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The Dynamics of Oocyte Growth during Vitellogenesis in the Rainbow Trout (*Oncorhynchus mykiss*)

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ABSTRACT

This report describes the dynamics of oocyte growth during vitellogenesis in a population of virgin female rainbow trout. Indices of ovarian development increased dramatically during the period of study: the gonadosomatic index (GSI) increased over 50-fold, reaching a peak of 20 just before ovulation; the mean oocyte diameter increased from less than 1 mm to 5.4 mm; and plasma levels of vitellogenin increased from less than 1.5 mg/ml to 25 mg/ml.

There were no changes in the numbers of developing oocytes (measuring 0.5 mm or greater in diameter) from the time when the majority of oocytes undergoing secondary development had entered vitellogenesis in August to ovulation in February (averaging 4000 oocytes per fish). The increase in ovary weight during vitellogenesis was, therefore, due to an increase in the size of oocytes rather than to recruitment of more maturing oocytes. The numbers of vitellogenic oocytes in the ovary during the entire study also suggested that atresia of vitellogenic oocytes does not play a prominant role in determining fecundity.

During early vitellogenesis, the volume of maturing oocytes within an ovary varied by as much as 250-fold. From September onwards, when all oocytes to be ovulated that season had entered vitellogenesis, a gradual uniformity in size began to develop, such that at ovulation, in February, all the eggs were very similar in size (there was less than a 2-fold variation in volume). The pattern of growth of oocytes in an ovary during vitellogenesis suggests that growth between oocytes is closely coordinated.

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INTRODUCTION

Egg production is an annual event imposing considerable metabolic demands on the rainbow trout, *Oncorhynchus mykiss*. Each year, the ovaries grow from less than 0.5% of the body weight up to approximately 20% just prior to ovulation. At ovulation, around 2000–3000 eggs per kilogram of body weight, each measuring approximately 5 mm in diameter, are concomitantly released into the body cavity. The sequence of events surrounding oocyte development can be broadly classified into 6 periods or phases according to the state of oocyte growth: (1) oogenesis, (2) primary oocyte growth, (3) cortical alveolus stage, (4) vitellogenesis, (5) maturation, and (6) ovulation (Vakaet, 1955; Yamamoto et al., 1965; Braekevelt and McMillan, 1967; Hurk and Peute, 1979; Khoo, 1979; Kagawa et al., 1981; Nagahama, 1983; Selman and Wallace, 1986). Although the nomenclature implies that these phases of egg development follow one another, this is not so, and often 2 or 3 phases occur simultaneously in an ovary. In fact, even within an oocyte, it is likely that there are prolonged periods when a number of these growth phases overlap (Selman et al., 1986).

The main growth of the oocyte occurs principally during the vitellogenic phase of development, when an oocyte increases in diameter from approximately 1 mm to 5 mm. Vitellogenesis therefore accounts for over 98% of the final volume of oocytes. This growth is largely achieved through the uptake of extra-ovarian proteins from the blood, predominantly the glycolipophosphoprotein, vitellogenin (VTG; Tyler et al., 1988a,b). During the month or so prior to ovulation, recent evidence suggests that water uptake also contributes to the increase in size of oocytes (Riazi and Fremont, 1988). Despite this pronounced growth, however, little is known about the dynamics of oocyte growth during vitellogenesis.

Rainbow trout are considered to be group synchronous spawners (reviewed by Scott, 1987), in that in the ovary at any one time there are 2 populations or cohorts of oocytes—a population of primary oocytes, from which the later phases are recruited, and a group of "synchronously" developing larger oocytes, which form the season's batch of eggs. The term "synchronous spawner" distinguishes this pattern of ovarian development from that of an "asynchronous spawner," in which oocytes develop and eggs are subsequently ovulated in batches. The literature implies that in the rainbow trout, oocytes, on reaching the vitellogenic phase of development, grow relatively uniformly so that at any time all vitellogenic oocytes are of a similar size and are subsequently ovulated together at a very similar size (Springate et al., 1985). Contrary to this, our observations have indicated considerable disparity in the size of developing oocytes during the early part of vitellogenesis and, thus, an asynchrony in development; it is not until later during vitellogenesis, as the oocytes approach full maturity, that a uniformity in size develops such that eggs are very similar in size at ovulation. In the rainbow trout, it is also unclear

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when the number of oocytes to be ovulated in a season's batch of eggs is determined. It may be at or before the time when oocytes are recruited into the cortical alveolus stage, at the time of their recruitment into vitellogenesis, or during either or both of these phases due to 'de-recruitment' or atresia. Questions of this nature can only be answered with the provision of quantitative data on the dynamics of oocyte growth. This study examined the dynamics of oocyte growth in the ovaries of a population of virgin female rainbow trout throughout vitellogenesis up to ovulation.

MATERIALS AND METHODS

Fish

A single population of female rainbow trout (of the Winthrop strain), maturing for the first time as 3 year olds, were used throughout this study. The fish were maintained under ambient conditions of photoperiod and temperature and fed on a commercially pelleted feed at a rate of 0.5% of their body weight daily. The study was conducted between June (when most of the year's cohort of developing oocytes had recently entered vitellogenesis) and February (the time of ovulation). At 28-day intervals, 8 randomly selected females were killed, blood samples were collected, and the progress of ovarian development (numbers and sizes of maturing oocytes) was determined.

Blood Sampling

Blood was collected from the Cuverian sinus into chilled, heparinized syringes containing 20 TIU/ml of the proteolytic enzyme inhibitor, aprotinin (Sigma Chemical Company, Dorset, Poole, U.K.). After centrifugation, the plasma was withdrawn and deep frozen. At the completion of the study in February, all of the plasma samples were assayed in a homologous rainbow trout VTG radioimmunoassay to determine the levels of circulating VTG (see Sumpter, 1985, and Copeland et al., 1986, for details of the assay).

Preparation of Oocytes for Analysis

At each sampling interval, the body weight and the weights of the left and right ovaries of each female were measured; from these, the gonadosomatic indices were determined (GSI; total ovary weight as a percentage of body weight). The right ovary was placed in Gilson's fixative (Simpson, 1951) in the proportion of 1 part ovary to 2 parts fixative (v/v) to separate the individual oocytes from the tissue matrix. During the period of fixing, samples were shaken frequently to aid fragmentation of the ovary and digestion of the connective tissues. Each sample was fixed for 3 mo, after which time all the oocytes had separated out from the ovary and the connective tissues remained as a fine slurry. The slurry was less dense than the oocytes and was washed to waste with running water. Oocytes below 0.5 mm in diameter (pre-vitellogenic oocytes) and any remaining fragments of connective tissue were then removed by passing the samples through a sieve with a 0.45-mm mesh. The oocytes retained, measuring 0.5 mm and above in diameter, would be expected to include all of those that were vitellogenic (Sumpter et al., 1984; Bromage and Cumaranatunga, 1988).

Assessing the Effect of Fixing on Oocyte Diameter

At each sampling interval, individual oocytes from the left ovaries were fixed separately in Gilson's fluid to examine the effects of fixing on oocyte size. Oocytes were carefully dissected from the fresh ovary, measured to the nearest 0.1 mm under a binocular microscope, fixed in Gilson's for 3 mo, and then re-measured to determine the change in diameter. The amount of shrinkage varied between 8% and 18%. There was no correlation, however, between the initial size of an oocyte and the amount of shrinkage it underwent; thus a mean shrinkage percentage of 10 was calculated for all sizes of fixed oocytes. Before detailed analyses of data on oocyte size were conducted, the diameters of fixed oocytes were multiplied by a factor of 1.1 to obtain an estimate of their size before fixing.

Counting and Sizing of Oocytes

The numbers and diameters of oocytes in the ovaries obtained from females sampled in the first 4 mo of the study (June to September) were analyzed automatically with a purpose-built egg analyzer (Witthames and Greer-Walker, 1987). This method of counting and sizing oocytes or eggs depends upon the passage of oocytes suspended in water through an optical sensor. In this study the sensor was connected to a HIAC Criterion PC-320 particle size analyzer that both counts and sizes the oocytes. The data were then transferred to an ACT1 Sirus microcomputer for storage and processing. The system integrated the oocyte counts into size-intervals of 20 μm, which were then pooled into 100-μm classes to standardize them with the samples analyzed manually (see below). A detailed description of the apparatus and its operating procedures are provided in Witthames and Greer-Walker (1987). The counting accuracy of the apparatus was determined in a series of 10 calibration runs, each using 2000 cellulose-acetate balls as standards. The accuracy was greater than 99%. To assess the accuracy of the apparatus in measuring the size of oocytes, 60 oocytes, varying in diameter between 0.5 and 2.5 mm, were carefully measured to the nearest 100 μm under a binocular microscope (oocytes were often not completely spherical; therefore, diameter measurements were taken as the mean of 2 axes) and run through the analyzer-sensor. The correlation coefficient (r) between oocyte diameters measured manually and automatically was 0.998.

The sensor in the automated egg analyzer had a maximal aperture size of 2.7 mm; therefore, ovaries collected from October onwards (which contained larger oocytes) were analyzed manually. In each ovary collected between October and February, individual oocytes were counted to de-

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termine oocyte numbers. The diameters of oocytes in an ovary and the numbers within a given 100-μm size class were then determined using a system of subsampling. The subsampling apparatus consisted of a 750-ml round-bottomed flask fitted with a detachable central core of glass tubing. Housed within the glass tubing was a plunger fitted with 2 rubber sections set a short distance apart. When the plunger was fully depressed, both rubber sections extended beyond the end of the central glass tubing to about 1 cm from the base of the flask. On withdrawal of the plunger, a 5-ml sample was removed from the flask and trapped between the rubber sections in the glass tubing. The central glass tubing could then be removed and the subsample taken out and analyzed. For each ovary analyzed in this way, the separated oocytes were placed in 500 ml of distilled water and added to the flask. The oocytes were held in suspension in the flask by a gentle shaking motion and the plunger was withdrawn to remove a 1% (5 ml) subsample. The diameters of all of the oocytes obtained thus were then measured to the nearest 100 μm under a binocular microscope as described above. Five 1% subsamples were taken from each ovary and data were pooled. The numbers of oocytes in the given 100-μm size classes in the whole ovary were then determined by multiplying the numbers of the subsample (which made up 5% of the ovary) by 20. The accuracy of the subsampling system in measuring the oocyte size frequencies in the whole ovary was examined in 3 ovaries, one obtained in each month of October, November, and December. In each of these ovaries, all the oocytes were measured manually under a binocular microscope and the resulting numbers of oocytes in the various size classes were compared to the distribution determined for the same ovaries with the subsampling system. In all cases, the numbers of oocytes within a given 100-μm size class did not vary by more than 4% between the two counting methods.

Analysis of the Data

In each fish, the number of oocytes (measuring 0.5 mm in diameter and above) counted in the right ovary was expressed in terms of number per unit of ovary weight; from this equation (the left ovary also had been weighed) the number of oocytes in the left ovary was estimated. The numbers of oocytes in each ovary were then added together. Mean numbers were calculated for each group of 8 fish.

Variability in oocyte diameter within an ovary and between the ovaries of different females was examined by the construction of oocyte size-frequency distributions. Plots for each fish expressed the numbers of oocytes within given 100-μm size classes as percentages of the total number of oocytes (of 0.5 mm in diameter and above) in the ovary. The average growth rate of the developing oocytes in the population was determined by calculating the mean of the most frequent size classes at each 28-day interval.

The variability in oocyte size within an ovary in the population at each sampling point was analyzed by expressing the size of oocytes in an ovary as a percentage of the median size class in that ovary. The data from all 8 fish were then combined in a single figure for each monthly interval. Again, the numbers of oocytes in the size intervals were expressed as percentages of the total numbers of oocytes in the ovary, centered around the median size class. In this analysis the sizes of oocytes were expressed in terms of their volumes (see *Discussion* for reasoning). Oocyte volumes were estimated by using the formula for a sphere ($\frac{4}{3} \pi r^3$).

RESULTS

During vitellogenesis, the mean GSI increased from less than 0.4 in June to 20 at ovulation in February (Fig. 1). Concomitantly, the plasma level of the yolk protein precursor, VTG, increased from approximately 1.5 to 25 mg/ml (Fig. 1). The body weight of the maturing females increased from a mean of approximately 400 g at the beginning of the study to over 1500 g at ovulation.

The mean number of oocytes per fish (measuring 0.5 mm or greater in diameter) varied between approximately 3000 and 5400 during the period of study (Fig. 2). There were statistically significant differences in the number of oocytes between some of the sampling times (analyses not shown), but no significant differences, however, in numbers between the time when the majority of the season's

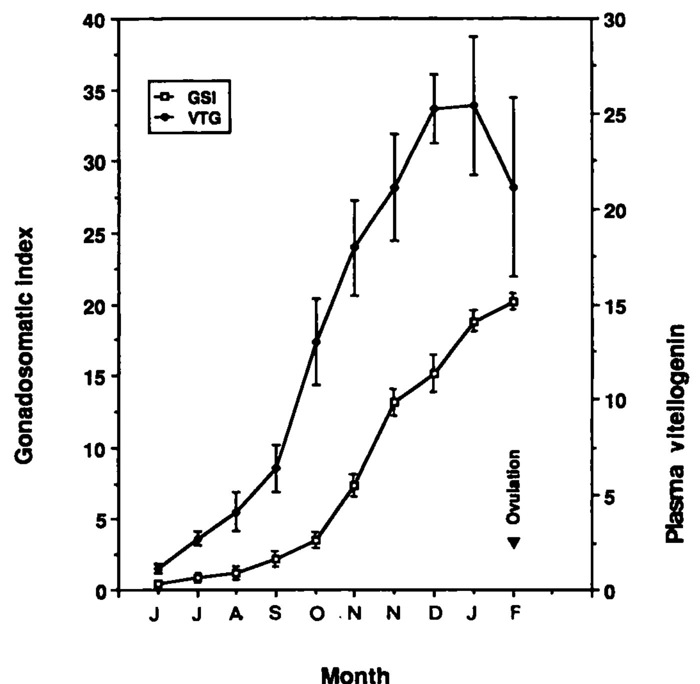

FIG. 1. Changes in the gonadosomatic index and plasma vitellogenin level (mg/ml) in the rainbow trout during vitellogenesis (vertical bars denote SEM). Fish were sampled at 28-day intervals, which accounts for the two samplings in November (one early, one late).

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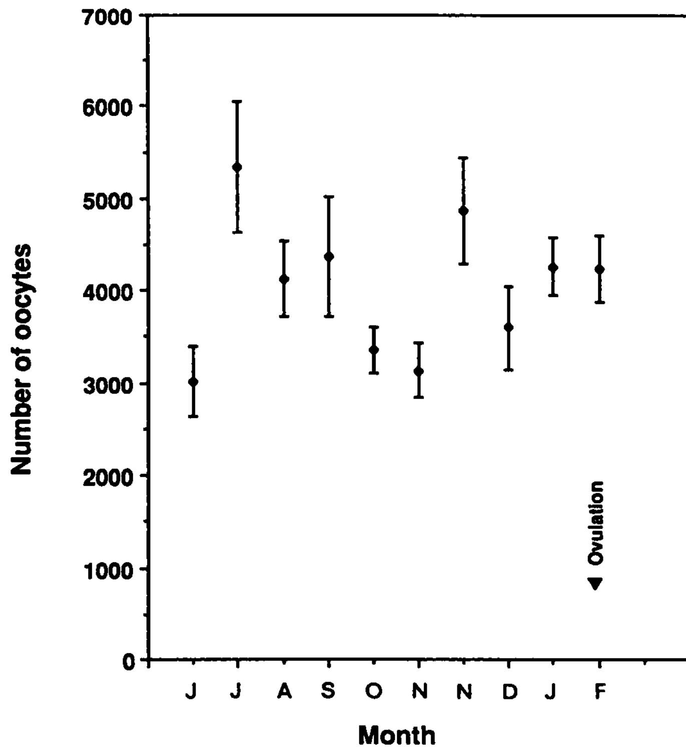

FIG. 2. Total numbers of oocytes (measuring 0.5 mm or greater in diameter) during vitellogenesis in the rainbow trout. Fish were sampled at 28-day intervals, which accounts for the two samplings in November (one early, one late).

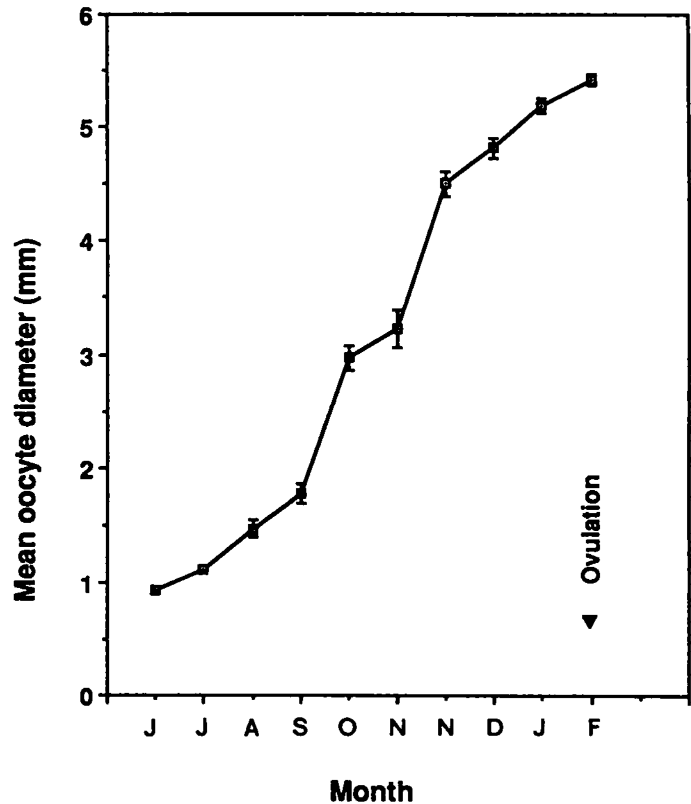

FIG. 3. Changes in the mean diameter of oocytes from female rainbow trout during vitellogenesis (vertical bars denote SEM). Some error bars are within the size of the symbol. Fish were sampled at 28-day intervals, which accounts for the two samplings in November (one early, one late).

batch of developing oocytes had been recruited into vitellogenesis in August and when the oocytes were ovulated 6 mo later in February (Student's t-test, $p > 0.05$; Fig. 2; see *Discussion* for an explanation of the results). At ovulation, 2629 ± 231 eggs per kg body weight were released into the body cavity.

The changes in the mean diameter of the most frequent size class of oocytes are shown in Figure 3. The oocyte diameter increased from 1.0 mm in June to 5.4 mm at ovulation in February. The rate of increase in oocyte size was maximal between September and late November, when the mean diameter of the most frequent size class increased by 2.7 mm, from 1.8 mm to 4.5 mm.

The percentages of oocytes in an ovary within the different size classes are shown in Figure 4. The plots presented are of individual fish and are derived from the median fish at each sampling. Recruitment of oocytes into the cohort to be ovulated that spawning season was completed by the end of September; in the following months, ovaries contained few, if any, oocytes between 0.5 mm and 1.0 mm in diameter. There was more variation in the diameter of oocytes within an ovary in the early part of vitellogenesis than later in development. This variation in size between maturing oocytes within an ovary is discussed more fully below.

The relative abundances of oocytes of varying diameters in the ovaries of all 8 fish sampled in June (beginning of the study), in October (half-way through vitellogenesis), and in February (at ovulation) are shown in Figure 5. This analysis was carried out to investigate the degree of synchrony of ovarian development within the population. In June, there was considerable variation in oocyte diameter within an ovary in all 8 fish, and the largest oocytes ranged between 1.2 mm and 1.4 mm. However, the population was fairly synchronized, the median oocyte size varying only between 0.8 mm and 1.3 mm. Approximately 4 mo later (in October), there was considerable variation in the sizes of vitellogenic oocytes between the ovaries of the different females. In the 8 fish, the smallest oocytes ranged from 1.9 mm to 3.0 mm and the largest from 2.9 mm to 3.8 mm. In contrast, at ovulation little variation was noted between the 8 fish; the smallest oocytes ranged from only 5.0 mm to 5.4 mm and the largest from 5.5 mm to 5.8 mm.

Figure 6 shows the variation in volume of oocytes in the ovaries of all 80 females sampled during the 9-mo period of study. The volume of oocytes in each size class was expressed as a percentage of the volume of the median oocyte size class within that ovary. Each monthly graph is a composite of the ovaries from all 8 fish. During the early part of vitellogenesis (in August), up to a 250-fold difference in volume was noted between the smallest and the largest oocytes. As development progressed, however, this

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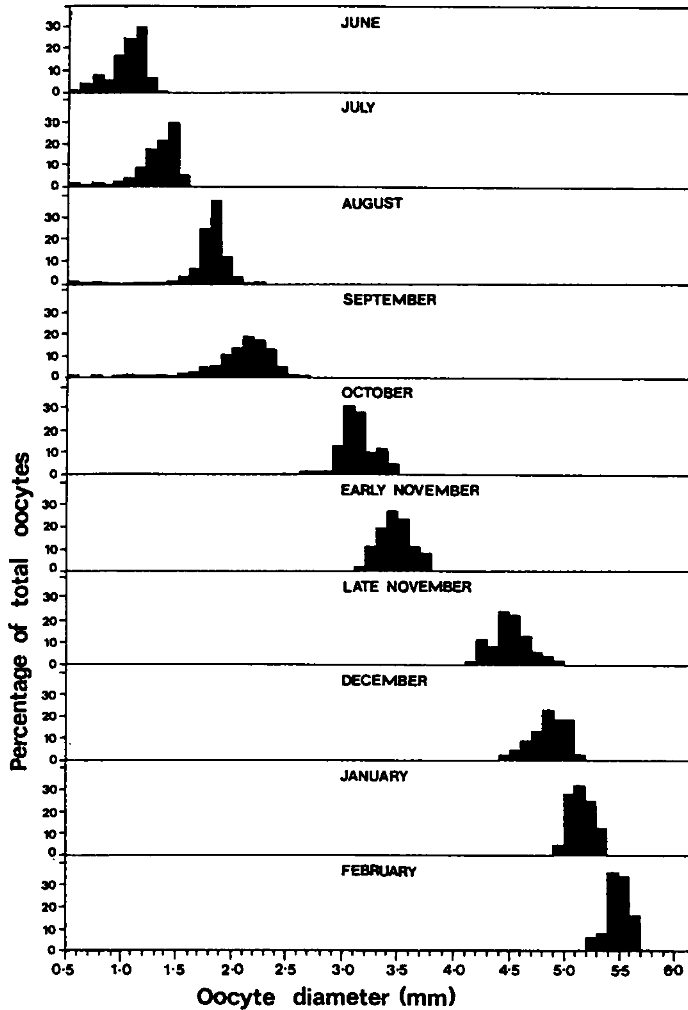

FIG. 4. Changes in the diameter of oocytes (measuring 0.5 mm or greater in diameter) in the ovaries of rainbow trout during vitellogenesis. The number of oocytes in an ovary in each 100-μm-diameter size class is expressed as a percentage of the total number of maturing oocytes in that ovary. The plots presented are the median for each sampling (see *Materials and Methods* for further details).

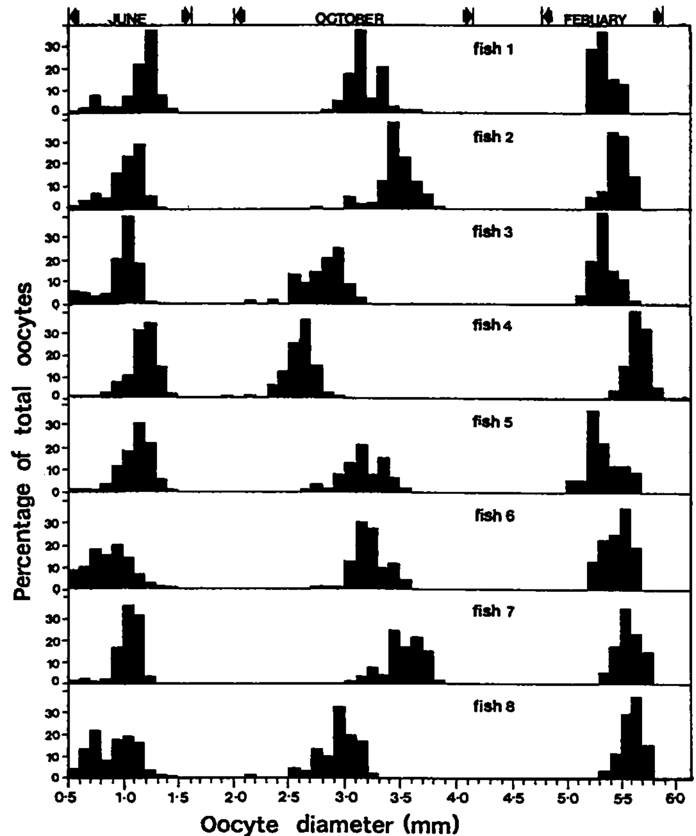

FIG. 5. Variation in the degree of sexual development between females during vitellogenesis. All 8 females sampled are represented: in June, at the beginning of the study; in October, half-way through vitellogenesis; and in February, at ovulation. The number of oocytes in an ovary in each 100-μm-diameter size class is expressed as a percentage of the total number of maturing oocytes in that ovary.

variability was considerably reduced; approximately half-way through vitellogenesis, in early November, this difference was only 5-fold. At ovulation (in February), the ovulated eggs were all of a remarkably consistent size, variation in volume being less than 2-fold.

DISCUSSION

In this study, we examined the dynamics of oocyte growth during vitellogenesis in the rainbow trout. Quantitative assessments were made on both the numbers of growing oocytes present in the ovary and their rates of growth. The synchrony in development of the growing oocytes was also examined, both within an individual ovary and between ovaries of different females in the population.

Both indices of ovarian growth (gonad weight and oocyte size) increased dramatically during vitellogenesis. The GSI underwent almost a 50-fold increase, from 0.4% to 20%, reaching the peak just before ovulation; similarly, the mean oocyte diameter increased from less than 1 mm in June to 5.5 mm at ovulation in February. These rates of growth of the ovary during vitellogenesis parallel those reported in other studies on rainbow trout (Scott and Sumpter, 1983) and indicate that considerable energy must be required to complete this period of reproductive development. The very high concentration of VTG (the major yolk precursor) in the blood (VTG can constitute over half the total plasma protein) clearly reflects the demands of the growing ovary. It should perhaps be pointed out that our study on oocyte growth encompassed both maturation and ovulation; in other teleosts, hydration can account for increases in oocyte/egg size during these final phases of development (Wallace and Selman, 1981). Available evidence suggests that in the rainbow trout water uptake begins during the final stages of vitellogenesis, before onset of maturation (Riazi and Fremont, 1988), and therefore contributes to the increase in oocyte size during this period. We do not know whether there is a period of hydration during or preceding ovulation in the rainbow trout.

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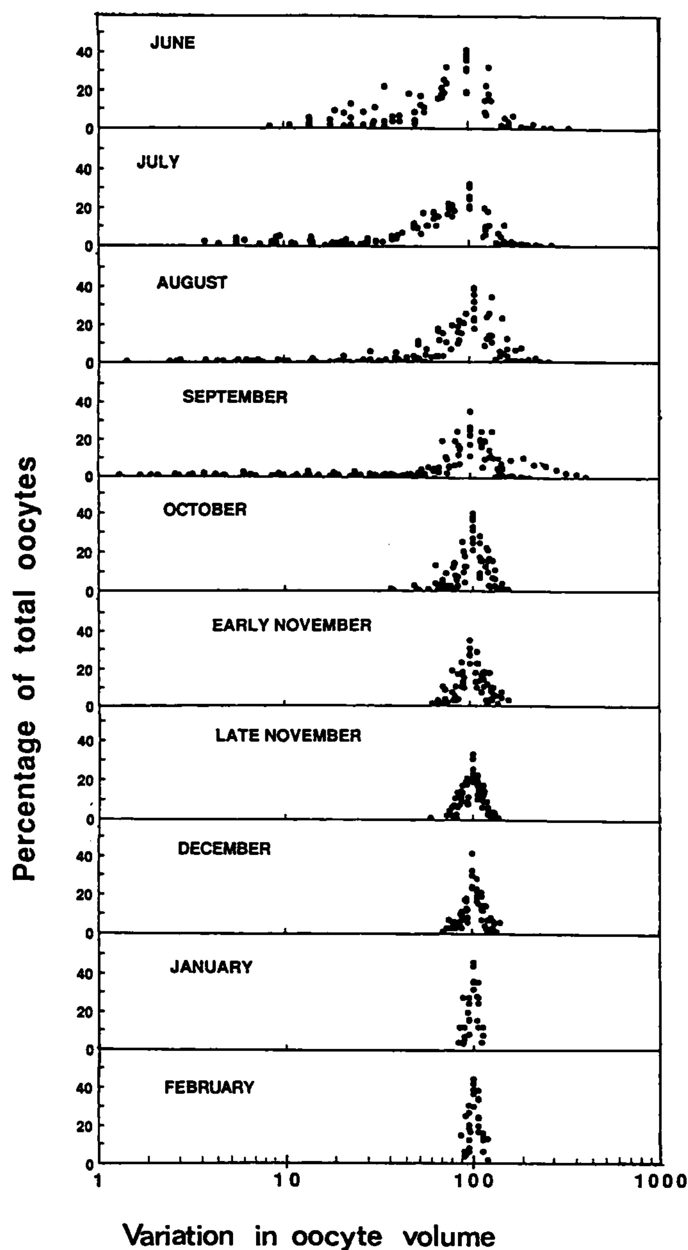

FIG. 6. Variation in oocyte volume in ovaries of rainbow trout during vitellogenesis. The volumes of oocytes in an ovary were plotted as a percentage of the mean oocyte volume in that ovary. Each monthly plot is a composite of the 8 females (see *Materials and Methods* for details of the analyses) centered around the median oocyte size class.

No difference in number of oocytes was detected between ovaries sampled in August, when almost all of the oocytes undergoing rapid growth had entered vitellogenesis, and ovaries sampled at ovulation in February, 6 months later (average: 4000 per fish). Earlier, in June, ovaries contained only approximately 3000 oocytes per fish, probably an underestimate of the number of oocytes that would have matured that reproductive year. It should be noted that we counted only oocytes that measured 0.5 mm or greater in diameter; in June, all oocytes that would provide the season's batch of eggs probably had not yet reached this size. By the next sampling time (July), nearly all the oocytes forming the season's batch of eggs were larger than 0.5 mm in diameter; hence, all were counted. The growth of the ovary during vitellogenesis was due, therefore, almost entirely to an increase in the size of the oocytes and not to an increase in their numbers. These data suggest that the processes of oocyte selection (including the parameters that determine fecundity in the rainbow trout) operate at stages of oocyte development before the onset of vitellogenesis—a full 9 months before ovulation.

In higher vertebrates, oocytes undergo degeneration, a process referred to as atresia (Ryan, 1981), at any stage of development; this plays a major role in determining the numbers of oocytes that are recruited into the successive stages of development and therefore determines the number of developing oocytes that eventually form mature eggs. Furthermore, it is suggested that atresia commonly occurs in all vertebrate groups (Byskov, 1978; Saidapur, 1978). A number of studies on atresia in teleosts have been published (reviewed by Saidapur, 1978), but in general these have been concerned with the cellular events and the physical characteristics of atretic oocytes rather than the numbers of regressing oocytes occurring at the various stages of sexual maturity. A quantitative study on the incidence of atresia in the rainbow trout by Bromage and Cumaranatunga (1988), however, led these authors to conclude that atresia plays a significant role in ovarian development, particularly in determining fecundity. They found an apparent decrease in the numbers of oocytes throughout the successive stages of oocyte development, including vitellogenesis, right up to ovulation, and they linked the reduction in number of maturing oocytes to atresia. Similarly, Tam et al. (1986) reported levels of atresia of up to 37% of the total oocyte complement during both the cortical alveolus stage and vitellogenesis in brook trout (*Salvelinus fontinalis*). Both of these studies were conducted on captive fish. In a study on a population of wild brook trout, Vladykov (1956) also indicated that oocyte atresia was an important process during ovarian development. In contrast to their observations, in this study we found no apparent decrease in the number of developing oocytes from the time when nearly all the oocytes had entered vitellogenesis (September) and ovulation in February, indicating little, if any, atresia. Continual recruitment could not compensate for atresia because, as we show, recruitment into the cohort of maturing follicles ceases shortly after vitellogenesis has begun. We should point out, however, that our study measured only the number of oocytes (no histological studies on developing ovaries were conducted) and the variability we observed in oocyte numbers on a month-to-month basis is large enough to hide a low incidence of atresia.

It is unclear why we found little (if any) atresia in our study (albeit that our evidence is indirect) whereas a number of other studies have suggested that atresia is wide-

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spread during vitellogenesis. One possibility may relate to the condition of the fish. Fish maintained well are likely to show less atresia than fish held under suboptimal conditions (Baganel, 1960). Indeed, Ball (1960) suggested that, contrary to the hypothesis that atresia has an important role in controlling fecundity, its incidence is largely caused by environmental stress. Our fish grew very rapidly throughout the study (they increased in body weight from a mean of 400 g in June to over 1500 g at ovulation, in February), indicating that they were maintained in good condition. In accordance with our study, Wallace and Selman (1979) suggested that atresia is probably an uncommon event in healthy fish and that it does not play a major role in follicular selection or derecruitment in normal ovarian physiology during vitellogenesis. It should be pointed out, however, that it is difficult to compare the low incidence (if any) of atresia seen in our study in captive salmonids to that in wild salmonids, which are subjected to greater environmental pressures and nutritional constraints during their reproductive development. In wild salmonids, as indicated by Vladykov (1956), oocyte atresia may well be an important process in ovarian development.

Another important factor in assessing the degree of atresia relates to the difficulty in distinguishing between an atretic follicle that failed to be ovulated at the previous spawning, even though it reached full size, and a developing oocyte that becomes atretic before it reaches full size. As noted earlier, our fish were spawning for the first time as 3-year-olds, and therefore would not have contained any oocytes that failed to be ovulated the previous year and were undergoing atresia. Some female trout do, however, mature for the first time as 2-year-olds, and then subsequently spawn annually. When we initially set aside our fish for the study, we found some mature 2-year-old fish, which we finclipped after their first spawning. We held these fish in our experimental population and sampled a few of them when they matured for the second time as 3-year-olds, and compared their ovaries to our virgin fish. We found that their ovaries contained considerable numbers of degenerating oocytes (between 700 and 2000 per fish), which originated from their first spawning, as well as the expected batch of growing oocytes. These degenerating oocytes from the previous season's spawning were easily distinguished from the current year's batch of growing oocytes during the early part of the study because they were much larger than the new batch of growing oocytes. Later in the year, however, these two groups of oocytes could not be distinguished on size alone. It is possible that some of the incidences of atresia reported in studies using nonvirgin female trout were degenerating oocytes from the previous sexual cycle.

In Figure 4, it can be seen that there is a tail of oocytes leading to the main mode in the first 4 months of the study, but not thereafter. This change in the distribution pattern of the smaller oocytes may be attributable (at least in part) to the change in the method of counting and sizing of oocytes between September and October rather than to a sudden disappearence of the smaller oocytes (samples up to and including September were counted with the automated system whereas the October samples onward were counted manually). Even if the smaller oocytes recorded in September had begun to undergo atresia, it is likely that they would still have been recorded in the manual sampling in October. Although the oocytes were carefully separated from the ovarian connective tissues, a very small percentage of stromal debris remained in the samples; this could easily be distinguished and ignored when oocytes were counted manually but may have been recorded and sized in the samples analyzed with the automated system. Stromal debris therefore may account for some of the smaller "oocytes" forming the tail of the frequency distributions between June and September. Nevertheless, we should emphasize that these anomalous "oocytes" represented only a few percent of the total and do not affect the results significantly.

In the majority of studies on oocyte development (at least in fish), the sizes of oocytes are expressed in terms of their diameters. Accordingly, the first part of our analysis on oocyte development expresses oocyte growth in terms of increases in oocyte diameter. However, volume increase provides a more meaningful measurement of growth than the diameter increases because it measures changes in mass. This is more useful because the volume of an egg provides a more direct measurement of the yolk reserves it contains (and that will sustain the embryo) and hence is a more physiologically relevent expression of size. Therefore, in our analysis of the dynamics of oocyte growth in an average ovary, we used oocyte volumes rather than diameters. Figure 6 shows that there was a broad spread of oocyte sizes during the early part of vitellogenesis; for example, the smallest and largest oocytes in an ovary varied in volume by up to 250-fold. It was not until late September/early October onwards, when all the required oocytes had been recruited into vitellogenesis, that a gradual uniformity in oocyte size began to develop, such that at ovulation in February all the growing oocytes were of a very similar size (varying in volume by less than 2-fold). The way in which oocytes that are very different in size during early vitellogenesis (see Fig. 6) eventually achieve a very similar size at ovulation is not known. Uniformity in size as oocytes approach ovulation cannot be achieved if the larger oocytes reach their maximal size earlier than the smaller oocytes and then remain quiescent until the smaller oocytes catch up, because, if this were the case, the distribution of oocyte sizes in the later part of vitellogenesis would be skewed to the right, and this clearly is not so (see Fig. 4). Thus, there appears to be coordination in growth rates between oocytes. How this growth between oocytes in an ovary is coordinated is not known. Specific growth factors may be involved in this regulation, but none have been positively identified, although their existence has been suggested (Tata, 1986). Di-

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rect evidence that vitellogenic oocytes within an ovary do not grow at the same rate in the rainbow trout comes from Tyler et al. (1990), who have shown that the rate of uptake of VTG (the major yolk precursor) into similarly sized oocytes varies both between different sites within the ovary (by up to 30%) and between the left and right ovaries (by up to 38%) of an individual fish. Kime et al. (1989), working on carp, *Cyprinus carpio,* suggested that local factors, as well as systemic signals, may bring about the synchronous maturation of oocytes. This theory would also help to explain the dynamics of oocyte growth during vitellogenesis in the trout ovary.

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