebrae (87 %) and pectoral spines (83.8 %) (Table 1). Also, otoliths had the lowest APE (1.36 %) and CV (1.93 %) values followed by vertebrae and pectoral spines. When otoliths age estimates were compared with other ageing structures (i.e., vertebrae and pectoral spines), the highest PA (87 %) and lowest APE (1.89 %) and CV (3.58 %) values were reported between otoliths and vertebrae (Table 1). Mean values of age estimates from different ageing structures, when compared using ANOVA followed by DMRT, showed that mean age estimates obtained from otoliths were significantly ($P < 0.05$) different from the values obtained from pectoral spines (Table 2). However, age estimates obtained from otoliths did not differ significantly ($P > 0.05$) to those from vertebrae. Also, the values of age estimates from vertebrae were comparable to those from pectoral spines ($P > 0.05$).

H. fossilis

Vertebrae were the most suitable structure for ageing *H. fossilis* based on PA, APE and CV values. The PA of age estimates between the two independent readers was the highest (90.8 %) for vertebrae followed by otoliths (80.3 %) and pectoral spines (73.7 %) (Table 3). However, APE (5.52 %) and CV (7.82 %) values were the lowest for vertebrae. When vertebrae age estimates were compared with other alternative structures, the highest PA and lowest APE and CV values were found between otoliths and vertebrae, while the lowest PA and highest APE and CV were reported between age estimates from otoliths and pectoral spines (Table 3). Mean values of age estimates from different ageing structures, when compared using ANOVA followed by DMRT, showed that maximum age estimates obtained from vertebrae were significantly ($P < 0.05$) higher from pectoral spines, but comparable ($P > 0.05$) to the values obtained from otoliths. The values of age estimates from otoliths and vertebrae did not differ significantly ($P > 0.05$) either (Table 2).

Wallago attu

Percent agreement of ages between two independent readers was higher for otoliths (86.7 %) than for pectoral spines and vertebrae in *W. attu* (Table 4). However, APE (2.21 %) and CV (3.14 %) values were the lowest for otoliths. When otoliths age estimates were compared with other alternative structures, the highest PA and lowest APE and CV values were found between otoliths and pectoral spines followed by otoliths and vertebrae age estimates (Table 4). Mean values of age estimates from otoliths were comparable ($P > 0.05$) to those from pectoral spines but significantly ($P < 0.05$) different to those from the vertebrae (Table 2).

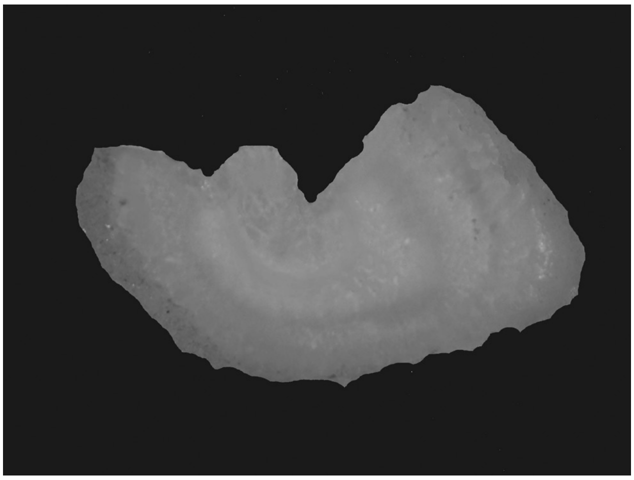

Fig. 2. Otolith from a *Clarias batrachus* specimen (four year old) showing translucent and opaque zones.

Fig. 3. Vertebrae from a *Heteropneustes fossilis* specimen (five year old) showing translucent and opaque zones.

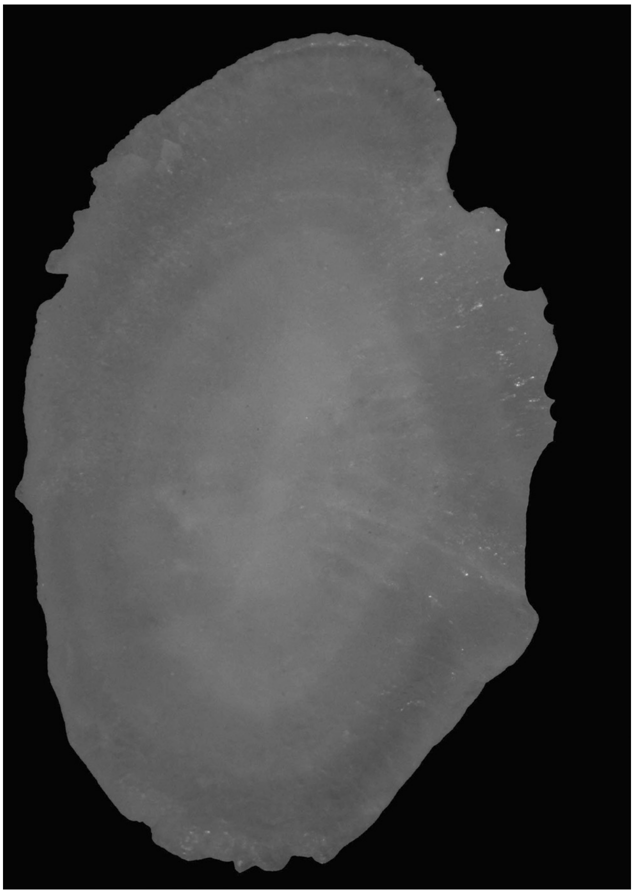

Fig. 4. Otolith from a *Wallago attu* specimen (three year old) showing translucent and opaque zones.

Discussion

Selection of the appropriate method for age and growth determination in fishes often requires balancing precision and accuracy of the method with sample size limitations (DeVries & Frie 1996, Zymonas & McMahon 2009). The degree of agreement among readers is a measure of the precision of determinations and not the accuracy of the technique (Polat et al. 2001). In a reliable age determination, structure with the lowest ageing error is to be preferred. In *C. batrachus* and *W. attu*, otoliths (Fig. 2 and Fig. 4, respectively) exhibited clear growth rings and their age estimates showed the highest percent agreement and lowest ageing error between independent readers. However, in *H. fossilis*, we found otoliths to be the second best structure for ageing, as evident from PA, APE and CV values between the two independent readers and among the ageing structures. Precise age estimation using otoliths is also supported by the fact that otoliths continue to grow and form annuli even as body growth slows and asymptotic length is reached, and annuli reabsorption does not appear to occur during periods of food limitation or stress (DeVries & Frie 1996). 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).

Vertebrae exhibited clear growth rings in *H. fossilis* (Fig. 3) and its age estimates were precise showing the highest percentage of agreement and lowest ageing error between independent readers. In corroboration with the observations of Li & Xie (2008) on *Glyptosternum maculatum*, we also observed that as compared to other structures (i.e. otoliths and pectoral spines) in *H. fossilis*, vertebrae had regularly formed annual rings and were more consistent and easier to handle. In *C. batrachus*, vertebrae were reported to provide age readings close to the otoliths as evident from PA, APE and CV values between ageing structures. Vertebrae were used for age determination of catfishes and studies have indicated that the reliability and consistency of vertebrae for age determination are virtually higher compared to other materials in *Ictalurus lacustris* (Appelget & Smith 1951) and *Psedobagrus vachelli* (Duan & Sun 1999). Vertebrae provided precise age estimates, similar to otoliths, in *Lota lota* (Guinn & Hallberg 1990) and in *Clarias gariepinus* (Khan et al. 2011b). The time required to process and read vertebrae made them less practical to use but that precision and accuracy involved with vertebrae made them the best among the three structures (vertebrae, otoliths, and scales) for the age estimation of *Oncorhynchus keta* (Clark 1987). Researchers have reported that rings present on vertebral centra were not very clear and showed numerous minute marks unrelated to cyclic events in fishes such as *Makaira nigricans* (Hill et al. 1989), *Boops boops* (Khemiri et al. 2005) and *Cirrhinus mrigala* (Khan et al. 2011a).

In the present investigation, pectoral spines were inferior to vertebrae and otoliths for ageing *C. batrachus* and *H. fossils*. They had the lowest percent agreement and highest average percent error and coefficient of variation values between readers. In contrast to *C. batrachus* and *H. fossilis*, pectoral spines provided better precision as compared to the vertebrae in *W. attu*. In many species, the spine nucleus may be reabsorbed and replaced by a hole (vascularization), which may eliminate the first rings (Kohli 1989, McFarlane & King 2001). If first annulus is not identified correctly, the fish age will be underestimated leading to an overestimation of growth and natural mortality coefficients, which in turn may have drastic implications to fish stock management advice and decisions (Leaman & Nagtegaal 1987, Casey & Natanson 1992). Several researchers have reported difficulty in the interpretation of annuli using pectoral spines in fishes such as, *Ictalurus punctatus* and *Pylodictis olivaris* (Sneed 1951, Turner 1982, Crumpton et al. 1987). As fish age, expansion of the central lumen erodes early annuli (caused by increasing amounts of vascularized tissue in the central part of the spine as it changes in structure with age), thereby causing the true age of older fish to be underestimated (Gonzalez-Garces & Farina-Perez 1983, Franks et al. 2000, Brusher & Schull 2009). Turner (1982) reported that some annuli in spines are composed of multiple growth rings. False marks are usually distinguishable in younger fish because of their proximity to true annuli; in older fish, however, false marks may become more problematic and lead to overestimation of fish age. Finally, in slow growing and old individuals, spine annuli near the edge tend to merge and may be indistinguishable (Lai et al. 1996, Kocovsky & Carline 2000), thereby increasing the chance of biased age estimates (Buckmeier et al. 2002). Kohli & Goswami (1989) studied the age and growth of *H. fossilis* using pectoral spines. However, in the present investigation, the annuli on pectoral spines in *H. fossilis* and *C. batarchus* showed less clarity as compared to other structures used for age estimation.

It may be concluded from the present study that otoliths were the most suitable ageing structure for *C. batrachus* and *W. attu* while vertebrae for ageing *H. fossilis* based on the lowest APE and CV values and highest PA values between age readers as compared to other ageing structures. The results of the present research work may be utilized by researchers, fishery managers, and policy makers for sustainable fishery management and conservation of the *C. batrachus*, *H. fossilis* and *W. attu* in Indian waters in general and the river Ganga basin in particular.

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