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Histological description of oocyte development in Atlantic Mackerel (*Scomber scombrus L.*) in the Gulf of St Lawrence

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by

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ABSTRACT

The reproductive stock size of the Gulf of St Lawrence mackerel is currently calculated using to the total egg production model. Female fecundity, one of the most important variables in this model, is determined as being the number of oocytes having a diameter greater than 140 µm just before spawning. This definition of fecundity is inadequate for indeterminate serial spawners such as anchovy and mackerel. The batch fecundity method involves defining the fecundity of these fishes by multiplying the number of oocytes expelled per batch by the number of batches spawned during the spawning season. To determine these variables, the different phases in the development of the oocyte must be examined and described. In the case of Gulf of St Lawrence mackerel, a histological study was carried out on gonads gathered during the 1991 spawning season. The histological structures identified are similar to those observed in European mackerel. Oocyte size frequency distributions are multimodal with overlapping modes, which is characteristic of indeterminate serial spawners. The simultaneous presence of hydrated oocytes and post-ovulatory follicles was noted. Since the presence of empty follicles is an indication that spawning has started, gonads having these two structures at the same time should not be included in the calculation of batch fecundity.

RÉSUMÉ

L'estimation de la taille du stock reproducteur de maquereau du golfe du Saint-Laurent se fait présentement à partir du modèle de la production totale d'oeufs. La fécondité des femelles, qui est une des variables importantes du modèle, est déterminée par le nombre d'ovocytes dont le diamètre est supérieur à 140 µm juste avant la fraie. Cette définition de la fécondité est inadéquate dans le cas des frayeurs multiples indéterminés comme l'anchois et le maquereau. La méthode de la fécondité en lots consiste à définir la fécondité de ces poissons en multipliant le nombre d'ovocytes expulsés à chaque ponte par le nombre de pontes effectuées au cours de la saison de fraie. Pour déterminer ces variables, la reconnaissance des différentes phases de développement des ovocytes est nécessaire. Dans le cas du maquereau du golfe du Saint-Laurent, un examen histologique a été effectué sur des gonades recueillies pendant la saison de fraie de 1991. Les structures histologiques identifiées se sont avérées identiques à celles observées chez le maquereau en Europe. Les distributions des fréquences de taille des ovocytes observées sont multimodales et se chevauchent, ce qui est caractéristiques aux frayeurs multiples indéterminés. La présence simultanée d'ovocytes hydratés et de follicules post-ovulatoires a aussi été notée. Puisque les follicules vides indiquent que la fraie est commencée, les gonades ayant au même moment la présence de ces deux structures ne devraient pas être incluses dans le calcul de la fécondité en lots.

INTRODUCTION

CALCULATION OF BIOMASS

The Atlantic mackerel (*Scomber scombrus L.*) population in the northwestern Atlantic is made up of two main groups or populations characterized by separate spawning areas. In the case of the northern, or Canadian, population, spawning generally takes place inside the Gulf of St Lawrence during June and July. The biomass of this group is currently estimated using a calculation of total annual egg production. This method has been preferred to sequential population analyses. These analyses, which are nevertheless the most commonly used in estimating fish stocks, fail to give satisfactory results for mackerel. Low mortality caused by fishing and the lack of a valid abundance index based on commercial catches render this type of analysis inadequate.

In 1897 Hensen and Apstein, quoted by Maguire (1981), were probably the first to suggest that the size of a stock might be calculated on the basis of its egg production. In a comparison of various fish stock calculation methods, Beverton and Holt (1956) state that estimating egg production is probably one of the best approaches to use because it is independent of any measurement of catches and fishing effort. Maguire (1979, 1981) was the first to use the calculation of egg production to assess mackerel in the Gulf of St Lawrence. This approach has, however, been criticized because of the sampling technique and type of gear used to gather base data (Ouellet 1987). These data, which came from an annual sampling program, were improved considerably beginning in 1979 by the adoption of a standard grid of stations, use of an oblique tow with a bongo net and a sampling period better synchronized with the maximum egg-laying period of mackerel. Based on the density of eggs collected between 1979 and 1986, Ouellet (1987) made some interesting modifications to the original method. For example, total daily egg production for the entire surface sampled is calculated using a stratified random sampling plan. This procedure is still in use and was recently re-examined (Grégoire 1992). Total daily production is combined with the seasonal production curve to determine total egg production. The ratio of this figure to the mean fecundity of females gives the number of females participating in spawning. Knowing the mean weight of these females and assuming the sex ratio to be equal, we can then assess the biomass.

ANNUAL OR POTENTIAL FECUNDITY

Because mackerel may release several successive batches of mature oocytes, Sette (1943) noted the importance of recognizing oocytes laid in the course of a year. In a study on the fecundity of mackerel in the southern, or American, population, Morse (1980) observed three types of oocytes. This author states that the presence of yolk in the oocyte is the criterion to use in determining whether or not it will be spawned. Since few oocytes with yolk are less than 250 µm in diameter, Morse considered that oocytes with diameters greater than this should be included in the fecundity calculation. This approach was used again by Maguire (1981) for the Canadian population. For the same population Laprise, quoted by Pelletier (1986), does however point out that the presence of cytoplasmic vacuoles and yolk in the oocyte is also a criterion confirming that the oocyte will be released. Vacuoles and yolk appear around 140 µm, a size

much smaller than that presented by Morse (1980) but closer to the values proposed in Europe. This value presented in Pelletier (1986) is still used in calculating the fecundity of the Canadian population.

The fecundity calculation as it is currently defined for mackerel fails to take into account possible oocyte losses through atresia in the course of the season. Annual fecundity is thus underestimated and represents instead potential fecundity. Some recent studies also point out that spawning in mackerel is indeterminate (Hunter and Macewicz 1985; Hunter *et al.* 1989; Priede 1990). The number of oocytes spawned cannot be determined before spawning because of the loss of oocytes caused by atresia or the subsequent gain caused by vitellogenesis. Since we do not know the proportions of oocytes linked to these two mechanisms and since the estimation of the biomass may be significantly affected, it seems essential to redefine fecundity in mackerel.

OBJECTIVE OF STUDY

The batch fecundity method was proposed to determine fecundity in indeterminate spawning fish. This method, which is currently being studied for use in evaluating the abundance of various species, was presented for the first time for anchovy, *Engraulis mordax*, on the U.S. west coast (Parker 1980). In addition to using only the daily egg production, this method, as opposed to the total egg production method, makes it possible to calculate confidence intervals for each biomass estimation.

Base calculations for a given species were based on the spawning frequency of females and the number of oocytes released each time. These two variables are determined by identification of certain histological criteria by which oocytes may be classified into different phases of development. With a view to applying batch fecundity to the Canadian population of mackerel, the primary objective of this report was to describe and compare the histological development of the oocytes of this species, in particular using the criteria established by Priede (1990).

MATERIALS AND METHODS

COLLECTION OF SAMPLES

From June 18-25, 1991, mackerel samples were collected in Chaleur Bay (Figure 1) using drift gill nets with a 73 mm mesh. At the time of capture, the sex of all fish was determined. The maturity stages of mackerel gonads were adapted from the scale proposed by Parrish and Saville (1965) for herring, *Clupea harengus harengus L.* Female Stage 3-6 gonads were placed at the time of sampling in a buffered 10% formaldehyde solution (Hunter 1985). These stages correspond to maturing gonads or those about to become mature. The respective number per maturity stage was 120, 36, 296 and 10. After a few months, the fish were thawed, measured and weighed (Table 1). The weight of frozen gonads and gonads kept in the formaldehyde solution were also recorded.

HISTOLOGICAL PREPARATION

A slice approximately 5 mm thick was taken from all Stage 4 and 6 gonads, 30 Stage 3 gonads and 50 Stage 5 gonads. The slices were taken from the central part of one of the two gonads and were placed in embedding cassettes and then dehydrated, cleared and impregnated with paraffin. Three sections 5-6 µm thick were taken from each block of paraffin. The sections were then stained with Harris haematoxylin then with Eosin Y. A few drops of acetic acid were added to increase the precision of the nucleus colouring (Luna 1968) and to obtain better solution conservation (Gabe 1968).

The various histological structures, as well as the main oocyte development stages were identified and compared to the observations presented by Hunter and Macewicz (1985), Isaac-Nahum *et al.* (1988), Morrison (1990) and Priede (1990). With a view to determining the relative proportions of different oocytes, a count of at least 100 oocytes per slide was taken on 30, 36, 50 and 10 slides corresponding to Maturity Stage 3 to 6 gonads. The diameter of oocytes from development phases 1 to 5 was taken on 50 slides selected at random during the sampling period. Measurements were taken using a 63X micrometer eyepiece unit (epu) at ten different locations on each slide. With the exception of Phase 5, when oocytes are hydrated and nucleus-free, only those oocytes that were relatively circular in form with a visible nucleus were selected. The longest diameter measurement was taken through the nucleus. As well, oocytes damaged by external factors, for example tears caused by the microtome blade, were not considered. The mean sizes of oocytes were compared using an ANOVA and the TUKEY test (Kirk 1982). Photographs were taken with a Leitz Aristoplan camera using Kodacolor 200 ASA film.

RESULTS

Phase 1 oocytes can be easily identified due to their small size and triangular or hexagonal shape (Appendix). They are wine-red in colour and the nucleus occupies a major part of the cytoplasm (Plate 1, figure 1). The second phase of development is characterized by the appearance of small vesicles first on the periphery of the cytoplasm then towards the centre of the oocyte (Plate 1, figures 1 and 2). At this phase, yolk granules may occasionally appear at various points in the cytoplasm. These granules are wine-red and their more marked appearance characterizes Phase 3 (Plate 1, figure 2; Plate 2, figure 3). In the course of Phase 4, the nucleus migrates towards the animal pole. Lipid droplets form around the nucleus and fuse (Plate 2, figures 3 and 4). The yolk granules expand and push the vesicles towards the periphery of the cytoplasm (Plate 2, figure 3). The next phase, the hydration phase, occurs when the yolk granules break (Plate 2, figure 4; Plate 3, figures 5 and 6). The same is true of the nucleus which, once it arrives at its site, releases its contents into the cytoplasm (Plate 3, figure 5). The hydrated oocytes are expelled during spawning and Phase 6 is characterized by the presence of empty follicles (Plate 4, figure 7). These follicles indicate that spawning is underway or has just taken place. For various reasons, the development of the oocyte may be altered and degeneration may follow (Plate 4, figure 8). Oocytes which present this phenomenon are identified as atresia. The follicles were differentiated from atretic oocytes by the following criteria: 1) the follicle walls are thin because of stretching caused by hydration, 2) the colouring of the follicle is less

pronounced because it contains no cytoplasm residue and 3) there has not yet been any proliferation of granules in the follicle.

The relative proportions of the different oocytes remained basically the same whatever the gonad maturity stage (Table 2). Phase 1 oocytes were the most numerous. They were followed by phase 2, 3 and 4 oocytes, in that order. Hydrated oocytes and follicles were present from maturity Stage 3 but their respective representation still remained low. Atresia were also found throughout gonad development.

There was a gradual increase in the size of the oocyte throughout its development (Table 3). However, when we compared the mean size of each oocyte phase for the various gonad maturity stages, only the mean diameter of the hydrated oocyte proved to be significantly different (ANOVA, $F= 76.50$, $P < 0.0001$). Moreover, these mean diameters were all different from one another (TUKEY, $P < 0.05$). The distribution of oocyte size frequencies was multimodal (Figure 2). Each mode represents a different phase, and this type of distribution was present whatever the maturity stage of the gonads. This multimodality, along with the overlapping observed between modes, is characteristic of indeterminate serial spawners (Hickling and Rutenberg 1936; Hunter and Goldberg 1980).

DISCUSSION

Oocyte development in mackerel is an ongoing process. Oocytes of varying stages of maturity may be present at any given time. Due to the indeterminate nature of spawning, there is thus no justification for calculating fecundity by the number of oocytes larger than a specified diameter. Identification of the different histological criteria presented in particular by Priede (1990) for mackerel in the northeastern Atlantic, enabled us to recognize the same development phases in Gulf of St Lawrence mackerel oocytes. The hydrated oocytes will be used to determine batch fecundity and the post-ovulatory follicles as an indication that spawning has just taken place. Despite agreement in identification of oocyte development phases, differences were observed in the formation of yolk granules, which, as observed by Landry and McQuinn (1988) in herring, by Morrison (1990) in cod and by Priede (1990) for mackerel, occurs first at the periphery of the cytoplasm. In our case, the formation of granules initially appeared uniformly (Plate 1, figure 2). As well, granules were observed to appear at the same time as the separation of the *zona radiata* from its follicle. Phase 3 and not Phase 2 as suggested by Priede (1990) was attributed to this observation. On some occasions only small granules were observed in Phase 2.

Differences with Priede (1990) were also observed in the range of sizes of various oocytes. It is possible that the size of fish sampled is responsible for this. Different types of fishing gear were used at different times in the course of the spawning season. As well, the sampling period was much longer in the case of Priede (1990) and a greater area was covered. In addition to a possible relationship between fish size and that of eggs released, it would be interesting to see whether the size of hydrated oocytes, as well as their number, vary from one batch to another. Different conservation times may also explain the differences observed in the size of oocytes. In future studies, the possible effect of conservation on the weight of gonads and the diameter

of oocytes should be measured. For Phase 5 oocytes, the absence of a nucleus may be due to the more pronounced differences observed. In the case of these oocytes, however, it was not possible to ensure that the measurement was made through the centre of the oocyte.

This study shows that macroscopic examination is not sufficient to determine actual female gonad development in Atlantic mackerel. A new scale might be proposed with a view to reconciling macroscopic and microscopic criteria.

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PLATE 1. Unhydrated oocytes.

FIGURE 1. Phases 1 and 2, X 40.

1. Oocyte Phase 1.

   A- Nucleus

   B- Nucleoli

   C- Cytoplasm

2. Early Phase 2 oocyte.

   A- Nucleus

   B- Nucleoli

   D- Nucleus membrane

   E- Formation of vesicles (cortical alveoli)

   F- Formation of *zona radiata*

   G- Theca and *granulosa*

3. Advanced Phase 2 oocyte.

4. Squamous epithelium (covering the ovigerous fold)

   H- Germinal cells

FIGURE 2. Phases 2 and 3, X 40.

1. Early Phase 2 oocyte.

2. Advanced Phase 2 oocyte.

3. Early Phase 3 oocyte.

   A- Nucleus

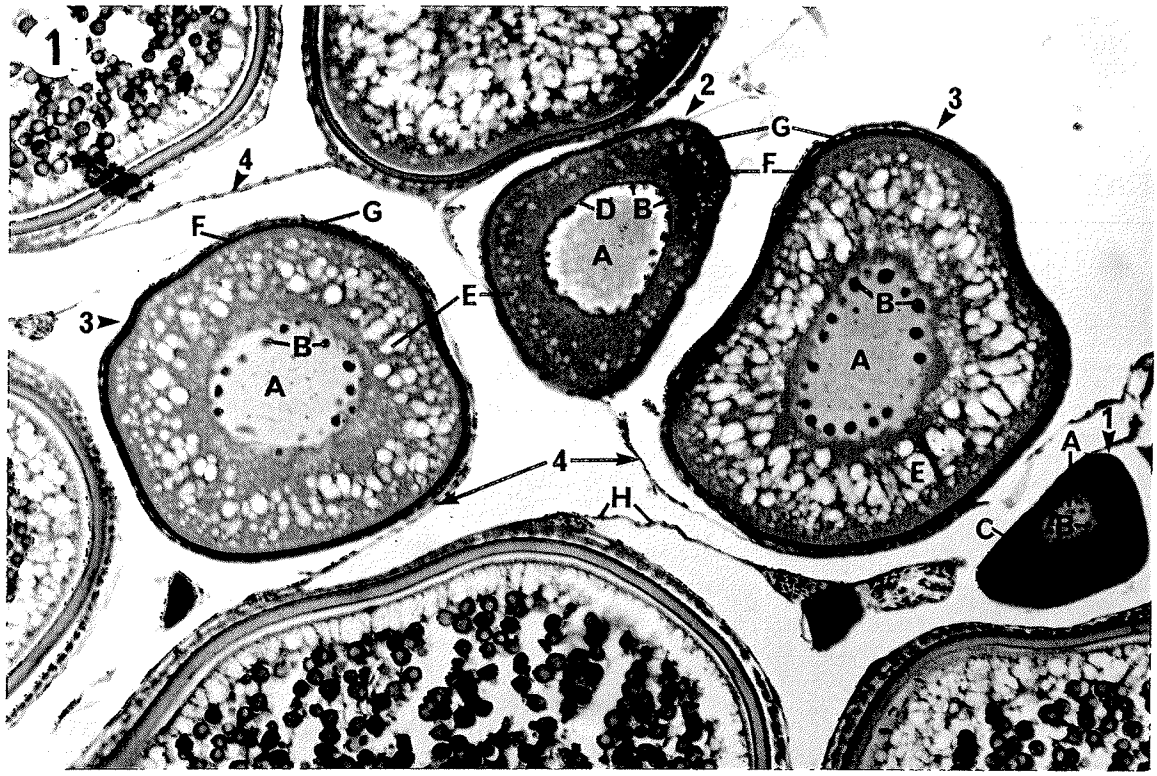
   B- Nucleoli

   C- Yolk granules

   D- Vesicles

   E- *Zona radiata*

   F- Theca and *granulosa*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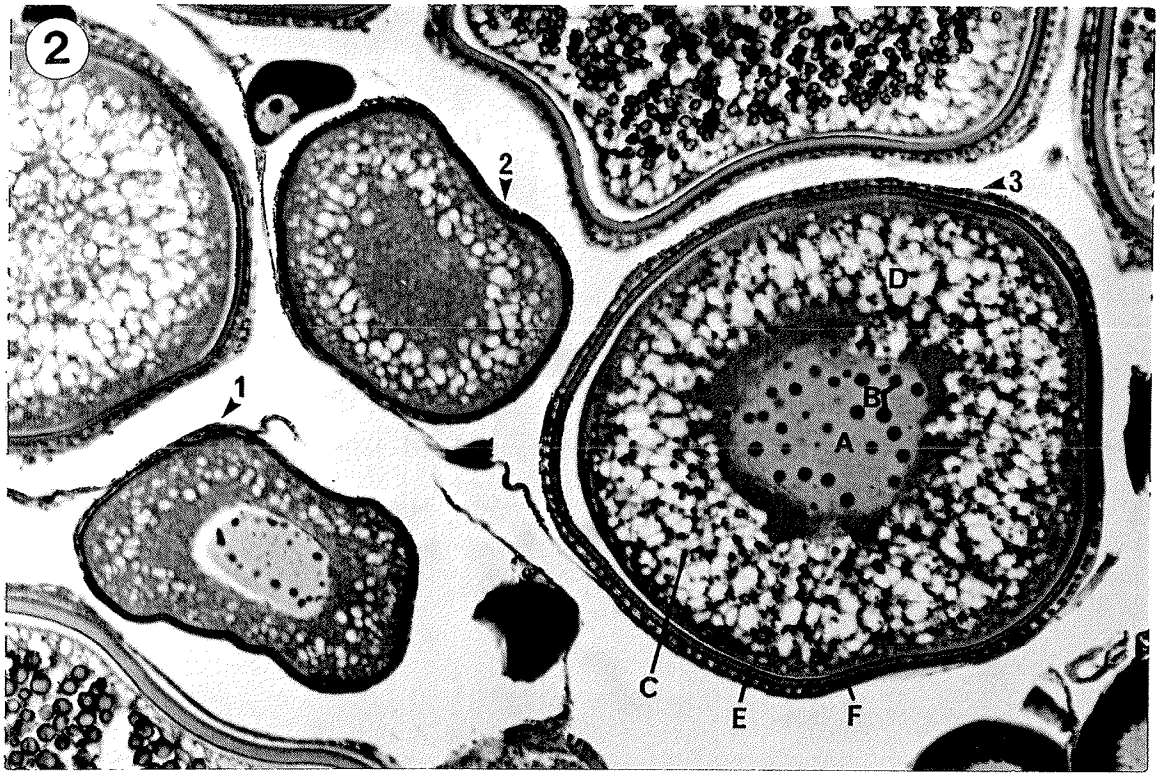
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PLATE 2. Unhydrated and hydrated oocytes

FIGURE 3. Phases 3 and 4, X 34.

1. Late Phase 3 oocyte.

2. Late Phase 4 oocyte.

A- Migrating nucleus

B- Nucleoli

C- Yolk granules

D- Lipid plates

E- Vesicles

F- Inner layer of *zona radiata*

G- Outer layer of *zona radiata*

H- Follicle

FIGURE 4. Phases 4 and 5, X 26.

1. Early Phase 4 oocyte

A- Nucleus

B- Nucleoli

C- Yolk granules

D- Lipid droplets

E- Vesicles

F- Inner layer of *zona radiata*

G- Outer layer of *zona radiata*

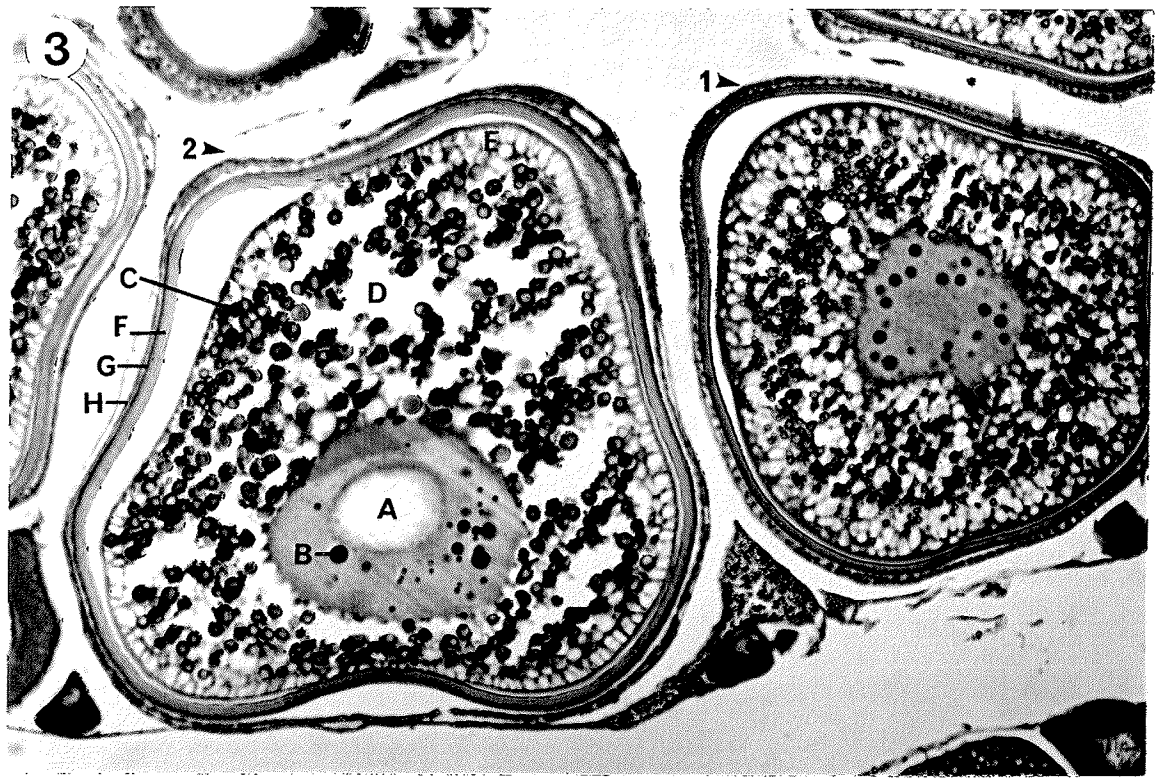
H- Follicle

2. Oocyte during hydration.

3. Ovary wall

I- Squamous epithelium

J- Smooth muscle



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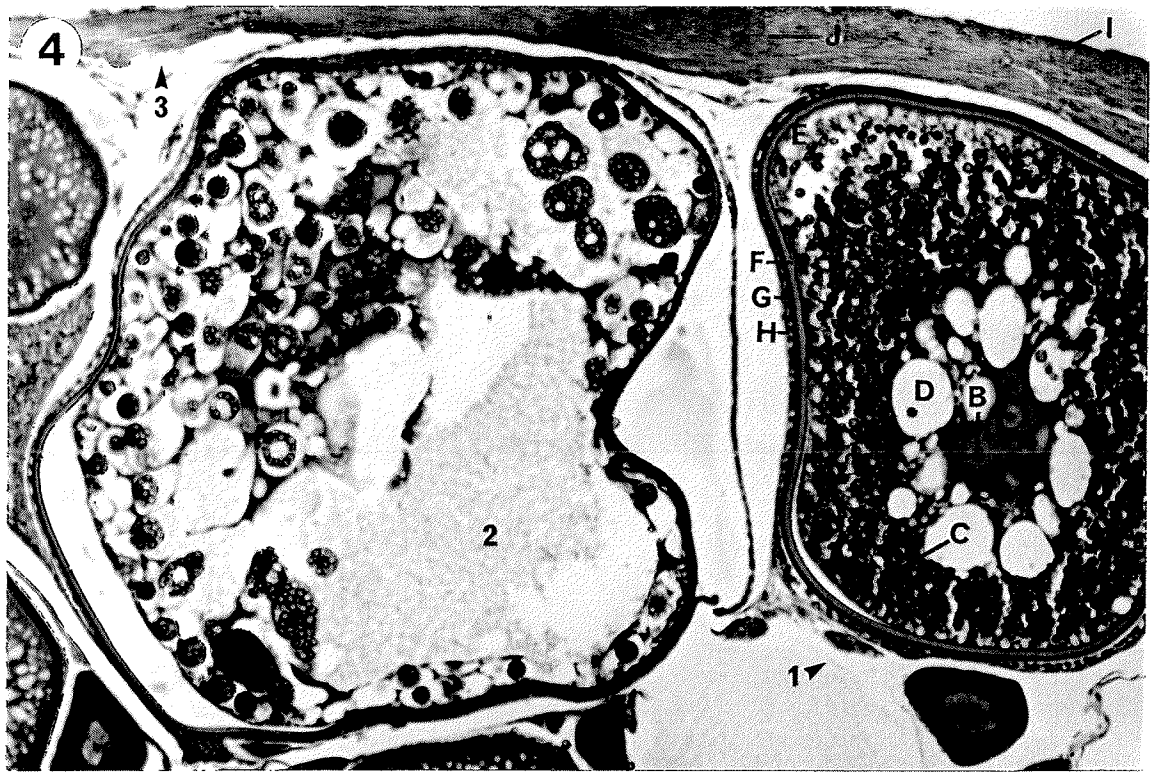

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PLATE 3. Hydrated oocytes.

FIGURE 5. Phase 5, X 27.

1. Oocyte at beginning of hydration.

A- Nucleus

B- Nucleoli

C- Lipid plates

D- Yolk granules

E- Yolk

F- Vesicles

G- *Zona radiata*

H- Follicle

2. Ovary wall.

I- Smooth muscle

J- Blood vessels

FIGURE 6. Phase 5, X 26.

1. Hydrated oocyte at end.

A- Hydration fluid

B- Vesicles

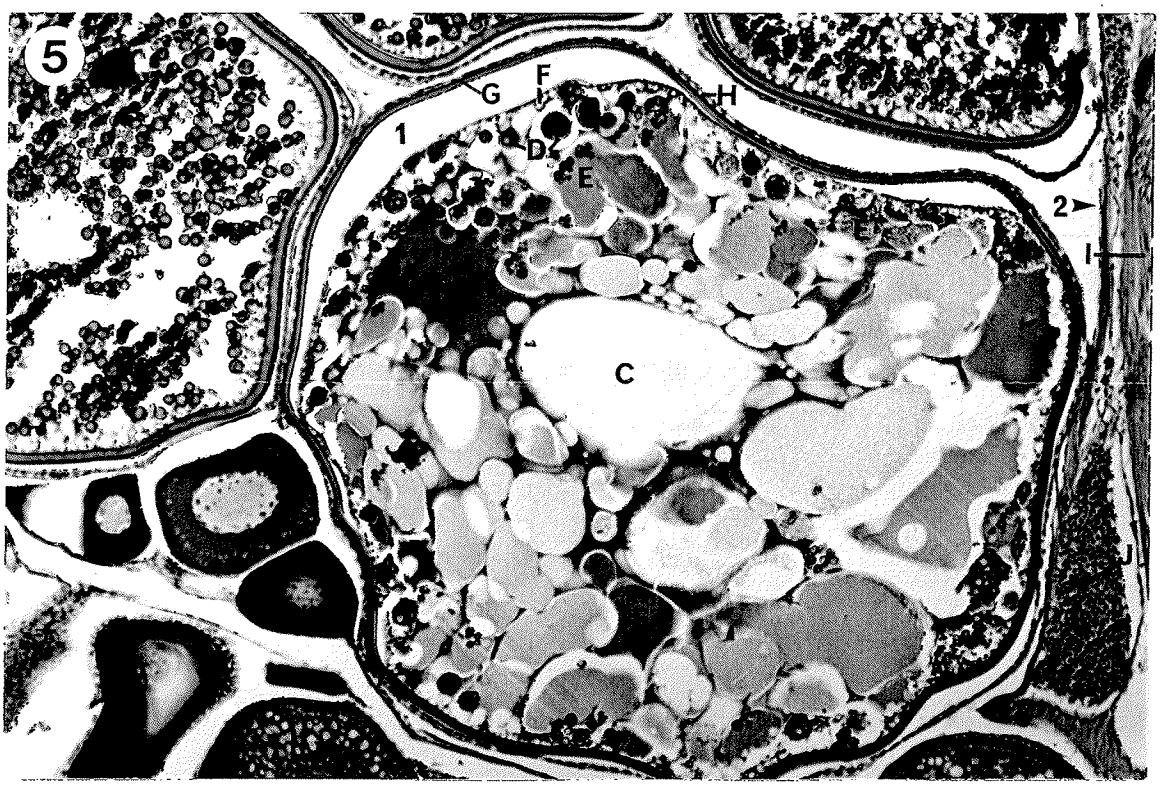
C- *Zona radiata*

D- Follicle

2. Extension of ovary wall.

E- Connective tissue

F- Blood vessels



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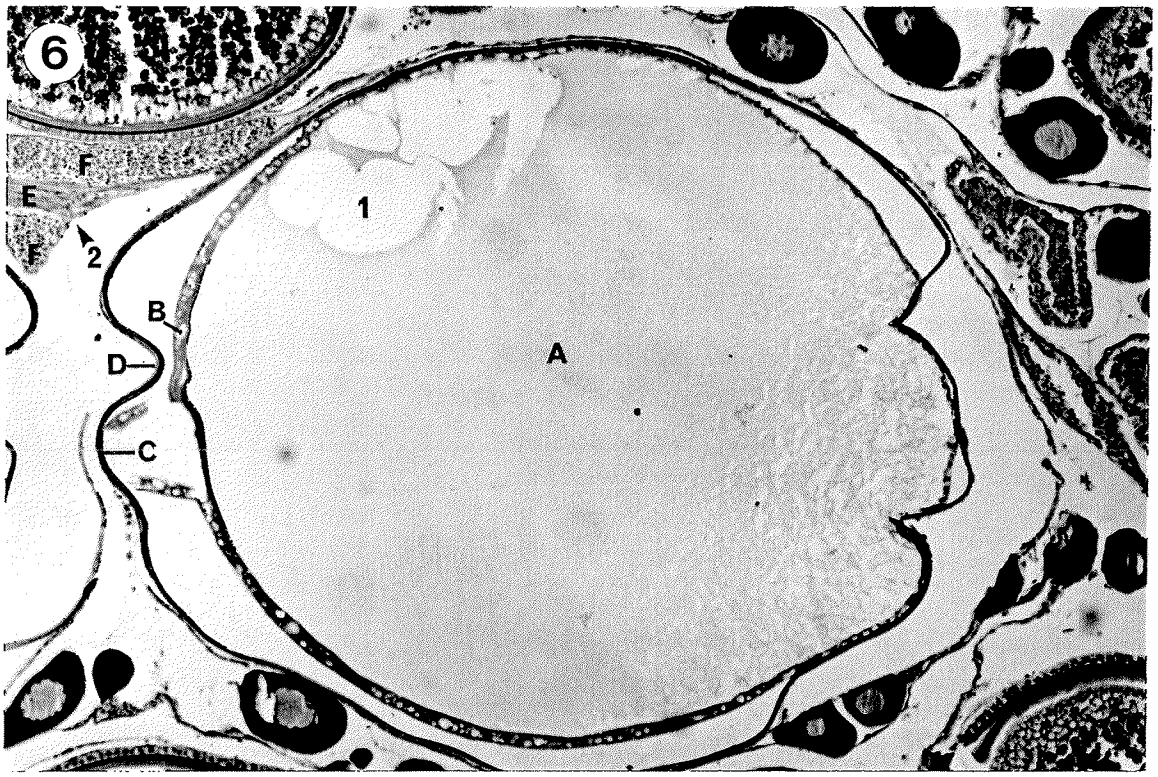


100 µm

PLATE 4. Post-ovulatory follicles and atresia.

FIGURE 7[1]. Phase 6, X 64.

1. Follicle.

A- Theca cells

B- *Granulosa* cells

C- Ovarian lumen

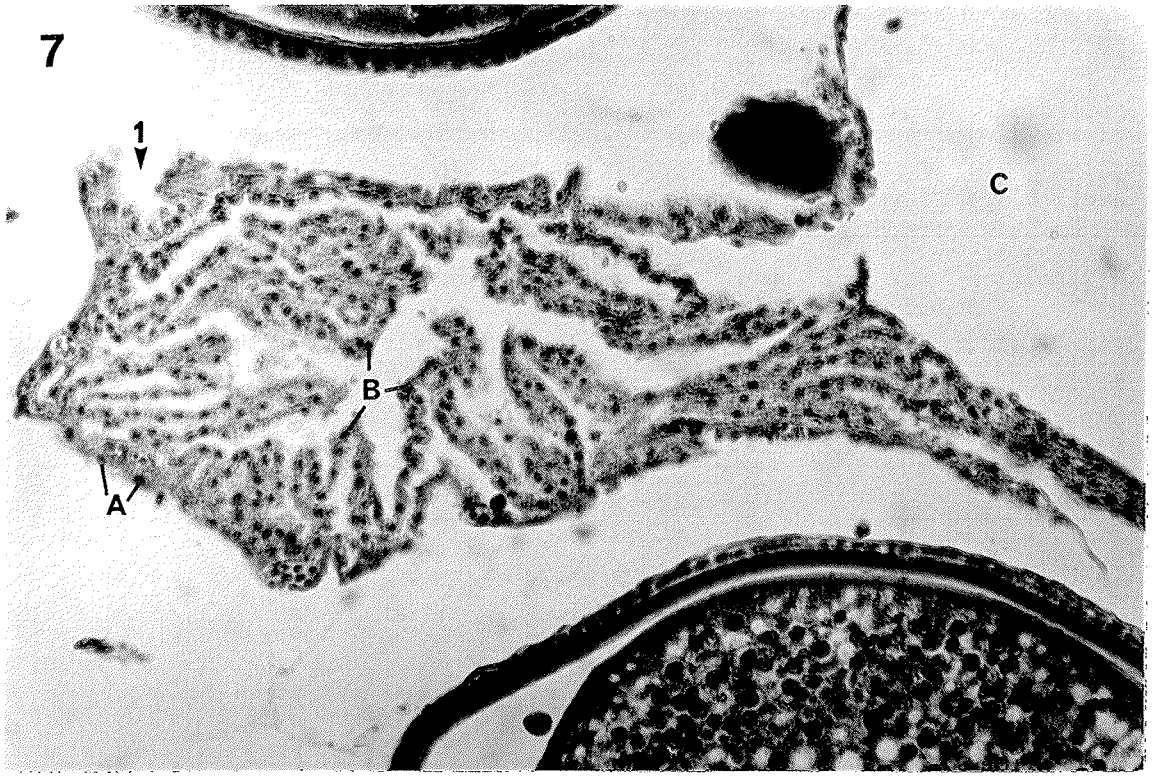
FIGURE 8. Phase 7, X 40.

1. Atretic oocyte.

A- Hydrated oocyte

B- Follicle folding

[1] The blue colouring of the follicle is due to photographic reproduction.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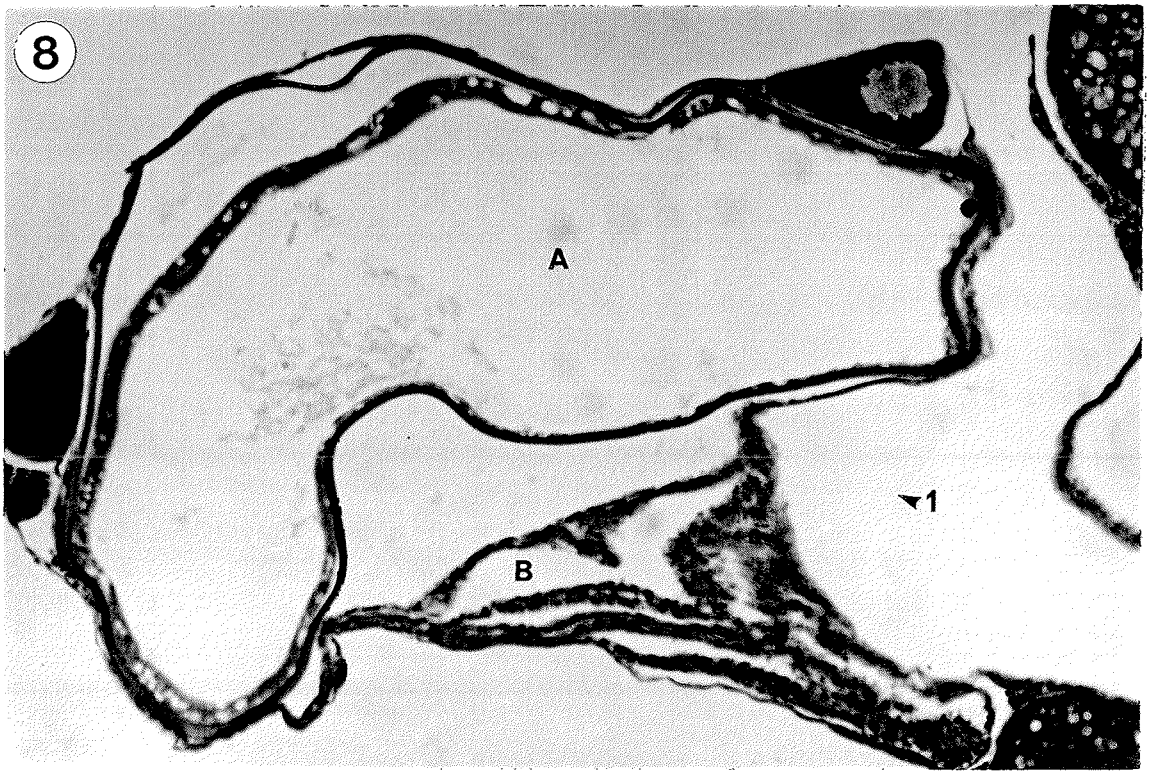
A
100 µm


8
A
1
B
100 µm

APPENDIX

DESCRIPTION OF OOCYTE MATURATION CYCLE

PHASE 1: **Previtellogenic.** Plate 1, Figure 1.

Oocyte wine-red, often triangular or hexagonal in shape. The nucleus, dark pink, occupies almost half the cytoplasm. Size at this phase does not exceed 175 µm (Table 3).

PHASE 2: **Appearance of vesicles.** Plate 1, Figures 1 and 2.

The dark colour of the cytoplasm and nucleus fades and small vesicles (known as cortical alveoli) appear at the periphery of the cytoplasm and larger ones near the nucleus.

The follicle begins to take shape, and granules can be seen. The early formation of the *zona radiata* (dark mauve) can also be observed.

The nucleoli move towards the periphery of the nucleus.

Occasionally, yolk granules will be seen to form throughout the cytoplasm. These oocytes measure 260 to 280 µm on average (Table 3).

PHASE 3: **Appearance of yolk granules.** Plate 1, Figure 2; Plate 2, Figure 3.

At this phase the yolk granules (wine-red) form, at first faint and uniformly distributed, then more pronounced and arranged concentrically in the cytoplasm.

At this phase, the *zona radiata* separates from the follicle. It takes on a pale pink colouring on the inner part and darker towards the outside. Lengthwise striations may be seen.

In this phase, the oocytes measure 350-625 µm (Table 3).

PHASE 4: **Migration of nucleus.** Plate 2, Figures 3 and 4.

The nucleus migrates towards the animal pole during this period.

This phase is characterized by the formation of lipid droplets around the nucleus which then fuse into a single plate.

The yolk granules also expand and push the vesicles closer to the periphery of the cytoplasm.

Oocytes measure an average of 630 to 670 µm (Table 3).

PHASE 5: **Hydration.** Plate 2, Figure 4; Plate 3, Figures 5 and 6.

The yolk granules break, first at the opposite pole then gradually through the whole cytoplasm, thus yielding a uniform pinkish tinge at the end of hydration.

The nucleus reaches its destination and breaks, releasing its contents into the cytoplasm.

Because the oocyte continues to expand, the vesicular zone, the *zona radiata* and the follicle become much thinner. Striations in the central layer (*zona radiata*) disappear.

Oocytes measure between 588 and 1338 µm (Table 3).

PHASE 6: **Post-ovulatory follicle.** Plate 4, Figure 7.

When the follicle breaks at a certain point and expels the ovule into the lumen of the ovary, it then contracts into two distinct parts; the *granulosa* convolutes towards the inside and the theca folds in over it.

PHASE 7: **Atresia.** Plate 4, Figure 8.

Oocytes which, at whatever phase in their development, fail to reach maturity due to various factors (environmental, physiological or physical) or which are degenerating during the post-ovulatory phase are known as "atretic".

TABLE 1. General statistics concerning the biological variables gathered in 1991.

DATE	VARIABLE	STATISTIC				
		N	MINIMUM	MEAN	MAXIMUM	S.D.
18-06	Length	126	308.00	338.60	394.00	1.65
	Fish weight	134	260.30	378.50	624.00	6.54
	Gonad weight	131	10.59	39.95	79.14	1.36
	Gonado-somatic index	131	2.71	10.70	23.19	0.36
19-06	Length	59	312.00	340.24	397.00	2.28
	Fish weight	59	269.30	376.46	638.10	10.25
	Gonad weight	59	14.82	45.09	96.07	2.22
	Gonado-somatic index	59	4.27	12.23	26.01	0.60
20-06	Length	144	310.00	354.24	405.00	2.08
	Fish weight	146	272.50	443.33	665.20	8.09
	Gonad weight	144	4.71	32.72	89.48	1.42
	Gonado-somatic index	144	1.48	7.46	30.39	0.34
21-06	Length	291	309.00	333.22	400.00	1.01
	Fish weight	291	250.00	364.32	657.90	4.02
	Gonad weight	281	15.96	44.65	100.47	0.94
	Gonado-somatic index	281	4.34	12.55	28.85	0.30
22-06	Length	109	304.00	334.46	399.00	2.23
	Fish weight	109	229.20	346.07	637.80	8.60
	Gonad weight	105	0.49	30.81	94.52	1.75
	Gonado-somatic index	105	0.14	8.88	20.70	0.44
24-06	Length	24	320.00	344.92	395.00	4.90
	Fish weight	24	274.50	382.53	638.60	19.83
	Gonad weight	24	10.75	36.92	65.27	3.12
	Gonado-somatic index	24	2.80	10.10	20.98	0.97
25-06	Length	447	296.00	350.02	409.00	1.43
	Fish weight	451	240.90	417.10	844.30	6.19
	Gonad weight	451	2.05	25.45	94.89	0.72
	Gonado-somatic index	451	0.64	6.27	18.63	0.17

TABLE 2. Mean number (%) of oocytes of the development phases 1 to 7 for some gonads stages of maturity.

GONAD MATURITY	DEVELOPMENT PHASE OF OOCYTE	STATISTIC				
		N	MINIMUM	MEAN	MAXIMUM	S.D.
3	1	30	44.34	62.26	85.29	9.20
	2	30	3.43	12.38	21.36	4.55
	3	30	2.38	8.76	19.00	3.74
	4	30	0.93	6.81	13.70	2.95
	5	30	0.00	1.11	4.63	1.46
	6	30	0.00	0.50	1.96	0.68
	7	30	3.67	8.19	15.09	2.65
4	1	36	37.76	56.49	73.51	8.32
	2	36	5.04	13.12	25.77	5.08
	3	36	5.61	10.86	16.95	3.42
	4	36	2.70	9.75	17.54	4.13
	5	36	0.00	2.10	11.21	2.84
	6	36	0.00	0.32	2.97	0.68
	7	36	2.63	7.36	15.13	2.66
5	1	50	42.76	58.49	78.31	9.75
	2	50	2.40	11.11	24.14	5.14
	3	50	0.00	8.12	17.91	3.50
	4	50	0.62	7.86	24.81	5.31
	5	50	0.00	4.45	12.80	3.16
	6	50	0.00	1.73	5.88	1.60
	7	50	1.85	8.23	21.12	4.02
6	1	10	47.93	58.62	77.42	10.10
	2	10	4.03	13.80	24.46	6.14
	3	10	5.77	9.21	11.57	1.83
	4	10	2.42	6.48	10.34	2.42
	5	10	0.00	1.58	5.17	1.85
	6	10	0.00	1.16	3.05	1.26
	7	10	4.48	9.14	14.50	3.21

TABLE 3. Oocyte diameter (µm) statistics for some gonads stages of maturity.

GONAD MATURITY	DEVELOPMENT PHASE OF OOCYTE	N	MEAN	S.D.	RANGE[1]
3	1	180	101.68	33.19	22.50 - 175.00
	2	180	259.23	62.89	137.50 - 438.80
	3	180	435.22	50.08	325.00 - 625.00
	4	180	629.27	75.90	400.00 - 862.50
	5	60	731.69	62.29	600.00 - 856.30
4	1	40	94.90	33.50	25.00 - 162.50
	2	40	278.61	58.57	156.30 - 385.00
	3	40	430.48	35.60	368.80 - 512.50
	4	40	670.26	84.77	537.50 - 843.80
	5	30	885.63	144.87	612.50 - 1150.00
5	1	180	85.03	33.51	22.50 - 162.50
	2	180	266.93	73.20	87.50 - 456.30
	3	180	435.27	50.45	306.30 - 612.50
	4	180	637.40	66.40	481.30 - 837.50
	5	180	950.88	128.76	587.50 - 1337.50
6	1	100	92.98	31.90	31.30 - 162.50
	2	100	264.79	74.15	80.30 - 431.30
	3	100	446.10	51.83	350.00 - 625.00
	4	100	651.61	59.59	516.30 - 806.30
	5	82	1 009.41	94.76	706.30 - 1241.30

[1] According to Priede (1990) : Phases range is
1 = 20 -170
2 = 170- 300
3 = 400- 500
4 = 600- 900
5 = 1000-1500

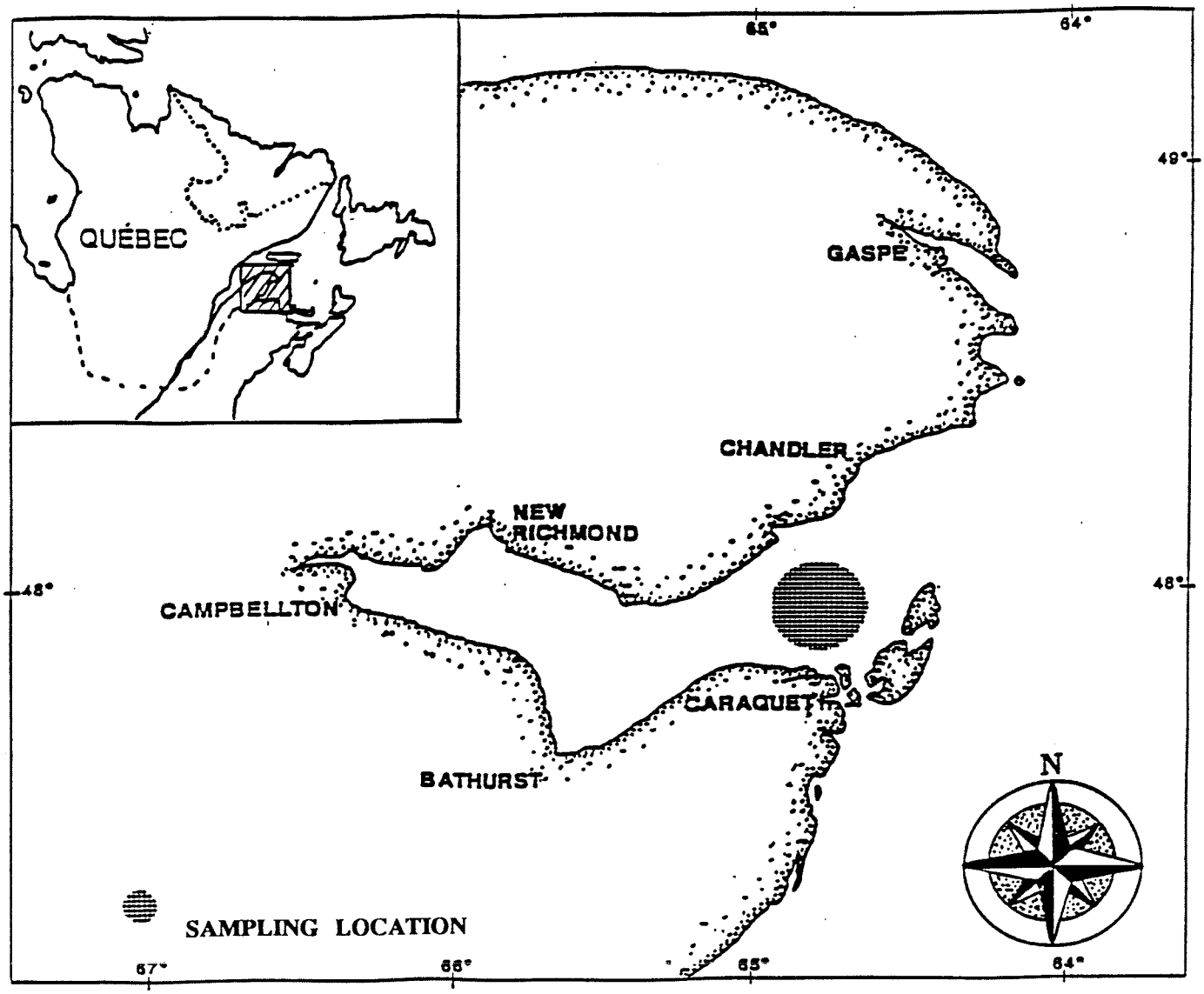


Figure 1. Sampling location of the mackerel gonads in 1991.

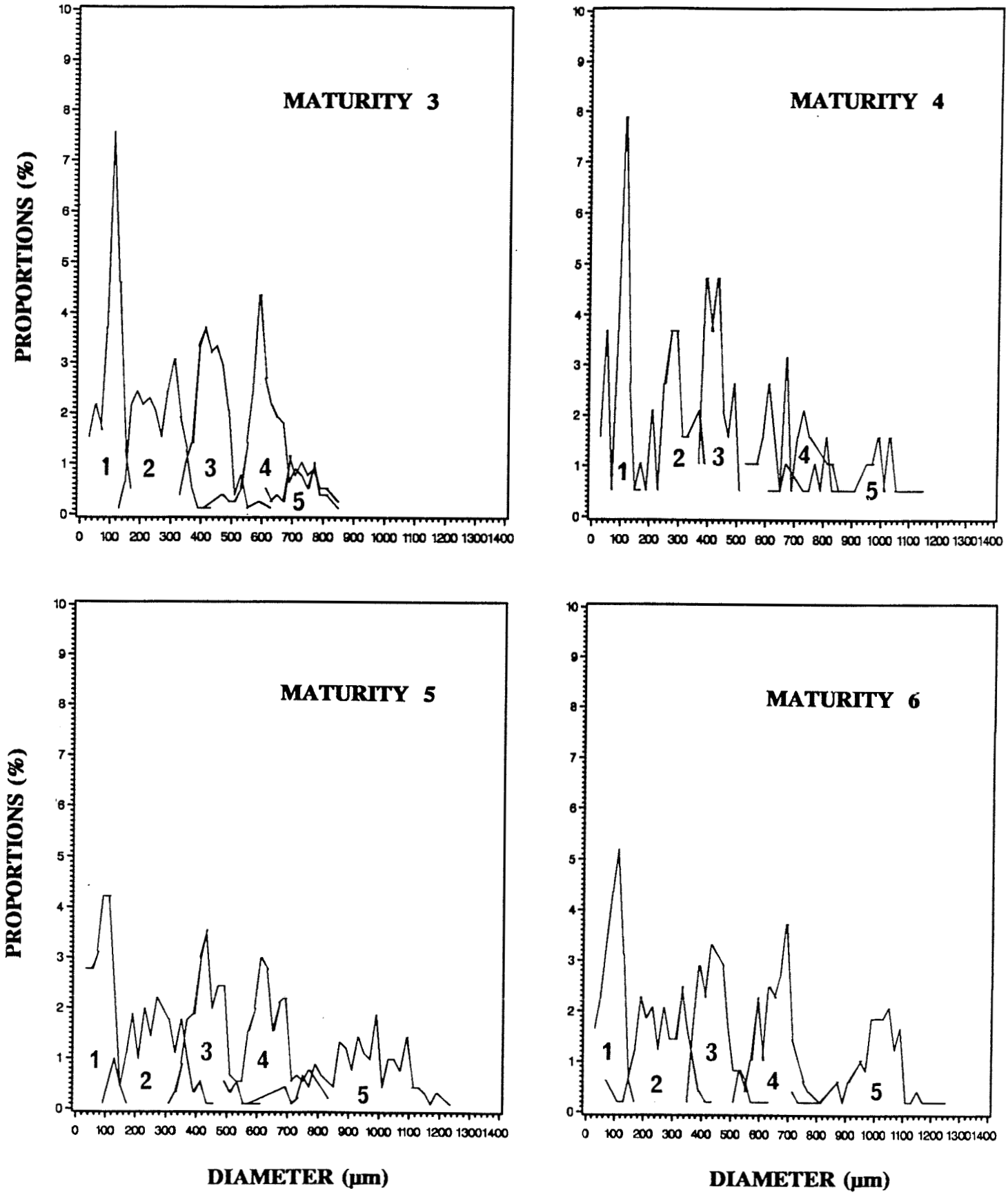


Figure 2. Frequency distribution of the oocyte diameter (µm) of phases 1 to 5 for different stages of gonad maturity.