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Gametogenesis and Sex Steroid Profiles in Cultured Coho Salmon (*Oncorhynchus kisutch*, Walbaum)

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ABSTRACT The gametogenesis of a 2-year-old coho salmon broodstock population cultured in a fish farm in southern Chile was studied. Gonadosomatic index (GSI), microscopic gonadal traits, and serum levels of estradiol-17β(E2), testosterone, and 17α,20β-dihydroxy-4-pregnene-3-one (17,20P) in both sexes were recorded beginning 9 months before spawning in bimonthly samplings. Maximum GSI means were reached during May, the month of spawning, with 16.8 ± 4.1% for females and 8.4 ± 0.8% for males, both values within the range described in the literature. GSI in males, however, was triple that of females during January, showing a faster rate of gonadal growth in males in early summer. Gonadal microscopy for both sexes showed stages corresponding to those described by different authors for other salmonids such as rainbow trout. The secondary vitellogenesis period was 4 to 5 months and corresponded with the short vitellogenesis model described in rainbow trout for broodstocks maturing at 2 years of age. The serum profiles of sex steroids in both sexes are consistent with those described in coho salmon and other salmonid species. In females, E2 and 17,20P show opposite profiles, reaching their maximum levels (E2: 45.13 ± 11.3 ng/ml; 17,20P: 24.47 ± 7.34 ng/ml) during vitellogenesis (March) and ovulation (May), respectively. In both sexes, testosterone concentration shows maximum levels in May (females: 61.68 ± 15.75 ng/ml; males: 107.8 ± 11.6 ng/ml), suggesting the physiological importance of this hormone during maturation, either directly or as a substrate for the synthesis of other hormones. In males, the maximum level of 17,20P (22.33 ± 4.5 ng/ml) also occurs in May during total spermiation, which confirms its role in semen production and semen fluid regulation as described in the literature about this hormone in salmonid males. On the basis of the data obtained, a reproductive pattern is proposed for 2-year-old salmon cultured in southern Chile. *J. Exp. Zool. 280:429–438, 1998.* © 1998 Wiley-Liss, Inc.

The silver or coho salmon, one of the six Pacific salmon species of the genus *Oncorhynchus*, is the most widely distributed both geographically and ecologically (Brannon et al., '82). Like all species of the Salmonidae family, it originated in the northern hemisphere, its native distribution range being the Pacific Ocean and its tributaries, from Monterey Bay in California to Point Hope in Alaska (McPhail and Lindsley, '70). The species, however, is more abundant between the south of Oregon and the southeast of Alaska (Laufle et al. '86). In Asia, coho salmon is found from near the Arctic Circle to north of Japan and Korea (Gordon et al., '87).

Like all other Pacific salmonids, the coho salmon is an anadromous species, that is, it starts its life cycle in freshwater, after which the juveniles migrate to the ocean, where they undergo a stage of intense growth, and finally return to freshwater, as sexually mature adults, to spawn and die (Alderdice, '88). This species, however, has been successfully transplanted into the Great Lakes in the United States, demonstrating its ability to grow and mature in freshwater (Gordon et al., '87).

In Chile, the introduction of coho salmon has been attempted since the beginning of this century. Diverse bodies of continental waters in southern Chile were sown on several occasions with coho salmon smolts from eggs imported from the northern hemisphere. The main coho salmon egg

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imports took place between 1905 and 1910 (Barrios, '61), in 1930 (Mann, '54) and between 1968 and 1972 (Vila et al., '78; Mendez and Munita, '89). None of these attempts, however, achieved the establishment of populations acclimated to Chile, in contrast to successful results with other salmonid species like brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) (Wetzlar, '79). The large-scale development of intensive salmon cultivation in Chile during the last decade, based mainly on net-pen rearing of salmonids in fjords and channels in regions X and XI, has made coho salmon, one of the most cultured and most commercially important species in Chile today. This rapid growth has brought about a greater understanding of the biology of salmonids cultured under the environmental conditions found in Chile.

Within the specific area of cultured salmonid reproduction, little work has been recorded in Chile (Caniggia, '90; Toledo et al., '94; Estay et al. '94, '97). The objectives of this study were to characterize the gametogenesis and serum levels of sex steroids in a cultivated population of coho salmon in the environmental conditions of southern Chile and to contrast these data with those described for the northern hemisphere, where coho salmon also spawn in the fall, but 6 months later because of opposite seasonality in the two hemispheres.

MATERIALS AND METHODS

Work was carried out at the facilities of the Fish Culture Complex of the Fisheries Development Institute in Region XI. Sample broodstocks were kept in net pens in Ensenada Baja, a small bay north to the Aysen Fjord (45°47´S; 72°45´W). Between 5 and 25 individuals of each sex were sampled bimonthly from September 1994 to May 1995. In May, the spawning season, sampling was performed in mature individuals at spawning time; in addition, three gonadal samples of individuals with preovulatory ovaries were collected for histological studies. At each sampling, the specimen's weight and length were recorded, a blood sample was withdrawn by puncturing the caudal venal sinus, and specimens were sacrificed for dissection of both gonads. Weight of fresh gonads was determined by means of a Sartorius digital scale at the last sampling; the weight of ovaries was estimated based on the total egg mass weight, after the extraction of celomic fluid, plus the weight of the remnants of ovarian stroma. The gonadosomatic index (GSI) was calculated as the percentage of gonads over body weight: GSI = (gonad weight/body weight + gonad weight) × 100.

Immediately after being weighed, the gonads were fixed in ALFAC (ethanol absolut: 850 ml; formaldehyde 40%: 100 ml; glacial acetic acid: 50 ml) for subsequent histological processing. Tissues were encased in paraffin, cut into 4-μm sections and stained with hematoxilin-eosine. Oocyte size was estimated by averaging the diameter of the five largest oocytes of a histological slide for three different females in each sampling. Measurements were made with a Nikon stereoscopic magnifier provided with a micrometer-graduated eyepiece.

Blood samples were kept in Eppendorf tubes for 8 h at ambient temperature (8°–12°C). Later, all the serum was extracted and transferred into new tubes that were kept frozen at –20°C until hormone determinations. Hormones measured were: estradiol-17β (E2), testosterone (T), and 17α,20β-dihydroxy-4-pregnene-3-one (17,20P). The latter hormone was not measured at the two first samplings. E2 and T determinations were made by radioimmunoanalysis (RIA), according to the methodology described by Llanos et al. ('85). Sensitivity of RIA trials was less than 10 pg, and the coefficient of intraatrial variation was 14% for E2 and 11% for T. Determination of 17,20P was made by means of high-pressure liquid chromatography (HPLC) according to the method described by Tcholakian et al. ('87). The serum extracted with dichloromethane was dried with N and resuspended in methanol. Quantification of 17,20P was made in a Merck-Hitachi HPLC system made up of an LC-Organizer injector, an intelligent LC-6200 pump, and a D-2500 chromatograph with a data integrator with variable wave length UV detector (progesterone detection was made at 240 nm). The column used was a uBondapak C18 (8 cm × 3.9 mm) with a reverse-phase system using hexane-ethanol (90:10) as a mobile phase; flow rate was 2 ml/min. The recovery of the extraction process for E2, T, and 17,20P was 86%, 90%, and 88%, respectively. Quantification of 17, 20P was performed according to the directions in the Merck-Hitachi handbook with the application of international standards; the method sensitivity was 2 ng.

RESULTS

1. GSI

The variations of the gonadosomatic index are shown in Figure 1. In four of the five samplings, IGS values are higher in females ($P < 0.01$). In January, however, these values were temporarily three times higher in males than in females (3.92 ± 0.27 vs. 1.36 ± 0.29, respectively) ($P < 0.01$).

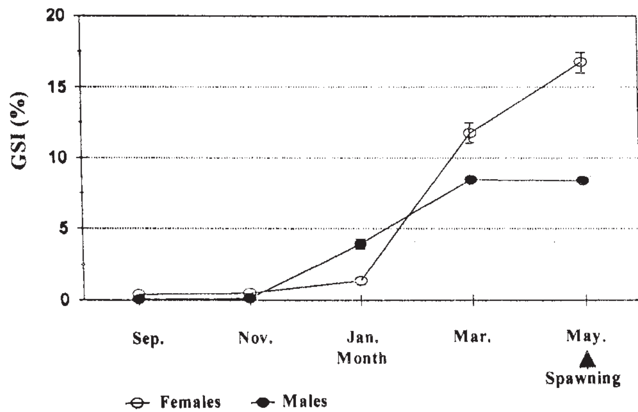

Fig. 1. Evolution of the gonadosomatic index (GSI) of coho salmon females and males, throughout their gonadal reproductive cycle.

Oocyte growth, estimated throughout the diameter means, is plotted in Figure 2.The growth rate of oocyte diameter increased markedly following the November measurement, when oocytes had reached a mean diameter of about 1 mm. The maximum mean reached exceeded 5 mm and occurred during May in the weeks before spawning.

2. Gonadal histology

Results of microscopic study of gonadal morphology through histological sections are summarized in Figures 3 and 4. In both sexes, the five samplings taken displayed the following different gonadal growth stages.

Ovaries

During September, oocytes are at an early stage of primary or endogenous vitellogenesis, presenting a mean diameter of 746.7 ± 38.6 µm (Fig. 3A).

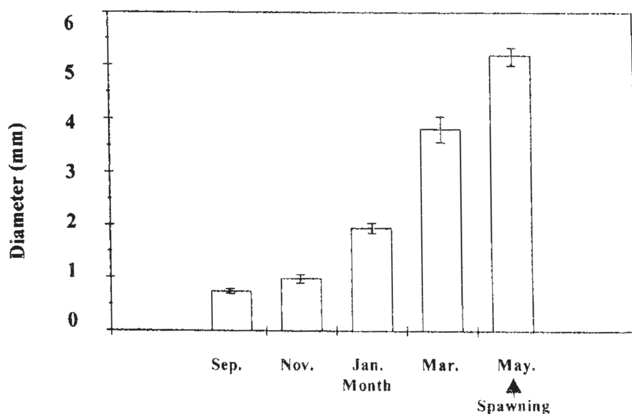

Fig. 2. Mean diameter growth of the oocytes during the oogenesis of coho salmon.

They characteristically show many yolk vesicles in the ooplasm and a large germinal vesicle with irregular contour lines. The different components of the follicle layers are well defined.

In November, oocytes lie in a stage of advanced endogenous vitellogenesis; they have attained a mean diameter of 980.0 µm (Fig. 3B). Yolk vesicles are more numerous, and the granules of endogenous yolk that they contain are quite conspicuous. Large lipid drops embedded in the ooplasm are also observed.

In January, oocytes are found in full secondary vitellogenesis and have attained a mean diameter of 1,950.0 ± 136.6 µm (Fig. 3C). The vitellin globules that occupy a large part of the ooplasm are quite evident, displacing the vitellin vesicles to the periphery (cortical alveolus). The germinal vesicle is proportionally smaller than in the previous stage and lies suspended in the middle of an aggregation of lipid drops.

In March, secondary vitellogenesis has progressed considerably, and oocytes have reached a mean diameter of 3,813.3 ± 236.3 µm. Yolk globules are held together more cohesively. The germinal vesicle is initiating the migration process (Fig. 3D).

In May, oocytes are found at the final maturation stage, with a mean diameter of 5,186.7 ± 236.3 µm (Fig. 3E). Yolk globule fusion has taken place, and the ooplasm is made up of a homogeneous yolk phase. The germinal vesicle has completed migration and lies in periphery position in contact with the inner wall of the oocyte plasma membrane, indicating the impending vesicle breakdown and ovulation. The latter has already taken place in many specimens.

Testes

In September, the seminiferous lobules appear covered with cysts containing numerous spermatogonia in an active proliferation process (Fig. 4A).

In November, the germ cells have initiated meiosis, and primary spermatocytes are seen at different stages of the meiotic prophase, as are some spermatids (Fig. 4B).

In January, spermatogenesis has progressed considerably, spermiogenesis has begun, and the first spermatozoa are already observed to be occupying the lobular lumen (Fig. 4C).

In March, there is active spermiogenesis, and a mass of spermatozoa can be seen occupying the lobular lumen, which covers more than 50% of the cross-section of the lobules (Fig. 4D).

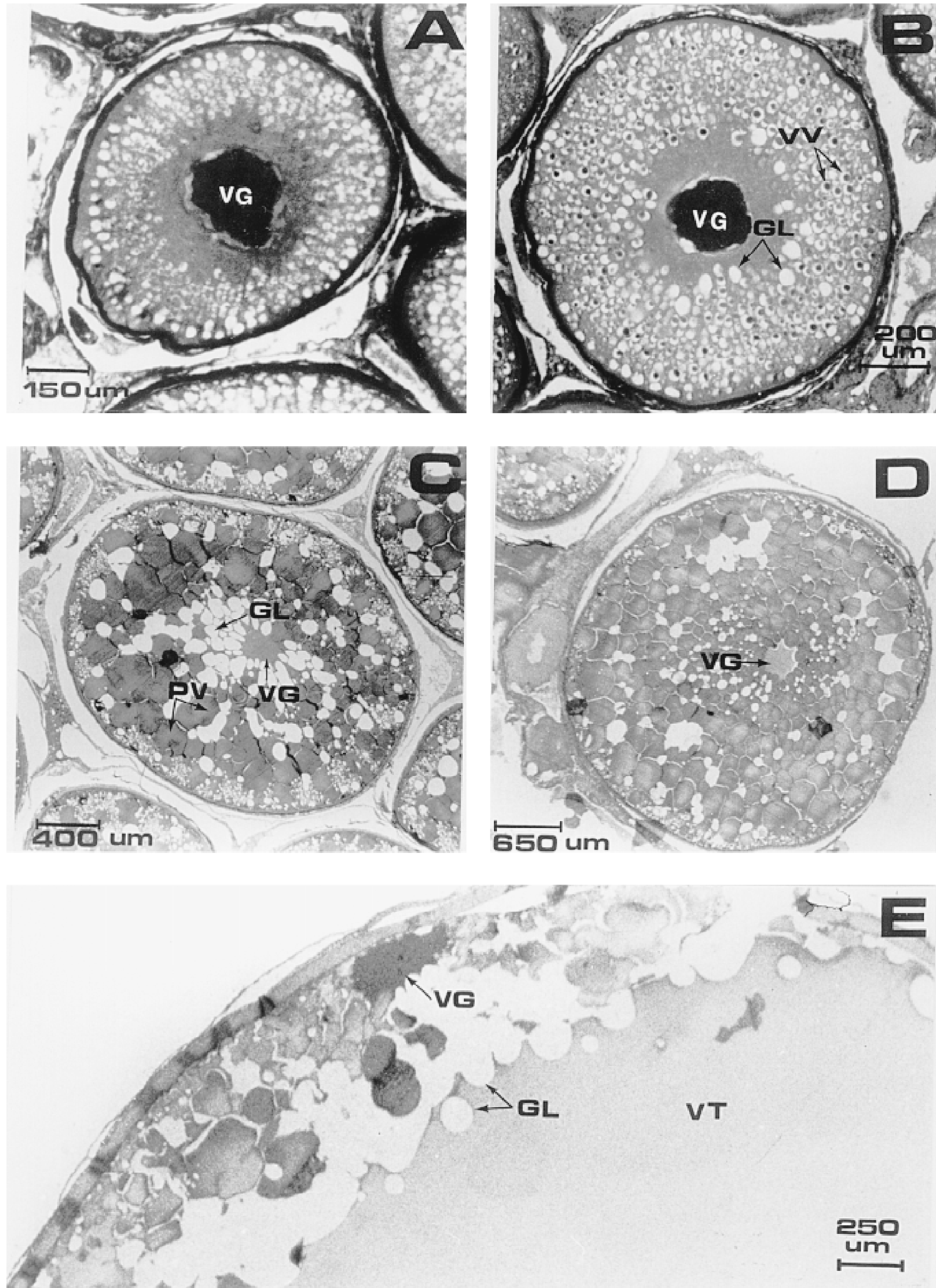

Fig. 3. Histological sections of ovaries at different stages of development during oogenesis. **A.** Oocyte in early endogenous vitellogenesis (September); **B.** Oocyte in advanced endogenous vitellogenesis (November); **C.** Oocyte in full exogenous vitellogenesis (January); **D.** Oocyte in advanced exogenous vitellogenesis. The migration of the germinal vesicle begins (March); **E.** Oocyte in final maturation, pre-ovulatory stage, germinal vesicle in peripheral position (May). VG = germinal vesicle; VV = yolk vesicles; GL = lipidic drops; PV = vitelline plaquets.

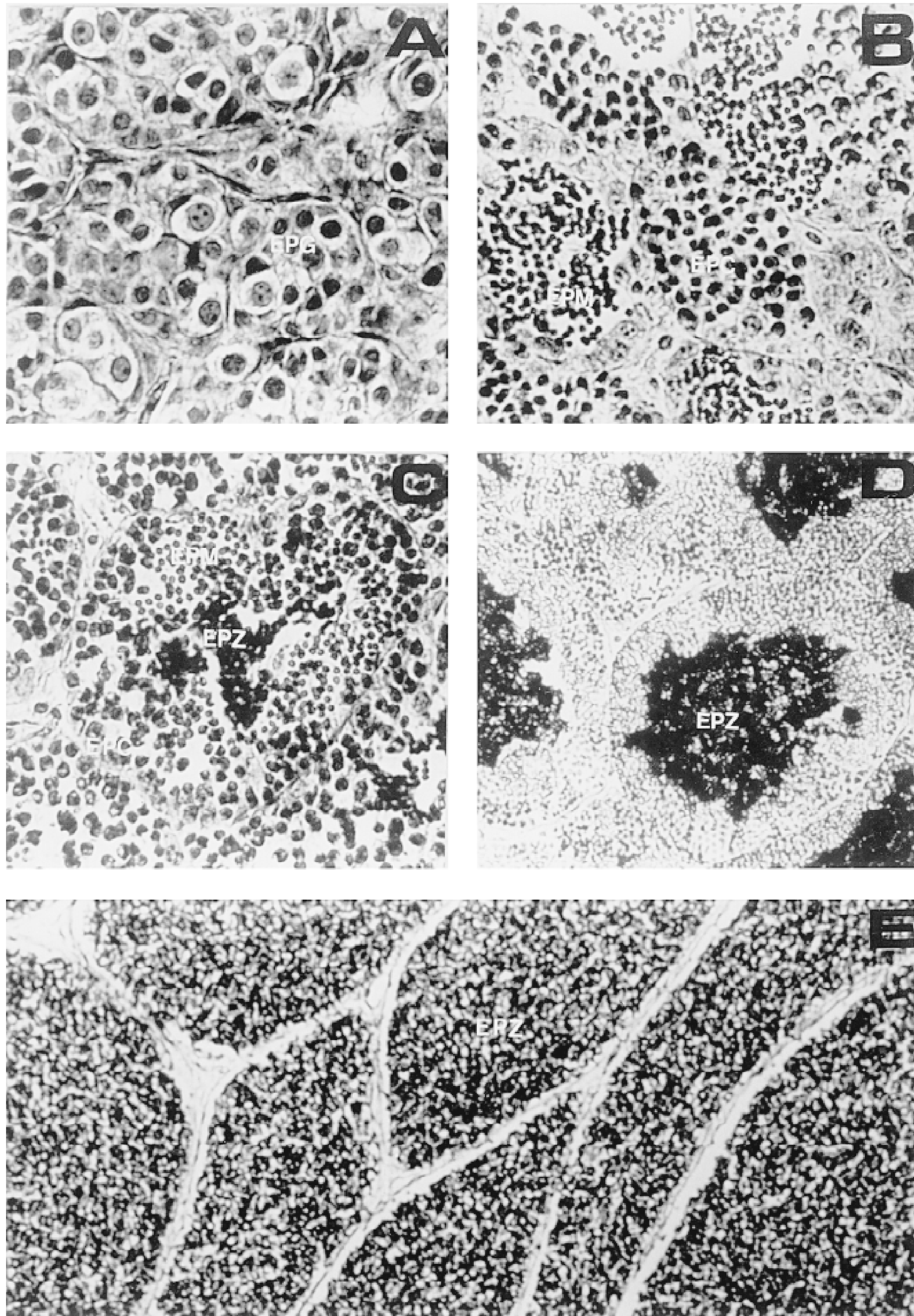

Fig. 4. Histological sections of testicles at different stages during spermatogenesis. **A.** Seminiferous lobules in spermatogonial proliferation (September); **B.** Seminiferous lobules with spermatocytes in different stages of the meiotic prophase (November); **C.** Beginning of spermiogenesis, the first spermatozoa appearing inside the lumen (January); **D.** Active spermiogenesis, large quantity of spermatozoa inside the lobular lumen (March); **E.** Total spermiation (May). EPG = spermatogonias; EPC = spermatocytes; EPM = spermatides; EPZ = spermatozoa.

In May, total spermiation as defined by Hurk and Peute ('79) has occurred, and the testicular tissue is entirely made up of a lobular web filled with mature and hydrated spermatozoa (Fig. 4E). This stage coincides with semen emission by males.

3. Sex steroid profiles

Mean values and S.D. of hormones measured for both sexes in this study are summarized in Table 1.

Females

E2 showed basal levels (<2 ng/ml) during spring, initiating an increase in January to reach its maximum levels during March. At spawning time, the levels of this hormone had returned close to the basal values. T levels remained low (<3 ng/ml) between September and March, but in May, during spawning, they increased markedly, reaching values above those of E2. Hormonal concentration of 17,20P was low during the January–March period, but it increased rapidly as spawning came approached, reaching its maximum during May.

Males

E2 remained at basal levels throughout the study period, except in March, when there was a slight and statistically nonsignificant rise ($P > 0.05$), which was sustained by only two of the individuals sampled. Conversely, T showed a consistent pattern of steady increase, presenting a marked rise, and reaching its maximum levels in May during total spermiation. Hormone 17,20P, in turn, exhibited a tendency very similar to that observed in females, with low and intermediate levels in the January–March period, as well as a significant increase ($P < 0.01$) and maximum values in May during total spermiation (Table 1).

DISCUSSION

Except for a few cases (Vladykov, '56; Tam et al., '86) in which brook trout (*Salvelinus fontinalis*) have been used to study the gonadal reproductive cycle of salmonids, most of these studies have concentrated on rainbow trout (Yamamoto et al., '65; Beams and Kessel, '73; Hurk et al., '78; Hurk and Peute, '79; Billard, '83, '92; Bromage and Cumaranatunga, '88; Estay et al., '90; Toledo et al., '94). We have not found a morphological description of the coho salmon's gonadal cycle in the literature, and the scarce reports on sex steroid profiles have only dealt with adult specimens returning to freshwater from the ocean during spawning time in the northern hemisphere (September–November) (Wright and Hunt, '82; Sower and Shreck, '82; Fitzpatrick et al., '86; Planas, '93). This is partly because rainbow trout are hardier and easier to manage under laboratory conditions. On the other hand, coho salmon in their native habitat (mainly in the United States and Canada) are cultured very little in closed systems that allow the sampling of specimens to be initiated many months in advance of spawning. Most of the populations are managed under open systems ("ocean ranching") in which, during the first stages of gonadal development, adults range freely in the ocean 100 km or even 1,000 km from the coast.

Body weight reached by wild coho salmon at maturation ranges from 2.5 to 6.0 kg, and their length varies from 60 to 90 cm (Drummond, '82; Gordon et al., '87), depending mainly on the length of time they have remained in the sea. Even though the specimens used in this study are cultured salmonids, their mean values of weight and length at spawning both in females (3.52 ± 0.53 kg, 62.3 ± 2.5 cm) and males (3.87 ± 0.66 kg, 66.0 cm), fell within these wide ranges defined for the species. The difference we observed for these variables between both sexes is consistent with the findings of Shapovalov and Taft ('54), who reported a moderate but consistent tendency for coho males to attain a larger size than females.

GSI is a useful index for monitoring the progression of the reproductive cycle of salmonids, because the gonadal mass attained in these species during maturation makes up a high percentage of their body biomass, especially in females. In the rainbow trout, for example, the ovaries rap-

TABLE 1. Serum concentrations of sex steroids in coho salmon

	Estradiol 17 (ng/ml)				Testosterone (ng/ml)				17,20 DHP (ng/ml)			
Month	Females	n	Males	n	Females	n	Males	n	Females	n	Males	n
Sep	0.36 ± 0.14	6	1.07 ± 1.14	5	1.54 ± 1.33	6	3.82 ± 0.97	5	–	–	–	–
Nov	1.35 ± 0.41	8	0.30 ± 0.01	5	3.14 ± 0.86	8	6.70 ± 0.35	5	–	–	–	–
Jan	5.44 ± 1.79	9	1.19 ± 1.17	13	7.45 ± 1.03	9	8.11 ± 2.00	13	5.25 ± 1.02	9	4.23 ± 0.72	13
Mar	45.13 ± 11.30	11	3.69 ± 3.85	9	2.83 ± 2.93	11	10.74 ± 2.27	9	6.67 ± 2.36	11	3.82 ± 0.44	9
May	3.46 ± 1.16	23	0.60 ± 0.18	5	61.68 ± 15.75	23	107.75 ± 11.58	5	24.47 ± 7.34	23	22.33 ± 4.45	5

idly increase in weight during the months before spawning, reaching a maximum GSI level of 15% to 20% in females and 5% to 13% in males (Nomura '63; Yamamoto et al., '65). In coho salmon, Sower and Shreck ('82) recorded GSI means from 15.87 ± 1.01% to 23.87 ± 3.21% for females, with oocytes showing either peripheral or ruptured germinal vesicles. In the present study, maximum GSI means obtained were 16.8 ± 4.1% for females and 8.42 ± 0.8% for males; both values were within the ranges given above for rainbow trout and coho salmon. The temporary advantage of the males' GSI over that of females during January (which we have not found described in the literature) should be noted here. It is evidence of the great rapidity with which spermiogenesis takes place and, along with it, the sudden increase of testicular biomass in the summer months, about 4 months before maturation.

Gonadal histomorphological aspects observed in this study are consistent with some general patterns found in the literature for the gonadal cycle of fishes as a whole (Wallace and Selman, '81; Grier, '81) and are consistent as well with the patterns established for salmonids, using rainbow trout as a model both in females (Bromage and Cumaranatunga, '88; Scott, '90) and males (Hurk et al., '79; Billard, '92).

The ovaries of females sampled in September and November exhibited oocytes in two stages of endogenous vitellogenesis equivalent to two moments in the "cortical alveolus stage" as defined by Scott ('90), or to the vesicle stages a and b, respectively, as defined by Bromage and Cumaranatunga ('88). Ovaries sampled in January and March exhibited oocytes in two stages of exogenous vitellogenic process, equivalent to two moments of the "vitelline globule stage" of Scott ('90), or to stages 5 and 6, respectively, of Bromage and Cumaranatunga ('88). Finally, the ovaries sampled in May presented oocytes with peripheral germinal vesicles or ovulated eggs corresponding to the "ovulation stage" of Scott ('90), or to stage 7 of Bromage and Cumaranatunga ('88). According to these authors' findings, the duration of secondary vitellogenesis differs according to the female's age, extending to 6 months in 2-year-old spawners, but to 10 months in 3-year-old spawners (Scott, '90). In turn, Bromage and Cumaranatunga ('88) indicate that this period starts 4 to 5 months before ovulation in 2-year-old spawners but at least 8 months before spawning in 3-year-old spawners. Our results agree with these tendencies described in rainbow trout, because the salmonids used in this study were 2-year-old specimens and the secondary vitellogenesis period observed was short (4–5 months). Vitellogenesis in 3-year-old salmon should hypothetically be longer.

Studies characterizing the testicular cycle according to the dynamics of cell changes, carried out by Hurk and Peute ('79), who defined three periods and by Billard ('92), who determined nine stages, will be used as references in analyzing the microscopic morphological characteristics of the coho salmon's testicular cycle observed in this study. During September, an active process of spermatogonial proliferation equivalent to the period of the same name described by Hurk et al., and to stage III described by Billard, was noticed. In November, an active spermatogenesis stage (equivalent to stage IV of Billard), in which germ cells at different stages of the meiotic prophase prevailed, was observed. In January, advanced spermatogenesis and the appearance of the first spermatozoa in the lobular lumen make up a stage that is included in the second period described by Hurk et al. and is equivalent to stage V of Billard. In March, spermiogenesis predominates, and there is a considerable amount of spermatozoa in the lobular lumen that keeps it within the second period of Hurk et al., and equivalent to stage VI of Billard. Lastly, in May, the testes are made up of a web of lobules filled with mature spermatozoa and are equivalent both to the total spermiation stage described by Hurk et al., and to stage VII of Billard.

Serum profiles of E2 and 17,20P observed during the reproductive cycle in the females in this study followed a reverse pattern, that is, while E2 presents a high level during the vitellogenesis period and a fall to basal levels during spawning, exactly the opposite takes place with 17,20P. This is consistent with the profiles shown for these hormones by Planas (pers. comm.), although in our females the 17,20P level increase is not as sharp, and the maximum level (24.5 ng/ml) is lower than that observed by Planas (64 ng/ml). In wild coho salmon, Fitzpatrick et al.('86) found high E2 levels in females that returned early from the ocean and whose oocytes showed central, migrating, or peripheral germinal vesicles, but these levels fell sharply toward the end of the cycle, during the vesicle breakdown and ovulation. In contrast, for 17,20P, these authors noticed a reverse pattern, where such levels were low in fish returning early and high in those sampled late.

These findings substantiate our observations and are consistent with the general pattern de-

scribed in salmonids, whereby functional significance is given to the regulation role of E2 during the vitellogenesis process and of 17,20P during final maturation and ovulation. The marked T increase observed in our female specimens during the latter periods has been noticed by other authors in coho salmon, and it suggests that T could play other roles in addition to being a reservoir for the production of other steroids (Fitzpatrick et al., '86). Regarding this, Nagahama et al. ('80) reported that, although not as effective as 17,20P, T induces the breakdown of the germinal vesicle in rainbow trout and amago salmon (*Oncorhynchus rhodurus*).

E2 levels in males were much lower than in females, remaining most of the time at values close to or lower than 1 ng/ml. This is consistent with the low E2 levels observed by Fitzpatrick et al. ('86) in coho salmon males. These authors point out that the role of E2 in males is still unknown.

Some years ago, it was demonstrated that spermatogenesis in fish occurs along with an important increase in 11-oxygenated androgens, particularly of 11-ketotestosterone (Fostier et al., '83; Prat et al., '85). Moreover, some authors attribute to this latter hormone the role of main regulating androgen in the reproductive cycle of salmonid males, with particular importance noted toward the end of the cycle during spermiation and development of secondary sex traits, when the hormone reaches its highest level (Gordon et al., '87). Even though 11-ketotestosterone was not measured in this study, T measurement may be considered an indirect indicator of the former, because it is synthesized from T (by means of the action of enzymes 11-hydrolase and 11-hydroxisteroid dehydrogenase by stimulus of GTH II), and plasmatic levels of these hormones in coho salmon males during sexual maturation are similar (Planas , '93). However, the T role may be of importance in other physiological events besides spermiogenesis (Fitzpatrick et al., '86). In our males, T suffered a marked increased during maturation, reaching a maximum level of 107.8 ± 11.58 ng/ml, a somewhat higher value than that recorded by Fitzpatrick et al.('86) for this same hormone (76–77 ng/ml) in coho salmon males during final maturation, but a little lower than that observed by Planas ('93) (140 ng/ml). Finally, 17,20P in males exhibited a pattern very much like that of females, increasing significantly during maturation (22.33 ± 4.45 ng/ml). Although lower than the maximum values obtained by Fitzpatrick et al.('86) (30–37 ng/ml) and by Planas ('93) (23 ng/ml) for 17,20P in coho salmon males, this value is consistent with the functional significance attributed to it in salmonid males, that is, its important role in semen production (Scott and Sumpter, '83; Ueda et al., '83), and in the control of the seminal fluid composition (Baynes and Scott, '85).

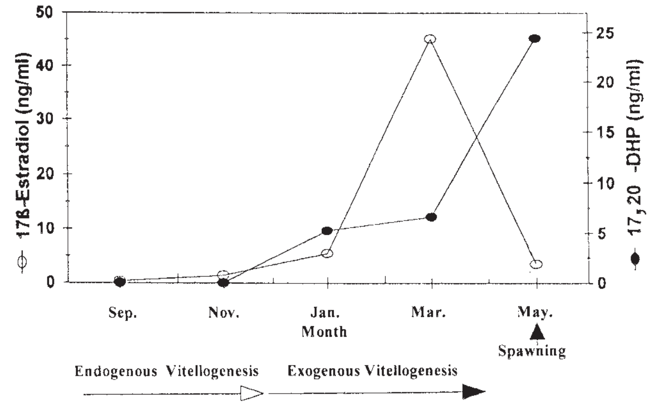

Fig. 5. Serum concentration of sex steroids in coho salmon females throughout their gonadal reproductive cycle.

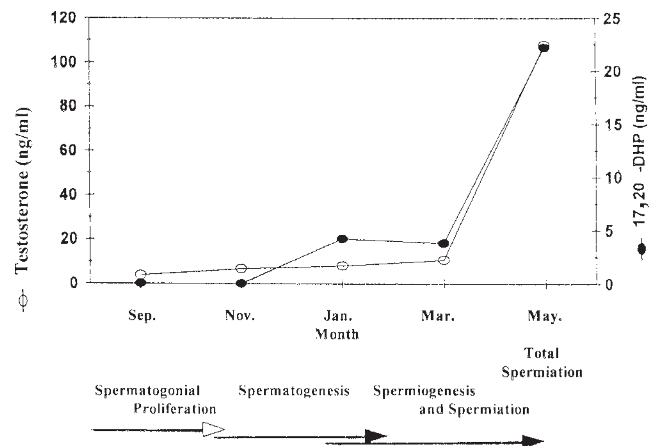

Fig. 6. Serum concentration of sex steroids in coho salmon males throughout their gonadal reproductive cycle.

On the bases of the hormonal and histomorphological findings recorded in this study, an attempt is made in Figures 5 and 6 to outline a synthesis of the gametogenesis of 2-year-old coho salmon cultured in southern Chile.

ACKNOWLEDGMENTS

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