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Identification of Female Cod (*Gadus morhua*) from Flemish Cap (Northwest Atlantic) at the Beginning of Ripening

by

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ABSTRACT

Ovaries of cod from Flemish Cap were sampled at three different times during the reproductive cycle. We study the use of the oocytes in the circumnuclear ring, cortical alveoli and vitellogenesis stages, the postovulatory follicles and gonosomatic index for the identification of females at the beginning of the ripening.

The results obtained indicate that there is a period of less than two months between the end of spawning and the beginning of the development of the cortical alveoli stage, and more than three months to the beginning of vitellogenesis in all mature females. It is necessary to wait for these periods to use these structures to identify all the females at the beginning of ripening.

Postovulatory follicles last a long time in the ovary, and their identification is possible seven months after spawning; the reconstruction of the last maturation ogive can be made three months after spawning.

The mean gonosomatic index of mature and immature females showed significant differences three months after spawning, but the overlap between the maximum and minimum values impedes the use of this index to identify all females at the beginning of ripening.

INTRODUCTION

A correct classification of the maturity stages of the gonads is necessary to calculate the proportion of mature and

immature females for a determined size or age. This classification is very easy in females during most of the annual reproductive cycle based on the macroscopic aspect of the ovary (Pitt, 1966; Morrison, 1990), but it is very difficult at the beginning of ripening in primiparous and multiparous females.

The problem of identification of females at the beginning of ripening is very frequent during fisheries research surveys, where it is necessary to estimate spawning biomass. These surveys are frequently carried out during the period when the macroscopic identification of mature and immatures females is more difficult, and it is necessary to use other techniques.

Some microscopic methods have been developed in cod (Gadus morhua), to classify ovaries in to maturity stages. Kjesbu (1991) used oocytes size and Morrison (1989) the presence of different kinds of oocytes in histological preparations, but the problem has not been totally resolved.

Cod on Flemish Cap (Northwest Atlantic) spawn in February and March, and the classification of mature and immature females is very difficult from the macroscopic aspect of the ovary three months after the end of spawning (July). Histological analysis of ovaries of Flemish Cap cod, sampled at three different times during the annual reproductive cycle, was carried out to study the value of three kind of oocytes, postovulatory follicles and gonosomatic index for the identification of mature and immature females at the beginning of ripening.

MATERIAL AND METHODS

During the Flemish Cap summer surveys from 1990 to 1992, and on board commercial trawlers, 557 cod ovaries were sampled. The gonads belong to three different periods of the annual reproductive cycle, and their distribution by months and sizes is presented in table 1.

Ovaries were immediately fixed after capture in 10% buffered formalin (Hunter, 1985), size and weight were recorded for each individual and otoliths obtained. Gonads

were weighed in the laboratory. Pieces 0.5 centimeters thick were embedded in paraffin and 6 microns sections stained with Harri's hematoxyline and eosine floxine b.

Circumnuclear ring (photograph 1), cortical alveoli (photograph 2) and vitellogenesis (photography 3, 4 and 5), and the postovulatory follicles (photography 2, 4, 6 and 7), were identified in each section, following the classification of Kjesbu and Krivi (1989) and Morrison (1990). The diameters of thirty oocytes in the cortical alveoli stage were measured in all the ovaries sampled in July 1990. The diameters of the oocytes sectioned through the nucleus were considered to represent the real diameters (Foucher and Beamish, 1980).

The gonosomatic index (IGS) was obtained by expressing gonad weight as a percentage of body weight (De Vlaming et al, 1982).

RESULTS

All sampled ovaries have oocytes in the circumnuclear ring stage. These oocytes have a ring of slender stained cytoplasm. The presence of this kind of oocyte in ovaries from females that, according their size must be immature, indicates that this stage lasts longer than one year, and they are not a good identifier of the spawners of the next breeding season.

In February, 29 ovaries were sampled (table 2), twelve in the spawning period, showing oocytes in nuclear migration or hydration stages. Six of them had completed spawning, lacked oocytes in vitellogenesis, and had a lot of postovulatory follicles. The rest of the ovaries did not spawn or were immature. Only three of the ovaries sampled in February had cortical alveoli oocytes: two of them were primiparous females and lacked postovulatory follicles, the third was a multiparous female which also had oocytes at the beginning of vitellogenesis.

Most of the ovaries sampled in summer show oocytes in the cortical alveoli stage. The percentage of ovaries with this kind of oocyte by size and age are show in tables 3 and 4.

Oocytes in the cortical alveoli stage are present in all ovaries with postovulatory follicles or oocytes in vitellogenesis.

The size distribution of oocytes in the cortical alveoli stage is near normal (figure 1). There is a low but significant correlation between length of the female fish and mean cortical alveoli oocyte diameter (r= 0.3).

In November, 34 ovaries were sampled (table 5) and only two of them have oocytes in the cortical alveoli stage; these ovaries also had fully yolked oocytes.

Cortical alveoli oocytes have a seasonal occurrence that starts after spawning; they are mainly present in June and July samples. The absence of cortical alveoli in females which have finished spawning in March indicates that there is a time period between the end of spawning and the formation of cortical alveoli.

All the females with postovulatory follicles or oocytes at the beginning of vitellogenesis also had cortical alveoli in June and July. The maturation ogive of maturation obtained with July 1990 samples (fig. 2) using the cortical alveoli stage as identifier of mature females gave similar results that obtained by De Cardenas (1992) for cod on Flemish Cap. In conclusion multiparous and primiparous females develop cortical alveoli in June and July, and at this time this structure is a good guide to those fish which will spawn the next breeding season.

The spawning season is in February and March, so the period between the end of spawning and the beginning of the cortical alveoli stage in mature females is less than two months.

The presence in July of females with oocytes in the cortical alveoli stage and without oocytes in the beginning of the vitellogenesis (tables 3 and 4) indicates that the period between the end of spawning and the beginning of vitellogenesis in all the females is longer than three months. The existence of this period prevents the use of vitellogenesis stages to identify the spawners of the next year for at least three months after spawning.

Less than 50% of the mature females have begun the vitellogenesis in July. The proportion of females in vitellogenesis in June and July was larger in the longest fish (table 3 and 4), and indicates that the period between the end of spawning and the start of vitellogenesis is shorter in the longest females.

New postovulatory follicles (POF) were observed in ovaries sampled in February, and more advanced stages of reabsorption of the POF were present in a high percentage of females sampled in summer and November (tables 3, 4 and 5). The POF are very persistent and can be identified in some females during most of the annual reproductive cycle (February to November).

The proportion of females with POF in summer and November increases with the size, reaching 100% for sizes at which all females have presumably spawned in the previous spawning season. The POF are useful during most of the year for identification of multiparous females.

Two maturation ogives were elaborated using samples of July 1990. For the first one, corresponding to the spawners in 1990, POF was used to identify mature females. For the second one, corresponding to the spawners in 1991, the presence of cortical alveoli was used as a criterion. The results are shown in figure 2; the 50% maturation ages, graphically obtained, were 5.6 years using POF and 3.8 years using cortical alveoli. Such a large difference in results is not inconsistent , since a one year difference is explainable because there is one year between spawning seasons. These results are very similar to those obtained by De Cárdenas (1992) in February 1992 with a 50% maturation length of 52 centimeters (five years).

Mean gonosomatic indices (GSI) of mature and immature females for July 1990 are represented in Figure 3; the presence of cortical alveoli was used to identify mature females. Mean IGS values were very similar, and larger for mature females. The mean IGS value for immatures is 0.56 (SD = 0.15) and 1.10 (SD = 0.56) for mature ones.

Mean IGS values of females without oocytes in the cortical alveoli stage, with cortical alveoli and no

vitellogenic oocytes, and with vitellogenic oocytes are showed in table 6. These values increase with the advance of ovary development, but the overlapping between maximum and minimum values of the ranges prevent their use to identify females in the initial stages of ripening.

DISCUSSION

Oocytes in the circumnuclear ring stage (OCR) .

This kind of oocyte has been observed in most of histological analysis on cod ovaries (Sorokin, 1957; Woodhead and Woodhead, 1965; Shirokova, 1977; Kjesbu and Kryvi, 1989; Morrison, 1990). The characteristic ring in the cytoplasm of this stage is a concentration of mitochondria (Morrison, 1990). The oocytes remain in this stage until the formation of the cortical alveoli.

It is frequently considered that the ovaries with OCR will spawn during the next breeding season (Woodhead and Woodhead, 1965; Shirokova, 1977; Holdway and Beamish, 1985; Kjesbu and Kryvi, 1989; Morrison, 1990), although there are no studies which analyze the moment of its formation and the duration of this stage.

In the ovaries analyzed, the OCR had a very variable aspect, and are present in different degrees of development in all ovaries studied. It is very improbable that all the females in the length range analyzed will be spawners in the next breeding season. This implies that the OCR last longer than one year, and is not useful to identify next year spawners.

Oocytes in cortical alveoli stage (OCA)

Cortical alveoli are the first structures to appear during the gonadotropin dependent growth, its content is synthesized within the oocyte, and is not yolk in a strict sense (Wallace and Selman, 1981). The function of the cortical alveoli is the formation of the perivitelline space after the fertilization of the egg (Yamamoto, 1961; Laale, 1980).

The cortical alveoli stage is considered to indicate the beginning of the ripening of ovaries that will spawn in the next year, Robb (1982) in *Melanogrammus aeglefinus*, Howell (1985) in *Limanda ferruginea* and Morrison (1991) in cod.

The OCA in Flemish Cap cod are seasonal. Their development begins before the end of spawning, with less than two month between the end of spawning and the start of their formation in all of the mature females, although this period may be shorter because we lack samples closer to the end of spawning.

The presence of OCA is the first sign of the start of ripening in females, and is the best criterion to identify ripening females before the beginning of vitellogenesis.

Vitellogenesis

Vitellogenesis is the next stage following cortical alveoli in the oocyte development, and involves the sequestration and storage of a hepatically derived vitellogenin into yolk protein (Wallace and Selman, 1981).

The beginning of the vitellogenesis in most Flemish Cap females cod was at least three months after the end of spawning. The storage of yolk during the vitellogenesis is the cause of most of the macroscopic characters used in the identification of ripening females (yellow color of the ovary, size of the oocytes and ovary). The existence of a delay between the end of spawning and the beginning of vitellogenesis is the reason for the difficulty, some months after spawning, of using the macroscopic aspect of the ovary to identify females at the beginning of ripening.

To use the presence of oocytes at the start of vitellogenesis to identify accurately the proportion of mature females in a population, it is necessary that all next year's spawners have initiated vitellogenesis. This does not occur in Flemish Cap cod before the fourth month after spawning (August).

The longest females frequently begin spawning early and produce larger eggs than smaller fish (Pitt, 1966;

Rinjnsdorp, 1989; Kjesbu, 1989; McEvoy, 1991). The shortest period between spawning and the start of vitellogenesis observed in the larger females indicates that these differences in relation to size observed during spawning are also detectable in the early stages of ripening in Flemish Cap cod.

Postovulatory follicles (POF).

After vitellogenesis maturation of the oocyte takes place, and consists of nuclear migration and hydration; following ovulation, the postovulatory follicle remains (Wallace and Selman, 1981).

The presence of postovulatory follicles in a ovary is indicative of a previous spawning, but the use of the POF to determine the proportion of females that have spawned is only possible if the duration of the POF in the ovary is known.

It is thought that the POF remain for a short time in the ovary since they are scarce in ovaries from natural conditions, and because their appearance in ovaries is short in species where reabsorption of the POF is well known (Hunter and Goldberg, 1980; Goldberg et al., 1984; Hunter and Macewicz, 1985a; Hunter et al, 1986).

The available information on reabsorption of the POF in cod is very scarce (Woodhead and Woodhead, 1965), and indicates that POF of cod can be identified in the ovary four and five months after spawning. The POF in Flemish Cap cod remain recognizable in the ovary during most the annual reproductive cycle, and their identification is possible in some females at the end of November, eight months after spawning (February-March).

The old POF can be confused with the last stages of reabsorption of oocytes (atresia) (Hunter and Macewicz, 1985), but the reconstruction of maturation ogives using the POF and the scarcity of atretic oocytes to justify the number of POF observed indicates that the POF has been correctly identified.

The POF are the best structure to determine the maturity stage of females during the first months after

spawning, when not all spawners of the next breeding season have developed oocytes in the cortical alveoli stage. The long permanence of the POF in the ovary also permits us to identify multiparous and primiparous females during most of the annual reproductive cycle.

The maturation ogives obtained using the cortical alveoli stage or the POF (Figure 2), show a difference of 1.8 years between ages of 50% maturations. One year difference is explainable due to the one year between the cortical alveoli stage and the POF, the rest of the difference (0.8) may be related to differences between cohorts in the age of 50% of maturation. Another possibility is that not all females that begin ripening of the ovary reach the stage necessary to spawn. The presence of nearly 30% non-reproductive adult females was identified in the Flemish Cap cod population for the years 1978-1985 (Walsh et al, 1986). Most of these non-reproductive females were 60 to 70 centimeters in length, which corresponds to ages 5, 6 and 7 years in our 1990 samples. The larger differences between females that begin ripening (mature according to the cortical alveoli stage) and finish spawning (matures according the POF) were observed in these ages.

Gonosomatic index (IGS)

The most important changes that occur in gonad weight are produced by yolk storage in the oocytes and by oocytes hydration before ovulation. These variations in gonad weight produce changes in the IGS that can reach values close to 20% of body weight, and allow the identification of mature and immature females. In this study we have tried to see if the small changes produced at the beginning of ripening, three months after spawning, are sufficient to classify females in to mature and immature by their IGS.

The results obtained show that the small changes produced in the ovary at the beginning of maturation are reflected in the IGS; and show significant differences between the mean values of mature and immature, although the overlapping of maximum and minimum values do not allow the correct identification of all females.

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Table 1. Date, range of sizes and number of ovaries sampled

Date	Range	Number
12-30 VII 1990	33-93	258
24/VI - 11/VII 1991	33-93	236
28-29 XI 1991	51-93	34
17/II - 10/III 1992	39-83	29

Table 2. Sampling by sizes of February and March 1992. Number of ovaries, number of ovaries with oocytes in cortical alveoli stage (COR. ALV), vitellogenesis (VITELLOG) and postovulatory follicles (POF)

Size	Number	COR.ALV	VITELLOG	POF
39-41	1	0	0	0
42-44	1	0	0	0
45-47	4	2	0	0
48-50	5	0	1	0
51-53	1	0	0	0
54-56	1	0	0	0
57-59	3	0	2	3
60-62	5	1	1	5
63-65	2	0	2	2
66-68	3	0	1	3
69-71	0			
72-74	1	0	1	1
75-77	0			
78-80	1	0	1	0
81-83	1	0	1	0

Table 3. Sampling by sizes of July 1990. Number of ovaries, percentage of ovaries with oocytes in cortical alveoli stage (% COR. ALV), vitellogenesis (% VITELLOG) and postovulatory follicles (%POF)

Size	Number	%COR.ALV	%VITELLOG	%POF
33-35	3	0.0	0.0	0.0
36-38	6	16.7	0.0	0.0
39-41	10	30.0	0.0	0.0
42-44	10	50.0	10.0	40.0
45-47	18	83.3	5.6	27.8
48-50	17	88.2	11.8	47.1
51-53	17	88.2	5.9	35.3
54-56	23	87.0	4.4	26.1
57-59	18	88.9	16.7	44.4
60-62	23	87.0	4.4	52.5
63-65	24	87.5	0.0	29.2
66-68	23	73.9	21.7	39.1
69-71	17	88.2	11.8	41.2
72-74	12	83.3	8.3	50.0
75-77	2	100	0.0	50.0
78-80	7	100	14.3	100
81-83	5	100	40.0	100
84-86	5	100	40.0	80.0
87-89	2	100	50.0	100
90-92	5	100	40.0	100
>93	11	100	36.4	100

Table 4. Sampling by sizes of June and July 1991 and 1992 Number of ovaries, percentage of ovaries with oocytes in cortical alveoli stage (% COR. ALV), vitellogenesis (% VITELLOG) and postovulatory follicles (%POF)

Size	Number	%COR.ALV	%VITELLOG	%POF
<33	2	0.0	0.0	0.0
33-35	2	0.0	0.0	0.0
36-38	1	0.0	0.0	0.0
39-41	2	0.0	0.0	0.0
42-44	6	33.3	0.0	0.0
45-47	2	0.0	0.0	0.0
48-50	9	44.4	22.2	33.3
51-53	13	69.2	15.4	46.2
54-56	26	84.6	19.2	42.3
57-59	26	92.3	34.6	57.7
60-62	35	100	40.0	97.4
63-65	25	100	56.0	100
66-68	28	96.4	50.0	92.9
69-71	24	87.5	33.3	79.2
72-74	8	100	37.5	100
75-77	10	100	40.0	90.0
78-80	4	75	50.0	75
81-83	2	100	0.0	100
84-86	4	100	25.0	75.0
87-89	0			
90-92	1	100	100	100
>93	6	100	33.3	100

Table 5. Sampling by sizes of november 1991, number of
ovaries, ovaries with oocytes in cortical alveoli stage (COR.
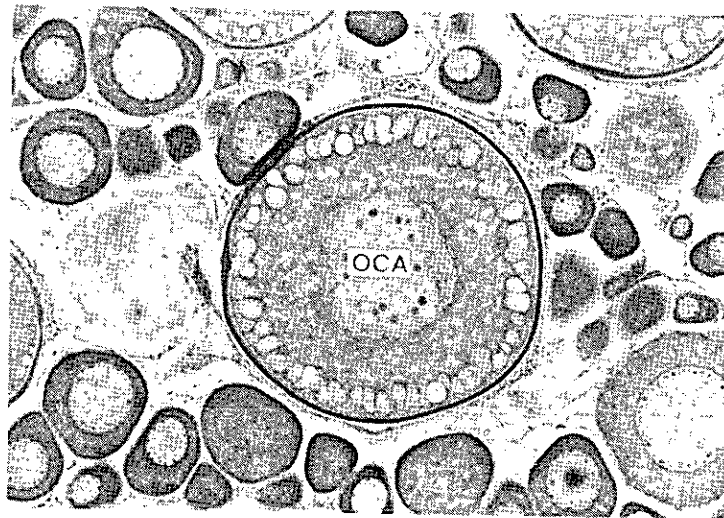ALV), vitellogenesis (VITELLOG) and postovulatory follicles
(POF).

Size	Number	COR.ALV	VITELLOG	POF
51-53	3	1	3	1
54-56	4	1	4	1
57-59	2	0	2	1
60-62	2	0	2	2
63-65	2	0	2	2
66-68	9	0	9	9
69-71	6	0	6	4
72-74	2	0	2	1
75-77	1	0	1	1
78-80	1	0	1	1
81-83	1	0	1	1
>93	1	0	1	1

Table 6. Mean gonosomatix index (IGS) by maturity stages for
July 1990, and maximum (Max) and minimum (Min) values.
Immature: ovaries without oocytes in cortical alveoli stage.
Mature (Cort.Alv): ovaries with oocytes in cortical alveoli
stage and without vitellogenic oocytes.
Mature (Vitell.): ovaries with oocytes in vitellogenesis.

|	Number	IGS	Max.	Min.	DT
Immature	45	0.56	1.0	0.1	0.15
Mature (Cort. Alv.)	179	1.03	6.2	0.4	0.55
Mature (vitell.)	30	1.53	3.6	0.9	0.60
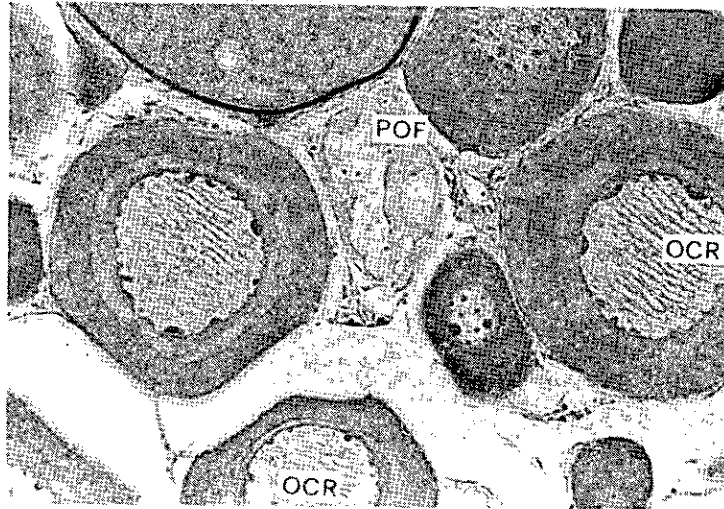
Photograph 1. Oocytes in circumnuclear ring stage (OCR) and postovulatory follicle (POF) of Flemish Cap cod in july 1990.
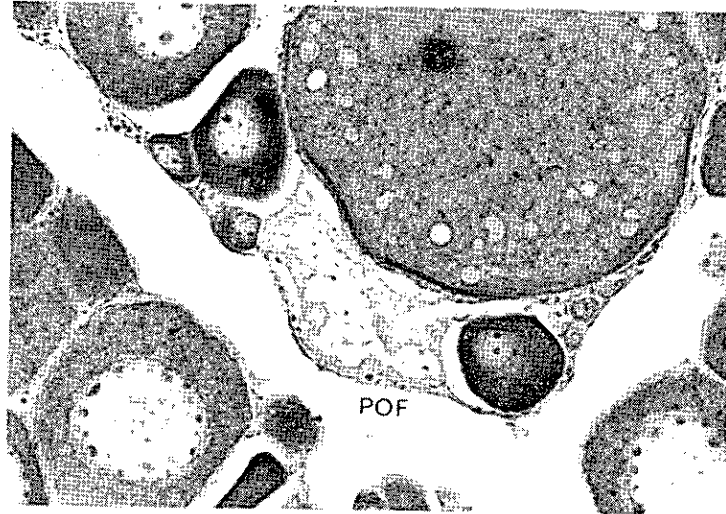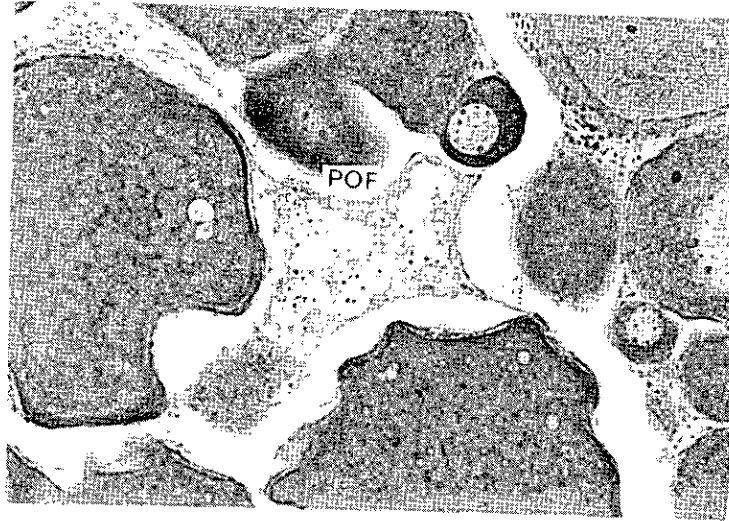

Photograph 2. Oocyte in cortical alveoli stage (OCA) of Flemich Cap cod in July 1990.
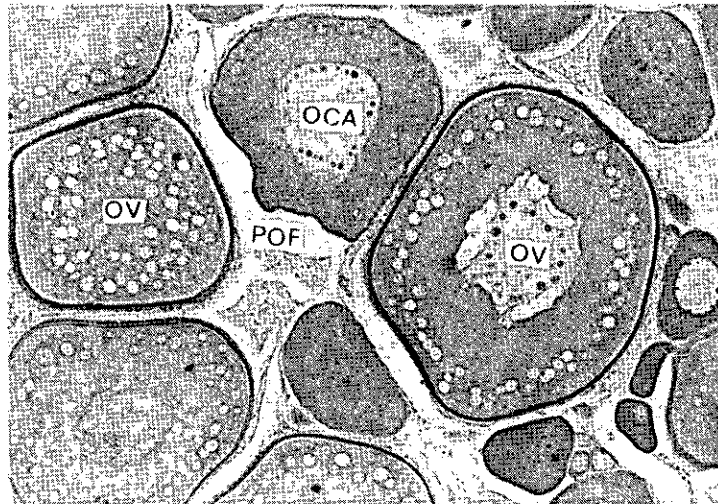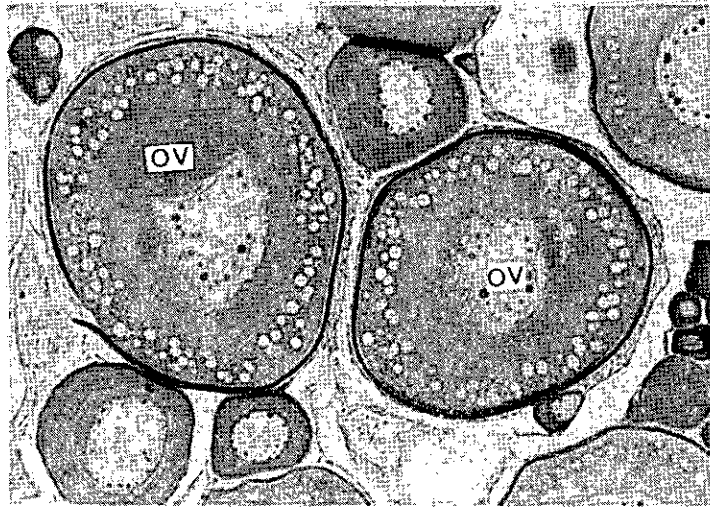
Photographs 3 and 4. Oocytes at beginning of the vitellogenesis (OV), oocyte in cortical alveoli stage and postovulatory follicles of Flemish Cap cod in July 1990.
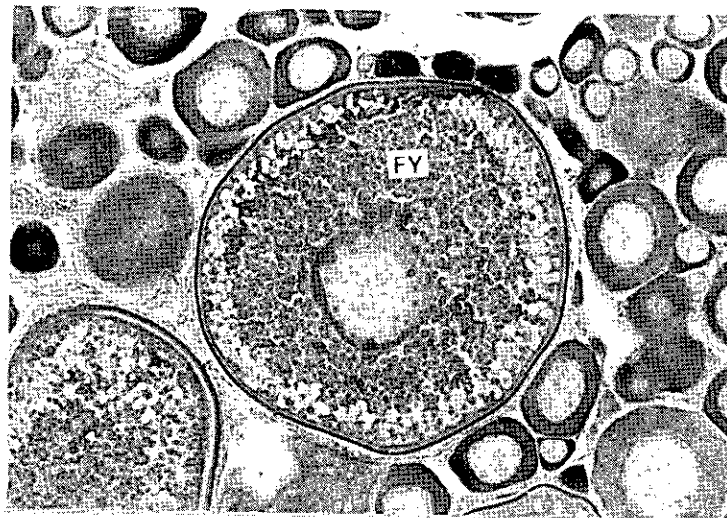
Photograph 5. Fully yolked oocyte (FY) of Flemish Cap cod in July 1990.

Photographs 6 and 7. Postovulatory follicles (POF) of Flemish cap Cod in July of 1990.
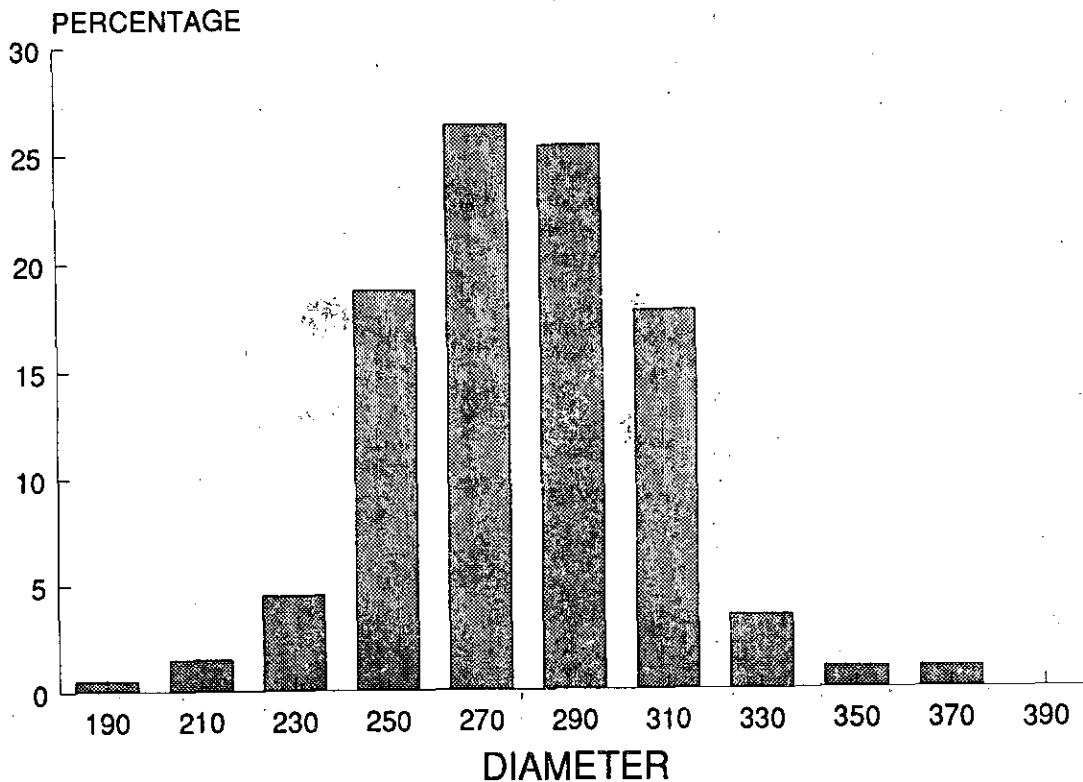
PERCENTAGE

Figure 1. Diameter distribution of oocytes in cortical alveoli stage of Flemish Cap cod in July 1990.
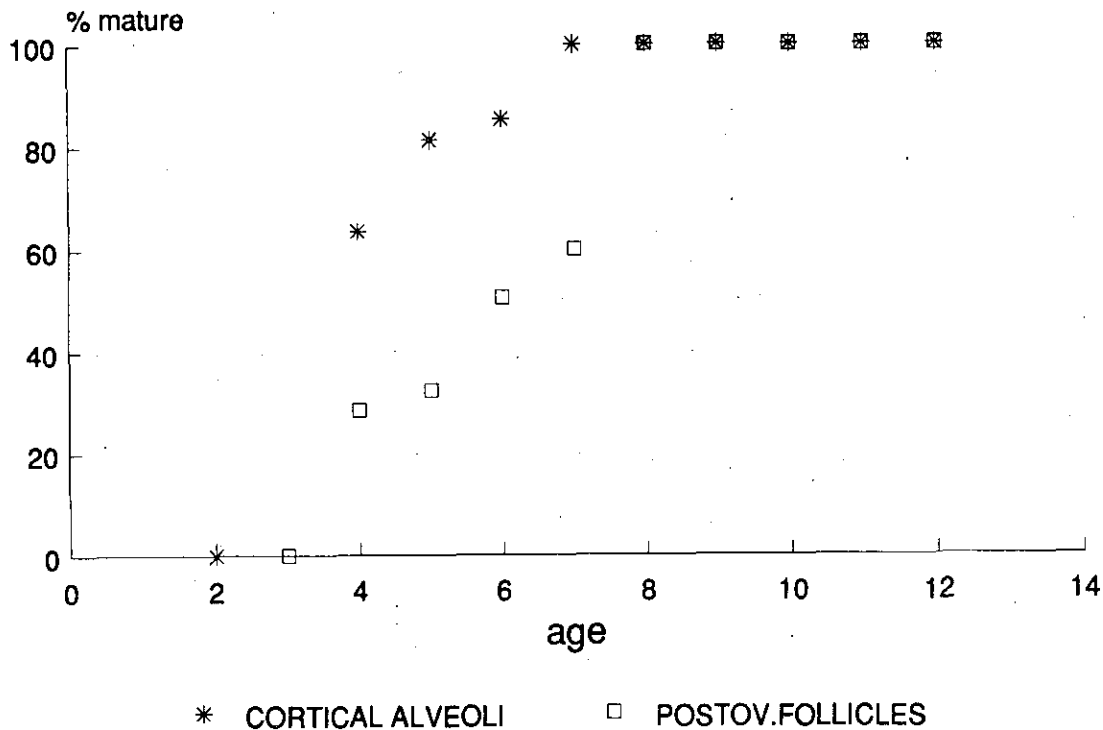

* CORTICAL ALVEOLI   □ POSTOV.FOLLICLES

Figure 2. Percentage by length of mature females (ovaries with oocytes in cortical alveoli stage) Flemish Cap cod in July 1990.
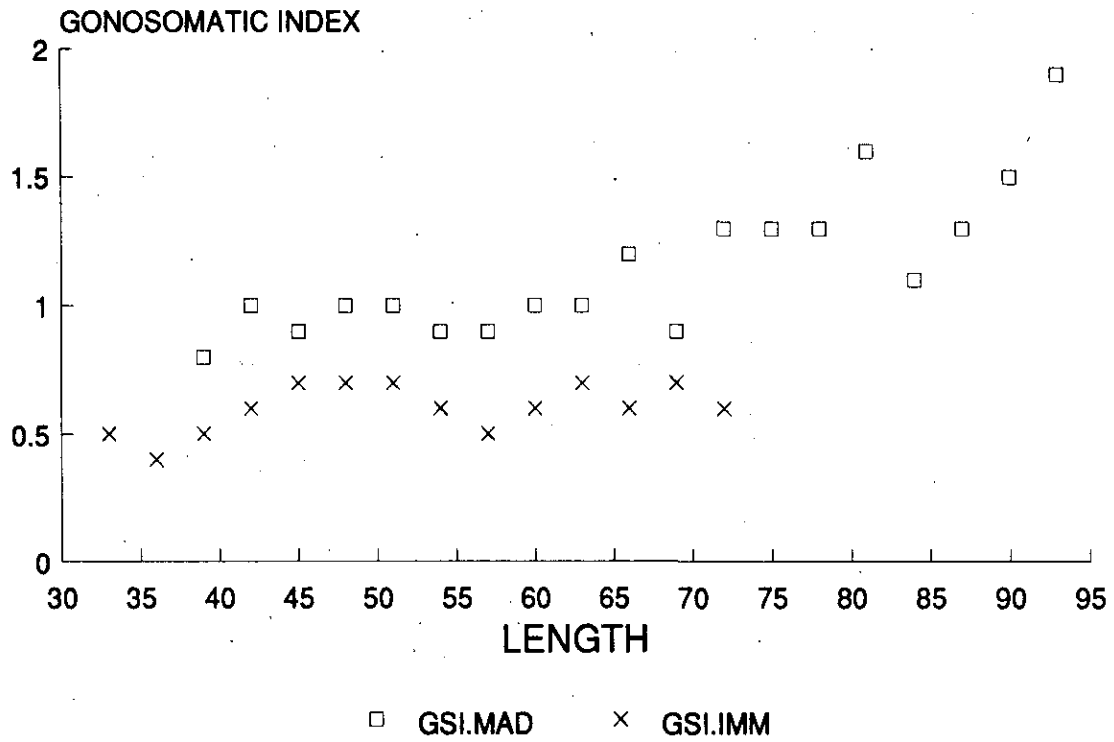
GONOSOMATIC INDEX

Figure 3. Mean gonosomatic index by length of mature and immature females of Flemish Cap cod in July 1990.