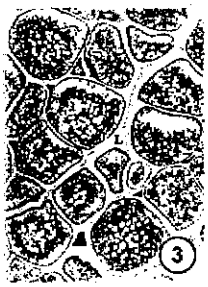
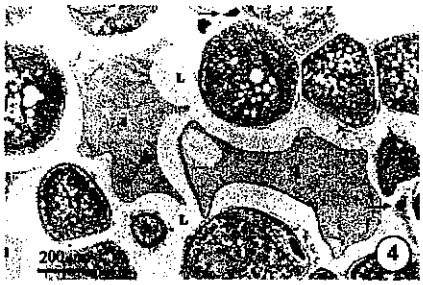
;grim, *Lepidorhombus whiffiagonis* (Walbaum, 1792).

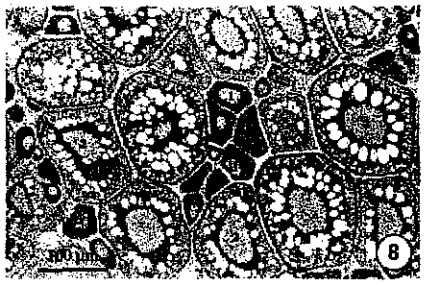
ñaki Quincoces & Paulino Lucio

CM 1998/OPEN:18

;ndi Irla s/n 48395, Sukarrieta, Bizkaia, Basque Country, Spain.

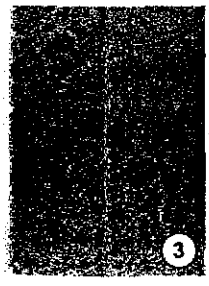


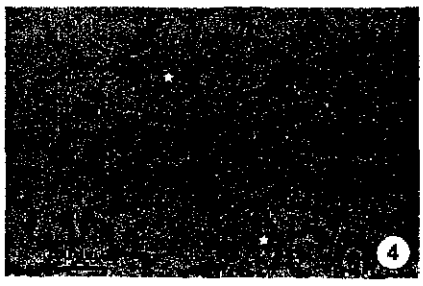


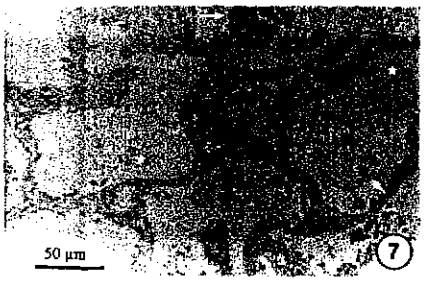


are stained with haematoxilyn-eosin. To do the identification of the structures easier they

6: advanced atretic oocyte; thick black arrow: blood vessel; L: lumen.







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INTRODUCTION

Megrim (*Lepidorhombus whiffiagonis*, Walbaum, 1792) is a pleuronectiform fish distributed from Faeroe Islands to Mauritania at depths ranging from 50 to 800 m., more precisely around 100-300 m.(Aubin-Ottenheimer, 1986). Multispecific fisheries catch this species in ICES Sub-areas IV to IX. Spain, France, Ireland and United Kingdom take the major proportion of these catches.

Three stocks have been recognised for assessment and management purposes: Megrim in ICES Sub-area VI, in Div. VIIb,c,e-k and VIIIa,b and in Div. VIIIc and IXa. The catches (landings and discards) from the three stocks amounted in 1996 for 4,708 t, 17,183 t and 329 t respectively (ICES CM, 1998 a,b). Mean annual landings in the Basque Country ports from the Bay of Biscay (Div. VIIIa,b,d) from 1994 to 1996 were around 200 t, most of them obtained by "baka"-trawlers.

There is a considerable number of studies about biology, ecology, and growth of Megrim in Sub-areas VI and VII. However, under ICES Div. VIIIa,b,d, little is known about the biology of this species. Alperi (1992) and Landa et al. (1996) carried out biological studies of Megrim in Div. VIIIc and VIIIa,b respectively.

However, for the first time, a histological study of the gonadal development of Megrim in Div. VIIIa,b,d (Bay of Biscay) is presented.

MATERIALS AND METHODS

During 1996-1997, an intensive sampling program was carried out on a monthly basis to obtain representative number of *L.whiffiagonis* in order to advance in the reproduction biology knowledge of the species. Biological samples were collected 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 brought to the laboratory. For histological studies, the aim of the sampling was to obtain the gonads of a maximum of 10 females and 5 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 (Technovitt 7100), sectioned at 2-3 µm, stained with Harris haematoxylin and 1% aquose yellowish eosin and mounted with Eukitt on slices with a coverslip on surface. At the end of the study, a total of 131 histological samples of male and 489 samples of female Megrim 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 Mature, IV Pre-spawning and Spawning, and V Post-spawning. The different development stages of the oocytes (Oogonia, Chromatin nucleolus stage, Perinucleolus stage, Vitellogenesis, Nuclear migration and Hydratation), and of the spermatozoa (Spermatocytes, Spermatids and Spermatozoa) have been also described. Due to the rather long period elapsed between the death of the specimina and the ovaries fixation, necrotic oocytes were found during the whole cycle. 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)-(7)] respectively. Structures have been marked with different labels (numbers, letters, black or white points, etc.)

DISCUSSION

The process of oocyte maturation in *L.whiffiagonis* is similar to other demersal fish described in previous works (Marrale, 1990).

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. In almost all the samples, the rate of necrotic oocytes was high. Post-spawning follicles were also observed but, as explained before, because of the rather long time elapsed between the ovary fixation and the death of the fish, the pictures obtained was only enough for their identification but not for their exposition.

The results of the histological analysis showned a very concrete spawning period for Megrim in Div. VIIIa,b,d that extends from January to March with a peak in February-March. 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 Megrim in this sea area

ACKNOWLEDGMENTS

This work was founded by the European Union DG XIV, the Basque Government and by the Spanish Ministry of Education and Culture through a fellowship to I. Quincoces.

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

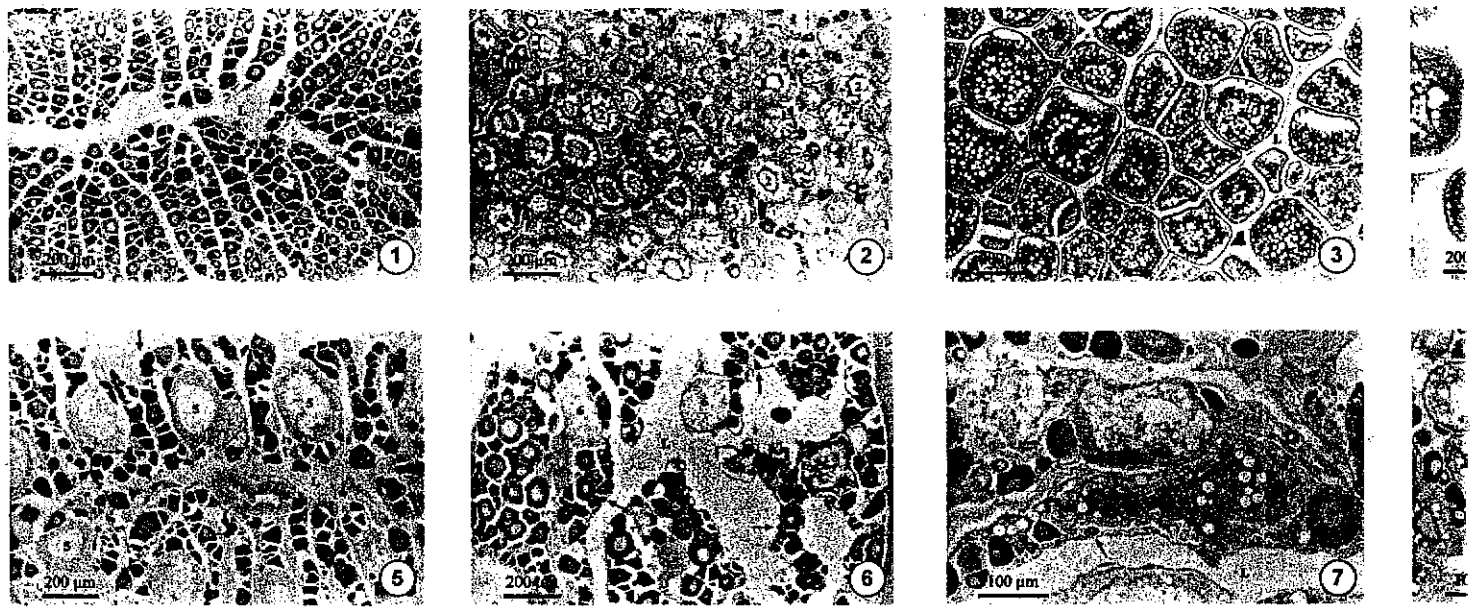
Gonad histology and its development in megrim, *Lepid*

AZTI

Marina Santurtún, Iñaki Quincoces

AZTI. Dept. of Fisheries Resources. Txatxarramendi Irla s/n 48395,

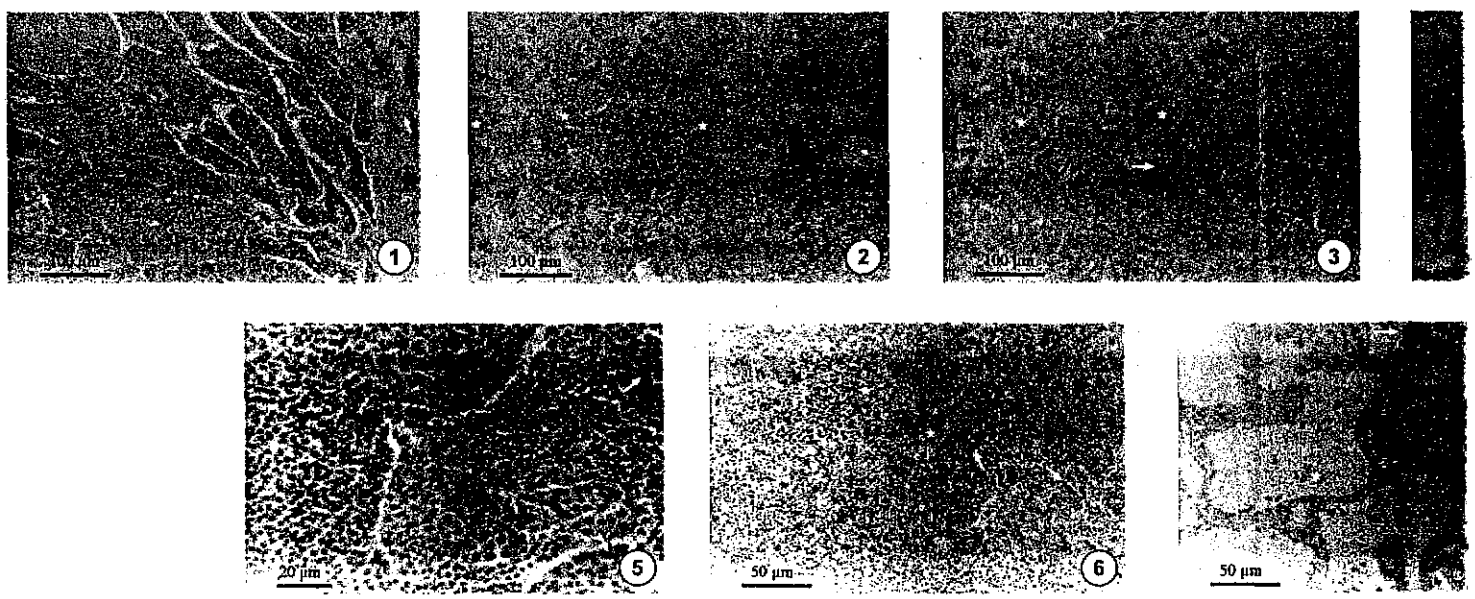
FEMALE



Light microscopy photomicrographs of histological structures of *L. whiffiagonis* ovaries in different stages of maturation. The histological preparations are stained with haematoxilyn-eosin. To have been marked with different labels (numbers, letters, or black or white points, etc.) and at different magnifications.

1) Ovary in stage I (Inmature or Virgin). Thin black arrow: Oogonia; 1: chromatin nucleolus stage; L: lumen.
2) Ovary in stage II (In maturation or resting). Thin black arrow: Oogonia; 1: chromatin nucleolus stage; 2: perinucleolar stage oocyte; L: lumen.
3) Ovary in stage III (Mature). Thin black arrow: Oogonia; 2: perinucleolar stage oocyte; 3: Vitellogenic oocyte; L: lumen.
4) Ovary in stage IV (Pre-spawning and spawning). Thin black arrow: Oogonia; 3: Vitellogenic oocyte; 4: hydrated oocyte; L: Lumen.
5) Ovary in stage V (Post-spawning). Thin black arrow: Oogonia; 1: chromatin nucleolus stage;5: early atretic oocyte; L: lumen.
6) Ovary in stage V (Post-spawning). Thin black arrow: Oogonia; 6: advanced atretic oocyte; L: Lumen.
7) Ovary in stage V (Post-spawning). Thin black arrow: Oogonia; 2: perinucleolar stage oocyte; 5: early atretic oocyte; 6: advanced atretic oocyte; thicl
8) Detail of ovary in stage II (In maturation or resting). Thin black arrow: Oogonia; 1: chromatin nucleolus stage; 2: perinucleolar stage oocyte.

MALE



Light microscopy photomicrographs of histological structures of *L. whiffiagonis* testes in different stages of maturation. The histological preparations are stained with haematoxilyn-eosin. To do t been marked with different labels (numbers, letters, or black or white points, etc.) and at different magnifications.

1) Testicle in stage I (Inmature or Virgin). White thin arrow: spermatogonia; black thin arrow: spermatocytes.
2) Testicle in stage II (In maturation or Resting). Black thin arrow: spermatocytes; white star: spermatozoids; white circle: seminiferous tubule.
3) Testicle in stage III (Mature). White thin arrow: spermatogonia; white star: spermatozoids.
4) Testicle in stage IV (Pre-spawning and Spawning). Black thin arrow: spermatocytes; white star: spermatozoids.
5) Detail of testicle in stage I (Inmature or Virgin). White thin arrow: spermatogonia; black thin arrow: spermatocytes.
6) Detail of testicle in stage IV (Pre-spawning and Spawning). White thin arrow: spermatogonia; white star: spermatozoids.
7) Testicle in stage V (Post-spawning). White thin arrow: spermatogonia; white star: spermatozoids.