AGE DETERMINATION OF LARVAL AND JUVENILE SMALL PELAGICS: THE IMPORTANCE OF A COMMON PROTOCOL

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Abstract
The sardine and anchovies' larvae and juveniles age interpretation criterials are defined based on otolith microstructure analysis. Included in SARDONE project, this comparative study was developed in order to understand the life history and recruitment of these key species in the pelagic ecosystem.
Keywords: Fishes, Growth

Introduction
Small pelagic fishes, such as *Sardina pilchardus* and *Engraulis encrasicolus*, are relevant constituents of the Mediterranean pelagic ecosystem. Their populations show evidence of important long-term natural fluctuations in their abundance, related, among other factors, to large-scale climate variability raising important scientific and economic concerns. It is commonly assumed that recruitment variability is largely determined by survival during the larval and juvenile stages. An important tool to decipher the initial life phases relies on them being encoded into otoliths both in the daily growth increments numbers, providing information about the age in days, and in the increment width, which is a proxy of fish growth. However, small pelagic otoliths have complex structures with numerous sub-daily units, that difficult the increment interpretation [1]. Therefore, and as part of SARDONE project (FP6-44294), it was necessary to establish a common age protocol before comparing three Mediterranean area's temporal and spatial patterns in small pelagic larvae and juveniles age and growth. This task was achieved by a step by step process based on the following: a) Interchange of otoliths images for joint otolith interpretation. b) An age interpretation Workshop, which was held in AZTI in 2008 knowledge gaps and which also lead to the establishment of a common protocol. c) A second round interchange of images. d) The 2nd Year meeting was held in the ICM in March 2009. In this meeting it was noted that the ageing was not performed in the same way by all the participants. The main discrepancies were found on the calibration of the images, the identification of the growth patterns and the otolith edge interpretation. e) A second otolith daily increments interpretation workshop, using real otoliths, was considered necessary. It was organized at IMEDEA on May 2009. Based on this iterative method a common criteria was established and applied. AGEING PROTOCOL In order to differentiate daily from sub-daily rings, the methodology suggests bringing the preparation into a focus where all growth structures can be clearly read and then slightly varying the focus, so that the sub-daily increments should disappear. This method could not be applied to the juveniles of small pelagics due to the presence of multiple wide rhythmic growth patterns in the central zone of otoliths (whiter and darker bands) and groups of multiple increments. The method, named Group Band Reading (GBR), consists on counting as one every repetitive cyclic set of growth bands or apparently groups of microincrements (usually 2 but occasionally more), assuming that they are sub-daily marks in postrostral zones corresponding to the early juvenile period [1].The otolith nucleus should be read at 1000 magnification, whilst the rest of the otolith at x100 for anchovy and X200 for sardine. When necessary to assess the growth pattern higher magnification may be used.The grouping of sub increments forming growth bands (GB) is common in all individuals and starts very early in the life history of the fish.The width of the GB is conservative, not sudden changes occurs. In some cases the sub increments are very clear and difficult the identification of the GB. However, once the GB is initiated, this pattern continues. Therefore the group banding interpretation has to be maintained.
Anchovy: The first increment corresponds to the hatch check with a radius between 3.5 and 5 µm [2], the following increments have a width around 1 µm. The GB starts very early in life, with double bands, once formed this pattern has to be kept in all the otolith.

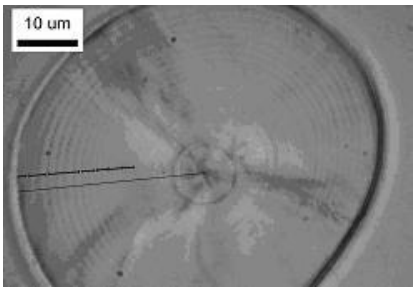

Fig. 1. Anchovy otolith showing the group band pattern age interpretation

Sardine:The hatching check appears at 5-7 µm, the following increments have a width around 1 µm. The GB appears at 40-80 increments after the hatching check, once formed this pattern has to be kept in all the otolith.

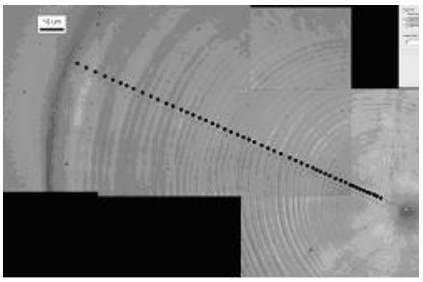

Fig. 2. Sardine juvenile showing the group band pattern age determination

References
1 - Cermeño, P.; Morales-Nin, B.; Uriarte, A., 2006. Juvenile European anchovy otolith microstructure. *Scientia Marina*, 70(3):553-557.
2 - Aldanondo, N; Cotano, U; Etxebeste, E. et al., 2008.Validation of daily increments deposition in the otoliths of European anchovy larvae (*Engraulis encrasicolus* L.) reared under different temperature conditions. *Fisheries Research*, 93: 257-264.