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Estimation of age and growth of juvenile hakes (*Merluccius merluccius* Linneaus, 1758) of the Bay of Biscay and Great Sole by means of the analysis of macro and microstructure of the otoliths

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ABSTRACT

The growth of juvenile and adult European hake from the Bay of Biscay and the Great Sole were studied analysing the sagittal otolith macrostructure and microstructure. The right and left sagittae otoliths were statistically symmetric in all studied dimensions. In the nucleus, 65 Daily Growing Increments (DGI) were identified. In the samples analysed a growth rate of 0.61 mm day^{-1} and a total length of 22.2 cm at the end of the first year were estimated. The comparative analysis of the results from several authors on hakes from similar areas showed that the growth rate obtained by tagging studies was almost half of that estimated by otolith microstructure analyses. These differences were probably due to the different size ranges of hake used in the studies. The increase and decrease of DGI width of three juveniles in Spring–Summer and in September coincided with the rise and fall of the water temperature in the Bay of Biscay and with a bloom of phytoplankton observed in March.

1. Introduction

The European hake (*Merluccius merluccius*) is one of the most heavily exploited fish species in Western European demersal fisheries, taken as part of mixed-species fisheries in the Northeast Atlantic (Casey and Pereiro, 1995).

The growth and age of commercial fish species are essential inputs in age-structured assessment models of fish stocks, which are useful tools in determining the impact of fishing on these stocks (Morales-Nin, 2000; Lombarte et al., 2003).

The estimation of age and growth of European hakes relies on counting the number of rings in the otoliths and in the interpretation of its morphology. However, the difficulty of reading the rings of the otoliths has been reported widely in the literature and one of the consequences is the problem of determining the length attained during the first year of life (Piñeiro and Pereiro, 1993; Morales-Nin and Aldebert, 1997, Morales-Nin et al., 1998). This complexity proceeds to the interpretation of the Daily Growth Increments (DGI) due to the difficulty to account and differentiate between the opaque and hyaline rings and sub-daily increments and "checks" (Morales-Nin and Moranta, 2004; Morales-Nin et al., 2005; Kacher and Amara, 2005). In this sense, difficulties in the assessment of hake stocks were noticed due to the problems of estimating the age of older fish (ICES, 2002). In an international otolith exchange programme in 2003 the average percent of error and coefficient of variation obtained among readers were 35% and 48%, while the values obtained in 2001 were 19% and 25%, respectively. In the international age-reading workshop held in 2004 (ICES, 2004), the participants concluded that ageing hakes older than 3 years old was not possible with an acceptable level of precision and therefore ages estimated previously using the internationally agreed criteria were overestimated (de Pontual et al., 2006; Piñeiro et al., 2008, 2009). Hence there is a need to make these criteria more uniform (García-Rodríguez and Esteban, 2002). In this sense, the results of recent tagging studies of European hake in the Bay of Biscay (de Pontual et al., 2003; Piñeiro et al., 2007) have questioned the accepted growth rate of this species.

The aim of the present study is to contribute to the knowledge of growth and age pattern of the juveniles and adults of Atlantic European hake based on the interpretation of otoliths both at macro structural and micro structural levels in order to aid clarification of the criteria to improve the assessments of the stocks.

2. Materials and methods

2.1. Sampling

Adult hake specimens were collected from commercial landings of the Basque trawl fishery from January to December 2005. Sam-

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pling was done according to a random stratified design at quarterly intervals covering the length range of hake in the Bay of Biscay and Great Sole (ICES Divisions VIIghj, VIIIabd) and the Cantabrian Sea (ICES Division VIIIc).

Juvenile hakes were collected from the 2nd to the 8th of November of 2005 in a tagging survey on board of a commercial trawler in ICES Divisions VIIIb and VIIIc. Total length (mm) and gutted weight (g) were recorded for all the specimens sampled.

2.2. Macrostructure

During 2005, 1482 adult hake were selected for the study. Once in the lab, sagittal otoliths were removed and stored in dry condition and the samples with broken or decalcified otoliths were rejected. Length and width (mm) of each otolith were determined twice using a *Nikon V-12* profile projector equipped with a *Nikon CM-6S* digital counter. Measurements that differed by less than 0.01 mm were accepted. Otolith weight (g) was determined with a *Metler Toledo* precision scale, calibrated with a ±0.001 g error.

The otolith preparation and age determination were in accordance with the methology described in the EU BIOSDEF (Anon., 1998) and DEMASSESS international projects (Anon., 2000). Each otolith was mounted in a black resin block and cut in a section of 0.5 mm by means of a Metkon Servocut A-250 automatic cutting machine. Sections were mounted on glass slides and read in a stereomicroscope with magnification of 20× with reflected light on a black background. Annual growth cycles consist of opaque and translucent or hyaline zones in a concentric pattern around the nucleus that are visible under reflected light. Otolith interpretation started from the nucleus towards the edge with the first of January adopted as the hake's birthday. Only the hyaline rings were counted.

Rings were counted twice by two readers trained according to the experience obtained from international workshops on hake ageing studies, and only otoliths in which the age estimates of the two readers coincided were accepted for analysis.

Total length (L_T) and eviscerate weight (W) relationships were estimated by fitting non-linear regressions to the data. The model fitted was $W = a \cdot L_T^b$ and the resulting parameters (a, b) were obtained using the Newton algorithm from the *Microsoft® Excel* Solver routine. Also statistical relationships were determined for the otolith dimensions and the hake's total length. For these analyses *Statgraphics Plus 5.0* and *R* statistical computing and graphics software were used.

2.3. Microstructure

The microstructure study was carried out with 300 juvenile hakes caught in tagging surveys in subdivisions VIIIc and VIIIb in the winter of 2005, but only specimens smaller than 25 cm were included in the microstructure analysis. The otoliths were removed on board and stored until the end of the survey. Later in the laboratory they were cleaned and otolith pairs not well conserved were rejected. Only one of the sagittal otoliths of each juvenile was prepared for the microstructure analysis. The otoliths were embedded into a plastic resin in silicone moulds forming solid blocks, orienting the long axis of the otolith parallel to the long axis of the mould. Blocks were mounted in slides with a thermoplastic resin, polished on the external side of the otolith until the core was reached and then mounted with the external side downward according to the technique described by Piñeiro et al. (1996). The internal side was then ground to obtain thin sections along the frontal plane. All mounts were ground and polished (alumina powder 0.1 μm) with frequent microscopic control, until the core plane was reached. The otoliths were viewed using a microscope equipped with a high-resolution video camera and *TNPC 3.2* PC image analysis system (Noesis, France, 2001).

Several structures such as sub-daily increments and "checks" are often confused with DGI (Campana, 1984; Piñeiro et al., 2009). DGI appear in the otolith as regular sequences which show weak transitions in width and contrast under the microscope, while sub-daily increments can occur in any part of the day and show different patterned widths. "Checks" have not been specifically described but it has been speculated that they may be due to stress of short duration, lunar cycles or interruptions of growth and so need a different focus from DGI for proper observation. Therefore, regions with sudden interruptions of otolith growth were excluded from the axis of reading.

As mentioned earlier, each otolith was counted for DGI by two readers. In order to minimize mistakes in the counting, each reader started one of the counts from the edge of the otolith to the core, and other from the first increment identified in the core to the edge and the counts were averaged (Morales-Nin and Aldebert, 1997). The average of the counts was compared between readers in order to check the precision and bias in the age estimation. If the average of the counts between readers differed more than 10% a third reading was performed according to the methodology of Arneri and Morales-Nin (2000).

For each otolith the average of the counts by two readers was considered the specimen's age in days.

The data of surface sea temperature in the Bay of Biscay and the surface chlorophyll/phytoplankton production (mg/m^3) in 2005 were obtained from the historical temperature series of the Aquarium of San Sebastian and from the MODIS sensor of the AQUA satellite respectively (MODISA Level-3 Standard Mapped Image, space resolution of 4 km).

3. Results

3.1. Macrostructure

The total length–gutted weight relationship obtained in the study was:

$$W = 0.0065 \cdot L_T^{2.9915} \quad (r^2 = 0.98).$$

For this study otoliths of 1482 hake from 13.1 cm to 82.1 cm were weighed and measured. Fig. 1 shows the relationship between the morphometry of the right and left otoliths (length, width and weight) and total length. The relationship between otolith weight and fish length, though exponential, showed a value of b of less than 2.5. The determination coefficients r^2 for all studied dimensions were always above 0.97, reaffirming sagittal otoliths as valid indicators of hake somatic growth. No differences in length, width and weight were found between the left and right otoliths (Mann–Whitney W test, $P > 0.05$).

Mean length at age estimates of this study were compared with historical estimates of hakes in Atlantic and Moroccan waters (Table 1). The mean lengths estimated in the present work of 28.4 cm at 2 years and 79.1 cm at 8 years were among the highest in the historical series.

3.2. Microstructure

For the DGI quantification study the best 47 processed otoliths of hakes from 11.5 to 18.9 cm total length were used and the rest were discarded due to difficulties during the polishing process. Sagittal otoliths showed the typical prismatic growth common to hake, with accessory growth centres around an irregular primordium that continues growing between prisms (Fig. 2).