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Precision of Age Estimates Obtained from Five Calcified Structure for Wels Catfish, *Silurus glanis*

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Abstract—In this study, vertebrae, otoliths (asteriscus and lapillus), sectioned lapillus and pectoral fin rays were evaluated for age determination of *Silurus glanis* L., 1758 inhabiting Siddikli Dam Lake. All calcified structures showed the variable annual ring formation. Ages obtained from calcified structures were compared using the percentage of agreement (PA), average percentage of error (APE), and coefficient of variation (*CV*). Sectioned lapillus showed the clearest annulus formation and highest PA (76.4%) between readings, followed by asteriscus (43.9%), whole lapillus (40.5%), vertebrae (36.0%) and pectoral fin ray (33.3%). When sectioned lapillus compared to other structures, there were high ages obtained from this structure. Especially, the ages from the whole lapillus were lower than sectioned lapillus. Also, ages obtained from pectoral fin ray were the closer to sectioned lapillus ages than the other calcified structures. Owing to the highest PA, lowest APE and *CV*, sectioned lapillus was recommended as the most reliable calcified structure for age determination of *S. glanis*. Our findings can be used for effective fisheries management and determination of the biological characteristics of wels catfish.

Keywords: age determination, sectioned lapillus, APE, accuracy, Siddikli Dam Lake

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INTRODUCTION

The wels catfish, *Silurus glanis* which is included in the Siluridae, is an economically important species. This species is normally inhabiting throughout in large rivers, lakes and coastal areas of low salinity. *S. glanis* distributed in North Sea, Baltic Sea, Caspian Sea, the Black Sea, the Aral Sea basin, northern areas of Sweden and Finland, the Aegean Sea basin and Turkey (Kottelat and Freyhof, 2007). It has also been reported in recent years that it has spread to UK in Western Europe. *S. glanis*, which is one of the 20 largest freshwater fish species worldwide, is particularly famous among European anglers. A few studies were posted on the environmental biology of introduced wels catfish populations and even less data is available on the species' effect on local biota and ecosystems in it brought European range (Copp et al., 2009).

Data on age and growth rates are vital for many aspects of fisheries management (De Vries and Frie, 1996). Growth in fish is a function of age. For this reason, it is essential that age information is determined with as few errors as possible (Gümüş and Polat, 1999). One of the most important problems encountered in determining age and growth parameters is choosing the appropriate calcified structure for age determination (Khan et al., 2011). The most reliable calcification formation to be used for age determination can vary not only between species but also with different populations of the same species (Chilton and Beamish, 1982). For the determination of reliable bony formation in age determination studies are very important to evaluate the formation of each calcification by multiple investigators or to read them repeatedly by a reader (Yilmaz, 2006). Also, the comparison of age estimates between structures is an alternative method to age validation (Sylvester and Berry, 2006).

There are very few studies on the age of this species done by evaluating more than one bones (Yilmaz et al., 2007; Saylar, 2009, 2014). These studies have very few calcified structures used in age determination. Therefore, our objective was to choose the most reliable calcified structures and to determine the reliability of this method in age determination by using the thin section otolith technique which will be applied for the first time for this species.

MATERIALS AND METHODS

A total of 203 samples were caught between September 2015 and August 2016 used by gill nets (18, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70 and 80 knot to knot) from Siddikli Dam Lake (Kirşehir, Turkey). For age determination, vertebrae, pectoral fin rays, asteriscus and lapillus was obtained from *Silurus glanis*. Operculum was collected from a few of the fish, but they could not be read. Preparation of vertebrae, asteriscus and lapillus for age determination was made according to Chugunova (1963) and the pectoral fin rays were adjusted to the age determination according to the method suggested by Burnett (1969).

Vertebrae (4th to 10th) was removed and kept for 4–5 min in beakers containing boiling pure water. Then, residual tissue, skin and marrow on the vertebrae were cleaned. The cleaned vertebrae were kept for 15 min in a drying oven which temperature was set at 103°C for removing oil and water droplets (Chugunova, 1963).

The upper and lower jaws of the catfish were separated from each other with the help of sharp scissors, and the skull bone, which is the continuation of the vertebrae in the upper part, was broken, and the lagenar (asteriscus) and utricular (lapillus) otoliths were removed with the help of a collet. After the otoliths were cleaned in alcohol, they were kept in a drying oven of 103°C for 15 minutes and made ready for examination (Chugunova, 1963). Both lapillus and asteriscus having unclear annual rings were ground using sandpaper to make the annuli more distinct. Otoliths were photographed before cutting. Asteriscus was not suitable for cutting because it has a very fragile structure. Therefore, the thin section otolith technique was applied to lapillus. Lapillus was embedded in epoxy and cut in a transverse section with a Buehler Isomet low-speed saw on the posterior end near the core (Secor et al., 1992). The cutting plane was adjusted to pass through the exact centre of otolith and along the lateral-medial line (0.5 mm thick). Each section was examined under a microscope, and when necessary, sanding was carried out to make age rings more distinctive.

Pectoral fin rays were cleaned from meat and skin fragments on them and kept in 96% ethyl alcohol for 1–2 min (Burnett, 1969). They were mounted in epoxy. The fin rays were then cut close to the base using Buehler Isomet low-speed saw. Thicknesses of the section were varied from 0.5 to 0.7 mm.

Age readings were repeated 3 times at different times by a reader under a stereo-binocular microscope at ×10 magnification. All calcified structures were subjected to the preliminary examination. The aim of preliminary examination has determining characteristics of age rings. No other data (length and weight) were considered, except for the time of capture and gonad status during age readings. The placement of the fish in the age class was made according to January 1. For example: samples (with 9 opaque and 9 hyaline rings) caught in December and fish (with 8 hyaline and 9 opaque rings) caught in August were placed in the same year class (8 year class).

As a result of repeated age readings, percentage agreement (PA) average percentage error (APE) and coefficient of variation (*CV*) were used to determine the age determination precision (Beamish and Fournier, 1981; Chang, 1982). PA, which is a traditional method of comparing age data, is used to determine the degree of similarity of repetitive age data obtained from any bony formation and is expressed as a percentage (Yazici et al., 2014).

APE and *CV* are considered as an indicator of incompatibility rather than compliance in age determination (Eltink et al., 2000). APE for any calcified structure was calculated by taking the average of individual APE values. The following equation is used for the individual APE.

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

Where APE_j is average percent error for jth fish, R is the number of readings, x_{ij} is the ith age of the jth fish and x_j is the mean age of the jth fish. *CV* for each a calcified structure was found by taking the average of the *CV* values calculated for all fish separately. *CV* for any fish is determined by the formula below.

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

Where CV_j is the age precision estimate for the jth fish, x_{ij} is the ith age of the jth fish and x_j is the mean age of the jth fish.

The reliability of calcified structures used in age determination was tested using the above formulas. The calcified structure with high PA and low APE and *CV* was chosen as the reliable calcified structure for age determination in the population of *Silurus glanis*. Also, a comparison between reliable calcified structure and other structures was evaluated using mean ages obtained from bony structures.

RESULTS

As a result of the observation of age rings throughout the year, some results have been obtained. The different formations in ring characteristics were observed during the reproduction periods, summer (when the growth was very good), and in the autumn (months when the growth started to slow down). There were hyaline zones in the margins of calcified structures in November, December, January, February, March, and April. Although the hyaline zone appeared in November and December, samples were included in a lower year class. Because we assumed the birth date of January 1 in the northern hemisphere, we must classify this way. In the reproductive period (April–June), hyaline rings have been observed in individuals who have reached reproductive maturity due to the slowing of growth. On the other hand, growth zones were observed in the margins of the calcified structures of individuals who had not reached reproductive maturity. However, a hyaline ring did not occur after this

Table 1. Sample numbers and percentages ($n/\%$) by age groups

Age, year	Aging structures, $n/\%$				
	vertebrae	whole lapillus	sectioned lapillus	asteriscus	pectoral fin ray
1	1/0.5	2/1.0	1/0.5	3/1.5	0/0
2	15/7.4	11/5.6	4/1.9	3/1.5	4/2.3
3	46/22.6	48/24.7	4/1.9	27/13.5	11/6.2
4	60/29.6	70/35.9	40/19.7	65/32.8	49/27.6
5	44/21.7	31/15.9	71/35.0	52/26.2	65/36.7
6	16/7.9	15/7.7	30/14.8	20/10.1	26/14.7
7	9/4.4	6/3.1	21/10.4	8/4.1	10/5.6
8	7/3.4	6/3.1	11/5.4	10/5.1	4/2.3
9	2/1.0	2/1.0	10/5.0	6/3.1	4/2.3
10	1/0.5	1/0.5	8/4.0	1/0.5	3/1.7
11	2/1.0	2/1.0	2/0.9	2/1.1	1/0.6
12	–	1/0.5	–	1/0.5	–
13	–	–	–	–	–
14	–	–	1/0.5	–	–
Total	203	195	203	198	177

"–"—no individual.

Table 2. Full age, ±1 year agreement values in calcified structures, average percentage of error (APE) and coefficient of variation (*CV*) values in the aging structures (total number of samples—203)

Aging structures	Number of readable samples	Full age agreement, %	±1 Year agreement, %	APE ± SE	*CV* ± SE
Vertebrae	203	36.0	82.75	9.58 ± 0.651	13.55 ± 0.070
Whole lapillus	195	40.5	80.51	10.12 ± 0.790	14.31 ± 0.184
Sectioned lapillus	203	76.4	92.11	3.25 ± 0.473	4.60 ± 0.076
Asteriscus	198	43.9	88.38	7.09 ± 0.539	10.03 ± 0.051
Pectoral fin ray	177	33.3	83.05	8.87 ± 0.628	12.55 ± 0.083

SE—standard error.

growth zone. These individuals were not placed in the upper-year class. With the increase in growth towards the end of the summer, opaque zones began to expand at the edges of the calcified structures. Then, towards the end of autumn, the growth slowed down and the hyaline region started to form.

As a result of repeated age analysis from 5 different calcified structures (whole lapillus, sectioned lapillus, asteriscus, vertebrae and pectoral fin ray) used in age determination, ages 1–14 were determined (Fig. 1). There was age class 12 in whole lapillus (Fig. 1b), sectioned lapillus (Fig. 1e) and asteriscus (Fig. 1d), 11 in the vertebrae (Fig. 1a) and 10 in the pectoral fin ray (Fig. 1c). In the sample, the 5-year-old individuals were predominant in the readings from the sectioned lapillus and pectoral fin ray, and the 4-year-olds were dominant in the readings from the asteriscus, whole lapillus and vertebrae (Table 1).

PA values obtained as a result of repeated age readings for five calcified structures in this sampling are shown in Table 2. The highest percentage agreement was obtained in sectioned lapillus (76.4%). Also, it was determined that the highest ± 1 age agreement value was again in the sectioned lapillus (92.11%).

Average percent error and coefficient of variation were lower in sectioned lapillus than other calcified structures. This indicates that different ages are observed in repeated age reading in calcified structures. In other words, age readings in sectioned lapillus were made with a lower error rate than other structures (Table 2).

According to the age determination analysis, the highest PA, the lowest APE and *CV* were obtained in the sectioned lapillus. This shows that sectioned lapillus provided more compatible age data than other calcified structures. For this reason, it was concluded that

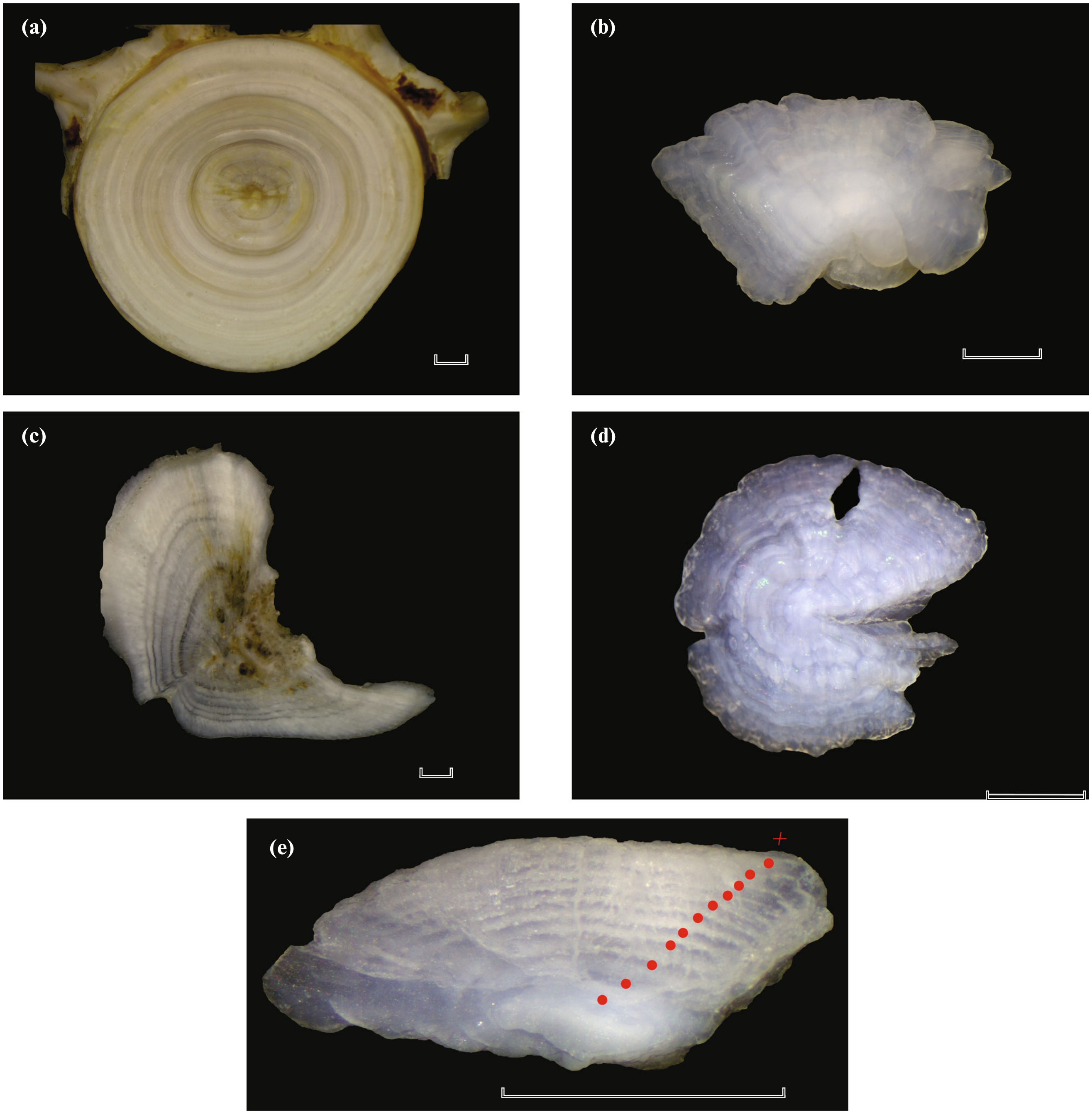

Fig. 1. Photographs of aging structure of the same individual: (a) vertebrae, (b) whole lapillus, (c) pectoral fin ray, (d) asteriscus, (e) sectioned lapillus. Scale bar: 1 mm.

the most reliable calcified structure for the age determination in the population of *Silurus glanis* is the sectioned lapillus.

The closest to age data obtained from the sectioned lapillus is the ages of pectoral fin ray (31.6%). The ages from whole lapillus were lower than sectioned lapillus. In other words, real age rings may be hidden due to thickening in the whole lapillus surface. Also, the ages obtained from the sectioned lapillus are higher than the ages read from other structures (Fig. 2).

DISCUSSION

Findings from this study demonstrated the first success application of thin-section otolith technique in *Silurus glanis*. Furthermore, we provided the first data on estimates of age using five calcified structure

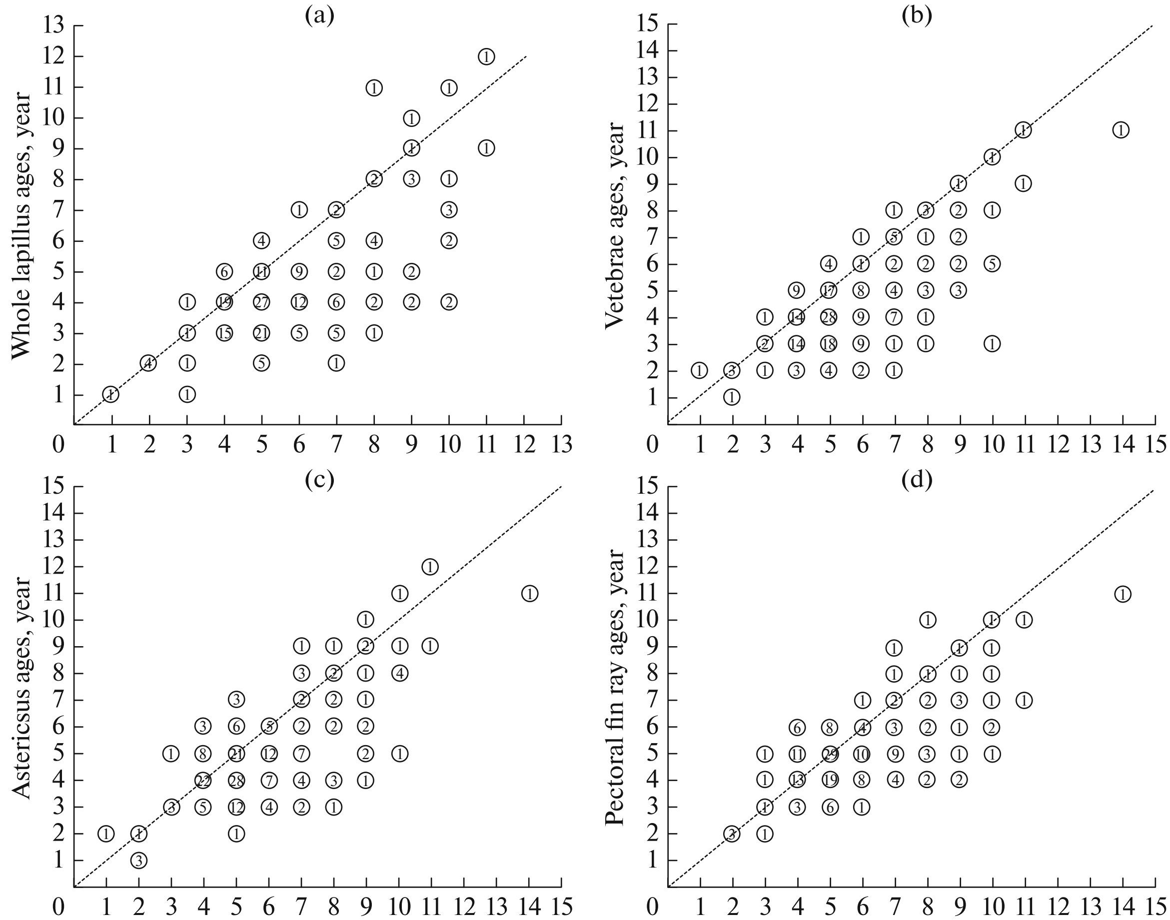

Fig. 2. Comparison of sectioned lapillus ages with other calcified structure: (a) vertebrae (PA—23.60% (±1 = 60.09%), *CV*—21.34, *n*—203); (b) whole lapillus (PA—21.03% (±1 = 59.48%), *CV*—21.53, *n*—195); (c) asteriscus (PA—29.30% (±1 = 67.67%), *CV*—16.71, *n*—198); (d) pectoral fin ray (PA—31.07% (±1 = 67.23%), *CV*—15.07, *n*—177). Numbers in circle—number of fish.

in *S. glanis*. Researchers have used vertebrae (Akyurt, 1988; Alp et al., 2004, 2011; Doğan Bora and Gül, 2004; Yilmaz et al., 2007; Saylar, 2009, 2014; Uysal et al., 2009; Yüngül et al., 2014) or pectoral fin rays (Harka, 1984; Harka and Bíró, 1990; Carol et al., 2009; Horoszewicz and Backiel, 2012) for age and growth studies on wels catfish. However, in this study, the most reliable structure for age determination was found as sectioned lapillus.

This study showed that vertebrae not provided precise age estimates in this sampling when compare with sectioned lapillus. Also, during repeated readings in the vertebrae; different ring structures varying from sample to sample, the difficulty of separation of the larval ring and the first age ring in the central region, the risk of damage to the growth zone or age ring in the operations performed for the purpose of removing tissue residues, illusions due to the concave and deep structure, microbial activity due to tissue residues, difficulty in distinguishing from other age rings due to observation of double-ring formation was determined. But some researchers reported that vertebrae should be used for age determination in *S. glanis* (Yilmaz et al., 2007; Saylar, 2009, 2014).

Lapillus have been used as the reliable calcified structure for age determination in many species (Escot and Granado-Lorencio, 2001; Gallardo-Cabello et al., 2006; Gümüş et al., 2007; Yazici et al., 2014). However, it has not been considered in age estimation research about this species. In this study, APE and *CV* values of the whole lapillus were higher than other structures. Therefore, it is a calcified structure which the most unreliable for age estimation. Especially the first age ring is not read due to thickening. This situation is an important disadvantage of this structure. This problem can be solved with various sanding techniques, but it is not enough. Similarly, Yilmaz et al. (2007) reported that whole lapillus of *S. glanis* inhabiting Altinkaya Dam Lake do not show a clear ring character.

Asteriscus, another type of otolith, has more regular age ring formations compared to the whole lapillus. Yilmaz et al. (2007) reported that asteriscus is suitable than the whole lapillus for age determination. Also, there were difficulties in storage and age reading due to a very fragile structure of asteriscus. According to both APE and *CV* values, the most reliable age determination structure after sectioned lapillus is asteriscus.

The most important disadvantage in pectoral fin rays is the risk of first age ring disappearing. Although it has a fixed cross-sectional thickness, this structure shows different age ring formation according to individuals. Pectoral fin ray has a very irregular structure especially in young individuals. It is very difficult to read the first few age rings in this structure. Yilmaz et al. (2007) and Saylar (2009) also reported a similar situation. Despite all these negativities, it is a remarkable advantage that the fish does not have to be killed during the removal of the structure. This structure can be evaluated especially in the age determination studies to be carried out in endangered species. Although pectoral fin ray is no reliable calcified structure in this study, it should be taken into consideration in the age determination as it provides the closest age data to the sectioned lapillus.

Our results showed that sectioned lapillus provide useful data for age determination of *Silurus glanis*. The relatively low APE (3.25) demonstrated that the readability of the sectioned lapillus was high. Reliable of sectioned lapillus presented satisfactory consistency in age determination. Because the estimated APE and *CV* values were below the precision point of 5.5% established by Campana (2001) for ageing studies. Also, percent agreement between readings in sectioned lapillus was higher than other structures. Khan et al. (2016) reported that agreement between readers was usually higher for otoliths prepared with thin-sectioned method than with the whole otolith method. In particular, it is essential to set the cross-section line to pass through the centre. Otherwise, there may be losses or overlaps in the age rings. Considering the sectioned lapillus in this study, it was determined that it shows a very clear age ring and the determination of the first age ring was made easily. Also, storage of samples is more practical compared to other structures. There are no studies in the literature using otolith section technique for age determination of wels catfish. However, successful results about otolith sectioned method have been reported for other species (Panfili and Tomàs, 2001; Li et al., 2009; Hosseini-Shekarabi et al., 2015; Koeda et al., 2016; Zhou et al., 2017). The otolith section technique is expensive and laborious than traditional techniques. In *S. glanis*, the removal of vertebrae and pectoral fin ray is easier than otolith. For these reasons, researchers may not have applied the thin section otolith technique.

Compared to the sectioned lapillus, the other age estimation structures underestimated fish age. It is clear the whole lapillus hides the age rings due to thickening. These results agree with other studies (Beamish, 1979; Campana, 1984) that suggested that sectioned otoliths generally produce higher age estimates than otoliths prepared by other methods. Age validation studies are needed to determine which structure gives accurate age data. If the ages obtained from the section of lapillus are closest to the real age of the fish, it turns out that ages of other calcified structures are found to be very lower than reality. Underestimated 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 (Ding et al., 2011). Therefore, age estimates not based on sectioned lapillus could present a danger to the fisheries management. For this reason, sectioned lapillus is recommended for age determination studies about *Silurus glanis*.

In this study, comments were made on the calcified structures used for age determination in catfish. Their positive and negative aspects were stated. As a result, we suggest that age determination should be made from sectioned lapillus in wels catfish. Age data should be obtained from the most reliable calcified structure, especially for effective fisheries management and accurate determination of the biological characteristics of wels catfish.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. Fish catching and laboratory procedures in this study were checked and approved by the animal experiments local ethics committee (document no. 68429034/05).

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