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Research Article

Differences between Whole Otolith and Broken-Burnt Otolith Ages of Red Mullet (*Mullus barbatus ponticus* Essipov, 1927) Sampled from the Black Sea (Samsun, Turkey)

Nazmi POLAT*, Derya BOSTANCI, Savaş YILMAZ
Department of Biology, Faculty of Science and Arts, Ondokuz Mayıs University, 55139 Kurupelit, Samsun - TURKEY
*E-mail: npolat@omu.edu.tr

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Abstract: Different bony structures as scales, vertebrae, otoliths, opercles and subopercles of 156 red mullet (*Mullus barbatus ponticus* Essipov, 1927) sampled from the Black Sea were removed for age determination. Otoliths were read once by two experienced readers. However, due to the difficulty of interpretation of annuli on the otolith edge, the broken-burnt method was applied to the 61 otoliths. Broken-burnt otoliths were also read once by the same two experienced readers. When whole otolith and broken-burnt otolith ages were compared, high agreement within structures and low precision between structures were observed. Finally, it was found that the age of fish was underestimated by a minimum of one year by whole otolith. Therefore, we recommend that the broken-burnt otolith method should be used in the age determination of red mullet.

Key Words: Black Sea, red mullet, otolith, age determination

Karadeniz (Samsun, Türkiye)'den Örneklenen Barbunya Balığı (*Mullus barbatus ponticus* Essipov, 1927)'nın Bütün Otolit ve Kırık Otolit Yaşları Arasındaki Farklar

Özet: Karadeniz'den örneklenen 156 barbunya balığı (*Mullus barbatus ponticus* Essipov, 1927)'nın pul, omur, otolit, operkül ve suboperkül gibi farklı kemiksi yapıları yaş tayini için alınmıştır. Otolitler yaş belirlemede tecrübeli iki okuyucu tarafından bir kez okunmuştur. Ayrıca otolit kenar bölgesindeki annulusların yorumlanmasında zorluk çekilen 61 otolite kırma-yakma metodu uygulanmıştır. Kırılıp-yakılan otolitler de aynı iki okuyucu tarafından bir kez okunmuştur. Bütün otolit ile kırık otolit yaşları karşılaştırıldığında, yapı-içi uyumun yüksek, yapılar-arası uyumun ise düşük olduğu gözlenmiştir. Sonuç olarak, bütün otolitlerin balık yaşını en az 1 yaş düşük gösterdiği bulunmuştur. Bu nedenle, barbunya balığının yaş tayininde kırık otolit metodunun kullanılması önerilmiştir.

Anahtar Sözcükler: Karadeniz, barbunya balığı, otolit, yaş tayini

Introduction

One of the significant aspects of obtaining accurate data on fish biology and population dynamics is to determine age with the lowest error (1). Use of inaccurate ages has caused serious errors in management and understanding of fish populations. Over- and underestimated ages for any fish, especially economic species, lead to major commercial losses (2).

The most accurate method of age determination in fish is to mark and recapture them in their natural environment (3,4) or to study fish of known age (5). However, its application is limited in fisheries due to a number of constraints such as time and money. The other method in age determination is length-frequency analysis. It is, however, considered a reliable method when the samples are the representative of a fish population that has short life, fast growth and reproduce once a year (4). The third method is the anatomical approach. It is possible to determine the age of fish by evaluation of the growth rings formed on bony structures. Ages of fish are estimated by comparison of the readings from various bony structures (6-12).

Red mullet (*Mullus barbatus ponticus* Essipov, 1927) is an economically important fish species. There are many studies on biology and population parameters of this species in Turkey. In these studies, age data are obtained from whole otoliths (13-16) or scales (17). However, there is no specific study on validation age determination of red mullet. Therefore, this study aims to examine different calcified structures, to determine its suitability for ageing, and to demonstrate the differences between whole otolith and broken-burnt otoliths of red mullet.

Material and Method

The study materials, consisting of 156 red mullet (*Mullus barbatus ponticus* Essipov, 1927) (99 females and 57 males), were provided from commercial fishery trawlers in the Black Sea (Samsun, Turkey) between April 2001 and October 2002. Five bony structures, i.e. scales, vertebrae, otoliths (sagitta), opercles and subopercles, from each fish were removed. Scales and vertebrae (18), otoliths (19), opercles and subopercles (20) were prepared for age determination by different techniques.

After examinations, scales, vertebrae, opercles and subopercles were excluded from age determination. It was because scales and vertebrae did not possess suitable annulus characteristic for ageing, and also opercles and subopercles did not have enough ossifying as the annuli were not observed clearly. Otoliths were read once by two experienced readers under a binocular microscope at 10 x 2 magnification with reflected light in a few drops of alcohol against a black background. Readers did not have any reference such as fish length, weight and sex, except the collection dates and gonad stages of the samples, and all readings were done independently.

The broken-burnt method (19) was applied to the 61 otoliths because of the difficulty of interpretation of annuli on the otolith edge. Ten samples of broken-burnt otoliths were not appropriate for age determination because they were broken from out of center. The remaining 51 broken-burnt otoliths were treated with a drop of vegetable oil and read once under a binocular microscope at 10 x 4 magnification with reflected light by the same two experienced readers.

The mean ages, percent agreement and ageing error were computed for age data. The graphics of age compositions for structures and age distribution between structures were made and compared.

The mean age for each structure-reader combination ($\overline{X}_{kl}$) was calculated by summing the age estimates ($\overline{X}_{ijkl}$) across fish (f) and replicates (n) and dividing by nf (total fish) (7):

$$\overline{X}_{kl} = \frac{\sum_{i}^{n} \sum_{j}^{f} X_{ijkl}}{nf} \quad [1]$$

The percent agreement (PA) and ageing error (AE) were used for precision of age determinations. For PA, two readings by the two readers for each sample were determined and implied as the percent of total sample number. The reproducibility index, called the ageing error (AE) was modified by Baker and Timmons (7) from Sharp and Bernard's (6) sampling standard error. AE was calculated for structure-fish combination. For this purpose, variance for each structure-fish combination [2] was estimated and AE was calculated by having the square root of variance valued [3].

$$V_{(\overline{X}_{jk})} = \frac{\sum_{i}^{n} (X_{ijk} - \overline{X}_{jk})^2}{(n - 1)} \quad [2]$$

$$AE = \sqrt{V_{(\overline{X}_{jk})}} \quad [3]$$

where $\overline{X}_{jk}$ is the mean age for J^{th} fish, k^{th} structure and n is number of replicates. Ageing error (AE) is analogous to the average percent error (APE) proposed by Beamish and Fournier (21) and the coefficient of variation (CV) proposed by Chang (22).

Results

For 156 fish, the mean age and ageing error from whole otolith readings were calculated as 2.72 and 0.43, respectively. The percent agreement of the two readers was recorded as 81%. Ages were observed between 2 and 4 in whole otolith counts and obtained 3 year-classes. Age-group 3 represented the dominant year-class (Figure 1).

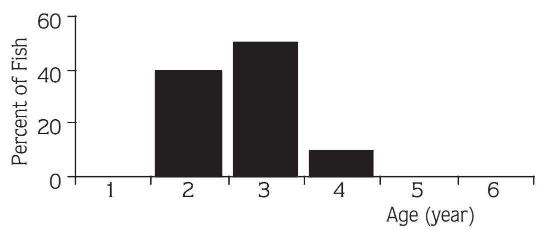

Figure 1. The mean ages of 156 red mullet based upon annuli counts of whole otolith.

For 51 samples, the mean age and ageing error were determined for whole otolith as 2.85 and 0.44, whereas these values for broken-burnt otolith were estimated as 3.79 and 0.37, respectively. The percent agreement of the age readings was recorded to be 80% for whole otolith and 87% for broken-burnt otolith (Table). Age difference between the mean ages of each different technique was approximately 1 year. This difference was found significant (ANOVA, $F = 34.89$, $P < 0,001$). In other words, ages of fish were underestimated by a minimum of 1 year with whole otolith.

Table. Comparisons of precision and mean ages for 51 red mullet.

Technique	No. of fish	Mean age	Percent agreement	Ageing error
Whole otolith	51	2.85	80	0.44
Broken-burnt otolith	51	3.79	87	0.37

Age compositions of different techniques were also dissimilar (Figure 2). Samples between 2 and 4 ages of whole otolith indicated 3 year-classes, while samples of broken-burnt otolith between 2 and 6 ages indicated 5 year-classes.

Although the rate of agreement within reading techniques was fairly high (Table), the rate of agreement between techniques was only 29% (15 fish). On the other hand, there was a disagreement of 71% (Figure 3). The majority of disagreement stemmed from the age difference of 1 year.

Discussion

It may be difficult to get accurate and reliable data for age determination from all bony structures. Therefore, it is necessary that all bony structures are examined and compared for getting accurate and reliable data. Initially, some bony structures may be discarded since they do not possess suitable annulus characteristics, and one or two bony structures may be approved for age estimation. After preliminary examinations, it has been determined that there are many rings ranged irregularly on scale and vertebra of red mullet. Determination of annuli from the other false rings is difficult, especially for inexperienced readers. On the other hand, the uncertainty of first annulus on scales and false rings on vertebra may cause errors in age estimations (4). Opercle and subopercle have not been included as annulus characteristics because they are not adequately ossified (23,24). Due to the above-mentioned reasons, scales, vertebrae, opercles and subopercles have been found unsuitable for age determination and they have not been evaluated in this study.

Our findings indicate that the broken-burnt otoliths are the best technique for age determination of red

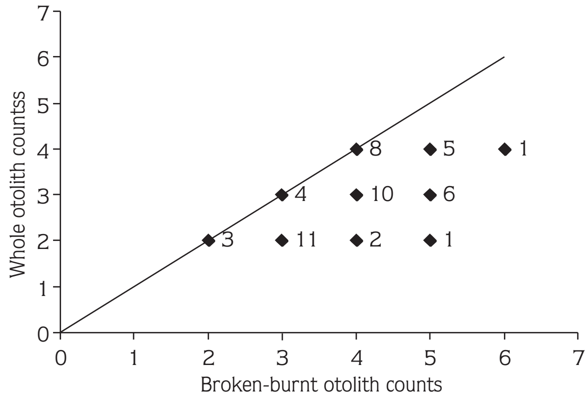

Figure 3. Comparison of whole otolith and broken-burnt otolith counts for 51 red mullet. Diagonal line represents whole otolith age equals to broken-burnt otolith.

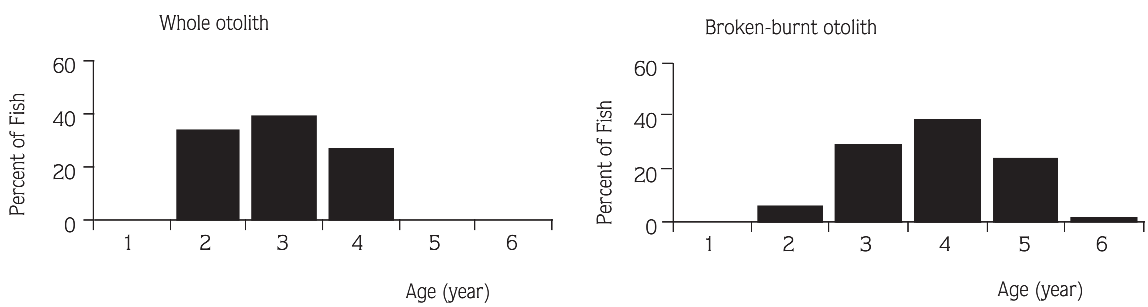

Figure 2. Age compositions of 51 red mullet derived from whole otolith and broken-burnt otolith.

mullet inhabiting the Black Sea. The broken-burnt otoliths are superior in all criteria we used to evaluate the two different techniques, in spite of its difficult preparation. They are clearer and easier to interpret than whole otoliths, and they have the highest between-reader agreement. Moreover, the whole otoliths underestimate the ages of fish (Figure 3). Our findings are new, and there are no previous studies concerned with the ageing of red mullet. However, similar results were reported by Campana (25), Skurdal et al. (26), McFarlane and Beamish (27), Polat and Gümüş (28) and Bostancı and Polat (10) for other species. In conclusion, we recommend that broken-burnt otoliths be used for age determination of red mullet (*Mullus barbatus ponticus* Essipov, 1927).

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