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### *Working Document*

## **Criteria for age estimation of anchovy otoliths in the Alborán Sea (Western Mediterranean Sea) based on the monitoring of the hyaline edge formation.**

Ana Giráldez & Pedro Torres

Centro Oceanográfico de Málaga, Instituto Español de Oceanografía, Puerto Pesquero s/n, E-29640  
Fuengirola (Málaga), Spain.

### **Abstract**

This study determines the seasonality in the formation of rings in the anchovy otoliths by monitoring hyaline edge in the Alborán Sea. Monthly samples were collected between October 1989 and December 1992 from the catches of the purse seine fleet. Two hyaline zones in the otolith are deposited per year, one in winter (primary) and a minor one during the summer spawning season. The formation of hyaline rings is significantly correlated with the temperature of seawater. The highest percentage of hyaline edge formation, recorded over the four year study, periodically occurred in February, when the temperature is at its lowest. When the nutritional status of fish is better, the percentage of individuals forming hyaline edge is lower. Considering these facts, a number of criteria for interpreting the growth rings on the anchovy otolith were developed. The population is subjected to a high rate of exploitation, so with the absence of older individuals in the area, it means that growth can only be accurately described in the first year. With some variations, depending on the year, anchovy grows between 12 and 13cm in their first year of life.

### **Introduction**

Due to its high market value, Anchovy (*Engraulis encrasicolus*) is the main target species of the purse seine fleet in the North of Alborán Sea. Catches have large fluctuations over the years (Abad et al., 1987), but since 1985 the catches have decreased dramatically causing serious economic problems to the fleet, so they have reduced their number of units. Specifically, in 1992 catches were the lowest recorded since studies begun in 1925. There was a slight recovery in 2001, but they are still at a very low level. The development of this fishery has been studied by Abad & Giráldez (1990, 1997) and Giráldez & Abad (1991, 2000). Aspects of reproduction have been studied by Giráldez & Abad (1995).

In the Western Mediterranean the closest anchovy growth studies are: Djabali et al. (1988) and Djabali (1991) in Algeria. Suau (1979) in Castellón (Spain), Pertierra (1987,

1992) and Morales-Nin & Pertierra (1990) in Catalonia (Spain), Campillo (1992) in the Gulf of Lion. In the Adriatic Sea, Gianetti (1985), Gianetti et al. (2003) and Sinovic (1988, 2000) and in the Black Sea, Erkoyuncu & Ozdamar (1989), and Karakam & Düzgünes (1990), Düzgünes et al. (1995); Basilone et al (2003, 2004) in the Strait of Sicily, Machias et al (2000) in Greece and Khemiri (2006) in Tunisia. Not all cases detail the criteria used for the otoliths readings and the birth date is not always specified.

This paper describes the criteria used for reading otolith ages taken from samples over a three years period (June 1989-December 1992) in the Alborán Sea. As a preliminary step to find out seasonality in the formation of rings, it has been monitored the edge of the otolith and discussed the possible relationship between the formation of rings and the temperature of the environment, as well as other biological parameters of the species as the condition factor (CF) and the gonad somatic index (GSI)

### Material and Methods

The samples were taken from commercial catches fished by purse seine vessels in the fish market of Malaga, Estepona and Marbella between June 1989 and December 1992 (Fig.1). The sagitta otoliths extracted (3918) were mounted on a black plastic plate, comprising a transparent and fluid synthetic resin (Eukitt) to increase the contrast. The results on the reproductive biology of this species based on the same samples were presented in Giráldez and Abad (1995).

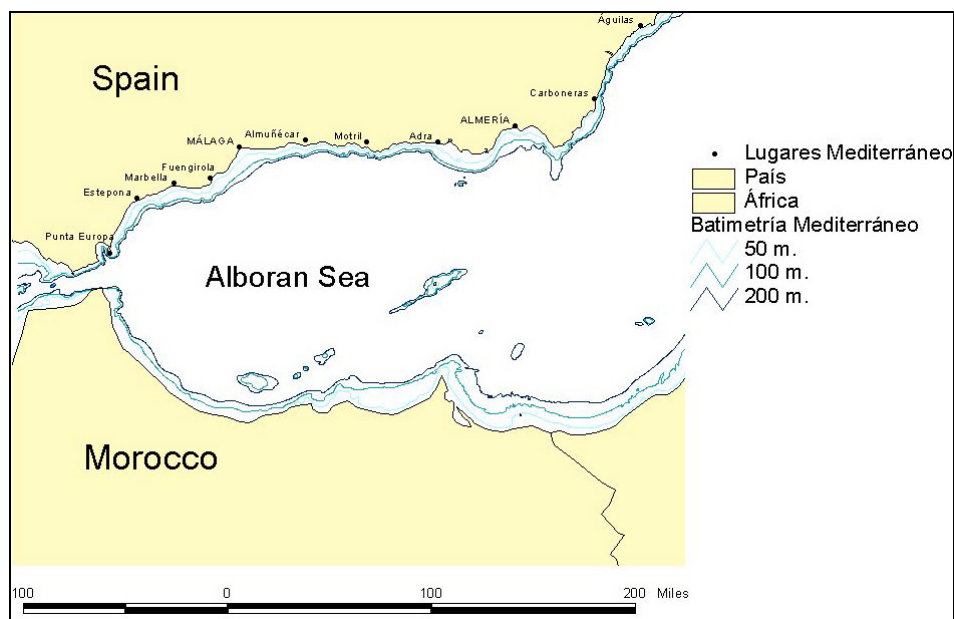


Figure 1. Geographical area where the anchovy samples were taken.

About 14% of the otoliths were discarded “a priori” as illegible due to their opacity, transparency, deformity, etc.

It has been necessary to establish criteria for interpreting the otolith edge, since the hyaline rings are thin and difficult to distinguish; only rarely, depending on the year, a clear wide band was found. The edge was considered opaque when it was white and continuous, and hyaline when it was transparent; in most cases it was discontinuous and disappeared by changing slightly the magnifying focus.

Monthly monitoring of the otolith edge was carried out in order to determine the seasonality in the formation of rings and to establish the reading criteria later used for ageing purpose.

It was estimated the percentage of hyaline edge (HE%) by month and the average yearly to find out the annual pattern. It was also calculated the average temperature of the seawater ( $T^a$ ), gonad somatic index (GSI) and Condition Factor (CF) in order to get a yearly pattern for these parameters too (Giráldez and Abad, 1995).

It has been observed the relationship between the percentage of hyaline edge and the average monthly temperature of the seawater. The GSI and the FC were analyzed with the correlation coefficient product-moment. Previously, the normality of the parameters was tested by means of D`Agostino Test (Martín and Luna del Castillo, 1990).

For the otoliths reading, a binocular microscope at 30 magnification and direct light was used. The otoliths of large individuals, fully opaque, were included in a two components resin and then polished to look at them and verify they had not any hidden ring.

## **Abbreviations**

CF: Condition factor; GSI: Gonad somatic index; HE%: percentage hyaline edge; OE%: Percentage Opaque edge.

## **Results and Discussion**

The monthly percentage of hyaline edge is shown in Figure 2. The highest percentage of hyaline edge occurs between December and February, with a maximum in February every single year. The minimum occurred between August and November. There are never a 100% of individuals forming a hyaline ring.

The formation of hyaline edge is significantly correlated with seawater temperature ( $r = -0.536$ ,  $p < 0.01$ ,  $n = 36$ ), this correlation is particularly strong in year 1990 ( $r = -0.65$ ,  $p < 0.01$ ,  $n = 12$ ). February has always been the month with the lowest temperature (Figure 2).

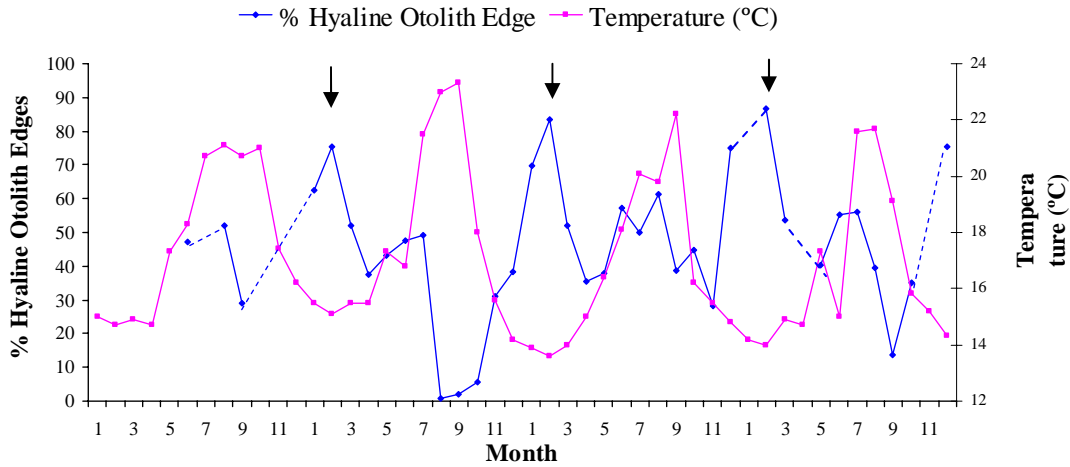


Figure 2. Monthly evolution of percentage of hyaline edge formation and seawater temperature (dashed line without data).

Figures 3 to 5 show the average HE% for the entire study period by  $T^a$ , GSI and CF respectively.

The correlation between HE% and  $T^a$  is shown in Figure 3. Figure 4 shows that coinciding with the spawning season (April-September) there is an increase in HE% that does not affect to all the individuals. Approximately half of them form this false ring. Eliminating the months of reproduction, the correlation between  $T^a$  and HE% increases up to  $-0.76$  ( $n=15$ ), suggesting that for this moment in the life-cycle the formation of the edge may be due to endogenous factors.

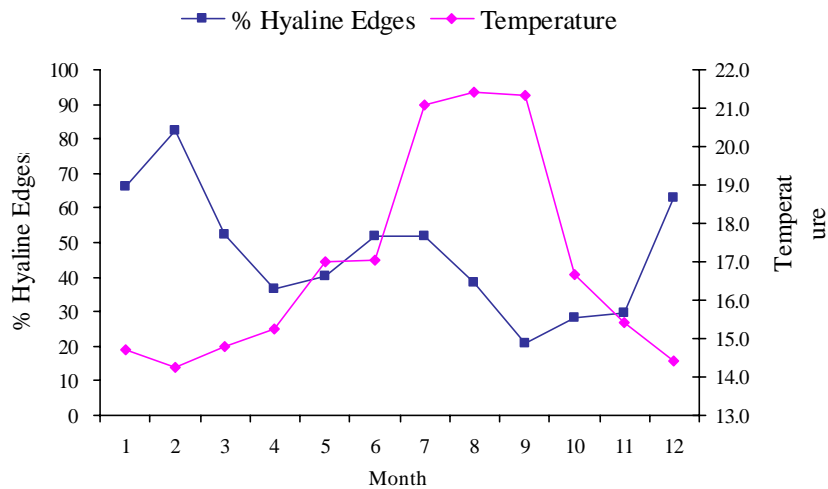


Figure 3. Evolution of percentage of hyaline edge formation (HE%) and seawater temperature ( $T^a$ ) for the entire study period.

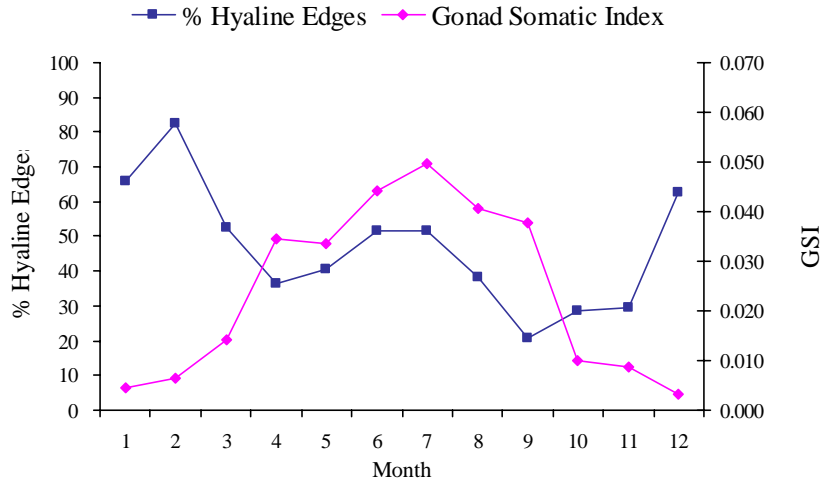


Figure 4. Evolution of percentage of hyaline edge formation (HE%) and gonad somatic index (GSI) for the entire study period.

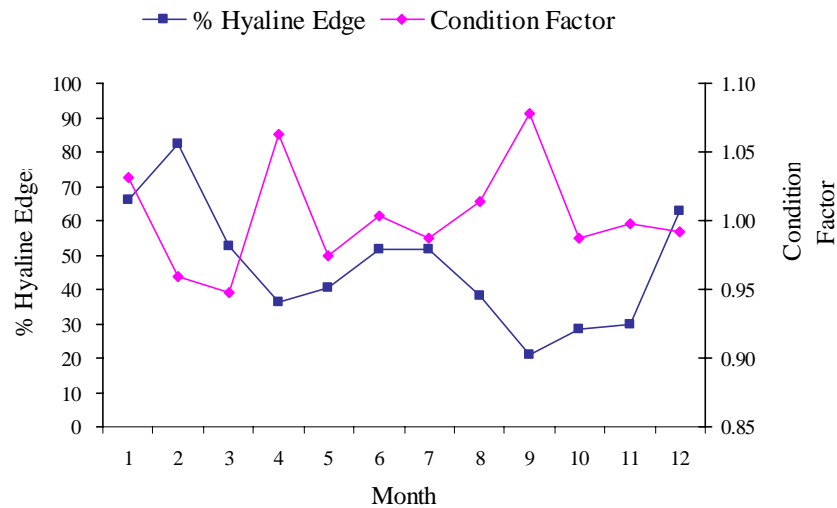


Figure 5. Evolution of percentage of hyaline edge formation (HE%) and condition factor (CF) for the entire study period ( $r = -0.48$ ,  $n = 12$ ).

Figure 5 shows the CF and the HE %. We note that for months with lower HE%, that is to say during opaque edge formation, anchovy shows a higher CF (better nutrition status). This suggests that the formation of the opaque edge is, in part, related to the time of fast growth of the fish.

Based on these facts, several criteria were established for the age-reading of anchovy otoliths in Alborán Sea. Based on the maximum of the gonad somatic index (GSI) (Giráldez and Abad, 1995), July 1<sup>st</sup> is considered the birth date for all the individuals. As a rule, if the fish was caught between February 1<sup>st</sup> and that birth date the hyaline rings only were counted if they were followed by a wide opaque zone (some juvenile are forming opaque ring after the winter hyaline ring formation, but they are usually

narrow). For individuals captured in the following months all the hyaline rings were counted. During the spawning season the hyaline ring was considered or not, depending on the distance that the winter ring was found.

Figure 6 shows the great complexity that can be found in the otoliths between August and November, since there is a great variability in the HE% formation (annual and interannual). This means that in many cases, in order to separate specimens with false rings, it is necessary to resort to common sense and of course to other characteristics of the otolith: less rounded shape and more dentate edge for the otoliths of older individuals.

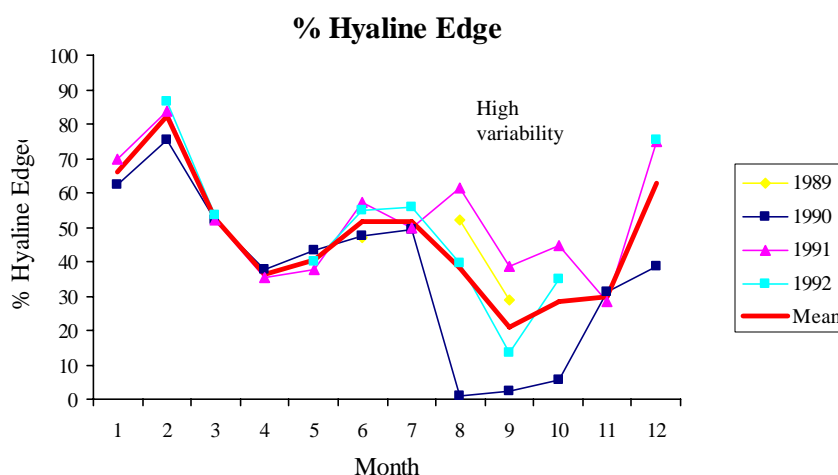


Figure 6. Evolution of percentage of hyaline edge formation (HE%) yearly and average for the entire study period.

The seasonal timing of formation of growth zones in otoliths has been studied by different authors. Beckman and Wilson (1995) conducted a review of 104 studies on seasonality in the formation of the opaque zone in the otoliths of fish, including 94 species and 36 families. They noted the existence of a general pattern in the formation of opaque zones in otoliths of species living in similar geographical areas. The formation of the opaque zone, both in the northern hemisphere and south, occurs during the spring-summer, although there is substantial variability in the formation time between individuals, populations and species. There is a not a clear association between the spawning season and the formation of the opaque zone. The formation of these rings would be associated with periods of high seasonality, or an increased of the seawater temperature, commonly associated with the fast growth of the fish; on the other hand the hyaline rings are formed at low temperatures during periods of slow growth.

The great variability in the timing of opaque zone formation in fish otoliths is further evidence that controlling factors are complex, mainly environmental and endogenous.

Our results agree with the conclusions of this review. The formation of rings in the otoliths of anchovy is linked to the temperature and, partly, to the fast growth of the

fish. Moreover, at the maximum of the gonad somatic index (GSI), half of the individuals are forming opaque edge. Figure 7 shows the annual pattern of formation opaque edge in anchovy otoliths in Alborán Sea.

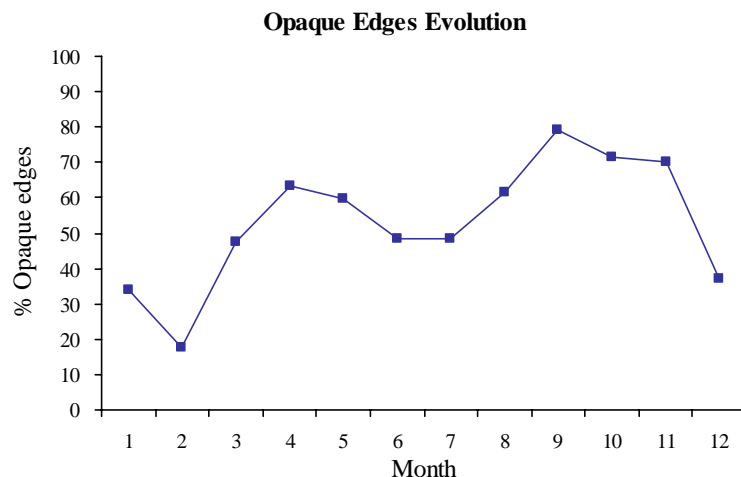


Figure 7. Annual pattern of opaque edge formation in Alborán Sea for the entire study period.

The results of age-reading of anchovy otoliths, using the criteria explained previously show that anchovy grows between 12 and 13 cm in their first year of life in the Alborán Sea, with some variation between years. This population is subjected to a high rate of exploitation, so with the absence of older individuals in the area, it means that growth can only be accurately described in the first year.

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