Ovarian cycle and plasma concentrations of estrogen and vitellogenin in brook trout (*Salvelinus fontinalis*, Mitchill)

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Oocyte development and plasma concentrations of estrogen and vitellogenin in brook trout have been investigated. Oocyte development and vitellogenesis resumed immediately after spawning in October, but, up until May, oocytes never developed beyond 1000 μm diameter and yolk deposition only resulted in the appearance of a relatively modest number of fine droplets and globules. A peak occurrence of atretic follicles of about 900 μm diameter was observed in March. In May, many cortical alveoli stage oocytes were seen developing from perinucleolar oocytes. From June to October, oocyte growth and vitellogenesis occurred at a faster rate. By the time of ovulation, the oocytes were about 2500 μm in diameter and became filled with a homogeneous mass of yolk. Throughout winter and spring, plasma level of estrogen was 1–5 ng/mL and that of vitellogenin was mostly below 5 μg/mL of alkali-labile protein phosphorus. In October, the plasma concentrations of estrogen and vitellogenin increased to 40 ng/mL and 96 μg/mL, respectively.

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Le développement des ovocytes et les concentrations plasmatiques d'estrogène et de vitellogénine ont été suivis chez l'omble de fontaine. Le développement des ovocytes et la vitellogenèse recommencent immédiatement après la fraye en octobre, mais jusqu'en mai, les ovocytes n'atteignent jamais plus de 1000 μm de diamètre et la production de vitellus est limitée et ne donne qu'un petit nombre de gouttelettes et de globules de petite tille. C'est en mars que les follicules atrésiques sont le plus nombreux et ils mesurent environ 900 μm. En mai, plusieurs ovocytes périnucléolaires se transforment et atteignent le stade d'ovocytes à alvéoles corticales. Entre juin et octobre, la croissance des ovocytes et la vitellogenèse subissent une accélération. Au moment de l'ovulation, les ovocytes ont environ 2500 μm de diamètre et sont remplis d'une masse homogène de vitellus. Durant tout l'hiver et le printemps, la concentration plasmatique d'estrogène est de 1 à 5 ng/mL et la concentration de la vitellogénine est en général de moins de 5 μg/mL de phosphore protéinique labile en solution alcaline. En octobre, les concentrations plasmatiques d'estrogène et de vitellogénine augmentent jusqu'à 40 ng/mL et 96 μg/mL, respectivement.

[Traduit par la Revue]

Introduction

Of the salmonids, most aspects of female reproductive physiology such as oocyte development, ovarian endocrine activities (van den Hurk and Peute 1979), and plasma levels of gonadotropin, estrogen, and vitellogenin (Bromage *et al.* 1982; van Bohemen and Lambert 1981) have only been thoroughly studied in rainbow trout. The picture is less complete in other species. For example, in brook trout, although the dependence of reproductive activities on photostimulation (Henderson 1963), the ability of some steroids to cause germinal vesicle breakdown (Duffey and Goetz 1980), and the thermal requirement for gamete and embryo development (Hokanson *et al.* 1973) have been documented, reproductive endocrinology in this commercially important species remains mostly unknown. The purpose of the present investigation is to study oocyte development and the changes in interstitial cell histology in brook trout throughout the year and to correlate these results with their seasonal changes in plasma levels of estrogens and vitellogenin. It is hoped that the results will shed more light on the reproductive endocrinology of salmonids.

Materials and methods

Animals

Maturing brook trout (434) were purchased from Goossens Trout Farm (43°0′ N) from June 1981 to May 1982 and from Linwood Acres Hatchery (43°30′ N) between June 1982 and August 1983. The Goossens fish were hatched in late December 1979 and the Linwood trout were all hatched in December 1980 In both locations, the fish were maintained in unheated buildings with water pumped from subterranean sources (pH 7.7–7.9, alkalinity total inflection point, 190.8 mg/L; minimal temperature, 6°C in February; maximal temperature, 10°C in September) and illuminated with natural lighting. The trout were fed daily with a commercial trout chow containing 40% protein (type 83G, Martin Feed Mills Ltd., Elmira, Ont.). The trout were sacrificed on location by stunning with a sharp blow to the head. Blood was obtained by caudal sectioning. The plasma fraction was collected after centrifugation and was stored at −30°C until used for the determination of vitellogenin and estrogen concentrations. The gonads were fixed in 4CF-1G diagnostic fixative (McDowell and Trump 1976) and gonadal weight was obtained after fixation. Depending on size, 10–97 oocytes of average size were taken from each fish, dissected free of extraneous tissue, and weighed to obtain the mean oocyte weight of the trout.

Histological technique

Most of the ovarian tissue was embedded in paraffin wax, sectioned at 10 μm, and stained with Gomori's trichrome. Each ovarian section contained approximately 40 cortical alveoli stage and vitellogenic oocytes. Because of their considerable size, the October sections contained only about 17 oocytes. The oocytes were divided into different types according to their state of development (see Results). To assess their rate of growth, the diameters of some of the largest oocytes of each type were measured with an ocular micrometer as the mean of two diameters set at right angles to each other. The histology of every type of oocyte was observed. However, as was frequently the case, many oocytes were not sectioned through the middle, but through a small peripheral region, making it difficult to distinguish between primary and early secondary vitellogenic stages and between chromatin nucleolar and perinucleolar stages. To avoid possible errors and to estimate the rate of progression through the major developmental stages, all oocytes of the different vitellogenic stages were counted and pooled together as a single vitellogenic group. The chromatin nucleolar and perinucleolar stages were pooled together in another category and the cortical alveoli stage oocytes were assigned to a third group. The proportions of these groups on each microscopic section were

TABLE 1. The proportion of different types of oocytes and atretic follicles in brook trout that have gone through at least one breeding season

	All vitellogenic stages (%)	Cortical alveoli stage (%)	Chromatin nucleolar and nucleolar stages (%)	Atretic follicles (%)
Linwood Acres				
Jan.	38.77±5.69(6)	42.81±5.56(6)	20.43±5.63(6)	3.94±3.52(6)
Feb.	37.46±10.46(4)	30.30±16.65(4)	18.58±4.06(4)	27.52±10.79(4)
Mar.	28.37±5.46(5)	17.84±4.73(5)	26.73±4.80(5)	37.31±9.93(5)
Apr.	36.34±9.03(4)	35.69±6.84(4)	20.72±6.02(4)	30.51±10.86(4)
May	52.74±7.73(7)	28.79±5.27(7)	18.47±4.58(7)	0(7)
Aug.	72.40±7.32(4)	17.34±7.72(4)	10.26±3.67(4)	0(4)
Sept.	66.12±3.15(4)	20.82±3.54(4)	13.07±2.79(4)	0(4)
Oct.	64.67±4.21(4)	26.22±6.01(4)	9.12±1.97(4)	0(4)
Nov.	29.08±6.54(9)	43.49±9.95(9)	27.78±5.08(9)	0(9)
Dec.	38.12±4.08(10)	32.92±3.19(10)	28.95±4.37(10)	0(10)
Goossens				
Jan.	55.21±6.52(3)	16.67±1.93(3)	19.36±2.35(3)	5.34±1.27(3)
Feb.	41.97(2)	41.52(2)	16.52(2)	0(2)
Mar.	39.11±6.28(6)	22.08±2.35(6)	25.79±4.23(6)	13.03±3.04(6)
May	31.12±4.81(3)	27.65±3.44(3)	41.22±5.50(3)	0(3)
July	58.49±7.67(4)	13.38±2.97(4)	25.36±3.41(4)	2.78±2.78(4)
Aug.	79.78±7.32(4)	13.33±5.52(4)	5.10±3.24(4)	1.79±1.79(4)
Oct.	88.69±2.15(3)	4.17±4.17(3)	7.14±4.13(3)	0(3)
Dec.	16.91±3.32(3)	38.48±2.65(3)	42.86±2.86(3)	1.75±1.75(3)

NOTE: Values are means ± SE, with the number of trout examined in parentheses.

calculated and tabulated in Table 1. Frozen sections (14 μm) of the fixed tissue were also made. The lipids were stained with Sudan black B and the section was counterstained with carmalum.

Determination of alkali-labile protein phosphorus

The amount of vitellogenin in 0.4 mL plasma was determined in duplicate as alkali-labile protein phosphorus according to the method of Wallace and Jared (1968) and the result was expressed as micrograms phosphorus per millilitre plasma. When an equal volume of male trout plasma was used, no alkali-labile portein phosphorus was detected.

Radioimmunoassay of estrogens

Antiestrogen serum was raised in male rabbits by repeated intradermal injection of 1 mg of 1,3,5,(10)-estratrien-3,17β-diol 17-hemisuccinate: bovine serum albumin (BSA) (Steraloids Inc., Wilton, NH) suspended in 0.5 mL saline and 1 mL of Freund's complete adjuvant (Gibco Laboratories, Grand Island, NY). The antiserum was used at 1 : 10 000 dilution and 0.05 mL of this diluted antiserum would specifically bind 30% of a given amount of [^{3}H]estradiol-17β (see below). Cross-reactivity was calculated as the ratio of the amount of any steroid to the amount of estradiol-17β necessary in each case to produce a 50% displacement of a given quantity of labelled estradiol-17β from the antibody (Orczyk *et al.* 1978). These values are as follows: estrone, 122.86%; estriol, 34.75%; 20α-hydroxy-4-pregnen-3-one, 0.05%; 20β-hydroxy-4-pregnen-3-one, 0.09%; aldosterone, 0.03%; cortisol, 0.03%; corticosterone, 0.02%; progesterone, androstenedione, testosterone, 17α-hydroxyprogesterone, and pregnenolone, all <0.0002%.

After approximately 1000 cpm of [^{3}H]estradiol-17β (specific activity, 104 Ci/mmol; Amersham Corp., Canada) was added as internal standard for the calculation of recovery, trout plasma (0.25 mL) was extracted with 2 × 2.5 mL ethyl acetate. After the ethyl acetate was evaporated under nitrogen, the residue was redissolved in a mixture of 0.125 mL ethanol, 2.5 mL benzene, and 2 mL petroleum ether (boiling point, 60–80°C). The phenolic estrogens were then extracted from the organic phase with 3 × 2 mL of a 0.4 mol/L solution of NaOH (Brown 1955), leaving the neutral steroids, lipids, and trimethylamine oxide in the benzene and petroleum. The NaOH solution was adjusted to pH 7.0 with glacial acetic acid and the estrogens were extracted with 3 × 3 mL ethyl acetate. The ethyl acetate was dried under nitrogen and the residue dissolved in the assay buffer (phosphate buffered saline with 8.30 mmol/L EDTA and 0.45% dialyzed BSA, pH 7.6). A suitable aliquot was taken for scintillation counting to estimate recovery. Extraction efficiency was 57.22 ± 1.21% (253). Either a one-tenth or one-fortieth aliquot of the plasma extract was removed in duplicate in 0.05 mL of assay buffer for radioimmunoassay (RIA). Approximately 15 000 cpm of [^{3}H]estradiol-17β in 0.05 mL assay buffer and 0.05 mL of the diluted antiserum were also added. The total volume in each assay tube was finally brought up to 0.2 mL with further addition of assay buffer. After being mixed by a vortex for 1 min, the assay mixture was incubated for 16 h at 4°C. Unbound antigen was sedimented after the addition of Dextran-coated charcoal suspended in 0.5 mL of assay buffer and centrifugation. The bound [^{3}H]estradiol-17β contained in 0.5 mL of supernatant was removed and counted in a Beckman liquid scintillation counter (model LS 255). After the specific binding of [^{3}H]estradiol-17β was calculated, the amount of estrogen in each assay tube was read off from a standardization curve constructed on the same day with known amounts of authentic estradiol-17β. Plasma estrogen concentrations were calculated to 100% recovery. When authentic estradiol-17β was added to male brook trout plasma to a concentration of 5 ng/mL, extracted, and assayed as outlined above, the assayed concentration was 5.28 ± 0.29 (6) ng/mL. When the 5 ng/mL male plasma extract was assayed at four different dilutions and the expected values (x) plotted against assayed values (y), the correlation line ($0.92x = y$, with 43 pairs of coordinates) passed through the origin and the correlation coefficient was significantly different from zero ($R = 0.8841$, $p < 0.01$). The intraassay and interassay coefficients of variation were 10.70 ± 1.43% (6) and 13.26% (6), respectively. The limit of sensitivity was 0.014 ± 0.002 ng (8). The method of RIA validation and quality control calculations were based on those of Rodbard (1974). Because of the cross-reactivity of the three common estrogenic steroids, the plasma estrogen concentrations were reported as nanograms estrogen per millilitre.

Statistical treatment

The results are expressed as mean ± SEM, with the number of trout used or the number of determinations in parentheses. Differences

TABLE 2. The number of oocytes per unit body weight and mean oocyte weight

	Age (months)	Linwood Acres		Goossens	
		No. of oocytes per gram body weight	Mean oocyte weight (mg)	No. of oocytes per gram body weight	Mean oocyte weight (mg)
June	18	6.7±0.4(10)	2.76±0.33(10)	6.1±0.6(12)	1.96±0.29(12)
July	19	6.3±0.8(5)	1.96±0.41(5)	5.5±0.6(5)	6.16±0.61(5)
Aug.	20	6.6±0.4(5)	6.73±0.73(5)	5.6±0.3(6)	11.02±0.63(6)
Sept.	21	6.6±0.2(9)	14.60±1.56(9)	—	—
Oct.	22	6.7±0.3(8)[a]	21.35±0.89(8)[b]	4.9±0.4(5)[a]	35.11±1.72(7)[b]
ANOVA		$p>0.5$		$p>0.05$	

NOTE: Values are means ± SE, with the number of trout examined in parentheses.
[a]Student's t-test, $p<0.01$.
[b]Regression analysis, $p<0.001$.

between means were calculated either by Student's t-test or analysis of variance (ANOVA). The significant difference between mean oocyte weight of the fish from the two hatcheries (Table 2) was calculated by comparing the slopes of their regression lines. Significant difference was taken at the 5% level.

Results

Ovarian cycle

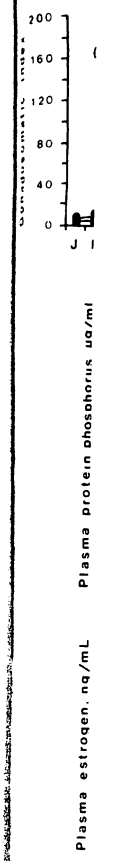
The gonadosomatic index (expressed as ovarian weight in milligrams per gram body weight) is given in Fig. 1*a*. Although the body weight of the Goossens fish (283–362 g) was heavier than that of Linwood fish (134–262 g) of the same age (18–22 months), the histological events of the ovaries were qualitatively identical. Their developing oocytes were divided into the stages described in detail by Yamamoto *et al.* (1965) and Wallace and Selman (1981). These were the chromatin nucleolar stage, perinucleolar stage, cortical alveoli stage, the vitellogenic stages, and finally the maturation stage. The vitellogenic stages consisted of the primary, secondary, and tertiary stages, according to whether yolk deposition occurred as droplets and globules, large polygonal masses, or as a homogenous mass filling the entire oocytes. Detailed changes in the maturation stage, such as the formation of the micropyle and the extrusion of the first polar body, were not studied in this investigation. The annual ovarian cycle was divided into the following phases.

November

This was the immediately postovulatory period and the brook trout used were all 23 months old. However, according to their ovarian histology, the brook trout could be subdivided into two categories. There were the ones whose ovaries contained no postovulatory follicle and in which the most advanced germ cells were the cortical alveoli stage oocytes. The largest of these measured about 500 μm in diameter (Fig. 1*b*). Numerous postovulatory follicles were found in the ovary of the other brook trout. Besides the corticol alveoli stage oocytes, a considerable number of oocytes in the early primary vitellogenic stage, having fine yolk droplets adjacent to the nucleus, were found (Table 1). A few more advanced primary vitellogenic oocytes were also seen. The yolk droplets in these oocytes had already condensed into peripheral yolk globules. In both types of fish, oocytes in the chromatin nucleolar (115.7 ± 8.0 μm (23) diameter) and perinucleolar (185.3 ± 14.7 μm (17) diameter) stages and slightly larger oocytes at the beginning of the cortical alveoli stage were frequently observed.

December–May

The brook trout used throughout this period were 24–29 months old. Again, up until May, postovulatory follicles could be found in the ovary of some but not all of the fish. In ovaries where postovulatory follicles were not found, cortical alveoli stage oocytes began to develop into the primary vitellogenic stage in January. The yolk droplets gradually increased in number and spread out to every part of the cytoplasm. In March, they began to condense into larger yolk globules in the peripheral cytoplasm (Fig. 2) and 2 months later, yolk globules could be found in most areas of the ooplasm (Fig. 3). In the ovaries where postovulatory follicles were found, yolk globules first appeared in December and they completely filled the oocyte as early as March. Once these primary vitellogenic oocytes became filled with yolk globules and reached a diameter of about 1000 μm, they apparently remained in this state until May (Fig. 1*b*). However, the number of oocytes that were filled with yolk globules probably changed. From November to January, the proportion of primary vitellogenic oocytes increased slightly and then decreased somewhat before it began to increase again in May (Table 1). During this period, the height of the membrana granulosa of the vitellogenic oocytes also underwent a similar pattern of wax and wane. It grew from a compact 5-μm layer to a vacuolated, 10-μm layer in February and then shrank back to 5 μm again in April and May (Fig. 4). In the larger yolky follicles, glandular cells were occasionally found amongst the otherwise typically fibroblastic cells of the thecal layer. These resembled the special thecal cells described by van den Hurk and Peute (1979) in rainbow trout. Throughout December–May, numerous interstitial cells could be seen in the brook trout ovary and they were characterized by their crenated nuclei and extensively vacuolated cytoplasm (Fig. 5). The postovulatory follicles were gradually resorbed and, by May, this criterion for distinguishing the two types of ovaries had vanished. Of the 42 fish examined between November and January, only 12 atretic follicles were found. However, the number of atretic follicles (Fig. 6) increased dramatically in March to 13% in the Goossens fish and 37% in the Linwood trout (Table 1). The size of the atretic follicles was fairly uniform and of the ones measured their diameter was 893.4 ± 70.5 μm (32), close to the size of the fully developed primary vitellogenic oocytes. These atretic follicles were not derived from the unovulated oocytes of the previous year, as they were found in ovaries where postovulatory follicles might or might not be found. Their zona radiata was also about the same thickness (10 μm) as that of the primary vitellogenic oocytes and not the 25-μm layer of the mature oocyte. In March, the number of chromatin nucleolar and perinucleolar stage oocytes



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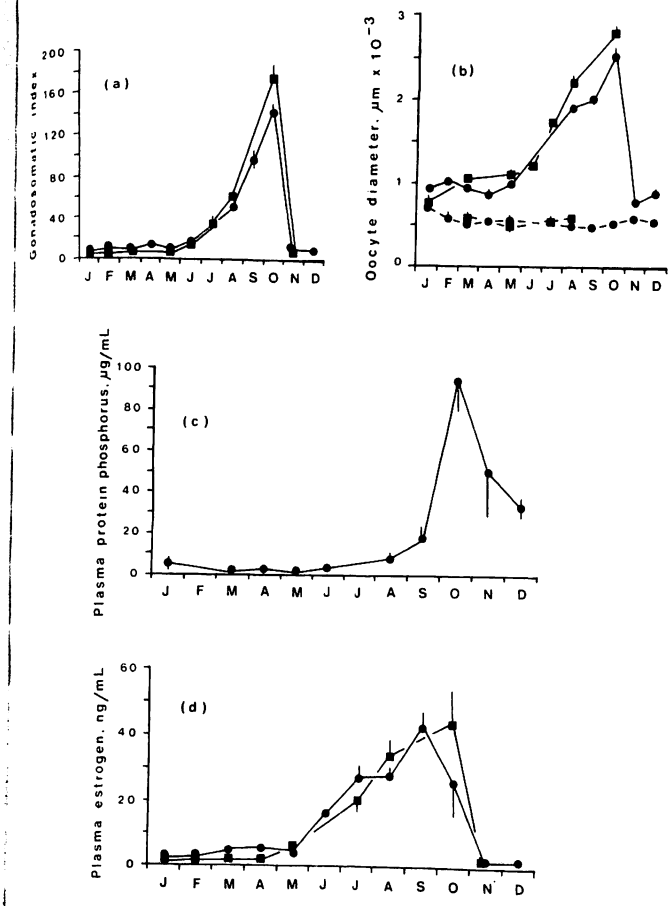

FIG. 1. Seasonal changes in various aspects of reproductive physiology in mature female brook trout. ●, brook trout from Linwood Acres Hatchery; ■, brook trout from Goossens Trout Farm. (*a*) Gonadosomatic index expressed as milligrams ovarian weight per gram body weight. (*b*) Diameter of vitellogenic stage (———) and cortical alveoli stage (- - -) oocytes. For the vitellogenic oocytes, the graph represents the maximal diameter of primary vitellogenic oocyte in November–May, of secondary vitellogenic oocytes in June–August, and of tertiary vitellogenic in September–October within the available histological materials. (*c*) Plasma vitellogenin concentration expressed as micrograms per millilitre protein phosporus. (*d*) Plasma estrogen concentration. Every point of the graphs represented the mean of 3–19 observations.

increased and in April and May a considerable number of perinucleolar stage oocytes were seen developing into the cortical alveoli stage (Fig. 3).

June–August

The brook trout used for observation during this period were either 18–20 or 30–32 months old. The younger fish could again be divided into two subgroups. In one subgroup, the most advanced oocytes remained in the cortical alveoli stage with a diameter of about 400 μm throughout this period. In other fish, the primary vitellogenic oocytes (1100 μm in diameter) already present in June developed into the secondary vitellogenic stage by rapid increase in size (Fig. 1*b*) and by the growth of the yolk globules into much larger polygonal masses (Fig. 7). This was also the manner in which the oocytes progressed in all the 30- to 32-month-old brook trout. By August, the secondary vitellogenic oocytes were about 2000 μm in diameter. During this period and within ovaries in which vitellogenic oocytes were found, the proportion of cortical alveoli stage oocytes decreased from about 30% to 13–17%, while vitellogenic oocytes increased from 50% to almost 80% (Table 1). The granulosa cell layer (Fig. 7) enveloping the vitellogenic oocytes again increased from 5 to 10 μm. Vacuoles appeared and, by July, the cytoplasm took on the appearance of a coarse network. From June onward, the special thecal cells became more or less detached from the thecal layer, making them indistinguishable from the nearby interstitial cells. The nuclei of the interstitial cells became rounded and the large vacuoles found in winter and spring were replaced by numerous fine vacuoles (Fig. 8). Only a few atretic follicles were seen from June to August. Relatively fewer chromatin nucleolar and perinucleolar stage oocytes were observed. Young developing cortical alveoli stage oocytes were also hardly encountered.

September–October

The brook trout used were 21–22 months old. Again, there were some fish in which the ovary contained oocytes no more advanced than the cortical alveoli stage. With the other fish, these 2 months were a continuation of the rapid growth phase that occurred between June and August. The secondary vitellogenic oocytes were rapidly transformed into the tertiary vitellogenic stage with further increase in size of the oocytes (Fig. 1*b*). Oocyte diameter of the Goossens fish was larger than that of the Linwood fish. This probably reflected the heavier ova of the brook trout from Goossens Trout Farm. The yolk content developed into a homogeneous mass filling the entire oocyte. The granulosa and interstitial cells remained almost unchanged compared with the situation in August. Ovulation occurred between the middle and the end of October. Within this age group and of the Linwood fish only 38 out of 60 females had eggs of ovulatory size and ovulated, but 28 out of 30 females from Goossens were sexually mature at the same age. By comparing the slopes of the regression lines, the mean oocyte weight of the Goossens trout was significantly heavier than that of the Linwood trout (Table 2). The total number of oocytes in a fish was estimated by dividing either the entire mass of the ovulated eggs or the ovarian weight with its own mean oocyte weight. The results (Table 2) showed that within the same hatchery there was no significant difference (ANOVA) between months, but at the time of ovulation, the number of oocytes per unit body weight in the heavier Goossens fish was significantly lower than that of the Linwood fish.

Plasma vitellogenin levels

Sufficient quantities of plasma for vitellogenin assay was available only from the Linwood Acres brook trout and, between June and October, only the plasma samples from fish with an ovarian weight to indicate ovarian maturation for the approaching season were used. The results of the assay are given in Fig. 1*c*. Despite the fact that there were two age groups (18 and 30 months old) in the June samples, the standard error of the mean for that month was very small. This probably showed that age at this range was not an important deciding factor on vitellogenin level. From June to September, vitellogenin concentration increased very slowly from 3 to 18 μg/mL phosphorus, but it made a dramatic five-fold increase to 96 μg/mL in October. After ovulation, plasma vitellogenin level decreased and by the following January it went down to the basal level of 5 μg/mL.

Plasma estrogen levels

Again, between June and October, the only plasma samples used for the radioimmunoassay were those from fish with active oocyte development as indicated by the ovarian weight. The results of the assays are plotted in Fig. 1*d*. From November to May, estrogen concentrations were low and did not exceed

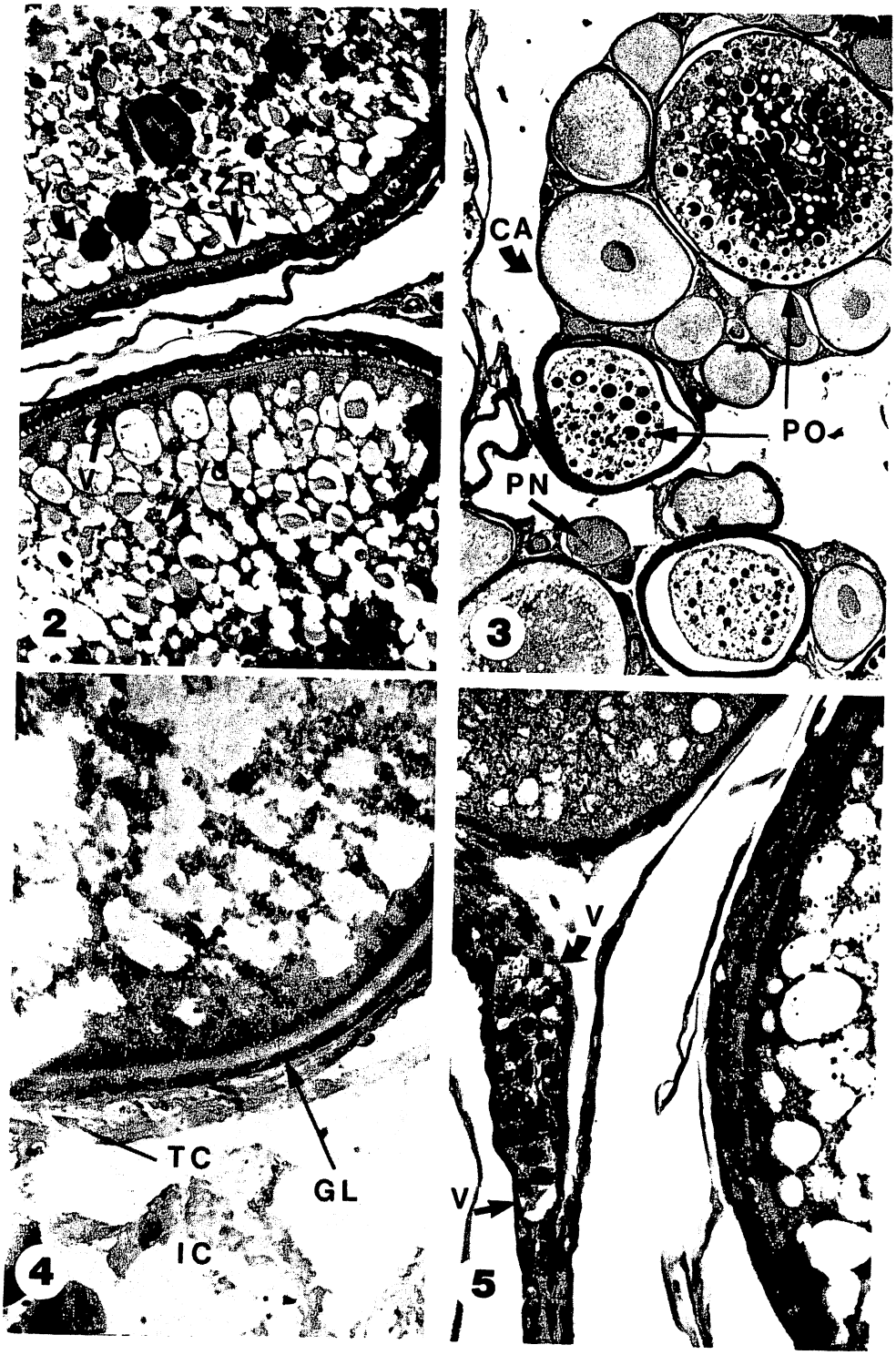

FIG. 2. Ovary in March. Both primary vitellogenic oocytes in this photomicrograph contained a relatively small number of fine yolk droplets (*yd*) and the larger yolk globules (YG). The thecal layer was not prominent, but the large vacuoles (V) in the hypertrophied granulosa cells were a common feature at this time of the year. ZR, zona radiata. Gomori's trichrome. ×145. FIG. 3. In May, in addition to the primary vitellogenic oocytes (PO), numerous chromatin nucleolar stage, perinucleolar stage (PN), and cortical alveoli stage (CA) oocytes could be found. Gomori's trichrome. ×33. FIG. 4. Ovary in April. The granulosa layer (GL) had almost reduced back to 5 μm thick and very fine lipid droplets could be found in the cytoplasm. Lipids were not detected in the thecal (TC) and interstitial cells (IC) at this time of the year. Yolk granules reacted positively with Sudan black. Sudan black B. ×300. FIG. 5. Throughout winter and spring, large vauoles (V) were found in all interstitial cells. The nuclei in many interstitial cells were also crenated. December ovary stained with Gomori's trichrome. ×290.

5 ng/mL. After May, plasma estrogen levels began to rise at a steady rate and reached a maximum of about 40 ng/mL in September and the first half of October. During the latter part of October, plasma estrogen concentration began to fall among the trout obtained from Linwood Acres even though none of them had ovulated. After ovulation and spawning in November estrogen levels fell precipitously to 5 ng/mL. It is worthy of note that despite differences in body and ovum weight between the trout from the two hatcheries plasma estrogen concentrations and seasonal patterns were virtually identical.

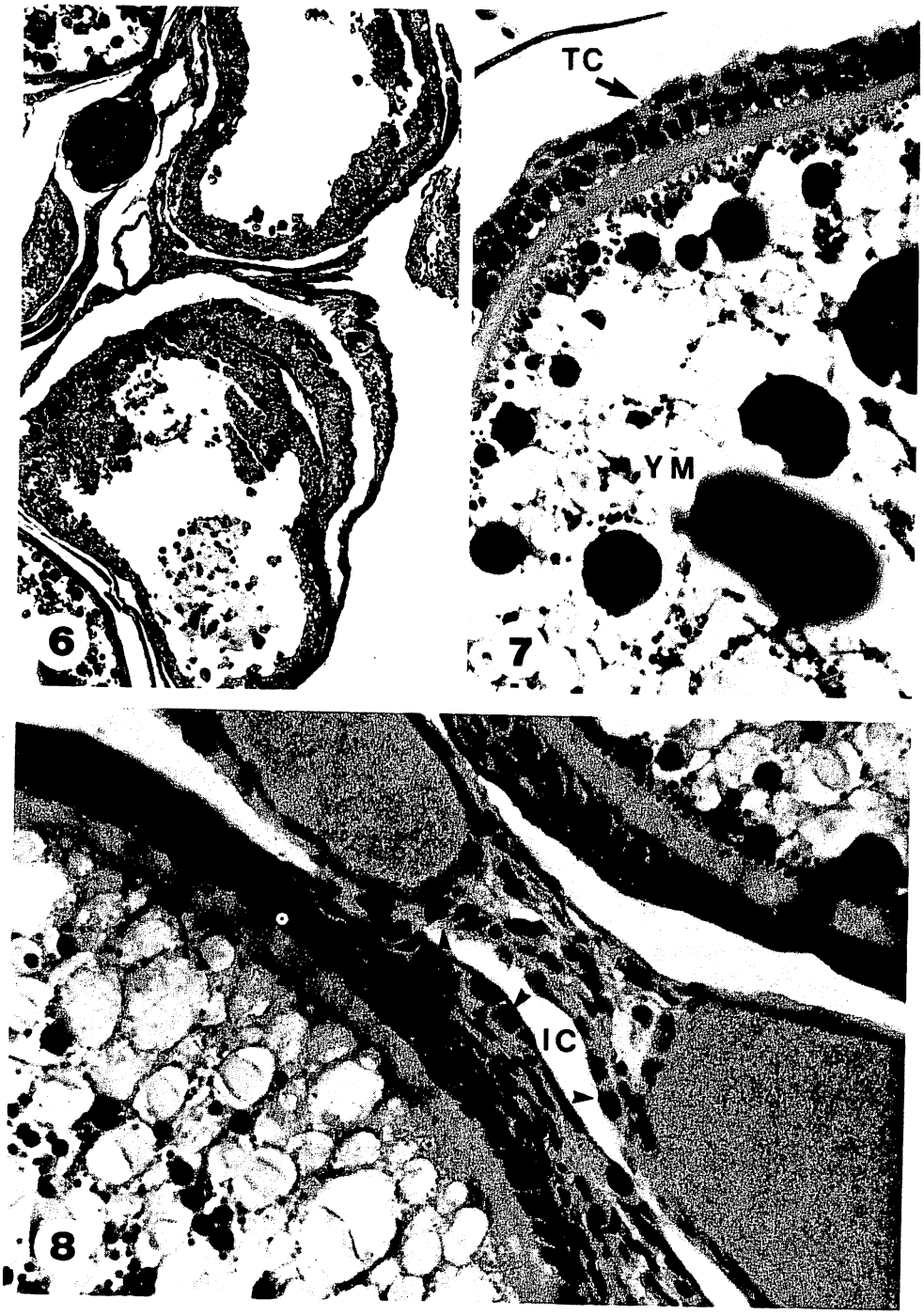

FIG. 6. Atretic follicles in March. Atretic follicles were characterized by their lack of content and by their thick, multilayered wall that could no longer be divisible into granulosa and thecal layers. Gomori's trichrome. ×78. FIG. 7. Large irregular masses of yolk (YM) began to appear in July in the cytoplasm of rapidly growing secondary vitellogenic oocytes. The granulosa cells became hypertrophied and vacuolated once more. The thecal layer appeared conspicuous and some of the thecal cells (TC) acquired large amounts of cytoplasm. Gomori's trichrome. ×330. FIG. 8. Interstitial cells in June. From this time of the year until spawning in October, the interstitial cells (IC) were more healthy looking than in winter and spring. Now their nuclei were no longer crenated and their large cytoplasmic vacuoles had disappeared. Gomori's trichrome. ×320.

Discussion

The results show that the ovarian cycle of the brook trout from the two hatcheries is essentially the same. It is very likely that nutritional status is responsible for the heavier fish, larger eggs, and higher proportion of mature females in the 1+-year-old brook trout obtained from the Goossens Trout Farm. However, when estimated as the product of body weight and number of oocytes per unit body weight, individual fish from both hatcheries ovulate the same number of eggs. It is also obvious that a very large proportion of hatchery brook trout becomes sexually mature when they are 1+ years old and all of them become mature at age 2+ years. For the 1+-year-old fish that fail to mature, the oocytes will remain in the cortical alveoli stage until the following reproductive season. For those that become mature in the previous year, the postovulatory follicles will remain until fully resorbed in the following May. Some of the young oocytes recruited at the end of the previous year also appear to continue on with their development into the following year. The recruitment of chromatin nucleolar stage to primary vitellogenic stage apparently goes on at all times of the year.

The results also seem to suggest that whenever female brook trout become sexually mature, two major waves of oocyte development occur in a year. The first wave occurs from November to March. The chromatin nucleolar stage and perinucleolar stage oocytes that are found in relative abundance in November probably develop into the cortical alveoli stage and primary vitellogenic stage oocytes observed during December–March. The proportions of vitellogenic oocytes and atretic follicles increase at the expense of oocytes at the cortical alveoli stage. However, at this time of the year, the vitellogenic oocytes never develop beyond 1000 μm in diameter and it was probably their degeneration that gave rise to the peak of atresia in March. Follicular atresia at this stage of oocyte development has also been reported in hatchery rainbow trout (van den Hurk and Peute 1979). Even the growth and subsequent reduction in the size of the granulosa cell layer suggest that many of the oocytes developed in winter may have been replaced in the spring by a new generation of oocytes. However, the crenated nuclei, large vacuoles, and lack of lipid did not suggest any secretory activity in the interstitial cells during this time of year. The work of Vladykov (1956) and Henderson (1963) were based on macroscopic examination of the gonads and therefore the histology of early oocyte development and their subsequent atresia were not observed. Both workers, however, agreed that the maximal oocyte diameter throughout winter and spring was 1000 μm.

The second wave of oocyte recruitment probably begins in April and May when numerous perinucleolar stage oocytes are seen developing into young cortical alveoli stage oocytes. These newly formed oocytes, probably together with the survivors of the previous wave of oogenesis, enter into the rapid growth phase that occurs between June and October. It is during this period that vitellogenic oocytes increase both in proportion and number, since the number of oocytes and body weight must increase simultaneously to maintain a constant ratio (Table 2). Unlike the last wave of oocyte development, rapid growth and vitellogenesis this time is accompanied by morphological changes in the granulosa cells, special thecal cells, and interstitial cells indicative of cellular activity. Maximum plasma estrogen levels in brook trout are comparable to those reported for rainbow trout (van Bohemen and Lambert 1981; Scott and Sumpter 1983). It is difficult to compare vitellogenin levels reported by various workers because of the widely different methods used for vitellogenin assay; but, in this report, it is evident that there is good correlation between plasma vitellogenin levels and yolk deposition in oocytes. However, it is of interest to note that in brook trout, maximum plasma levels of estrogen and vitellogenin occur simultaneously with maximum oocyte development immediately prior to ovulation in October. In rainbow trout, maximum plasma estrogen and vitellogenin concentrations have been reported to occur 1–3 months before the oocytes achieved their maximal size (Lamber *et al.* 1978; van Bohemen and Lambert 1981) and before ovulation (Bromage *et al.* 1982; Scott and Sumpter 1983).

The development of the oocytes and the seasonal changes in granulosa and interstitial cell histology and in plasma vitellogenin and estrogen concentrations provide a good indication of the annual reproductive endocrine changes. As in rainbow trout (van den Hurk and Peute 1979), a certain amount of yolk formation can take place during the sexually inactive part of the year. In brook trout, oocyte development and yolk formation is relatively slow in November–May, when blood concentrations of both estrogen and vitellogenin are low. There is, of course, extensive atresia in February, March, and April that cannot be attributed to fluctuations in food supply and temperature. These hatchery fish are fed and maintained at a fairly constant temperature throughout the year. The only ovarian cellular activity that can be associated with the formation of yolk droplets and globules during this time of the year is that of the granulosa cells. Yolk formation and oocyte growth occur at a much faster rate between June and October when circulating levels of vitellogenin and estrogen are high. This wave of activity can be correlated not only to the increased activity of the granulosa cells, but also to those of the special thecal cells and interstitial cells. It has been suggested that of the two pituitary gonadotropic hormones, vitellogenic hormone stimulates the uptake and deposition of vitellogenin as yolk in the oocytes (Ng and Idler 1983) and maturation hormone enhances estrogen production and oocyte maturation (Idler and Ng 1983). Estrogen, in turn, stimulates vitellogenin synthesis by the liver. Thus, it is very possible that basal levels of these gonadotropins are secreted by the pituitary from November to May, allowing a modest amount of yolk formation to take place. Without higher levels of gonadotropins, many of the primary vitellogenic oocytes formed earlier on in the annual cycle would undergo atresia in February–April. When higher levels of gonadotropins were eventually available throughout May–October, especially that of the maturation hormone, yolk deposition and oocyte maturation occur cumulating in ovulation in October. From the rather clear relation between the appearance of yolk in the oocyte and granulosa cell activity, it appears that one of the main functions of the granulosa cells in brook trout is directly associated with yolk deposition. The close correlation between interstitial and special thecal cellular activity and elevated plasma levels of estrogen in the summer and fall suggests that these cells are involved in steroid synthesis.

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BOHEMEN, CH. G. VAN, and J. G. D. LAMBERT. 1981. Estrogen synthesis in relation to estrone, estradiol, and vitellogenin plasma levels during the reproductive cycle of the female rainbow trout, *Salmo gairdneri*. Gen. Comp. Indocrinol. **45**: 105–117.

BROMAGE, N. R., C. WHITEHEAD, and B. BRETON. 1982. Relationships between serum levels of gonadotropin, oestradiol-17β, and vitellogenin in the control of ovarian development in the rainbow trout. Gen. Comp. Endocrinol. **47**: 366–376.

BROWN, J. B. 1955. A chemical method for the determination of oestriol, oestrone and oestradiol in human urine. Biochem. J. **60**: 185–193.

DUFFEY, R. J., and F. W. GOETZ. 1980. The *in vitro* effects of 17α-hydroxy-20β-dihydroprogesterone on germinal vesicle breakdown in brook trout (*Salvelinus fontinalis*) oocytes. Gen. Comp. Endocrinol. **41**: 563–565.

HENDERSON, N. E. 1963. Influence of light and temperature on the reproductive cycle of the eastern brook trout (*Salvelinus fontinalis* (Mitchill)). J. Fish. Res. Board Can. **20**: 859–897.

HOKANSON, K. E. F., J. H. MCCORMICK, B. R. JONES, and J. H. TUCKER. 1973. Thermal requirements for maturation, spawning, and embryo survival of the brook trout, *Salvelinus fontinalis*. J. Fish. Res. Board Can. **30**: 975–984.

HURK, R. VAN DEN, and J. PEUTE. 1979. Cyclic changes in the ovary of the rainbow trout, *Salmo gairdneri*, with special reference to sites of steroidogenesis. Cell Tissue Res. **199**: 289–306.

IDLER, D. R., and T. B. NG. 1983. Teleost gonadotropins: isolation, biochemistry, and function. Fish Physiol. 1969–1983, **9**: 187–221.

LAMBERT, J. G. D., G. I. C. G. M. BOSMAN, R. VAN DEN HURK, and P. G. W. J. VAN OORDT. 1978. Annual cycle of plasma oestradiol-17β in the female trout *Salmo gairdneri*. Ann. Biol. Anim. Bioch. Biophys. **18**: 923–927.

MCDOWELL, E. M., and B. F. TRUMP. 1976. Histologic fixatives suitable for diagnostic light and electron microscopy. Arch. Pathol. Lab. Med. **100**: 405–414.

NG, T. B., and D. R. IDLER. 1983. Yolk formation and differentiation in teleot fishes. Fish Physiol. 1969–1983, **9**: 373–404.

ORCZYK, G. P., M. HICHENS, G. ARTH, and H. R. BEHRMAN. 1978. Progesterone and 20α-dihydroprogesterone. *In* Methods of hormone radioimmunoassay. 2nd ed. *Edited by* B. M. Jaffe and H. R. Behrman. Academic Press, New York. pp. 701–713.

RODBARD, D. 1974. Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays. Clin. Chem. (Winston-Salem, N.C.), **20**: 1255–1270.

SCOTT, A. P., and J. P. SUMPTER. 1983. A comparison of the female reproductive cycles of autumn-spawning and winter-spawning strains of rainbow trout (*Salmo gairdneri* Richardson). Gen. Comp. Ednocrinol. **52**: 79–85.

VLADYKOV, V. D. 1956. Fecundity of wild speckled trout (*Salvelinus fontinalis*) in Quebec lakes. J. Fish. Res. Board Can. **13**: 799–841.

WALLACE, R. A., and D. W. JARED. 1968. Studies of amphibian yolk. VII. Serum phosphoprotein synthesis by vitellogenic females and estrogen-treated males of *Xenopus laevis*. Can. J. Biochem. **46**: 953–959.

WALLACE, R. A., and K. SELMAN. 1981. Cellular and dynamic aspects of oocyte growth in teleosts. Am. Zool. **21**: 325–343.

YAMAMOTO, K., I. OOTA, K. TAKANO, and T. ISHIKAWA. 1965. Studies on the maturing process of the rainbow trout, *Salmo gairdneri irideus*. I. Maturation of the ovary of a one year old fish. Bull. Jpn. Soc. Sci. Fish. **45**: 31–36.