Working Document 17 presented to the ICES Working Group on the
Biology and Assessment of Deep Sea Fisheries Resources

ICES WGDEEP - Copenhagen 2-8 March 2011

Morphometric and morphological studies of Red seabream (*Pagellus bogaraveo*) otoliths from the Strait of Gibraltar: Exploratory analysis of its application for ageing.

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Abstract

In the last years, the otoliths have become a useful tool for the determination of ichthyic species, because these structures present a high morphologic specificity. Besides, its shape should change between the sampled ages. Thus, our study deals with several features of the otoliths (sagitta) related with the age of the individuals. 235 (Morphometry) and 53 (Morphology) otoliths from Red seabream samples, 2003 – 2008, of the Strait of Gibraltar were analyzed. The combined use of both features (morphometrics and morphological) resulted in a discriminant function which an ageing success higher than 70%.

1. Introduction

The Red seabream is found in the NE Atlantic, from South of Norway to Cape Blanc, in the Mediterranean Sea, and in the Azores, Madeira, and Canary Archipelagos (Desbrosses, 1938). Adults inhabit depths ranging around 300-700 m. The vertical distribution of this species varies according to individual size (Desbrosses, 1938; Guegen, 1974; Silva *et al*., 1994; Gil, 2006).

The knowledge of the fish age is a major task in the analytical stocks assessment. Traditionally, the method used to estimate ages in fish is based on the study of bony fishes hard parts as otoliths, scales, spines... (Bermejo, 2007). In our case, the Spanish fishery of the Strait of Gibraltar, ALKs were obtained by three agreed otoliths readings collected from 2003 onwards. In the last ICES WGDEEP assessment the combined ALK (2003-2007) was obtained by 1242 three agreed readings from otoliths collected from 2003 onwards. It covers lengths from 24 to 62 cm. Combined ALK comprises ages between 3 and 10.

The age estimate from the reading of growth rings with binocular lens is complex, requires a lot of time-consuming and depends on the reader experience (Boehlert and Yoklavich, 1998). Besides is also a subjective method that requires reading agreements from more than a reader which is even greater cost and time investment. Therefore, the ideal would be to have an objective, reliable and accurate for the age estimates minimizing time and cost. Recently, several researchers have directed their efforts towards the analysis of changes in the otolith shape and its application in different fields of research, mainly stocks identity. Our proposal differs significantly because the otoliths study came from the population of the Strait of Gibraltar only, so a stock identity study is not acceptable. However, the study of possible differences between different age classes from the fishery is quite interesting, especially the practical usefulness it might have on the criteria adopted for the estimation of the Red seabream growth in the Strait of Gibraltar.

2. Material and methods

Table I presents the primarily otolith samples used for this study (266) by year and age class. From those, 235 were used in morphometrics, but only 156 can be used in Discriminant Analysis (the first ages have not a normal distribution). Morphometrics variables taken in account are: weigth (precision scale), thickness and curvature (gauge) and others by image analysis as: Area, Maxferet, MinFeret, EqDiameter, Circularity (Figure 1). For digital image capture and its analysis has been used NIS-Elements AR 3.2 NIKON Software.

Morphological variables were created transforming the 20 Fourier harmonics in Principal Components which could describe morphological variations.

Kolmogorov-Smirnoff and t de Student test were used to compare left and right otoliths measures. Also, mono and multivariant statistics tools were applied in the work development.

3. Results and discussion

Shape and structure of the otoliths has taxonomical specificitions (Volpedo and Echevarría, 2000). Morphology, morphometry and composition of the *sagitta* otoliths could be used for fish stock identification (Bori, 1986; Campana *et al.*, 1996; Bermejo, 2007). The shape and structure also varies with the individual's age because the otolith growth is a result of the interaction between fish growth rate and the effects of environmental conditions (Campana and Nelson, 1985, Gutierrez and Morales-Nin, 1986; Radtke and Shafer, 1992). These clear morphological changes can be observed *de visu*. In general, there is an increase in the otolith size with age (Figure 2): Variables such area, perimeter, maximum otolith length (MaxFeret), width (MinFeret) or thickness increases gradually with age. While circularity decreases from the Red seabream early ages and since 3 years old the otolith is taking a more oblong shape (increasing the rate MaxFeret/MinFeret to reach the maximum value at older ages: 9-10).

The result of the statistical tests is the absence of significant differences ($p > 0.05$) between left and right otolith morphometrics. So only the left otolith measures were taken for the purposes of this work.

Morphometry

Figure 3 shows the trend of the different morphometric variables by age like the Von Bertalanffy growth function. Almost all the variables present an asymptotic increasing of the values with age. Circularity was the only exception. This variable does not show any clear trend, although its lower values appear at older ages (6-10 years).

Area and EqDiameter had an average upward trend: from 10.54 mm^2 to 91.33 mm^2 and from 3.66 mm^2 to 10.78 mm^2, respectively. Besides, the intervals between the upper and lower limits by age are clearly defined and there is no overlap between age range values.

However, a classification system (regression function) using only one morphometric variable does not seem the most appropriate. Time (in this case age) is a continuous variable and, in some ways, we are "discretizing" it comprising in a single age class individuals born from January to December of each year.

Table II shows the Discriminant Analysis final results. The total reclassification reaches 61.1% of success, lower than the successful value proposed by Palmer *et al*. in 2004 and Galley *et al*. in 2006. The highest percentage, more than 75%, was obtained at older ages, age classes 9 and 10 while age class 7 was the one that had the lowest number of matches, below 50%. However most of the otoliths that are not well classified by its morphometric variables are located below or above the agreed readings otolith estimates.

Morfology

Otolith shape is a phenotypic character and should vary between ages, sexes and cohorts in marine teleosts (Smith et *al*., 2002). These shape changes may be caused by the rate of growth, as in the case of Atlantic cod (Campana and Casselman, 1993). Experimental studies with freshwater fish (Reznick, 1989; Secor and Dean, 1989) suggest that such morphological changes have a strong environmental component related with growth rates differences. Fourier Analysis obtained harmonics for the Red seabream of the Strait of Gibraltar were the inputs of a Principal Components Analysis which results into 9 functions (or Principal Components: PCs). The first five (PC1 to PC5) explain the 81% of the observed variance in the otoliths shape and its scores were used as new morphological variables. Figure 4 shows the morphological variation described in each PC from the average values and its variation (mean ± 2 standard deviation). The left side of the figure reflects the shapes overlapping image.

Galley *et al*. in 2006 propose the idea of a Discriminant Analysis performance combining morphometric variables with Fourier shape descriptors. Table III shows the results of this kind of joint analysis (7 morphometric variables and 5 Fourier PCs) with otoliths from Red seabream samples of the Strait of Gibraltar. The total reclassification reaches 85.3% of success, which clearly exceeds the value adopted by Palmer *et al*. in 2004 and also Galley *et al*. in 2006 (70%). The maximum percentage (100%) was obtained in age classes 6, 9 and 10. Age class 5 is the only one with the least number of matches, below 70%.

Throughout this work morphometric and morphological differences between the age classes considered have been patents both, so it may be appropriate its future use as helpful tool to estimate ages of the Red seabream of the Strait of Gibraltar.

4. Conclusions

The Discriminant Analysis combining morphometric and morphological variables obtained the highest percentage of reclassification success (85.3%), well above from the 70% adopted by other authors.

Changes in the otolith shape could be related with the growth rate, so that might strongly influenced by environmental component. Therefore, future work should be done including the analysis of such influence through interannual variations.

Acknowledgments

We would like to express our most sincere gratefulness to Jorge Tornero Nuñez (called *Richar*) for his help and patience in the teaching of Image Analysis stuff.

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Table I. Red seabream otoliths from the Strait of Gibraltar: Number of samples primarily considered, by age class and year.

AGE/YEAR	1997	2003	2004	2005	2006	2007	2008	TOTAL
0	17							17
1	5							5
2		10	1			10	1	22
3		8	5	5	5	6	12	41
4		7	5	5	4	5	8	34
5		5	5	5	5	6	9	35
6		5	5	9	5	3	3	30
7		5	5	9	5	5	4	31
8		7	5	4	4	5	3	28
9		4	2		3	4	3	16
10		2	2			3		7
TOTAL	23	53	35	35	31	47	43	**266**

Table II. Red seabream otoliths from the Strait of Gibraltar: Reclassification success percentage from Discriminant Analysis with morphometric variables.

Age	Predicted belonging group						
	4	5	6	7	8	9	10
4	**66,7**	23,3	10,0	0,0	0,0	0,0	0,0
5	17,2	**69,0**	13,8	0,0	0,0	0,0	0,0
6	0,0	17,2	**51,7**	31,0	0,0	0,0	0,0
7	0,0	3,4	31,0	**44,8**	17,2	3,4	0,0
8	0,0	0,0	0,0	17,4	**65,2**	8,7	8,7
9	0,0	0,0	0,0	0,0	8,3	**75,0**	16,7
10	0,0	0,0	0,0	0,0	0,0	20,0	**80,0**

Table III. Red seabream otoliths from the Strait of Gibraltar: Reclassification success percentage from Discriminant Analysis combining morphometric and morphological variables.

Age	Predicted belonging group						
	4	5	6	7	8	9	10
4	**83,3**	16,7	0	0	0	0	0
5	33,3	**66,7**	0	0	0	0	0
6	0	0	**100,0**	0	0	0	0
7	0	0	0	**83,3**	16,7	0	0
8	0	0	0	20,0	**80,0**	0	0
9	0	0	0	0	0	**100,0**	0
10	0	0	0	0	0	0	**100,0**

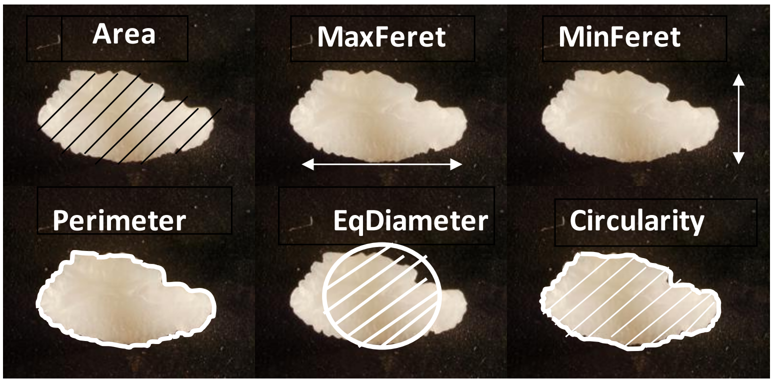

Figure 1. Red seabream otoliths from the Strait of Gibraltar: Description of the morphometric variables measured by image analysis.

Figure 2. Red seabream otoliths from the Strait of Gibraltar: Examples from different agreed age estimates.

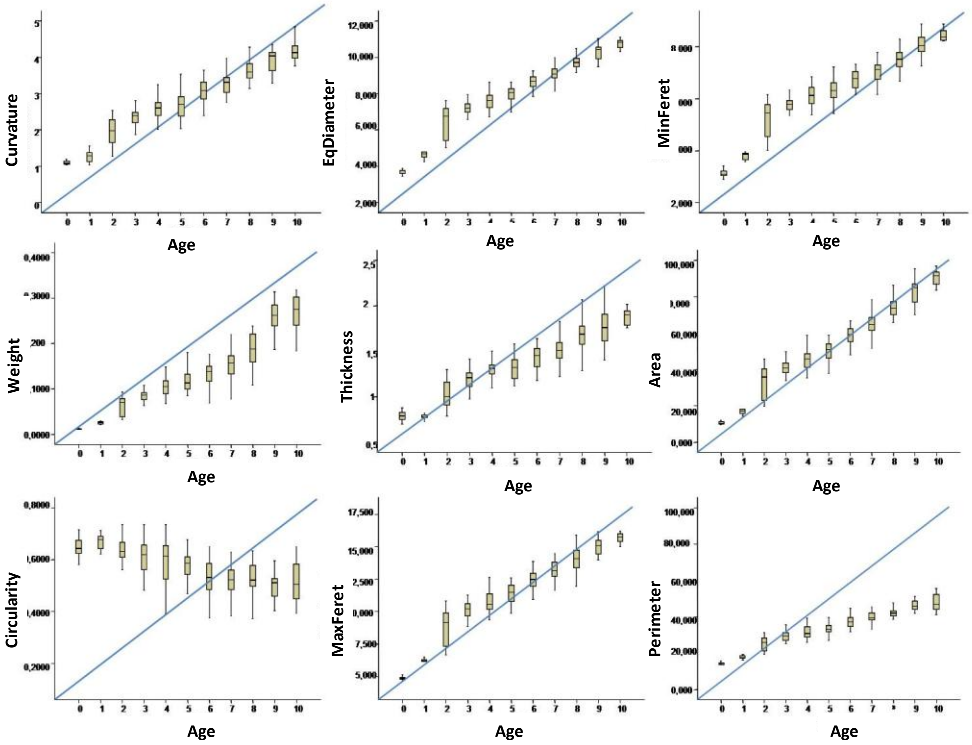

Figure 3. Red seabream otoliths from the Strait of Gibraltar: Descriptive statistics for the morphometric variables considered.

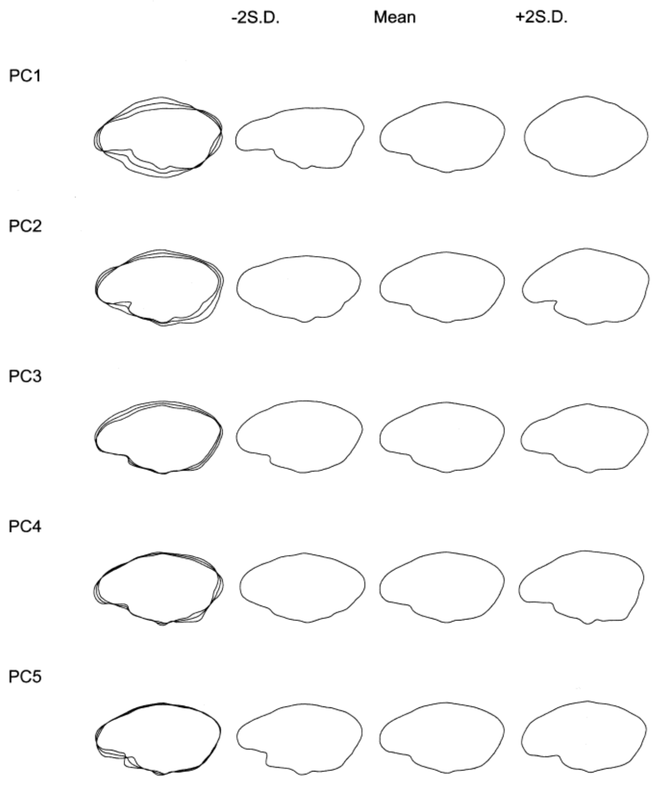

Figure 4. Red seabream otoliths from the Strait of Gibraltar: morphological variation by PC from Fourier Analysis results.