



## Otolith microstructure of a hatchery reared European hake (*Merluccius merluccius*)

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### Abstract

European hake (*Merluccius merluccius*) were reared from eggs up to 245 days of age in temperature and salinity controlled stable conditions. The lapillus and sagitta of one of these fish were examined for microstructural features. The age derived from the increments support the daily nature of the hake sagittal increments starting the formation at day 8, probably related to the start of exogenous feeding. The lapillus shows a later increment formation. The sagitta otolith increments show rhythmical groupings and banding that are similar to wild fish. The accessory growth centres appear to be related to pectoral fin development and demersal habitat. The growth rates obtained are discussed.

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### 1. Introduction

European hake *Merluccius merluccius* L. is a major demersal finfish in the marine ecosystem of the North Atlantic and Mediterranean Seas. This species is heavily exploited and in recent years assessment has raised concern about the state of the stocks (ICES, 2003). Knowledge of hake's biological traits (especially growth and maturity) is considered insufficient to improve the assessment of stocks.

The age interpretation of European hake is unusually complex due to the presence of a multiplicity of growth rings, that at least in the Mediterranean are not formed with seasonal periodicity (Morales-Nin et al., 1998). Despite the effort carried out to develop standard ageing criteria for North Atlantic hake (Piñeiro and Saínza, 2003) a recent mark and recapture experiment has raised doubts about the actually accepted growth rates, indicating that possibly growth is much faster than expected (Pontual et al., 2003).

In the Mediterranean, a series of studies on European hake juvenile age and growth have used otolith daily growth increments (DGI) and length frequency analysis (Morales-Nin and Aldebert, 1997). The daily

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nature of the increments has been validated indirectly for juvenile hake in the Adriatic (Arneri and Morales-Nin, 2000) and increments have been used to determine growth rates and hatch date distribution for juveniles (Morales-Nin and Moranta, 2004) and larvae (Palomera et al., 2005).

A pioneer project carried out at Austevoll Aquaculture Research station (Norway) successfully reared for the first time European hake from eggs to 1 year of age. The eggs incubated at 12 °C hatched approximately after 4 days of fertilization, within 6 days after hatching the larvae started to feed and at around 30 days the larvae metamorphosed. The rearing conditions and larvae development are described in Skiftesvik and Bjelland (2004).

The aim of the present work is to take advantage of this successful rearing to carry out the first direct validation of European hake daily growth increment periodicity. Although the rearing conditions may affect fish development and only a single fish was available for study, the information provide insights in the otolith formation and age attribution on a relevant species with otoliths particularly difficult to interpret.

## 2. Material and methods

Adult hake caught on the spawning grounds west of FlorØ (Norway) were used to obtain gametes (Skiftesvik and Bjelland, 2004). The experimentally reared fish hatched on August 31, 1999. The larvae transferred from the incubator to a tank at day 45. They were feed *Artemia* and rotifers until day 55, when they were weaned to formulated feed and feed ad libitum. They were transferred to progressively larger tanks on days 210 and 226.

The water supplied to the rearing units was pumped from a depth of 110 m, sand filter, heated and aerated. Salinity was a constant 34.5. Water temperature was measured daily, mean temperatures from incubation to hatching were  $12.3 \pm 0.5$  °C and afterwards the temperature was maintained at approximately 12 °C. The light was maintained constant and no seasonality was present in the environmental parameters.

One European hake was sacrificed after 245 days corresponding to a total length of 135 mm. The sex was not identified due to undifferentiated gonads. The

sagitta and lapillus otoliths were extracted, cleaned and stored dry until preparation.

The otoliths were mounted in slides with a thermoplastic resin, polished on the external side until the core was reached and then mounted with the external side downward. The internal side was then ground to obtain thin sections along the frontal plane. All mounts were ground and polished 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 a PC with an image analysis system. Otoliths were viewed on the screen using an immersion oil objective at  $\times 1000$  magnification and GI were selected by fine focussing for their definition and continuity. GI were enumerated from the otolith core to the edge following for sagitta the dorsal prism, which provides the most complete increment sequence (Morales-Nin and Aldebert, 1997) and the maximum radius for lapillus. GI were counted twice from the nucleus to the dorsal margin and vice versa. The mean of the readings was considered the specimen's age in days.

## 3. Results

### 3.1. Sagitta

The otolith showed the typical prismatic growth common to hake otoliths (Fig. 1a) with accessory growth centres laid down around an irregular primordium which had an anterior–posterior diameter of 461.28  $\mu\text{m}$ . The primordium continues growing between the prisms (Fig. 1b box) with overlapping in the increments (19 GI). The increments composed of a bright unit and a dark wide discontinuous unit, were enumerated in the primordium till the first increment coincident with the accessory growth centre (Fig. 1b inbox), after the increments were enumerated only in the prism.

Hake otoliths have a central core of 1.94  $\mu\text{m}$  (Fig. 2a) surrounded by narrow GI which have a variable thickness (mean = 2.24  $\mu\text{m}$ , S.D. = 0.88; Fig. 2b). This central area is composed by 64 GI. A luminance profile along the same radius where the GI measurements were taken (Fig. 2a–c) shows that the translucent and opaque bands are not related to the formation of narrower GI.

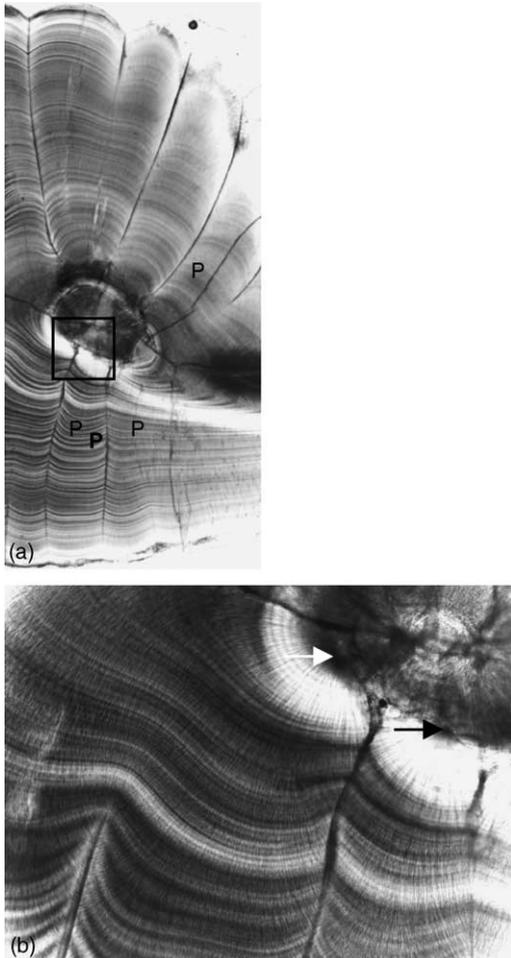


Fig. 1. (a) Dorso-ventral area of the frontal sagitta section showing the primordium and the prismatic growth ( $\times 50$ ). (b) Magnification of the transition zone (square in a) between the primordium and the prisms ( $\times 1000$ ). Arrows, accessory growth centres; P, prisms.

The primordium last increments are thick and dense with a clear discontinuity, around which accessory centres are seen (Figs. 1a, b and 3a). These accessory centres gave origin to prisms, characteristic of hake otoliths (Morales-Nin and Aldebert, 1997), in which the increments are laid down in regular patterns with some rhythmical grouping (Fig. 3b).

The GI in the prisms initially are narrower (mean  $4.68 \mu\text{m}$ , S.D. 1.6,  $n = 21$ ), getting more wide in the intermediate zone (mean  $5.33 \mu\text{m}$ , S.D. 1.82,  $n = 23$ ) and had decreasing width towards the otolith edge (mean  $2.45 \mu\text{m}$ , S.D. 0.79,  $n = 44$ ).

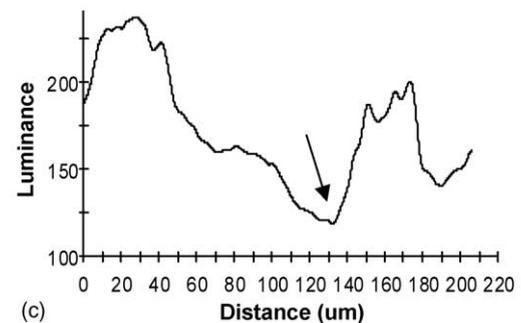
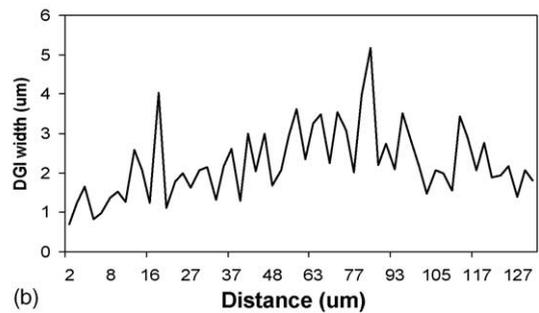
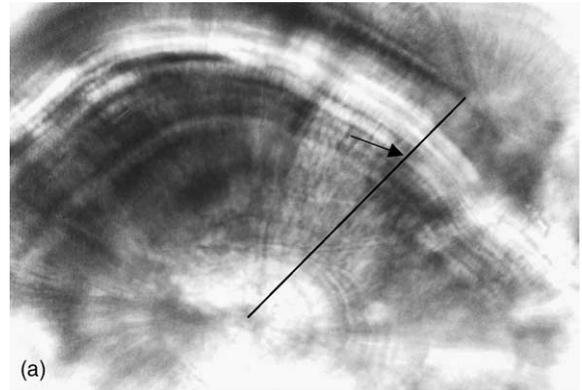


Fig. 2. (a) Core and central area of the sagitta otolith. The line indicates the measurement radius. (b) Evolution of the daily growth increment (DGI) width along the radius. (c) Luminance profile along the radius. Note that the DGI were measured only in the inner area before the sharp transition in luminance (arrows a and c).

The total GI number on the prismatic zone was 173 added to the 64 of the primordium gave an age of 237 days.

### 3.2. Lapillus

The lapillus had a maximum radius of  $193.04 \mu\text{m}$  with regular GI around a circular core (Fig. 4). There is

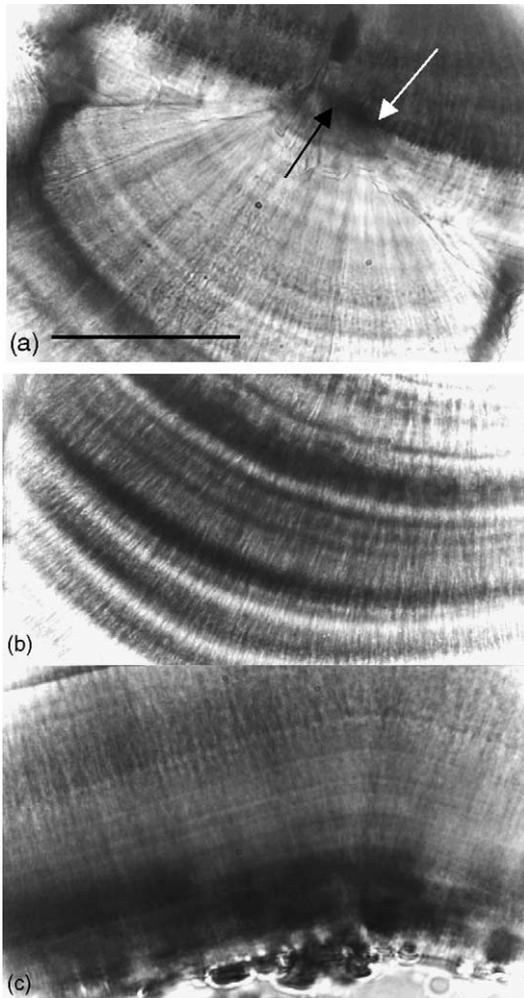


Fig. 3. Prismatic sagitta growth. (a) Accessory growth centre (black arrow) and edge of the primordium (white arrow). (b) Growth patterns of wide DGI. (c) Marginal otolith area with narrow DGI. Scale bar 50  $\mu\text{m}$ .

not a complex structure similar to the sagitta. The width of the increments decreased from 1.25 (S.D. = 0.06) to 0.92  $\mu\text{m}$  (S.D. = 0.01) towards the edge. Some six GI groupings were present in part of the lapillus. The total number of increments was 208.5.

#### 4. Discussion

The GI had a poor contrast in the lapillus and in some sagitta otolith areas, probably introducing some

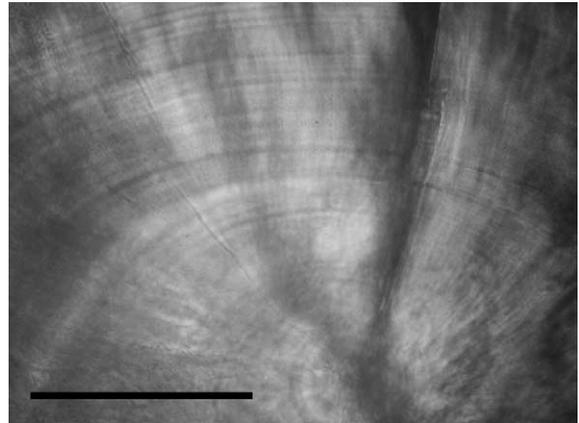


Fig. 4. Growth increments in the lapillus. Scale bar 50  $\mu\text{m}$ .

error in the increment enumerations. The most difficult area was the central part of the sagitta, where the discontinuity between the primordium and the prisms complicate both the otolith preparation and interpretation. Moreover, the increments depending of the focus plane (Fig. 5) show a different appearance with a very wide discontinuous zone in which in some cases narrow increments can be discerned introducing error. The lapillus is more easy to prepare and observe, but there is discrepancy in the number of GI in both otoliths, being superior in the sagitta. The correspondence between the number of days in the rearing experiment and the number of increments suggests that the lapillus start increment formation later than the sagitta (29 days), while the GI are first formed in the sagitta at the age of 8 days. The start of feeding at 6 days of hatching, suggest that the exogenous feeding might be related to the start of the increment formation.

The sagitta hake otoliths show a great variability in translucency that make difficult to obtain good uniform images. These changes have been associated to the presence of organic material (Morales-Nin, 1986) or to structural changes (Arneri and Morales-Nin, 2000). In the reared fish, similar banding was found. Another common feature on hake otoliths is the presence of 7, 14 and 28 GI groupings, with subtle changes in translucency and increment width, which have been related with the vertical migrations and with tidal influences (Morales-Nin, 1987). These groupings were also found in the reared specimen; although the number of increments more frequent was 7 and 9 (Fig. 6), there

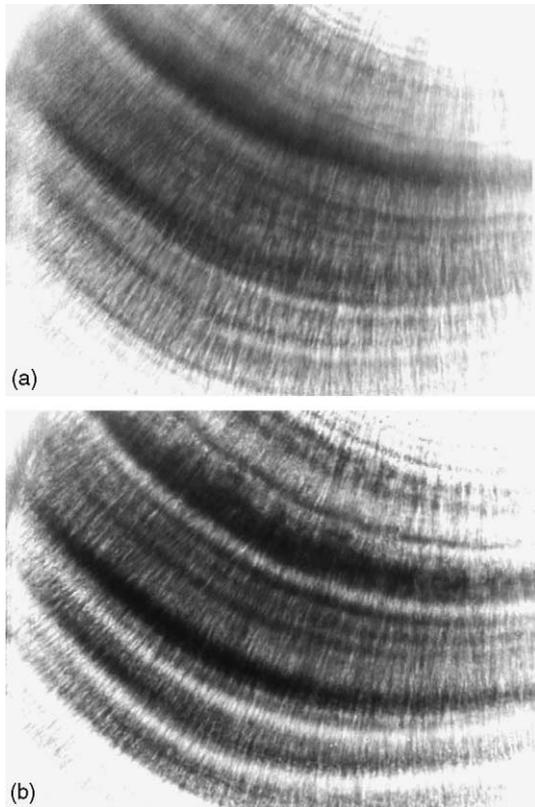


Fig. 5. (a and b) Differences in focussing in a prismatic zone ( $\times 400$ ).

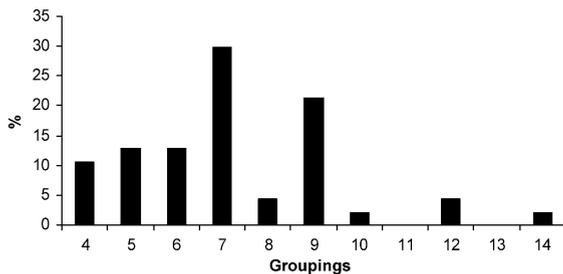


Fig. 6. Plot of the frequency in increment groupings in the prismatic zone of the sagitta.

Table 1

Otolith features from the reared European hake and from wild fish ( $n=150$ ) caught on the Western Mediterranean (Arneri and Morales-Nin, 2000)

Otolith feature	Core ( $\mu\text{m}$ )	First increments mean width ( $\mu\text{m}$ )	Number of DGI in the primordium	Number of accessory centres	DGI mean width in the prisms	Fish growth ( $\text{cm month}^{-1}$ )
Reared hake	1.94	1.39	64	4	3.9	1.2
Wild hake	8	1.4	62.7	2–6	2.5–4	1.2–2.5

were groupings with fewer increments that suggest that the lack of an external signal had weakened the inner growth rhythm. In the lapillus, some grouping of six DGI was also observed.

The accessory centres or primordia in hake have been related to the transition to the demersal mode of life; they are absent in pelagic larvae of 1.1 cm standard length (Palomera et al., 2005) but they are present in demersal fish of 1.6 cm total length (Arneri and Morales-Nin, 2000). Our results show that they are related to the metamorphosis and to the formation of the pelvic fins, corresponding to a bottom dwelling behaviour in the juveniles (Bjelland, 2001). Probably these changes on the otolith correspond to the necessity to detect different sound signals (Lombarte and Popper, 2004).

A comparison of otolith features of the reared fish with wild Mediterranean hake (Table 1) shows some particularities that probably are related to the different environmental conditions. The Mediterranean hake recruits on the outer part of the shelf between 100 and 150 m depth, where the water temperature at the recruitment depth is almost constant ( $13.5\text{--}14\text{ }^{\circ}\text{C}$ ) and similar to the rearing one. However, although there was not seasonality in the rearing conditions, the light regime was quite different from the natural one.

The reared specimens ( $n=4$ ) reached a length of 12 cm at 200 days post hatch representing a monthly increase in length of about 1.8 cm. A growth rate of  $1.15\text{ cm month}^{-1}$  has been reported for juvenile hake in the NW Mediterranean (Morales-Nin and Aldebert, 1997) and between  $0.7$  and  $1.2\text{ cm month}^{-1}$  for the Ligurian Sea (Orsi Relini et al., 1989). These estimates were obtained by modal progression analysis of length frequencies and refer to specimens larger than 6–8 cm whose absolute ages were unknown. Growth rates obtained from otolith readings in the Adriatic (Arneri and Morales-Nin, 2000) and Catalan Sea (W-Mediterranean) (Morales-Nin and Moranta, 2004) how great growth plasticity at individual and seasonal levels, with

intense growth rates depending of the season but with a mean growth superior to  $1.6 \text{ cm month}^{-1}$ . These quite fast growth rates are directly confirmed in the Atlantic hake by a recent tagging experiment (Pontual et al., 2003). Thus, the reared hake were growing at similar rates than wild fish or possibly at slower rates. Although food was provided ad libitum, direct observations showed that the fish eat once a day until gorging.

A hake age determination has a great degree of uncertainty and there is a lack of standard ageing criteria for North Atlantic hake (Piñeiro and Saínza, 2003). The first annulus is identified based on its relative size because the presence of multiple macrostructure rings obscures the identification. These rings are present in the reared specimen and do not seem to be caused by narrow GI (at least in the first one, probably corresponding to the first false ring).

The results provided, although only based in a single fish, support the growth rates from wild fish. However, a larger sample size is necessary before drawing firm conclusions on which otolith provides the better estimate of hake age and on the daily periodicity of the GI.

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