SEASONAL CHANGES IN THE OVARIES OF ADULT YELLOWTAIL FLOUNDER, *LIMANDA FERRUGINEA*

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ABSTRACT

Seasonal changes in both macroscopic and microscopic appearance of yellowtail flounder ovaries are described, as well as seasonal changes in the gonosomatic index. Oocytes pass through seven cytologically different developmental stages. By examining seasonal changes in the relative abundance and sizes of these stages, it was found that oogenesis occurs in two broad phases, each lasting about 1 year. The previtellogenic phase begins as a new stock of oogonia arises each year, principally in the summer months following spawning. These rapidly develop into early perinucleolus oocytes, which in turn develop into resting stage oocytes by the fall. Most oocytes remain in this stage until the following spring, when they then develop into late perinucleolus oocytes. The vitellogenic phase begins as these late perinucleolus oocytes, now about 1 year old, become transformed into early maturing oocytes through the accumulation of yolk. This occurs during the late spring and summer months. Through the following fall and winter the cytoplasm completely fills with yolk as oocytes reach the late maturing stage. Shortly before spawning the following spring, the final hyaline stage of development is reached.

These results indicate that there are two synchronous groups or populations of oocytes present in the ovary at any given time. Members of the vitellogenic group are in their second year of development, and will be released in the upcoming spawning season. These are recruited from a previtellogenic group which developed during the previous year.

Seasonal changes in the microscopic appearance of the ovaries were well correlated with seasonal changes in both gonosomatic index and macroscopic appearance.

Yellowtail flounder, *Limanda ferruginea*, range along the Atlantic coast of North America from the Gulf of St. Lawrence to the Chesapeake Bay (Bigelow and Schroeder 1953). Over much of this range the species supports important commercial fisheries. Despite their economic importance, comparatively little is known of their reproductive biology. Spawning season is latitudinally dependent, with most spawning occurring from April through June in the southern portion of the range, and from May through July in the northern portion (Bigelow and Schroeder 1953; Royce et al. 1959; Pitt 1970; Smith et al. 1975; Able 1978; Colton et al. 1979). Females generally mature between 30 and 40 cm TL (total length), which is reached in 2-4 yr in the southern portion of their range (Royce et al. 1959) and in 5-8 yr further north (Scott 1954; Lux and Nichy 1969; Pitt 1970). The fecundity of Grand Bank, Newfoundland, yellowtail flounder has been related to total length and age by Pitt (1971), and Howell and Kesler (1977) related fecundity of southern New England yellowtail flounder to total length, age, and ovary weight.

The purpose of this research was to examine seasonal changes in both the macroscopic and microscopic appearance of the ovary, to examine sea-

sonal changes in gonosomatic index (GSI), and to describe histologically the process of oogenesis. Specific questions addressed were: 1) What developmental stages does an oocyte pass through from oogonium to fully ripe egg? 2) How long does this process take, and when do the changes from one stage to the next occur? 3) How do seasonal histological changes relate to seasonal changes in both GSI and the macroscopic appearance of the ovary? These data provide baseline information against which either experimental results or field data can be compared, and which are useful in identifying environmental variables that may effect eventual fecundity.

MATERIALS AND METHODS

Data were collected from commercially landed fish at the Pt. Judith, R.I., Fisherman's Cooperative at approximately monthly intervals from June 1977 through November 1978 (Table 1). On each sampling date adult females were randomly selected from the combined catches of several vessels that had been fishing southeast of Block Island, R.I. In addition to the data collected in Rhode Island, 15 immature females were examined. They were collected by the National Marine Fisheries Service on Georges Bank in April 1979.

All specimens were measured to the nearest 0.1 cm

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TABLE 1.—Collection dates and number of yellowtail flounder examined.

Sampling date	No. of specimens examined		
	For length, weight, and maturity stage	For ovary weight	Histologically
26 Jan. 1978	90	50	25
27 Feb. 1978	90	50	25
3 Apr. 1978	100	50	25
1 May 1978	100	50	25
31 May 1978	100	50	25
27 June 1977	139	50	25
28 July 1977	106	50	25
21 Aug. 1978	100	50	25
6 Sept. 1977	50	50	25
4 Oct. 1977	100	50	25
15 Nov. 1977	7	7	7
20 Nov. 1978	50	50	25
19 Dec. 1977	46	46	25
Total	1,078	603	307

TL, then eviscerated and weighed to the nearest gram wet weight. Both ovaries from a subsample of the examined specimens were weighed to the nearest 0.1 g. A GSI was calculated for these individuals as ovary wet weight (g) divided by eviscerated wet weight (g) and expressed as a percentage.

Based on the macroscopic appearance of the ovaries, fish were classified into one of five maturity stages following a modification of the International Scale of Sexual Maturity first proposed by Hjort (1910) (Table 2).

A portion of ovarian tissue was removed from the anterior third of the eyed-side ovary from 25 fish on each sampling date (except November 1977) and fixed in Davidson's solution. The histological technique employed was developed for fish gonads by the Florida Department of Natural Resources (reported in Yevich and Barszcz 1977). Tissues were embedded in Paraplast,[2] sectioned at 6 μm, and stained using Harris' hematoxylin and Eosin Y.

Slides of tissue were prepared and scanned at 400X magnification. The first 200 oocytes encountered were classified based upon the degree of chromatin condensation, size and staining characteristics, number and placement of nucleoli, and morphological appearance of the cytoplasm and follicle cells. In 10 fish randomly chosen from the 25 individuals of each sampling date, diameters of the first 50 oocytes encountered were measured to the nearest micrometer using an ocular micrometer. Measurements were taken only on oocytes sectioned through the nucleus. Such measurements have been shown to be representative of true oocyte diameters (Foucher and Beamish 1980). The mean diameter of each oocyte type was calculated from the 10 fish in every sample. A one-way analysis of variance (ANOVA) was used to test the null hypothesis that no significant differences in mean diameter were present between samples. When significant differences were found, Student-Newman-Keuls test was employed to examine the differences between samples.

Percent frequency distributions of the different oocyte types were calculated for each sample by dividing the total number of that type by the total number of oocytes examined in the sample. These fractions were then expressed as percentages. Since the probability of an individual oocyte being cut in a

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[2]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

TABLE 2.—Macroscopic and microscopic characteristics of the different maturity stages in yellowtail flounder. Stages modified from Hjort (1910). Oocyte developmental stages are described in text.

Maturity stage	External appearance	Histological appearance
I. Immature virgin	Ovaries small (2-6 cm), slender, conical, pinkish, and generally translucent. Enveloped in a layer of silvery epithelium. No oocytes visible to the naked eye.	Mainly Stage II and IV oocytes, few >50 μm. A few Stage I and III oocytes also present. All oocytes with lightly basophilic cytoplasm. Ovarian wall from 25 to 75 μm thick.
II. Developing virgin, recovering spent	Ovaries relatively small (6-12 cm), rounded, reddish, and translucent. Ovarian wall thick. Vascularization slight.	Stage II, IV, and V oocytes predominate. Stage IV oocytes are abundant early in this maturity stage, but tend to decline as Stage V oocytes increase later in the period. Most oocytes <150 μm diameter. Ovarian wall from 250 to 400 μm thick.
III. Developing, maturing	Ovaries larger in size (>12 cm) and occupying most of ovarian cavity. Visible oocytes large, yellowish in color, and opaque. Ovarian wall thin, translucent, and granular in texture due to underlying developing oocytes.	Mainly Stage III and VI oocytes, but small numbers of Stages I, II, IV, and V also present. Stage VI oocytes increase in diameter to reach about 370 μm just prior to the ripe/running stage. Ovarian wall from 100 to 200 μm thick.
IV. Ripe	Ovaries very large and distending the body wall. Some oocytes yellowish and opaque, others transparent (hyaline) producing a speckled appearance. Vascularization heavy. Ovarian wall thin. Ova run from vent upon slight pressure.	Stage III, VI, and VII oocytes predominate. Stage VII oocytes are irregular in shape, about 400 μm in diameter, and often present in the lumen of the ovary. Ovarian wall from 50 to 100 μm thick.
V. Spent	Ovaries flaccid, bloodshot. All visible remaining oocytes clear.	Many empty and collapsed follicles with relatively few oocytes. Stage III and IV oocytes predominate, with many in transition between these two stages. Small numbers of Stage I, II, and resorbing oocytes also present. Ovarian wall from 250 to 400 μm thick.

section is proportional to its size as well as its abundance, large oocytes tend to be overestimated and small oocytes underestimated when percent frequencies are calculated. The calculated percentages, therefore, may not be exact, but they do provide indications of seasonal changes.

Size-frequency distributions of oocyte diameter were constructed for each sample by dividing the diameters into 10 μm categories. The number per category was then divided by the total number of measurements and expressed as a percentage.

To test the hypothesis that no differences existed in either frequencies of oocyte types or diameters between different regions within an ovary, or between ovaries of the same fish, chi-square contingency tests were employed to compare both size- and percent-frequency distributions from portions of tissue taken from the anterior, middle, and posterior regions of both ovaries from eight fish collected in October. Since no significant differences were found ($P>0.05$), the anterior portion of the eyed-side ovary was assumed to be representative, and this region was used exclusively in the study.

Two samples were collected in November; one in 1977 and one in 1978 (Table 1). In order to facilitate data analysis it was desirable to combine them. Mean length, weight, and GSI for both samples were compared using a Student's t-test (Snedecor and Cochran 1973). Since no significant differences were found ($P>0.25$), the samples were combined for all further analyses.

RESULTS

Macroscopic Structure and Maturity Stages

The paired ovaries lie, one on either side, in an ovarian cavity between the haemal spines and the body wall musculature. Each is shaped like an elongated cone, with the apex oriented toward the tail and the enlarged anterior end protruding slightly into the abdominal cavity. They are anchored by both connective tissue fascia and a suspensory ligament that runs from the cleithrum to the anterior end of each ovary. Very short oviducts arise from the anterior end of each ovary and terminate at the cloaca.

Percentages of fish in each of the different maturity stages are given in Table 3. Stage I (Immature virgin) females were observed only in the collection made in April by the National Marine Fisheries Service. None were found among those fish landed commercially, since fishermen generally discard small immature fish. Stage II (Developing virgin, recovering spent) females were seen from May through early October. Their percentage was relatively low in May, but increased to 66% by June. In July and August all fish were in this category. A rapid decline in the percentage of Stage II females occurred from September (81%) to October (7%). Stage III (Developing, maturing) females were present from September through May. In early September, 19% of the fish were in this stage. The percentage increased to 93% by October, and all fish were in this stage from November through at least February. Percentages then declined from 91% in April to only 7% in late May. Stage IV (Ripe) females were observed only in April (9%), early and late May (17 and 31%, respectively), and June (24%). Stage V (Spent) females were seen only in May and June.

Gonosomatic Index

When the relationship between GSI and fish length was examined using functional linear regression (Ricker 1973), a significant positive relationship was

TABLE 3.—Seasonal changes in gonosomatic index (GSI) and percentages of yellowtail flounder in the different maturity stages. Stages are modified from Hjort (1910).

Sampling date	GSI (mean ±2 SE)	Stage II (developing virgin, recovering spent)	Stage III (developing, maturing)	Stage IV (Ripe)	Stage V (Spent)
1/26/78	11.79±1.10	—	100	—	—
2/27/78	11.26±1.58	—	100	—	—
4/3/78	18.54±1.70	—	91	9	—
5/1/78	13.49±2.29	4	68	17	11
5/31/78	11.53±2.78	12	7	31	50
6/27/77	3.32±0.51	66	—	24	10
7/28/77	2.46±0.17	100	—	—	—
8/21/78	3.81±0.22	100	—	—	—
9/6/77	3.20±0.22	81	19	—	—
10/4/77	3.87±0.34	7	93	—	—
11/15/77 and 11/20/78	4.95±0.37	—	100	—	—
12/19/77	6.96±0.62	—	100	—	—

found ($P<0.001$). Since this variable was dependent on fish length, and since mean fish length was significantly different between samples (ANOVA, $P<0.05$), the sample means of GSI were adjusted for length using analysis of covariance (Snedecor and Cochran 1973).

Adjusted mean monthly GSI values showed a seasonal pattern (Table 3). Values were highest in early April, then fell sharply to their lowest value by late July. From July through September GSI values remained low, then began to rise gradually to reach about 11% by the following January and February. A sharp increase was observed from late February through early April.

Histology of the Ovaries

The ovarian wall is comprised of three tissue layers (Fig. 1A). The outermost layer (tunica albuginea) is fibrous connective tissue. Internal to this are two layers of smooth muscle tissue. Fibers of the external layer are oriented perpendicularly to the long axis of the ovary, while fibers of the inner smooth muscle layer run parallel to the long axis. Ovigerous folds (lamellae) arise from inner layers of the ovarian wall and extend into the lumen. Margins of these folds are covered by epithelial cells. The entire ovary is enclosed in a thin peritoneum.

Oogenesis was divided into seven developmental stages based upon the cytological characteristics of the cells. Although the developmental stages through which teleost oocytes pass are quite similar from species to species (Wallace and Selman 1981), the nomenclature used, and the number of stages defined, differs considerably between investigators. The terminology used here generally follows that of Yamamoto (1956a). The developmental stages are defined as follows:

Stage I—Oogonia (Fig. 1B): Small (5-29 μm), spherical to slightly oval in shape. Nucleus spherical and large, occupying most of cell. Chromatin material appearing as thin threads. One prominent, deeply basophilic nucleolus located at periphery of nucleus. Nucleoplasm clear. Cytoplasm very thin and faintly basophilic. Usually associated with a single potential follicle cell. Most often found in small groups, or "nests" of 4 or 5.

Stage II—Early perinucleolus (Fig. 1B): Small (10-78 μm), and angular to round in shape. Nucleus spherical and large. Chromatin material forming chromosomes characteristic of meiotic prophase. Two to five deeply basophilic large nucleoli located near periphery of nucleus. Nucleoplasm slightly basophilic. Cytoplasmic volume greater than in oogonia and deeply basophilic. Surrounded by a single layer of flattened follicle cells (theca).

Stage III—Resting (Fig. 1C): Small to intermediate in size (23-140 μm), and spherical in shape. Nucleus spherical and large. Chromatin dispersed, granular, and lightly basophilic. Three to ten deeply basophilic nucleoli arranged peripherally just inside nuclear envelope. Cytoplasm divided into two concentric zones; the inner deeply basophilic and dense, and the outer only slightly basophilic and less dense. Boundary between zones usually poorly defined. Single layer of flattened follicle cells surround oocyte (theca).

Stage IV—Late perinucleolus (Fig. 1D): Small to intermediate in size (39-174 μm) and spherical in shape. Nucleus spherical and large. Chromatin material dispersed, causing nucleoplasm to appear granular. Five to twenty deeply basophilic nucleoli arranged peripherally around inner surface of nuclear envelope. Cytoplasm lightly basophilic. Oocyte surrounded by a single layer of flattened follicle cells (theca).

Stage V—Early maturing (Fig. 2A, B): Intermediate to large in size (52-260 μm), and spherical in shape. Nucleus large and spherical. Chromatin material dispersed. Ten to twenty basophilic nucleoli arranged peripherally, just inside nuclear envelope. Cytoplasm containing either yolk vesicles (Fig. 2A) or yolk globules (Fig. 2B) in its outer region. Follicle is composed of a thin inner acidophilic zona radiata and two outer layers of follicle cells; an inner granulosa and an outer theca.

Stage VI—Late maturing (Fig. 2C, D): Large (104-474 μm), and spherical in shape. In early stages (Fig. 2C), nuclear envelope is distinct, nucleoplasm is lightly acidophilic, and numerous nucleoli are located peripherally in nucleus. Cytoplasm is completely full of yolk globules. In later stages (Fig. 2D), nuclear envelope is indistinct, and nucleus is irregularly shaped. Nucleoli dispersed throughout the slightly acidophilic nucleoplasm. Lampbrush chromosomes often apparent. Nucleus may be located either centrally or toward the periphery of the cell. Zona radiata thick, and radial striations are apparent. Two layers of follicle cells are located external to the zona radiata; an inner granulosa and an outer theca.
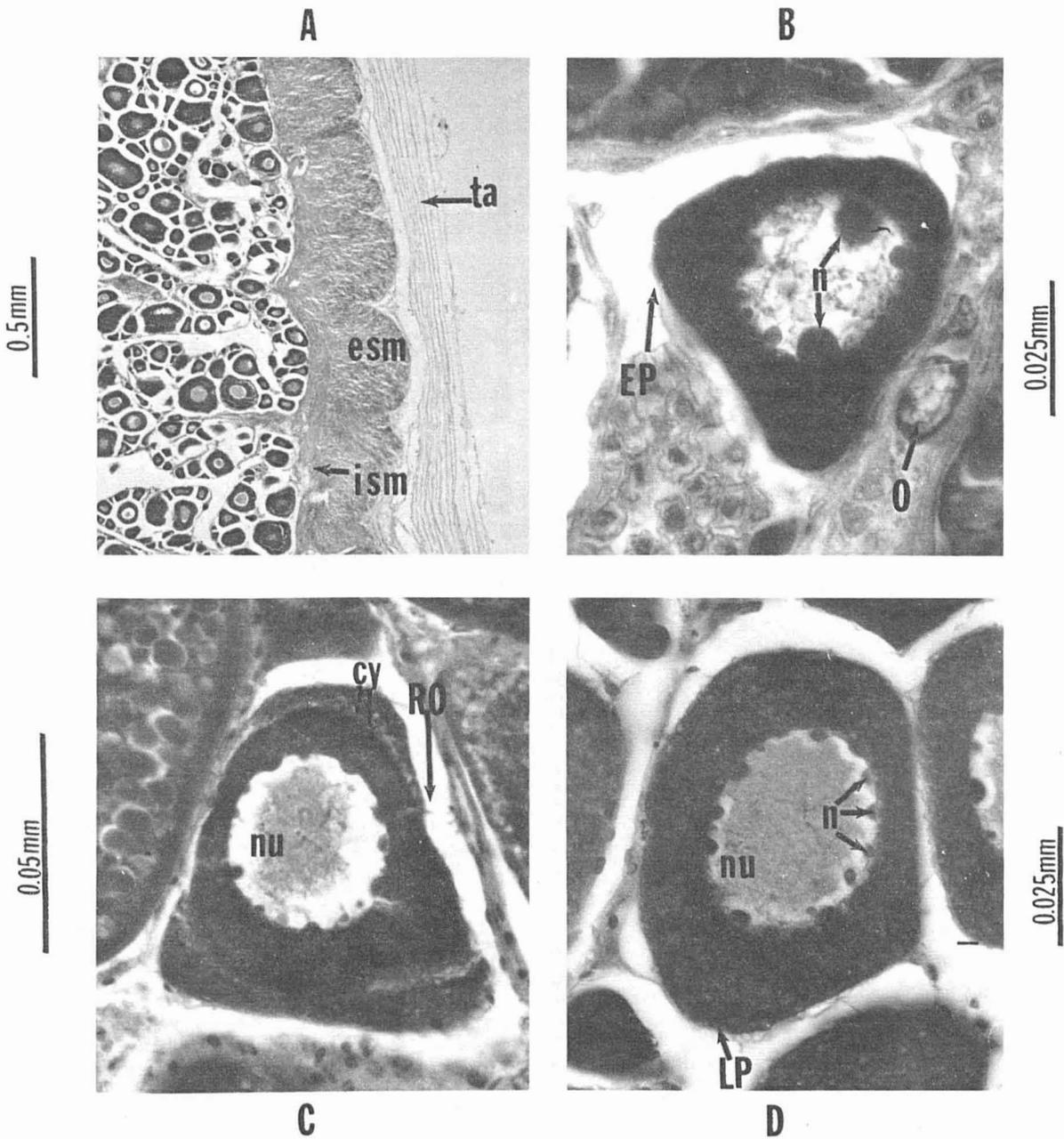
FIGURE 1.—A. Cross section of the ovary wall in a postspawning yellowtail flounder. B. Oogonia and early perinucleolus stage. C. Resting oocyte stage. D. Late perinucleolus stage. cy = cytoplasm, EP = early perinucleolus oocyte, esm = external smooth muscle, ism = internal smooth muscle, LP = late perinucleolus oocyte, n = nucleolus, nu = nucleus, O = oogonia, RO = resting oocyte, ta = tunica albuginea.
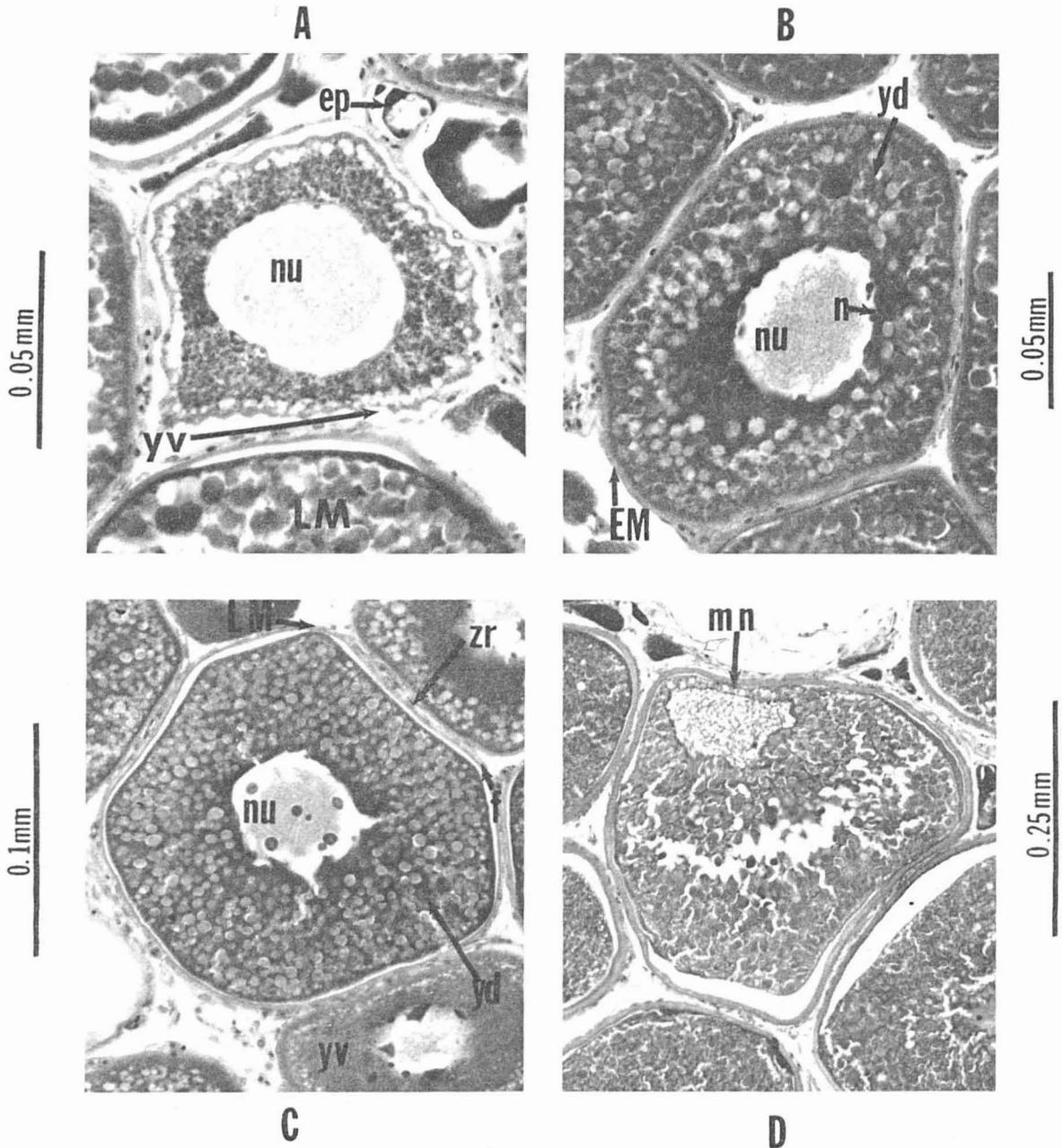
FIGURE 2.—A. Early maturing stage of the yellowtail flounder with yolk vesicles. B. Early maturing stage acquiring yolk. C. Late maturing oocyte. D. Migrating nucleus of late maturing oocyte. EM = early maturing oocyte, ep = early perinucleolus oocyte, f = follicle cell layer, LM = late maturing oocyte, mn = migrating nucleus, n = nucleolus, nu = nucleus, yd = yolk droplet, yv = yolk vesicle, zr = zona radiata.

Stage VII—Hyaline (Fig. 3A): Large (about 400 μm) and irregularly shaped, presumably due to histological processing. Nucleus, seen only at the beginning of this stage, is irregularly shaped and located near margin of cell. Early in this stage the yolk globules begin to break up, allowing the yolk to coalesce. At conclusion of this stage, interior of cell is completely homogeneous, with no yolk globules or nucleus apparent. Zona radiata is thin, and no radial striations are seen. Follicle layer external to the zona radiata is absent.

In addition to the above seven developmental stages, there were two types of regressing oocytes:

Type I—Corpora atretica (Fig. 3B): Large and irregularly shaped. Size variable, but generally from 150 to 300 μm. Characteristics similar to either early maturing or late maturing oocytes except that the zona radiata is broken and collapsed inward. Follicle cells are hypertrophied, lightly basophilic, and invade the cytoplasm. Comparatively few yolk globules remain, and those present are indistinct. Found only in prespawning fish.

Type II—Resorbing (Fig. 3C): Cytoplasmic characters similar to those of either late maturing or hyaline oocytes. Entire cell is collapsed inward, and zona radiata is broken in numerous places. Found only in postspawning fish. Size variable, but generally from 350 to 400 μm.

Seasonal Changes in Microscopic Appearance

When the relative abundances of the different developmental stages of oocytes were calculated for each sampling date, seasonal differences were apparent (Table 4; Figs. 4, 5). The mean diameter of some oocyte types also showed seasonal changes
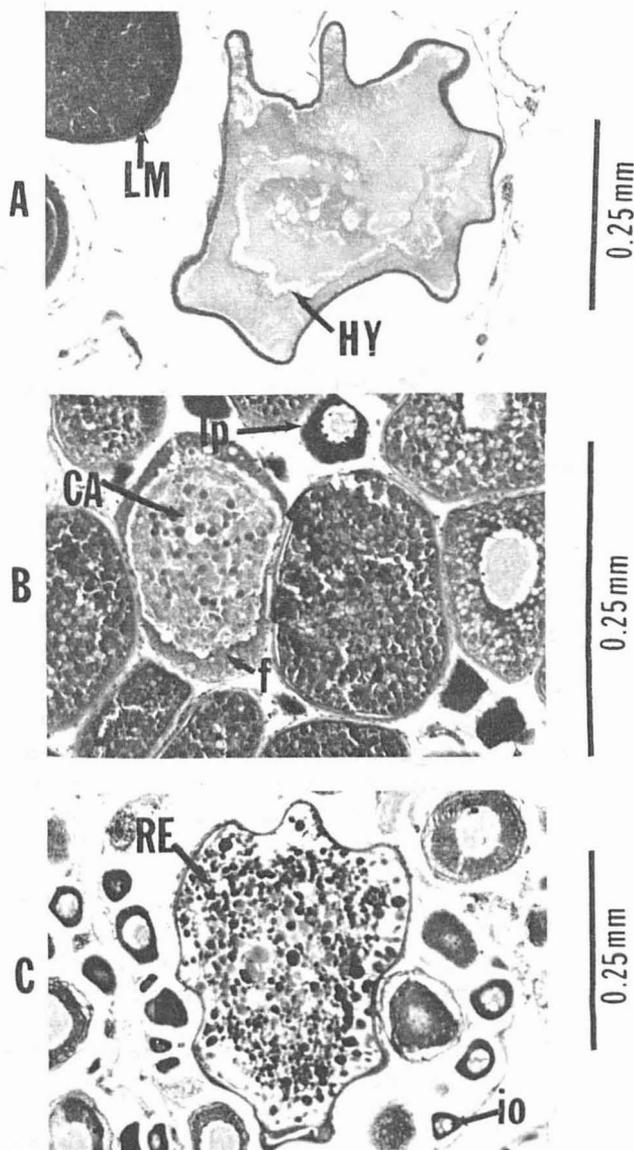
FIGURE 3.—A. Hyaline oocyte of the yellowtail flounder. B. Corpora atretica in a ripening fish. C. Resorbing oocyte in a spent fish. CA = corpora atretica, f = hypertrophied follicle layer, HY = hyaline oocyte, io = immature oocyte, LM = late maturing oocyte, lp = late perinucleolus oocyte, RE = resorbing oocyte.

TABLE 4.—Seasonal changes in frequency (%) of different oocyte developmental stages in yellowtail flounder. n = number of cells examined. Developmental stages and regressing type are described in text.

Sampling date	n	Developmental stages							Regressing type
		I	II	III	IV	V	VI	VII	I
1/26/78	5,000	1.42	4.86	17.90	4.94	—	69.54	—	1.28
2/27/78	5,000	0.52	3.88	23.84	—	0.68	69.28	—	1.80
4/3/78	5,000	2.70	5.04	24.00	1.24	1.08	63.64	2.02	0.28
5/1/78	5,000	2.28	8.28	13.86	8.04	8.44	57.44	1.60	0.06
5/31/78	5,010	5.17	7.12	32.29	1.34	18.14	26.91	8.98	0.04
6/27/77	5,000	2.34	26.64	1.92	38.42	22.20	8.06	0.30	—
7/28/77	5,000	2.96	39.94	—	27.38	29.72	—	—	—
8/21/77	5,000	8.08	41.22	—	22.28	28.42	—	—	—
9/6/77	5,000	4.66	44.46	—	16.98	33.90	—	—	—
10/4/77	4,978	11.05	12.17	25.15	7.31	9.68	34.63	—	—
11/15/77 and 11/20/78	6,400	5.59	11.89	14.81	8.75	6.66	52.19	—	0.11
12/19/77	4,900	2.38	5.35	20.75	7.06	3.71	60.55	—	0.14
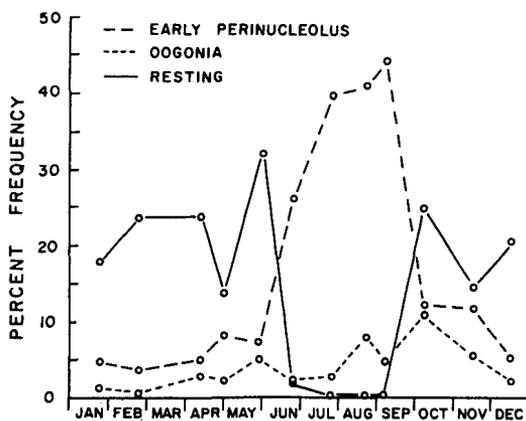
FIGURE 4.—Seasonal changes in the percent frequencies of oogonia, early perinucleolus, and resting stage oocytes in yellowtail flounder.

FIGURE 5.—Seasonal changes in the percent frequencies of late perinucleolus, early maturing, and late maturing stage oocytes in yellowtail flounder.

(Table 5; Figs. 6, 7), as did their size-frequency distribution (Fig. 8).

Oogonia were present year-round in variable percentages (Table 4), but showed a tendency to be more abundant during the summer and early fall (Fig. 4). No significant differences in mean diameter were apparent between months ($P>0.05$).

Early perinucleolus oocytes were also present on all sampling dates, but marked seasonal changes in abundance were apparent (Table 4). Relatively few were seen from October through May, but their percentage increased sharply from June through September (Fig. 4). Although their size stayed fairly constant over the year, a significant decrease in mean diameter was noted between September and October, and April and May ($P<0.05$), while a significant increase ($P<0.05$) was seen between February and April (Fig. 6). Most early perinucleolus oocytes were from 21 to 30 μm in diameter in all months ex-

cept June, July, and September when their modal size increased to 31-40 μm (Fig. 8).

Resting oocytes were present only from October through June (Table 4). From September to October their percentage increased sharply, then fluctuated from about 14 to 32% through the following May when they began a sharp decline in abundance (Fig. 4). The percentages of early perinucleolus and resting oocytes were inversely related. As the percentage of early perinucleolus oocytes declined from September to October, the percentage of resting oocytes increased. An opposite situation was noted from May to June (Fig. 4). From October through December the mean diameter of resting oocytes increased (Table 5). It remained fairly constant through April, and then began a gradual decline through June (Fig. 6). The size-frequency distributions of resting oocytes widely overlapped those of early perinucleolus, late perinucleolus, and early maturing oocytes. Modal diameter was relatively small in October, increased

from November through April, and then decreased in May (Fig. 8).

Although resting oocytes remained histologically similar from October through February, changes began to occur in April and May. The boundary between the cytoplasmic zones became very indistinct and eventually disappeared as the inner zone became less basophilic. Accompanying this transition, the number of nucleoli increased, and in some oocytes a peripheral ring of yolk vesicles appeared in the cytoplasm.

Late perinucleolus oocytes were observed in all months except February (Table 4). Their abundance increased sharply during June and then began a gradual decline through October (Fig. 5). Coincidentally, there was a decrease in the percentage of resting oocytes (Fig. 4). The mean diameter of late perinucleolus oocytes showed little seasonal change with the exception of a significant ($P<0.05$) increase in size from July to August followed by a significant decline from August to September (Fig. 7). The size-frequency distributions of this oocyte stage overlap-

ped those of all other oocyte types except for late maturing. Beginning in April some late perinucleolus oocytes had a narrow, clear area in the cytoplasm just inside the follicle layer.

The percentage of early maturing oocytes increased fairly steadily from April through September, and then declined through December (Fig. 5). As their abundance increased from May to June, the percentage of resting oocytes decreased (Fig. 4). Although the percentages of both early maturing and late perinucleolus oocytes increased from May to June, the percentage of late perinucleolus oocytes peaked in June and then declined, while the peak percentage of early maturing oocytes was not seen until September, about 3 mo later (Fig. 5). The mean diameter of early maturing oocytes increased steadily from April through August, declined from August to October, and then remained fairly constant through February (Fig. 7). The size-frequency distributions of early maturing oocytes overlapped those of both resting and late perinucleolus oocytes in the months when they were present together. A continuous size-

TABLE 5.—Seasonal changes in mean diameter (μm) (± 1 SD) of different oocyte developmental stages and regressing types in yellowtail flounder. Developmental stages and regressing types are described in the text.

Sampling date	Developmental stage							Regressing type	
	I	II	III	IV	V	VI	VII	I	II
6/27/77	13.47±1.97	36.20±4.62	71.62± 8.74	78.17± 5.39	129.74± 8.05	372.03±45.44	413.43±36.65	—	397.43±35.88
7/28/77	10.23±1.71	31.90±4.22	—	76.59± 6.84	142.00± 9.93	—	—	—	—
8/21/78	11.47±3.53	36.31±6.11	—	95.50±10.57	151.79±13.43	—	—	—	—
9/6/77	12.99±1.69	36.87±4.86	—	77.70±10.21	133.15±22.10	—	—	—	—
10/4/77	12.64±1.58	28.55±5.39	59.57±13.37	67.50±12.22	102.73± 9.45	170.98±32.35	—	—	—
11/15/77 and 11/20/78	11.58±2.60	32.40±4.21	65.79± 4.19	79.81±12.09	105.68±15.30	204.46±26.44	—	171.63±48.48	—
12/19/77	9.56±1.19	29.57±6.58	77.68± 2.55	77.10± 5.80	121.60±19.69	249.58±19.81	—	229.20±38.56	—
1/26/78	12.75±3.31	35.16±5.73	76.85± 8.79	70.89±22.24	—	294.43±19.94	—	203.67±30.39	—
2/27/78	10.00±1.43	28.41±6.31	78.35± 6.86	60.10±13.46	116.27±21.08	293.71±23.15	—	234.00±36.77	—
4/3/78	13.15±3.09	37.86±8.30	76.85± 8.79	—	92.90± 6.65	324.43±37.84	404.18±37.43	246.11±33.75	—
5/1/78	12.65±0.61	21.14±3.32	74.89± 8.48	76.35±14.35	96.53± 9.26	313.55±18.90	403.00±33.00	231.33±45.52	—
5/31/78	11.45±3.52	26.41±2.52	73.02± 9.82	78.75±17.32	108.87±20.10	349.12±12.90	399.00±32.71	228.80±41.75	366.36±51.29
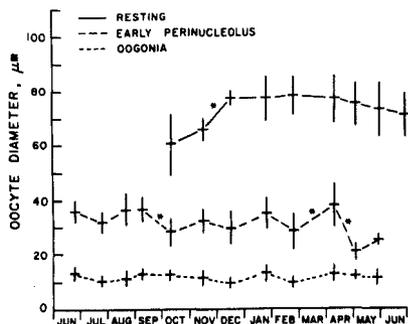
FIGURE 6.—Seasonal changes in diameter (mm) of ogonia, early perinucleolus, and resting stage oocytes in yellowtail flounder. Horizontal bar = mean, vertical bar = mean ± 1 SD, *indicates a significant difference (ANOVA, Student-Newman-Keuls, P<0.05) between adjacent means.
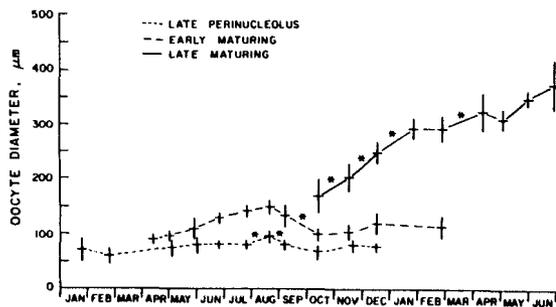
FIGURE 7.—Seasonal changes in diameter (mm) of late perinucleolus, early maturing, and late maturing stage oocytes in yellowtail flounder. Horizontal bar = mean, vertical bar = mean ± 1 SD, *indicates a significant difference (ANOVA, Student-Newman-Keuls, P<0.05) between adjacent means.

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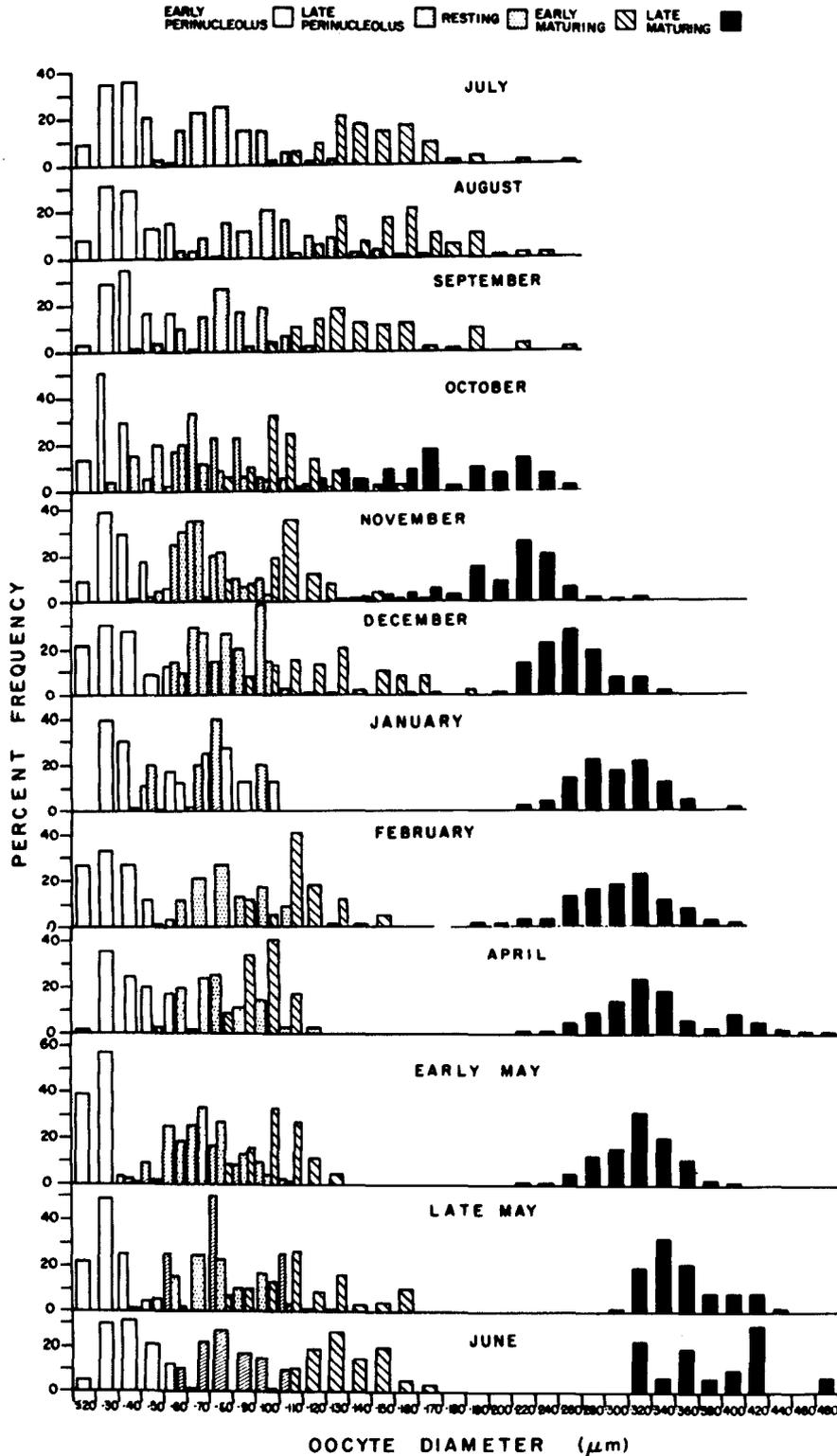
FIGURE 8.—Size-frequency distributions of yellowtail flounder oocytes from July through June.

frequency distribution was seen between early and late maturing oocytes from October through December. The continued growth of late maturing oocytes caused the distributions to become discontinuous by February (Fig. 8).

Late maturing oocytes were present from October through June (Table 4). Their abundance increased steadily from September through January, remained fairly constant through April, and then declined sharply in May and June (Fig. 5). Their mean diameter increased from October through June (Fig. 7). This increase was reflected in their progressively larger size-frequency distributions (Fig. 8).

Hyaline oocytes were present in relatively small percentages from April through June (Table 4). Their mean diameter was about 400 μm (Table 5). Corpora atretica (Regressing Type I) were seen from November through May in very small percentages (Table 4). There was a slight tendency for them to be more abundant in January and February.

DISCUSSION

The developmental events observed in yellowtail flounder oocytes are very similar to those described for most other teleosts (see review by Wallace and Selman 1981). Development can be divided into two broad phases. In the first, or previtellogenic phase, growth is slow and comparatively few cytoplasmic changes occur. The second, or vitellogenic, phase is characterized by rapid growth and the deposition of large amounts of yolk in the cytoplasm. The previtellogenic phase includes the oogonia, early perinucleolus, resting, and late perinucleolus developmental stages. While oogonia were found throughout the year, their abundance tended to be somewhat higher from August through October. Similar patterns of year-round presence, with peak abundances in postovulatory fish, have been reported by many others (Barr 1963; Crossland 1977; Htun-Han 1978; Khoo 1979). Since oogonia represent the initial stage in the process of oogonesis, and thus the reserve from which all oocytes will eventually develop, the timing and location of their production are of considerable interest (see review by Tokarz 1978). Braekevelt and McMillan (1967), studying the brook stickleback, *Eucalia inconstans*, suggested that they arose mitotically from residual oogonia that remained in the ovary from year to year. Bowers and Holliday (1961) concluded that in the herring (*Clupea harengus*), oogonia were derived annually from primary germ cells, while others including Wheeler (1924), Yamamoto (1956a), and Foucher and Beamish (1980) working with *Pleuronectes* (= *Limanda*) *limanda*,

Liopsetta obscura, and *Merluccius productus*, respectively, concluded that at least some oogonia arose from follicle cells following ovulation. In addition to the site of production, the life history stage during which production occurs may differ from species to species. Many investigators (Barr 1963; Shirokova 1977; Htun-Han 1978; Monaco et al. 1978) have observed mitotic activity in oogonia of mature fish, suggesting that a new stock of oogonia arises during each reproductive cycle. Hickling (1935) and Yamamoto (1956b) saw no evidence of mitotic activity and concluded that the total reserve stock of oogonia had been produced prior to sexual maturity. While no mitotic divisions were apparent in this study, the fact that oogonia were usually present in small groups is an indication that such divisions were occurring but were overlooked due to the very small size of the oogonia. Furthermore, if the total reserve fund of oogonia, representing all future oocytes, were present in the ovary of a fish as fecund as yellowtail flounder (Howell and Kesler 1977), it seems likely that their abundance would have been considerably higher than observed. Because of these observations, and the seasonal changes in the abundance of oogonia, it seems reasonable to conclude that a new stock of oogonia is produced each year in yellowtail flounder, primarily in the months following spawning.

The sharp increase in abundance of early perinucleolus oocytes following spawning indicates that oogonia were rapidly being transformed into early perinucleolus oocytes at this time. Since few intermediate types were observed, it must be assumed that the transition was rapid.

The coincidental decline in the percentage of early perinucleolus oocytes and the increase in resting oocytes seen in September indicate that some early perinucleolus oocytes are converted into resting oocytes at this time. This is further indicated by their overlapping size-frequency distributions and their cytological similarity. This transformation was accompanied by a division of the cytoplasm into two concentric zones. Similar cytoplasmic zonation has been noted in a variety of species including *Clupea harengus* (Bowers and Holliday 1961), *Gadus morhua callarias* (Shirokova 1977), *Gadus merlangus* and *G. esmarkii* (Gokhale 1957), *Liopsetta obscura* (Yamamoto 1956a), and *Pleuronectes* (= *Limanda*) *limanda* (Wheeler 1924). Recent studies (see review by Guraya 1979) suggest that this apparent zonation may be due to aggregates of ribonucleoprotein particles having been extruded through the nuclear membrane. When these aggregates become surrounded by cytoplasmic organelles (not seen in this study) they are variously known as "yoke nuclei" or

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"Balbiani bodies." While the function of these remains unknown, Guraya (1979) has suggested that they act as centers for the formation, multiplication, and accumulation of organelles and materials needed for yolk deposition.

The mean diameter of resting oocytes increased from October to December, remained fairly constant from December to April, and then declined from April through June. The increase was presumably caused by progressively older, and therefore larger, early perinucleolus oocytes entering the resting stage. The decline in mean size seen during the spring was probably due to the larger ones having been transformed into late perinucleolus oocytes.

Resting oocytes rapidly declined in abundance from May to June and were absent by July. Coincident with this decline was an increase in late perinucleolus oocytes. This observation, combined with their similarities in mean size and overlapping size-frequency distributions, indicates that resting oocytes were transformed into late perinucleolus oocytes. During this transformation the zonation in the cytoplasm was lost, the number of nucleoli increased, and the cytoplasm became less basophilic.

The vitellogenic phase of oogenesis contains the early and late maturing types as well as hyaline oocytes. It begins as the late perinucleolus oocytes develop into early maturing oocytes. As indicated by their relative changes in seasonal abundance and size-frequency distributions, this changeover occurs primarily during the late spring and summer months. In large late perinucleolus oocytes a ring of vacuole-like structures is seen near the periphery of the cytoplasm. Oocytes of this type have alternatively been described as yolk vesicle (Yamamoto 1956a; Khoo 1979), early or primary vitellogenic (Monaco et al. 1978; Htun-Han 1978) or vacuolated (James 1946) oocytes. This stage marks the beginning of vitellogenesis during which the oocyte rapidly grows in size and accumulates yolk. Yolk vesicles apparently originate from the Golgi complexes (Yamamoto and Onozata 1965; Yamamoto and Oota 1967) and contain mucopolysaccharides which represent the first form of yolk inclusions (Yamamoto 1956a; Malone and Hisaoka 1963; Khoo 1979). Yamamoto (1956c) and Khoo (1979) have reported that in the later stages of vitellogenesis the yolk vesicles are displaced to the periphery of the oocyte and gave rise to the cortical alveoli which, after fertilization, contribute to water hardening of the egg. Simultaneous with the appearance of these vesicles the beginning of the zona radiata was seen between the follicle cells and the cytoplasm. As early maturing oocytes continued to develop, yolk globules became in-

terspersed with the yolk vesicles near the periphery of the cytoplasm. These globules represented the second form of yolk inclusions which have been shown in other species to contain proteins, phospholipids, and fats (Yamamoto 1957; Khoo 1979).

As yolk continued to accumulate toward the nucleus the mean diameter of early maturing oocytes continued to increase. The significant decrease in mean diameter of this stage noted from September to October was due to the larger early maturing oocytes being classified as late maturing. This is demonstrated in their size-frequency distributions where it can be seen that size classes formerly dominated by early maturing oocytes had become predominantly late maturing oocytes. As expected, the percentages of early maturing oocytes declined as the percentages of late maturing oocytes increased. Following this transformation, mean oocyte diameter increased rapidly.

Beginning in April late maturing oocytes began to be transformed into hyaline oocytes preparatory to their release from the follicle. At this time the yolk globules began to break open allowing the yolk to coalesce. Accompanying this was an increase in size, presumably due to the absorption of fluid, which caused the zona radiata to become thin.

The low percentage of hyaline oocytes observed is an indication that spawning is intermittent during the breeding season, with only a portion of the late maturing oocytes taking in fluid and being discharged at one time. Following the expulsion of the ripe ovum, the remaining follicle collapses into an irregular mass, decreases in size, and disappears shortly after spawning.

The year-round presence of some oogonia and early perinucleolus oocytes indicates that a small amount of oogonia production and subsequent development into early perinucleolus oocytes occur throughout the year. Although not established, it is assumed that those early perinucleolus oocytes produced during the late fall and winter enter the resting stage until the following spring. Small percentages of late perinucleolus and early maturing oocytes were also present year-round. The small percentage of late perinucleolus oocytes from October through January indicates that not all of them had developed into early maturing oocytes over the late spring and summer months. Presumably these would have begun to accumulate yolk during the late fall and early winter months. This would account for the small percentages of early maturing oocytes seen at this time.

The percentages of the two types of regressing oocytes were very small. Corpora atretica (Regressing Type I) were seen only in prespawning fish, and

were formed as either early or late maturing oocytes ceased to develop and began to be resorbed. Even in the samples where most abundant (January and February), they accounted for <2% of the oocytes examined. The persistence of corpora atretica and corpora lutea in certain species has led some investigators to conclude that they are the source of ovarian hormones (see review by Ball 1960). The very small percentages of corpora atretica seen in this study suggest that in yellowtail flounder these structures either disappear rapidly or are formed very infrequently. Similar low percentages have been reported in numerous other teleosts (Wheeler 1924; Yamamoto 1956a; Barr 1963; Davis 1977). Resorbing oocytes (Regressing Type II) were observed even more infrequently, and only in postspawning fish. Because of their scarcity in the samples, no percentages were calculated. The infrequency of the two types of regressing oocytes indicates that the vast majority of oocytes which reach the vitellogenic phase continue to develop and are released during spawning. Those few which cease to develop or remain in the ovaries after spawning are quickly resorbed.

These data indicate that the development of a fully mature yellowtail flounder egg requires 2 yr. During the first year, which begins after spawning, oocytes pass through the previtellogenic phase which includes oogonia, early perinucleolus, resting, and late perinucleolus developmental stages. In the second year of development, oocytes pass through the vitellogenic phase and are then released. This phase begins as late perinucleolus oocytes, now about 1 yr old, develop into early maturing oocytes during the spring and summer. By the fall of their second year the oocytes have accumulated large amounts of yolk in the cytoplasm and have reached the late maturing stage. Over the following winter and early spring months they continue to accumulate yolk and increase in size. During the spawning season, batches of late maturing oocytes enter the hyaline stage and are then released. This apparently occurs intermittently throughout the breeding season until virtually all late maturing eggs become hyalinated and are released.

At any given time then, there are two populations or year classes of oocytes present within adult yellowtail flounder ovaries. These include a population of small, previtellogenic oocytes which develops over 1 yr, and a second population of larger vitellogenic oocytes which are recruited from the previtellogenic population. Members of the vitellogenic population are in their second year of development and will mature and be released in the upcoming breeding season. Ovaries such as this have been described as "group synchronous" (Wallace and Selman 1981).

The events described in the process of oocyte maturation fit closely with seasonal changes in both GSI and the macroscopic appearance of the ovaries. Maturity Stage II (Developing virgin, recovering spent) ovaries were seen from May through October. These ovaries contained only oogonia, early and late perinucleolus, and early maturing oocytes. The relatively small size of these oocyte developmental stages (<150 μm diameter), and the absence of late maturing and hyaline oocytes, resulted in these ovaries being relatively small and translucent. As expected, in postspawning months when fish with ovaries of this type accounted for >50% of the sample (June-September), mean GSI values were very low. Stage III (Developing, maturing) fish were observed from September through May. Due to the presence of large early and late maturing oocytes, their ovaries were enlarged, yellowish in color, and granular. As the percentages of fish in this maturity stage increased from September to October, mean GSI values increased. From November through at least February, all fish had ovaries in this maturity stage. Mean GSI values rose during this time due to the increasing size of the late maturing oocytes present. Ripe fish (Stage IV) were present from April through June. Mean GSI was highest in April since all fish examined were either still maturing or ripe. The first spent fish (Stage V) were found in early May, and by the end of May 50% of the fish had spawned. Loss of eggs through spawning caused these ovaries to be flaccid and bloodshot, and mean GSI values decreased as the spawning season progressed.

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