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Shorte Note / Araştırma Notu

A Comparison of Ageing Techniques to Improve Precision of Age Estimation from Fish Scales

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Özet: ***Balık pullarından yapılan yaş tahminlerinin doğruluğunu arttırmada yaş tayini tekniklerinin bir karşılaştırması.*** Kızılkanat, *Scardinius erythrophthalmus* (L., 1758), gümüşi havuz balığı *Carassius gibelio* (Bloch, 1782) ve kızılgöz, *Rutilus rutilus* (L., 1758) balıklarının pulları iki farklı habitattan elde edildi. Tüm balık türlerine iki yaş tayini yöntemi uygulandı. Bu yöntemler arasında uyuşmazlıklar bulundu. Farklı okuyucuların yaş tayinleri arasındaki uyum, pul basma yönteminde pul temizleme metodundakinden daha iyiydi. Pul basma metodu yaş hesaplamalarının doğruluğu bakımından en iyi sonuçları ortaya çıkardı. Bunun yanı sıra hızlı kullanım, verileri saklama, ve kolay geri-hesaplama gibi birçok avantajları görüldü.

Anahtar Kelimeler: Balık, yaş tahmini, baskı, temizleme, pul.

Abstract: Scales of rudd, *Scardinius erythrophthalmus* (L, 1758), prussian carp, *Carassius gibelio* (Bloch, 1782) and roach, *Rutilus rutilus* (L., 1758) were sampled from two different habitats. Two methods of determining age from scales of fish were applied for all fish species. There was disagreement between methods. The agreement between the determinations of different age readers was better with the impressing scale method than cleaning scale method. Impressing method produced the best results in terms of precision of the age estimates besides it had many advantages such as quick - apply, storing data and easy back-calculation.

Key Words: Fish, age estimation, impression, cleaning, scale.

Introduction

Fish age determination is a fundamental task in fisheries biology and particularly in stock assessment, where most models used in temperate waters are age-structured (Hilborn and Walters, 1992). The success of this task may, therefore, have a strong influence on the precision and accuracy of estimated stock parameters (Gulland, 1955). Scales have been used widely for ageing because they are collected, prepared and read easily. The greatest difficulty in the determination of fish age is to identify annual annuli in the scale, following objective criteria which must be validated and periodically calibrated between readers. This is helped by a clear visualisation of scale patterns. Several ageing techniques of fish scales have been used for this purpose (Lea and Went, 1936, Campbell and Arthur, 1953; Smith, 1954, Nakamura et al., 1998). Even though it has been mainly distinguished two common techniques as cleaning and impressing scale of fish for the ageing, they have not been compared qualitatively, yet.

In the present study a statistical comparison of age estimates for these two common ageing techniques was undertaken in order to determine the most precise method for ageing of fish from scales.

Roach, *Rutilus rutilus* (L.), rudd, *Scardinius* erythrophthalmus (L.) and Prussian carp, *Carassius gibelio* (Bloch, 1782) scales were chosen for this work because of their availability and also because their structure is similar to that of many other commercial species. It is likely, therefore, that the best ageing methods for these would also be suitable for other species.

Materials and Methods

The study material consisted of 30 roach, 30 rudd and 30 prussian carp provided randomly from two localities (Sapanca Lake and Ömerli Reservoir). Scales were taken from the preferred area between the left lateral line and dorsal fin approximately five to ten.

Two ageing methods were applied as following;

(a) Cleaning scale; about 10 scales were soaked in 4 % KOH solution for 24 hours. After they were rinsed with distilled water and then they were subsequently put in 96 % alcohol for 10-15 min. They were then mounted between two glass slides and dried. Scales were observed with a binocular microscope at 10x magnification with transmitted light and scales with small circular nuclei were regarded as original scales (Geldiay and Balık 1996).

(b) Impressing scale; about six scales from each specimen were placed on a 1 mm thick polycarbon plastic plate and pressed at a roller press (Fig. 1). After the scales were removed, their prints were left on the plate. The prepared plates bearing the prints of scales were read using a Microfish Reader (V.A.G. MPF-System 2400 R) (Lagler 1956).

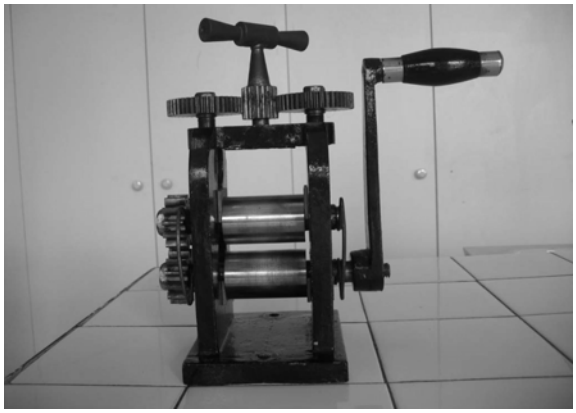

Figure 1. External view of the roller press.

An opaque zone (summer) and following translucent zone (winter) were together regarded as a year's growth, and the age was determined as the number of translucent zones. A January 1st birthday was assumed (Chilton and Beamish, 1982).

The methods were examined by four readers three times each for the three species. The readers did not have any reference to any information such as fish length, weight and sex, except of collection date of the sample. Readers aged the samples independently.

Agreement among readers was expressed as Index of Average Error (Beamish and Fourner 1981), calculated as; $IAE = 1/N \Sigma(1/R\Sigma(|Xij - Xj| / Xj))$, where N is the number of fish aged, R the number of times each fish is aged, Xj the average age for the jth fish, and Xij the ith reading of the jth fish.

Similarity of estimates between methods was tested by Wilcoxon's test on the ranked values (H_0: no difference in age reading). Large absolute Z value results in rejection of H0. Differences between methods and replicates were tested with ANOVA. Student's t – test was used to compare average ages of the sampled fish by each method and reader (Zar, 1996).

Results and Discussion

The ANOVA results for all fish species and replicates mostly were identical. Significant differences were found in impressing method for Reader C and D and in cleaning method for Reader A and C (Table 1). Average ages estimated with two aging methods for all fish species and readers were identical and mostly not significant between each other (Table 2). For all four readers (A, B, C, and D) the comparisons by two age determination methods were similar, although the differences were higher for roach (Table 3).

For cleaning method IAE (Index of Average Error) was considerably higher than impressing method for all readers except of Reader B (Table 4). The lowest IAE was found in impressing method with 4.46 (Reader A) and highest IAE was found in cleaning method with 14.81 (Reader C).

These results show that, depending on the ageing method used, the determined ages can differ significantly, due to reader ability to distinguish more or fewer annuli. Therefore, growth modelling and estimation of other parameters involving age are likely to be influenced by the procedure followed for age estimation. Also, the lack of significant differences between replicates and of a significant interaction between replicates and methods, suggest that consistent criteria for age estimation were followed in this work.

Table 1. Results from ANOVA for the differences between replicates for both methods. o: p>0.05, x: p<0.05, xx: p<0.01, xxx: p<0.001.

Reader	Rudd		Prussian carp		Roach	
	Cleaning	Impression	Cleaning	Impression	Cleaning	Impression
A	x	o	o	o	xxx	x
B	o	o	o	o	o	xxx
C	x	xx	o	xxx	xxx	xxx
D	x	xx	o	xxx	xxx	xxx

Table 2. Mean ± S.D. age calculated for each method and fish species.

Fish species	Method		
	Cleaning	Impression	*p*
Rudd			
Reader A	6.66 ± 1.18	6.67 ± 1.38	>0.05
Reader B	5.44 ± 1.13	6.01 ± 1.32	<0.05
Reader C	7.88 ± 1.57	7.48 ± 1.31	>0.05
Reader D	6.54 ± 1.88	6.10 ± 1.28	>0.05
Prussian carp			
Reader A	5.22 ± 1.22	5.44 ± 1.57	>0.05
Reader B	5.48 ± 1.63	5.80 ± 1.94	>0.05
Reader C	6.53 ± 2.12	6.46 ± 1.43	>0.05
Reader D	5.34 ± 2.01	5.68 ± 1.91	>0.05
Roach			
Reader A	5.86 ± 0.68	4.89 ± 0.63	<0.05
Reader B	4.38 ± 0.96	4.34 ± 0.81	>0.05
Reader C	6.09 ± 0.84	5.09 ± 0.81	<0.05
Reader D	4.88 ± 0.79	5.51 ± 0.84	<0.05

Table 3. Reader comparison of two ageing methods. Percentage of similar age determinations (%), Wilcoxon-test parameters (Z) and significance (*p*).

Fish species	Reader			
	A	B	C	D
Rudd	60 % Z = 0 *p* = 1	40% Z = 1.786 *p* = 0.074	36% Z = -1.070 *p* = 0.285	53% Z = -1.027 *p* = 0.304
Prussian carp	43% Z = 0.419 *p* = 0.675	60% Z = 0.696 *p* = 0.486	63% Z = -0.167 *p* = 0.867	57% Z = 0.252 *p* = 0.801
Roach	13% Z = -6.029 p<0.005	57% Z = -0.145 *p* = 0.885	10% Z = -4.697 *p*<0.005	23% Z = 3.110 *p*<0.005

Table 4. Index of Average Error (IAE) calculated for each method and fish species.

Reader	Rudd		Prussian carp		Roach	
	Cleaning	Impression	Cleaning	Impression	Cleaning	Impression
A	7.69	4.46	7.52	7.95	10.11	6.26
B	6.00	6.05	5.89	9.35	7.78	11.82
C	8.63	7.40	14.81	6.90	8.57	9.67
D	9.14	8.47	13.08	7.54	12.00	8.36

The precision of age estimates is most likely to be correlated with the sharpness of the annuli pattern in the scales; hence the most precise estimates in terms of IAE should correspond to the method that performed best. This was the impressing scale method (Table 4).

A clear visualization of the scale pattern is important not only for an effective counting of annual annuli, but also to allow the reader to apply pre-defined criteria in distinguishing between annual and false annuli, which is very important for accurate age estimation. Although the methods tested here are expected to act in the same way on annual and false annuli, enhancing the visualization of the scale pattern will help to put in practice established criteria.

Although cleaning scales is probably still common method to facilitate fish age determination, it performed less well than impressing method in this study. Moreover, in cleaning method, scales need to be treated by several chemicals because mud and slime may remain on scales and interfere with the reading of the ages of fish. These practices result in waste of time and material.

In view of the clarity and distinctness of the image which is obtained from impressing the scales of fish, age determination can be done more rapidly. The preparations obtained by impressing scales are also convenient for back-calculation of rate of growth and for other work connected with the scales of fishes, as well as for storing data.

In conclusion, impressing scale method is easier to apply and gives more precise estimates compared cleaning scale method, which is a good reason to use it routinely.

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