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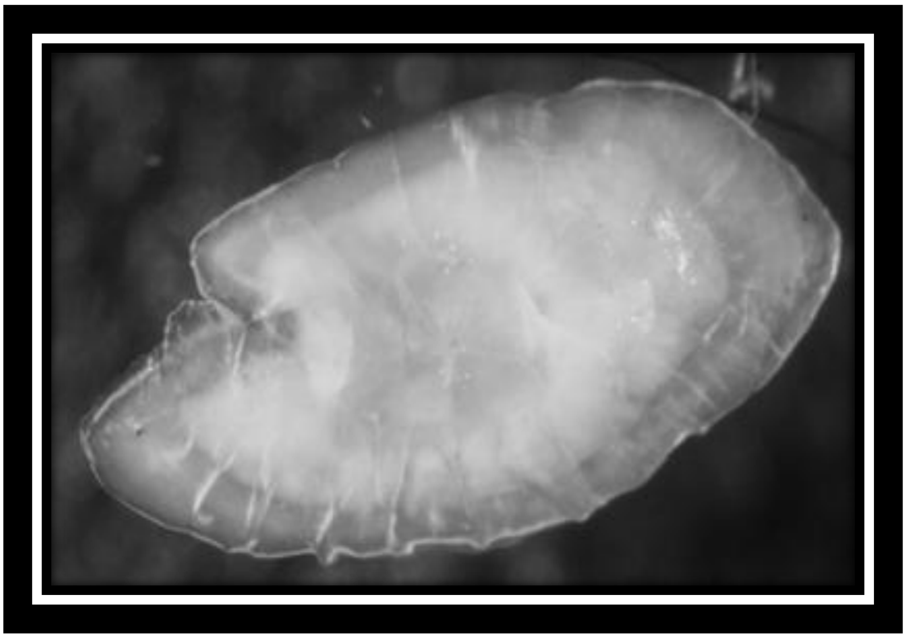
Setting up interpretation criteria for juvenile and adult European anchovy otoliths from Canary Islands



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Setting up interpretation criteria for juvenile and adult European anchovy otoliths from Canary Islands

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ABSTRACT

A standardisation of annual age determination in juveniles and adults for this species (*Engraulis encrasicolus*) is proposed. A total of 150 sagitta otoliths were selected for the analysis and annual increments were read in the postrostrum and antirostrum axes under a dissecting microscope on 25X. It was used three different treatments: water, alcohol and glycerine in order to get the best ring appreciation. All otoliths were aged three times by a single reader. The results not show high differences between the readings for the same one, or any treatments. Consequently, it is suggested bearing the economic part in mind to use water for later readings of the annual age determination of anchovy. Likewise one proposes the otolith validation by means of a new methodology where to determine the daily increments, checking that the determination realized in this work is correct.

Keywords: *Engraulis encrasicolus*, otolith, juvenile, adult, validation

RESUMEN

-Establecimiento de un criterio para la asignación de edad mediante el análisis de otolitos de juveniles y adultos de anchoa europea en Gran Canaria- Se propone una metodología con el fin de estandarizar la lectura de los incrementos anuales en juveniles y adultos de anchoa europea (*Engraulis encrasicolus*). Para ello se eligieron un total de 150 otolitos *saggita*, los cuales se leyeron por la parte cóncava con una magnificación de 25X. Se usaros tres tratamientos diferentes, agua, alcohol y glicerina a fin de obtener la mejor apreciación de los anillos. Se realizaron tres lecturas por un mismo lector para cada tratamiento. Los resultados no muestras grandes diferencias entre las lecturas para un mismo, ni entre los distintos tratamientos. Por consiguiente, se sugiere teniendo en cuenta la parte económica el utilizar agua para posteriores lecturas de los incrementos anuales de anchoa. Asimismo se propone la validación de los otolitos mediante una nueva metodología donde poder determinar los anillos diarios, verificando que la determinación realizada en este trabajo es correcta.

Palabras clave: *Engraulis encrasicolus*, otolito, juveniles, adultos, validación

1. INTRODUCTION

The genus *Engraulis*, include eight species, three of which are commercial important: the European anchovy, *Engraulis encrasicolus,* Linnaeus, 1758; Japaneses anchovy, *Engraulis japonicus*, Temminck and Schlegel 1846; and Peruvian anchovy, *Engraulis ringens,* Jenyns, 1842 (Inoue et al, 2001). The anchovies represent at least 15 % of 80 million tons of fish captured annually in the world (FAO, 2009). This species is also an essential element of marine food web due to its significant biomass at intermediate levels (Palomera et al, 2007). The European anchovy (*Engraulis encrasicolus*) is widely distributed along the North Eastern and Central Atlantic (coasts of Europe, about South Bergen, Norway, but absent in the Baltic and it is rare in the North sea); in the whole Mediterranean and Black and Azov seas, southward along the coast of west Africa to South Africa (Whitehead et al, 1988). In the Canary Islands water there is one of the main pelagic resources for artisanal fleets. Anchovies are well represented in the catch throughout the year, in the Canary Islands coastal. Despite the importance of the anchovy fishery in the Canary Islands, there is not information available, either on the age structure or on the growth of this species in the area.

The last knowledge of daily growth microincrements in fish otoliths (Pannella, 1971) opened new possibilities for studies on fish population dynamics through age and growth estimation (Methot and Kramer, 1979), duration of larval staged and hatching dates (Moksness and Fossum 1992), individual growth analysis (Moksness and Wespestad 1989), and recruitment patterns based on age structure of juveniles (Methot, 1983). Given the importance of accurate ages for determining growth trajectories, age compositions, the primary goal of the current work was to evaluate the best way to read the annulus of the European anchovy otolith.

2. MATERIAL AND METHODS

Sampling

Juveniles and adult European anchovy otoliths were obtained from fishes captured from August 2011 to April 2012 (smale scale fishery of Gran Canaria; Hernández-Castro, 2012). In total, the length range of the anchovy juveniles and adults analyzed was 46 to 140 mm. The number of anchovy otoliths used in this study was selected by fish-length-ranges (Table 1).

Table 1. Summary of samples used in this study.

	Fish number		
Lenght-ranges (mm)	August-January	February-April	Total
46-50	5	3	8
51-55	5	5	10
56-60	5	5	10
61-65	5	5	10
66-70	5	5	10
71-75	5	5	10
76-80	5	5	10
81-85	5	5	10
86-90	5	5	10
91-95	5	5	10
96-100	5	5	10
101-105	3	5	8
106-110	5	5	10
111-115	5	1	6
116-120	5		5
121-125	5		5
126-130	4		4
131-135	3		3
136-140	1		1
Total	86	64	150

Otolith processing

Otoliths were extracted in the laboratory from anchovy that were frozen at sea, and processed at University of Las Palmas de Gran Canaria. After otoliths were extracted they were rinsed in water to remove tissue and stored dry for subsequent ageing.

For surface ageing, otoliths were placed under a plate with 3 different treatments: water, alcohol and glycerine, and were photographed using a camera connected to a dissecting microscope at 25X magnification, using reflected light. Against a dark background, opaque zones appeared light, translucent zones appeared dark. The outer ring was considered to be either opaque or translucent only when the entire edge demonstrated this characteristic. Annuli were defined as the area consisting of one opaque zone (summer growth) and one translucent zone (winter growth). Annuli were counted from the core to the post-rostrum on the distal face of the whole otoliths along the antero-posterior axis using reflected light.

The age was determined by interpreting growth rings on the otoliths. All otoliths were aged three times by a single reader, without prior information on length, sex or time of capture. The readings were done in a random order.

Data analysis

The bias and precision of annulus counts were compared between readings using the coefficient of variation (CV) of Chang (1982) and the average percent error index (IAPE) of Beamish and Fournier (1981). The age-biased plot was also used to compare the readings (Campana *et al*. 1995, Campana 2001).

3. RESULTS

Otolith's analysis

A total of 150 otoliths were examined. The otoliths analyzed under reflected light show an opaque zone, a high content of calcium carbonate, impedes the step of the light, and a hyaline zone, where the light throughout with high facility. These zones are distributed concentric around the core Figure 1.

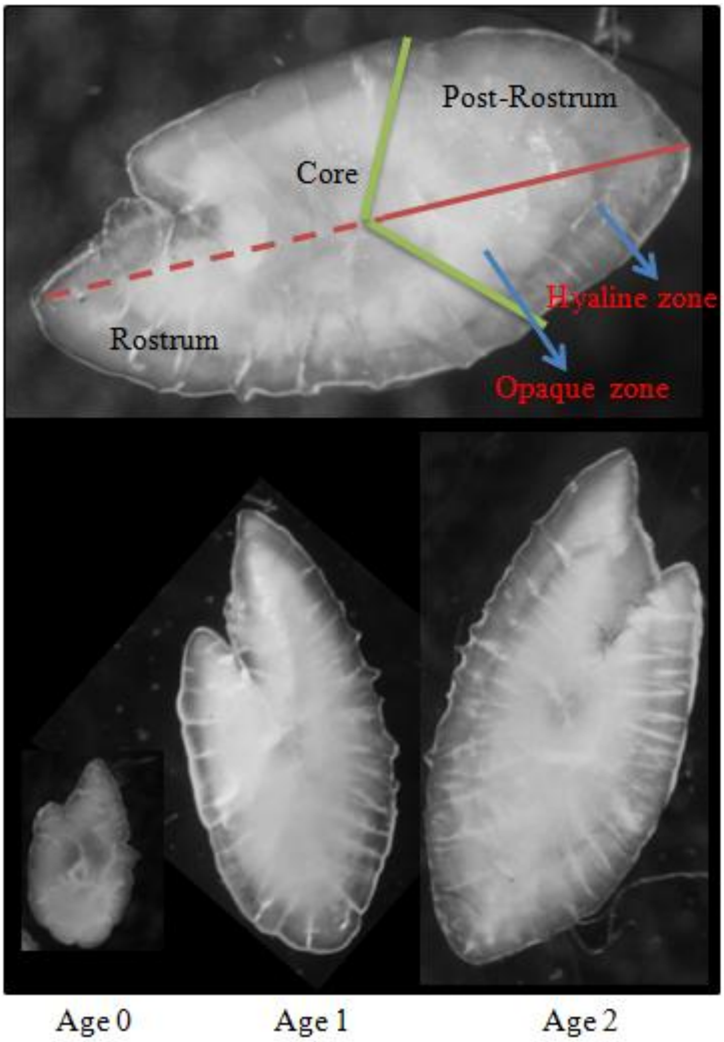

Figure 1.- Top: Otolith's part; Bottom: Otolith of *Engraulis encrasicolus* used in this study.

Precision of age interpretation

The index of average percent error (APE) (Beamish and Fournier, 1981) and the mean coefficient of variation (CV) (Chang, 1982) were calculated to estimate the relative precision of the readings. Both authors suggest that these indexes are adapted so much to compare assignment methods of age as to compare between readers (Hoening et al, 1995).

The indexes of the precision of each treatment are shown in the Table 2.

Table 2.- Precision index estimated to each treatment.

Treatment	Number of fish	APE	CV
Water	150	1.14	2.09
Alcohol	150	1.20	2.85
Glycerine	150	1.55	2.20

Age-bias plots provide a clear, simple means of detecting bias and are excellent for matched pair relationships. The figure 2 show the age-bias plot using the information presented in table 3. Matched pairs are compared by plotting the mean counts of one treatment for the samples corresponding to each of the age categories of the other treatment.

Table 3.- Age frequency tables summarizing pairwise comparisons of age estimates from three different otolith samples. Data are numbers of fish.

Age (years) estimated by:	0	Age years 1	2	Total	% agreement
Water		**Alcohol**			
0	60			60	100
1		44		44	
2			28	28	
Water		**Glycerine**			
0	60	0		60	97
1		42		42	
2		4	28	32	
Glycerine		**Alcohol**			
0	60			60	97
1	1	43	3	47	
2			24	24	

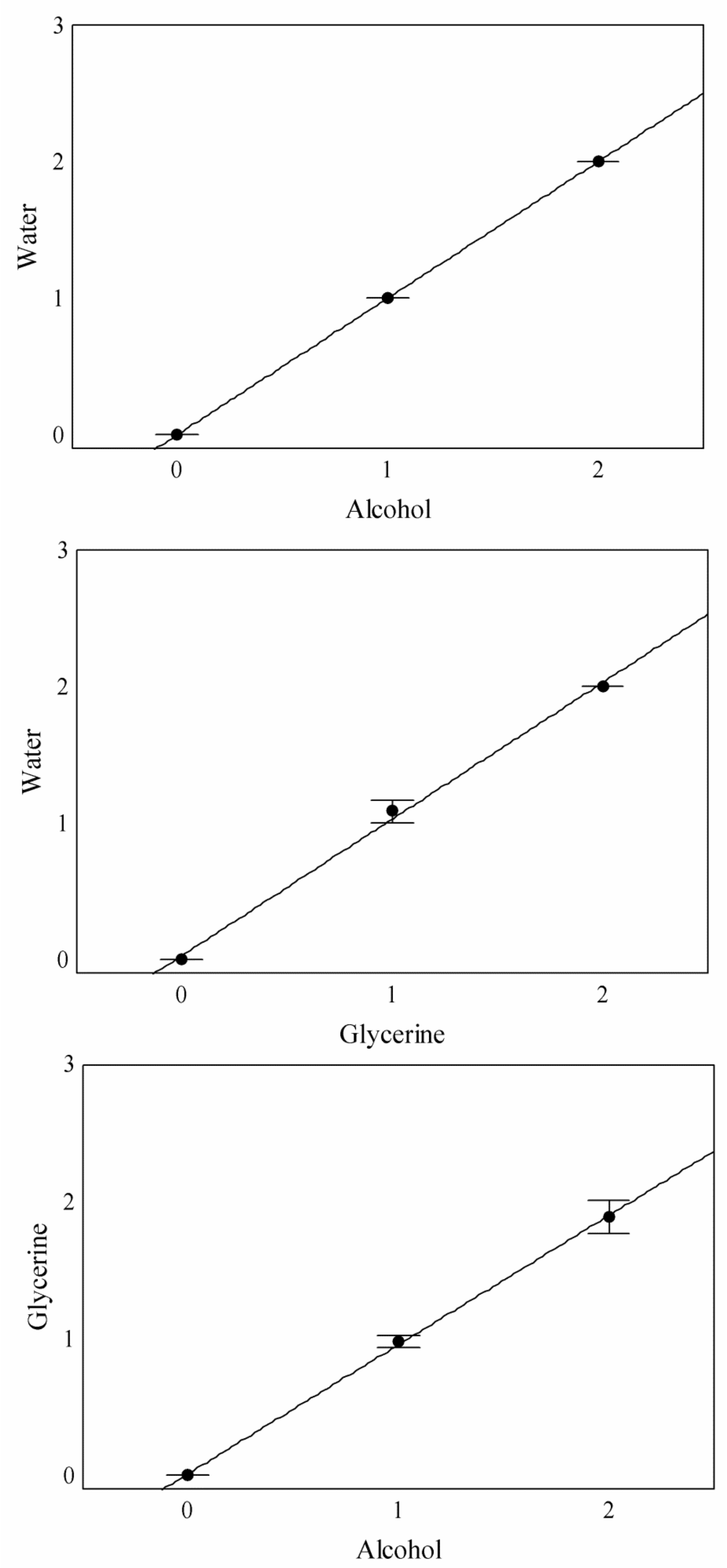

Figure 2.- Age-bias plot for each of the three pairwise age comparisons presented in Table 3. Each error bar represents the 95% confidence interval. The 1:1 equivalence (solid line) is also indicated.

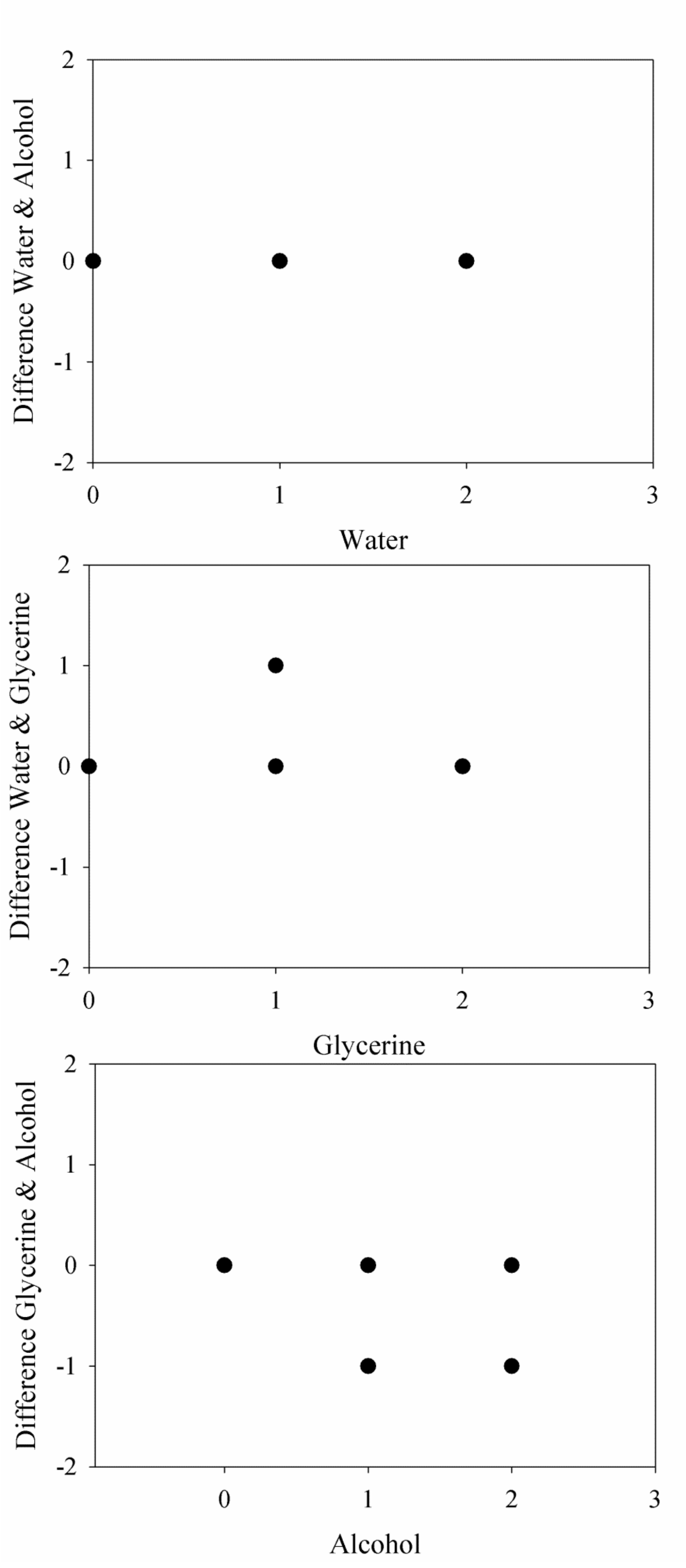

Figure 3.- Differences (years) in age estimates between paired age treatment for each of the three pairwise age comparisons in Table 3.

Engraulis encrasicolus otoliths

The age readings of young *Engraulis encrasicolus* produced relatively less difficulty than the older fish. Age-bias plot comparing between treatments indicate the goodness of the ageing procedure adopted and a reasonable level of consistency.

The differences in age estimates revealed no bias in age estimates between water alcohol, water-glycerine and glycerine-alcohol, shown in the figure 3, for European anchovy. In summary, there are not important biases using any treatment.

4. DISCUSSION

All otoliths were aged three times by a single reader. The grade of subjectivity, using a single reader, is due to the fact that point of comparison does not exist between readings. It is possible a systematic bias in the readings that the single reader does not identify.

Nowadays, it is very common to find works where the interpretation of the same structure is realized by two laboratories (Campana, 2001; ICES, 2010), a complete independence being achieved between readings and readers. In this case, it is suggested the participation of a second reader.

According to the indexes of precision APE and CV for each treatment the readings are precise; nevertheless, the age-bias plot shows that older fish have more variability in age estimation, contrary to young fish that revealed no bias in age estimation between any treatments (Campana, 2001; ICES WAKRA Report, 2009).

In this study the index of average percent error and the mean coefficient of variation were less than 5% and 7.5% respectively, so, the readings between each other are acceptable according to Campana (2001). In conclusion, it suggests using water as only treatment to analyses the otoliths of *Engraulis encrasicolus* in Canary Islands waters. Nevertheless, that this study is precise it does not mean that it is exact, so, the indexes of precision are not a tool that allows to know the exactly fish age. (Campana, 2001)

The author recommended implements this work with a new methodology of validation annual periodicity of ring formation in otoliths. The used of this validation method will improve the accuracy of the age estimates.

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