Zoology and Ecology, 2014
Vol. 24, No. 1, 16–25, http://dx.doi.org/10.1080/21658005.2014.896145

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Precise age estimation and growth of three Schizothoracinae fishes from Kashmir valley

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(*Received 11 November 2013; accepted 12 February 2014*)

Studies on the precision of age estimates from different structures/readers and growth assessment were undertaken in selected Schizothoracinae fishes. Ageing structures were prepared and read following standard protocols. The maximum ages of the observed *Schizopyge curvifrons* and *Schizopyge niger* specimens were 6 years and 5 years of *Schizothorax esocinus* specimens. Based on the highest percentage of agreement (PA) and the lowest average percentage of error as well as on the values of coefficient of variation between readers, otoliths provided a precise age estimate of *S. curvifrons* and *S. niger* individuals, while vertebrae showed a precise age estimate of those of *S. esocinus*. Among structures, the highest PA was found between otoliths and opercular bones in *S. curvifrons*, between otoliths and scales in *S. niger*, and between vertebrae and opercular bones in *S. esocinus*. Comparable values of mean age estimates were obtained from different structures in specimens of the following species: from otoliths, opercular bones and vertebrae in *S. curvifrons*; from otoliths, scales, vertebrae and opercular bones in *S. niger*; and from vertebrae, opercular bones, scales and otoliths in *S. esocinus*. Based on precise age estimates, the von Bertalanffy growth equation was $L_t = 49.8\ (1 - e^{-0.263(t+0.34)})$ for *S. curvifrons*, $L_t = 44.8\ (1 - e^{-0.255(t+1.42)})$ for *S. niger* and $L_t = 66.6\ (1 - e^{-0.278(t+0.34)})$ for *S. esocinus*.

Pagal kelių tyrėjų išmatuotas įvairias žuvų kūno dalis įvertintas Schizothoracinae (azijinių priekalnių ūsuočių) amžiaus nustatymo tikslumas bei augimo greitis. Matavimai atlikti pagal standartinę metodiką. Maksimalus *Schizopyge curvifrons* ir *Schizopyge niger* individų amžius buvo šeši, *Schizothorax esocinus* – penki metai. Pagal dviejų tyrėjų nustatyto žuvų amžiaus sutapimą, mažiausią vidutinę procentinę paklaidą ir variacijos koeficientų reikšmes gauta, kad iš otolitų tikslus žuvų amžius buvo įvertintas *S. curvifrons* ir *S. niger*, iš stuburo slankstelių – *S. esocinus* individams. Vienodžiausiai žuvų amžius *S. curvifrons* buvo nustatytas iš otolitų ir žiaunadangčių, *S. niger* – iš otolitų ir žvynų, *S. esocinus* individams – iš stuburo slankstelių ir žiaunadangčių. Panašios vidutinės amžiaus reikšmės *S. curvifrons* buvo gautos analizuojant otolitus, žiaunadangčius ir stuburo slankstelius, *S. niger* – stuburo slankstelius ir žiaunadangčius, *S. esocinus* individams – stuburo slankstelius, žiaunadangčius, žvynus ir otolitus. Gautos von Bertalanffy augimo lygtys $L_t = 49.8\ (1 - e^{-0.263(t+0.34)})$ *S. curvifrons* individams, $L_t = 44.8\ (1 - e^{-0.255(t+1.42)})$ *S. niger* individams ir $L_t = 66.6\ (1 - e^{-0.278(t+0.34)})$ *S. esocinus* individams.

Keywords: ageing precision; growth; *Schizothorax esocinus*; *Schizopyge niger*; *Schizopyge curvifrons*

Introduction

Schizothoracinae are widespread in different rivers, lakes and tributaries throughout the Himalayas extending to the confines of China, eastern Afghanistan, Pakistan, Turkistan, Nepal, Ladakh, Tibet, Bhutan and north-east India. In India, Schizothoracinae are the most important food fish in the Himalayan region including Kashmir, Himachal Pradesh, Uttarakhand, the Uttar Pradesh foothills (Terai area), Assam, etc. (Day 1958; Bahuguna, Negi, and Upadhyay 2009). Freshwater fishes in India are under threat of extinction for several reasons, but primarily due to unsustainable and unethical fishing practices. Other common threats that affect freshwater fish populations include habitat loss due to dredging of lakes and rivers, filling, alteration of river courses, dams, irrigation canals, etc. Of the three species selected for the study, two species are listed as Vulnerable (*Schizopyge curvifrons* and *Schizopyge niger*) and one as Lower risk-near threatened (*Schizothorax esocinus*) (Molur and Walker 1998). Studies of biological parameters of threatened fishes are highly significant to the management and conservation of populations in natural water bodies (Sarkar, Deepak, and Negi 2009; Hossain et al. 2012; Khan, Khan, and Miyan 2012).

Age and growth analyses of fish species are used to determine longevity, mortality, productivity, yield and population dynamics, which in turn are essential for responsible management (Holden 1973; Hale and Lowe 2008). Fish age estimation has been undertaken using different hard structures (Koch and Quist 2009); however, age estimation is often accompanied by several sources of error that can have significant effects on estimates of many population parameters. For example, age underestimation may result in optimistic estimates of growth and mortality rates leading to serious overexploitation of the population and its eventual collapse (Campana 2001). Scales have been widely used for ageing fishes because they are collected, prepared and read easily (Gursoy et al. 2005). However, several researchers have reported that scales can provide unreliable estimates of fish age

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Published online 17 Mar 2014

(DeVries and Frie 1996; Maceina and Sammons 2006), particularly in older individuals. Thus, scientists have used other calcified structures such as otoliths, opercular bones, vertebrae, fin rays and spines, cleithra, etc. for fish age determination. Studies into the precision and accuracy of age determination from bony structures are assuming immense significance due to the fact that the most reliable ageing method may exhibit variations not only among species but also within the same species. For instance, opercular bones were the most suitable structure for age determination in *Catla catla,* while in case of *Labeo rohita* (Khan and Khan 2009) the most suitable structure was scales.

Growth is one of the most important processes in fish life history influencing population dynamics (Zhan 1995). Assessment and management of sustainable fisheries stock require reliable growth estimates. The von Bertalanffy Growth Function (VBGF) is one of the most studied and most widely used of all length-age growth models in fisheries, and its parameters are particularly useful in describing general fish growth (Chen, Jackson, and Harvey 1992; Quinn and Deriso 1999), deriving fisheries reference points (Clark 1991; Williams and Shertzer 2003) and estimating life history parameters (Beverton and Holt 1959; Beverton 1992; Cope and Punt 2007). Therefore, for a given fish species, we need to have a precise age estimate that can be utilized for studying a number of population parameters including the development of length-at-age growth models.

There are a few studies on the age and growth of Schizothoracinae fishes: *Schizopygopsis younghusbandi younghusbandi* (Chen, Chen, and He 2009), *Ptychobarbus dipogon* (Li et al. 2009), *Schizothorax waltoni* (Qiu and Chen 2009), *Schizothorax o'connori* (Yao et al. 2009; Ma et al. 2010) and *Oxygymnocypris stewartii* (Jia and Chen 2011; Huo et al. 2012). However, for the selected fish species, there is no published information on the precision of age estimates. Also, no earlier study has utilized precise age estimates for developing VBGF equation. The objectives of the current study, therefore. were: (i) to determine the precision of age estimates from different hard structures (otoliths, opercular bones, vertebrae, scales and cleithra) in order to select the most suitable ageing structure and (ii) to develop VBGF equation and describe precise growth-utilizing age estimates.

Materials and methods

A total of 173 *Schizopyge curvifrons*, 126 *Schizopyge niger* and 199 *Schizothorax esocinus* specimens were collected from Jhelum River and Lake Dal in the Kashmir valley, India from June 2011 to June 2012. The total length (TL) was recorded for each individual to be nearest to 0.1 cm and weight (W) nearest to 0.1 g. Identification of fishes was done following Day (1878) and Kullander et al. (1999).

Age reading techniques

Scales, opercular bones, otoliths, vertebrae and cleithra were examined to compare their age estimates as per standard protocols (Khan and Khan 2009; Khan, Khan, and Miyan 2011a).

Collection and preparation of scales

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).

Collection and preparation of opercular bones

The opercular bones were removed and dipped in boiling water for a few minutes to remove the extraneous tissue. A bristle brush was used to remove the remaining tissues. The cleaned opercular bones were dried at room temperature and examined under transmitted fluorescent light with the naked eye (Phelps, Edwards, and Willis 2007; Khan and Khan 2009).

Collection and preparation of otoliths

Otoliths (Sagittae) were removed with a pair of fine forceps, rinsed with distilled water and stored dry in labelled envelopes. Otoliths were read whole by immersing them in glycerol and examined under a microscope using reflected light. Otoliths with unclear annual rings were ground with sandpaper to make the annuli more distinct for age reading (Tandon and Johal 1996; Khan and Khan 2009).

Collection and preparation of vertebrae

Vertebrae (4th–10th) were removed and placed in boiling water for 10–15 min to remove the attached muscles. A bristle brush was used to remove the remaining tissues. Vertebrae were examined by shining a fibre-optic light near the bottom of the structure to illuminate annuli under a dissecting microscope (Phelps, Edwards, and Willis 2007; Khan and Khan 2009).

Collection and preparation of cleithra

Cleithra were removed from fresh specimens, and the muscles were separated by dipping them in boiling water for 5 min. The cleaned and dried cleithra were examined under transmitted fluorescent light with a dissecting microscope (Euchner 1988).

Measures of precision

Each structure was examined for age estimation independently by two readers. Age assessments of all fish samples were done in random order and without prior information on fish length, weight or date of collection. In *S. curvifrons* and *S. niger* specimens, each alternative structure (scales, opercular bones, vertebrae and cleithra) was paired with otoliths (showing high percent agreement [PA], low average percent error [APE] and coefficient of variation [CV] values) and in case of *S. esocinus* specimens, each alternative structure was paired with vertebrae (showing high PA, low APE and CV values).

Age readings were tested for bias and precision. Age bias plots were constructed to identify trends and sources of bias in discrepancies between age estimates among successive readings. Precision between pairs of structures was quantified as an APE, CV and PA between the readers and between the pairs of ageing structures (Campana, Fowler, and Jones 1994). APE was derived using the formula presented by Beamish and Fournier (1981):

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

where x_{ij} is the ith age determination of the jth fish, x_j is the mean estimate of the jth fish and R is the number of times each fish is aged.

CV expressed as the ratio of standard deviation to the mean, was computed as follows Chang (1982):

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

where CV_j is age precision for the jth fish. Low values of CV and APE indicate high levels of precision.

PA between structures was also calculated to further interpret precision and was estimated as the proportion of each age on which both readers agreed.

Growth analysis

To evaluate variability in growth, the recorded length-at-age data based on otoliths in *S. curvifrons* and *S. niger* individuals and vertebrae in *S. esocinus* were fitted to the VBGF (Ricker 1975) by non-linear least squares regression. The VBGF is represented as:

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

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; and
t_0 = the hypothetical age at which the fish had zero length.

VBGF equation was also computed using one of the most common and non-lethal ageing structures, scales, in order to compare VBGF parameters to the one obtained using the precise ageing structure.

The concept of growth performance index introduced by Pauly and Munro (1984) makes it possible to directly compare growth performance of different populations of the same species based on von Bertalanffy growth parameters. The performance index ($\varnothing$) is calculated as:

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

The performance index was estimated using growth parameters L_∞ and K obtained for the target fish species.

Data analysis

PA was calculated using the "Templates for calculating ageing precision" by Sutherland (2006). One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was used to test the degree of variation among age readings from various ageing structures (Gomez and Gomez 1984; Khan, Khan, and Miyan 2011b). Growth Parameters were estimated using non-linear regression methods as implemented in a Microsoft Excel-based application developed by Cope and Punt (2007).

All statistical analyses were done using MS Excel and SPSS (version 12.0).

Results

Age estimation

Schizopyge curvifrons

The age of *S. curvifrons* specimens ranged from 1 to 6 years. The highest PA between two independent readers was recorded for otoliths (95.4%) followed by opercular bones (93.6%), vertebrae (91.3%), scales (86.1%) and cleithra (82.7%) (Table 1). Between structures, the highest PA was found between otoliths and opercular bones (88.4%), followed by vertebrae (82.1%), scales (74.0%) and cleithra (68.8%) (Table 2). In *S. curvifrons*, otoliths and opercular bones showed the lowest values of APE and CV as compared to other structures substantiating high precision in detecting annuli. There was no ageing bias observed between readers for otoliths, while some ageing bias was found between age estimates from scales and cleithra as shown by the age bias graph (Figure 1).

The comparison of the mean values of age estimates from different structures using ANOVA followed by DMRT showed that the highest ($p < 0.01$) values of age readings were obtained from otoliths followed by opercular bones, vertebrae, scales and cleithra (Table 3). Age readings from otoliths, opercular bones and vertebrae did not show any significant ($p < 0.01$) differences.

Schizopyge niger

In *S. niger*, PA between readers was higher for otoliths (94.6%) than scales (92.3%), vertebrae (84.6%), opercular bones (82.3%) and cleithra (80.0%) (Table 1). When otolith age estimates were compared with those from other structures, the highest PA was found between

Table 1. Comparison of percent agreement (PA), average percent error (APE) and coefficient of variation (CV) between age readings of two independent readers in *Schizopyge curvifrons*, *Schizopyge niger* and *Schizothorax esocinus*.

Between readers	PA	APE	CV
Schizopyge curvifrons ($N = 173$)			
Otoliths	95.4	0.92	1.30
Opercular bones	93.6	1.18	1.68
Vertebrae	91.3	1.62	2.30
Scales	86.1	2.59	3.48
Cleithra	82.7	3.36	4.75
Schizopyge niger ($N = 126$)			
Otoliths	94.6	1.49	2.10
Scales	92.3	2.88	4.07
Vertebrae	84.6	5.08	7.19
Opercular bones	82.3	7.27	10.29
Cleithra	80.0	8.13	11.49
Schizothorax esocinus ($N = 199$)			
Vertebrae	96.0	1.57	2.22
Opercular bones	95.0	1.68	2.98
Scales	93.5	1.89	2.67
Otoliths	90.5	3.57	5.05
Cleithra	87.4	3.98	5.63

Note: N – total number of samples.

Table 2. Comparison of percent agreement (PA), average percent error (APE) and coefficient of variation (CV) of ages assigned between structures in *Schizopyge curvifrons*, *Schizopyge niger* and *Schizothorax esocinus*.

Between structures	PA	APE	CV
Schizopyge curvifrons ($N = 173$)			
Otoliths-opercular bones	88.4	1.75	2.47
Otoliths-vertebrae	82.1	2.40	3.37
Otoliths-scales	74.0	4.23	5.86
Otoliths-cleithra	68.8	4.91	7.12
Schizopyge niger ($N = 126$)			
Otoliths-scales	86.9	3.61	5.10
Otoliths-vertebrae	83.8	4.41	6.23
Otoliths-opercular bones	78.5	7.60	10.75
Otoliths-cleithra	73.8	9.46	13.37
Schizothorax esocinus ($N = 199$)			
Vertebrae-opercular bones	87.9	3.47	4.91
Vertebrae-scales	86.4	3.75	5.31
Vertebrae-otoliths	80.9	5.60	7.93
Vertebrae-cleithra	73.9	8.83	12.48

Note: N – total number of samples.

otoliths and scales (86.9%) followed by vertebrae (83.8%), opercular bones (78.5%) and cleithra (73.8%) (Table 2). Otoliths and scales showed the lowest values of APE and CV as compared to other structures. No age bias was present between readers for otoliths, scales and vertebrae, while some ageing bias was found in opercular bones and cleithra (Figure 2).

The mean values of age estimates from different structures showed that age estimates obtained from otoliths were significantly ($p < 0.05$) higher than those from cleithra but comparable ($p < 0.05$) to the values from scales, vertebrae and opercular bones (Table 3).

Schizothorax esocinus

Between-readers agreement for vertebrae was higher (96.0%) than for opercular bones (95.0%), scales (93.5%), otoliths (90.5%) and cleithra (87.4%) (Table 1). When age estimates from vertebrae were compared with other structures, the highest PA was found between vertebrae and opercular bones (87.9%) followed by scales (86.4%), otoliths (80.9%) and cleithra (73.9%) (Table 2). Vertebrae, opercular bones and scales showed the lowest values of APE and CV. No indication of reader bias was noted for any structure (Figure 3).

Mean values of age estimates from different structures showed that values obtained from cleithra were significantly ($p < 0.05$) lower than the readings obtained from all other structures except otoliths (Table 3). Also, age readings from vertebrae, opercular bones, scales and otoliths were comparable ($p < 0.05$) with each other.

Growth

The von Bertalanffy growth parameters as well as growth performance index ($\o'$) of the selected fish species are presented in Table 4.

S. curvifrons

The following growth equation was obtained using otoliths as an ageing structure:

$$L_t = 49.8\left(1 - e^{-0.263(t+0.34)}\right)$$

S. niger

The von Bertalanffy growth curve was fitted to the TL-at-age data using otoliths. The following growth equation was obtained:

$$L_t = 44.8\left(1 - e^{-0.255(t+1.42)}\right)$$

S. esocinus

The VBGF parameters obtained using vertebrae are presented in the following equation:

$$L_t = 66.6\left(1 - e^{-0.278(t+0.34)}\right)$$

The von Bertalanffy growth curve is shown in Figure 4. Greater predicted lengths were estimated for older scale-aged specimens than for otolith-aged specimens. The von Bertalanffy growth parameters constructed from scale data had a higher asymptotic length and a lower growth coefficient than those found in the model based on otolith in *S. curvifrons* and *S. niger* and vertebrae in *S. esocinus*. The growth performance index was found to be the highest in *S. esocinus* specimens (3.09) followed by *S. curvifrons* (2.81) and *S. niger* (2.71), respectively.

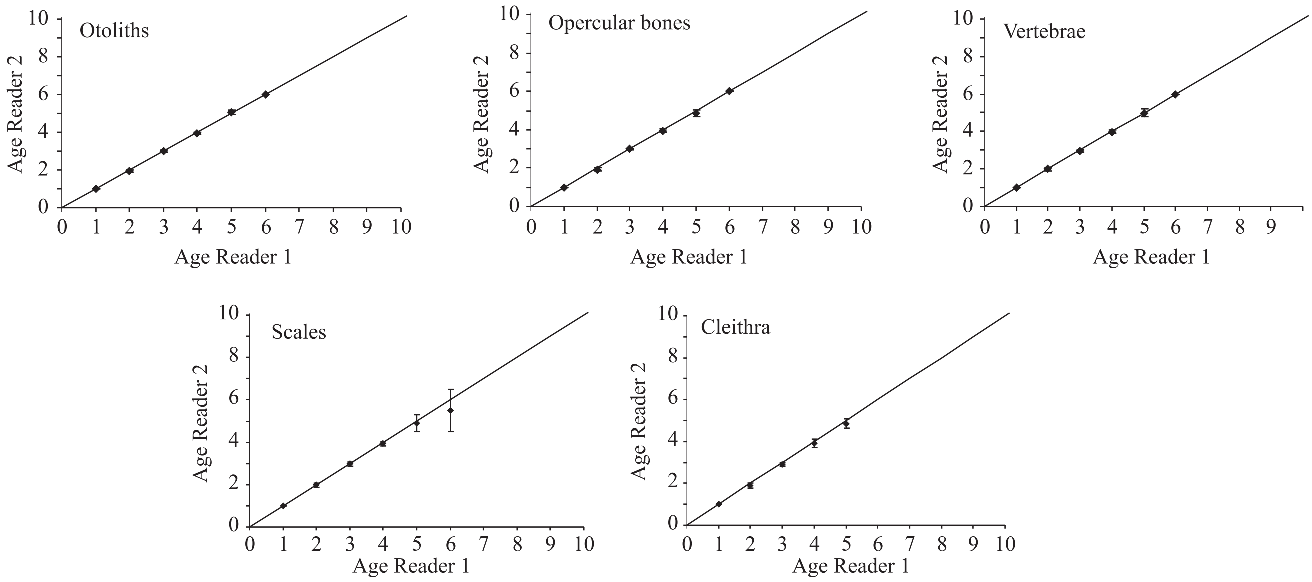

Figure 1. Age bias plots for comparison of age estimates between readers for scales, opercular bones, otoliths, vertebrae and cleithra in *Schizopyge curvifrons*.

Table 3. Comparison of mean values of age estimates from different bony parts of *Schizopyge curvifrons*, *Schizopyge niger* and *Schizothorax esocinus*.

	Mean values of age estimates[1]		
Bony parts	*Schizopyge curvifrons* ($N = 173$)	*Schizopyge niger* ($N = 126$)	*Schizothorax esocinus* ($N = 199$)
Scales	2.9538^{a}	2.3308^{b}	2.8191^{b}
Opercular bones	3.1734^{b}	2.1538^{ab}	2.8392^{b}
Otoliths	3.2023^{b}	2.3846^{b}	2.5729^{ab}
Vertebrae	3.1676^{b}	2.1923^{ab}	2.8492^{b}
Cleithra	2.8671^{a}	1.9615^{a}	2.4724^{a}

[1]Values having similar superscripts in each column are insignificantly different ($p > 0.05$) from each other.
Note: N – total number of samples.

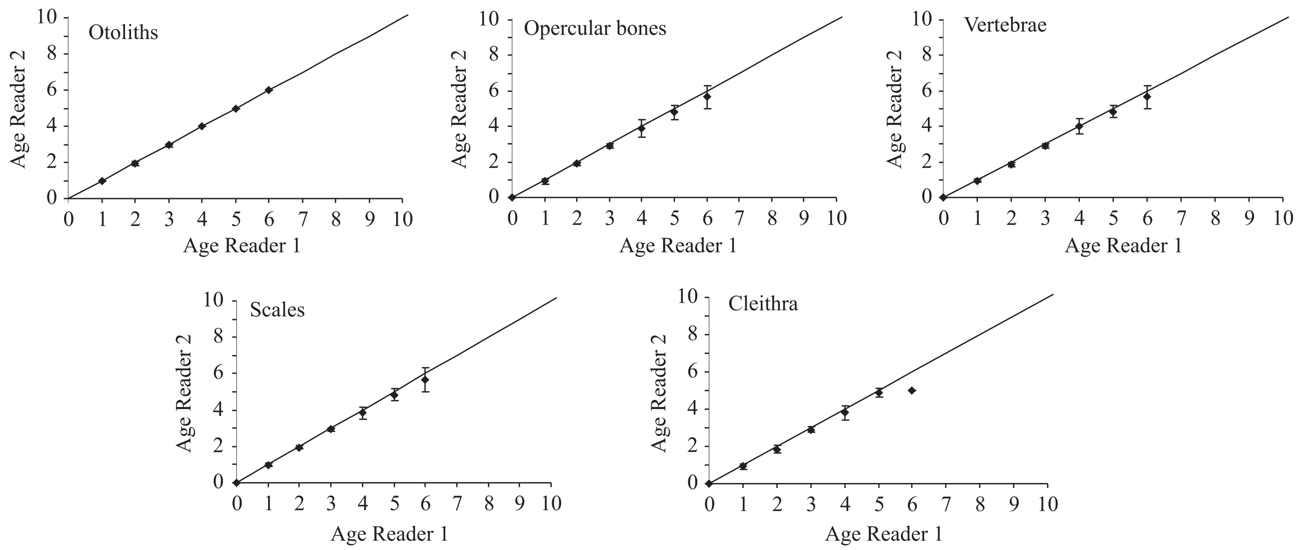

Figure 2. Age bias plots for comparison of age estimates between readers for scales, opercular bones, otoliths, vertebrae and cleithra in *Schizopyge niger*.

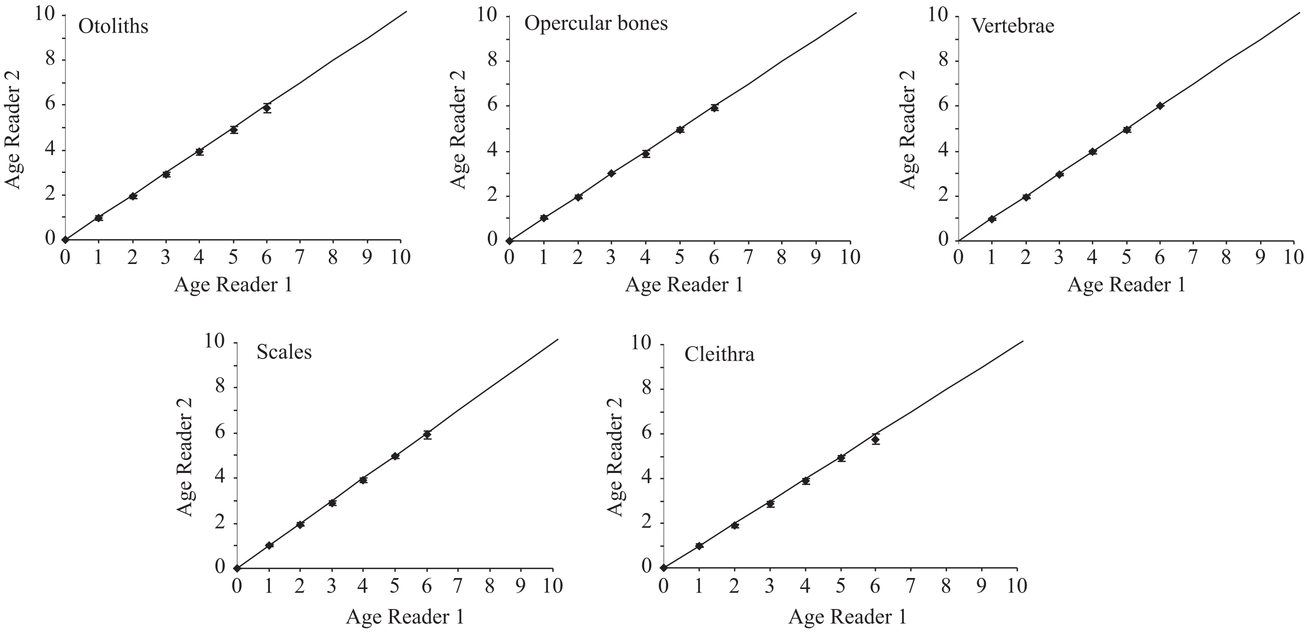

Figure 3. Age bias plots for comparison of age estimates between readers for scales, opercular bones, otoliths, vertebrae and cleithra in *Schizothorax esocinus*.

Table 4. von Bertalanffy growth parameters along with growth performance index ($\varnothing'$) in *Schizopyge curvifrons*, *Schizopyge niger* and *Schizothorax esocinus*.

Species	Method of age determination	L_∞ (cm)	Max. TL recorded (cm)	K (cm year^{-1})	T_o	$\varnothing'$	Max. age
Schizopyge curvifrons	Otoliths	49.8	42.9	0.263	−0.34	2.81	6
	Scales	57.0		0.216	−0.42	2.84	
Schizopyge niger	Otoliths	44.8	37.3	0.255	−1.42	2.71	5
	Scales	47.2		0.254	−1.25	2.76	
Schizothorax esocinus	Vertebrae	66.6	61.1	0.278	−0.34	3.09	6
	Scales	74.0		0.230	−0.45	3.10	

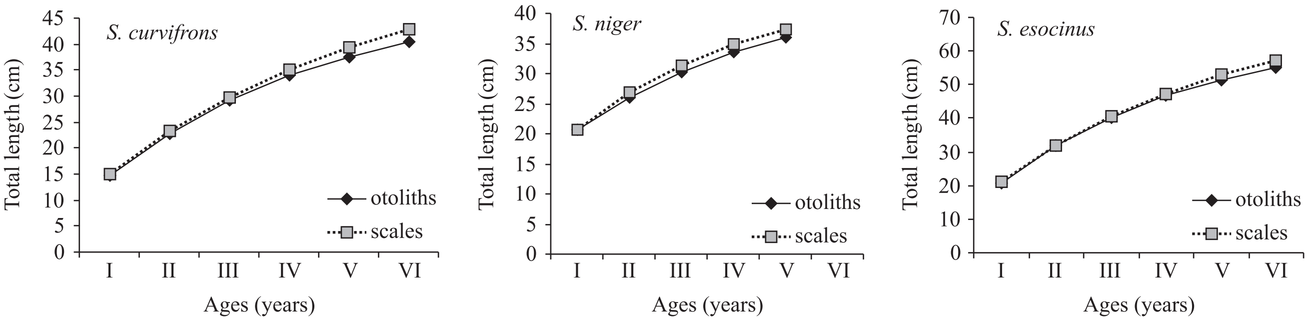

Figure 4. The von Bertalanffy growth curves for *Schizopyge curvifrons*, *Schizopyge niger* and *Schizothorax esocinus*. Symbols represent back-calculated length at estimated age.

Discussion

Ageing precision

Imprecise age estimates suggest variability in ageing criteria or the presence of unclear annuli that are difficult to distinguish and count, while inaccurate age estimates bias population parameters such as growth and mortality (Quinn and Deriso 1999). Despite the call for more statistically robust and consistent analysis of ageing data in recent years (Beamish and Fournier 1981; Chang 1982; Campana, Fowler, and Jones 1994), many studies still report population data without any mention of the precision of their age estimates.

Otoliths yielded the most precise age estimates of *S. curvifrons* and *S. niger* specimens. Studies have consistently shown higher precision in ages determined from otoliths than from scales. This is in accordance with a mounting body of evidence that the scale method of age estimation in fishes belonging to the subfamily Schizothoracinae may be unreliable (Chen, Chen, and He 2009; Li et al. 2009; Ma et al. 2011). Otoliths were reported to provide the most reliable age estimation, while annuli on vertebrae and opercular bones were not very clear in *Gymnocypris selineuoensis* (Chen, He, and Chen 2002; Chen, He, and Duan 2002; Ma et al. 2011). Otoliths continue to grow and form annuli even when body growth slows down and asymptotic length is reached, and annuli reabsorption does not appear to occur during periods of food limitation or stress (DeVries and Frie 1996; Maceina and Sammons 2006). Accurate age estimation using otoliths is based on the premise that otoliths are metabolically inert and thus do not reflect physiological changes that may occur throughout the life of fish (Phelps, Edwards, and Willis 2007). Further, otoliths do not show reabsorption and their growth is acellular rather than by calcification (Secor, Trice, and Hornick 1995). In Transcaucasian barb, *Capoeta capoeta umbla* (Heckel) (Ekingen and Polat 1987) and Atlantic horse mackerel, *Trachurus trachurus* (Linnaeus) (Polat and Kukul 1990), otoliths were reported to be the most reliable ageing structure. Isermann et al. (2003) reported that otoliths provide the most time-efficient and precise approach for age estimation in walleyes, *Stizostedion vitreum* (Mitchill) compared to scales, sagittal otoliths and dorsal spines.

Among all age determination structures, vertebrae were found to be the most suitable for *S. esocinus*. In corroboration of the present findings, vertebrae have been reported to be the most suitable ageing structure giving precise age estimates in *Alosa pontica* (Yilmaz and Polat 2002) and *Cyprinus carpio* (Yilmaz and Polat 2008). Ma et al. (2011) reported that age estimates of *S. o'connori* from vertebrae and otoliths matched well, while age estimates from opercular bones appeared to underestimate age. But Chatwin (1956) maintained that vertebrae are not practical for commercially caught fish due to the time required for processing the structure and the damage caused to the fish carcass during sampling. Thus, it is suggested that if collection of the most suitable structure (e.g. otoliths/vertebrae in the present study) is not affordable due to any reason, another suitable ageing structure should be selected on the basis of exhibiting insignificant values, minimal error and bias of age estimates compared to the most suitable ageing structure.

Scales have been the most widely used ageing method for the majority of cyprinids (Kamilov 1984; Phelps, Edwards, and Willis 2007) primarily due to advantages such as ease of their collection and preparation, and, more importantly, because of the method being non-destructive to fish (DeVries and Frie 1996). Scales have been reported to provide precise age estimate when compared to other structures in several fish species, such as *Morone saxatilis* (Welch et al. 1993), *L. rohita* and *C. marulius* (Khan and Khan 2009) and *C. mrigala* (Khan, Khan, and Miyan 2011a). However, in some scientific reports, the use of scales has been criticized mainly because of the frequent underestimation of age in older fish (Beamish and McFarlane 1987). The imprecise age determination from scales has been accounted for reabsorption and deposition of false annuli due to stress and food limitation, and for the fact that annuli become obscure because scale growth tends to cease as fish grow older (Beamish and McFarlane 1987; Maceina and Sammons 2006).

When compared with other ageing structures (i.e. scales, opercular bones, vertebrae and cleithra), age estimates from otoliths in *S. curvifrons* were found to closely match those from opercular bones. Khan and Khan (2009) suggested that annuli in opercular bones provided precise age estimation of *C. catla*. Opercular bones were reported to be more advantageous compared to scales for the age estimation of common carp (McConnell 1952). The determination of age and growth of fish from opercular bones is well established in fishes of temperate waters and has also been found to be more satisfactory than other methods such as scales, vertebrae, spines or other hard parts in *Esox lucius* (Linnaeus) (Frost and Kipling 1959). However, in some fishes opercular bones were found to be less reliable than other structures such as otoliths and vertebrae in *Schizothorax o'connori* (Ma et al. 2011) and scales and otoliths in *L. rohita* and *C. marulius* (Khan and Khan 2009). Gumus et al. (2010) rejected opercular bones for age estimation in *M. mastacembelus* due to the loss of most of their primary annuli that represent the early period of life history.

Cleithra have been used for fish age estimation by various researchers (Casselman and Crossman 1986; Sharp and Bernard 1988; DeVries and Frie 1996; Quist et al. 2007). Govind and Gopal (1966) found valid growth rings in the cleithral bone of *Silonia childrenii*. Laine, Momot, and Ryan (1991) noted that scales are not so well-suited for pike-age determination as cleithra. Brennan and Cailliet (1989) evaluated a variety of calcified age structures (pectoral fin rays, opercula, clavicles, cleithra, medial nuchals and dorsal scutes) used to age white sturgeon *Acipenser transmontanus* and found that ages estimated from these structures did not vary significantly. However, in the present study, the mean values of age estimates based on cleithra were found to be significantly different from those of all other structures except scales in *S. curvifrons*, opercular bones in *S. niger* and otoliths in *S. esocinus*.

Growth parameters

Nothing has been published yet on the development of VBGF equation using precise age estimates in the selected fish species. VBGF has only three parameters, making it statistically robust compared to other fish growth models with more parameters (Booth 1997). The growth coefficient (k) is a useful index for estimating the potential vulnerability of stocks to excessive exploitation and for comparing life history strategies (Pratt and Casey 1990; Musick 1999). Branstetter (1987) categorized k values as 0.05–0.10/year for slow-growth 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 was in the range 0.20–0.30/year. 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 *S. niger* was higher than the value of the observed maximum size, which was likely due to the smaller number of large specimens. Growth model estimates are greatly affected by the lack of very young or old individuals (Cailliet and Goldman 2004; Ma et al. 2010). In *S. curvifrons* and *S. esocinus,* due to relatively large sample size and rather good agreement of L_∞ with the observed maximum length, values appear to be more reliable and clearly underline the trend for higher growth coefficients in smaller sized species (Frisk, Miller, and Fogarty 2001).

In general, growth parameters need to be checked for quality and validity (Karlou-Riga and Sinis 1997). A negative value (close to zero) of t_0 is a good indicator of the determined ages' reliability (Kerstan 1985); the estimate of t_0 in the present study varied from −0.30 to −1.45.

VBGF parameters were generated using otoliths and scales both in *S. curvifrons* and *S. niger in* order to compare the results obtained from the two structures. Otoliths were selected because they were found to be the most suitable ageing structure in these two species. Scales were selected because they are often the most common structure for age estimation in fishes having scales for the reasons described elsewhere. The length-at-age data derived from otoliths and scales were significantly different in *S. curvifrons* (t-test for paired comparison; $t = 3.159$, df = 5, $p < 0.05$), but no significant differences were found in *S. niger* ($t = 2.588$, df = 4, $p > 0.05$). In *S. esocinus*, VBGF parameters were generated using vertebrae and scales in order to compare the results from the two methods. The mean TLs derived from vertebrae and scales were compared but no significant differences emerged (t-test for paired comparison; $t = 2.562$, df = 5, $p > 0.05$). Estimates of the asymptotic length derived from scales were higher for both species together with a moderate growth coefficient that shows that their size increased at similar ages. Thus, scale-age estimates could be utilized for developing VBGF equation in the selected fish species, except *S. curvifrons*, without affecting the outcome of growth parameters.

Acknowledgements

Authors are thankful to the Chairman of the Department of Zoology, Aligarh Muslim University, Aligarh, India for providing necessary facilities for the study. The Animal Care Committee of the Department has approved the research of food fishes. The first author gratefully acknowledges the financial assistance in the form of UGC non-NET Fellowship.

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