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Histological and Visual Observations on Oogenesis and Sexual Maturity of Flemish Cap Female Cod

by

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INTRODUCTION

Wells (MS 1979) reported on observations of sexual maturity in female cod on the Flemish Cap during pre-spawning season (January-February 1979). Thirty-five percent of mature females sampled were classified as having spawned in the previous year (spent L) but were not showing signs of development necessary for the upcoming spring spawning session. This category of fish was found in the length range 49-88 cm with major concentrations in the 60-70 cm length range (see Table 9; Wells 1979). The effect of this non-spawning category of mature fish, also present in the 1978 sample, on the observations used in the calculation of a spawning ogive was to produce a bimodal series. The accuracy of determining maturity stages of fish at sea is essential to understanding the reproductive cycle and the onset of maturity and spawning in cod. If the accuracy of visual assessment of mature female cod designated as "spent in the previous year" was correct then a large segment of the mature stock would not spawn in 1979.

A study was undertaken to compare accuracy of maturity determination for female cod. Four methods are commonly used to determine reproductive state of fish: 1) staging of ovaries by gross anatomical features (visual analysis); (2) calculation of a gonosomatic index; (3) estimating mean diameter of eggs in the advance stage of development; and 4) classifying ovaries by histological analysis of oocyte development. Of all these methods histological classification is considered superior (Hunter and Macewicz, 1985). Consequently results of visual analysis were compared to results obtained from analysis of histological sections of ovaries using the descriptions of Sorokin (1957).

The intention of this study was to: 1) test accuracy of assigning maturity stages of female cod at sea; 2) to elucidate the problem of female fish that are apparently failing to mature for spawning in the year of observation; and 3) to increase understanding of events that constitute the reproductive cycle of cod prior to spawning.

MATERIALS AND METHODS

A total of 269 ovaries was collected from female cod in the length range 30-91 cm during the years 1981 (109 ovaries), 1982 (36 ovaries), 1983 (60 ovaries), and 1985 (64 ovaries). Samples were collected during January and February bottom trawl surveys of the Flemish Cap by the GADUS ATLANTICA. A maximum of 5 gonads was collected per 3 cm grouping. Additional samples of ovaries that were doubtful i.e. spent L or immature were included in the spent L gonad samples. Length measurements were recorded as fork lengths to the nearest cm and otoliths were removed for ageing. The ovaries were assigned a maturity stage according to gross anatomical features, following Templeman et al.'s (1978) classification for haddock, and then preserved in Bouin's fluid.

In the laboratory tissues excised from the anterior lobe of the gonad were dehydrated, embedded in wax, and stained with Harris haematoxylin and eosin and a variant of Mallory's triple stain. In 1985 a sample of fish was iced at sea and 35 mm pictures were taken of the whole gonad representing each visual stage of maturity.

Measurements of oocyte diameters and ovarian wall transverse thickness, both in microns (u), were made at twenty different sites using a calibrated eye piece micrometer following the

procedure outlined by Htun-Han (1978). Oocytes were divided into groups by size and appearance. Mean ovarian wall thickness of immature, mature, and spent in the previous year fish were compared using analysis of variance and Sheffe's multiple range test (= .05).

RESULTS

Histological stages of maturity were assigned to each ovary according to Sorokin's (1957) description of cod in the Barents Sea (Table 1). By visual examination at sea, 57.99% (156 ovaries) were classified as immature, 23.05% (62 ovaries) were mature, and 18.96% (51 ovaries) were designated as spent in the previous year. Histologically 96% (149 ovaries) of visually immature fish were designated as correct (Stage II) while the remaining 4% (7 ovaries) were maturing (Early Stage III) (Table 2, Fig. 1A&B). There was total agreement between visual and histological assessed mature fish (62 ovaries) (Early and Late Stage III) (Table 2, Fig. 2A). Thirty-seven percent of ovaries designated visually as spent in the previous year were classified histologically as immature (Stage II) while the remainder (63%) were classified as maturing (Early Stage III) (Table 2).

Evidence of oocyte degeneration, referred to as atresia, in oocytes entering vacuolization phase (endogenous vitellogenesis) was seen in 37% (19 ovaries) of the 51 ovaries designated visually as spent in the previous year. These fish were entering Early Stage III of histological maturity (Fig. 2B). No evidence of atresia was seen in the other ovaries designated as spent in the previous year and these were either classified as Stage II or Early Stage III based on oocytes characteristic of these stages (Table 2).

Microscopically there was a significant difference in the mean thickness of the ovarian wall in the three visual designated maturity categories immature, mature, and spent in the previous year fish (p .05) (Table 3). This difference also existed in the mean ovarian wall thickness of histologically described ovaries: Stage I-II, Stage III-IV, and atretic ovaries (p .05) (Table 4).

Macroscopically gonads of fish designated as spent last year had distinguishing characteristics that separated them from immature and mature fish. Immature females had a bright pinkish color, a thin transparent glossy ovarian wall, no eggs visible and a compact appearance with length and width measurements of the gonad being approximately the same (Fig. 3A). Spent in the previous year females had a dull pink color with a grayish cast in some cases, a shrunken wrinkled ovarian wall without a glossy appearance, no eggs visible, and length of gonad was usually longer than width (and on the average slightly longer than immatures of comparable size), giving it a flaccid appearance (Fig. 3B). Mature females, on the other hand, had a pink to whitish color, a thin glossy ovarian wall and eggs visible to the naked eye (Fig. 3C).

SEXUAL MATURITY

Length maturity ogives were constructed for both visual and histological data for each year of sampling. In the visual data all fish designated as spent L were considered mature while in the histological data they were considered either immature (Stage II) or mature (Stage III), based on oocyte development. The curves, drawn by eye, showed good consistency in agreement of M50, length at which 50% of sample were mature (Fig. 4). M50 had increased over the time period indicating faster growing fish. Not enough data were available for construction of age-at-maturity ogives.

Female cod were sexually maturing at age 4 years in 1981, 1982, and 1985 but not till age 5 years in 1983. Mean length of sexually mature 4 year olds showed a gradual increase over time (Table 5). Absence of age 4 from the 1983 sample was probably due to no samples of gonads being taken in this age group. Spent L females first appears in age group 4 in all years except 1983 when they appeared in age 5 group (Table 5). The 1981 sample had a large range of ages with spent L ovaries appearing in all age groups and size ranges.

The occurrence of females designated Spent L was first noted in 1978. Analysis of fish samples taken at sea in the years 1978-85 showed that on the average, 1/3 of sexually matured females would miss spawning in those years (Table 6).

DISCUSSION

Accuracy of visual staging of female cod at sea based on gross anatomical characteristics was extremely high when contrasted with histological classification of immature and mature fish. This agreement is further seen in the construction of maturity ogives for the years 1981, 1982, and 1983. The 1985 sample had only 12 fish that were considered either mature (3) or spent in the previous years (spent L) (9) and 5 of these fish were histologically classified as mature. Agreement probably would be much closer with a larger sample size. All fish designated as having spawned in the previous year (spent L) are considered to be post-mature non-reproductive fish.

The occurrence of this maturation condition has been noted previously in other fish most notably: hake (Hickling 1930). American plaice (Bagenal 1957); Norway pout (Gokhale 1957); Greenland halibut (Federov 1971; Walsh and Bowering, 1981) and winter flounder (Burton and Idler, 1984). Saidapur (1978) reviewed much of the literature on follicular atresia in ovaries of non-mammalian vertebrates, but most of the work cited dealt with oocytes that had already completed vitellogenesis.

In this study atresia occurred at the onset of endogeneous vitellogeneous (vaculoziation stage - Early Stage III). It was not seen in all specimens classified as post-mature non-reproductive fish. The asynchronous nature of development of oocytes commences at the period of vitellogenesis, which brings about the non-simultaneous ripening of the oocytes destined to spawn (Sorokin 1957). This asynchronization is probably the reason why atresia was not seen in all post-mature non-reproductive females. Since there is no yolk to reabsorb phagocytosis of the cytoplasm of the oocyte by granulosa cells is more rapid. Hence not all fish would have shown signs of atresia and thus would be classified histologically as either immature (Stage II) or mature (Stage III) based on oocyte types only. It is believed that those categorized in Stage II had the process of atresia completed while for those categoried in Early Stage III the process had not fully begun. Research on winter flounder by Burton and Idler (1984) showed that measurements of the ovary wall was the best parameter to consider in their distinction of immature, mature and postmature non-reproductive fish. Visually and microscopically the ovarian wall of these fish is thicker than immature fish and thinner than mature fish and this is an excellent characteristic to distinguish these fish in the field and in the laboratory.

The failure of gonads to mature could be due to a pathological condition, a life-cycle adaption to a limited feeding season, or finally a feature of senescence (Burton and Idler, 1984). Senescence can probably be ruled out here since non-reproductive females were found in the lowest age category of mature cod. Atresia was not found in Sorokin's (1957) work on Barents Sea cod. Woodhead and Woodhead (1965) reported atretic eggs in the majority of cod examined in their samples from the Barent Sea. They reported that numbers of atretic eggs rarely formed more than one percent of ripening eggs in the ovary and didn't affect spawning. Templeman et al. (1978) reported the incidence of spent L in haddock sampled prior to spring spawning was rare. Federov (1971) reported that all instances of non annual spawning known to the present time may be divided into two large groups. Missed spawning due to effect of adverse environmental factors on the concluding processes of development of gametes resulted in mass resorption of mature sexual products. The second group comprises instances in which the sexual cycle of the gonads is extended to several years for some reason or another. Federov's (1971) samples of Greenland halibut in the Barents Sea which failed to spawn fell in the second category. He presented evidence to show that sexually mature females missing spawning were characterized by continuous elimination of oocytes entering the phase of trophoplasmic growth (vitellogenesis). Tyler and Dunn (1976), investigating the effect of food ration on laboratory-held winter flounder, suggested that in the face of food shortage, a flounder's adaptive strategy is to sacrifice egg production and maintain body size. Investigations on the Flemish Cap have shown no adverse environmental conditions occurring over the time period 1978-85 (S. Akenhead, Department of Fisheries and Oceans, Science Branch, P. O. Box 5667, St. John's, pers. comm.). The presence of these post-mature non-reproductive fish may be an adaptive strategy by cod experiencing food shortage with the result of energy used for maturation of the oocytes being diverted to maintenance of somatic tissue. The prey spectrum of cod on the Flemish Cap is relatively narrrow with juvenile redfish year-classes being a major prey item which along with juvenile cod, mcytophids, hyperiids and shrimp varies annually (Lilly, MS 1985). The increase in M50 point from the maturity ogives showed an increase in growth rates of females cod but the percentage of mature cod in the spawning stock had dropped considerably in the last 3 years (Fig. 4; Table 6). Overfishing of the stock could account for most of this drop but also the quantity and quality of food consumed by adult cod should be considered.

CONCLUSIONS

Detailed histological analysis of Flemish Cap female cod during prespawning season confirms Wells (MS 1979) hypothesis that fish designated in the maturation condition of spent in the previous year would not spawn in the upcoming spring spawning period. There is no evidence to support a fall spawning period on the Flemish Cap. During the 1978-85 period on the average 1/3 of the spawning population would not have spawned, seriously limiting the amount of viable eggs/larvae production that one might expect. The stock on the Flemish Cap shows a declining trend in the number of mature fish present in over all composition. The mechanism for reduction in the number of mature fish in the population is probably related to overfishing and poor recruitment while the exact mechanism for disruption in the spawning cycle of female cod can only be speculated as shortage of quantity and quality of food items.

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Table 1. Descriptive stages of maturity used for visual and histological analysis of female cod ovaries.

Visual- Templeman et al. 1978	Histological - Sorokin 1957
1. Immature: Ovary small, grey to pink in color; membrane thin and translucent; eggs not visible to naked eye.	1. Oocytes in protoplasmic growth stage with nest of oogonia present - beginning of minor growth.
2. Spent L: Ovary thick-walled with no new eggs visible to naked eye; spent in previous (L = last) year.	2. Oocytes in the monolayer follicular phase constitute greater mass of ovary along with a number of oocytes not having completed minor growth and also oocytes in the synoptic phase; ovarian wall thin (3 generations of oocytes.)
3. Maturing A-P: Eggs visible to naked eye in ovary itself, all eggs opaque; maturing to spawn in present (P) year.	3E. Early Stage III: Oocytes entering trophoplasmic (major) growth phase: oocytes at commencement of the phase of vacuolization and primary yolk accumulation (Vitellogenesis).
4. Maturing B-P: Opaque and clear eggs present with less than 50% of the volume being clear eggs; maturing to spawn in present (P) year.	3L. Late State III: Oocytes entering intensive trophoplasmic growth with heavy yolk deposition mixture of early and late oocytes and some Stage I and Stage II ovarian membrane thin.
5. Maturing C-P: 50% or more of the volume are clear eggs; this stage also includes the ripe condition where the ovarian content is almost liquid with clear eggs; to spawn or spawning in present (P) year.	4. Most developed oocytes are filled with yolk while others are in late Stage III, radial situations are seen in the chorion (Zona radiata). Stage II oocytes (monolayer follicular) which are the next generation are also present.
6. Partly Spent P: Ovary not full as in MAT C-P; some eggs extruded but many clear eggs remaining.	5. Flowing or shedding stage: Older oocytes have hydrated and the appearance of flowing sexual products is noted; shedding of eggs has begun; other oocytes still completing trophoplasmic growth.
7. Spent P: Spawning completed in present year but possibly a few clear eggs remaining; no new opaque eggs visible to the naked eye, ovarian wall slack, opaque, thick, and wrinkled.	6. Spent Stage: Ripened oocytes shed. The ovary has a soft, flabby appearance. The ovarian wall contracts, thickens, and becomes opaque.

Table 2. Comparison of visual assessed maturity stages with histological assessed maturity stages. All years combined. Female cod.

Visual maturity	No. of samples	Histological analysis Immature (Stage II)	Mature Stage III	No. of fish showing atresia
Immature	156 (58%)	149 (96%)	7 (4%)	0
Mature	62 (23%)		62 (100%)	0
Spent L	51 (19%)	19 (37%)	32 (63%)	19 (37%)

Table 3. Analysis of variance and Scheffe's multiple comparison of mean ovarian wall thickness of three visual assessed maturity stages. (α = 0.05). All years combined. Measurements are in microns (μ). * Indicates significance at .05 level.

Maturity stage	N.	Mean	Range Standard error	Min.	Max.	Significance value
Immature	156	59.48	0.5360	5.00	228.00	*
Spent Last Year	51	113.02	2.0930	12.00	560.00	*
Mature	62	213.51	3.770	14.00	790.00	*

Table 4. Analysis of variance and Scheffe's multiple comparison of mean ovarian thickness of three histological maturity stages (α = 0.05). All years combined. Measurements are in microns (μ). * Indicates significance at .05 level.

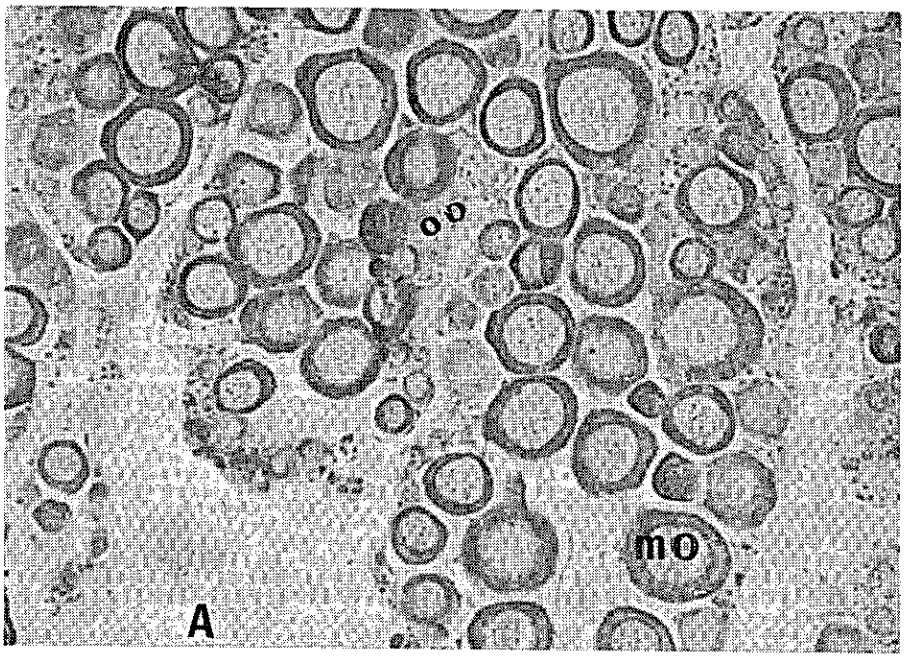
Maturity stage	N.	Mean	Range Standard error	Min.	Max.	Significance value
Immature (Stage I-II)	169	61.16	0.5421	5.00	246.00	*
Atretic Ovaries	19	145.61	3.9633	21.00	560.00	*
Mature (Stage III-IV)	81	186.86	3.1778	14.00	790.00	*

Table 5. Mean length-at-age for female cod by maturity stage for each year of the samples. (No age data for 3 fish out of 269 sampled).

1981 N	Mean length	Range	1982 N	Mean length	Range	1983 N	Mean length	Range	1985 N	Mean length	Range	Maturity condition	Age
												Immature	8
						1		70					7
1		63											6
1		47	2	62.50	62-63								5
24	47.50	39-55	13	50.77	42-56				45	47.89	32-67		4
17	35.71	32-43				29	45.86	30-56	7	34.00	30-38		3
			8	32.50	30-34	6	31.50	30-33					2
29	81.55	62-92	1		80							Mature	8
12	69.58	58-75										+ Spent L.	7
17	64.82	57-74				5	75.80	67-85					6
4	57.00	56-58	5	60.00	57-63	18	66.50	61-75	4	73.00	64-79		5
3	51.33	49-53	6	56.00	50-61				8	59.38	43-66		4
3	85.33	83-89										Spent L	8
3	70.00	66-74											7
9	66.56	57-74				1		67					6
2	57.00	56-58	2	57.50	57-58	12	65.75	61-70	2	78	77-79		5
3	51.33	49-53	6	56.00	50-61				7	61.71	57-66		4

Table 6. Incidence of Spent L from fish sampled at sea; 1978-85.

Maturity condition	1978	1979	1980	1981	1982	1983	1984	1985
Immature	5625(63%)	1122(70%)	1023(62%)	1754(75%)	340(72%)	280(92%)	1553(91%)	1911(92%)
Mature	1663(19%)	315(20%)	456(28%)	415(18%)	114(24%)	18(6%)	106(6%)	94(5%)
Spent L	1701(19%)	170(11%)	161(10%)	174(7%)	18(4%)	8(3%)	45(3%)	63(3%)
Total	8989	1607	1640	2343	472	306	1704	2068
% Mature Inc. Spent L	3364(37%)	485(30%)	617(38%)	589(25%)	132(28%)	26(8%)	151(9%)	157(8%)
% Mature fish Destined to spawn	1663(49%)	315(65%)	456(74%)	415(70%)	114(86%)	18(69%)	106(70%)	94(60%)
% Mature Non-Reproductive	51%	35%	26%	30%	14%	31%	30%	40%



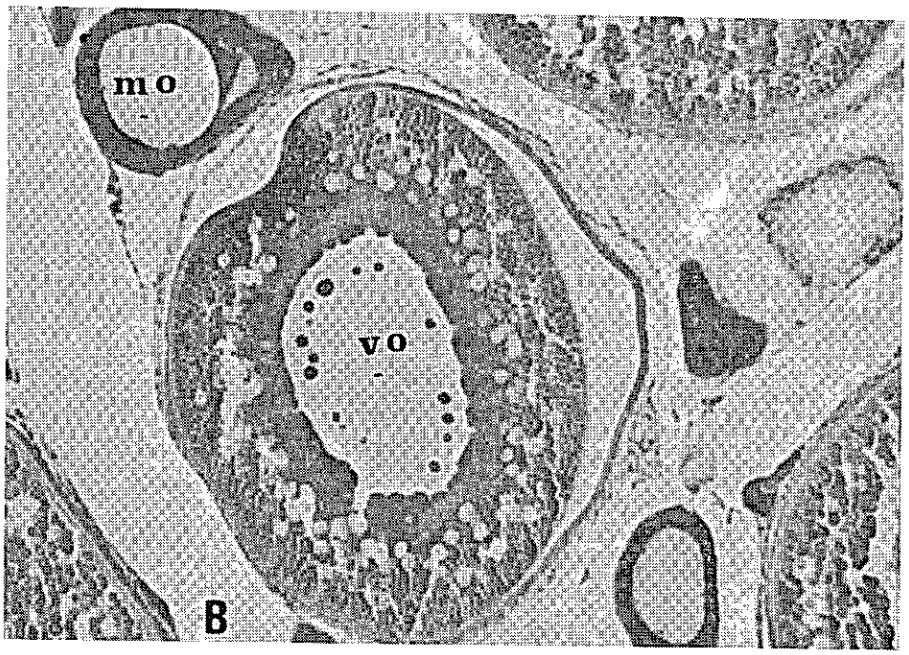
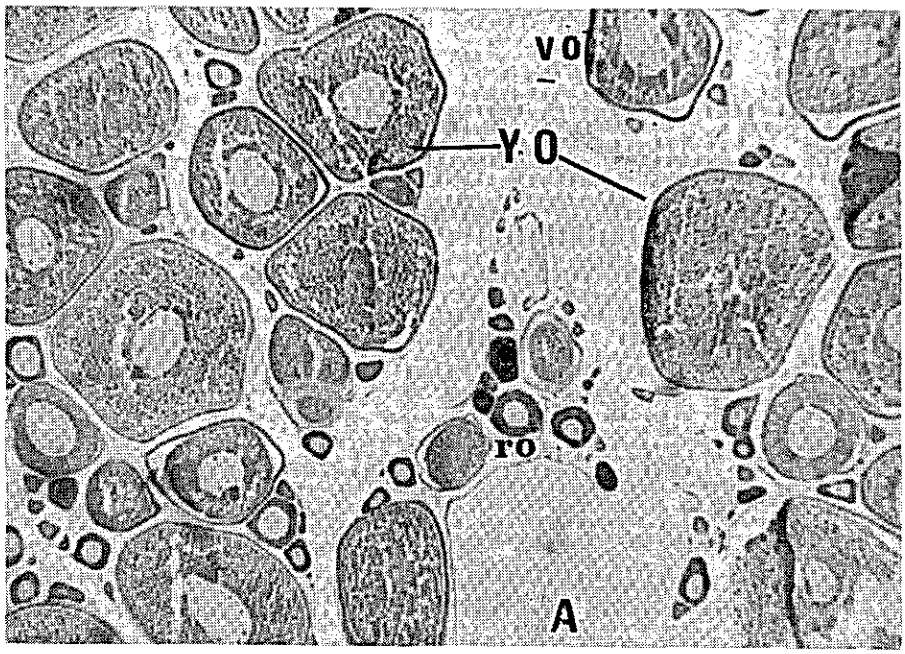


Fig. 1. Oocytes in monolayer follicular phase (mo) with oogonial nest present (oo). Typical of Stage II: Immature fish (34 cm), 200X magnification.

Fig. 1B. Oocyte entering period of trophoplasmic growth (endogeneous vitelogenesis) showing oocyte entering vacuolization and primary yolk accumulation (vo). Oocytes in monolayer follicular phase present (mo). Early Stage III: Maturing fish (75 cm), 200X magnification.



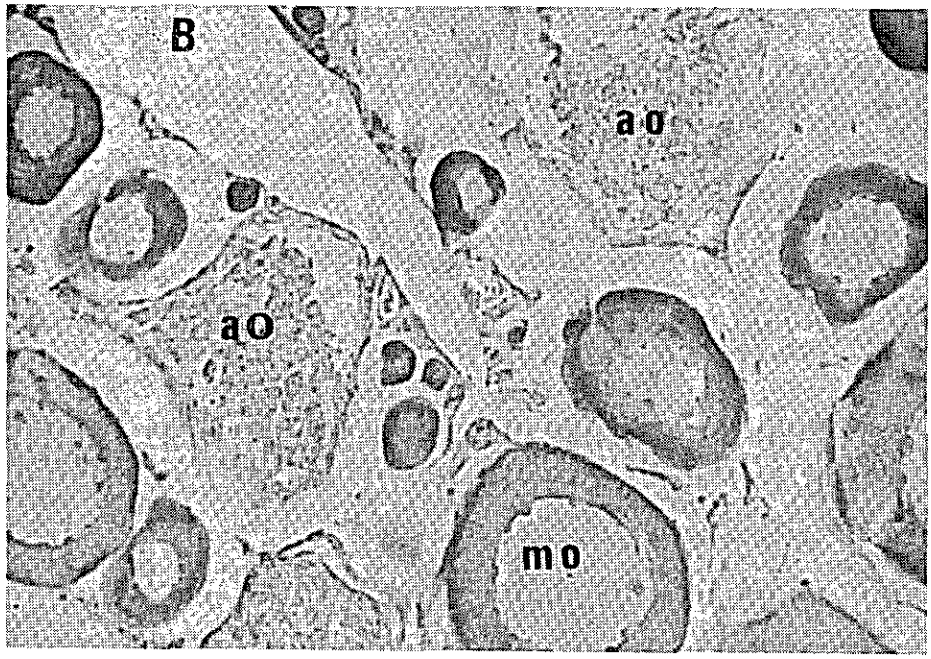


Fig. 2A. Oocytes entering intensive trophoplasmic growth with oocytes filling with yolk (yo). Oocytes of the vacuolization stage (vo) and resting oocytes (ro) of the next generation present. Late Stage III: Mature fish (63 cm), 250X magnification.

Fig 2B. Atretic oocytes (ao) undergoing degeneration. Monolayer follicular oocytes (mo) present. Early Stage III: Spent-L fish (83 cm), 200X magnification.

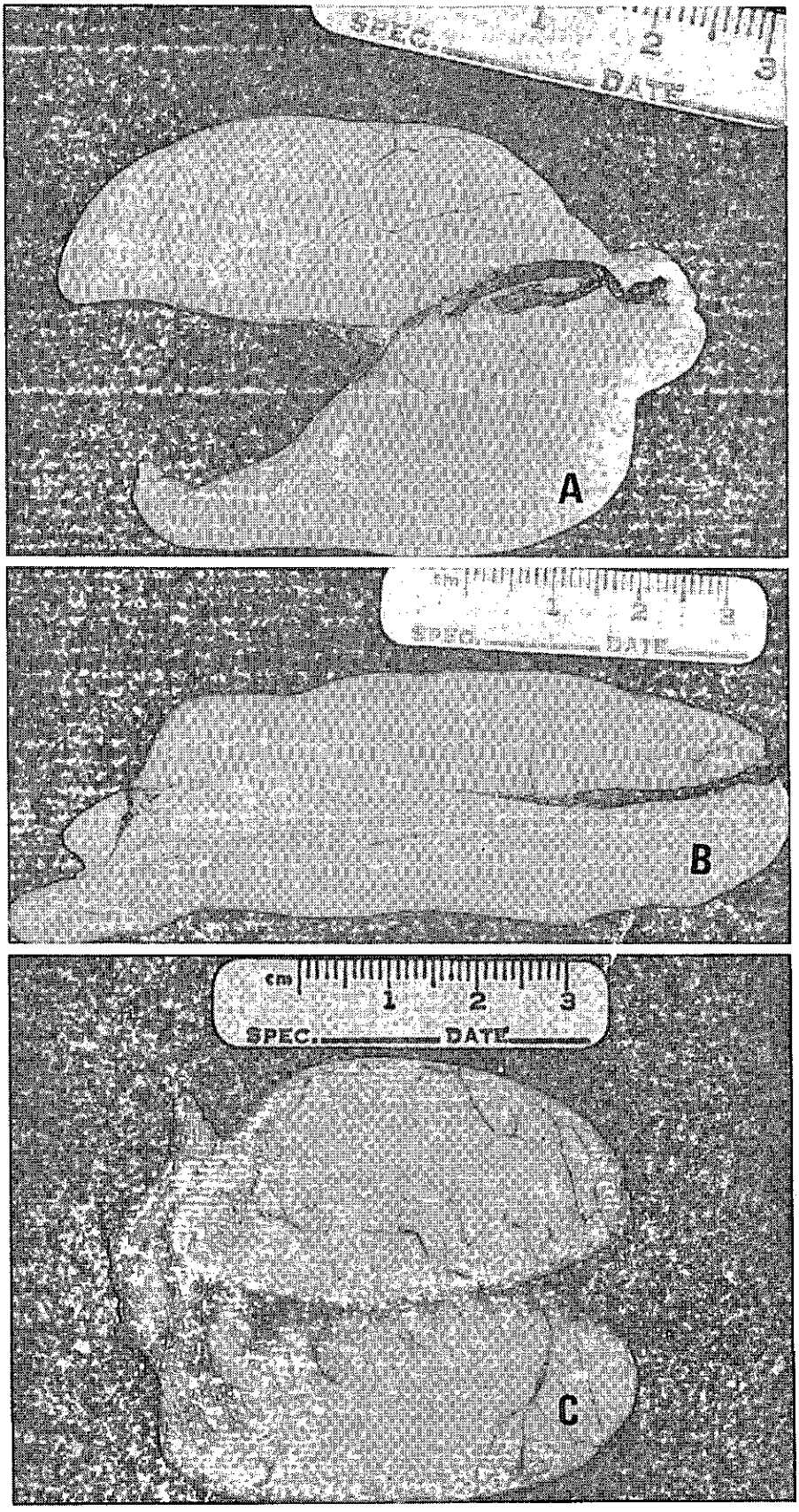


Fig. 3. Gonads of (A) immature fish (51 cm), (B) spent-L (non-reproductive fish (62 cm), and (C) mature fish (45 cm).

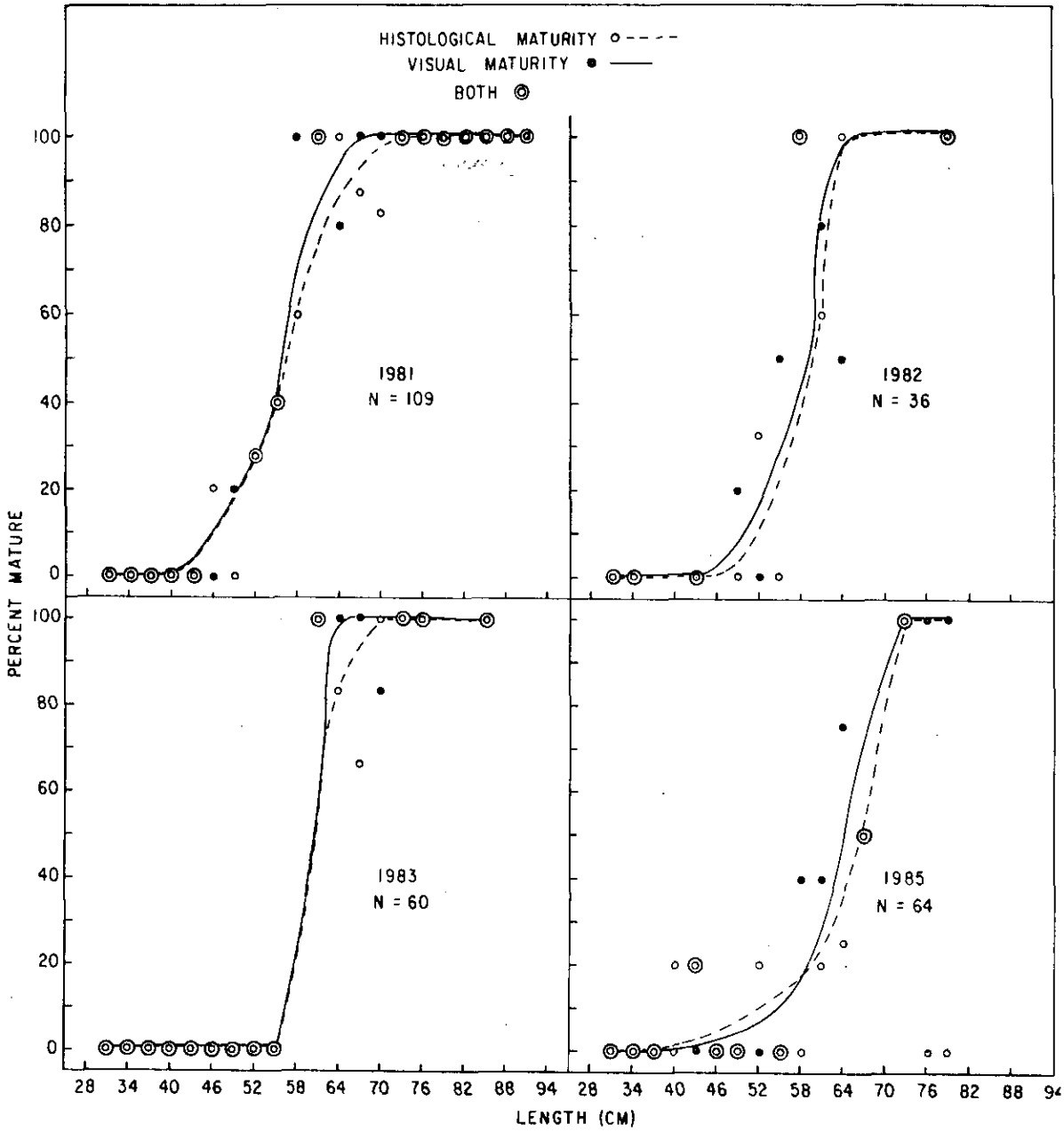


Fig. 4. Maturity ogives of female cod derived from visual stageing and histological stageing. Flemish Cap, NAFO Div. 3M.