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Precision of age estimations from scales, otoliths, vertebrae, opercular bones and cleithra of two loaches, *Misgurnus anguillicaudatus* and *Paramisgurnus dabryanus*

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Abstract. Characteristics of annual rings on scales, otoliths, vertebrae, opercular bones and cleithra of two loaches, *Misgurnus anguillicaudatus* and *Paramisgurnus dabryanus* collected from Zhengzhou city, Henan Province, China, and the precision of age estimations from the five different structures of these two loaches were investigated in this study. Results obtained here showed that: the scale and otolith were suitable for age estimation in *M. anguillicaudatus* and *P. dabryanus*; percent agreements of age estimations based on these two different structures for *M. anguillicaudatus* and *P. dabryanus* were respectively 81.5 % and 79.5 %; *M. anguillicaudatus* and *P. dabryanus* had similar age compositions including five age groups; while the vertebra, opercular bone and cleithrum were not suitable for age estimation of *M. anguillicaudatus* and *P. dabryanus* because of their unclear annual rings.

Key words: two freshwater fishes, age essessment, different structures

Introduction

Misgurnus anguillicaudatus and *Paramisgurnus dabryanus* respectively belonging to *Misgurnus* and *Paramisgurnus*, Cobitidae, Cypriniformes, are widely distribute in Asia (You et al. 2009, Zhang et al. 2014). Due to high protein content, low fat, delicious taste and traditional Chinese medicine value, these two loaches known as "water ginseng" are very popular in the aquatic products markets of some East Asia countries like China, South Korea and Japan (Kiros et al. 2011, Gao et al. 2012, Zhang et al. 2014). Recently, because of market demands and huge decreases of their wild resources, prices of these two loaches are getting higher and higher, causing these two loaches become two most important freshwater commercial fishes in China (Fisheries Bureau of the Ministry of Agriculture of China 2014).

Researches about *M. anguillicaudatus* and *P. dabryanus* mostly focused on the breeding physiology (You et al. 2008), nutrition (Gao et al. 2014), development (Liang et al. 1988), and toxicology (Li et al. 2003). Age estimations of these two loaches were poorly understood. There are many different structures for fish age estimation, such as scales, otoliths, vertebrae and opercular bones (Khan et al. 2013, Ilies et al. 2014, Lozano et al. 2014). To date, only the scale was used to estimate ages of these two loaches (Wang et al. 2001, 2009, Huang et al. 2015), unconcerned with other age estimation structures. The objective of this study was to compare the feasibilities and precisions of age estimations of these two loaches using five different structures which were scale, otolith, vertebra, opercular bone and cleithrum. Results obtained here can enrich basic biological data of these two loaches.

Material and Methods

Fish collection

M. anguillicaudatus (291 individuals) and *P. dabryanus* (355 individuals) were collected from Zhengzhou

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city, Henan Province, China, using cages. Followed by body length measurements, the scales and otoliths were taken out from 200 *M. anguillicaudatus* and 200 *P. dabryanus* selected randomly. Meanwhile, the vertebrae, opercular bones and cleithra were removed from 50 *M. anguillicaudatus* and 50 *P. dabryanus*.

Making specimens of age estimation structures

About 10 to 20 scales of each fish were sampled from the region between the third line near dorsal-fin base and lateral line. Scales were soaked and digested in distilled water for 24 hours at room temperature (20-25 °C), then washed, dried and mounted between two glass slides. The mounted scales were labelled. Before photographing, the mounted scales were immersed in anhydrous ethanol and then the slides surfaces were dried. Scales observation and photographing were performed in liquid phases (Huang et al. 2014). Observations for annual rings on scales were conducted by using stereo microscope (Leica MZ75, Germany) and photographs of scales were taken and saved (Leica DFC300 FX).

Fish heads were dissected and immersed in a 95 % solution of alcohol. Otoliths were extracted using fine tweezers, rinsed in water, fixed with nail enamel and air-dried. After drying at room temperature, the otoliths were hand sanded using sandpaper with increasingly fine grit of 1000#, 1500# and 2000# and polished smooth. Otoliths were then mounted with nail enamel to prevent oxidation.

The vertebrae, opercular bones and cleithra obtained from fish specimens were dipped in a 1 % sodium hydroxide of boiling water for several minutes to remove connective tissues and the attached muscles, and then immersed in 10 % hydrogen peroxide for 24 hours and air-dried to prepare for microscopic observation.

Observations of annual rings on scales and otoliths were conducted by using stereo microscope (Leica MZ75, Germany) and photographs of scales and otoliths were taken and saved (Leica DFC300 FX). The other three age estimation structures (namely vertebrae, opercular bones and cleithra) were immersed in dimethylbenzene to become transparent, and then photographed and saved.

Age estimations

Each scale was examined three times by two people without reference to the growth information of fish. When ages estimated through the same scale specimen appeared in three different values the result was excluded. The observed age estimated through each scale, or the observed age for each fish, was determined by the modal age. The method of age estimation based on otoliths was as same as that based on scales. Annual rings were not observed on the vertebrae, opercular bones and cleithra of these two loaches. Different age estimation structures (namely scales and otoliths) from the same fish should be observed at least after half a month hiatus.

Data analyses

Data were analyzed by using SPSS 16.0. Photoshop software was used to process photos. The long

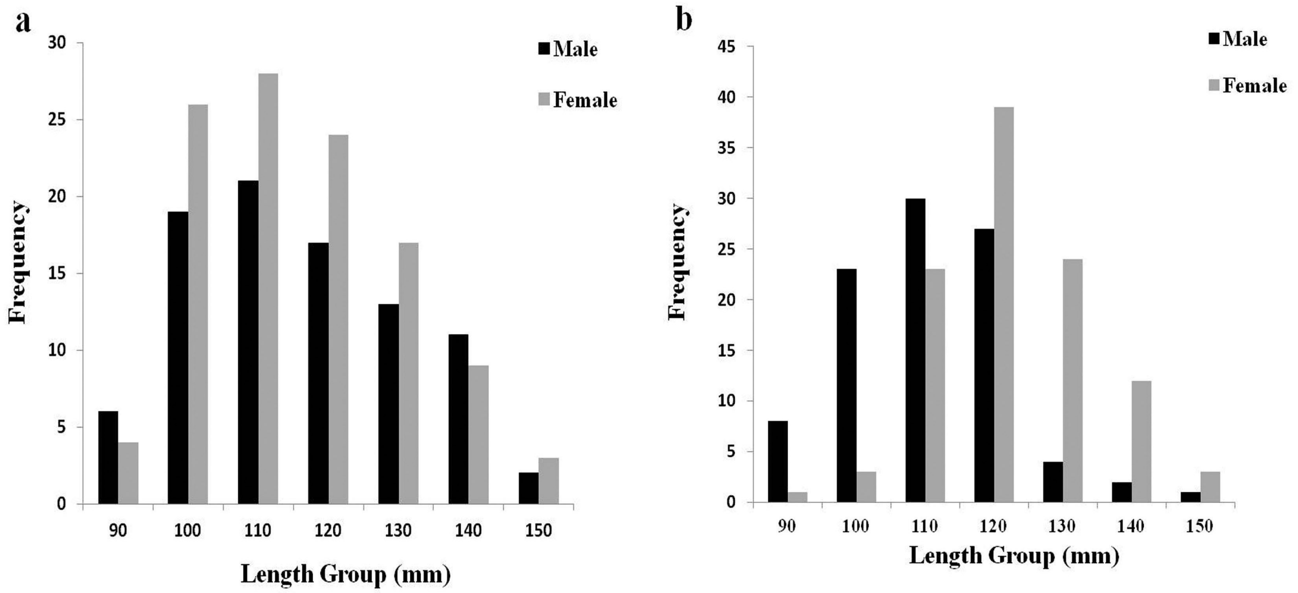

Fig. 1. Body length frequency distributions of *M. anguillicaudatus* and *P. dabryanus*. a) *M. anguillicaudatus*, b) *P. dabryanus*.

diameter of scale was defined as the longest diameter through the scale focus, while the short diameter of scale was defined as the widest diameter perpendicular to the long diameter. The long and short diameter of 50 scales of *M. anguillicaudatus* and 50 scales of *P. dabryanus* were respectively measured to analyze their scale diameter radios. The mean coefficient of variation was used to compare the similarity of age estimations based on different structures (Chang 1982). Age bias plots (Campana 2001) were used to visually detect systematic bias between structures.

Results

Body length frequency distributions

The body length of *M. anguillicaudatus* ranged from 9.04 to 15.99 cm (Fig. 1a). The average body length of *M. anguillicaudatus* was 12.02 cm (n = 200). The average body length of male *M. anguillicaudatus* was 11.96 cm (n = 89), while the average body length of female *M. anguillicaudatus* was 12.06 cm (n = 111). The body length of *P. dabryanus* ranged from 9.20 to 15.75 cm (Fig. 1b). The average body length of *P. dabryanus* was 12.14 cm (n = 200). The average body length of male *P. dabryanus* was 11.50 cm (n = 95), while the average body length of female *P. dabryanus* was 12.71 cm (n = 105). Body length distributions of *M. anguillicaudatus* and *P. dabryanus* (Fig. 1) met normal distributions.

Characteristics of annual rings on different structures

Annual rings on the scale and otolith of *M. anguillicaudatus* and *P. dabryanus* were distinct, while unclear or no annual rings were observed on the vertebra, opercular bone and cleithrum of these two loaches (Fig. 2).

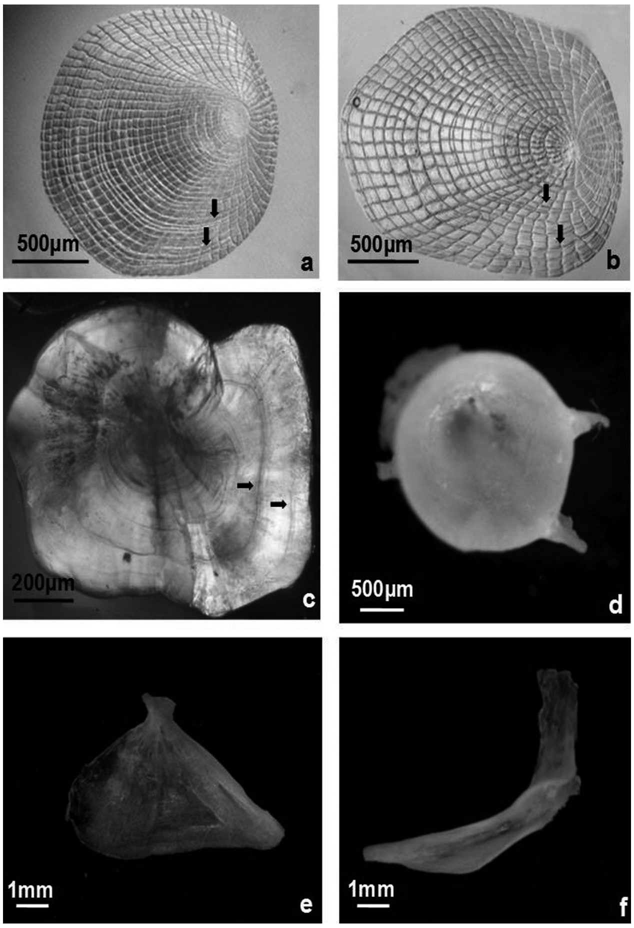

Fig. 2. Age estimation structures of *M. anguillicaudatus* and *P. dabryanus* (arrows indicate annual rings). a) a scale of *M. anguillicaudatus*, b) a scale of *P. dabryanus*, c) a lapillus otolith of *M. anguillicaudatus*, d) a vertebra of *P. dabryanus*, e) an opercular bone of *M. anguillicaudatus*, f) a cleithrum of *P. dabryanus*.

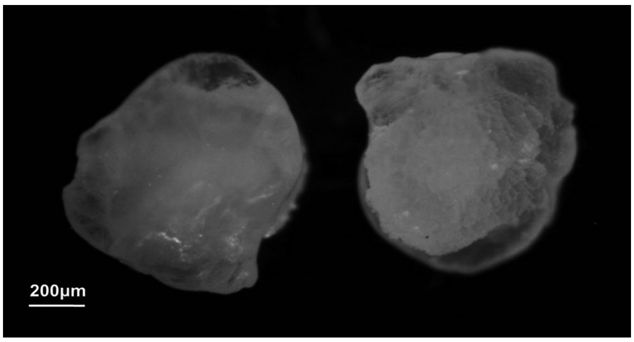

Fig. 3. The back (left) and ventral (right) of the lapillus otolith of *P. dabryanus*.

Scale specimens

Morphologies and characteristics of scale specimens of *M. anguillicaudatus* and *P. dabryanus* were similar. Each scale could be divided into four parts which were the top area, a base region and two lateral regions, with a smooth margin and the scale focus base. Scales of *M. anguillicaudatus* were near round with dense annuli (Fig. 2a), while scales of *P. dabryanus* were oval with sparse annuli (Fig. 2b). Ratios of the long diameter to short diameter in *M. anguillicaudatus* and *P. dabryanus* were respectively 1.03 ± 0.09:1 and 1.37 ± 0.13:1. The average diameter of scales of *P. dabryanus* tended to be longer than *M. anguillicaudatus* when these two loaches had same body length. Scale grooves of these two loaches consisted of circular grooves surrounded the scale focus and vertical grooves perpendicular to circular grooves. Vertical grooves were divided into primary and secondary radii on the basis of the original source. The former came from the focus of the scale, while the latter was initiated by somewhere away from the focus. The location of secondary radii with dense circular grooves turned dark under an incident light.

As a result, annual rings of these two loaches could be distinguished through the location of secondary radii and the density of circular grooves.

Otolith specimens

Both *M. anguillicaudatus* and *P. dabryanus*, had three pairs of otoliths: lapillus otoliths, asteriscus otoliths and sagittal otoliths. Since the sagittal otoliths were fragile, we did not obtain them successfully. Asteriscus otoliths were flaky, so only the lapillus otolith with a clear pattern of alternating zones could be used for age estimation. Lapilli with no significant differences between these two loaches were defined as dorsal and ventral section or anterior and posterior section according to the direction of fish body. The dorsal side with slightly inward depression was smooth, while the ventral side formed by small particulate crystals was very protruding (Fig. 3). The location of annual rings was near to the depression of dorsal side. Annual rings could be distinct by sanding the bumps of the ventral side (Fig. 2c). There was a darker central core in the central section of lapillus otolith. Annual rings were dark, while growth bands were bright under a transmission light. Ages were read counting the number of dark zones. Annual rings on otoliths were often clear enough to provide reliable age estimations, but the sampling and processing of otoliths specimens were harder and more complicated than in other age estimation structures.

Vertebrae specimens

No significant differences were found in the number and morphology of vertebrae between these two loaches. Vertebrae presented a double concave, with a first concave part shallower towards the head and a second concave part deeper towards the tail. Wide translucent bands and narrow opaque bands with dense black spots were showed in *M. anguillicaudatus* when a second concave part towards the tail was observed using a stereoscopic microscope. Narrow opaque bands acted as annual rings. Only 46 % vertebrae presented annual rings in *M. anguillicaudatus*. There were no obvious wide translucent bands and narrow opaque bands found in vertebrae of *P. dabryanus* (Fig. 2d). Therefore, vertebrae were not suitable for age estimations of these two loaches.

Opercular bone and cleithrum specimens

Opercular bones and cleithra from these two loaches had similar morphological characteristics. Opercular bones presented irregular triangular (Fig. 2e), and the latter part of cleithra presented semiterete (Fig. 2f). There were no annual rings observed on opercular bones and cleithra in these two loaches. So, opercular bones and cleithra could not serve as the structures of age estimations of these two loaches.

Comparisons of age estimations between scales and otoliths

Percent agreements of age estimated by scales and otoliths for *M. anguillicaudatus* and *P. dabryanus* were 81.5 % and 79.5 %, respectively. Age bias plot of ages estimated from scales compared to ages estimated from otoliths for *M. anguillicaudatus* revealed a smaller discrepancy (Fig. 4). The mean value of coefficients of variation (CV) between age readings of scales and age readings of otoliths for *M. anguillicaudatus* was 6.77 %. Age estimated bias of scales and otoliths for *M. anguillicaudatus* showed more variations in age group 1 and age group 5. For *P. dabryanus*, age bias plot of ages estimated from scales compared to ages estimated from otoliths revealed a larger discrepancy (Fig. 4). The CV value between age readings of scales

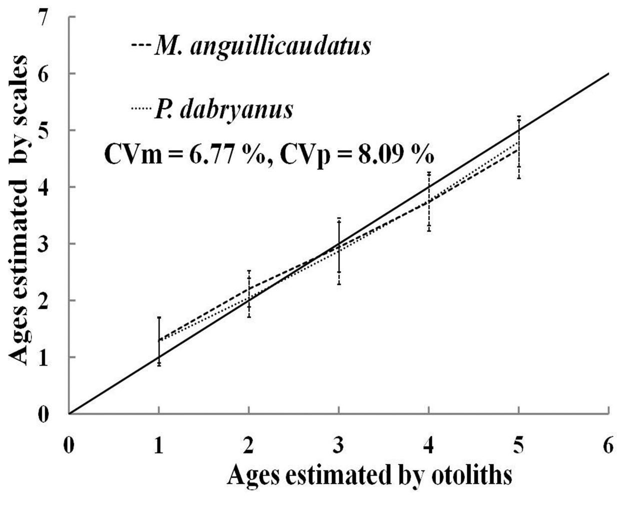

Fig. 4. Age bias plot comparing age estimates using scales and otoliths in *M. anguillicaudatus* and *P. dabryanus*.

Table 1. Age compositions of *M. anguillicaudatus* and *P. dabryanus*.

Age groups	Number of *M. anguillicaudatus*		Number of *P. dabryanus*	
	male	female	male	female
1	6	4	7	4
2	23	26	32	30
3	37	48	39	45
4	21	29	17	21
5	2	4	0	5
Total	89	111	95	105

and age readings of otoliths for *P. dabryanus* was 8.09 %. Age estimated bias of scales and otoliths for *P. dabryanus* showed more variations in age group 1 and age group 5. Least age variations between age readings of scales and age readings of otoliths for *M. anguillicaudatus* and *P. dabryanus* were observed in age group 3. Age estimates of scales were higher than those of otoliths for loach ages under or equal to three years. Age estimates of scales were lower than those of otoliths for loach ages above to three years.

Age compositions

Age compositions of *M. anguillicaudatus* and *P. dabryanus* were obtained by analyzing the scales and otoliths specimens, as shown in Table 1.

Discussions

Fish age estimation is one of the important contents of fisheries resources research (Younger et al. 1975, Casselman 1983, David et al. 2013). Age data is the basic for analyses of fish growth, sexual maturity, and changes in population structures (Gallagher & Nolan 1999). Therefore, obtaining reliable and accurate age data by age estimation is crucial for growth analysis, population dynamics and proper management. Comparison of age estimations from different bony structures has been studied in a number of fishes to identify the most suitable structure (Khan & Khan 2009).

In addition to the scale which was easily sampled and processed, the researchers attached more and more attention to using other anatomical structures in age estimations, like otoliths (Deng et al. 2010), opercular bones (Hua et al. 2005, Li et al. 2009, Wang et al. 2011), vertebrae (Hua et al. 2005, Cai et al. 2011), cleithra (Li et al. 2009), fin rays (Xiong et al. 2006), and so on. Historically, the major part of previously published studies of age estimations for *M. anguillicaudatus* and *P. dabryanus* were made with scales, which had great limitations. By comparative analyses of five age estimation structures (scales, otoliths, vertebrae, opercular bones and cleithra), this study concluded scales of loaches (small fish) had a strong applicability for age estimation in loaches, which were easily sampled and observed. More accurate age estimations were obtained when otoliths were used. Vertebrae generally served as auxiliary age estimation structure. However, it appeared to be unreliable for these two loaches' age estimations, since annual rings on vertebrae of *M. anguillicaudatus* were obscure and those of *P. dabryanus* were not presented. Annual rings on opercular bones and cleithra were poorly defined, making age reading difficult. As a result, opercular bones and cleithra could not be used for age estimations of these two loaches. It could be concluded that scales and otoliths were the best age estimation structures of *M. anguillicaudatus* and *P. dabryanus*.

Age compositions of these two loaches were similar, showing approximate normal distribution. The number of age 1 was low, which may be related to the sampling methods (like cage fishing) by which small individuals were not easy to get. Meanwhile, the proportion of elder group dropped, which to a large extent was the result of long-term development and over utilization of natural resources of these two loaches.

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