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Steroid and Thyroid Hormones during Sexual Maturation of Coho Salmon (*Oncorhynchus kisutch*) in Seawater or Fresh Water[1]

STACIA A. SOWER[2] AND CARL B. SCHRECK

Oregon Cooperative Fishery Research Unit,[3] Oregon State University, Corvallis, Oregon 97331

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Osmolality and circulating levels of estradiol-17β, thyroxine, androgens, progesterone, and sodium are measured concomitantly with determination of oocyte maturation in coho salmon (*Oncorhynchus kisutch*) during the spawning season. Male and female salmon are held in both fresh water (their natural environment) and seawater (an unnatural environment) throughout the season. During the early stages of final gonadal maturation, sodium levels and osmolality of fish in seawater increase substantially, which suggests osmoregulatory difficulties. Also, there is a higher mortality rate of fish held in seawater. The circulating levels of all hormones measured differ between coho salmon in fresh water and those in seawater. We conclude from these differences in hormone levels, the dehydrated eggs, incomplete ovulation, and high adult mortality of fish in seawater that reproductive function is compromised in salmon which are retained in seawater during the spawning season.

Pituitary gonadotropin(s) and gonadal steroids consistently are considered to be major endocrine mediators in the control of reproduction in fish (Donaldson, 1973; Fontaine, 1976). Gonadotropin(s) released from the pars distalis are thought to stimulate various gonadal functions (de Vlaming, 1974). Both biochemical and histophysiological methods have been used in attempts to answer the question of whether one or two gonadotropins occur in teleosts (Chestnut, 1970; Idler *et al.*, 1975a, b; Pierce *et al.*, 1976). Other pituitary hormones that may exert indirect or direct effects on reproduction in salmon are thyrotropin, prolactin, and corticotropin, although their precise roles remain unclear and available information is often contradictory (Reinboth, 1972; Fontaine, 1976; Jalabert, 1976).

Jalabert (1976) concluded from *in vitro* studies in rainbow trout (*Salmo gairdneri*) that the gonadotropic action of the pituitary is mediated through steroid hormone action on oocyte maturation. The steroid responsible for this action is believed to be 17α-hydroxy-20β-dihydroprogesterone (17α,20β-dihydroxy-4-pregnen-3-one) which is produced by the follicle after stimulation by gonadotropin (Jalabert, 1976). This steroid has been found in the blood of both the Pacific salmon at the time of spawning (Schmidt and Idler, 1962) and the rainbow trout during oocyte maturation (Campbell *et al.*, 1980).

Corticosteroids and estrogens are additional steroids which may exert either indirect or direct effects on final gonadal maturation, although the precise role of these steroids in final maturation of salmonids is unclear. Elevated estrogens have been demonstrated during maturation in Atlantic salmon, *S. salar* (Cedard *et al.*, 1961), brown trout, *S. trutta* (Crim and Idler, 1978), coho salmon (Jalabert *et al.*, 1978), and rainbow trout (Fostier *et al.*, 1978). Elevated blood corticosteroids have been recorded in salmonids during sexual mat-

[1] Oregon State University Agricultural Experiment Station technical paper No. 5826.

[2] Present address: Department of Zoology, NJ-15, University of Washington, Seattle, Washington 98195.

[3] Supported cooperatively by Oregon State University, Oregon Department of Fish and Wildlife, and the U.S. Fish and Wildlife Service.

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uration (Donaldson and Fagerlund, 1968; Schmidt and Idler, 1962). On the basis of their studies of catfish (*Heteropneustes fossilis*), Sundararaj and Goswami (1977) have suggested that corticosteroids are responsible for induction of oocyte maturation through direct action on the oocytes.

Coho salmon, an anadromous species, undergo two migrations during their life cycle. The first occurs in the spring when the salmon (about 18 months old) migrate downstream and undergo morphological and physiological changes referred to as smoltification which prepare the fish for life in seawater (see review by Hoar, 1969). After 18 to 24 months at sea, the fish return as adults to fresh water to undergo sexual maturation. Not only do the salmon undergo final maturation upon entrance into fresh water, but they also experience many morphological and physiological changes that enable them to survive in fresh water (Woodhead, 1975). Coho salmon artificially held in seawater for maturation, experience difficulties, such as dehydration of eggs and osmoregulatory problems, and often die without spawning (Sower, 1980). Changes in salmon that normally occur upon their entrance into fresh water ensure the fish's survival and may also influence final maturation.

The present investigation was undertaken to understand the fish's apparent inability to survive in seawater during final stages of maturation and ovulation. The main objective was to describe the hormonal changes of the coho salmon and relate its reproductive development in seawater to that in fresh water during final maturation. Plasma and serum levels of estradiol-17β, progesterone, androgens, and cortisol were measured in order to further elucidate the process of sexual maturation. Gonadosomatic index and oocyte development in females were determined to establish the degree of reproductive development of the salmon. Time of spermiation was noted for male salmon. The interrelationships between osmoregulation and maturation may be such that any disturbance of either system affects the other system. When coho salmon are retained in seawater during maturation, the effects of osmoregulatory adjustments may be inhibitory or antagonistic to reproductive functions (Sower, 1980). To further aid in understanding the interrelationship between maturation and osmoregulatory processes of fish, serum sodium, osmolality, and thyroxine were measured in fish in fresh water and seawater during the spawning season.

MATERIALS AND METHODS

The studies were conducted at the seawater facility of Ore-Aqua Foods (Weyerhaeuser, Newport, Oreg.) during 1978 and 1979 and at Weyerhaeuser's Jefferson facility, Springfield, Oregon in 1979.

1978 studies. Starting on September 22, two or three female coho salmon, which averaged 2.5 kg body weight, returning to Newport facility were killed and sampled on Days 0, 6, 14, 24, 28, 34, 40, and 45 for plasma and gonads. Final maturation of the gonads occurred from Days 6 through 40 while ovulation occurred around Day 45. These fish remained in seawater during final maturation and ovulation. Blood was collected in heparinized syringes from the caudal vessel and centrifuged; the plasma was drawn off and frozen at $-20°$ until analyzed for progesterone, estradiol-17β, and cortisol. Gonads were weighed to determine the gonadosomatic index,

$$\text{GSI} = \frac{\text{weight of one gonad} \times 2}{\text{weight of fish}} \times 100,$$

and fixed in Stockard's fixing agent (4% glacial acetic acid and 5% formaldehyde dissolved in 0.9% NaCl salt solution) for histological examination to determine the stage of oocyte maturation. Stages of oocyte maturation in adult coho salmon, as described by Jalabert *et al.* (1976) for rainbow trout, were defined as follows: (1) premigrating germinal vesicle, (2) migration of germinal vesicle and segregation of lipid drops, (3) germinal vesicle against chorion with distinct contour, and (4) vitelline maturation, coalescence of lipid drops, and germinal vesicle breakdown.

1979 studies. Ten female coho salmon were killed and sampled every 2 weeks for serum and oocytes from fish held in either seawater or fresh water from September 14 until December 5. These fish were considered the early entry group. Comparative data were collected also from fish returning in middle (September

26) and late (October 15) parts of their spawning run. The results for the middle and late entry groups were similar to results obtained from the early entry group and are not reported here. Ten males were also killed and sampled for serum during each of these sampling times. The fish in seawater were at Weyerhaeuser's Newport facility, while the fish in fresh water were at Weyerhaeuser's Jefferson facility. The temperature and salinity of seawater at Newport ranged between 9 and 15° and 30‰ and 32‰, respectively, throughout the study. The temperature of fresh water at Jefferson averaged 14°.

The fish sampled from fresh water and seawater were allowed to suffocate (left out of the water for about 10 min) before serum and oocytes were taken. To determine if this sampling procedure affected the hormone levels, blood was taken from ten males that were killed immediately by a blow to the head and ten that were allowed to suffocate. Suffocation did not substantially alter osmolality or the levels of sodium, estradiol-17β, progesterone, or thyroxine; however, androgen levels were significantly higher in males that suffocated (107.96 ± 19.38 ng/ml) than in those fish that were killed immediately (35.65 ± 13.02 ng/ml).

The blood collected was held on ice for 30 min. It was then centrifuged, and the serum was drawn off and divided into two equal portions. One portion was frozen at −20° until it was analyzed for estradiol-17β, progesterone, androgens, and thyroxine; the other portion was kept briefly on ice, and analyzed for sodium and osmolality on the day of collection. Samples of eggs from the fish were fixed in Stockard's fixing agent and analyzed immediately by eye, or within 2 days under a dissecting microscope. The gonadosomatic index was determined for each female salmon.

Assays. Cortisol was assayed in 15 μl of plasma by competitive protein-binding assay as described by Strange and Schreck (1978). Strange and Schreck (1978) showed consistently that over 75% of the hormone activity measured by this assay represented cortisol; the remainder of the hormone activity was represented by other corticoids. The cortisol assay has been validated only for juvenile salmon. Thyroxine was determined in 25 μl of plasma by radioimmunoassay (RIA) as described by Dickhoff *et al.* (1978). The intraassay and interassay coefficients of variation for the thyroxine RIA of coho salmon plasma were 6.1 (n = 6) and 21.0% (n = 5), respectively. The antibody binding in the absence of unlabeled thyroxine ranged between 47 and 56% in the assays.

Estradiol-17β, progesterone, and androgens were determined by RIA as described by Korenman *et al.* (1974), Koligian and Stormshak (1977), and Vernon Gay (Department of Physiology, University of Pittsburg, Pa.), respectively. Each RIA was validated for adult coho salmon plasma and serum. All assays were modified by reducing the amount of phosphate-buffered saline–gelatin (PG) added to each extract. Each steroid RIA followed a standardized procedure. Each blood sample was extracted twice with diethyl ether for estradiol-17β or hexane:benzene (2:1) for progesterone and androgens after 1000 dpm of appropriate [^{3}H]steroid in PG (used to estimate extraction recovery) had been incubated with the plasma for 2 hr. The aqueous phase was frozen in liquid N_2 and the extracts were decanted, combined, and dried under air. The extracts were reconstituted in 0.25 or 0.2 ml of PG (when recovery efficiency was not determined). For the determination of recovery efficiency, 0.05 ml PG-extract solution was counted. Extraction efficiencies were determined only initially, and then were not determined after it was established with great reliability that efficiencies exceeded 85%. Antiserum at 0.1 ml in the proper dilution of PG was added to each extract and to the standards which ranged from 7.8 to 2000 pg of the steroid. In addition, 0.1 ml of α-globulin (1.8% solution) was added to each tube in the estradiol-17β assay. After the samples were incubated for 30 min, 0.1 ml of [^{3}H]steroid (10,000 dpm in PG) was added to each tube. Samples were incubated at room temperature for 60 min (for androgens and progesterone) or 90 min for estradiol-17β, placed in an ice bath for 15 min after addition to each tube of dextran charcoal suspension (0.625% charcoal and 0.4% dextran in PG buffer; 1.0 ml for androgens, progesterone, and 0.5 ml for estradiol-17β). The samples were then centrifuged, decanted into Insta-gel scintillation fluid, and counted in a Packard liquid scintillation spectrophotometer.

Estradiol-17β was determined in 100 μl of plasma or serum. Antiestradiol-17β (S-244) was obtained from Dr. G. Niswender (Colorado State University, Fort Collins, Colo.) and diluted 1:85,000 in PG. This assay is highly specific for estradiol-17β. The intraassay and interassay coefficients of variation for the estradiol RIA of coho salmon plasma were 4.5 (n = 6) and 8.2% (n = 6), respectively. The lower limit of detection was about 6 pg/tube. The antibody binding ranged between 47 and 56% in the assays.

Progesterone was determined in 100 μl of plasma or serum. Antiprogesterone (11-BSA) was obtained from Dr. G. Niswender and diluted 1:40,000 in PG. Less than 0.5% cross-reactivity was found with 17α-hydroxyprogesterone. The intra-assay and interassay coefficients of variation for the progesterone RIA of coho salmon plasma were 3.6 (n = 8) and 5.4% (n = 6), respectively. The antibody binding ranged between 36 and 46% in the assays. The lower limit of detection was about 5 pg/tube.

Androgens were determined in 10 μl of plasma. Antitestosterone (11-BSA) was obtained from Dr. G. Niswender. The assay cross-reacts with testosterone (100%), 11-ketotestosterone (112%), and dihydrotestosterone (69%). The intra-assay and interassay coeffi-

cients of variation for the RIA of coho salmon plasma were 7.4 (n = 9) and 26.7% (n = 7), respectively. The antibody binding ranged between 39 and 58% in the assays. The lower limit of detection was about 12 pg/tube. Sodium and osmolality were determined by flame spectrophotometer and osmometer by personnel at Weyerhaeuser's Ore-Aqua Springfield facility, Oregon.

Statistics. Data for hormone concentrations were analyzed by a Student–Newman–Keuls test, which was used for computing the significances of differences for samples of unequal sizes after preliminary analysis of variance. In all tests, the level of significance was $P < 0.05$.

RESULTS

1978

Most of the fish that had entered the facility by the end of September had completed vitellogenesis; the germinal vesicle was subperipheral in the oocytes. The female salmon underwent oocyte maturation during October 6 to November 1, and ovulation occurred by November 6 (Table 1). The GSI for females increased from 11.2 ± 0.3% ($\bar{X}$ ± SE) on September 22 to 23.9% on November 1. Estradiol-17β concentrations in plasma increased just before ovulation, paralleling the increases in GSI, and then decreased by the time of ovulation. Progesterone levels were low during the final stages of maturation, ranging from 0.32 ± 0.15 to 1.04 ± 0.06 ng/ml (Table 1). Cortisol levels decreased slightly just before and during ovulation. Because adult salmon were difficult to obtain, only two or three salmon were sampled at each of these times, thus resulting in considerable variation in hormone concentrations, especially cortisol.

1979

Peak spawning time for the salmon was during December 5 and 6, about three weeks later than in 1978. The GSI and egg diameter increased through final maturation in fish held in fresh water and seawater, with few exceptions (Table 2). The egg diameters were more variable in fish held in seawater than in those held in fresh water.

In most cases, ovulation of fish in seawater was incomplete. In some instances, half of the eggs in one ovary (skein) were overripe (semitransparent), whereas the eggs in the other ovary had not yet ovulated but appeared to be mature (germinal-vesicle breakdown). Overripe eggs are those that presumably have been held in the body

TABLE 1
1978 RESULTS FOR FEMALE COHO SALMON ENTERING SEAWATER FACILITIES

Date		Day	n	Stage of oocyte maturation	GSI[a] ($\bar{X}$ ± SE) (%)	Hormone concentrations (ng/ml) ($\bar{X}$ ± SE)		
						Progesterone	Estradiol-17β	Cortisol
September	22	0	2	Unknown	11.21 ± 0.28	0.84 ± 0.45	1.09 ± 0.14	154
	28	6	2	Unknown	9.55 ± 3.55	0.79 ± 0.36	0.99 ± 0.01	82 ± 15
October	6	14	3	MGV[b]	13.73 ± 2.19	0.77 ± 0.16	1.32 ± 0.23	145 ± 17
	16	24	2	MGV	19.39 ± 2.09	0.32 ± 0.15	1.52 ± 0.98	106 ± 1
	20	28	3	PGV[c]	15.87 ± 1.01	0.40 ± 0.15	1.27 ± 0.29	219 ± 32
	26	34	2	PGV	16.40 ± 3.41	1.04 ± 0.06	1.07 ± 0.37	201 ± 59
November	1	40	2	PGV, GVBD[d]	23.87 ± 3.21	0.77 ± 0.57	1.59 ± 0.42	97 ± 28
	6	45	1	OV[e]	No data	0.66	0.48	141

[a] GSI, mean gonadosomatic index.
[b] MGV, migrating germinal vesicle.
[c] PGV, peripheral germinal vesicle.
[d] GVBD, germinal vesicle breakdown.
[e] OV, ovulation.

TABLE 2
MEAN GONADOSOMATIC INDEX AND EGG DIAMETER FOR FEMALE COHO SALMON IN 1979 HELD IN SEAWATER OR FRESH WATER DURING FINAL MATURATION AND OVULATION

Date		Egg stage[a]	GSI (%) ($\bar{X}$ ± SE) (n)	Egg diameter (mm) ($\bar{X}$ ± SE) (n)
Seawater				
September	17	PMGV, MGV	10.1 ± 0.2 (10)	5.1 ± 0.1 (10)
October	2	MGV	15.5 ± 0.2 (10)	6.4 ± 0.2 (10)
	9	MGV	17.0 ± 0.2 (10)	5.7 ± 0.2 (10)
	23	PGV	na[a]	na
November	13	OV	na	na
Freshwater				
October	1	PMGV	11.4 ± 0.8 (10)	5.3 ± 0.2 (10)
	11	na	15.2 ± 0.5 (10)	6.1 ± 0.1 (10)
	25	PGV	16.8 ± 2.1 (10)	6.3 ± 0.2 (10)

Note. Stages of oocyte maturation include premigrating germinal vesicle (PMGV), migrating germinal vesicle (MGV), peripheral germinal vesicle (PGV), germinal vesicle breakdown (GVBD), and ovulation (OV). Number in (n) refers to numbers of female salmon in that sample. Egg diameter was averaged from 6 eggs from each female.

[a] na, no data.

cavity for an extended period after ovulation.

Fish entered the seawater facility in Newport from the ocean on September 17. On October 9, both male and female coho salmon in seawater had significantly higher sodium levels than those of male and female fish in seawater on October 2 (Figs. 1 and 2). The values for October 2 are considered the normal values seen in salmon from seawater.

The sodium levels and osmolality for males were slightly higher than those for females held in either fresh water or seawater (Figs. 1 and 2). Sodium and osmolality both increased significantly in salmon

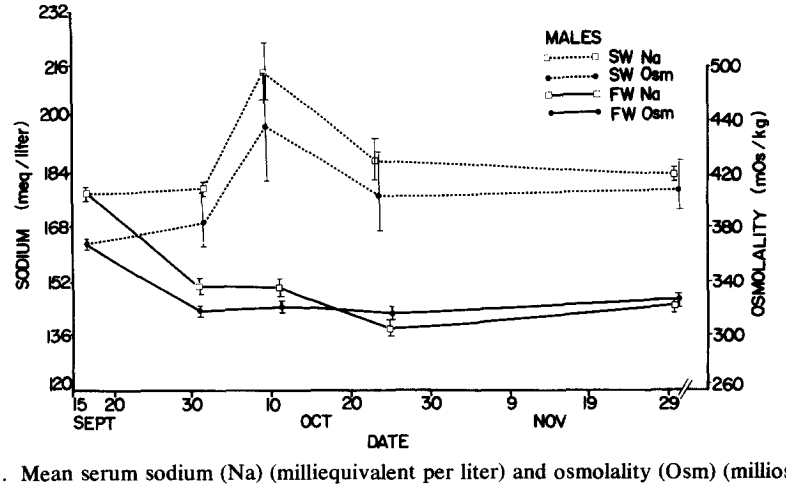

FIG. 1. Mean serum sodium (Na) (milliequivalent per liter) and osmolality (Osm) (milliosmole per kilogram) concentrations for male coho salmon held in seawater (SW) or fresh water (FW) during the final maturation of the spawning season from September 15 to December 5, 1979. First data point represents fish in seawater and end point represents spermiation.

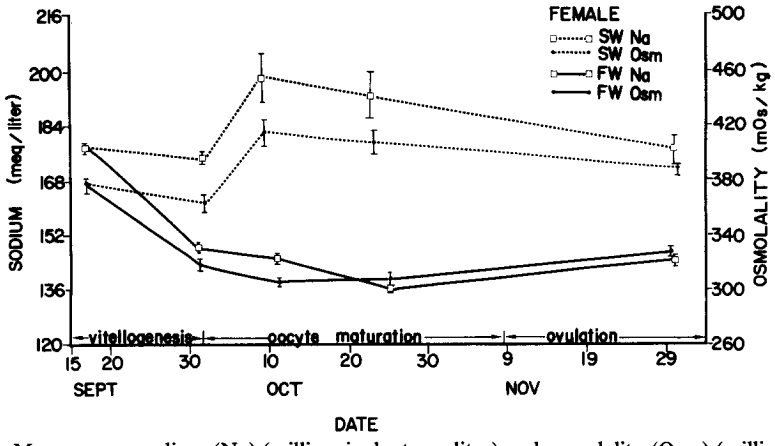

FIG. 2. Mean serum sodium (Na) (milliequivalent per liter) and osmolality (Osm) (milliosmole per kilogram) concentrations for female coho salmon held in seawater (SW) or fresh water (FW) during the final maturation of the spawning season from September 15 to December 5, 1979. First data point represents fish in seawater and end point represents ovulation.

held in seawater during early October. Mortalities began to increase at this time and remained high after this period. Osmolality and sodium changed little in female or male salmon held in fresh water.

The serum concentrations of estradiol-17β in female salmon showed an overall decrease from the time they entered the seawater facility to the time of ovulation, regardless of whether they were held in fresh water or seawater (Fig. 3). Estradiol-17β tended to fluctuate, however, during oocyte maturation. Estradiol-17β levels in female coho salmon in seawater during the early stages of oocyte maturation were significantly higher (6.38 $\pm$ 0.52 ng/ml) than those in salmon in fresh water (3.96 $\pm$ 0.55 ng/ml; Fig. 3). At ovulation, estradiol-17β was sig-

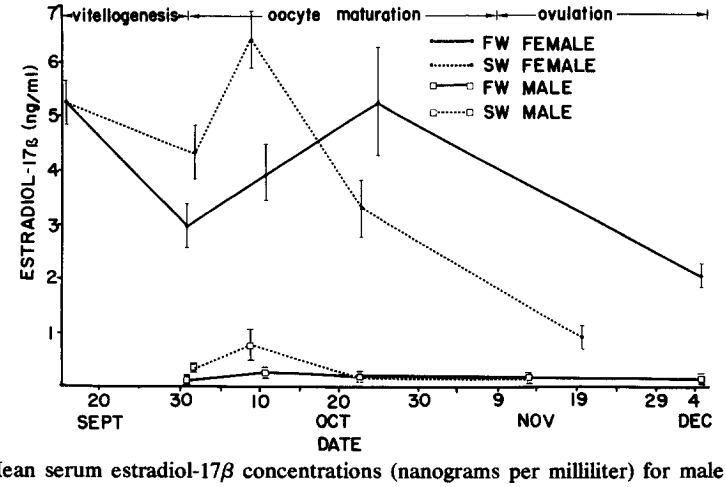

FIG. 3. Mean serum estradiol-17β concentrations (nanograms per milliliter) for male and female coho salmon held in seawater (SW) or fresh water (FW) during the final maturation of the spawning season from September 15 to December 5, 1979. First data point for female salmon represents fish in seawater.

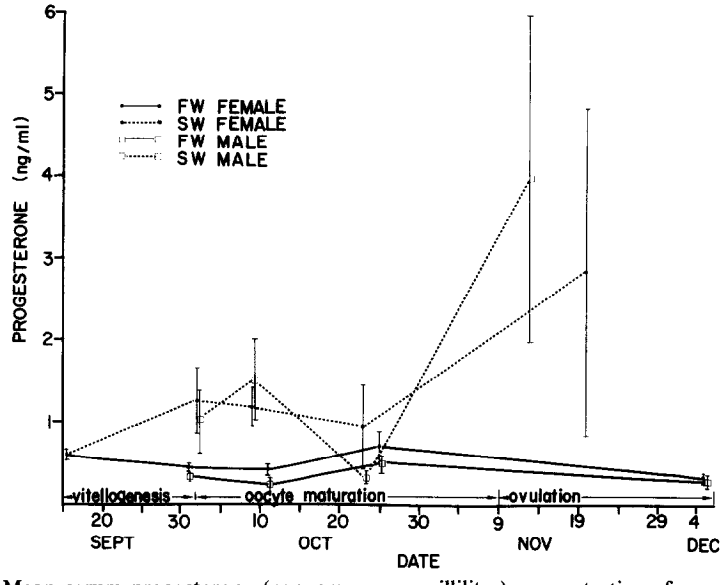

FIG. 4. Mean serum progesterone (nanograms per milliliter) concentrations for male and female coho salmon held in seawater (SW) or fresh water (FW) during the final maturation of the spawning season from September 15 to December 5, 1979. First data point represents females in seawater.

nificantly lower in the females held in seawater than in those held in fresh water (Fig. 3). Serum estradiol-17β levels in males were lower than in females and were not significantly different in fresh water and seawater fish throughout the sampling period.

Progesterone levels of both males and females in fresh water were low, and covaried within a range of 0.26 $\pm$ 0.03 to 0.72 $\pm$ 0.21 ng/ml (Fig. 4). Similar to estradiol-17β, progesterone during the early stages of final maturation in female and male salmon was higher in fish held in seawater than in fish held in fresh water. At spermiation and ovulation, progesterone levels were higher in male and female coho salmon in seawater compared to salmon in fresh water (Fig. 4).

Thyroxine levels in serum were higher in males than in females, in both seawater and fresh water (Fig. 5). In the final stages of maturation, thyroxine levels were significantly higher in male salmon in fresh water compared to those found in female salmon in fresh water (Fig. 5). Thyroxine decreased throughout the spawning season in both male and female coho salmon in fresh water (Fig. 5). At ovulation, thyroxine levels in females were higher in fish held in seawater than in fish held in fresh water. Similarly, thyroxine at spermiation was significantly higher in males in seawater than in those in fresh water.

During the later stages of final maturation, androgen levels were significantly higher in males in fresh water than in those in seawater (Fig. 6). Before ovulation and spermiation, androgen levels in both males and females in fresh water increased, whereas no elevation occurred in males and females held in seawater. Greater variation of androgen levels occurred in the female compared to male salmon (Fig. 6).

DISCUSSION

Sodium and osmolality in fish in seawater increased substantially during the early stages of final gonadal maturation. This increase is highly suggestive that osmoregulatory difficulties may have been the base for the high adult mortality observed in fish held in seawater. There appear to be strong interrelationships between os-

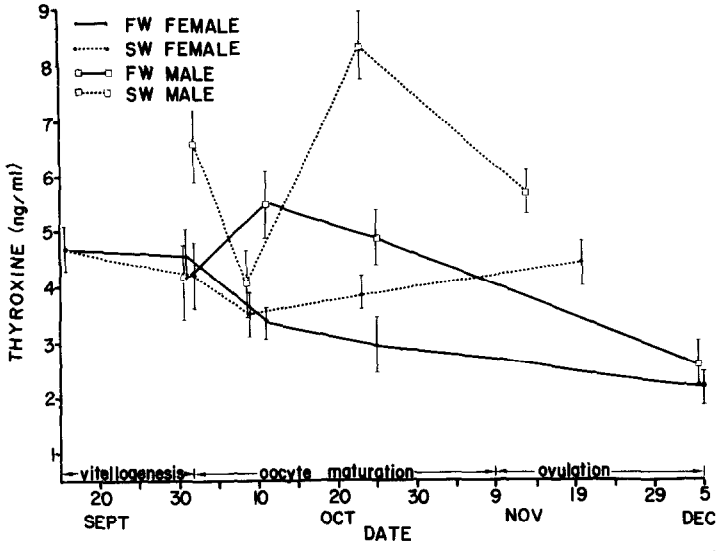

FIG. 5. Mean serum thyroxine concentrations (nanograms per milliliter) for male and female coho salmon held in seawater (SW) or fresh water (FW) during the spawning season from September 15 to December 5, 1979. First data point for female salmon represents fish in seawater.

moregulatory and reproductive factors in female coho salmon remaining in seawater to spawn. For example, hormone profiles of estradiol-17β, thyroxine, androgens, and progesterone differed between coho salmon in fresh water and those in seawater. These different profiles of hormones of maturing salmon in seawater coincided with collapsed or dehydrated eggs, ovaries that were not completely ovulated, and low egg survival. Some of these abnormalities may have been the result of incomplete oocyte maturation due to an ion imbalance in the egg (Sower, 1980). Therefore, osmoregu-

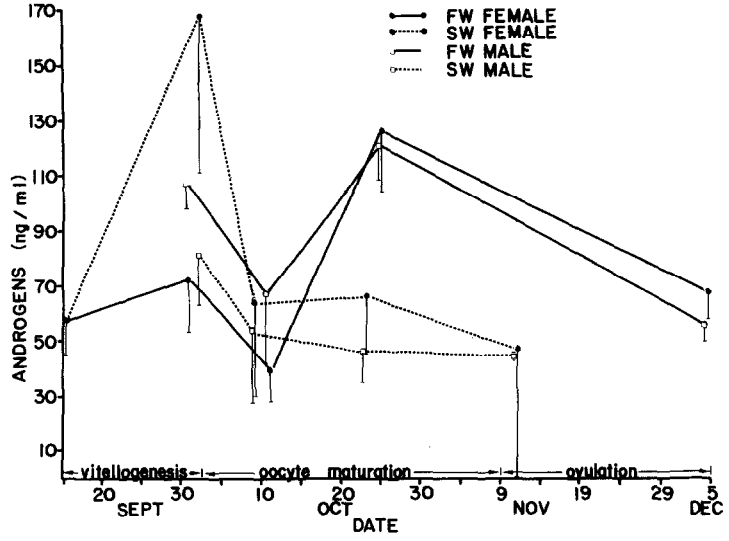

FIG. 6. Mean serum androgen concentrations (nanograms per milliliter) for male and female coho salmon held in seawater (SW) or fresh water (FW) during the spawning season from September 15 to December 15, 1979. First data point for females represents fish in seawater.

latory factors may inhibit or antagonize reproduction. The response of the ovary may also be desensitized by the hypersaline environment. Different responses of the ovary to gonadotropin after exposure to different temperatures in goldfish (*Carassius auratus*) were demonstrated by Cook and Peter (1980). When the salmon normally enter fresh water from the ocean, they must undergo osmotic changes; these osmotic changes affect, or are effected, by the endocrine system (Woodhead, 1975). Thus, if the returning salmon are prevented normal entry into fresh water, the endocrine system may not be able to respond properly.

Levels of estradiol-17β in females in fresh water decreased at the end of vitellogenesis, increased during the first stages of oocyte maturation, and then decreased at ovulation. In contrast, estradiol-17β levels in females in seawater decreased during the first stages of oocyte maturation, and decreased ever further at ovulation. In both males and females in seawater, estradiol-17β was elevated during the early stages of final maturation, an elevation that may be due to hemoconcentration, as suggested by the increased sodium and osmolality levels, rather than an actual increase in hormone concentrations. The role of estrogen during final maturation and ovulation is unknown. In coho salmon (Jalabert *et al.*, 1978) and rainbow trout (Fostier *et al.*, 1978), estradiol-17β levels were elevated just before ovulation, and decreased at ovulation. The same trend was shown by salmon in fresh water in the present study, including an increase in estradiol in the early stages of oocyte maturation. Estradiol-17β is believed to play a prominent role in yolk synthesis, although Fostier *et al.* (1978) indicated that no generalization can be made about the role of estrogen during final maturation and ovulation. Steroid hormones, especially estradiol-17β, however, have been implicated in modulating the changes of responsiveness to luteinizing hormone-releasing hormone during reproductive cycles of mammals (reviewed by Peter, 1978). Furthermore, it has been established in the rat that estradiol-17β enhances the responsiveness of follicular cells of gonadotropins, which causes an increase in luteinizing hormone receptors (Richards, 1979). Although the role of estradiol-17β has not been established in oocyte maturation and ovulation in fish, it is evident from studies of higher vertebrates that estrogens can influence the responsiveness of gonadotropins, and thus may be involved in final maturation. One can speculate that estradiol-17β plays an unknown yet important role in final maturation. Therefore, the different hormone levels of coho salmon in seawater associated with incomplete ovulation suggest improper functioning of the endocrine system during final maturation and ovulation.

Progesterone concentrations in females and males in seawater were higher and extremely variable at ovulation, compared with those in fish in fresh water. Progesterone was also elevated during the early stages of final maturation, perhaps due to hemoconcentration, as suggested by the increased sodium and osmolality levels. Campbell *et al.* (1980) believed that in rainbow trout 17-α-hydroxy-20β-dihydroprogesterone or 17α-hydroxyprogesterone, rather than progesterone, are the active mediators in oocyte maturation as demonstrated by their high plasma levels during this time in addition to their effects *in vitro*. Campbell *et al.* (1980) measured low progesterone levels which were only slightly higher than the progesterone levels measured in male and female salmon in this study.

Androgen concentrations in females and males in fresh water were similar and covaried, increasing during the first stages of maturation and decreasing at ovulation and spermiation. Androgen levels in females and males in seawater were also similar and covaried, except during the early stages of maturation when the androgen levels were higher in females than

in males. Androgen levels in fish in seawater remained stable during the first stages of maturation with lowered levels at ovulation and spermiation. Schmidt and Idler (1962) and Campbell *et al.* (1980) have shown high testosterone levels in female Atlantic salmon (32 ng/ml) and rainbow trout (187 ng/ml) and high 11-ketotestosterone and testosterone in male Atlantic salmon (48 and 108 ng/ml, respectively) and rainbow trout (98 and 59 ng/ml, respectively) during the spawning season. On the basis of these elevated levels, Campbell *et al.* (1980) suggested that the active steroid in gonad maturation and spermiation in male rainbow trout was 11-ketotestosterone but that this steroid was probably not the major androgen in female rainbow trout. It is unclear from our study which androgen, 11-ketotestosterone or testosterone, was higher in female or male salmon, since the antibody used binds both androgens.

Thyroxine levels in male and female salmon in fresh water generally decreased during the spawning season to low levels at the time of spermiation and ovulation. Thyroxine levels in male salmon in seawater increased during the early stages of maturation and decreased at spermiation, but the concentration present at spermiation was significantly higher in fish in seawater than those in fresh water. Thyroxine in female salmon in seawater varied slightly, and remained essentially the same by the time of ovulation. At ovulation, thyroxine was higher in females in seawater than in those in fresh water. The role of thyroxine in ovarian development is unknown. Thyroxine has been shown to enhance the effectiveness of SG-G100 in maintaining the ovary in goldfish (reviewed by Lam *et al.*, 1978). Studies on Atlantic and Pacific salmon have shown a general decrease in thyroid activity as the fish migrate upstream in fresh water until they spawn (reviewed by Woodhead, 1975). Thyroxine which has been implicated in the process of parr–smolt transformation of juvenile salmon (Dickhoff *et al.*, 1978) may also play a prominent role in determining the fish's readiness for entry into fresh water. Thyroxine did not decrease in salmon that remained in seawater. If one interprets that high concentrations of this hormone are indicative of biological function, it is possible that thyroxine in salmon in seawater may be involved in osmoregulation and/or general metabolism. As with the other hormones measured, however, the different levels of thyroxine in coho salmon in fresh water versus seawater may be a reflection of osmoregulatory difficulties of fish retained in seawater.

It is apparent from the differences in hormone profiles, dehydrated eggs, small amounts of seminal fluid, incomplete ovulation, low egg survival, and high adult mortality of salmon that are retained in seawater during the spawning season that reproductive function was compromised. Although functions of the various hormones in this study have not been established clearly, some of them are probably active in reproduction. As stated earlier, the response of the ovary may be desensitized by the hypersaline environment, which was indicated by generally higher circulating levels of hormones of salmon in seawater. Also, hormonal treatments that were effective in inducing ovulation in salmon in fresh water were less effective or in some cases ineffective in inducing ovulation in salmon in seawater (Sower, 1980), again suggesting gonadal insensitivity. Therefore, it is highly suggestive that osmoregulatory factors strongly influence the maturational processes of coho salmon in a hypersaline environment, resulting in hindered reproductive development.

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