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# The Ovarian Histology of English Sole (*Parophrys vetulus*)

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## ABSTRACT

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We collected ovaries from mature English sole in Hecate Strait from 1986-1990. We prepared sections from these for microscopic examination. We observed six distinct histological stages of oocytes, characterized by size and morphology, over an annual cycle. We relate these to macroscopic maturity stages previously described for Pacific coast flatfish species. The microscopic stages represent the sequence of biological events occurring in oogenesis; (production of primary germ cells, mitosis, vacuolization, deposition of yolk, hydration/ovulation, and atresia). We present photomicrograph examples of the different stages.

## RÉSUMÉ

Fargo, J. and T. Sexton. 1991. The ovarian histology of English sole (*Parophrys vetulus*). Can. Manuscr. Rep. Fish. Aquat. Sci. 2133: 19 p.

Nous avons recueilli des ovaires de soles anglaises matures du détroit d'Hecate entre 1986 et 1990. Des coupes ont été préparées pour examen au microscope. Nous avons observé chez les ovocytes six stades histologiques distincts correspondant à des tailles et à des caractéristiques morphologiques différentes et couvrant un cycle annuel. Nous établissons des relations entre ces stades et les stades de maturation macroscopiques établis pour diverses espèces de poissons plats de la côte ouest. Les stades microscopiques correspondent à la séquence des processus biologiques de l'ovogenèse (production des cellules germinales primordiales, mitose, vacuolisation, formation du vitellus, hydratation et ovulation, et atrésie). Des photomicrographies des différents stades sont présentées.

## INTRODUCTION

English sole (*Parophrys vetulus*) is an important component of the bottom-trawl fishery in Hecate Strait, British Columbia. Its abundance has fluctuated widely over the last 40 years, largely due to interannual variation in recruitment (Fargo 1991). We wanted to investigate recruitment mechanisms to be able to forecast recruitment and estimate sustainable yield for stock assessment. To do this we needed detailed information concerning oocyte maturation that we could relate to the length of the reproductive cycle and the time of spawning for this species.

We reviewed the literature for information on the reproductive cycle of English sole, but could find no specific information. A study by Johnson et al. (1988) examined effects of contaminants on ovarian development in English sole, but they used information modified from Wallace and Selman (1981) on teleosts to characterize, generally, some stages of oogenesis. They did not consider the time frame for the reproductive cycle or provide descriptions of distinct stages in the English sole reproductive cycle.

We then designed a systematic sampling scheme, over several years, that would provide us with the samples necessary for describing the reproductive cycle of English sole. The study was spread over several years to ensure adequate sampling coverage and to investigate the possibility that the reproductive cycle might be longer than one year in duration.

This paper presents information concerning the microscopic ovarian histology of English sole. We do not cover folliculogenesis or the biochemical and molecular aspects of oogenesis. Some information on these aspects associated with reproduction in English sole has been presented by Collier et al. (1986), and Varanasi et al. (1982). More detailed information concerning analysis of oocyte size composition data with reference to the length of the reproductive cycle and time of spawning for English sole in Hecate Strait will be the subject of a separate publication.

## MATERIALS AND METHODS

We obtained ovary samples from English sole from research cruises and commercial trawlers from Hecate Strait on the northern coast of British Columbia. We dissected the right ovary of each fish sampled, and preserved it in a buffered formal saline solution (Foucher et al. 1989). Ovaries were then soaked in Davidson's fixative for approximately 24 hours, and sections were sliced from their anterior portions. The sections were embedded in paraffin wax, sectioned at 5μ, stained with haematoxylin and counterstained with eosin (Yasutake and Wales 1983). To ease the cutting process, the ovaries were soaked in 60% alcohol and glycerin (of equal parts) before sectioning.

Prepared sections were examined with a light microscope to determine features characteristic of maturity stages previously assigned for flatfish at the macroscopic level (Harling et al. 1982). Suitable examples were then photographed. Oocyte diameters were measured with a light microscope calibrated to the nearest 5μ or with a projection microscope calibrated to the nearest 4μ. A sample size of approximately 300 oocytes/fish was used for oocyte measurements. Only oocytes whose nuclei were included in the sections were measured (Foucher and Beamish 1980).

## RESULTS

In describing the histological stages in the reproductive cycle, our terminology is the same as that used by Bowden et al. (1990). We used samples taken at five different months over a period of three years (Table 1) to illustrate the different stages.

The stage 1 ovary (Figure 1) has a characteristically thin ovarian wall and minute germ cells have developed from the germinal epithelium. The lamellae in the ovary wall are well developed. Germ cells and small oogonia have large nuclei that occupy most of the cell's volume. Some of the oogonia have divided mitotically to form small previtellogenic oocytes. The nuclei of these oocytes were located slightly off-centre. Oocytes in the stage one ovary range from minute germ cells to small previtellogenic oocytes (10μ to 50μ diameter).

The stage two ovary (Figure 2) contains large previtellogenic oocytes. Euvitelline nucleoli are arranged around the periphery of the nuclei of these cells and some of them show signs of vacuolization. A connective tissue layer, containing blood supply, has formed around the oocytes. Oocytes in the stage two ovary range in diameter from 50μ to 200μ.

Oocytes in the stage three ovary (Figure 3a) undergo vacuolization. A band of vacuoles forms around the periphery of the cytoplasm. The ovary wall thickens, and blood vessels are prominent in it. Lipid vesicles appear in the cytoplasm, and the external oocyte membrane has formed (Figure 3b). The nuclei of these oocytes are somewhat convoluted. Oocytes in the stage three ovary range in diameter from 180μ to 250μ.

In the stage four ovary the wall appears thin and stretched. The oocytes are large enough to be visible macroscopically. Vacuolated oocytes undergo vitellogenesis and protein yolk granules accumulate in the outer cortex of the oocytes. The nucleus is extremely convoluted and a band of chromatin is visible surrounding it (Figure 4). Most of the oocyte growth occurs during this stage. Oocytes in the stage four ovary range in diameter from 200μ to 400μ.

In the stage five ovary, oocytes are hydrated and translucent. There is some evidence of atresia and the band of chromatin surrounding the nucleus has condensed to form dark patches. Yolk granules are spread throughout the cortex of the oocytes (Figure 5). Ovulation occurs at this stage. Ova in the stage five ovary range from 375μ to 550μ.

In the stage six ovary, (Figure 6a) yolk granules and nuclei in mature oocytes that have not been spawned breakdown and are resorbed . Smaller oocytes that never fully matured are also resorbed (Figures 6b-e). The resorbing oocytes become atretic bodies (Figure 6f). New oocytes for the next year's spawning are produced from the germinal epithelium (Figure 6g).

## DISCUSSION

We observed that the nuclei of small oocytes, in stage one ovaries were located slightly off centre. Similar observations have been reported by Negonovskaya (1982) in a study of trout (*Salmo ischchan*) and Bowden et al. (1990) in a study of Pacific cod (*Gadus macrocephalus*). The reason for this phenomenon is unknown. Bowden et al. (1990) reported an unidentified grey-staining band surrounding the nucleus in vitellogenic oocytes. We identified a similar band around the nucleus in vitellogenic oocytes in English sole as chromatin. This band appeared just after hydration had occurred, prior to spawning. In post-spawning Pacific cod, Bowden et al. (1990) reported the presence of phagocytic macrophages. The function of these cells was to "clean" the ovary of all disrupted oocytes and ova. They imparated a blackish appearance to the ovary by their accumulation of melanin granules. We did not observe the presence of these cells in English sole. We did not observe any dark discoloration of the ovary in spent English sole either. However, the spawning period for English sole is protracted (Kruse and Tyler 1983) and we may not have sampled the post-spawning period adequately. We observed the presence of collapsed follicles in the ovaries of spent fish. Previous investigators have suggested that these may differentiate to form new oogonia (Foucher and Beamish 1977).

## ACKNOWLEDGEMENTS

Serge Villeneuve, Christina Horvath, and Corinne Kikegawa prepared the histological sections used for this study and measured diameters of oocytes on the prepared slides. Corinne Kikegawa produced the photomicrographs used in this report. John Bagshaw supervised the histological work and helped in interpretation of the histological sections.

Graham Gillespie and Claudia Hand reviewed the manuscript and provided a number of suggestions which improved the content of the paper.

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Table 1. English sole specimens used for photo micrographs.

| Stage | Fish no. | Date | Figure |
|---|---|---|---|
| 1 | 5A | 870319 | 1 |
| 2 | 36 | 880505 | 2 |
| 3 | 47 | 880505 | 3a |
| 3 | A13 | 870603 | 3b |
| 4 | 7 | 880602 | 4 |
| 5 | 6 | 881027 | 5 |
| 6 | 16 | 881027 | 6a |
| 6 | 5 | 901019 | 6b |
| 6 | 59 | 871105 | 6c |
| 6 | 49 | 881105 | 6d |
| 6 | 56 | 880316 | 6e |
| 6 | 48 | 901103 | 6f |
| 6 | H2 | 870113 | 6g |

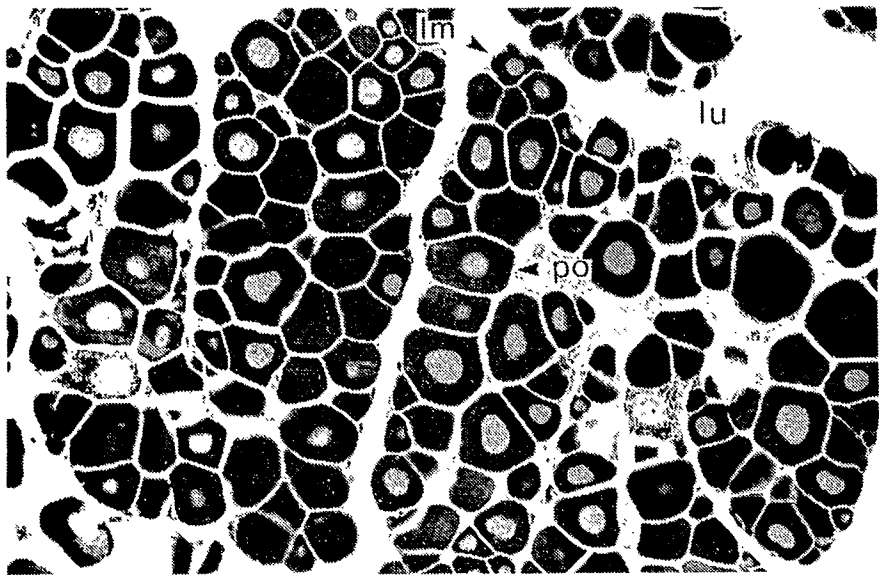


Fig. 1. Cross section of ovary with stage 1 oocytes. (lm) lamella, (lu) lumen, (po) pre-vitellogenic oocyte.

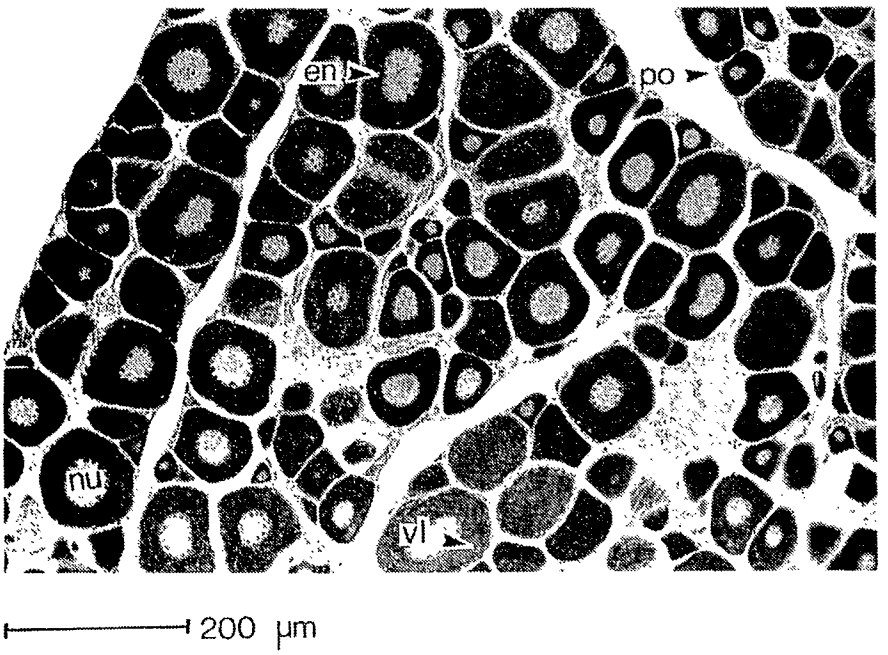


Fig. 2. Cross section of ovary with stage 2 oocytes. (en) euvitelline nucleolus, (nu) nucleus, (po) pre-vitellogenic oocyte, (vl) vacuole.

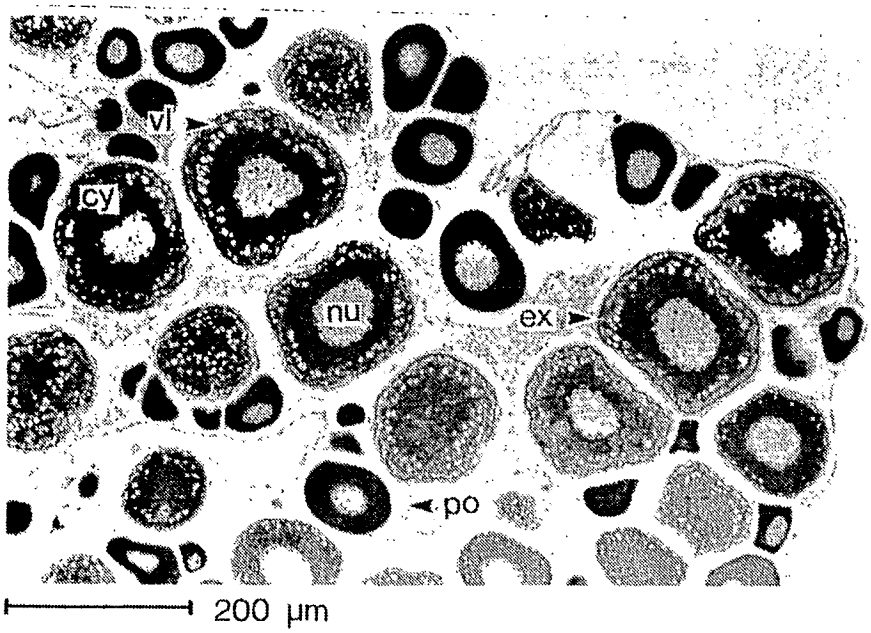


Fig. 3a. Cross section of ovary with stage 3 oocytes. (cy) cytoplasm, (ex) external oocyte membrane, (nu) nucleus, (po) pre-vitellogenic oocyte, (vl) vacuole.

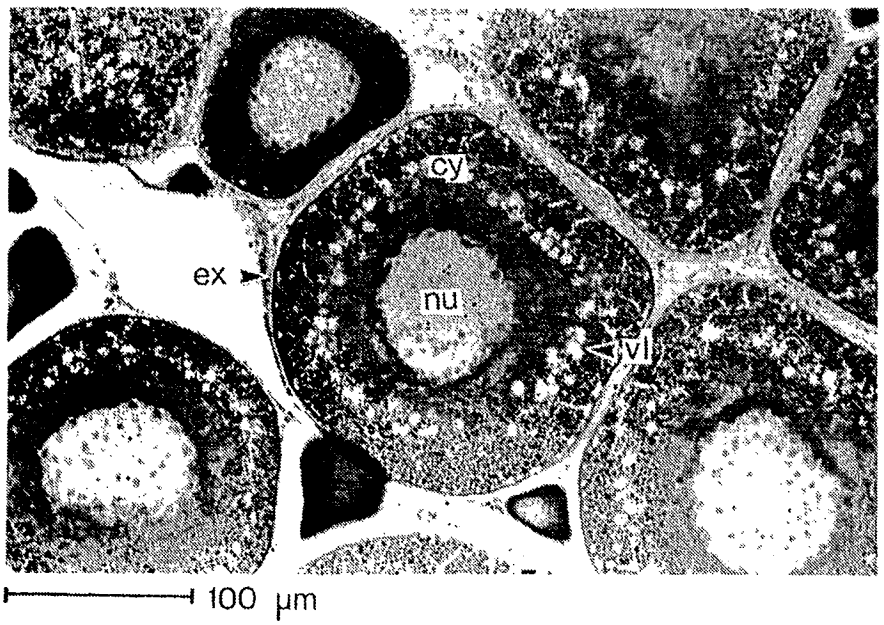


Fig. 3b. Vacuolated oocyte. (cy) cytoplasm, (ex) external oocyte membrane, (nu) nucleus, (vl) vacuole.

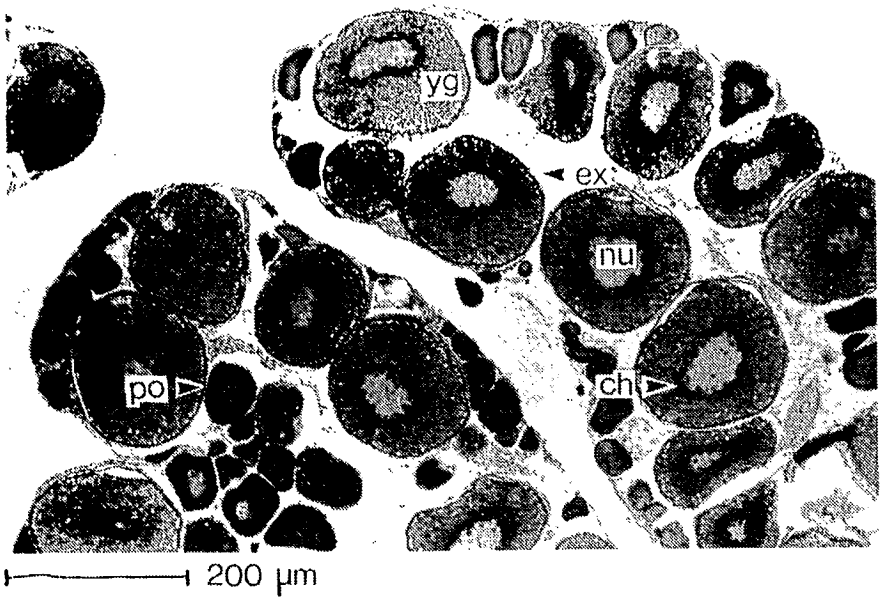


Fig. 4. Cross section of ovary with stage 4 oocytes. (ch) chromatin, (ex) external oocyte membrane, (nu) nucleus, (po) pre-vitellogenic oocyte, (yg) yolk granules.

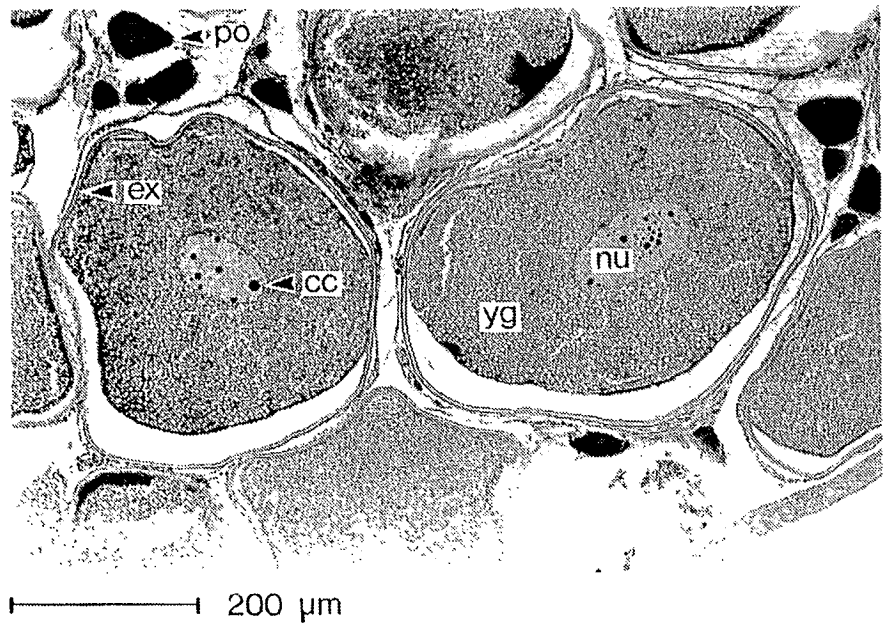


Fig. 5. Cross section of ovary with stage 5 oocytes. (cc) condensed chromatin, (ex) external oocyte membrane, (nu) nucleus, (yg) yolk granules, (po) pre-vitellogenic oocyte.

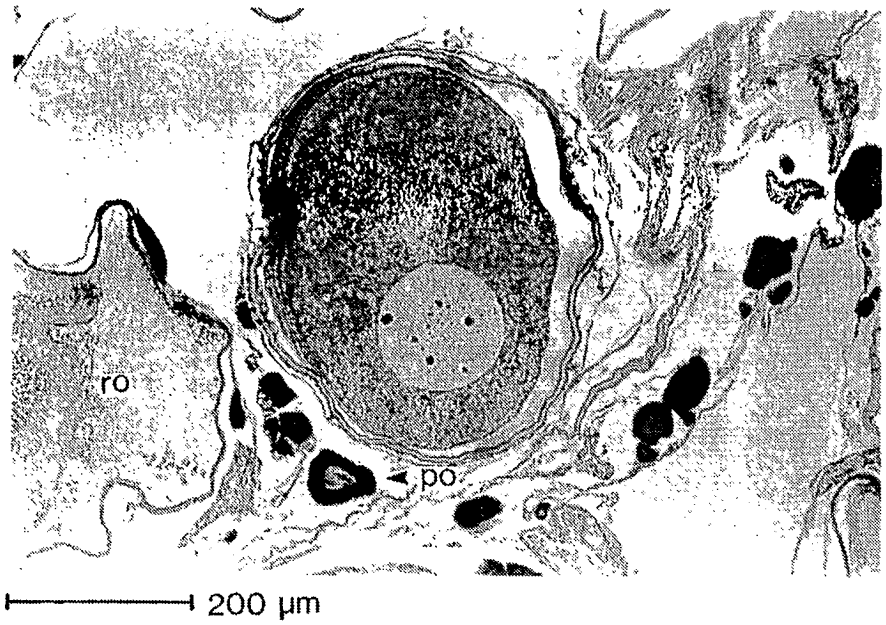


Fig. 6a. Cross section of an ovary from post-spawning fish. (po) pre-vitellogenic oocyte, (ro) resorbing oocyte.

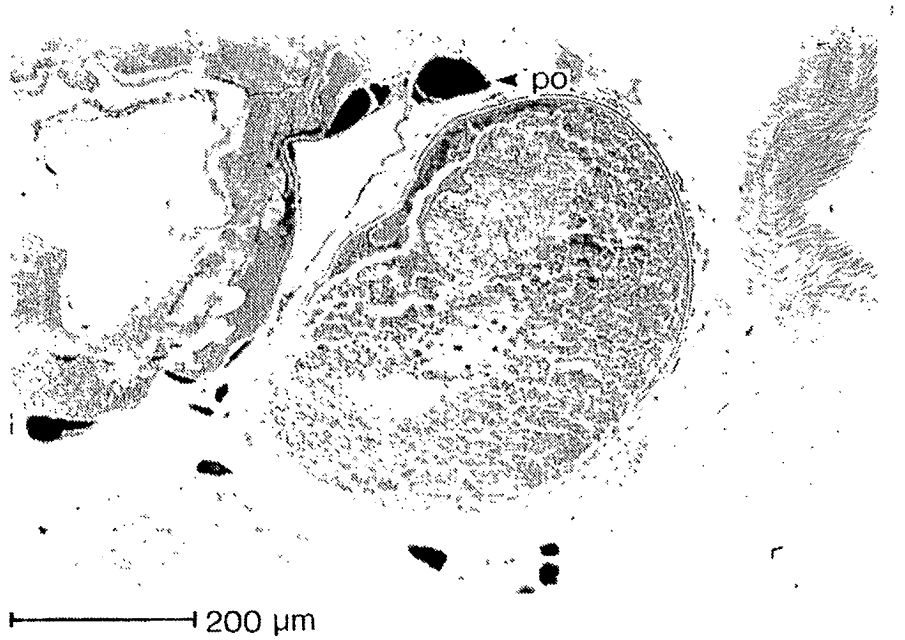


Fig. 6b. Mature oocyte in beginning stage of resorption after spawning has occurred. (po) pre-vitellogenic oocyte.

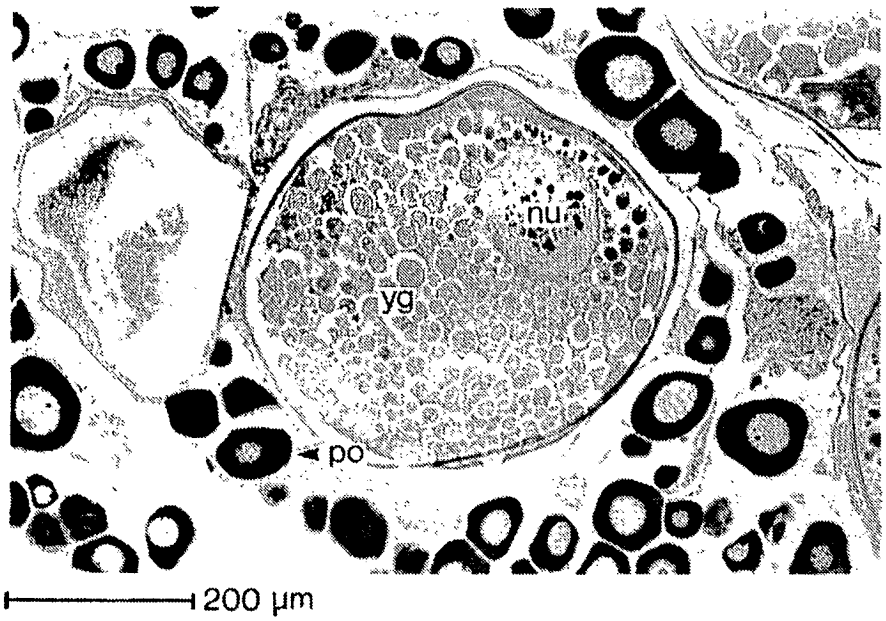


Fig. 6c. Yolk granules breaking down in mature oocyte after spawning has occurred. (po) pre-vitellogenic oocyte, (yg) yolk granules, (nu) nucleus.

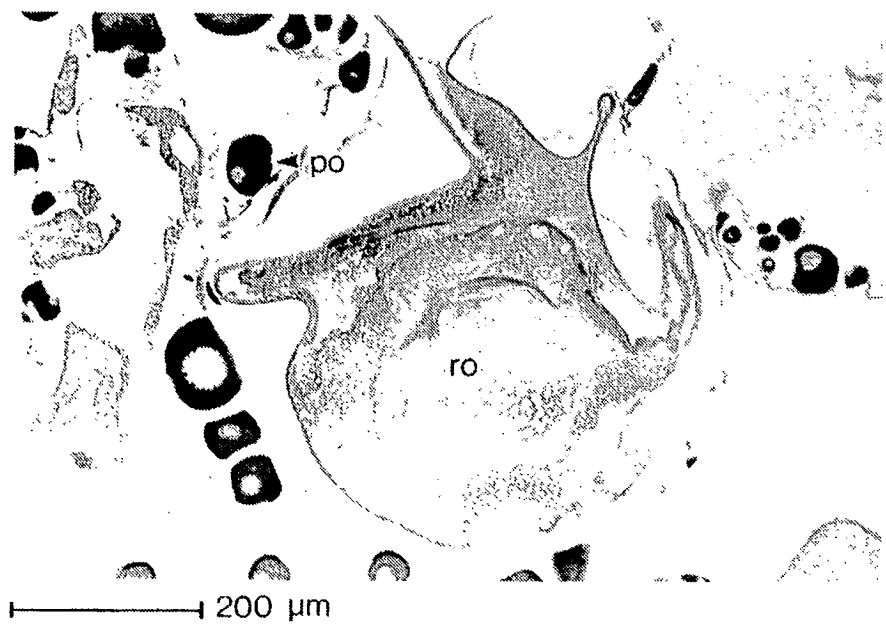


Fig. 6d. Resorption of mature oocyte is almost complete following spawning. (po) pre-vitellogenic oocyte, (ro) resorbing oocyte.

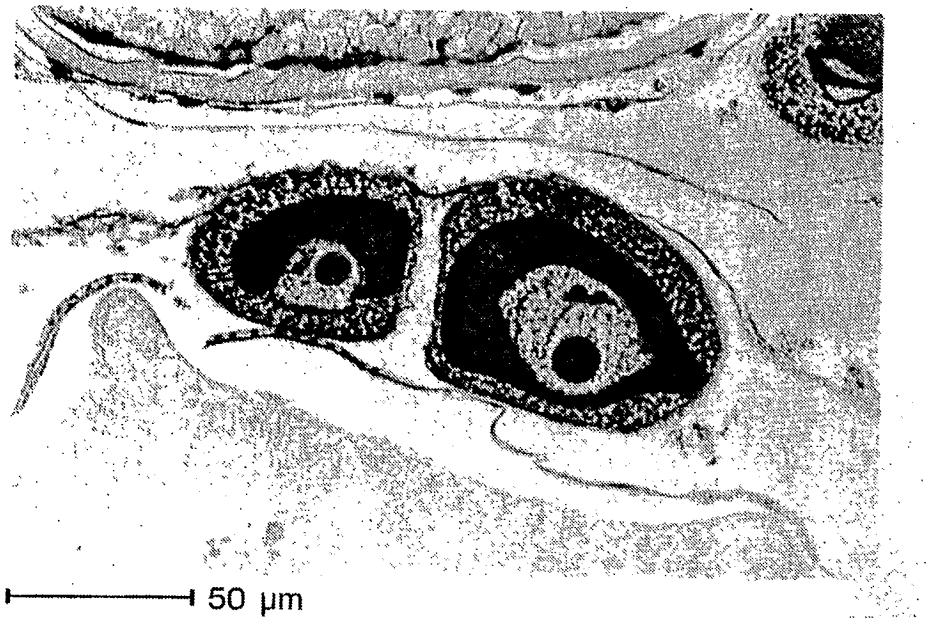


Fig. 6e. Resorption of smaller mature oocytes.

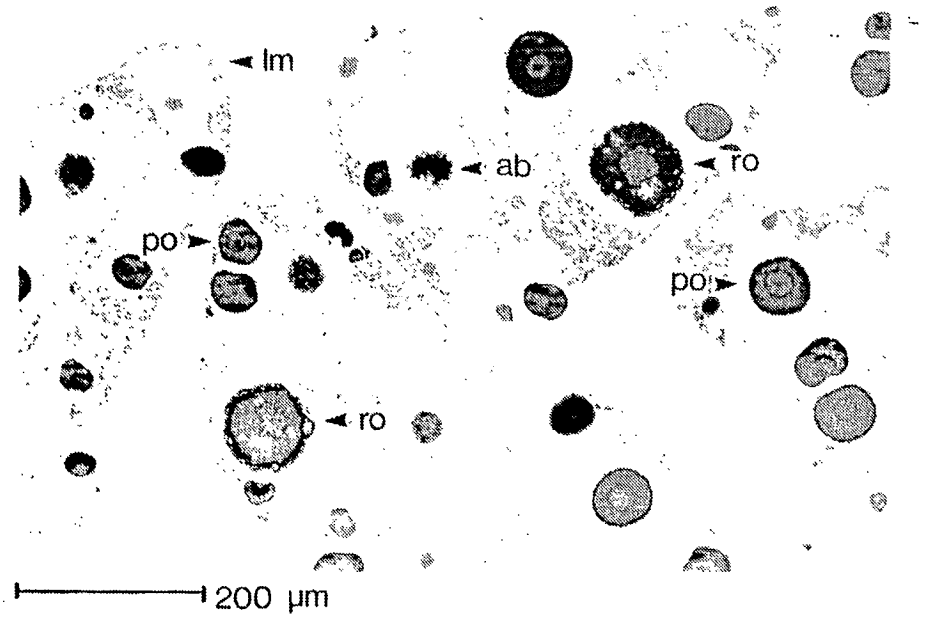


Fig. 6f. Cross section of ovary from a fish just following spawning. (ab) atretic body, (lm) lamella, (po) pre-vitellogenic oocyte, (ro) resorbing smaller mature oocyte.

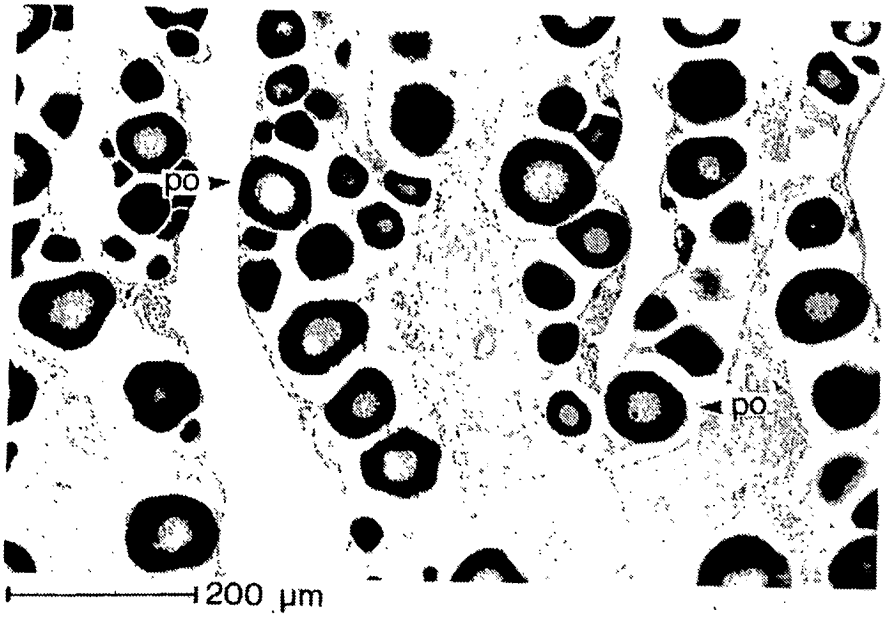


Fig. 6g. Cross section of ovary from a fish following spawning. (po) pre-vitellogenic oocyte.