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# Age determination in European anchovy (*Engraulis encrasicolus* L.) otoliths in the Bay of Biscay (NE Atlantic).

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## Introduction

This study arises from the two recommendations given in the Workshop on Age reading of European anchovy, WKARA 2009, due to the difficulties encountered by various experts readers in annual age to determinate between first annual ring and the check formed before its first winter ring.
Two methods were used, in the first, age was determined by identifying and measuring growth rings formed on sagitta otoliths (Morales-Nin, 1988). In order to support the identification of the first annual ring, the otolith radius of the first hyaline ring was measured and used as a gauge for exclude the first check in ageing older individuals
In the second, a method for age corroboration was used by means of the otolith microstructure and fish ages were determined by daily increment counts (Campana *et al*., 1985; Campana, 2001). Total number of daily increments in otoliths was counted to test whether identified macroscopically hyaline area is, in fact, a check or the first annulus (Waldron et al., 1998, Waldron et al., 2001).
We compare the results of age determination using whole otoliths with those determined through counts of daily increments in an attempt to corroborate the above method.

## Material and Methods

### Analysis of whole otoliths

A total of 358 otoliths were collected from *Engraulis encrasicolus*, in the total length (TL) range 11.7-20.5 cm. The fish were caught in the Bay of Biscay between 2010 and 2011. Whole otoliths were examined after immersion in distilled water. Otoliths were viewed on a black background, under a reflected light, using a microscope applied to an image analyzer (NIS-Element). The radius (µm) was measured across the widest part of the otolith, along the same axis used to estimate the age of the fish, the presumed check and annual growth ring distances were measured between the core and the inner edge of hyaline rings (Figure 1), although this is usually measured between the outer edges, we considered more convenient to measure in this way, as in many of the specimens

studied had not formed completely hyaline ring and because the inner edge of the hyaline ring is usually well marked in otoliths and can be measured accurately.
Once all the increments (hyaline rings) on otoliths have been identified, an expert reader established the age of fish by counting the number of seasonal increments on an annual basis (Uriarte et al., 2002).

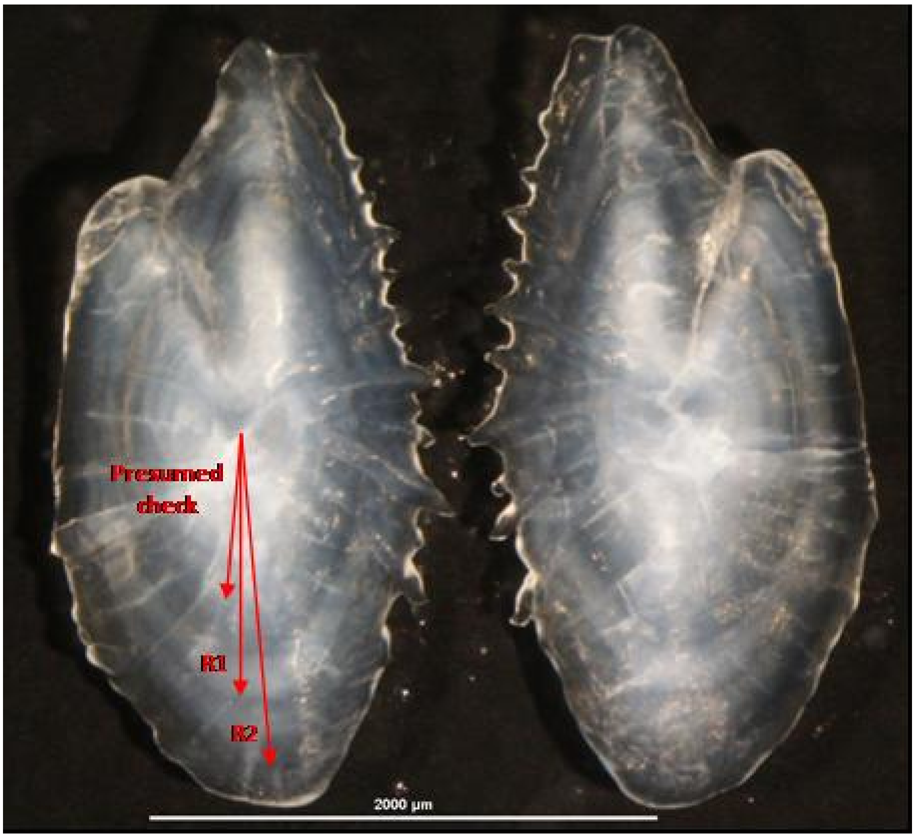



**Figure 1. Measurements taken from anchovy otoliths. Presumed check= false ring, R1= 1st annual hyaline ring, R2= 2nd annual hyaline ring.**

### Analysis of otolith sections

To identify if the formation of the first ring is a check or an annulus daily increment structure was examined. A total of 6 otoliths from fish caught in March, April and June in the total length (TL) range 13.0 -14.5 cm, in which the expert age reader determined the presence of a presumed check were mounted on microscope slides with the proximal side face up, using thermoplastic glue Crystal Bond. Otoliths were then ground on both sides with a Buehler Metaserv rotating wheel covered with a grinding paper (grit 3 µm/5µm) at 200-500 rpm until core and primary growth increments were visible. Otoliths were analyzed using a light microscope coupled with an image analyzer (Visilog 6.4/ TNPC 4.2, Software, Ifremer, France). Daily growth increments were examined at 1000x magnification for the core area and the outer part of the otolith was analysed at x100 and x200 magnification. The criterion named Group Band Reading

(GBR) was used for daily increment interpretation (Cermeño et al., 2003, Cermeño et al., 2008). Growth increments were counted from the core, beginning at hatch increment (Aldanondo et al., 2008), to the outer edge of otolith. Otolith radius, presumed first check and 1st annual ring were measured along the same axis. Each otolith was read at least 2 or 3 times until a consistent increment count was obtained. When error in reading precision was more than 10%, a third reading was taken. If the discrepancy persisted, the otolith was discarded. Also an otolith was rejected if the primordium was not visible or if more than 10% of the increment count was only possible by interpolation (Waldron et al., 1998, Waldron et al., 2001).

## Results

### Analysis of whole otoliths

The results showed that increments widths have a normal distribution (Kolmogorov-Smirnov test, Presumed Check, R1, R2 and R3 values p > 0, 05) with a falling rate of otolith growth with age (Figure 2, Figure 3a). This linearly decreasing interval between increments is a verification criterion that forms the basis of age estimation (May, 1965).

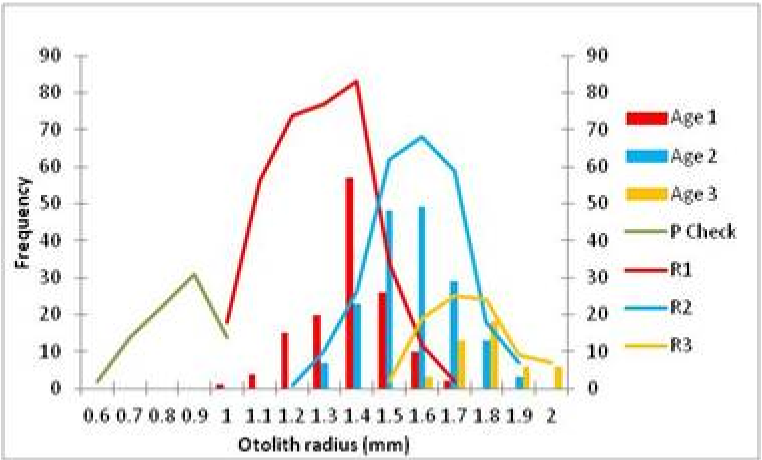



**Figure 2. Frequency distribution of rings distances presumed check, R1, R2, R3 and tree annual age ring distances.**

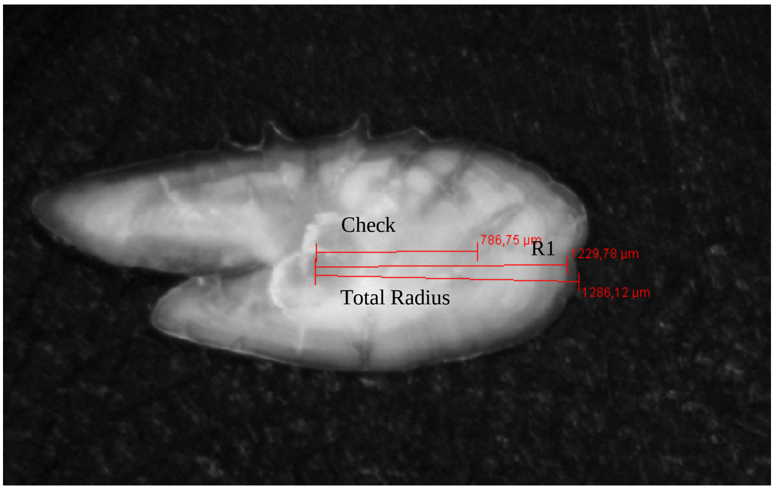
In cases where the distance from the core to the first visible ring was < 852± 100 µm, this ring was assigned as a presumed check. Based on the measurements made, a guideline table for age interpretation was established (Table 1).

| Rings | Average (µm) | s.d | Minimun (µm) | Maximun (µm) |
|---|---|---|---|---|
| Presumed Check | 852 | 100 | 593 | 999 |
| R1 | 1295 | 147 | 1000 | 1675 |
| R2 | 1589 | 133 | 1202 | 1936 |
| R3 | 1750 | 124 | 1523 | 2047 |

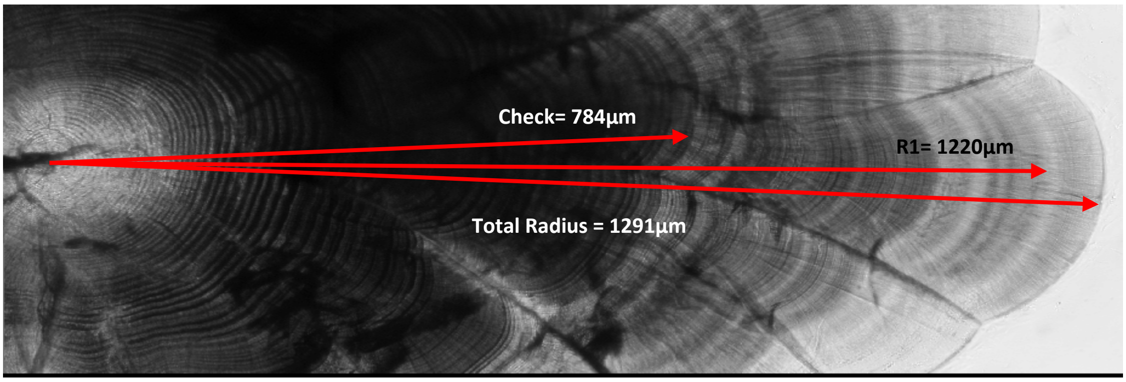
**Table 1 Guidelines for age estimation and the distance between the core to the beginning of the presumed check and winter rings (µm): average, standard deviation (s.d.), and minimum and maximum values observed.**

## Analysis of otolith sections

The growth of the entire sequence of opaque and translucent zones was analysed for a subset of selected otoliths in which the daily increment structure to the check and to the 1st annual ring (R1) was clear (Figure 3b). To ensure good-quality results, rigorous rejection procedures were applied to the otoliths, for this reason so far only six otoliths have been analyzed and read with confidence (Waldron et al., 1998, Waldron et al., 2001).




(a)




(b)

**Figure 3. Measurements taken from the same anchovy otolith, (a) whole otolith and (b) otolith section. Check= false ring, R1= 1st annual hyaline ring and Total Radius. (Daily increments to Check = 73 days, Daily increments to R1= 185 days, Total Daily increments= 211 days)**

For otoliths analysed the average distance between the core to the beginning of the check was 800 ± 69 µm at a mean age of 94±27 days (Table 2 and 3)

| | **1** | **2** | **3** | **4** | **5** | **6** | **Average (µm)** | **s.d.** |
|---|---|---|---|---|---|---|---|---|
| **Radius to Check (µm)** | 915 | 800 | 714 | 784 | 756 | 833 | **800** | **69** |
| **Radius to R1 (µm)** | 1396 | 1424 | 1200 | 1220 | 1400 | 1496 | 1356 | 119 |
| **Total Radius (µm)** | 1632 | 1774 | 1596 | 1291 | 1520 | 1637 | 1575 | 162 |

**Table 2. Otolith radius to Check, R1 and Total obtained by daily increment counts.**

| | **1** | **2** | **3** | **4** | **5** | **6** | **Average (days)** | **s.d.** |
|---|---|---|---|---|---|---|---|---|
| **Increments to Check (days)** | 138 | 117 | 74 | 73 | 82 | 80 | **94** | **27** |
| **Increments to R1 (days)** | 276 | 279 | 124 | 185 | 282 | 284 | 238 | 68 |
| **Total Increments (days)** | 353 | 394 | 225 | 211 | 309 | 338 | 305 | 73 |

**Table 3. Otolith Ages (days) to Check, R1 and Total obtained by daily increment counts.**

## Conclusion and Discussion

The results obtained through the analysis of otolith microstructure indicated that the hyaline zone macroscopically identified as a check is not an annual growth area because they had less than 365 daily increments in otoliths before deposit of this ring.

If we analyze the otolith macroscopically we found that the first check was 852± 100 µm and if we look through the otolith microstructure it is at an average distance of 800±69 µm, the observed difference in both cases, apart from the difference in the number of specimens analyzed is due mainly to the difficulty in identifying macroscopically the exact position of the nucleus, it also would explain the high difference between maximum and minimum ring distances (Table 1). One recommendation to solve this problem would be place one of the otolith with the proximal face up (sulcus face) when the reader measure otolith rings distances, as the core is best located on this side. Thus we could obtain more accurate measures and average distance values would be more fitting.

This study has confirmed that the estimated ages read using whole otoliths with those determined through counts of daily increments are generally correct up to the age of one year, supporting that the reader can distinguish correctly between rings and false rings and fish can be assign to the correct year class.

Based on the corroboration of age estimates presented here two recommendations to annual readers for ageing routines has been established,

1 - It is recommended to anchovy annual readers to measure the distances between the core and the inner edge of the hyaline rings, it is usually well marked in the otoliths and can be measured accurately.

2 - From the validation of daily ring formation in anchovy larvae and juveniles (Cermeño et al., 2003; Aldanondo et al., 2008), and corroboration of the position of the first hyaline ring (check) formed before their first winter annual ring, through the counts of micro-increments, is suggested to measure the first hyaline ring (check) and all hyaline rings that are at a distance less than 850 µm (± 100µm) should be considered as a check.

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