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A Comparative Study on Age Determination of Carp (*Cyprinus carpio* Linnaeus, 1758) in Lake Eğirdir Using Otolith, Vertebrae and Scale Counts

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ABSTRACT

This study, a comparative age determination study was carried out between May and November of 2013 in order to estimate most reliable bony tissue for ageing of the common carp in Lake Eğirdir. A total of 78 specimens aged between 1 and 11 were used in the study, having weights between 191 and 8685 g and fork lengths between 19.5 and 76 cm. Scales, vertebrae and otoliths were used by one reader via 5 replicates for ageing. Highest agreement among the bony tissues was found in otoliths (10.67%) and most reliable bony tissue was determined as otoliths for age determination in Lake Eğirdir carps.

Keywords: *Cyprinus carpio*; Age; Otolith; Vertebrae; Scale; Eğirdir Lake

Eğirdir Gölü Sazan (*Cyprinus carpio* Linnaeus, 1758) Balıklarında Otolit, Omur ve Pullardan Karşılaştırmalı Yaş Tayini Üzerine Bir Araştırma

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ÖZET

Bu çalışma, Eğirdir Gölü sazan balıklarının yaş tayininde en güvenilir kemiksi dokunun belirlenmesi amacıyla karşılaştırmalı bir yaş tayin çalışması olarak Mayıs-Kasım 2013 tarihleri arasında gerçekleştirilmiştir. Çalışmada toplam 78 adet yaşları 1 ile 11, ağırlıkları 191 g ile 8685 g ve çatal boyları 19.5 cm ile 76 cm arasında değişen bireyler kullanılmıştır. Yaş tayini tespitinde pullar, omur ve otolitler kullanılmış ve 5 tekrarlı okuma yapılmıştır. Kemiksi yapılar arasında en yüksek uyum (% 10.67), otolit olarak belirlenmiş ve Eğirdir Gölü sazan balıklarında yaş tayini için en güvenilir kemiksi yapının otolit olduğu tespit edilmiştir.

Anahtar Kelimeler: *Cyprinus carpio*; Yaş; Otolit; Omur; Pul; Eğirdir Gölü

1. Introduction

Various bony structures including scales, otolith, vertebrae, fin rays and spines, opercular bones, cleithra have been utilized to estimate the age of different fish species (Das 1994; Worthington et al 1995; Göçer & Ekingen 2005; Khan et al 2011; Başusta et al 2014). There are numerous studies on usage of this practice for age determination of the common carp also (Lubinski et al 1984; Yerli 1997; Brown et al 2004; Balık et al 2006; Demirkalp 2007; Phelps et al 2007; Yılmaz & Polat 2008; Apaydın Yağcı et al 2008a; 2008b; Amouei et al 2013; Mert & Bulut 2014).

Ambrose (1989) stated that age determination is the most critical issue in fisheries biology studies and Chalanchuk (1984) reported age estimation with high precision is among the things to obtain accurate data on population dynamics during fish biology studies (Türkmen et al 2005). However, it is reported that peculiar features of each species like the timing of annulus formation, latent period of growth, breeding and migration activities should be known prior to age determination, and since the ideal bony structure or age determination method may vary among species, even age classes and populations of the same species, it is advised to perform confirmation studies on age determination which can be affected by a number of variables for the sake of the reliability of the method applied (Polat 2000).

Being an indispensable data source in fisheries management, reliable and precise age determination

is of utmost importance in monitoring fish populations. The present study focuses on age determination by using three different bony structures of common carp in Eğirdir Lake.

2. Material and Methods

2.1. Sampling of the fish

The study material consisted of 78 *Cyprinus carpio* specimens collected with trammel nets having the mesh sizes 55, 60, 65, 70 and 80 mm from different parts of Eğirdir Lake between May and November 2013. Fork lengths (FL, mm) and weights (g) were recorded for each specimen caught.

2.2. Scales preparation

After measurements, scales were removed from above left lateral line under dorsal fin from near head region (Chugunova 1963). Scales from each fish were kept in petri dishes with warm water for 10-12 hours. Following the cleansing of mucus and the pigment layer via a brush, the scales were placed into 5% NaOH for 2 hours, then washed with distilled water and put into 96% ethanol for minutes to get rid of the water. Afterwards, the scales preserving structural unity and intact nucleus under light were placed between two slides and preparations were made. The study was performed via a Nikon Profile Projector V-10 imaging device and under a constant magnification rate (10x) (Figure 1).



Figure 1- Scale preparations and annulus

2.3. Vertebra preparation

All vertebrae between the fifth to tenth were taken out after removal of the internal organs and cleansing (Chugunova 1963), kept in boiling water for 3 minutes, cleaned from tissue remains and air dried. The polyester molds prepared with 100 g polyester (styrene (100-42-5)) and 2 g hardener (methyl ethyl ketone peroxide) under 70 °C in a pre-heated incubator for two hours (Figure 2), instead of the suggested method using 100 g polyester with 20-40 drops of catalyst under 35 °C incubator treatment (Metin 2001).

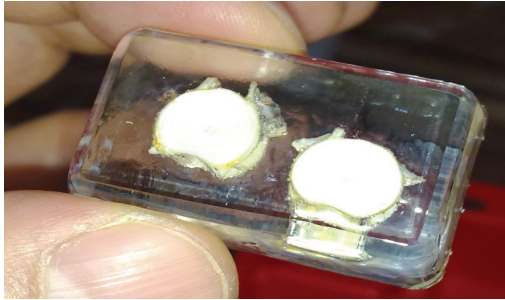


Figure 2- A mould filled with common carp vertebrae fixed in clear polyester resin

A Micracut 201 precision cutter was used for sectioning. Prior to sectioning, centre of each vertebra was marked. 0.5 mm sections were cut via two blades with 40-50 $\mu\text{m sec}^{-1}$ feed rate and 400-500 rpm speed (Figure 3).

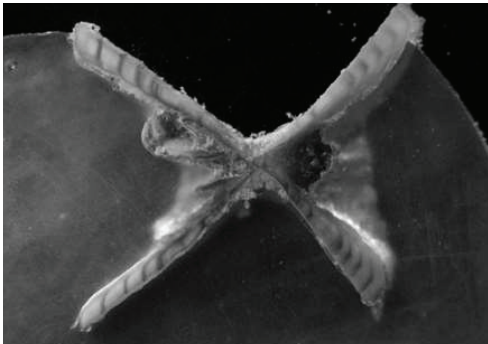


Figure 3- Translucent and opaque bands in vertebral section

Scratches were removed with 200 μ sandpaper. Readings were made under binocular microscope illuminated from the top and sides with the same magnification rate (10x). The images were captured imaging system and ageing was made through images.

2.4. Otolith preparation

Head portions of the fish were cut from midline and the otoliths were removed from otolith capsules with pincers (Chugunova 1963), the otoliths were cleaned in 96% ethanol and placed into Eppendorf tubes with glycerin. After three weeks, otoliths were studied under binocular microscope illuminated from the top and sides with the same magnification rate (10x). The images were captured imaging system and ageing was made through images (Figure 4).

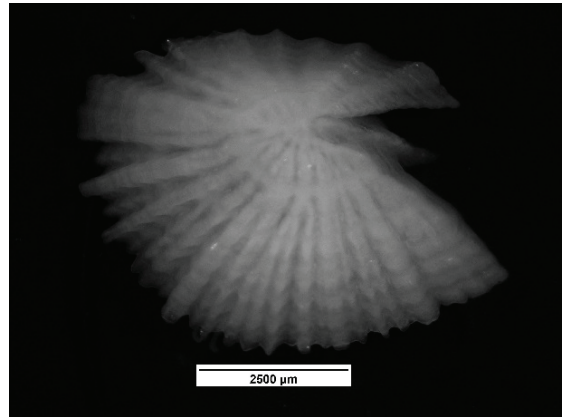


Figure 4- Translucent and opaque bands in carp otolith

Readings were made by a single reader and 5 replicates at different times. Last complete annulus were taken into account for ageing, determination of last annual ring based on 1st January accepted as the birthday for fishes of northern hemisphere.

2.5. Calculation of mean age

According to Baker and Timmons (1991), calculation of mean age aims to determine over-or underestimations. To calculate mean age Equation 1 was used (Bostancı 2005).

$$X_{kt} = \frac{\sum_i^n \sum_j^f x_{ijklt}}{n \cdot f} \tag{1}$$

Where; x_{ijklt} , j fish; i, readings; l, age value of reading; n, replicate; f, total number of specimens. Although not without certainty about reliability of a certain structure over other, mean age gives a preliminary idea about presence of a reading over or below normal values (Bostancı 2005).

2.6. Agreement of otolith, vertebrae and scales

This value helps to determine the criteria to evaluate annuli. If all 3 readings give the same age in a study with 3 replicates, the similarity will be zero or in other words agreement is 100%. Higher agreement is preferred, although this doesn't prove the reliability of a certain structure. In short agreement is an important index for age analyses, although solely not enough to ensure reliability (Bostancı 2005). Rate of agreement over 5 readings can be expressed as 5/5, 4/5, 3/5, 2/5, 1/5, and demonstrated as a percentage (%) of the specimen number in the expression to all specimens. The structure with highest agreement is accepted as the most reliable (Yılmaz 2000). Five replicates were applied in our study.

2.7. Variance analysis of otolith, vertebrae and scales

Age estimation data obtained from 5 different readings for each structure was subjected to variance analysis (Equation 2). Fowler (1990) describes variance as the sum of squares mean age estimates minus mean age over degrees freedom (Yılmaz 2000).

$$V_t = \frac{\sum_i^n (X_i - \bar{X})^2}{n - 1} \tag{2}$$

Where; V_t , the variance for the structure t; n, number of replicates; X_t , first reading for each sample; \bar{X} , mean of replicates.

2.8. Ageing error

For each age estimation of a certain bony structure after 5 replicates, error rate was calculated after variance analysis. Ageing error (S_t , standard deviation) is expressed as the square root of the variance (Equation 3) (Yılmaz 2000). Lowest standard error gives the most reliable structure (Yılmaz 2000).

$$S_{t=\sqrt{V_t}} \tag{3}$$

2.9. Data analysis

Minimum age, maximum age, mean age, standard deviation, standard error, variance and CV values were analyzed using JMP 8 package program.

3. Results and Discussion

Distribution of weights in the 78 carps caught from Lake Eğirdir ranges between 191 and 8685 g, while the fork lengths range between 19.5 cm and 76 cm.

3.1. Age composition of otolith, vertebrae and scales

According to 5 reading replicates, age composition estimated for each bony structure is different. Scale readings of 78 carps give age distribution as 1-11, of 63 specimens (as in 15 samples no annulus formation was detected) vertebrae readings give it as 2-11 and according to readings from 75 specimens (as 3 otoliths were broken upon removal) it is found as 2-10 (Table 1).

Table 1- Age composition derived from different bony structures readings

| Bony structure | Age | | | | | | | | | | | Total |
|----------------|-----|----|-----|----|----|----|-----|------|----|---|----|-------|
| | I | II | III | IV | V | VI | VII | VIII | IX | X | XI | |
| Scales | 1 | 10 | 6 | 7 | 10 | 12 | 11 | 12 | 4 | 3 | 2 | 78 |
| Vertebrae | - | 7 | 9 | 2 | 4 | 11 | 13 | 11 | 3 | 2 | 1 | 63 |
| Otolith | - | 7 | 9 | 12 | 7 | 8 | 14 | 12 | 5 | 1 | - | 75 |

When the average estimates were compared for each bony structure, were found to be around 5, no statistical difference was observed ($P>0.05$). Standard errors and reading errors for bony structure-reader combinations are given in Table 2. Accordingly, lowest standard error and ageing errors are calculated in otoliths.

Table 2- Mean ages, standart and ageing errors in different bony structures for common carp

| Bony structures | Scales | Vertebrae | Otoliths |
|-----------------|--------|-----------|----------|
| Mean ages | 5.76 | 5.88 | 5.64 |
| Standard errors | 0.28 | 0.30 | 0.25 |
| Ageing errors | 2.44 | 2.38 | 2.21 |

When the agreement among otoliths, vertebrae and scales of carps in Lake Eğirdir were investigated to find the most reliable bony structure, according to 5 different readings at different times, the evaluations of the percentages of shared readings for all five replicates over all readings are given in Table 3.

Table 3- Precision of readers on different bony structures (%)

| Bony structure | Agreement of readers/number of total readings | | | | | Total |
|----------------|---|-------|-------|-------|-----|-------|
| | 5/5 | 4/5 | 3/5 | 2/5 | 1/5 | |
| Scales | 7.69 | 29.49 | 38.46 | 24.36 | - | 100 |
| Vertebrae | 7.94 | 25.40 | 34.92 | 31.74 | - | 100 |
| Otolith | 10.67 | 28.00 | 36.00 | 25.33 | - | 100 |

This study is the first to be based on comparison of methodologically different bony structures in Lake Eğirdir fishes. As in similar studies, the most reliable age estimation is based on the lowest reading error and otolith readings had the lowest error (2.21) (Table 2). According to 5 different readings of the same reader, the highest agreement was found otolith (10.67) and the lowest in scales (7.69) (Table 3). Polat et al (2001) in Black Sea flounders and Polat et al (2004) in Derbent Dam perch found the highest reader agreement and the lowest reading error in vertebrae. Likewise, in a study on comparison of 6 different structures in carps of Altinkaya, Derbent Dams and Balık Lake (Bafra), Yılmaz & Polat

(2008) have found the highest reader agreement and the lowest reading error in vertebrae for each lake. On the other hand, Temizer & Şen (2008) found among Keban Dam mirror carps the highest reader agreement between scales and vertebrae (86.68%).

There are many studies on use of scales to obtain the most suitable bony structure and process for age determination (Yerli 1997; Campana 2001; Balık et al 2006; Apaydın Yağcı et al 2008a, b). However, otoliths were found as the most reliable ageing structure in *Carassius gibelio* from Lake Eğirdir (Bostancı 2005), whereas vertebrae were more precise as compared to otoliths in *Alosa pontica* (Yılmaz 2000) and in *Carassius gibelio* in Lake Bafra (Bostancı 2005).

4. Conclusions

In conclusion, according to the highest agreement and lowest ageing error values, otolith is the ideal bony structure for precise age determination in Lake Eğirdir carps.

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