



Age and growth in different stocks of striped snakehead *Channa striata* (Bloch, 1793) inhabiting the three rivers of Gangetic river system

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ABSTRACT

Age was estimated using different types of otoliths (sagittae, lapilli and asterisci) collected from 486 specimens of *Channa striata* (Bloch, 1793) from three Indian rivers, the Ganga, Gomti and Yamuna, during the period November 2012 - August 2015. The size range of the specimens was 15-70 cm total length (TL). The age estimates were compared and assessed for potential bias between readers and between pairs of aging structures. Due to the highest percentage of agreement and lowest average percentage of error and coefficient of variation values between readers, sagittae were found to be the most suitable aging structure for *C. striata*. When sagittae age reading were compared with those from lapilli and asterisci, the percentage of agreement was found to be highest between estimates of sagittae and lapilli. Mean values of age estimate from sagittae were comparable ($p > 0.05$) to the values obtained from lapilli but significantly ($p < 0.05$) different with that of asterisci. Thus, sagittae emerged as the preferred otolith for age estimation in *C. striata* followed by lapilli. The growth of *C. striata* was assessed by examining annual growth increments on sectioned sagittae. The parameters of von Bertalanffy growth equations for the fish in the selected rivers were: $L_t = 94.4 (1 - e^{-0.15(t+1.07)})$ for river Ganga, $L_t = 93.7 (1 - e^{-0.15(t+0.96)})$ for river Yamuna and $L_t = 96.9 (1 - e^{-0.14(t+1.02)})$ for river Gomti. The growth performance index was highest in fish stocks collected from river Ganga (3.13) followed by river Gomti (2.96) and river Yamuna (2.38). Marginal increment analysis suggested annual periodicity of the growth band formation.

Keywords: Ageing precision, *Channa striata*, Marginal increment analysis, Otoliths, VBGF

Introduction

Fish population analysis and assessment requires information on fish age estimation (Maceina and Sammons, 2006). Otoliths, scales, opercular bones, vertebrae, fin rays, spines and cleithra are some of the calcified structures that have been used to determine the age of fish. Otoliths have been the preferred ageing structure owing to higher precision in age estimation (Hoxmeier *et al.*, 2001). Error prone age estimates may lead to flawed population parameters estimates, for example, a fish population may be subjected to serious overexploitation and may eventually collapse if fish age is under estimated (Campana, 2001).

A reliable growth estimate is necessary for successful fish stock assessment and management. Growth is among the most examined aspects of fish biology as it reflects the wellbeing of individual as well as the population of a fish. A popular fisheries growth model is the von Bertalanffy growth function (VBGF) and its variables are quite effective in understanding overall fish growth in natural environment. (Quinn and Deriso, 1999; Khan and

Khan, 2014). Various studies have successfully employed the VBGF model to estimate growth in a variety of fish species, such as *Gerres* sp. (Kanak and Tachihara, 2006), *Nelussetta ayraudi* (Miller *et al.*, 2010), *Oxygymnocypris stewartii* (Jia and Chen, 2011), *Schizopyge curvifrons*, *Schizopyge niger* and *Schizothorax esocinus* (Sabah and Khan, 2014).

As the most suitable method for age estimation may vary among species, it is necessary to evaluate precision and accuracy of the age estimates from a selected hard structure (Polat *et al.*, 2001). Since accuracy of age estimates does not ensure its precision (Maceina and Sammons, 2006), thus generating information on precise age estimates will not only help to minimise error but increase the utility of age and growth data (DeVries and Frie, 1996). Sylvester and Berry (2006) suggested to compare age estimates from different structures as an alternate form of validation, which could help to understand the accuracy and bias of age data. However, to validate age estimates from a hard anatomical structure, one of the most widely used technique is marginal increment analysis (MIA) (Okamura *et al.*, 2013).

The striped snakehead *Channa striata* (Bloch, 1793) (Family: Channidae) is distributed in the freshwaters of India as well as many other countries in the Asian continent. Because of its flavour and taste, lack of intramuscular spines and therapeutic properties, this species is a desirable food fish with a reasonable and affordable market price (Haniffa *et al.*, 2004). Numerous studies have compared the age estimated from several calcified hard structures for *e.g.* *C. carpio* (Phelps *et al.*, 2007), *Labeo rohita*, *Catla catla* and *Channa marulius* (Khan and Khan, 2009), *Salvelinus confluentus* (Zymonas and McMahon, 2009), *Clarias gariepinus* (Khan *et al.*, 2011), *Clarias batrachus*, *Heteropneustes fossilis* and *Wallago attu* (Khan *et al.*, 2013a), *Channa argus argus* (Gu *et al.*, 2013), *Channa punctata* (Khan *et al.*, 2013b), *Schizopyge curvifrons*, *Schizopyge niger* and *Schizothorax esocinus* (Sabah and Khan, 2014), *Hoplias malabaricus* (Lozano *et al.*, 2014), *Labeo bata* (Khan *et al.*, 2015) and *C. punctata* (Khan *et al.*, 2019b). In one of our earlier studies, sections of *sagittal otoliths* provided precise age estimate in *C. striata* in comparison to whole sagittae, scales, opercular bones, vertebrae, cleithra and fin rays (Khan *et al.*, 2017). Sagittal otoliths were used in the above study on account of being largest in size, without any consideration of clarity and ease of annuli reading on other otoliths; also age estimates were not validated. The *C. striata* population from the river Ganga and its two tributaries, rivers Gomti and Yamuna have been delineated into three separate stocks (Khan *et al.*, 2019a, Khan *et al.*, 2021). In the present study, all the otoliths *viz.* *sagittae*, *lapilli* and *asterisci* were compared in order to

determine the type of otolith giving precise age estimate. The age estimates in *C. striata* were validated using MIA. This was followed by developing stock-specific VBGF equations using sectioned sagittae (the structure giving precise age estimate) and scales (the structure commonly used for age estimation).

Materials and methods

Fish samples (N=486) were collected from Narora (28.1968°N; 78.3814°E) in the Ganga River; Agra (27.1767°N; 78.0081°E) in the Yamuna River and from Lucknow (26.8467°N; 80.9462°E) in the Gomti River (Fig. 1) during November 2012-August 2015. Fish were collected using cast nets and drag nets of varying mesh size to capture fish of different size-class. It was ensured that the ageing structures were not damaged in the selected samples. Fish samples having damaged aging structures were not included in the study. Sampled fishes were identified based on the descriptions given by Jayaram (1999) and Talwar and Jhingaran (1991). Fish were measured to the nearest 0.1 mm for total length. The size range of the specimens was 15-70 cm total length (TL).

Otoliths (*sagittae*, *lapilli* and *asterisci*) were removed from the fish and prepared for ageing following standard protocols (David *et al.*, 1994). All otoliths were examined under a stereozoom microscope under reflected light (Fig. 2). Although annual growth is represented by an opaque and a translucent zone, we counted the total number of translucent zones to determine the fish age (Fig. 3). The clarity of annuli on *sagittae*, *lapilli* and *asterisci* were assessed on a five point scale given by Paul and

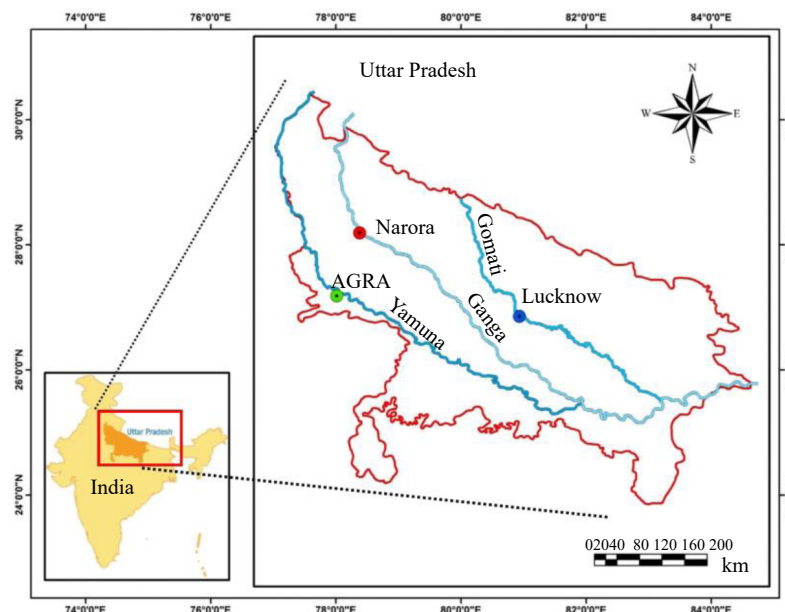


Fig. 1. Map showing sampling sites: Narora (Ganga River), Agra (Yamuna River) and Lucknow (Gomti River)

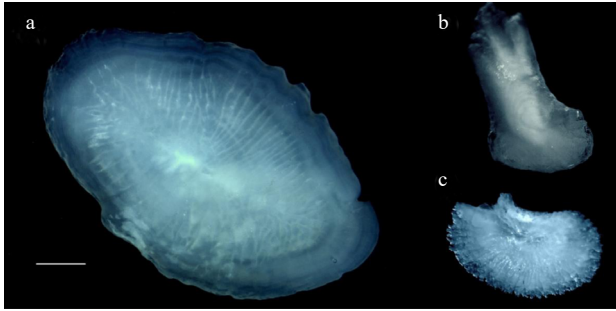


Fig. 2. Otoliths in *C. striata*: (a) sagitta, (b) lapillus and (c) asteriscus. Scale bar = 1000 μ m

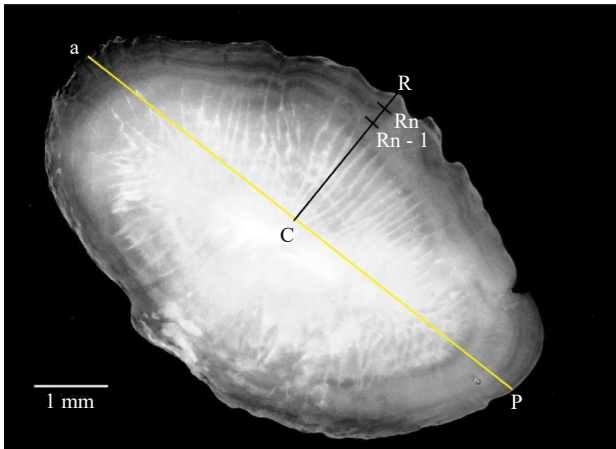


Fig. 3. Photograph of whole otolith of *C. striata*, under reflected light. C = core; A = anterior; P = posterior. White line indicates the measurement axis of annual rings. R = Otolith radius, R_n = Radius of the last complete growth ring and R_{n-1} = Radius of the previously completed growth ring

Horn (2009): 1 rated as “excellent”; 2 rated as “good”; 3 as “acceptable” (little uncertainty in deciphering the true rings for some of the annuli); 4 as “poor” (some annuli are poorly seen and difficult to interpret) and 5 as “almost unreadable”.

Calculations and statistical analysis

Average percentage error (APE), coefficient of variation (CV) and percent agreement (PA) were calculated between age readers as well as between the structures used for age estimation. Following formula (Beamish and Fournier, 1981) was used to calculate APE:

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

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 computed as follows:

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

where, CV_j is the coefficient of variation for the j^{th} fish.

APE and CV are considered as quite useful and are widely used parameters to measure precision of age estimates (Campana, 2001). However several researchers have not considered PA as a suitable measure of precision (Beamish and Fournier, 1981; Chang, 1982; Campana *et al.*, 1995) despite many published reports incorporating it as one of the precision parameters (Hoxmeier *et al.*, 2001; Sabah and Khan 2014). PA was depicted as percentage of number of similar age data over the total number of observations. It was computed using the template developed by Sutherland (2006). Any bias in age readings between readers as well as structures was assessed by plotting age bias graphs as explained in Campana *et al.* (1995).

The significance of variation in mean age readings of different structures were analysed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) (Gomez and Gomez, 1984). Once we select the structure giving precise age estimate, mean values of age data when subjected to DMRT may help us to understand relative under or over estimation of age without any consideration of size-class. So, in absence of the most suitable ageing structure, we can select an appropriate alternative structure for fish age estimation.

Growth analysis

To assess growth pattern, length-at-age data obtained from sectioned sagittae (Khan *et al.*, 2017) were fitted to the von Bertalanffy growth function equation (Ricker, 1975):

$$L_t = L_\infty (1 - e^{-k(t-t_0)})$$

where, L_t = total length (cm) of fish at age t ; L_∞ = asymptotic mean length; k = rate constant that determines the rate at which L_t approaches L_∞ ; t = time or age of the fish; t_0 = the hypothetical age at which the fish had zero length.

We also developed VBGF equation utilising the length-at-age data from one of the most commonly used and non-lethal structure, the scales, to get a comparative account of the growth parameters from the structure providing precise age estimate. Comparison between observed and calculated mean length-at-age (VBGF) were tested using Student's t-test (Zar, 1996). The back calculated lengths (VBGF) at estimated ages between non-lethal ageing structure (scales) and the most suitable

ageing structure were compared using Student's t-test. Growth performance index (Pauly and Munro, 1984) was used to compare the growth performance of different populations of fish based on von Bertalanffy growth parameters. The performance index (ϕ') was calculated using the following equation:

$$\phi' = \log_{10} K + 2 \log_{10} L_{\infty}$$

Growth parameters were estimated using non-linear regression methods in a Microsoft Excel-based application developed by Cope and Punt (2007).

Marginal increment analysis

Validation of the annual periodicity of growth ring formation was assessed by MIA using the formula given by Hayashi (1976):

$$MIA = R - R_n / R_n - R_{n-1}$$

where, R is the otolith radius, R_n the radius of the last complete growth ring and R_{n-1} is the radius of the previously completed growth ring. Measurements were made from the focus to the outer edge of each growth ring (R_n) using image analysis software. Monthly mean marginal increment ratio (MIR) were plotted to reveal periodic trends in annulus formation (Lessa *et al.*, 2006).

Results and discussion

Sagittal otoliths exhibited highest PA followed by lapilli and asterisci. Age readings from sagittae showed least values of CV and APE in all the fish stocks (Table 1).

Age bias graphs between two readers for sagittae, lapilli and asterisci are presented in Fig. 4. There was no age bias between readers for sagittae but minor bias occurred in case of lapilli. As evident from larger standard error bars in case of older fish specimens, age estimates from asterisci showed proportionate variation with increasing age. While comparing sagittal age estimates to that from other otoliths, the sagittae-lapilli showed highest PA followed by sagittae-asterisci (Fig. 5). Analysis of mean age data using ANOVA followed by DMRT exhibited significantly ($p < 0.05$) higher values for sagittae compared to asterisci but insignificantly different for lapilli (Table 2). Moreover, sagittal otoliths showed clarity and sharpness in annuli, and thus error was minimal during age estimation. Majority of sagittae were readable with a good (63.5%) score, while 46.7% of the asterisci were scored as unreadable (Table 3).

The annulus was formed on striped snakehead sagittal otoliths once a year in the Ganges river system, during June to September. The annual marks consist of a binary set of bands, a narrow translucent band formed in monsoon season (June to September) and a wide opaque band formed over the rest of the year. Monthly mean marginal increment ratio for striped snakehead sagittal otoliths are presented in Fig. 6.

The following von Bertalanffy growth function equations were derived for the three stocks of *C. striata*: $L_t = 94.4 (1 - e^{-0.15(t+1.07)})$ for river Ganga; $L_t = 93.7 (1 - e^{-0.15(t+0.96)})$ for river Yamuna and $L_t = 96.9 (1 - e^{-0.14(t+1.02)})$ for river Gomti. Growth performance index was highest for the fish stock from river Ganga (3.13) followed by river Gomti (2.96)

Table 1. Average percent error (APE), coefficient of variation (CV) and percentage of agreement (PA) between two independent readers and between pairs of structures in *C. striata* collected from three rivers

Ageing structure	River Ganga			River Yamuna			River Gomti			Pooled data		
	APE	CV	PA	APE	CV	PA	APE	CV	PA	APE	CV	PA
Between readers												
Sagittae	2.11	4.04	84.4	2.52	4.48	82.5	2.41	4.27	83.5	2.14	3.89	85.0
Lapilli	15.5	16.9	38.7	15.6	17.1	38.1	15.4	16.9	38.9	15.2	16.5	40.3
Asterisci	18.2	20.1	34.4	18.6	19.9	35.0	18.4	19.7	35.8	18.2	19.4	37.0
Between structures												
Sagittae-lapilli	12.1	13.6	54.3	11.8	13.8	54.1	12.0	13.2	54.9	11.7	12.8	56.4
Sagittae-asterisci	15.7	15.8	51.4	17.3	17.0	49.8	15.3	15.5	52.1	15.0	15.2	53.7

Table 2. Mean age data from sagittae, lapilli and asterisci were subjected to ANOVA followed by DMRT

Ageing structure	Mean age estimates*			
	River Ganga	River Yamuna	River Gomti	Pooled data
Sagittae	4.06 ^a	3.99 ^a	4.01 ^a	4.08 ^a
Lapilli	3.59 ^{ab}	3.42 ^{ab}	3.53 ^{ab}	3.64 ^{ab}
Asterisci	3.41 ^b	3.32 ^b	3.42 ^b	3.45 ^b

*Values having dissimilar superscripts are significantly ($p < 0.05$) different from each other.

Table 3. Distribution of readability scores for different otoliths of *C. striata*; the values are percentages of the sample size ($n = 486$)

Ageing structure	Readability scores				
	1	2	3	4	5
Sagittae	6.7	63.5	19.9	5.7	3.9
Lapilli	0.8	18.7	4.7	31.8	43.8
Asterisci		2.2	8.4	42.5	46.7

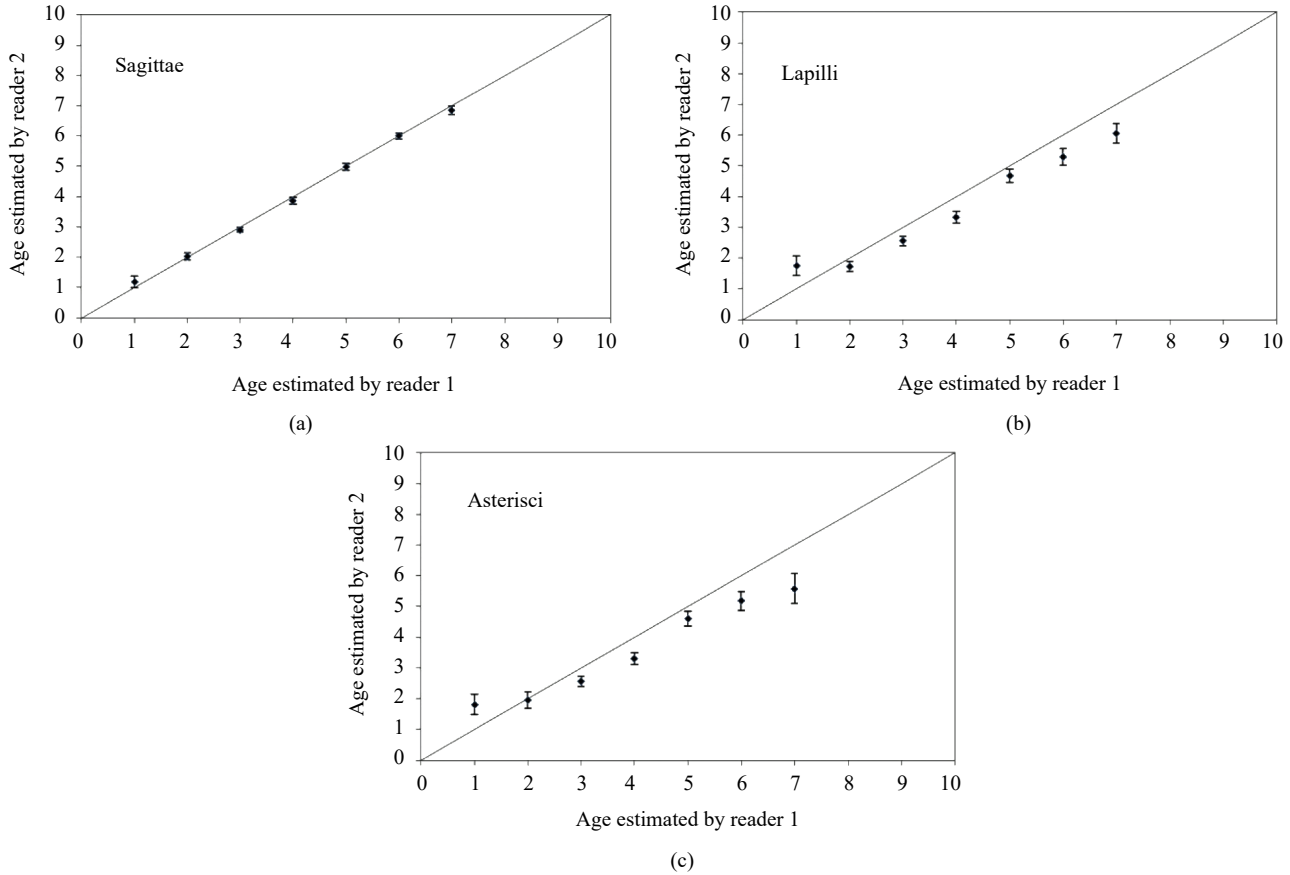


Fig. 4. Age data (error bars showing 95% confidence interval) reported by two independent readers shows bias (if any) for sagittae, lapilli and asterisci. Over or under estimation of fish age can be understood by position of the points above or below the theoretical 1:1 agreement line

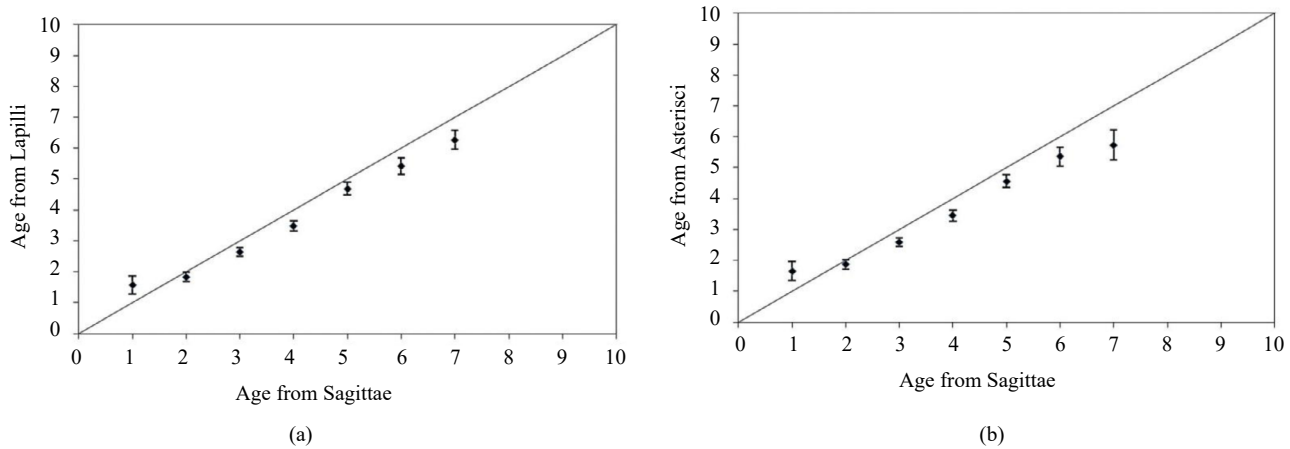


Fig. 5. Age data (error bars showing 95% confidence interval) when compared between the structures shows bias (if any) for sagittae-lapilli and sagittae-asterisci. Over or under estimation of fish age can be understood by position of the points above or below the theoretical 1:1 agreement line

and river Yamuna (2.38) (Table 4). There were no significant differences between calculated lengths and observed lengths in all the fish stocks: $R^2 = 0.9981$; $t = 1.84$, $df = 6$, $p > 0.05$ for river Ganga, $R^2 = 0.9986$;

$t = 2.02$, $df = 6$, $p > 0.05$ for river Yamuna and $R^2 = 0.9931$; $t = 1.86$, $df = 6$, $p > 0.05$ for river Gomti. A comparison of mean total lengths derived separately from scales and sectioned otolith varied significantly ($p < 0.001$) with each

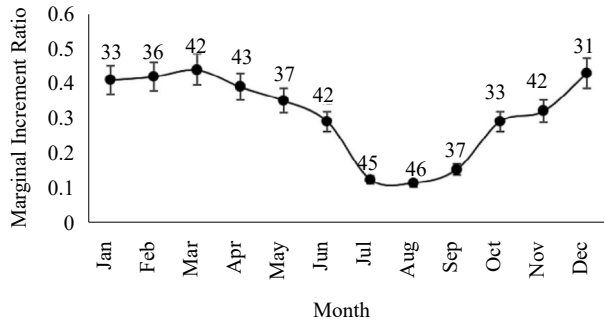


Fig. 6. Monthly mean marginal increment ratio for striped snakehead sagittae. Sample numbers are indicated at respective points

other in each of the three fish stocks of *C. striata* (Fig. 7). The von Bertalanffy growth curves using precise age estimates of *C. striata* fish stocks from the three rivers showing significant difference ($p < 0.05$) are presented in Fig. 8.

Otoliths are often preferred for age estimation on account of them providing higher precision compared to other structures (Sharp and Bernard, 1988; Hoxmeier *et al.*, 2001; Khan *et al.*, 2013b, 2017). Sagittae provided the most suitable age estimates in *C. striata* compared to other otoliths. The fact that otoliths do not show reabsorption and acellular growth rather than by calcification

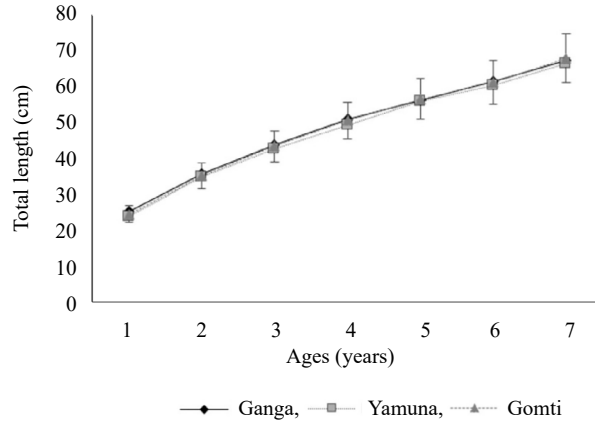


Fig. 8. The von Bertalanffy growth curves for *C. striata* fish stocks sampled from rivers Ganga, Yamuna and river Gomti showing significant difference ($p < 0.05$)

contributes to their potential for age determination (Secor *et al.*, 1995). Further, otoliths being metabolically inert, do not represent physiological changes occurring during a fish life (Phelps *et al.*, 2007). When body growth slows down and asymptotic length is achieved, otoliths keep growing and does not seem to show reabsorption even during food shortage or stress (DeVries and Frie, 1996). In a number of fish species, sagittae provided most

Table 4. Parameters of VBGF and the growth performance index (ϕ') in *C. striata*

Species	Selected river	Sampling site	Structure used	L_{∞} (cm)	Max TL (cm)	k	t_0	Age	ϕ'
<i>C. striata</i>	Ganga	Narora	Sectioned otoliths	94.4	70	0.15	-1.07	7	3.13
			Scales	99	70	0.14	-1.18	7	3.14
<i>C. striata</i>	Yamuna	Agra	Sectioned otoliths	93.7	68.5	0.15	-0.96	7	2.38
			Scales	95	68.5	0.14	-1.11	7	3.10
<i>C. striata</i>	Gomti	Lucknow	Sectioned otoliths	96.9	69	0.14	-1.02	7	2.96
			Scales	98.1	69	0.15	-1.05	7	3.16



Fig. 7. The von Bertalanffy growth curves for *C. striata* fish stocks sampled from the rivers (a) Ganga; (b) Yamuna and (c) Gomti showing significant variation ($p < 0.001$) in back-calculated length at estimated age using sectioned otoliths and scales

suitable age estimate, for example in *Pylodictic olivaris* (Nash and Irwin, 1999); *Ictalurus punctatus* (Buckmeier *et al.*, 2002) and *C. gariepinus* (Khan *et al.*, 2011). In *C. batrachus*, the most suitable age estimates were provided by sagittal otoliths in comparison to other structures (Khan *et al.*, 2013a). The current investigation suggested that ages estimated from the lapillus otoliths showed poor clarity of rings close to the primordia. The clarity of growth rings on lapilli of *Scomberomorus sierra* were hindered and difficult to interpret due to thickness of the structure (Cabello *et al.*, 2011). In contrast, Escot and Granado-Lorencio (1998) suggested that the lapillus is the precise structure for ageing in *Barbus sclateri*. Similarly, asterisci were also very difficult to interpret in *C. striata*. In a study from Pongolapoort Dam, South Africa on *Hydrocynus vittatus*, whole asteriscus were delicate, thin, not easy to process with poor repeatability in age readings (Soekoe *et al.*, 2013). On the other hand, David *et al.* (1994) suggested asteriscus as the best ageing structure in *Sciaenaps acellatus*. Age validation has frequently been undertaken using scales and otoliths in teleost by MIA in order to confirm annul periodicity of growth ring formation, which is being interpreted as annulus (Tsikliras *et al.*, 2005). In *C. striata* otoliths, marginal increments were minimum during June to September (Fig. 6). There are several causes of annuli formation in fishes like water temperature, feeding, reproduction, light exposure, metabolism, endocrine system and the environment (Morales-Nin and Panfili, 2005). Spawning has often been considered as one of the major causes for annulus formation on ageing structures of a fish (Takahashi *et al.*, 1995; Baeck and Huh, 2004; Shimose and Tachihara, 2006). In the present study, translucent zone was formed on *C. striata* otoliths during the spawning season. Annulus formation in *C. striata*, *i.e.*, alternating occurrence of the opaque and translucent zones occurred once a year and the rings observed were considered annuli (Fig. 3). In conclusion, sagittae may be considered as the structure giving precise age estimate based on least APE, CV and maximum PA values. Annuli on sagittae were validated for periodicity using MIA.

According to Branstetter (1987), rapid fish growth is exhibited by k values of 0.20 to 0.50 per year; moderate growth occurs when $k = 0.1$ to 0.2 per year; and fish shows slow growth when $k = 0.05$ to 0.10 per year. All the three stocks of *C. striata* showed moderate growth as evidenced by k values ranging from 0.12 to 0.15 per year. A high growth coefficient infers a high metabolic rate and such fish may show early maturity at a relatively larger size than their asymptotic length (Qasim, 1973). The parameter, k is considered important to understand relative life history strategies and potential vulnerability of the population to overexploitation and varied habitat

conditions (Musick, 1999). Disparity in representative samples of different size/age class greatly influence the growth model estimates (Ma *et al.*, 2010). The computed value of L_{∞} in *C. striata* was 94.4, 93.7 and 96.9 cm for rivers Ganga, Yamuna and Gomti, respectively. Sanguttuvan and Sivakumar (2010) reported $L_{\infty} = 102.4$ cm for the same species inhabiting in Ukkadam Lake, Coimbatore, Tamil Nadu, India. Karlou-Riga and Sinis (1997) suggested to check the quality and validity of growth parameters for their efficient application in natural population. Kerstan, (1985) found that the ages determined could be considered reliable if t_0 has a negative value (close to Zero). In *C. striata* stocks, t_0 ranged between -0.96 to -1.07. Sparre and Venema (1998) had reported stock-specific as well as species specific variation in growth parameters due to differing environmental conditions such as temperature, water quality and food availability. Low temperature can reduce the growth rate of ectotherms (Angilletta *et al.*, 2004).

The reliability of growth parameters is often indicated by the growth performance index that exhibits little variation among stocks (Ama-Abasi *et al.*, 2004). Sparre *et al.* (1989) suggested that the growth performance index is one of the appreciated growth indices because it has minimum variance. It is quite useful parameter for comparing fish population growth from same or different environments (Akombo *et al.*, 2015). In the present study, the growth performance index (ϕ') of *C. striata* was 3.13, 2.38 and 2.96 for the three different fish stocks collected from the rivers Ganga, Yamuna and Gomti respectively. It suggests that river Ganga is better for *C. striata* stock growth followed by the rivers Gomti and Yamuna. The growth performance index could be affected by a number of factors such as genetics, species growth potential, over exploitation, predation, and diet type and abundance (Gatabu 1992). It has been observed that the growth performance of a fish species increases with increase in L_{∞} value.

According the Wootton (1990), a fish species having different geographical distribution may also show variation in individual growth rate. The back-calculated lengths (VBGF) using the age estimates from sectioned otoliths (most suitable ageing structure) and scales (non-lethal) showed significant variation with each other. Therefore, in *C. striata*, scales cannot be used for reliable growth estimation. It has been commonly known that scales or spines/rays are commonly used for fish age estimation as a non-lethal structure. It may be suggested that while developing a growth equation for a fish stock, precise age data must be used in order to avoid misleading interpretation of age and growth dependent population characteristics (Khan, 2013).

The findings of the present study may be utilised for formulating scientific and efficient strategies for the management of *C. striata* fish stocks inhabiting the rivers Ganga, Yamuna and Gomti.

Acknowledgements

Authors thank the Chairperson of the Department of Zoology, Aligarh Muslim University, Aligarh, for extending the facilities required for the study. The Council of Science and Technology, Lucknow is gratefully acknowledged for funding the study and providing financial assistance as “Research Assistantship” to the first author. We also acknowledge the anonymous reviewers for their comments that helped in improving the overall presentation of the manuscript.

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