Production of Nonviable Oocytes by Pacific Hake (*Merluccius productus*)

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Ripening ovaries of Pacific hake from the Strait of Georgia contained several size-classes of oocytes. Only those from the group of the largest yolked oocytes became hydrated and were eventually released to be fertilized. Examination of ovaries from recently spawned fish showed that smaller, residual yolked oocytes were being resorbed. Yolked oocytes not released at spawning may represent a potential for an increase in fecundity of Pacific hake in the Strait of Georgia. The failure of some developing oocytes to be released suggests that traditional methods of estimating fecundity will not accurately estimate the number of gametes produced by each female. Cytological observations suggested that the number of potential oocytes is not fixed at first maturity.

Key words: Pacific hake, fecundity, oocytes, spawning, Strait of Georgia

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Les ovaires en voie de maturation de merlus du Pacifique capturés dans le détroit de Géorgie contenaient plusieurs classes de taille d'ovocytes. Seuls ceux provenant du groupe qui contenait le plus de vitellus s'hydratèrent et furent ensuite libérés pour être fécondés. Un examen des ovaires de poissons ayant frayé récemment indique que les petits ovocytes avec vitellus résiduaire sont résorbés. Les ovocytes contenant du vitellus qui ne sont pas libérés au moment de la fraie sont une source possible d'augmentation de fécondité du merlu du Pacifique dans le détroit de Géorgie. Le fait que quelques ovocytes en voie de développement ne sont pas libérés donne à penser que les méthodes traditionnelles d'estimation de la fécondité ne donnent pas de résultats précis quant au nombre de gamètes produites par une femelle. Des observations cytologiques suggèrent que le nombre d'ovocytes potentiel n'est pas fixé lors de la première maturité.

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STATE of maturity observations of Pacific hake in the Strait of Georgia during the spawning period revealed that oocytes did not exhibit synchronous development, suggesting that not all oocytes would be released as viable gametes at the same time. This study was undertaken to demonstrate whether the unspawned oocytes were resorbed or continued to develop and become viable in some subsequent spawning period. The term viable is used to identify developing oocytes which are spawned.

Materials and Methods

Pacific hake were collected in the Strait of Georgia (49°N; 123°W) throughout all seasons during 1974–78, by midwater and bottom trawls. Results of cruises and methods of collecting have been described in a series of data records published by the Fisheries and Marine Service (Beamish et al. 1978a; Weir et al. 1978; and others). Ovaries were selected to represent all the maturity states of hake described by Foucher and Beamish (1977). Samples were preserved in Bouin's fixative (1 part glacial acetic acid, 5 parts formalin, 15 parts 1.2% saturated aqueous solution of picric acid) and stored in 95% ethanol. Cross sections, 6 μm in thickness, were cut from the middle portion of the ovary mounted in 61°C paraffin. Sections were stained for 4 min in Harris' haemotoxylin followed by a 30-s counterstain in 0.5% eosin in 95% ethanol.

Oocyte diameters were calculated as the mean of the minimum and maximum diameters. These were measured either from a television screen connected to a closed-circuit, television-microscope system or from photographic enlargements. Cell measurements made using these two methods were compared by measuring the same sample of cells using each technique. The precision of the diameter measurements was checked by having each of two technicians measure cells from one slide. The Kolmogorov-Smirnov two-sample test (Fisz 1963) was used to determine if significant differences existed between the two measurements.

To ensure that measured oocyte diameters would be relatively close to the true oocyte diameters, we measured only oocytes whose nuclei were included in a section (except for resorbing and translucent oocytes whose nuclei were broken down). An empirical test of the error involved in using the diameter of a cell measured from a section as a measure of its true diameter was made by examining serial sections from the same ovary. Cells that had been

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sectioned through the nucleus were measured from one section and their true diameters were determined from adjacent serial sections.

Because the nucleus of resorbing oocytes and the nuclear membrane of hydrated oocytes deteriorated quickly, all resorbing and hydrated (translucent) oocytes were measured with the knowledge that measurement error (underestimation of actual diameter) was increased.

The probability of an oocyte being in a section and it being included in the results (nucleus also in section) is proportional to the diameter of its nucleus which increases much less than the cell's diameter. If cells not sectioned through the nucleus had been included, the probability of a cell being measured would be proportional to its diameter and the larger cells would have been favored. By including only cells showing a nucleus, bias towards larger cells was minimized as the increase in diameter of the nucleus was relatively small.

Results

The accuracy of the diameter measurement was tested by determining the true diameter from serial sections of 41 oocytes. The average underestimate of the true diameter by measuring any cell that contained a nucleus was found to be 5.4%. Thus the procedure of using measurements of only those cells having nuclei to approximate oocyte diameters was considered representative of the true diameter of the oocyte. Because the difference in diameter of cells in distinct modes is commonly 100–200 μm or more this error does not appreciably affect the relative position of the modes. The very small error associated with these measurements also indicates that the nucleus in the fixed cell tends to be at the center of the cell.

A comparison of diameter measurements from photographs and the closed-circuit television (Fig. 1A) indicated there was no significant difference between the two methods, $0.75 < P < 0.90$ (Kolmogorov-Smirnov test). There also was no significant difference in measurements made by two technicians (Fig. 1B) from the same slide, $0.50 < P < 0.75$ (Kolmogorov-Smirnov test).

Approximately 4000 mature female hake were checked for maturity from 1974 to 1978 and ovaries from 313 of these specimens were examined histologically. Oocyte diameters measured from a number of these show several modes (Fig. 2). The smallest mode consists of a large number of oocytes 50–150 μm in diameter and containing no yolk. These cells represent the reserve stock of young oocytes from which, each year, a proportion proceed with further growth and maturation. Expansion of the ovary due to an increase in diameter of the growing oocytes results in the small, nondeveloping oocytes being distributed through a considerably greater volume so that fewer of them occur in a section as the ovary matures. Because of the time required to measure the diameter of the large number of reserve oocytes they were included in only one figure (Fig. 2C). Oocyte measurements from each ovary were arranged according to advancing maturity

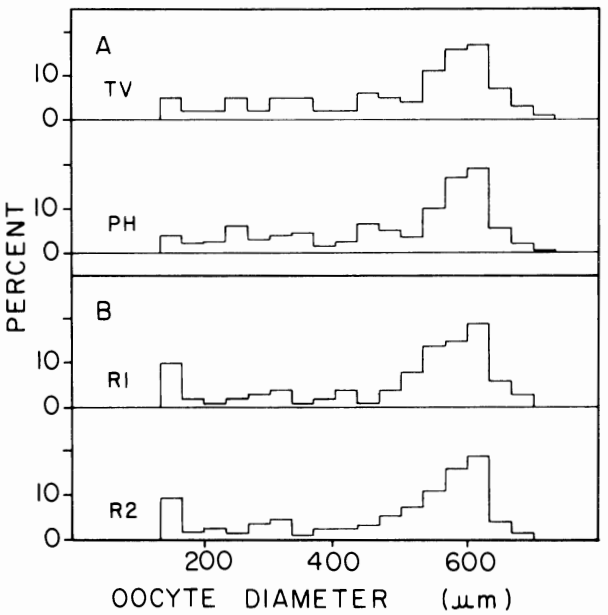

FIG. 1. A, Comparison of frequency (%) of Pacific hake oocytes measured from photographs (PH) and from closed-circuit television (TV). B, Comparison of frequency (%) of Pacific hake oocytes measured by two readers (R1 and R2).

rather than by time of sampling and were expressed as percent. A considerable range in maturity states, assigned macroscopically, was evident in individual samples (Fig. 3) and in some fish development was 2–3 mo later than most of the population (Fig. 2D, H, I).

Ovaries in early stages of development have a mode (150–300 μm) of small oocytes (Fig. 2A to D) which have begun to accumulate yolk. These are cells which have recently grown from the stock of reserve oocytes in preparation for spawning. They continue to increase in size (Fig. 2E to I) until the ripe stage at which there is a distinct mode of cells around 600 μm in diameter (Fig. 2J to Q). Prior to the ripe stage, it appears that asynchronous growth results in the appearance of modes of smaller yolked oocytes (300–450 μm).

Following the ripe stages, the more advanced oocytes hydrate and undergo a great increase in volume and diameter (Fig. 2S). This dilutes their contents causing them to become translucent in appearance. Pacific hake oocytes ready for release average slightly more than 1 mm in diameter and contain an oil globule about one third of a millimetre in diameter. The translucent oocytes, having undergone ovulation in preparation for spawning form a loose mass in the ovary. Gonad maturity data from an extensive collection in the Strait of Georgia during 1974–78 show a change from predominately ripe in late March to spent and recovering in late May (Fig. 3). Spawning begins in March and

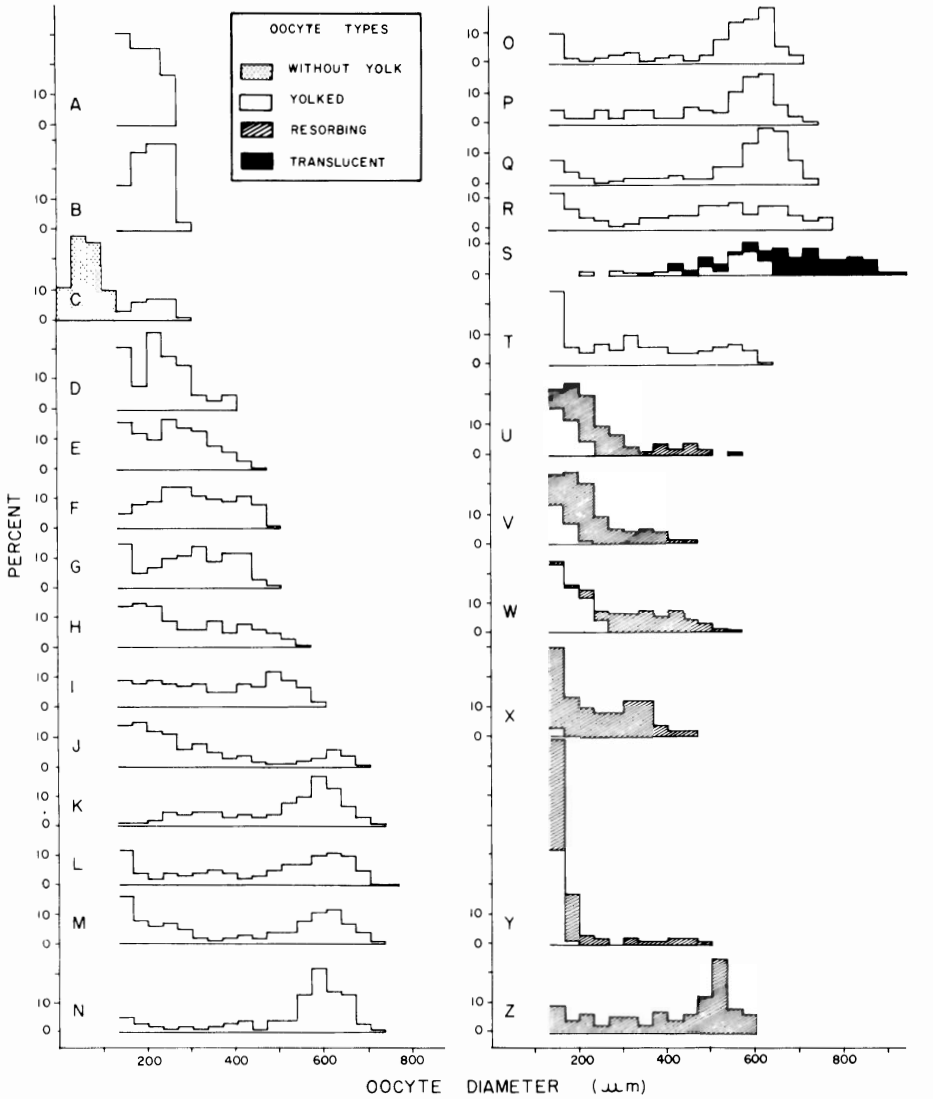

FIG. 2. Frequency (%) of Pacific hake oocytes diameters arranged in approximate order of increasing ripeness. Cells less than 140 μm not included except in C which shows relation of developing oocytes to reserve oocytes in early development. Sample sizes and dates indicated. A, Jan. 16, 1976, $n = 213$; B, Dec. 9, 1975, $n = 90$; C, Same section as B but with reserve oocytes included, $n = 359$; D, Apr. 2, 1976, $n = 39$; E, Jan. 13, 1976, $n = 482$; F, Jan. 16, 1976, $n = 275$; G, Jan. 16, 1976, $n = 99$; H, Apr. 2, 1976, $n = 591$; I, Apr. 2, 1976, $n = 791$; J, Mar. 25, 1976, $n = 563$; K, Mar. 25, 1976, $n = 482$; L, Mar. 30, 1976, $n = 417$; M, Mar. 25, 1976, $n = 542$; N, Mar. 30, 1978, $n = 358$; O, Mar. 31, 1978, $n = 272$; P, Mar. 30, 1978, $n = 368$; Q, Mar. 30, 1978, $n = 333$; R, Mar. 25, 1976, $n = 199$; S, Mar. 25, 1976, $n = 150$; T, Mar. 31, 1978, $n = 260$; U, May 30, 1977, $n = 255$; V, May 30, 1977, $n = 938$; W, May 30, 1977, $n = 328$; X, May 30, 1977, $n = 364$; Y, May 30, 1977, $n = 180$; Z, June 10, 1976, $n = 85$.

peak spawning occurs in April or May. Ichthyoplankton studies indicate that a peak concentration of hake eggs in 1979 occurred in the first week of April (J. C. Mason, personal communication, Pacific Biological Station, Nananimo, B.C.), supporting the spawning period suggested by macroscopic observations of ovaries.

Observations of recently spawned ovaries indicated that a large volume of macroscopically visible, nonhydrated oocytes (400–600 μm in diameter) frequently

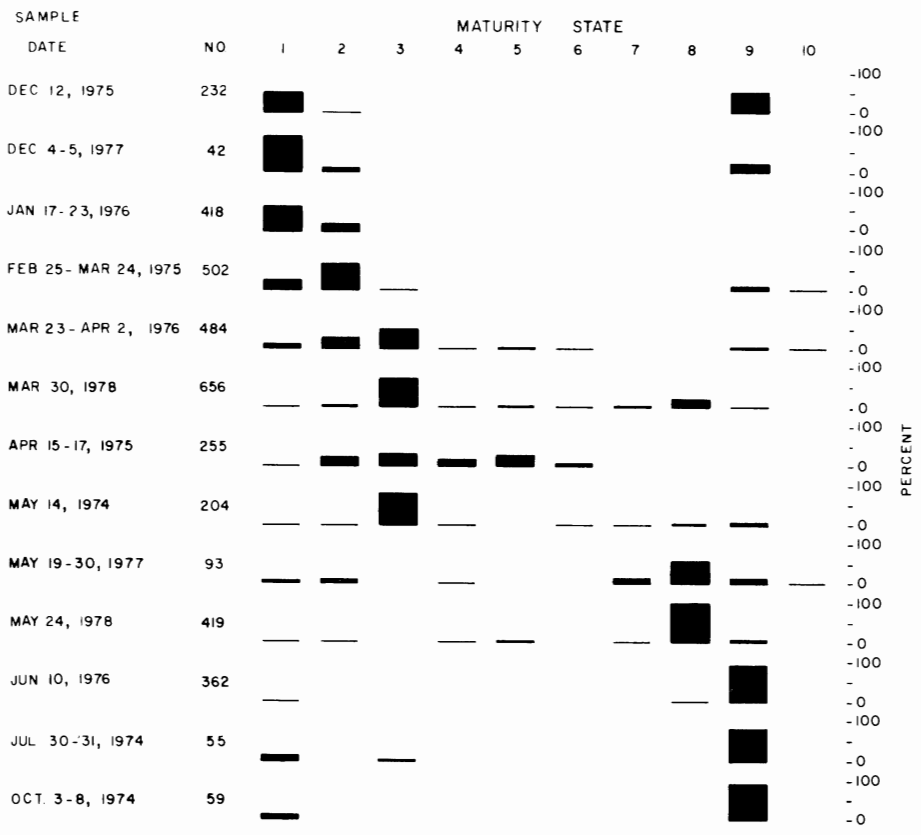

FIG. 3. Pacific hake maturities (%) by sample, 1974–78 Strait of Georgia. Maturity states: 1, early development; 2, advanced development; 3, Ripe; 4, less than one half the oocytes hydrated; 5, more than one half hydrated; 6, sex products flow freely from vent; 7, Spent; 8, Recovering; 9, Resting; 10, Resorbing (resorption without spawning).

remained after spawning (Fig. 2T). These ovaries are similar in appearance to those in early stages of development but may be distinguished microscopically by the few remaining translucent oocytes and numerous empty follicles. Soon after spawning resorption of all remaining yolked oocytes begins. Recovering ovaries examined from fish sampled in May contained many resorbing oocytes of various sizes (Fig. 2U to Y). Resorption of most yolked oocytes and empty follicles is fairly rapid but a few form temporary structures thought to have possible endocrine functions and a role in preventing development for a second spawning (Christiansen 1971).

Occasionally ripe ovaries from fish that had not spawned were collected soon after most of the stock had spawned; these were completely resorbing their ripe ovaries (Fig. 2Z).

Once resorption is complete all that remains is the smallest oocytes, making up the reserve stock for future spawnings. At this point the ovary enters a resting state, common from June to October (Fig. 3). Of 42 ovaries examined that were resting or just beginning development (collected from May to early December), none was found to contain oocytes greater than 225 μm in diameter.

After each follicle bursts at ovulation to release its enclosed oocyte it collapses into an irregular mass and is usually quickly resorbed. In some spent ovaries, however, empty follicles were seen which had shrunk to a smaller size while retaining their spherical shape (Fig. 4A). An apparent increase in the number of cells in the follicle results in a much thickened follicular layer and the formation of large masses of cells, each cell being 4–8 μm in diameter with no cytoplasm, but visible chromatin (Fig. 4B). The obvious concentration of chromosomes in many of these cells suggests that they are in the synaptic stage of early meiosis (Fig. 4C). In some recovering ovaries these 4–8 μm diameter cells, which might be termed follicular oocytes, occupied the greater part of the volume of the ovary (Fig. 4D). Follicular oocytes were seen in spent, recovering, and resting ovaries sampled from March to June and were often associated with the occurrence of empty follicles, the remains of unspawned translucent oocytes, and resorbing, yolked oocytes. A few ovaries sampled in December contained these cells, perhaps due to mixing of stocks or anomalous development of individual fish.

Diameter-frequencies of two resting ovaries sampled in June (Fig. 5A, B) contain two modes. The smaller mode (10–20 μm) appears to be made up of follicular

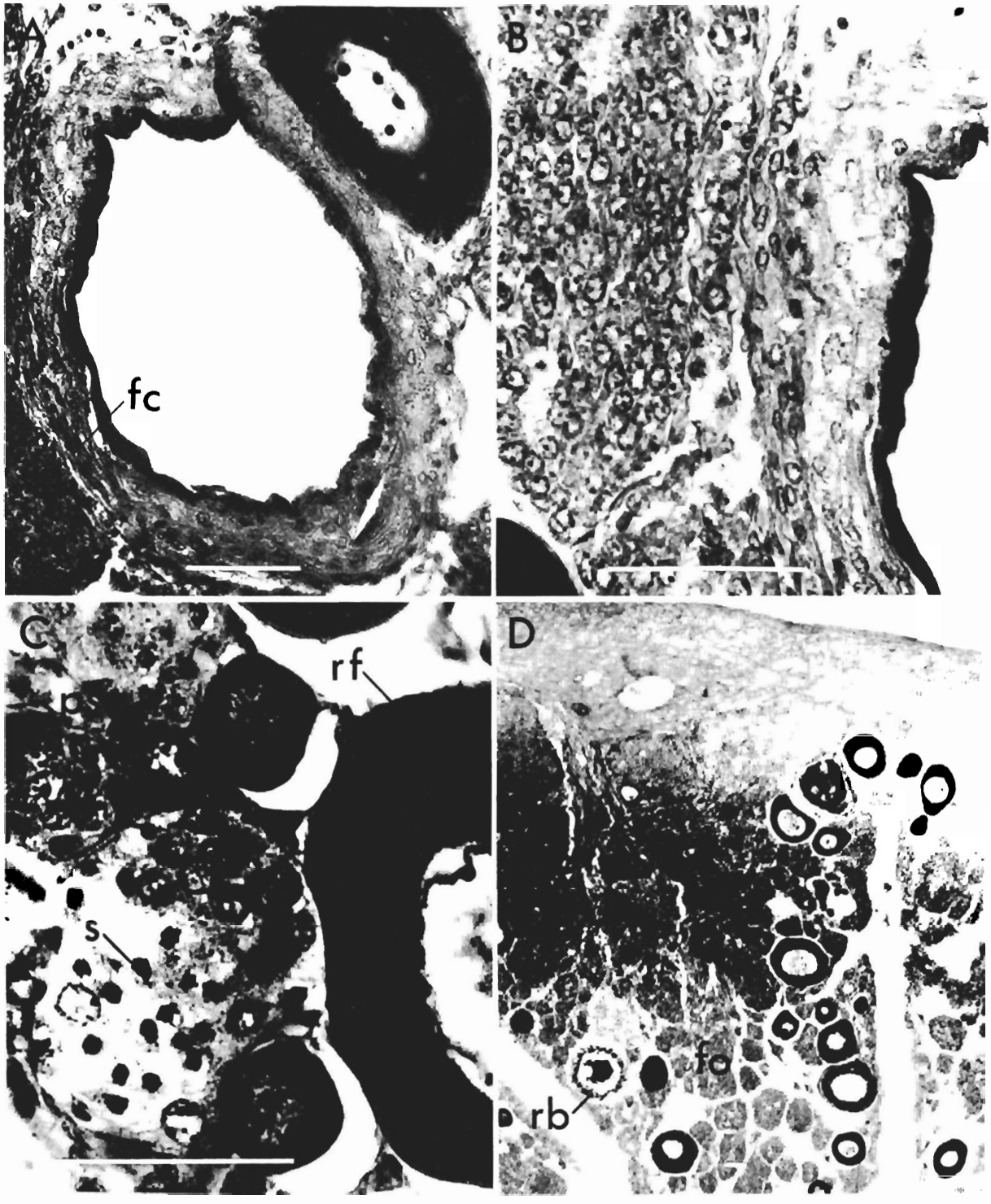

FIG. 4. A, Thickened layer of follicle cells (fc) from empty follicle not undergoing usual collapse and resorption. B, Mass of cells appearing to be derived from cells of empty follicles. C, Oocytes of follicular origin in synaptic stage (s) and post-synaptic oocytes (ps) growing to join reserve cells (rf). D, Groups of follicular oocytes (fo) filling most of ovary which also contains reserve oocytes and a few resorbing structures (rb). Bar = 50 μm.

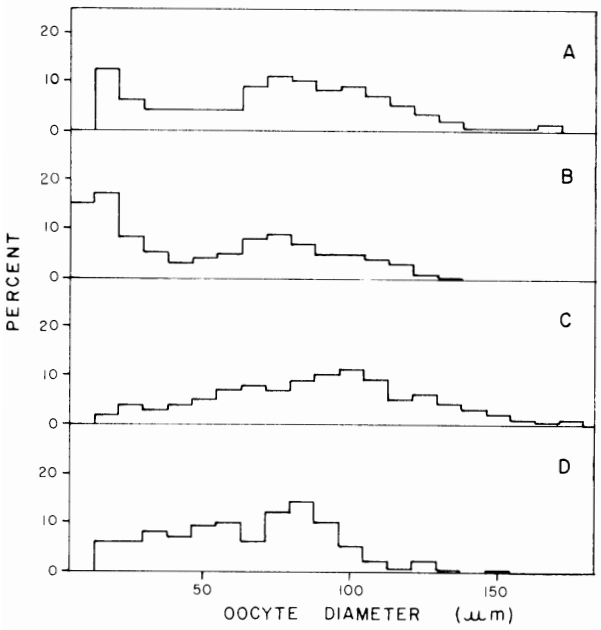

FIG. 5. Frequency (%) of oocyte diameters in undeveloped ovaries. Smallest cells (not included in Fig. 2) were measured at higher magnification. A, resting, June 1976, $n = 279$; B, resting, June 1976, $n = 459$; C, resting, Aug. 1977, $n = 491$; D, immature, Dec. 1975, $n = 507$.

oocytes which are passing, or have passed, through synapsis, one of the early stages of the meiotic process, and are increasing in size to join the stock of reserve oocytes. In a resting ovary sampled in August (Fig. 5C) the smaller cells of the first mode are missing, and presumably have grown to join the mode of larger reserve oocytes from which the next year's crop will later develop.

Discussion

Multiple size modes of oocytes have been interpreted in some studies as indicating multiple spawnings in one year or the presence of "year-classes" of oocytes within the ovary (Nikolskii 1969). Ermakov et al. (1974) concluded that Pacific hake found off the west coast of the northern United States spawn in stages with two clutches of eggs relased each year. They measured oocyte diameters from 45 ovaries collected in November and found that a single mode occurred in 22% of the samples, 65% were bimodal and 6% were trimodal. MacGregor (1966; 1971) also reported that oocyte diameters from Pacific hake collected off California were multimodal. Fish sampled in April, after spawning, had retained their small, yolked oocytes and it was stated that these must be resorbed although no histological evidence was presented. MacGregor concluded that the apparently poor condition of the fish after spawning would make it improbable that these fish could produce the volume of yolk material necessary for the development of the smaller oocytes for a second spawning. Although data on condition were not reported, the apparent condition and lack of any evidence of a second spawning in the plankton resulted in the conclusion that Pacific hake in these regions spawn only once a year. The study of oocyte development of Pacific hake from the Strait of Georgia clearly showed that relatively large numbers of small and large yolked oocytes remained in some ovaries after spawning but they were completely resorbed so that a second spawning did not occur and the remaining oocytes were not "stored" in the ovary for subsequent spawning.

The number of oocytes which are not released and are finally resorbed may be related to the general condition of the Pacific hake stock in the Strait of Georgia. Pacific hake in this area are unexploited and have been noted to be slower growing after maturity than Pacific hake found offshore (Beamish 1979). Increases in fecundity in other species have been related to an improved food supply due to a reduction in stock size from a commercial fishery (Woodhead 1960; Tyler and Dunn 1976). It is possible, then, that a future fishery for Pacific hake in the Strait of Georgia will reduce the stock size, resulting in an increase in the individual production of viable oocytes and a simultaneous decrease in production of nonviable oocytes.

Although it seems probable that individual Pacific hake found off the west coast of North America do not spawn more than once a year, it has been reported (Christiansen and Cousseau 1971) that *Merluccius merluccius hubbsi* in the Argentine Sea spawn more than once a year. While Christiansen and Cousseau (1971) believed that a portion of the summer (October–March) spawning stock spawned again in the winter (June–July), they acknowledged the possibility that they might be studying more than one stock. It was not actually demonstrated that individual hake spawned more than once a year and there is the possibility that more than one stock is involved.

Stocks of Pacific hake in the Strait of Georgia do exhibit variation in spawning times. Some fish from what appears to be a small stock of much larger Pacific hake in the Gulf Islands near Yellow Point (49°03′N, 123°45′W) have been observed to spawn 5–6 mo prior to the main stock (Beamish et al. 1978b) indicating there can be extreme variability of spawning times within a small area. Thus, although there was no evidence in the present study that any individual fish spawned more than once in one year, it is possible that multiple spawnings within the same area occur in one year. Our results show that it is necessary to examine the oocyte development of individuals from the same stock throughout the year before cases of multiple spawnings of individual fish can be confirmed.

The occurrence of large, yolked oocytes remaining in the ovaries of Pacific hake from the Strait of Georgia after spawning may introduce an error (overestimate) in estimates of oocyte production by conventional tech-

niques of evaluating fecundity. Fecundity has been defined as "the number of eggs (oocytes) for the generation of that year present in the ovaries, i.e. the number that should be laid in that year" (Nikolskii 1965). The difficulty with this and similar definitions is that it may not be possible to differentiate the oocytes that are "for the generation of that year" since many may be retained in the ovary after spawning. Absence of an obvious relation between the number of oocytes counted and the number of viable oocytes that are ultimately released indicates that the assumptions used when making fecundity measurements must be clearly identified when applying these measures to Pacific hake and perhaps other species. Fecundity should be defined as the number of oocytes that are actually released to be fertilized, i.e. the number of viable oocytes. Because it is difficult to differentiate and count viable oocytes prior to spawning, studies of fecundity should include, along with the traditional counting methods, a histological examination of spent and recovering ovaries from fish of the same stock to determine the relationship between the proportions of viable and non-viable oocytes. An increase in fecundity might be brought about by an increase in the proportion of developed oocytes that become viable and are released rather than by an increase in the number of oocytes which began developing that season. Since the fecundity methods now in use cannot distinguish viable from non-viable oocytes they might not detect such an increase.

It is not known how the total fecundity over the reproductive lifespan of a fish is controlled. Although various theories exist (Foucher and Beamish 1978), it is not certain whether new gametes are produced to supplement the stock of reserve oocytes after first maturity or if the total number of germ-cells are present at first maturity.

In hake, oogonial divisions are completed before first maturity and the newly mature ovary is full of the resulting resting oocytes (Hickling 1930). Many of these young oocytes grow to form a reserve from which a portion develop for spawning in each year after maturity. Results of this study indicate a growth of young oocytes to join the reserve stock in the mature resting ovary. The question, often raised, is whether the number of resting oocytes present is sufficient for the replacement of oocytes removed annually from the reserve stock for reproduction. Hickling (1930) theorized that there exists a vast number of resting oocytes within the ovigerous lamellae of the newly mature ovary of *M. merluccius* sufficient in number to maintain the reserve stock throughout the fish's reproductive lifespan so that a further production of oocytes is unnecessary. Wheeler (1924) suggested that the reserve stock of oocytes are added to by growth of certain of the follicle cells, known to be sister-cells of the oocytes. Cytological evidence presented in the current study indicates the possibility that, in Pacific hake, the reserve stock is supplemented by cells that are produced from the remains of empty follicles. Observations on time of occurrence of this production of follicular oocytes suggest an annual process. Because it is not always seen in recovering ovaries, it is either a short-term event or it may not occur in all fish each year. Although little is known of the conditions for, or frequency of, occurrence of this process, it is a possible mechanism for a complete restocking of the reserve stock of oocytes. That is, the number of gametes is not fixed at the time of first maturity, but is restocked, perhaps on an annual basis.

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