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SHORT COMMUNICATION

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# Precision of age estimates in striped snakehead *Channa striata* (Bloch, 1793) from the Ganga River and its tributaries (rivers Gomti and Yamuna)

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## 1 | INTRODUCTION

Precise age estimation is crucial for fisheries management. Age composition and parameters such as growth, mortality, maturity, recruitment and estimates of longevity are significant indicators to assess stock status. Ageing studies use various calcified structures including otoliths, scales, opercular bones, vertebrae, fin rays and others (Campana, 2001; Khan, 2014). Inaccuracy in ageing fish can lead to errors in fisheries management decisions (Beamish & McFarlane, 1983). Ageing errors can originate from lack of accuracy, or the precise reproducibility of repeated measurements on a given structure (Kalish et al., 1995). Age underestimates may result in high growth calculations and mortality rate estimates leading to serious overexploitation of populations and their eventual collapse (Campana, 2001).

The freshwater striped murrel, *Channa striata*, (Channidae) is distributed throughout India and other Asian countries. It is a valuable food fish (Haniffa, Marimuthu, Nagarajan, Jesu Arokiaraj, & Kumar, 2004). Several studies have compared age estimates from different bony structures to obtain precise data, avoiding potential biases associated with each structure. Well-studied species include *Cyprinus carpio* (Phelps, Edwards, & Willis, 2007), *Labeo rohita*, *Catla catla*, and *Channa marulius* (Khan & Khan, 2009), *Channa punctata* (Khan, Khan, & Miyan, 2013b), *Hoplias malabaricus* (Lozano, Vegh, Doma′ nico, & Ros, 2014), and *Labeo bata* (Khan, Khan, & Miyan, 2015). Otoliths have been previously used for age and growth studies in *C. striata* (Jutagate, Phomikong, Avakul, & Saowakoon, 2013). However, there are no publications on the precision of age estimates from various calcified structures in *Channa striata*. Therefore, the objective of this study was to compare and evaluate the age estimates from different calcified structures (otoliths, scales, vertebrae, opercular bones, cleithra and fin rays) in order to identify the most suitable calcified structure exhibiting a precise age estimate in *C. striata*.

## 2 | MATERIALS AND METHODS

### 2.1 | Sample collection

*Channa striata* ($N$ = 486) were collected monthly from November 2012 to August 2015 in the River Ganga ($N$ = 175) and two of its tributaries, the River Gomti ($N$ = 160), and the River Yamuna ($N$ = 151) in India (Figure 1). Fish were collected using cast nets (mesh size = 25 mm) and drag nets (mesh size = 28 mm), ensuring that the ageing structures were not damaged in the selected samples. Total length (TL) of each fish was measured to the nearest cm and body weight recorded to the nearest gram as total weight (TW) including gut and gonads. Scales, otoliths, opercular bones, vertebrae, cleithra and fin rays were removed from the fish and prepared for ageing according to Khan & Khan, 2009; Khan, Nazir, & Khan, 2016 and Sabah & Khan, 2014;. All structures were examined under a stereozoom microscope with 10×, 20× and 40× magnification under transmitted light (Figure 2). Each calcified structure was examined by two readers independently (486 samples × 7 structures × 2 readers × 1 replicate). Neither reader had prior information on the fish length, weight or sex, except for the collection date. An opaque and a translucent zone together were regarded as a year's growth; age was considered to be the total number of translucent zones.

### 2.2 | Calculations and statistical analysis

Age estimates were compared by calculating the average percentage error (APE), coefficient of variation (CV), and percentage agreement

 

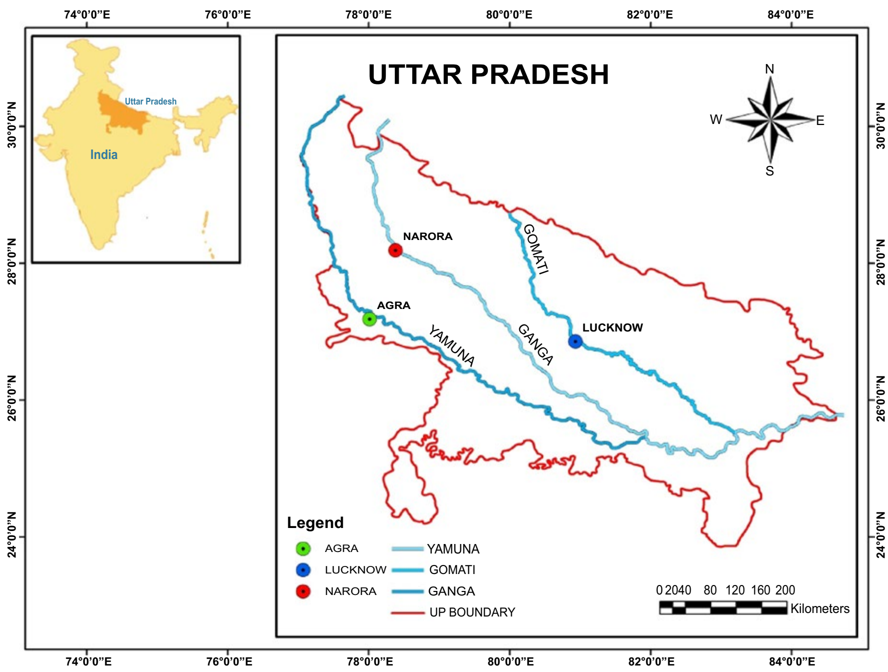


**FIGURE 1** Map indicating the three sampling sites

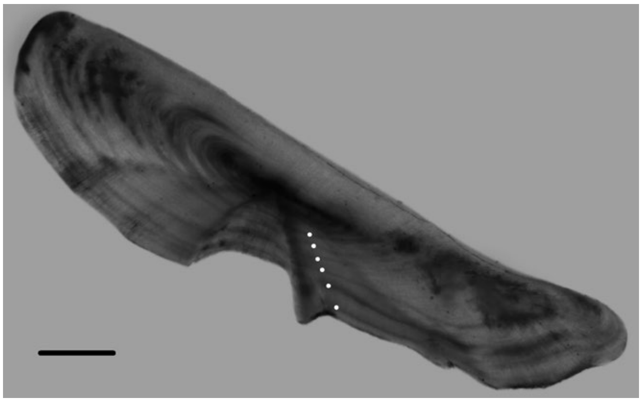

**FIGURE 2** Sectioned otolith of *Channa striata* under transmitted light (dots indicate annuli). Scale bar = 1000 µm

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

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

where $x_{ij}$ is the $i$th age determination of the $j$th 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, which 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.

Both APE and CV have been widely used as statistically sound measures of ageing precision in fishes (Campana, 2001). However, although PA is used as an index of ageing precision in fish by many researchers (Hoxmeier, Aday, & Wahl, 2001; Sabah & Khan, 2014; Stolarski & Hartman, 2008) it is not considered to be a suitable measure of precision by several authors (Beamish & Fournier, 1981; Campana, Annand, & McMillan, 1995; Chang, 1982). Percentage 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. Percentage agreement was calculated using the templates for calculating ageing precision (Sutherland, 2006). Age bias graphs (Campana et al., 1995) were constructed to examine potential biases between readers and between pairs of ageing structures.

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 & Gomez, 1984), in order to explain whether the readings from different bony structures of the same species showed significant differences among themselves (Khan & 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 a structure irrespective of fish size-class. This may prove useful in selecting the structure(s) that could be used for age estimation in the absence of the most suitable ageing structure.

## 3 | RESULTS

Average percentage error (1.28%) and CV (2.59%) values were lowest for the age estimates of sectioned otoliths followed by whole otoliths, scales, opercular bones, vertebrae, cleithra and fin rays. Percentage agreement between readers was highest for sectioned

Table 1 Comparison of percentage of agreement (PA), average percentage of error (APE) and coefficient of variation (CV) between the age readings of two readers and between pairs of ageing structures in *C. striata* collected from three Indian rivers $N = 486$

| Hard parts | APE | CV | PA |
|---|---|---|---|
| Sectioned otoliths | 1.28 | 2.59 | 89.7 |
| Whole otoliths | 2.14 | 3.89 | 85.0 |
| Scales | 3.66 | 5.15 | 79.2 |
| Opercular bones | 6.14 | 7.35 | 71.2 |
| Vertebrae | 8.75 | 9.92 | 60.9 |
| Cleithra | 11.2 | 12.62 | 53.5 |
| Fin rays | 14.8 | 15.4 | 43.2 |
| Between structures | | | |
| Sectioned otoliths—whole otoliths | 0.86 | 1.14 | 95.3 |
| Sectioned otoliths—scales | 1.98 | 2.03 | 91.4 |
| Sectioned otoliths—opercular bones | 3.99 | 3.63 | 86.4 |
| Sectioned otoliths—vertebrae | 5.77 | 5.26 | 80.5 |
| Sectioned otoliths—cleithra | 7.05 | 7.80 | 71.4 |
| Sectioned otoliths—fin rays | 10.10 | 8.76 | 70.6 |

otoliths (Table 1). Ageing structures exhibited variation in estimates of age composition for fish collected from three Indian rivers (Figure 3). When sectioned otoliths age estimates were compared with other ageing structures (i.e. whole otoliths, scales, opercular bones, vertebrae, cleithra and fin rays), the least variation was observed between sectioned otoliths versus whole otoliths age estimates, as indicated by lowest APE and CV values followed by sectioned otoliths versus scales, sectioned otoliths versus opercular bone, sectioned otoliths versus vertebrae, sectioned otoliths versus cleithra and sectioned otoliths versus fin rays (Table 1).

Age bias graphs between age estimates from sectioned otoliths and each of the other ageing structures are presented in Figure 4. Age estimates from sectioned otoliths were in good agreement with those

Table 2 Comparison of mean values of age estimates from different bony parts in *C. striata*

| Ageing structure | Mean values of age estimates |
|---|---|
| Sectioned otoliths | $4.1255^{b}$ |
| Whole otoliths | $4.0864^{b}$ |
| Scales | $4.0473^{b}$ |
| Opercular bones | $3.9918^{ab}$ |
| Vertebrae | $3.9568^{ab}$ |
| Cleithra | $3.9444^{ab}$ |
| Fin rays | $3.7922^{a}$ |

Mean values with similar superscripts are insignificantly different to each other.

from whole otoliths but differed substantially when compared to the age estimates from other ageing structures. 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 from dorsal fin rays but comparable ($p > 0.05$) to the values obtained from whole otoliths, scales, opercular bones, vertebrae and cleithra (Table 2).

## 4 | DISCUSSION

Whole otoliths in *C. striata* exhibited clearer annuli that were easier to read than other structures. However, when otoliths were sectioned the annuli became more visible, requiring less time and effort by the readers. Sectioned otoliths have also been reported to provide precise age estimates in *Paralichthys dentatus* (Sipe & Chittenden, 2001) and *Sperata aor* (Khan et al., 2016). The suitability of otoliths for age estimation is supported by the fact that otoliths do not show reabsorption and their growth is acellular rather than by calcification (Secor, Trice, & Hornick, 1995). Otoliths are also reported to be metabolically inert and thus do not reflect physiological changes that might occur throughout the life of the fish (Phelps et al., 2007). Otoliths continue to grow and form annuli, even when the body growth slows and the asymptotic length is reached; annuli reabsorption also does not seem to occur during periods of food limitation or stress (DeVries & Frie, 1996). Otoliths were reported to be

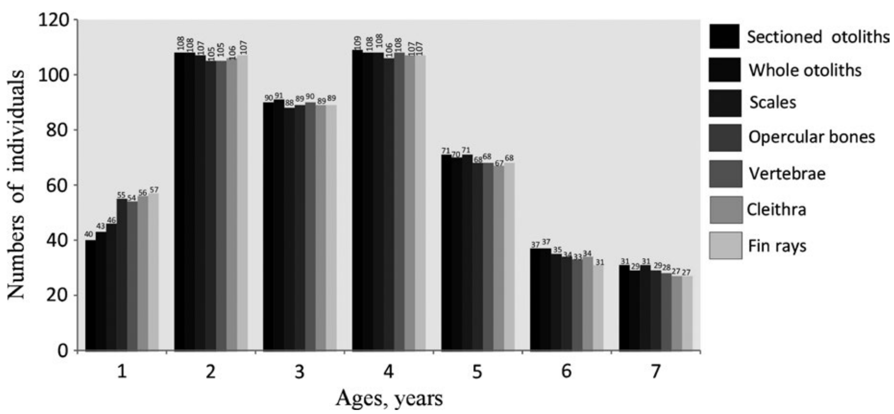


**FIGURE 3** Age composition of *C. striata* derived from readings of different ageing structures. Total number of samples ($N = 486$) remains the same for each ageing structure

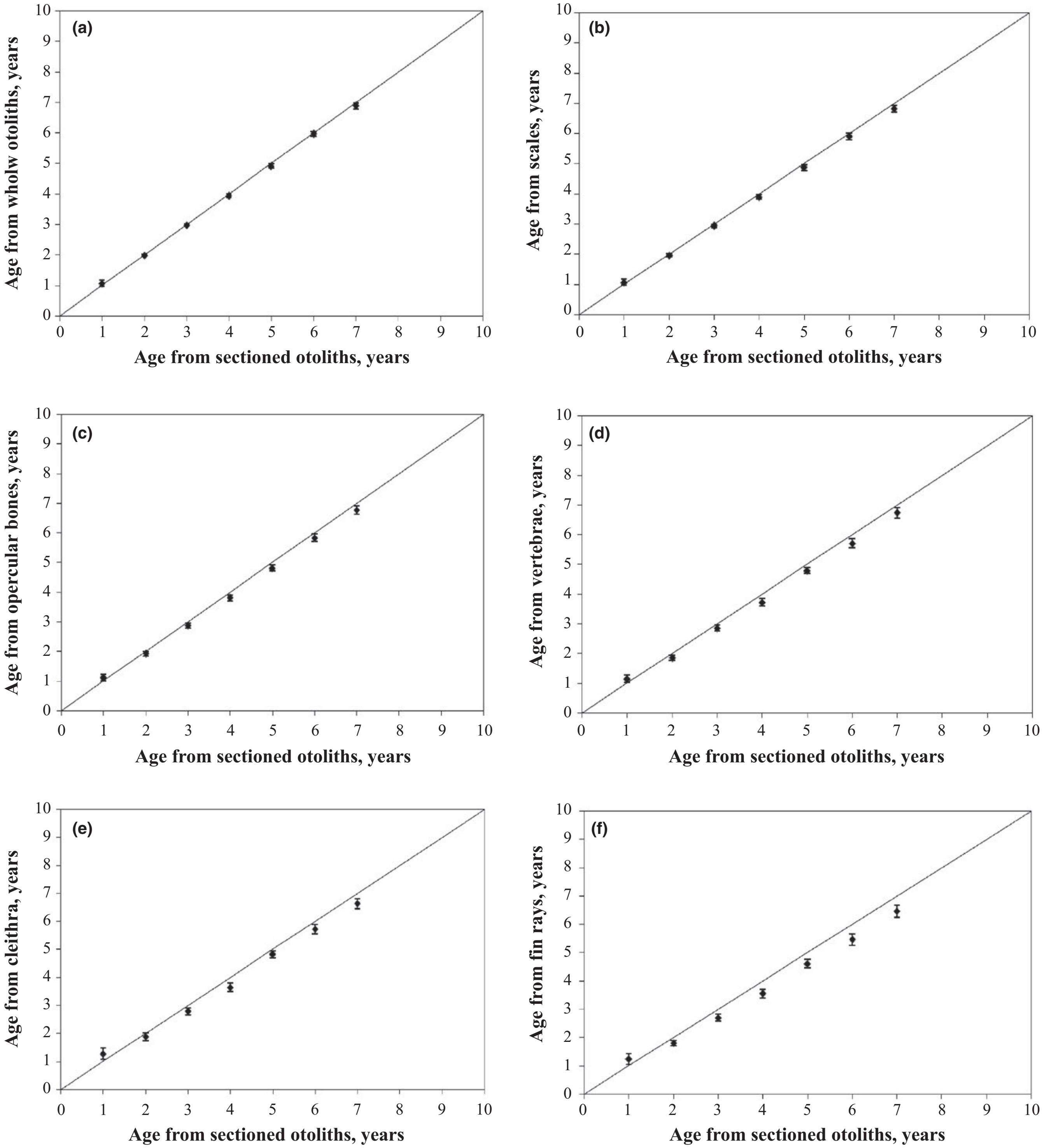


**FIGURE 4** Age bias graphs for C. striata (N=486) age estimates from sectioned otoliths versus (a) whole otoliths, (b) scales, (c) opercular bones, (d) vertebrae, (e) cleithra and (f) fin rays. Each error bar represents the 95% confidence interval. The 1:1 equivalence (solid) line is also indicated. Points above the line indicate ages that were overestimated whereas a point below the line indicates ages that were underestimated

the most suitable ageing structure in a number of fish species such as *Pylodictis olivaris* (Nash & Irwin, 1999), *Ictalurus punctatus* (Buckmeier, Irwin, Betsill, & Prentice, 2002), *Clarias gariepinus* (Khan, Khan, & Miyan, 2011) and *Channa punctatus* (Khan et al., 2013b). Khan, Khan, and Miyan (2013a) reported otoliths as the most time-efficient and precise means of age estimates in *Clarias batrachus* compared to vertebrae and pectoral spines. In the present study scales also showed a good value of agreement between age readers because annuli were clear and easy to read and have been the most widely used ageing practice for the majority of cyprinids (Kamilov, 1984), primarily due to advantages such as the ease of collection and preparation, and more importantly, because the method is non-destructive to fish

(DeVries & Frie, 1996). In the published literature on Indian freshwater fishes, scales have been exclusively used for age studies in *Catla catla* (Natarajan & Jhingran, 1963) and *C. mrigala* (Kamal, 1969). According to Kamal (1969), *Cirrhinus mrigala* scales showed clear annulations. In the first published report on precision of age estimates from different ageing structures of Indian freshwater fishes, it was observed that in *L. rohita*, *C. catla*, and *C. marulius*, among different structures (scales, otoliths, opercular bones, vertebrae, and dorsal fin rays), scales were the most suitable structure for ageing *L. rohita* and *C. marulius*, but that opercular bones were best for ageing *C. catla* (Khan & Khan, 2009). In corroboration with the observations of Beamish and McFarlane (1987), we also noted an underestimation of age by scales in older (5+ years) *C. striata*; however, annuli on the scales of younger fish were quite clearly legible. Opercular bones also provided age readings, as evident from the PA, APE, and CV values between structures. In the present study opercular bones did not show clear annuli in older fishes, possibly due to an overgrowth of dense bone near the hyomandibular socket. Similarly, in the longnose sucker (*Catostomus catostomus*) this problem was observed by Perry and Casselman (2012), who concluded that when using the whole operculum technique, interpreters should be aware that as fish become older, increments and annuli located close to the origin become obscured. Also, in some fishes the opercular bones were found to be less reliable than other structures, e.g. in *Schizothorax o'connori* (Ma, Xie, Huo, Yang, & Li, 2011) *Labeo rohita* and *Channa marulius* (Khan & Khan, 2009). Khan et al. (2015) determined that the opercular bones were the second most suitable ageing structure for *Labeo bata* in the Ganga River. In the present study, rings on the vertebral centra were not very clear and showed numerous minute marks unrelated to cyclical events. Because we used whole vertebral centra for age estimation in *C. striata*, the degree of clarity and sharpness of the growth rings were low. Several researchers have reported that the rings on vertebral centra were not very clear and showed numerous minute marks unrelated to cyclic events in a number of fish species such as *Makaira nigricans* (Hill, Calliet, & Radtke, 1989), *Labeo rohita*, *Catla catla*, and *Channa marulius* (Khan & Khan, 2009), *Channa punctata* (Khan et al., 2013b). In the cleithra of *C. striata* it was very difficult to identify true rings because of the presence of numerous marks. Similarly, cleithra was the least preferred ageing structure in *Schizopyge curvifrons*, *Schizopyge niger* and *Schizothorax esocinus* compared to applying otoliths, scales, opercular bones, and vertebrae (Sabah & Khan, 2014). Fin rays underestimated age in *C. striata* and more so in the older individuals. Many investigators rejected fin rays because of difficulties in sample preparation, identification of the first annulus, and distinction between true and false annuli (Khan & Khan, 2009). Quinn and Ross (1982) questioned the reliability of age estimates from pectoral fin rays in fish older than 7 years because of the lack of annulus formation and the difficulty in interpreting fin ray sections. Thus, suitability of fin rays for ageing may vary among species and, potentially, among populations (Zymonas & McMahon, 2009). In conclusion, otoliths may provide precise age estimates in *C. striata*, especially when the otoliths are sectioned and polished.

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