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SHORT REPORT

Assessing the accuracy of using whole and sectioned vertebrae to determine the age of an endemic sisorid catfish, *Glyptosternon maculatum*, in Tibet, China

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Abstract The purpose of this study was to develop a reliable method of age estimation for *Glyptosternon maculatum* (Sisoridae, Siluriformes) in the Yarlung Tsangpo River basin of Tibetan Plateau using whole and sectioned vertebrae. Sectioned vertebrae showed that translucent bands were separated by distinct opaque bands through the focus to the margin. Although whole vertebrae showed clear band patterns in the intermediate region, such band patterns are obscure around the region of the focus of the vertebrae in older fish. The whole and sectioned vertebrae methods produced consistent ages until age 6, but there was a trend towards greater differences between the two methods as age increased. These findings indicate that sectioned vertebrae are suitable for age estimation of this species.

Keywords Age determination · *Glyptosternon maculatum* · Sectioned vertebra · Whole vertebra

Introduction

Accurate determination of age is essential in order to understand the dynamics of fish populations; however, inaccurate age determinations can cause severe problems in fisheries management (Beamish and McFarlane 1983). For example, age underestimation may result in overly optimistic estimates of growth and mortality rates, leading to the serious overexploitation of the population and its eventual collapse (Campana 2001).

Glyptosternon maculatum is an endemic and demersal catfish (Sisoridae) that is distributed in the Yarlung Tsangpo River basin of the Tibetan Plateau, with the highest elevation of its distribution reaching 4,200 m. This species also supports important commercial fisheries for Tibet. Li and Xie (2008) compared sectioned otoliths and whole vertebrae in order to estimate the age of individuals of this species. They found that its otoliths were irregularly shaped and difficult to process, while whole vertebrae exhibited the best structure and allowed them to age fish up to 13 years of age. Although vertebrae have proved a useful calcified structure for age determination in fish, they have previously always been used for the age determination of short-lived fish species (Appelget and Smith 1951; Sturm 1984; Yoneda et al. 2002; Li et al. 2006), and numerous authors have shown that whole vertebrae tend to underestimate the ages of older individuals (Lee et al. 1983; Prince et al. 1985). Thus, the objective of this study was to compare age determinations of *G. maculatum* based on whole and sectioned vertebrae, and to assess whether the use of sectioned vertebrae can improve the accuracy of age estimates.

Materials and methods

Fish sampling and data collection. Fish samples were collected from the Yarlung Tsangpo River basin in November 2005 and from March to September 2006 by floating gill net and set net. In the laboratory, all fish were

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measured to the nearest 0.1 mm in standard length (SL), and weighed to the nearest 0.1 g in total weight (W). Preliminary observations indicated that the fifth vertebra was optimal for age determination, so this vertebra was selected from 200 fish from 69.7 to 283.0 mm in SL.

Preparation and examination of age structures. Whole vertebrae were cleaned of excess tissue and soaked in a 4% sodium hydroxide solution for 2 days to remove remaining tissue. After cleaning, each vertebra was soaked in 95% ethanol for 30 min and 30% hydrogen peroxide for 15 min successively. Digital images of each whole vertebra were obtained under reflected light using a Leica DC100 digital camera attached to a Leica GZ6 stereomicroscope. After these images had been collected, each vertebra was sagittally sectioned adjacent to the center of its focus with a small handsaw, and then mounted onto a glass slide with resin and burnished into a 0.5 mm slice. The resulting "bow-tie" sections were photographed with a Leica DC100 digital camera attached to a Leica GZ6 stereomicroscope using transmitted light.

Vertebral images were aged in a blind/independent manner three times by two experienced readers. The band marks on the vertebral images were read along a counting path from the focus to the dorsal edge; only band pairs including one completely opaque band and one completely translucent band represent a whole year's growth. Two adjacent bands were considered annuli and counted.

To detect if the first annulus had disappeared, the radii of the first annuli of the whole and sectioned vertebrae were compared with the radius of young-of-year (YOY) vertebrae. The first annuli of the whole and sectioned vertebrae were measured along the axis used for aging. The radius of YOY vertebrae (r_1) was obtained by measuring five individuals (SL <80 mm) captured in April–July at the time of first annulus formation.

Data analysis. The growth band periodicities and the times of annuli formation on sectioned vertebrae were determined by marginal increment ratio (MIR) analysis using the following equation (Natanson et al. 1995):

$$\mathrm{MIR} = (VR - r_{\max})(r_{\max} - r_{\max-1})^{-1}$$

where VR is the radius of the sectioned vertebra, $r_{\max}$ is the ring radius for the the last annulus, and $r_{\max-1}$ is the ring radius for the penultimate annulus (Fig. 1). Samples were separated into an immature (1–6 years old) group and a mature (7–18 years old) group based on reproduction information for this species (Ding et al. 2010), and then MIR analysis was performed on the immature and mature fish.

To assess the precision of the age determination for an individual, the average percent error (APE) (Beamish and Fournier 1981) was calculated for both whole and sectioned vertebrae:

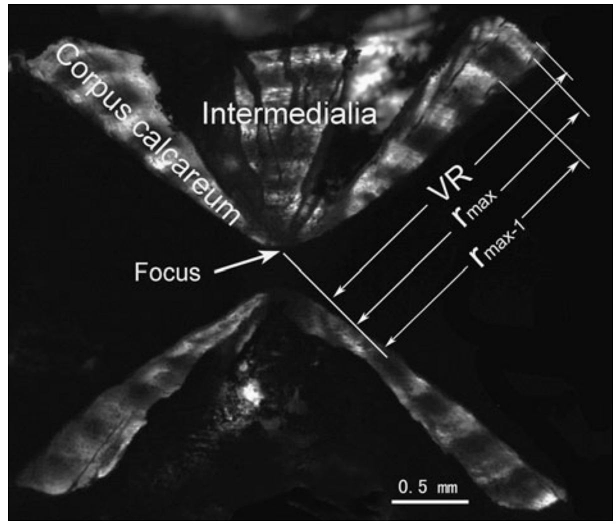

Fig. 1 An example of the measurement of a sectioned vertebra of *Glyptosternon maculatum*, and associated nomenclature. *VR* vertebral radius, r_{max} ring radius for the last annulus, r_{max-1} ring radius for the penultimate annulus

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

where x_{ij} is the ith age determination of the jth fish, x_j is the mean age estimate for the jth fish, and R is the number of times each fish is aged. The upper limit for the APE was arbitrarily set at 15%. If the APE index exceeded 15%, the vertebra was not used in the analysis because it was considered to be unreadable. When averaged across all of the samples, the APE becomes the index of average percent error (IAPE). For vertebrae with statistically acceptable APE indices, the estimated age was the rounded mean of six readings.

Results

Annular characteristics of whole and sectioned vertebrae. Band patterns of whole vertebrae are clear in the intermediate region, compassed along the margins, and obscured in the regions near the focii of whole sectioned vertebrae from older individuals (Fig. 2a). In the sectioned vertebral images, a banding pattern from the focus to the margin was readily distinguishable in sectioned vertebrae; opaque bands alternated with translucent opaque bands on the sectioned vertebrae (Fig. 2b).

Figure 3 shows the relationship between the radii of the first annuli of whole and sectioned vertebrae and the estimated age based on sectioned vertebrae. The mean first radius of YOY vertebrae (r_1) was 426 μm. There was no variation across all age groups of sectioned vertebrae, and no significant difference was detected between the radius

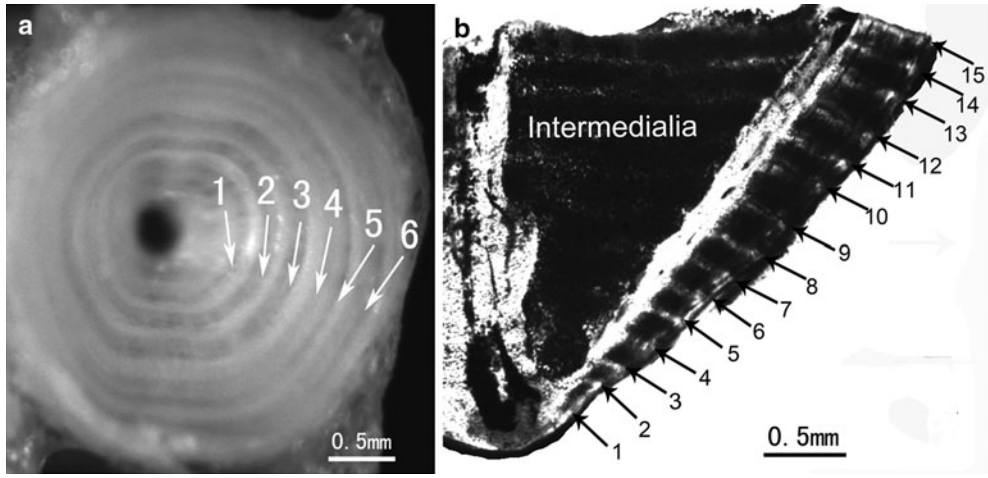

Fig. 2 Whole (**a**) and sectioned (**b**) vertebrae of *Glyptosternon maculatum* viewed under reflected and transmitted light, respectively. Whole-vertebral image (**a**) from an individual (178 mm SL) that was assigned an age of 6 years, and a sectioned-vertebral image (**b**) from an older individual (257 mm SL) that was assigned an age of 15 years. *Numbers* and *arrows* show annuli

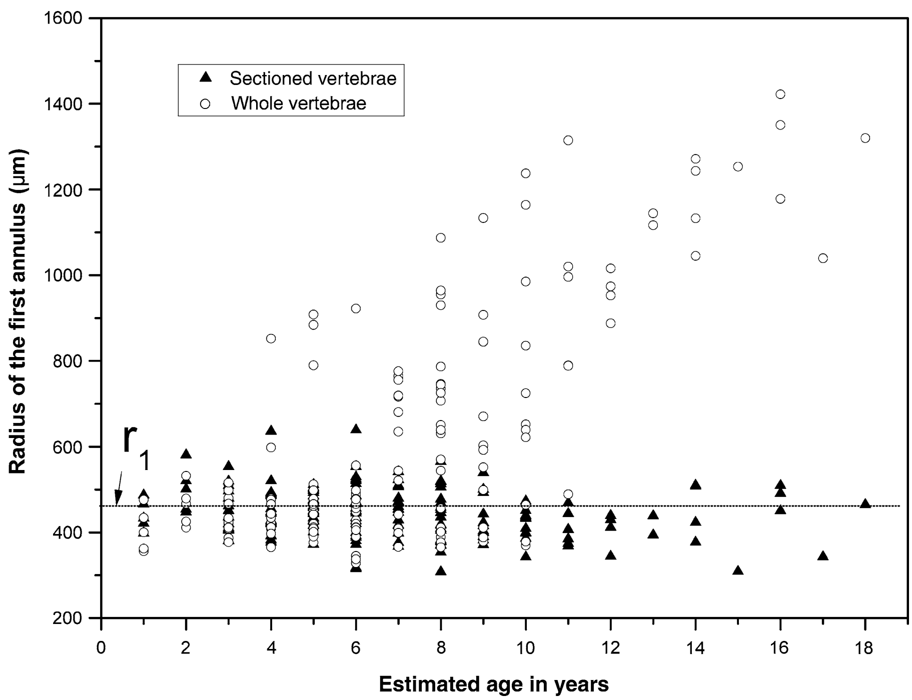

Fig. 3 The relationship between age in years estimated using sectioned vertebrae and the radii of the first annuli of whole and sectioned vertebrae in *Glyptosternon maculatum*. *Horizontal line* represents the radius of the first annulus (r_1) (426 μm), as obtained by measuring the radius of young-of-year vertebrae at the time of annulus formation

of the first annulus from sectioned vertebrae and r_1 (t test, $P > 0.05$). However, for the whole vertebrae, there was a significant trend for an increased radius of the first annulus with age (t test, $P < 0.05$).

Annular periodicity and precision of age estimates. MIR analysis was performed on immature (1–6 years old) and mature (7–18 years old) fish separately (Fig. 4), but the results of the analysis did not differ between immature and mature fish. MIR analysis showed that the annuli mainly form annually between April and July; the smallest MIR occurred in May, followed by a consistent increase in MIR, and a peak in November and March. MIR analysis confirmed that one translucent and one opaque band were deposited each year.

Estimates of APE ranged from 0 to 32% for whole vertebrae and from 1 to 22.5% for vertebrae. Of the 200 processed vertebrae, 183 whole vertebrae were readable and 189 sectioned vertebrae were readable, resulting in IAPEs of 6.3 and 4.9%, respectively. These IAPEs indicated that the precision of the age estimation process was acceptable.

Age bias between whole and sectioned vertebrae. A comparison of the ages estimated from whole and sectioned vertebrae indicated that the two methods produced consistent ages until age 6 (paired-sample t test, $P > 0.05$); thereafter, the whole vertebrae tended to yield more underestimated ages than those estimated via sectioned vertebrae (paired-sample t test, $P < 0.01$), and there was a

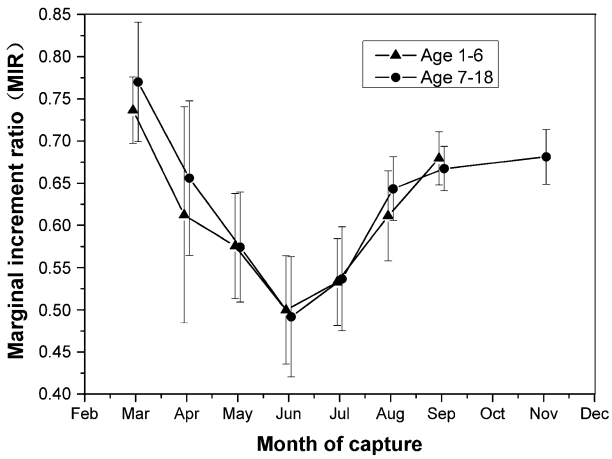

Fig. 4 Monthly changes in mean marginal increment ratio for specimens in the 1–6 and 7–18 year old classes of *Glyptosternon maculatum*. *Vertical lines* indicate standard errors

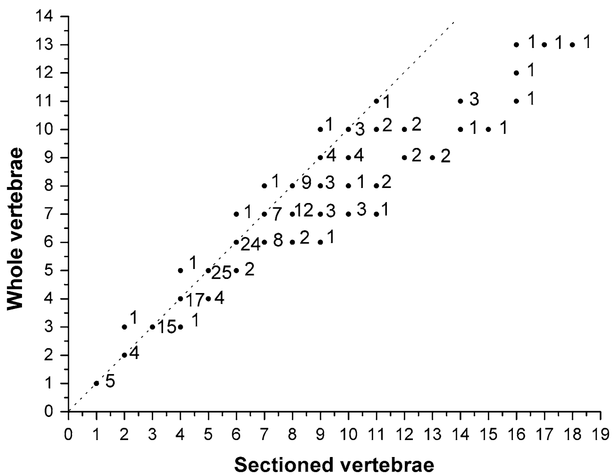

Fig. 5 The relationship between ages estimated from whole and sectioned vertebrae of *Glyptosternon maculatum*. The deviation from the *1:1 line* shows the extent of the aging bias. The *numbers to the right of the dots* indicate numbers of specimens

trend for the difference between the two methods to increase as the age increased (Fig. 5). The maximum estimated ages were 13 and 18 years for the whole and sectioned vertebrae, respectively.

Discussion

This study showed that sectioned vertebrae provide a useful approach for determining the ages of individuals of *Glyptosternon maculatum*. The relatively low IAPE (4.9%) demonstrated that the readability of the sectioned vertebrae was high. Marginal increment ratio analysis supported the hypothesis that the mechanisms that lead to band pairs on sectioned vertebrae from immature or mature fish occurred once per year, and that the opaque band was deposited during the summer, when the water temperature increased from 7.9 to 13.9°C (wild survey). The mechanisms of annuli formation on sectioned vertebrae closely resemble those of sectioned otoliths from fish that occur in the Tibetan Plateau (Chen et al. 2002; Qiu and Chen 2009). Otoliths are the structures that are most frequently used to estimate fish age (Casselman 1987), but the sagittal and lapillar otoliths of *G. maculatum* are both very small and irregular in shape. Therefore, otoliths are not suitable for age determination in this species.

In this study, compared to the sectioned vertebrae method, the traditional method of aging whole vertebrae from *G. maculatum* was shown to underestimate fish age above an age of 6 years (Fig. 5). Age underestimation for older individuals of long-lived species has been demonstrated in a number of species of fish when the estimates were based on whole hard structures; for example, whole otoliths led to underestimated ages of Pacific ocean perch (*Sebastes alutus*) (Beamish 1979), Baltic herring (*Clupea harengus*) (Peltonen et al. 2002) and yellowtail flounder (*Limanda ferruginea*) (Dwyer et al. 2003), and whole vertebrae to underestimated ages of Atlantic bluefin tuna (*Thunnus thynnus*) (Prince et al. 1985) and blue sharks (*Prionace glauca*) (Macneil and Campana 2002). Underestimated fish ages can lead to overestimated growth and mortality rates, which can in turn result in a fisheries management strategy that causes overfishing of the stock. Therefore, age estimates based on whole vertebrae could present a danger to the fisheries management of *G. maculatum*.

Comparing the characteristics of whole and sectioned vertebrae, we can infer that there are two reasons why whole vertebrae may lead to underestimated ages for older individuals. The most prominent of these is the fact that the surface of the central area of the whole vertebra is calcareous, so some annuli near the focus will be obscured and miscounted. Since the spinal hole of the whole vertebra gets thicker as the fish grows, this problem would appear to become more and more serious with age. This inference was demonstrated by the trend for an increased first annular radius with age (Fig. 3). Thus, a significantly high of first annular radius is more likely to relate to the whole vertebrae from individuals older than 6 years, and these whole vertebrae more generally result in underestimated ages than the corresponding sectioned vertebrae. On the other hand, an inspection of the sectioned and whole vertebrae from older individuals found that high magnification of the section images allowed for easy resolution of the thinnest bands near the vertebral edge, whereas these bands are somewhat compressed on the curved surfaces of whole vertebrae, which is the second reason for the aging bias in these fish.

Although numerous authors have used sectioned vertebral centra of elasmobranchs to estimate age (Cailliet and

Goldman 2004; Licandeo et al. 2006), the use of sectioned vertebrae has not previously been reported as an approach to aging freshwater fish. Our study indicates that the sectioned vertebrae method affords more accurate age estimation than the whole vertebrae method, especially for older *G. maculatum*. Therefore, we suggest that the sectioned vertebrae method should be considered as a viable alternative for aging freshwater fish, especially long-lived species.

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