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Age and Growth of Spotted Snakehead, *Channa punctata* from the Ganga River[1]

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Abstract—The present study was undertaken to estimate age and growth of the spotted snakehead *Channa punctata* (Bloch, 1793) from the Ganga River, based on 390 fish samples collected during August 2016 to July 2017. Each of the 3 otoliths (lapillus, asteriscus and sagitta) were dissected out, cleaned and observed to identify the annual rings. Based on clarity of annuli and ease of reading, sagittae (whole as well as sectioned) were selected for age estimation. Standard procedures were followed to prepare and study the age structures viz., the otoliths and scales. The alternating opaque and translucent bands present on the ageing structures were interpreted as annuli. Amongst the 3 methods used for age estimation, the sectioned otolith exhibited highest percentage of agreement (92.4) between readers, and least values of average percent of error (2.68) and coefficient of variation (2.36). Age estimates from sectioned otoliths were taken to develop the von Bertalanffy growth equation [$L_t = 31.5(1 - e^{-0.29(t + 1.06)})$] for the target fish species. The observed and calculated lengths in the fish did not vary significantly. Thus, the sectioned otoliths may be used for precise age estimation as well as calculation of various age-based population parameters of *C. punctata* inhabiting the river Ganga.

Keywords: ageing precision, otoliths, Ganga River, scale, von Bertalanffy growth function

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INTRODUCTION

The spotted murrel, *Channa punctata* (Bloch, 1793), is a warm-water teleost widely available in lakes, ponds and rivers as well as in confined water bodies of southeast Asia (Jayaram, 1999). *C. punctata* contributes to considerable landings among inland fishes available in local markets and fetches a good price due to its peculiar taste and fewer intramuscular bones (Haniffa et al., 2003). Studies on various biological parameters of a fish species are of immense significance not only to academics and research but more importantly to the fishery resource management of the target fish species. This requires precise determination of fish age to develop a relationship between its age and various other parameters of fish population viz., growth analyses of fish, longevity, mortality, productivity, yield and population dynamics. Many hard anatomical structures have been used to estimate the age of fishes, including scales, otoliths, vertebrae, fin rays, spines, opercular bones, cleithra, urohyal bone, and hyomandibular bone. One of the main problems in age and growth studies is the selection of the most suitable ageing structure that can ensure minimum error and bias across broader size range of the fish. The hard structure providing most suitable age reading may vary from one fish species to the other, for example scales in *Labeo rohita* and *Channa marulius* (Khan and Khan, 2009), *Cirrhinus mrigala* (Khan et al., 2011a) and *Labeo bata* (Khan et al., 2015), otoliths in *Ictalurus punctatus* (Buckmeier et al., 2002; Colombo et al., 2010); *Clarias gariepinus* (Khan et al., 2011b); *C. batrachus* and *Wallago attu* (Khan et al., 2013a); *C. punctata* (Khan et al., 2013b), *Sperata aor* (Khan et al., 2016) and *Channa striata* (Khan et al., 2017) and so on. Even within the same species there are certain structures found unsuitable for ageing all the life stages of a fish, e.g. scales in older largemouth bass, *Micropterus salmoides* and smallmouth bass, *M. dolomieu* (Maceina and Sammons, 2006) and spines in *Clarias gariepinus* (Clay, 1982). In case of scales of older specimens, the annuli near the margins are usually placed so closely to each other that a clear distinction become difficult. However, in case of spines of older fish specimens, the lumen of spines usually enlarges with age and obscures initial growth increments, resulting in a consistent underestimation of age. Otolith in most cases emerge as the most suitable ageing structure on account of exhibiting unique characteristics of being metabolically inert, growing throughout a fish life and not getting resorbed even during starvation. In one of our recent study (unpublished data), sagittae gave pre-

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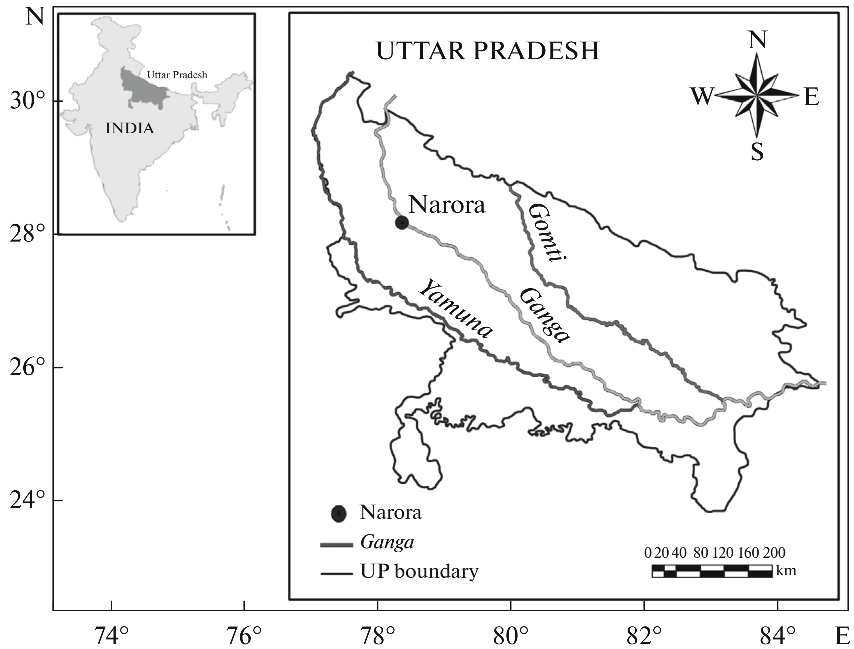

Fig. 1. Map showing sampling site of *Channa punctata*.

cise age estimate in *C. pucntata* compared to lapilli and asterisci. Earlier, a comparison of age estimates from scales, whole otoliths, opercular bones and vertebrae showed otoliths to provide precise age estimate in *C. punctata* (Khan et al., 2013b). Since *C. punctata* has scales of decent size, it forms one of the easy, popular and non-lethal method for age estimation. Growth has been one of the most intensively studied aspects of fish biology as it indicates the health of the individual and the population. von Bertalanffy Growth Function (VBGF) is a widely used growth model in fisheries, and its parameters are particularly useful in describing general fish growth (Chen et al., 1992; Quinn and Deriso, 1999). Several researchers have successfully used von Bertalanffy growth model for the estimation of growth in different fish species such as *Cyprinus carpio* (Vilizzi and Walker, 1999), *Gerres* sp. (Kanak and Tachihara, 2006), *Clarotes laticeps* (Abowei and Davies, 2009), *Nelusetta ayraudi* (Miller et al., 2010), *Schizopyge curvifrons, Schizopyge niger* and *Schizothorax esocinus* (Sabah and Khan, 2014), etc. The objective of this study was to provide a detailed information on the age and growth of *C. punctata* in waters of river Ganga, which may prove to be of great utility in its stock evaluations and fisheries management.

MATERIALS AND METHODS

Study site. Fish samples (n = 390) were collected from the Ganga River at its Narora site (28.1968° N, 78.3814° E) (Fig. 1). The specimens were identified according to Talwar and Jhingaran (1991). 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. In *C. punctata*, lapillus otoliths were acicular and fragile, and asteriscus otoliths flaky, so only the sagittae with a clear pattern of alternating zones could be used for age estimation (Fig. 2). Otoliths were removed, cleaned, and stored dry in labeled tubes. After setting in epoxy resin, otoliths were sectioned through the nucleus using Buehler's low speed IsoMet saw (Buehler, Lake Bluff, United States) (Khan et al., 2016). A minimum of 10 scales were removed with forceps from under the anterior part of the dorsal fin. Scales were cleaned by first removing the extraneous matter and mucous by washing them with tap water and then rubbing them in between the fingertips. They were then mounted between two glass slides and studied with the help of a compound microscope (Tandon and Johal, 1996). An opaque and a translucent zone together were regarded as a year's increment, age was estimated as the total number of translucent zones. To minimize bias, the ageing structures (Fig. 3) were independently observed by two readers without prior knowledge of fish and sampling date. Each fish scale, sectioned otolith and whole otolith were scored for clarity on a five-point scale: 1, excellent; 2, good; 3, acceptable (few annuli not clear, or little uncertainty in distinguishing 'true' annuli from other rings); 4, poor (some annuli not clearly defined and alternative counts possible); 5, virtually unreadable as per the description provided by Paul and Horn (2009).

Calculations and statistical analysis. Age estimates were compared by calculating the average percentage error (APE), coefficient of variation (CV), and per-

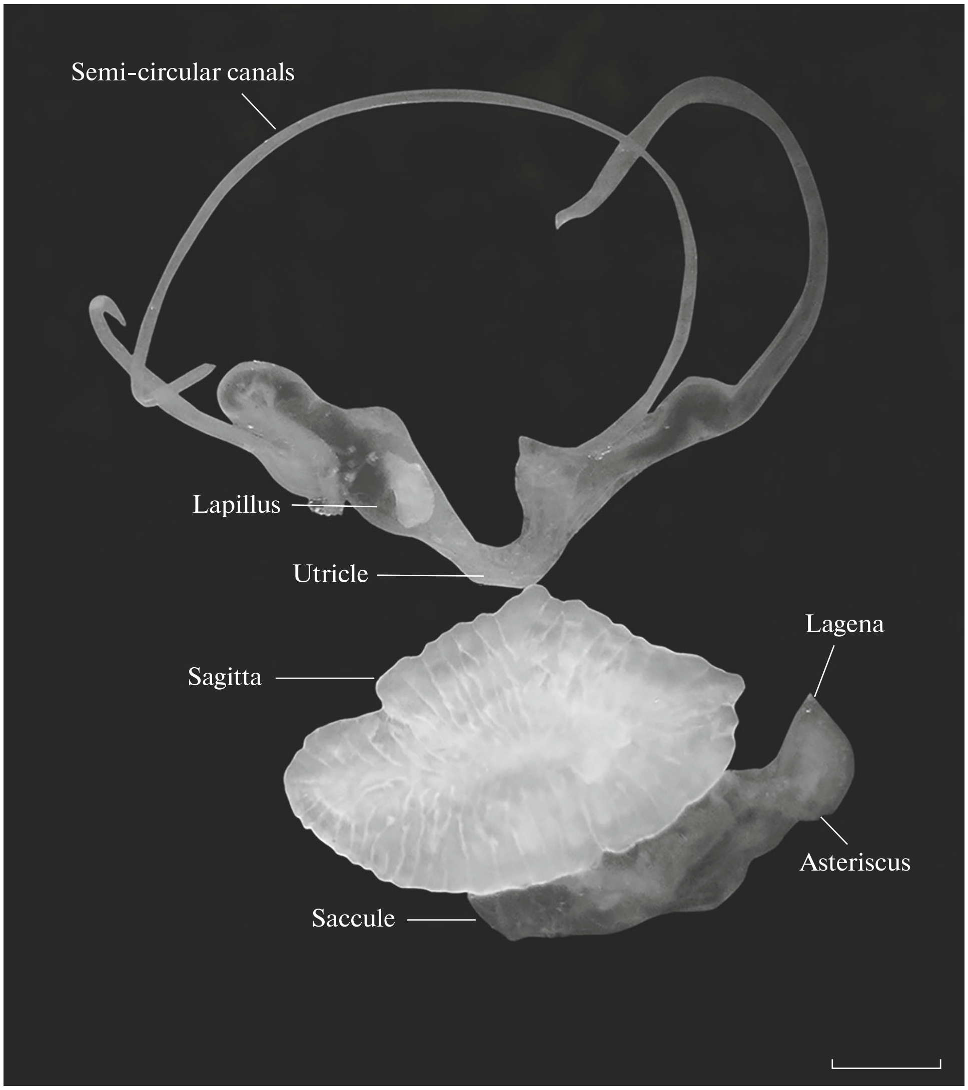

Fig. 2. Internal ear of *Channa punctata* showing the 3 types of otoliths.

centage agreement (PA) between the readers and between the pairs of ageing structures. To calculate APE, the formula of Beamish and Fournier (1981) was used:

$$\mathrm{APE}_j = 100\% \times \frac{1}{R}\sum_{i=1}^{R}\frac{\left|x_{ij} - x_j\right|}{x_j}.$$

Where x_{ij} is the i_{th} age determination of the jth fish, 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

$$\mathrm{CV}_j = 100\% \times \frac{\sqrt{\sum_{i=1}^{R}\frac{\left(x_{ij} - x_j\right)^2}{R-1}}}{x_j}.$$

Where, CV_j is the age precision estimate for the jth fish. Both APE and CV have been widely used as statistically sound measures of ageing precision in fishes (Campana, 2001). But PA, although used as an index of ageing precision in fish by many researchers (Hoxmeier et al., 2001; Stolarski and Hartman, 2008; Sabah and Khan, 2014) is not considered as a suitable measure of precision by several authors (Beamish and Fournier, 1981; Chang, 1982; Campana et al., 1995). Percent agreement may be expressed as the 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 template for calculating ageing precision by Sutherland (2006). Age bias graphs (Campana et al., 1995) were constructed to examine potential biases between readers and between pairs of ageing structures.

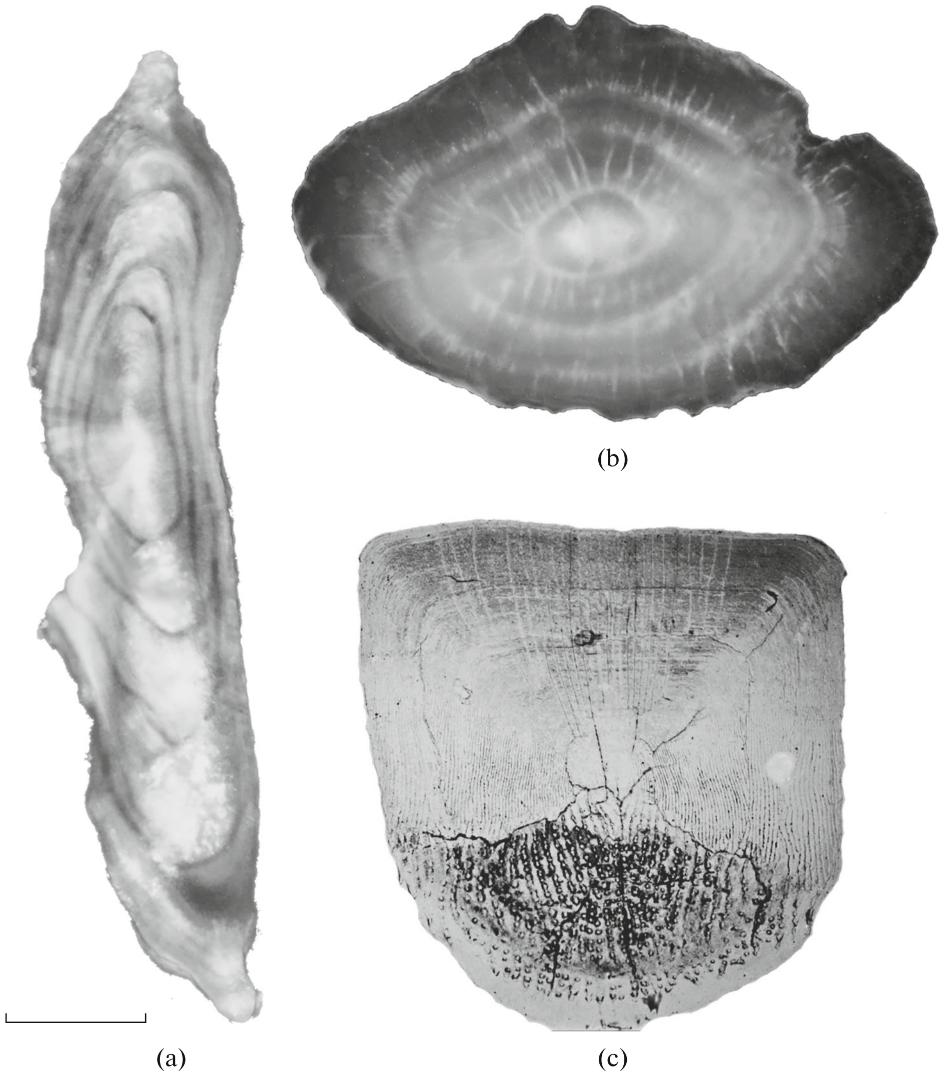

Fig. 3. Sectioned otolith (a), whole otolith (b) and scale (c) in *Channa punctata* showing annuli. Scale bar: 1 mm.

Mean age readings obtained from various bony structures 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 the degree of association among the age estimates obtained from different methods (Khan and Khan, 2009). Although the 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 each method in comparison to the structure giving precise age estimate. This may prove useful in selecting the structure(s) that may be used for age estimation in absence of the most suitable ageing structure.

The observed length-at-age data based on sectioned otoliths was fitted to the von Bertalanffy growth function (VBGF, von Bertalanffy, 1938) using the following equation:

$$L_t = L_\infty\left[1 - e^{-k(t - t_0)}\right].$$

Where, L_t = total length (cm) of fish at age t; L_∞ = asymptotic mean length; k = growth coefficient that determines growth rate at which L_t approaches L_∞; e = base of natural logarithm; t = time or age of fish, t_0 = age at which the fish had zero length.

To compare the observed and calculated mean length-at-age obtained from VBGF, the goodness of fit was assessed using coefficient of determination (R^2) and also by subjecting the data to paired Student's t-test (Zar, 1996).

RESULTS

Average percent error (2.68%) and coefficient of variation (2.36%) values were lowest for the age estimates from sectioned otoliths. Percent agreement

Table 1. Comparison of age estimates (A) reported by two independent readers and (B) from the most suitable ageing structure with other structures used for age estimation in *Channa punctata*

Hard parts	APE	CV	PA
(A) Between Readers			
Sectioned otoliths	2.68	2.36	92.4
Whole otoliths	2.88	3.22	89.9
Scales	3.94	5.42	82.3
(B) Between Structures			
Sectioned otoliths—Whole otoliths	4.66	6.88	79.7
Sectioned otoliths—Scales	10.6	11.0	68.4

Measures of precision include percentage agreement (PA), average percentage error (APE) and coefficient of variation (CV).

between the age readings of two readers was highest for sectioned otoliths (92.4%) followed by whole otoliths (89.9%) and scales (82.3%) (Table 1). Age bias graphs between two readers as well as between structures viz., sectioned otoliths, whole otoliths and scales are presented in Figs. 4 and 5. In case of sectioned otoliths and whole otoliths, no age bias was found between readers, while small error was noticed in the estimates from scales. Disagreement between readers increased with age for scales, as indicated by larger standard error bars for older fishes. In case of scales, there was a consistent pattern of underestimation of ages (Figs. 4 and 5). Annuli were clearer and sharper in sectioned otoliths thereby producing fewer errors in age estimation. More sectioned otoliths were scored as good in readability (68.2%) compared to whole otoliths and scales. However, the unreadability score (48.7%) was maximum in case of scales (Table 2). Mean values of age estimates from different structures, when compared using ANOVA followed by DMRT, showed that maximum age estimates obtained from sectioned otoliths were significantly ($p < 0.05$) higher than scales but comparable ($p > 0.05$) to the values obtained from whole otoliths (Table 3). The age estimates from scales and whole otoliths were insignificantly different ($p > 0.05$) from each other.

The von Bertalanffy growth equation derived for *C. punctata* from the selected river was

$$L_t = 31.5\left(1 - e^{-0.29(t + 1.06)}\right).$$

The observed and calculated total lengths of *C. punctata* have been presented in Fig. 6. No significant differences were found between calculated lengths and observed lengths ($R^2 = 0.995$; $t = 1.23$, $df = 4$, $p > 0.05$) of the fish.

Table 2. Distribution of readability scores for different ageing methods in *Channa punctata*; the values are percentages of the sample size ($n = 390$)

Hard Parts	Readability scores				
	1	2	3	4	5
Sectioned otoliths	4.8	68.2	23.0	3.33	0.5
Whole otoliths	1.5	41.2	39.4	15.1	3.33
Scales		11.0	15.1	24.6	48.7

Table 3. Comparison of mean values of age estimates from different ageing methods in *Channa punctata*

Ageing Structure	Mean values of age estimates
Sectioned otoliths	2.68^{a}
Whole otoliths	2.56^{b}
Scales	2.15^{b}

Values having similar superscripts are insignificantly different ($p > 0.05$) from each other.

DISCUSSION

In the present study sectioned otoliths emerged as the best structure for ageing the spotted snakehead. Sectioned otoliths had the shortest reading times and were consistently clearer and easier to read than whole otoliths and scales. These findings are new for spotted snakehead because no published studies have used sectioned otoliths to age this species. These findings generally agree, however, with many studies on other species that have found sectioned otoliths to be the best ageing structure (for example, Lowerre-Barbieri et al., 1994; Khan et al., 2016, 2017). One of the limitations of our study was not having age estimates validated by a direct or an indirect method. However, until validation is done, we feel there is sufficient evidence to recommend the sectioned otoliths to replace the current practice of using whole otoliths and scales for ageing spotted snakehead. The suitability of otoliths for age estimation 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). 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

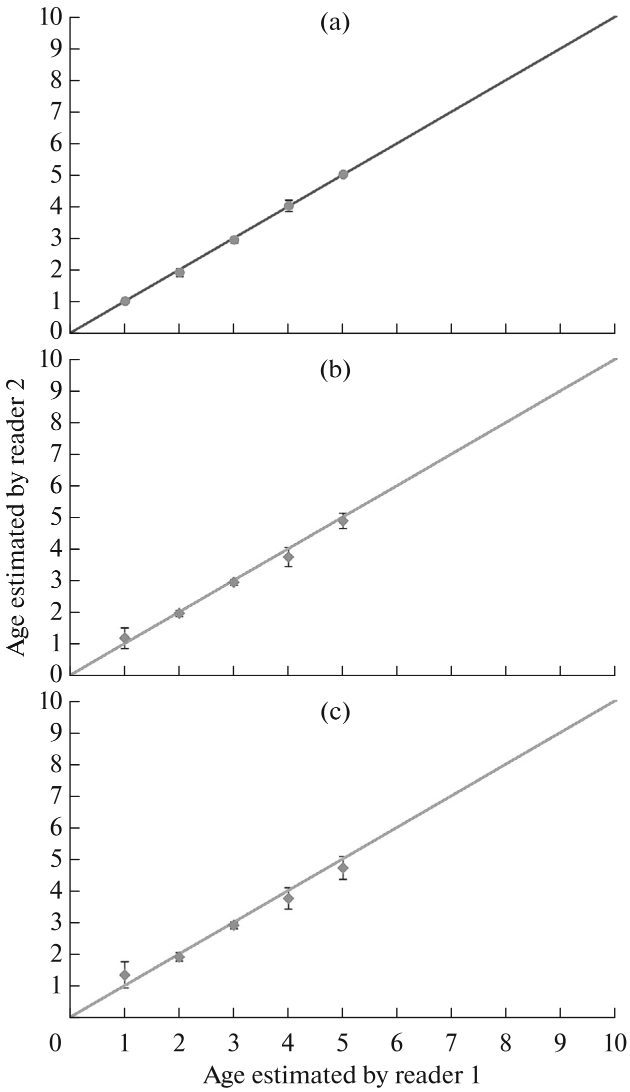

Fig. 4. Age bias plots for comparison of age estimates between readers for sectioned otolith (a), whole otolith (b) and scales (c) in *Channa punctata*. The 1 : 1 equivalence (solid) line is also indicated. Points above the line indicate ages that were overestimated whereas a point below the line indicates ages that were underestimated.

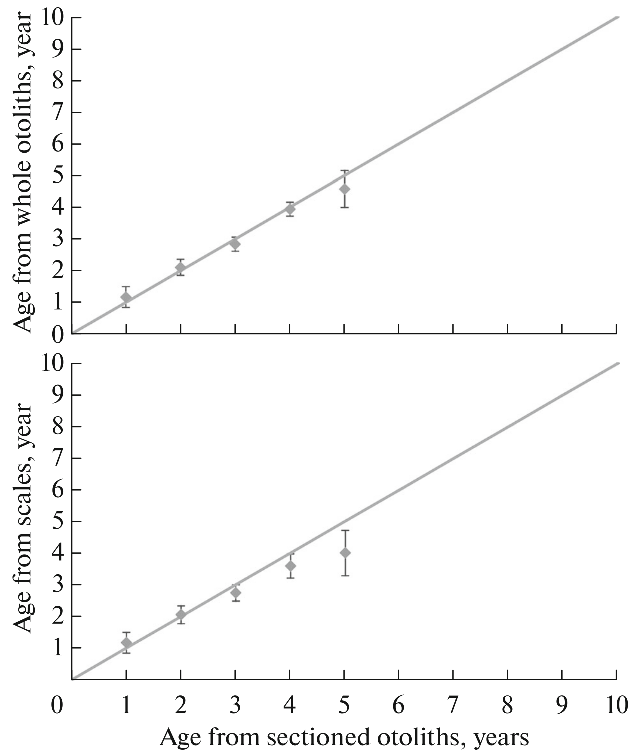

Fig. 5. Age bias plots for comparisons of age estimates between structures in *Channa punctata*. The 1 : 1 equivalence (solid) line is also indicated. Points above the line indicate ages that were overestimated whereas a point below the line indicates ages that were underestimated.

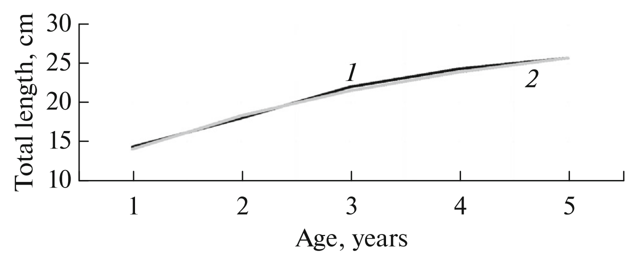

Fig. 6. Observed total length (*1*) and calculated total length (*2*), cm (using VBGF equation) of *Channa punctata*; $L_t = 31.5\ (1 - e^{-0.29(t + 1.06)})$.

stress (DeVries and Frie, 1996). 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 sectioned otoliths (Abecasis et al., 2006; Khan et al., 2016).

Branstetter (1987) categorized the k values as 0.05–0.10/year for slow growing species, 0.10–0.20/year for species with moderate growth, and 0.20–0.50/year for rapid growth. In the present study, the value of k calculated for *C. punctata* was 0.29/year suggesting a moderate growth rate. A high value of k indicates a high metabolic rate and such fishes mature at an early age or at a size which is large in relation to their asymptotic length (Qasim, 1973). The L_∞ value of *C. punctata* in the present study was higher than the maximum observed length, which was likely due to less number of large specimens. The growth coefficient (k) is a useful index for estimating the potential vulnerability of stocks to excessive exploitation and for comparing life history strategies (Musick, 1999).

Growth model estimates are greatly affected by the lack of very young or old individuals (Ma et al., 2010).

Sabah and Khan (2014) found high value of L_∞ than the observed maximum length in *Schizothorax niger*, which was likely due to the less number of large specimens. In general, growth parameters need to be checked for quality and validity (Karlou-Riga and Sinis, 1997). A negative value (close to zero) for t_0 is a good indicator for the reliability of the determined ages (Kerstan, 1985); the estimate of t_0 in the present study is -1.06. We found that the estimated maximum age was 5 years, and the k value was around 0.29, suggesting that *C. punctata* is a moderate growing, short-lived species. Therefore, it is necessary to establish reasonable management practices for this species to allow its sustainable use.

CONCLUSIONS

It may be concluded from the present study that the sectioned otoliths are the most suitable ageing structures for *C. punctata* and VBGF equation suggested a moderate fish growth in the selected river. The results of the present research work may be utilized by researchers, fishery managers and policy makers for sustainable fisheries management and conservation of the selected fish species in Indian waters in general, and the Ganga River basin in particular.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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