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Precision of age estimates from different ageing structures in selected freshwater teleosts

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Abstract

The present study was undertaken with a view to compare the precision of age readings obtained from different ageing structures of some important freshwater teleosts viz., *Hypophthalmichthys molitrix*, *Mastacembelus armatus* and *Ompok pabda*. Standard procedures were followed to study the ageing structures. Based on the highest percent agreement and lowest average percentage of error and coefficient of variation values, precise age estimates were exhibited by opercular bones in *H. molitrix* and vertebrae in the remaining two fish, *M. armatus* and *O. pabda*. When precise age estimates were compared among the age estimates of other ageing structures, highest percent agreement and lowest average percent error and coefficient of variation values were exhibited by vertebrae (versus opercular bones) in *H. molitrix* and opercular bones (versus vertebrae) in both *M. armatus* and *O. pabda*. When mean age estimates from different ageing structures were compared, vertebrae and opercular bones exhibited comparable values in *H. molitrix*. In *M. armatus*, mean values of precise age estimates from vertebrae were significantly different from the values of other ageing structures. However, in *O. pabda*, vertebrae as well as opercular bones showed insignificantly different age readings.

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Introduction

Studies on ageing precision have offered a rather new perspective to fish biology research. Currently, information on ageing precision is available only for a limited number of fish species. However, because of its significance, researchers have developed stock assessment models based on precise age estimates (Dorval *et al*., 2013). Ageing precision attains further importance owing to its utility in age based calculations of growth estimates. Selection of appropriate method for age and growth determination in fish often requires balancing precision and accuracy of the method with sample size limitations (DeVries and Frie, 1996; Zymonas and McMahon, 2009). The measure of precision is a valuable means of assessing the relative ease of determining age of a particular structure or assessing the reproducibility of an individual's age estimations (Campana, 2001).

The most reliable ageing method may vary among species. Thus, studies on the precision of ageing structures for selected fish species become highly significant (Polat and Gumus, 1995). Calcified body structures may not always form a complete growth sequence through the life of the subject; false checks can be laid down; or annuli can overlay each other, especially in older fish at the margin, thereby making interpretation difficult (Campana, 2001). The effect of errors in determination of age structure of a fish population can have serious repercussions for management decision-making, as biological productivity of fishery will be either under or overestimated (Campana, 2001). Errors in determining the age of fish need to be minimized, wherever possible, by incorporating a validation method. However, comparison of age estimates between structures is an alternative technique to validation that may provide useful information on the accuracy and bias of age estimating structures (Sylvester and Berry, 2006).

Studies on comparison of age estimates from various structures have been reported for many fish species, viz., *Acipenser oxyrinchus* (Stevenson and Secor, 1999); *Hemisorubim platyrhynchos* (Howland *et al*., 2004); *Catostomus commersonii* (Sylvester and Berry, 2006); *Cyprinus carpio* (Phelps *et al*., 2007, Yilmaz and Polat., 2008); *Salvelinus confluentus* (Zymonas and McMahon, 2009); *C. carpio* (Weber and Brown, 2011); *Lepidotrigla argus* (van der Meulen *et al*., 2013) etc. The selected fish are economically important species having good market value across the country. Due to the increased pressure of fishing (overexploitation) and human interventions leading to habitat deterioration, the fish population structure experiences great variation over time. However, the basic information needed to develop scientifically sound management policies is still warranted, particularly those related to precise age estimation and growth pattern in changing environments across all the major habitats of the fish. In earlier reports from India on age and growth studies, age has been usually estimated with only one ageing structure. These studies did not focus thoroughly on the clarity of annual rings in different ageing structures with respect to different size class and ageing errors. In India, few studies were carried out in our laboratory for a limited number of fish species, *Labeo rohita*, *Catla catla* and *Channa marulius* (Khan and Khan, 2009); *Cirrhinus mrigala* (Khan *et al.,* 2011a); *Clarias gariepinus* (Khan *et al.,* 2011b); *Channa punctata* (Khan *et al.,* 2013a) and *Wallago attu, Clarias batrachus* and *Heteropneustes fossilis* (Khan *et al.,* 2013b). Based on the literature study, it was observed that no published reports are available on the assessment of precision of age estimates, using different ageing structures in selected fish species, *Ompok pabda* and *Mastacembelus armatus*. However, ageing precision in *Hypophthalmichthys molitrix* has recently been reported from Midwestern U.S. Rivers (Seibert and Phelps, 2013). Therefore, the present study was undertaken with the objective to evaluate and compare different ageing structures (i.e., scales, otoliths, vertebrae, opercular bones and spines) so as to identify and quantify the differences in precision between readers and among the pairs of ageing structures in *H. molitrix, M. armatus* and *O. pabda*, collected from river Ganga.

Materials and Methods

Fish samples were collected from river Ganga at Narora, U.P., India. The study material consisted of fish species, *Hypophthalmichthys molitrix* (N=180), *Mastacembelus armatus* (N=85) and *Ompok pabda* (N=118). Fish were sampled using gill nets/cast nets/drag nets of various mesh sizes during the period January 2010 to February 2012. Total length (TL) of fish was measured to the nearest cm while body weight was recorded to the nearest gram as total weight (TW) including gut and gonads. Ageing structures were prepared following the methods adopted by Khan and Khan (2009) and Khan *et al*. (2011b).

Collection and preparation of ageing structures : In *H. molitrix*, scales were washed in tap water and dried on a filter paper. Five to eight scales from each fish were mounted dry between two glass slides, fastened each end by means of cello tape and examined for age reading.

In *H. molitix*, *M. armatus* and *O. pabda,* opercular bones were detached with the help of scalpel and dipped in boiling water for few minutes to remove extraneous tissues. A bristled brush was used to clean the opercular bones. In *H. molitix* and *O. pabda,* opercular bones were examined under transmitted fluorescent light with naked eye while in *M. armatus,* opercular bones were examined dry under transmitted light and in water, with the help of reflected light on dark background (Sipe and Chittenden, 2001).

Sagittal otoliths were removed from otic capsules by opening the otic bulla. In *H. molitix,* otoliths, with unclear annual rings, were ground with sandpaper to make the annuli more distinct and examined under microscope using reflected light. In *M. armatus*, cleaned and dried otolith was immersed in xylene and observed under microscope while in *O. pabda*, whole otoliths were submerged in a petri dish with black base filled with water and viewed under microscope in reflected light (Kowalewski *et al.,* 2012). Vertebrae (4th to 10th) were placed in boiling water for 10−15 min to clear the attached muscles and then dried for 2 weeks, after which annual rings were examined under microscope. Pectoral fin spines were removed at the point of articulation, air-dried and sectioned using a jeweller's saw. Some of the sections were polished with sandpaper, and a drop of immersion oil was used to improve the clarity. Sections were placed on a microscope slide and aged under microscope.

Calculations and statistical analysis : All the otoliths, scales, vertebrae, opercular bones and sections of fin spines were aged independently by two readers without the knowledge of fish length, weight and date of collection. 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 percentage of agreement, coefficient of variation, and average percentage of error between the readers and between the pairs of ageing structures. Percent agreement was calculated using the "Templates for calculating ageing precision" by Sutherland (2006). Average percentage of error was calculated using the formula presented by Beamish and Fournier (1981). The coefficient of variation (Camapana, 2001) was calculated as the ratio of standard deviation over the mean.

Mean age readings obtained from various bony parts were subjected to one-way analysis of variance (ANOVA) followed, by Duncan's multiple range test (DMRT) (Gomez and Gomez, 1984). All calculations and statistical analyses were done using MS-Excel and SPSS (version 17.0).

Results and Discussion

In this study, variations were observed in age estimates from different ageing structures. In *H. molitrix*, percent agreement between the age estimates of two independent readers was highest for opercular bones followed by vertebrae, scales and otoliths (Table 1). Opercular bones, showed lowest values of average percentage of error and coefficient of variation. When opercular bone age estimates were compared with other alternative structures, highest percent agreement and lowest average percentage of error and coefficient of variation values were found between opercular bones vs. vertebrae, followed by opercular bones vs. scales and opercular bones vs. otoliths (Table 1). When mean values of age estimates from different structures were compared using ANOVA followed by DMRT, mean age estimates from vertebrae were significantly ($P < 0.05$) different from otoliths and scales but comparable ($P > 0.05$) to the values obtained from opercular bones (Table 2). Also, the values of age estimates from scales and otoliths did not differ significantly ($P>0.05$). Opercular bones were found to be superior to other ageing structures in *H. molitrix* and the second best ageing structure in *M. armatus* and *O. pabda*. Several researchers have used opercular bones for age estimation in different fish species, such as *Labeo rohita*, *Catla catla* and *C. marulius* (Khan and Khan, 2009); *Mastacembelus mastacembelus* (Gumus *et al.,* 2010); *Schizothorax o'connori* (Ma *et al.,* 2011); *Cirrhinus mrigala* (Khan *et al.,* 2011a) and *Channa punctata* (Khan *et al.,* 2013a). Rings on opercular bones of younger age group fishes were clearer and more easily identifiable than in the older age groups in *H. molitrix*. Pazira *et al.* (2005) suggested vertebrae and opercles as appropriate structures for age estimation rather than otoliths in *Mastacembelus mastacembelus*. However, in some fish opercular bones were reported to be less reliable as compared with other structures, such as otoliths and vertebrae in *Schizothorax o'connori* (Ma *et al*., 2011) and scales and otoliths in *Labeo rohita* and *Channa marulius* (Khan and Khan, 2009).

In *M. armatus* and *O. pabda* (Table 1), percent agreement between age readings of two independent readers was highest for vertebrae, followed by opercular bones and pectoral spines. Coefficient of variation and average percentage of error values were lowest for age estimates from vertebrae. When vertebrae age estimates were compared with other alternative structures, highest percent agreement and lowest average percentage of error and coefficient of variation values were found between vertebrae vs. opercular bones, followed by vertebrae vs. pectoral spines in *M. armatus* (Table 1) and *O. pabda* (Table 1). In *M. armatus*, when mean values of age estimates from different ageing structures were compared using ANOVA followed by DMRT, age estimates from vertebrae were significantly ($P<0.05$) different from pectoral spines and opercular bones. The values of age estimates from opercular bones and pectoral spines were insignificantly ($P > 0.05$) different from each other (Table 2). In *O. pabda*, the mean values of age estimates from different ageing structures, when compared using ANOVA followed by DMRT, showed that mean age estimates obtained from vertebrae were significantly ($P < 0.05$) different to the values obtained from pectoral spines (Table 2). However, the values of age estimates from vertebrae were comparable to those from opercular bones ($P > 0.05$). Vertebrae exhibited clear growth rings in *M. armatus* and *O. pabda* and their age estimates were unbiased and precise, showing the highest percentage of

Table 1 : Comparison of percent agreement (PA), average percent error (APE), and coefficient of variation (CV) between the age readings of two independent readers and between pairs of hard anatomical structures in *Hypophthalmichthys molitrix, Mastacembelus armatus* and *Ompok pabda.*

	Between readers									
	Hypophthalmichthys molitrix				***Mastacembelus armatus***			***Ompok pabda***		
	Scales	**Otoliths**	**Vertebrae**	**Opercular bones**	**Opercular bones**	**Vertebrae**	**Pectoral spines**	**Opercular bones**	**Vertebrae**	**Pectoral spines**
Percent agreement (%)	61.3	47.5	70	89.4	68.6	80.7	62.9	75.5	80	57.9
Average percentage of error (%)	7.30	10.42	5.59	2.98	6.48	3.81	8.25	5.11	4.04	9.66
Coefficient of variation (%)	10.33	14.74	7.91	4.21	9.20	5.40	11.3	7.04	5.59	13.9

	Between structures						
	Hypophthalmichthys molitrix			***Mastacembelus armatus***		***Ompok pabda***	
	Opercular bones vs.scales	**Opercular bones vs.vertebrae**	**Opercular bones vs. otoliths**	**Vertebrae vs.Pectoral spines**	**Vertebrae vs.Opercular bones**	**Vertebrae vs.Pectoral spines**	**Vertebrae vs.Opercular bones**
Percent agreement (%)	55.4	84.4	53.8	53.6	69.3	59.2	72.5
Average percentage of error (%)	9.01	3.17	9.61	10.33	6.70	11.2	5.12
Coefficient of variation (%)	12.34	4.40	13.31	14.96	9.48	15.6	8.42

Table 2: Comparison of mean values of age estimates from different ageing structures in *Hypophthalmichthys molitrix, Mastacembelus armatus* and *Ompok pabda*

Ageing structures	**Mean values of age estimates**^{1}		
	H. molitrix	***M. armatus***	***O. pabda***
Opercular bones	3.2157^{a}	2.8169^{b}	2.2254^{a}
Vertebrae	2.8431^{a}	3.2676^{a}	2.4648^{a}
Otoliths	2.1569^{b}	-	-
Scales	2.3333^{b}	-	-
Pectoral spines	-	2.5493^{b}	1.7887^{b}

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

agreement and the lowest ageing error between independent readers. Many studies have indicated that the reliability and consistency of vertebrae for age determination are relatively higher as compared to other materials (Duan and Sun, 1999, Mc Auley *et al*., 2006). Polat *et al*. (1995) compared different bony parts of *Pleuronectes flesus luscus* for age determination and reported vertebrae as the most reliable structures having minimal ageing error. In corroboration with the observations of Li and Xie (2008) on *Glyptosternum maculatum*, it was observed that, as compared to other structures in *M. armatus* and *O. pabda* vertebrae had regularly formed annual rings and were more consistent and easier to handle.

Vertebrae have rarely been used to study age estimation in fishes which show clear annual rings in other structures that cause negligible or no damage to the fish.. Vertebrae were reported to provide reliable age estimates in *Ophiodon elongatus*, but the author maintained that they are not practical for commercially caught fish due to the time required for processing the structure and the damage to the fish carcass caused during sampling.

In the present study, scales were inferior to other bony structures for ageing *H. molitrix*. Scales are one of the most frequently used fish ageing structures due to their ease of collection and because fish can be released alive (DeVries and Frie, 1996). Assemment of ageing precision from different ageing structures (scales, opercular bones, otoliths, vertebrae and fin rays/spines) revealed scales to be the best structures for age estimation in *Pomoxis nigromaculatus* (Kruse *et al*., 1993), *Morone saxatilis* (Welch *et al*., 1993), *L. rohita* and *C. marulius* (Khan and Khan, 2009) and *Cirrhinus mrigala* (Khan *et al*., 2011a). In *H. molitrix*, annual rings present on scales were not clear as compared to opercular bones and vertebrae. Several studies revealed that scales were not reliable for age determination in *Micropterus salmoides, Micropterus dolomieu* and *Perca flavescens* (Maceina and Sammons, 2006); *Ptychobarbus dipogon* (Li *et al.,* 2009); *Ptychobarbus dipogon* (Chen *et al.,* 2009) and *Schizothorax o'connori* (Ma *et al.,* 2011). Moreover, different environmental conditions may also result in the formation of checks which sometimes are very difficult to distinguish from true annuli and may lead to some errors in age studies.

In *H. molitrix*, *M. armatus* and *O. pabda*, otoliths were extremely small, and emerged less reliable than other alternate structures. Johal *et al*. (2000) experienced difficulty in age reading of *H. molitrix* using otoliths which were small, hazy and fragile. To use otoliths or any other ageing structure for age determination, the deposition of regular visible age marks is important. Morales-Nin *et al*. (1998) found that factors such as identification of the otolith nucleus, development of annual or false rings and an extended spawning period, all contribute to the discrepancies between different otolith readings. 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). In older fish, as deposition occurs across the entire otolith surface and otolith thickness increases, identification of annual ring became more difficult. It seems plausible that the use of whole otoliths could have lead to the underestimation of ages, which corroborates the findings on *Helicolenus dactylopterus* (Abecasis *et al*., 2006). Several researchers have reported otoliths to be the most suitable ageing structure in *Ictalurus punctatus* (Buckmeier *et al*., 2002; Colombo *et al*., 2010); *Clarias gariepinus* (Khan *et al*., 2011b); *Channa punctata* (Khan *et al.,* 2013a); *Wallago attu* and *Clarias batrachus* (Khan *et al*., 2013b) etc.

In the present investigation, pectoral spines were inferior to other structures for ageing *H. molitrix, M. armatus* and *O. pabda*. They had the lowest percent agreement and highest average percentage of error and coefficient of variation values between readers. Due to difficulty in the correct identification of annuli in fin spines, fish age may be underestimated. In this investigation, the annuli on fin spines showed less clarity than other structures for age estimation. Several researchers have reported difficulty in the interpretation of annuli using spines in *Morone saxatilis* and *Salmo trutta* (Graynoth, 1996); *Acanthocybium solandri* (Franks *et al.,* 2000); *Epinephelus itajara* (Brusher and Schull, 2009). A reduction in accuracy of spine age estimates, as a result of annulus loss, was reported in *Morone saxatilis* and *Salmo trutta* (Welch *et al*., 1993; Graynoth., 1996). Buckmeier *et al*. (2002) reported that the underestimation and lack of precision for ageing ictalurids using spines occur due to expansion of the central lumen, which obliterates early formed annuli, the appearance of multiple growth rings, and poor sectioning techniques. The imprecise age estimates by spines in the present study could also be attributed to relatively poor quality of sections using jeweller's saw, as compared to the low speed isomet saw.

It may be concluded from the present study that opercular bones are the most suitable ageing structure for *H. molitrix,* and they can also be used as an alternative to the most precise ageing structure in *M. armatus* and *O. pabda*. Vertebrae are found to be the best structure for ageing in *M. armatus* and *O. pabda*. They can also be used as an alternative to the best ageing structure in *H. molitrix.* 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 selected fish species in Indian waters in general, and the river Ganga basin in particular

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