

# Development of hake *Merluccius merluccius* (L. 1758)

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## INTRODUCTION

Hake (*Merluccius merluccius* L. 1758) is a gadiform fish widely distributed from Iceland to Mauritanian waters, in the Mediterranean and Black Sea. It is found between 70-370 m depth, but also occur from inshore (30 m) to 1000 m. In the Northeast Atlantic multispecific European fisheries catch this species from ICES Sub-area IV to IX. Spain and France fish about 80% of total catches; other countries involved in the fishery are United Kingdom, Ireland and Portugal. For Spain and France Hake is the basis of a very economically important fishery.

Two stocks have been recognised for assessment and management purposes: the northern stock (Div. IVa, VIa, Sub-area VII and VIIIa,b) and the southern one (Div. VIIIc and IXa). The catches (landings and discards) of the two stocks amounted in 1996 for 47,754 t and ca. 8,900 t respectively (ICES CM, 1998). In the Bay of Biscay (Div. VIIIa,b,d) 17,800 t of Hake were caught in 1996 mainly by longliners, "baka", "bou"- and VHVO (very high vertical opening) pair-trawlers.

Due to the economical importance of this species there are abundant studies about the biology, ecology, and growth. This information is referred mainly to its morphology, taxonomy and geographical distribution (Whitehead et al., 1986; Smith et al., 1986; Agnew, 1989; Sánchez et al., 1991). There are also some studies about reproductive and other biological aspects in the Bay of Biscay and in adjacent waters (Perez & Pereiro, 1981; Martín, 1991).

In this work the results of a histological study on reproduction of Hake, *M. merluccius*, carried out on a monthly basis sampling in 1996-1997 in the Bay of Biscay (Div. VIIIa,b,d) are presented.

## MATERIALS AND METHODS

During 1996-1997, an intensive sampling program was carried out on a monthly basis to obtain representative number of *M. merluccius* in order to advance in the reproduction biology knowledge of the species. Biological samples were collected at sea and at the main fishing ports of the Basque Country from vessels that operate in Div. VIIIa,b,d.

The gonads (ovaries and testes) were collected after fish were caught. For histological studies, the aim of the sampling was to obtain the gonads of a maximum of 10 females and 6 males by 1 cm length range and by month, along a "biological year" in the [1996-1997] period. Gonads were fixed in a 4% formaldehyde buffered solution. Later, in the laboratory, the gonad samples were embedded in glycol methacrylate resin (Technovit 7100), sectioned at 2-3 µm, stained with Harris haematoxylin and 1% aqueous yellowish eosin and mounted with Eukit on slides with a coverslip on surface. At the end of the study, a total of 163 histological samples of male and 266 samples of female Hake were prepared, examined and described. The reading of the histological samples were made by means a binocular microscope under transmitted light at different magnifications. Some of the more representative samples of the different maturation stages were photographed.

## RESULTS

A total of 5 gonad development stages for both females and males were considered: I Immature or Virgin, II In Maturation or Resting, III Maturing, IV Spawning, and V Post-spawning. The different development stages of the oocytes (Oogonia, Chromatin nucleolus stage, Perinucleolus stage, Vitellogenesis, Nuclear migration and Hydration), and of the spermatozoa (Spermatocytes, Spermatids and Spermatozoa) have been also described. Post-ovulatory follicles and atretic oocytes are also described in spent females (see Table 1).

The structures identified for each maturity stage assigned to females and males are shown in Column "FEMALES" [(1)-(8)] and Column "MALES" [(1)-(6)] respectively. Structures have been marked with different labels (numbers, letters, black or white points, etc.)

## DISCUSSION

The process of oocyte maturation in *Merluccius merluccius* is similar to other demersal fish described in previous works.

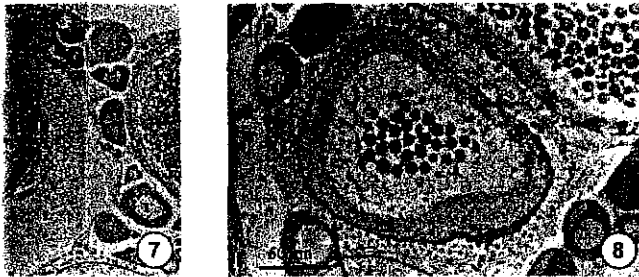
Oogonias were observed in all maturity stages. Chromatin nuclear and perinucleolar stage were described in immature and maturing females. Vitellogenic oocytes appeared in pre- and spawning females while hydrated oocytes were observed in spawning females. Necrotic oocytes were observed in post-spawner females. Post-spawning follicles were also observed although there are not shown.

The results of the histological analysis showed a very expanded spawning period for Hake in Div. VIIIa,b,d that extends from January to May with a peak in January-February-March. The long duration of this period must be caused by the addition of several factors: different period of the year and different sea areas of the Bay of Biscay in which adult (large and medium-sized) Hake spawn. The identification of the different structures observed coincided with the macroscopical analysis deployed to establish the spawning period. Thus, histology appears as the validation method for the maturity stages assigned *de visu* to Hake in this sea area.

## ACKNOWLEDGMENTS

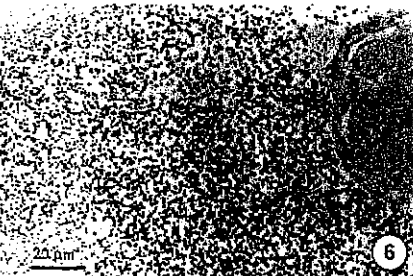
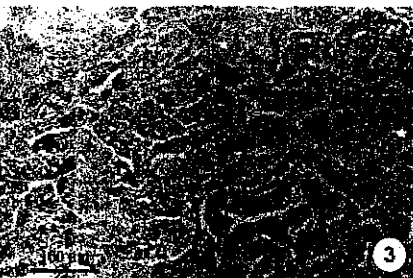
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Histological Maturity Stages	Females	Males
I Immature or Virgin	Only Oogonia, Chromatin nucleolus stage.	Spermatogonia and Spermatoocyte present, Spermatozooids absent.
II Maturing	Oogonia, Chromatin nucleolus stage, Perinucleolus stage, early vitellogenesis.	Spermatogonia and Spermatoocyte present, few Spermatozooids.
III Mature or Pre-spawning	Vitellogenesis, early nucleus migration.	Spermatozooids predominant, Spermatoogonia and Spermatoocyte present only in the testis cortex.
IV Spawning	Migration nucleus stage and Oocyte hydration. Oogonia and the other immature stages are also present.	Spermatozooids predominant.
V Post-spawning	Post-ovulatory follicles, follicular atresias and atretic oocytes. Oogonia and Chromatin nucleolus stage.	Empty seminiferal ducts, residual Spermatozooids and few Spermatoogonia.



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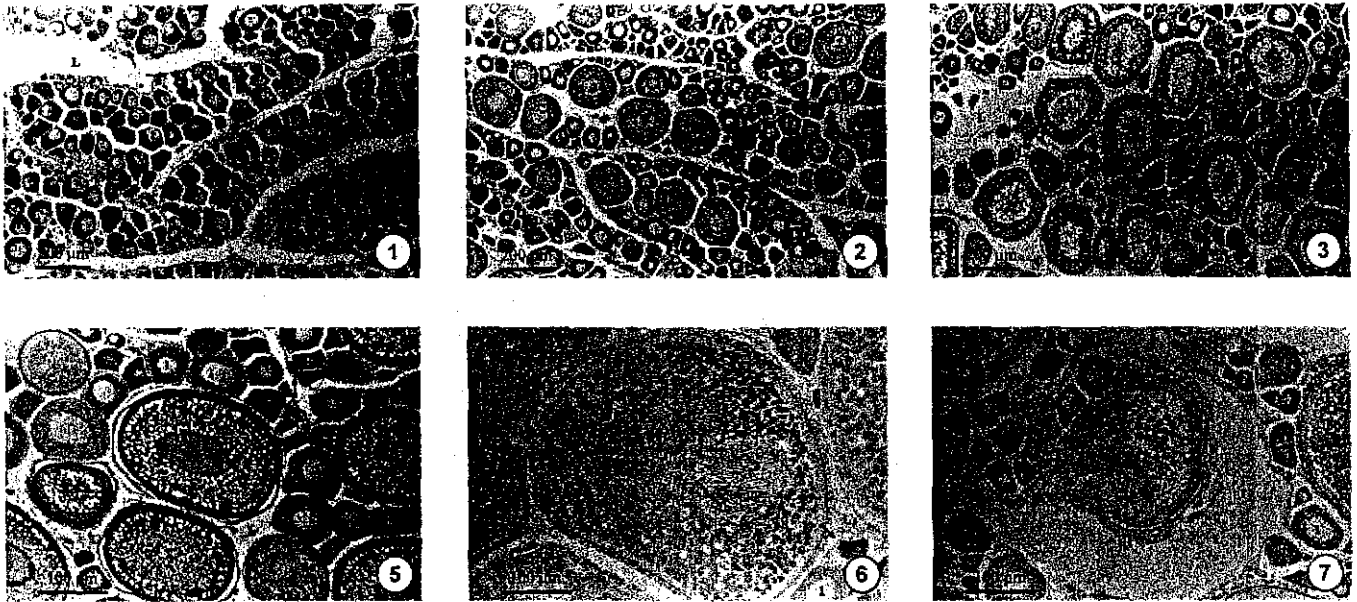
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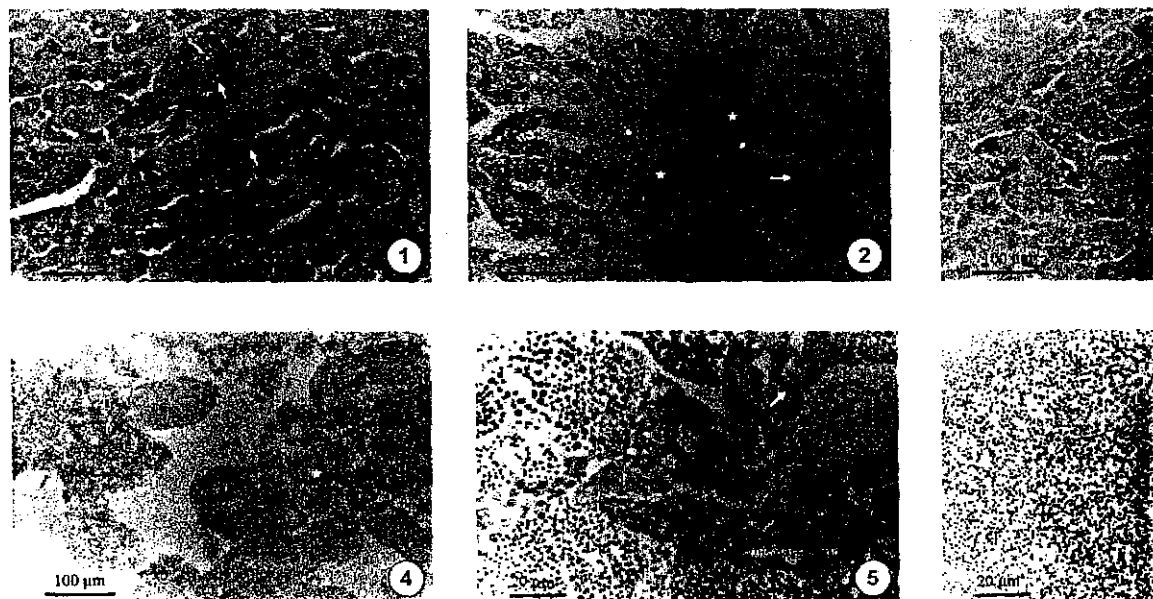
## FEMALE



Light microscopy photomicrographs of histological structures of *M. merluccius* ovaries in different stages of maturation. The histological preparations are stained with haematoxylin-eosin and marked with different labels (numbers, letters, black or white points, etc.) and at different magnifications.

- 1) Ovary in stage I (Immature or Virgine). Thin black arrow: Oogonia; 1: chromatin nucleolus stage; L: lumen.
- 2) Ovary in stage II (Resting). Thin black arrow: Oogonia; 1: chromatin nucleolus stage; 2: perinucleolar stage oocyte; L: lumen.
- 3) Ovary in stage III (Maturing). Thin black arrow: Oogonia; 2: perinucleolar stage oocyte; 3: early vitellogenic oocyte; L: lumen.
- 4) Ovary in stage IV (Spawning). Thin black arrow: Oogonia; 3: early vitellogenic oocyte; 4: hydrated oocyte; L: lumen.
- 5) Detail of a Ovary in stage II (Resting). Thin black arrow: Oogonia; 1: chromatin nucleolus stage; 2: perinucleolar stage oocyte; L: lumen.
- 6) Detail of a Ovary in advanced stage III (Maturing). Thin black arrow: Oogonia; 1: chromatin nucleolus stage; 3: advanced vitellogenic oocyte (nucleus migration); L: Lumen.
- 7) Ovary in stage V (Post-spawning). Thin black arrow: Oogonia; 1: chromatin nucleolus stage; 5: atretic oocyte; L: Lumen.
- 8) Ovary in stage V (Post-spawning). Thin black arrow: Oogonia; 1: chromatin nucleolus stage; 3: Vitellogenic oocyte; 5: atretic oocyte; L: Lumen.

## MALE



Light microscopy photomicrographs of histological structures of *M. merluccius* testes in different stages of maturation. The histological preparations are stained with haematoxylin-eosin and marked with different labels (numbers, letters, black or white points, etc.) and at different magnifications.

- 1) Testicle in stage I (Immature). White thin arrow: spermatogonia; black thin arrow: spermatocytes.
- 2) Testicle in stage II (Resting). White thin arrow: spermatogonia; black thin arrow: spermatocytes; white star: spermatozooids; white circle: seminiferous tubule.
- 3) Testicle in stage III (Maturing). White thin arrow: spermatogonia; black thin arrow: spermatocytes; white star: spermatozooids.
- 4) Testicle in stage IV (Pre-spawning or spawning). White star: spermatozooids.
- 5) Detail of a testicle in stage III (Maturing). White thin arrow: spermatogonia; black thin arrow: spermatocytes.
- 6) Detail of a testicle in stage IV (Pre-spawning or spawning). White star: spermatozooids.