Estonian Journal of Ecology, 2011, **60**, 3, 183–193 doi: 10.3176/eco.2011.3.02

Comparison of age estimates from otoliths, vertebrae, and pectoral spines in African sharptooth catfish, *Clarias gariepinus* (Burchell)

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Received 20 October 2010, revised 11 January 2011

Abstract. Otoliths, vertebrae, and pectoral fin spine sections were compared to ascertain the best ageing structure in *Clarias gariepinus*. Standard procedures were followed to prepare and study the age structures. All ageing structures showed alternating opaque and translucent bands that were interpreted as annuli. Age estimates were evaluated for comparison between readers and among structures. Among all structures otoliths showed highest (95.6%) agreement between readers, followed by vertebrae (91.2%) and pectoral spine sections (79.7%). Due to the highest values of percent agreement and lowest average percent error and coefficient of variation values between two readers, otoliths were considered to be the most suitable structure for ageing *C. gariepinus*. When otoliths' ages were compared with other bony structures, viz. vertebrae and pectoral spine sections, the highest percent agreement and lowest average percent error and coefficient of variation values were found between otoliths and vertebrae age estimates (90.7%). Mean age estimates from otoliths were comparable ($P > 0.05$) to the values obtained from vertebrae but significantly ($P < 0.05$) different to those from pectoral fin spine sections.

Key words: ageing, bony structures, *Clarias gariepinus*, percent agreement, average percent error.

INTRODUCTION

Estimates of fish ages provide important demographic parameters to analyse and assess fish populations (Maceina & Sammons, 2006). Many structures have been used to estimate the age of fishes, including scales, otoliths, vertebrae, fin rays and 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 structure to age the fish. Ages of fish are estimated by the comparison of age estimates from various bony structures and different readers (Barnes & Power, 1984). The most suitable ageing method may vary among species. Thus, the evaluation of the precision of bony structures by readers should be studied (Baker & Timmons, 1991). A measure of precision is a valuable means of assessing the relative ease of determining the age of a particular structure, of assessing the reproducibility of an individual's age determinations, or of comparing the skill level of one ager relative to that of others (Campana, 2001). Furthermore, ageing errors must be considered before deciding on the most reliable bony structure for the ageing of fish (Kimura & Lyons, 1991). Comparison of age estimates between structures is an alterative technique to validation that may provide useful information on the accuracy and bias of age estimating structures (Sylvester & Berry, 2006). Several studies have focused on comparing ages estimated from different bony structures in an attempt to quantify the most suitable age estimate and to identify possible bias associated with each structure. Comparisons of age estimates from various structures have been performed for many fish species, including black crappies, *Pomoxis nigromaculatus* (Kruse et al., 1993); yellow perch, *Perca flavescens* (Niewinski & Ferreri, 1999); river carp suckers, *Carpiodes carpio* (Braaten et al., 1999); channel catfish, *Ictalurus punctatus* (Buckmeier et al., 2002); thinlip grey mullet, *Liza ramada* (Gocer & Ekingen, 2005); common carp, *Cyprinus carpio* (Phelps et al., 2007); Tibetan catfish, *Glyptosternum maculatum* (Li & Xie, 2008); bull trout, *Salvelinus confluentus* (Zymonas & McMahon, 2009); as well as rohu, *Labeo rohita*; catla, *Catla catla*; and giant snakehead, *Channa marulius* (Khan & Khan, 2009).

The African sharptooth catfish, *Clarias gariepinus* (Burchell, 1822) is a benthopelagic, dioecious, omnivorous fish widely tolerant to extreme environmental conditions (Yalcin et al., 2002). Several researchers have studied its age and growth estimation by using different ageing structures such as spines (van der Waal & Schoonbee, 1975; Bruton & Allanson, 1980; Quick & Bruton, 1984), vertebrae (Pivnicka, 1974; Willoughby & Tweddle, 1978), and otoliths (Bruton & Allanson, 1980; Quick & Bruton, 1984). To the best of our knowledge the only paper available on comparison of age estimation in *C. gariepinus* deals with comparing age estimates obtained from pectoral spines and vertebrae (Clay, 1982). In a recent study (Weyl & Booth, 2008) saggital otoliths were validated for ageing *C. gariepinus*. There are, however, no published reports available on comparison of age estimates from otoliths, vertebrae, and pectoral spines, which are widely used for age estimation in *C. gariepinus*.

Therefore, the present study was undertaken with the following objectives: (1) to evaluate and compare age estimates of different structures (i.e., otoliths, vertebrae, and pectoral spines) between readers and between pairs of ageing structures and (2) to quantify potential biases of age estimates between readers and between pairs of ageing structures in order to select the most suitable bony structure for age estimation of *C. gariepinus*.

MATERIALS AND METHODS

Samples of *Clarias gariepinus* ($N = 182$) were collected monthly from the local fish market at Aligarh, U.P., India, during the period from May 2008 to April 2010. Total length (TL) of each fish was measured from the tip of snout to the longest fin ray of the caudal fin (in mm). Body weight (in grams) was recorded as total weight (TW) including gut and gonads. For each fish, annuli were counted on the ageing structures independently by two readers without prior knowledge of fish length, weight, date of collection, and age estimates from other bony structures.

Collection and preparation of structures for age estimation

Sagittal otoliths were removed, cleaned, immersed in ethanol, and examined with a dissecting microscope in whole view on a black background with reflected light. Otoliths with unclear annual rings were ground with sandpaper to make the annuli more distinct for age reading (Tandon & Johal, 1996). Vertebrae from the fish were extracted, placed in boiling water for 2–3 minutes to remove soft tissue, cleaned, air dried, and examined in xylol under microscope (Yalcin et al., 2002). Pectoral spines were sectioned using a jeweller's saw. Sections for each spine were mounted on microscope slides and aged under dissecting microscope (Buckmeier et al., 2002).

Calculations and statistical analysis

Age estimates were compared by calculating the average percent error (APE), coefficient of variation (CV), and percent agreement (PA) between the readers and between the pairs of ageing structures. To calculate APE we used the formula presented by Beamish & Fournier (1981):

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

where x_{ij} is the ith age determination of the jth fish, x_j is the average age calculated for the jth fish, and R is the number of times each fish was aged.

The coefficient of variation (Camapana, 2001) was calculated as the ratio of standard deviation over the mean, and can be written as

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

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 (Welch et al., 1993; Hoxmeier et al., 2001; Stolarski and Hartman, 2008; Koch et al., 2009), is not considered as a suitable measure of precision by several authors (Beamish & 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 "Templates 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. Age readings from each alternative structure (i.e., vertebrae and pectoral spine sections) were paired with the otoliths readings (which were validated for age estimation in *C. gariepinus* by Weyl & Booth, 2008) to calculate PA, APE, and CV.

Mean age readings (consensus data) 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 underestimation of age by a structure irrespective of fish size class. This may prove useful in selecting the structure(s) that may give statistically indifferent readings when size class is not taken into account.

RESULTS

The age composition of the sampled fish specimens based on different bony structures exhibited variation in their age estimates (Fig. 1). The PA of age readings between the two independent readers was the highest and the CV and APE the lowest for otoliths followed by vertebrae and pectoral spine sections (Table 1). The age estimates from otoliths by the two readers did not differ. In the age estimates between the readers from vertebrae no differences were observed up to 5 years of fish age, while slight differences in age readings were noticed in the fish of 6 years of age (Fig. 2). Differences in age estimates between the readers were found for pectoral spine sections after age 2, and these increased with fish age as indicated by larger standard error bars. Comparison of age estimates from the different bony structures revealed the highest PA and lowest APE and CV values between age estimates from otoliths and vertebrae while the lowest PA and highest APE and CV were observed between age estimates from otoliths and pectoral spine (Table 1). Age readings

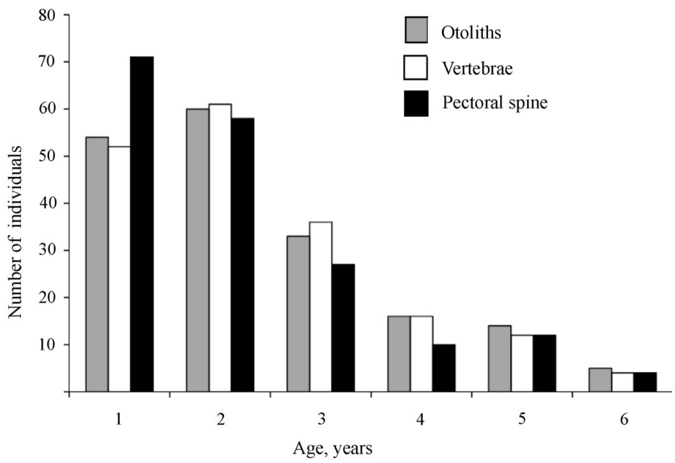

Fig. 1. Age composition derived from the readings of different ageing structures in *Clarias gariepinus*.

from otoliths and vertebrae were in good agreement while those of otoliths and pectoral spine sections differed substantially (Fig. 3). Mean values of age estimates from different structures, when compared using ANOVA followed by DMRT, showed that the mean age estimates from otoliths (2.33 ± 0.09) were significantly ($P < 0.05$) different from the values obtained from pectoral spine sections (2.01 ± 0.09). However, age estimates obtained from otoliths were comparable ($P > 0.05$) to those from vertebrae (2.13 ± 0.09). The values of age estimates from vertebrae and pectoral spine sections did not differ significantly either ($P > 0.05$).

Table 1. Comparison of percent agreement (PA), average percent error (APE ± standard error), and coefficient of variation (CV ± standard error) between the age readings of two independent readers and between pairs of hard anatomical structures in *Clarias gariepinus*

Hard part	PA	APE	CV
Between readers			
Otoliths	95.6	1.31 ± 0.00	1.86 ± 0.01
Vertebrae	91.2	2.43 ± 0.01	3.44 ± 0.01
Pectoral spine	79.7	6.85 ± 0.01	9.70 ± 0.02
Between structures			
Otoliths–vertebrae	90.7	2.72 ± 0.01	3.78 ± 0.01
Otoliths–pectoral spine	66.5	8.76 ± 0.09	12.65 ± 0.14

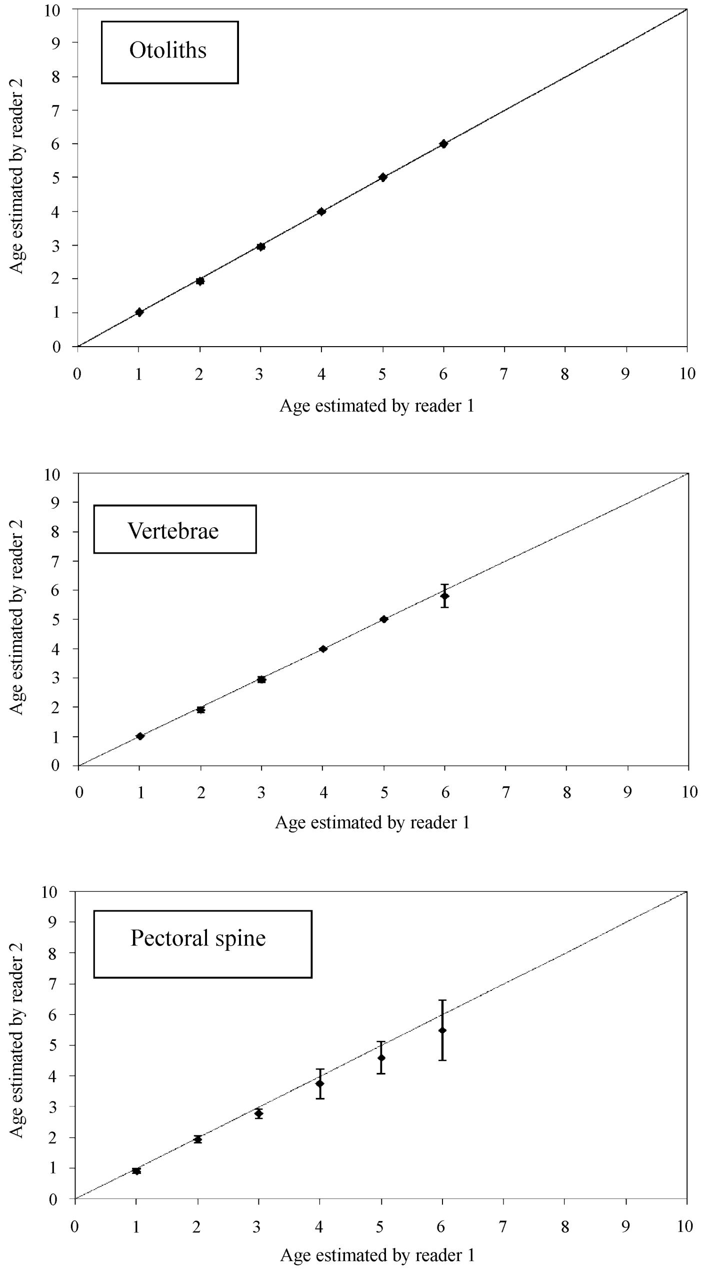

Fig. 2. Age bias graphs between two independent readers in age estimates from otoliths, vertebrae, and pectoral spines. Each error bar represents the 95% confidence interval. The 1 : 1 equivalence (solid) line is also indicated. Points above the line indicate ages that were overestimated, whereas a point below the line indicates ages that were underestimated.

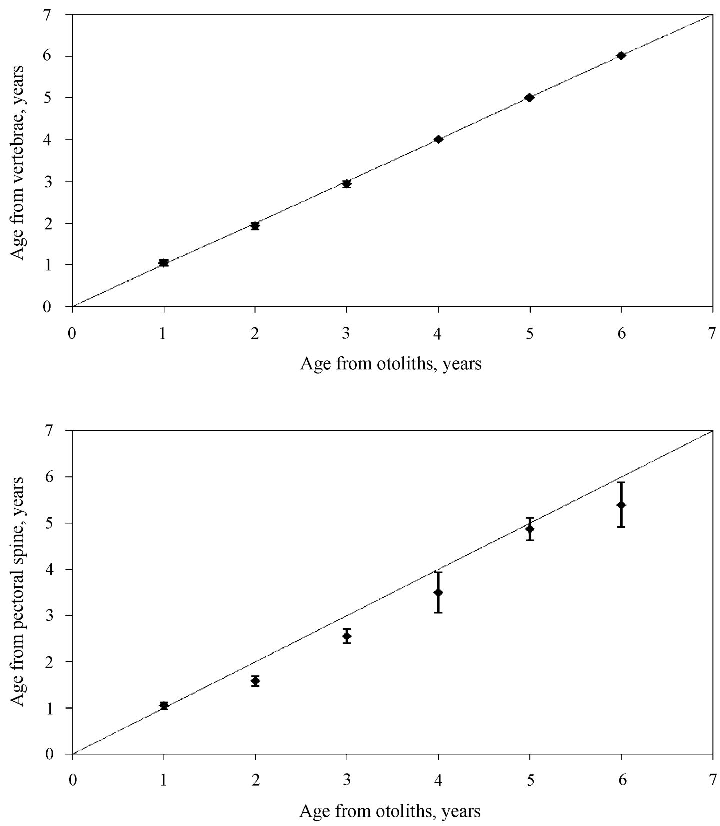

Fig. 3. Age bias graphs between age estimates from otoliths and vertebrae and from otoliths and pectoral spines. Each error bar represents the 95% confidence interval. The 1 : 1 equivalence (solid) line is also indicated. Points above the line indicate ages that were overestimated, whereas a point below the line indicates ages that were underestimated.

DISCUSSION

Comparison of age estimates from the three ageing structures within the current study revealed that otoliths provided the most suitable age estimates in *C. gariepinus*. 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) and also because otoliths are reported to be metabolically inert and thus do not reflect physiological changes that may occur throughout the life of fish (Phelps et al., 2007). Otoliths continue to grow and form annuli even as body growth slows and asymptotic length is reached, and annuli reasbsorption does not appear to occur during periods of food limitation or stress (DeVries & Frie, 1996). Otoliths were reported to be the most reliable ageing structure in a number of fish species such as *Chelidonichthys kumu* (Staples, 1971), *Capoeta capoeta umbla* (Ekingen & Polat, 1987), *Trachurus*

trachurus (Polat & Kukul, 1990), *Pylodictis olivaris* (Nash & Irwin, 1999), and *Ictalurus punctatus* (Buckmeier et al., 2002; Colombo et al., 2010).

Vertebrae provided age readings that were very close to those from otoliths as evident from the PA, APE, and CV values between the structures (Table 1). In other studies with other fishes, vertebrae and otolith age readings gave similar results for the age of burbot, *Lota lota*, and thus both the structures were recommended for age and validation studies of the fish (Guinn & Hallberg, 1990). However, vertebrae, although giving the most suitable estimates of age for lingcod, *Ophiodon elongate*, were not considered practical for commercial fish due to the time required for the processing of this bony structure and damage caused to the fish carcass during sampling (Chatwin, 1956). Previous researchers have used the vertebrae for age determination of *C. gariepinus* (Pivnicka, 1974; Willoughby & Tweddle, 1978; Yalcin et al., 2002). The rings on vertebrae were reported as a better indicator of growth (according to length frequency data) than those of pectoral spines in *C. gariepinus* (Clay, 1982). Due to paucity of information on the most suitable structure for ageing *C. gariepinus*, the majority of researchers have selected the ageing structure of their choice with the assumption of getting precise age estimates, which form the basis for the development of basic biological information. For instance, vertebrae reportedly provide reliable age estimation in *C. gariepinus* and have been used for the study of age and growth of the species from the Asi River, Turkey (Yalcin et al., 2002). Vertebrae were reported as the most suitable ageing structure showing the smallest ageing error as compared to scales and otoliths in *Pleuronectes flesus luscus* and it was recommended that studies involving rate of survival, growth, mortality, age composition, and reproduction rate of this species should use vertebrae as the most reliable structure for age determination (Polat et al., 2001). In a study on comparison of vertebrae, otoliths, and scales for ageing fall chum salmon, *Oncorhynchus keta*, Clark (1987) suggested that the time required to process and read vertebrae (twenty times as long as scales) made them less practical to use but the precision and accuracy involved with vertebrae made them the best of the three structures researched.

Within the current study, age estimates based on readings from pectoral spine sections showed significantly different values from those of otoliths. This observation was further supported by respective PA, APE, and CV values. In old fish, the lumen of the pectoral spine enlarges with age and obscures initial growth increments, resulting in a consistent underestimation of age in *C. gariepinus* (Clay, 1982; Quick & Bruton, 1984). In many species, the spine nucleus may be reabsorbed and replaced by a hole (vascularization), which may eliminate the first rings (Kohli, 1989; McFarlane & King, 2001). If first annulus is not identified correctly, the fish age will be underestimated leading to an overestimation of growth and natural mortality coefficients, which in turn may have drastic implications to fish stock management advice and decisions (Leaman & Nagtegaal, 1987; Casey & Natanson, 1992). A reduction in the accuracy of spine age estimates as a result of annulus loss was reported in adult striped bass, *Morone saxatilis* (Walbaum) and brown trout, *Salmo trutta* L. (Welch et al., 1993;

Graynoth, 1996). Buckmeier et al. (2002) reported that the underestimation and lack of precision for ageing ictalurids using spines occur due to the expansion of the central lumen, which obliterates early formed annuli, the appearance of multiple growth rings, and poor sectioning techniques.

It may be concluded from the study that otoliths are the most suitable ageing structure for *C. gariepinus* exhibiting the lowest APE and CV and the highest PA between age readers as compared to other bony structures. The information generated will be useful to fisheries managers and researchers to select the most appropriate structure for age estimation in the selected fish species.

ACKNOWLEDGEMENTS

The authors are thankful to the Chairman of the Department of Zoology, Aligarh Muslim University, Aligarh, India, for providing the necessary facilities for the study. The authors are also thankful to the anonymous reviewer and the editorial team of the Journal for their comments and suggestions that improved the presentation of the manuscript.

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Otoliitide, selgroolülide ja rinnauimekiirte alusel määratud vanuse võrdlus Aafrika sägal *Clarias gariepinus* (Burchell)

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Selgitamaks vanuse määramiseks sobivaimat luustruktuuri kalaliigil *Clarias gariepinus*, uuriti standardprotseduuri alusel võrdlevalt otoliite, selgroolülide ja rinnauimekiirte lõike, kasutades kahe spetsialisti vanusemääranguid. Ilmnes, et kõigil uuritud luustruktuuridel vaheldusid opaaksed ja hüaliinsed tsoonid, mida interpreteeriti kui aastaid. Kuna vanusemäärangute suurim kokkulangemine (95,6%) ja väikseim keskmine viga ning variatsioonikoefitsient registreeriti otoliitide puhul, siis töö tulemusena soovitatakse kala vanuse määramist otoliitide alusel. Erinevate luustruktuuride alusel määratud vanused olid sarnased ($P > 0{,}05$) otoliitide ja selgroolülide puhul ning oluliselt erinevad ($P < 0{,}05$) otoliitide ja rinnauimekiirte korral.