

Changes in Plasma Thyroxine, Estradiol-17 β , and 17 α ,20 β -dihydroxy-4-pregnen-3-one during Smoltification of Coho Salmon

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Circulating levels of plasma estradiol, 17 α ,20 β -dihydroxy-4-pregnen-3-one (17 α ,20 β PROG), and thyroxine were measured during smoltification of coho salmon in two successive years (1984 and 1985). Two elevations of thyroxine occurred in both years, the first occurred in April followed by a second increase in May. Estradiol levels were dynamic with variable changes particularly in postsmolts when estradiol levels were either depressed in 1984 or high in 1985. However, elevations of estradiol preceded the second elevation of thyroxine in May in both years when the salmon were undergoing the later stages of smoltification. There were no consistent changes of plasma 17 α ,20B PROG during this period. Although, in 1984, there were significant elevations and depressions of 17 α ,20B PROG, and in 1985, there was a major unexplained depression of 17 α ,20B PROG on April 24. There were no sexual differences noted for plasma steroid levels in the juvenile fish. These data suggest that there are seasonal changes of estradiol coinciding with other developmental changes during smoltification. © 1992 Academic Press, Inc.

Coho salmon (*Oncorhynchus kisutch*) are anadromous and undergo two migrations during their life cycle. During the first migration, juvenile fish undergo morphological and physiological changes prior to seaward entrance referred to as smoltification (Hoar, 1976). The second migration occurs after approximately 18 months at sea when the salmon return as adults to fresh water to complete their final maturation and spawning, after which they die.

During their life cycle, the salmon progress through distinct and defined maturational stages. Histological studies have demonstrated that gonads of coho salmon are rudimentary prior to smoltification with subsequent stages of gonadal development occurring in the smolts upon seawater entry (Fontaine, 1954; Chestnut, 1970). In earlier

studies, Hoar *et al.* (1952) and Baggerman (1960) suggested a possible influence of gonadal steroids on migratory behavior in juvenile salmon. However, data on circulating sex steroids during smoltification in salmon are conflicting. Increasing plasma levels of estradiol coinciding with increases of thyroxine were demonstrated in coho salmon during smoltification (Sower *et al.*, 1984a). The significance of this estradiol increase is not known; however, coordinated changes in reproduction associated with smoltification are one possible explanation. In other studies, Patino and Schreck (1986) failed to demonstrate any changes of estradiol in coho salmon and Nagahama *et al.* (1982) were unable to detect plasma estradiol in amago salmon (*Oncorhynchus rhodurus*) during the period of smoltification. Coho salmon spawn only once in their lifetime such that the reproductive physiology of salmon of a certain year class develops in

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a synchronous pattern. Reproductive development would in all probability be coordinated with other developmental changes such as smoltification. Annual cycles in the gonads and thyroid gland and their interactions have been described for numerous species of vertebrates. Further studies on plasma sex steroids and gonadal histology correlated with plasma thyroxine are necessary to determine any possible changes of sex steroids during smoltification.

Our major objectives were to measure plasma estradiol, $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one ($17\alpha,20\beta$ PROG), thyroxine, and activity of the gonad to define any possible relationship between changes in sex steroids and thyroxine.

MATERIALS AND METHODS

Experimental animals. Juvenile coho salmon were maintained in circular concrete pools (8 m in diameter) under natural photoperiod in well water ranging from 5° to 16° throughout the sampling period at the New Hampshire Fish and Game Milford Hatchery, New Hampshire. Fish were fed commercial diets several times a day at a prescribed percentage of body weight.

1984 and 1985. Juvenile coho salmon were reared in two successive years (1984 and 1985) at Milford Hatchery for 15 months until their release into the Lamprey River for ocean migration in April. A few hundred fish were held back in fresh water until June.

In both experiments, at bimonthly intervals from January until June, 20 fish each were randomly sampled by use of a cast net from two ponds. The fish were then sampled for length, weight, blood, and gonads.

Radioimmunoassays and histology. Fish were not fed during the 24-hr period before sampling. Fish were stunned by a blow to the head and blood was collected immediately from the severed caudal blood vessels into lithium-heparinized tubes. Blood was centrifuged and plasma collected and stored at -20° until assayed for thyroxine, estradiol, and $17\alpha,20\beta$ PROG. Equal-sized plasma samples taken from two or three fish (either all male or all female) were pooled for estradiol and $17\alpha,20\beta$ PROG determinations. Plasma thyroxine (T_4) was measured by the method of Dickhoff *et al.* (1978) as modified by McCormick *et al.* (1987). Plasma (100 μ l) was measured for estradiol by radioimmunoassay as described by Sower and Schreck (1982) as modified by Sower *et al.* (1984a,b) with the lower limit of detection at 6 pg/ml. [3 H] $17\alpha,20\beta$ PROG was prepared as described by Scott *et al.* (1982). The radio-

immunoassay for $17\alpha,20\beta$ PROG was validated for juvenile coho salmon plasma as described by Scott *et al.* (1982) with the lower limit of detection at 60 pg/ml. Assay procedures for $17\alpha,20\beta$ PROG followed the progesterone procedure as described by Sower and Schreck (1982). The antibody binding efficiencies for estradiol and $17\alpha,20\beta$ PROG ranged from 33 to 43% and 31 to 49%, respectively. The interassay coefficients of variation for estradiol and $17\alpha,20\beta$ PROG were 3.6% ($n = 6$) and 11.3% ($n = 10$), respectively.

Gonadal tissues were preserved in Bouin's solution, embedded in paraffin, sectioned at 10 μ m, and stained with hematoxylin and eosin for histological examination to determine sex and analyze gonadal development. Degree of gonadal development was based on criteria of Beams and Kessel (1973), Funk *et al.* (1973), and Yasutake and Wales (1983). Any testis exhibiting precocious male sexual development as characterized by advanced stages of spermatogenesis was not used in the final analysis.

Data for hormone concentrations were analyzed by a Student-Newman-Keuls test after preliminary multiple analysis of variance. In all tests, the levels of significance for differing groups were $P < 0.05$.

RESULTS

The data for the fish from the two ponds were pooled together since the data were not significantly different. In addition, because there was no significant sexual differences in various plasma hormone levels, the values for male and female salmon were also pooled within groups. Temperature ($^\circ$ C) generally was very low during February through April with increasing temperatures occurring in early May 1984 and in late April 1985 (Fig. 1).

With respect to condition factor and morphological criteria (Sower *et al.* 1984a), stages of early smoltification were evident in April with most of the fish smolts by June (Table 1).

Gonadal Histology

Histological examination of gonads revealed slight changes in gametogenesis during the experimental periods. All ovaries were in the perinucleolar stage of oocyte development. Early stages of spermatogen-

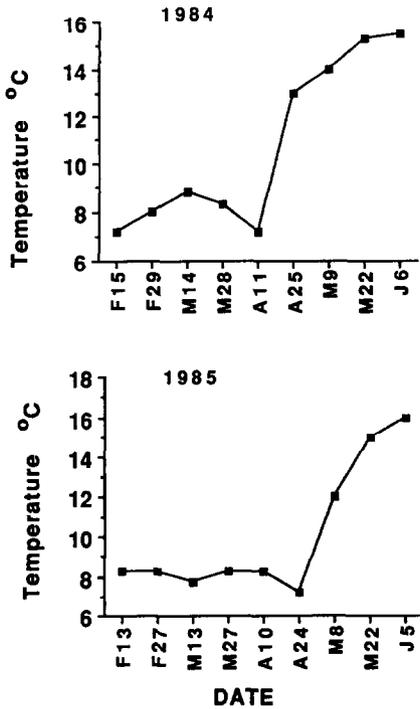


FIG. 1. Water temperature profile (°C) in Experiment 1 and Experiment 2.

esis (spermatogonial stage) were prevalent in the testes of males.

1984: Plasma Thyroxine, Estradiol, and $17\alpha,20\beta$ PROG

Plasma thyroxine changed significantly ($P < 0.05$) through time with two peaks occurring April 11 and May 22 (Fig. 2). Plasma thyroxine gradually increased from 0.2 ± 0.01 SE ng/ml on February 15 to 4.3 ± 0.4 ng/ml on April 11 followed by a decline to 1.9 ± 0.1 ng/ml on April 25 with another increase to 4.34 ± 0.55 ng/ml on May 22.

Plasma estradiol levels fluctuated significantly through time with a significant elevation occurring May 9 at 132 ± 12 pg/ml (Fig. 2).

Plasma $17\alpha,20\beta$ PROG varied significantly through time. There were significant elevations occurring March 28 and May 22

TABLE I
MEAN CONDITION FACTOR OF COHO SALMON

Date	Condition factor ($\bar{X} \pm \text{SE}$)
1984	
February 15	1.10 ± 0.01
February 29	1.07 ± 0.01
March 14	1.05 ± 0.01
March 28	1.03 ± 0.01
April 11	0.97 ± 0.02
April 25	0.92 ± 0.01
May 9	0.92 ± 0.01
May 22	0.94 ± 0.01
June 6	0.95 ± 0.01
1985	
February 13	1.09 ± 0.01
February 27	1.13 ± 0.01
March 13	1.15 ± 0.01
March 27	1.00 ± 0.03
April 10	1.00 ± 0.02
April 24	0.95 ± 0.02
May 8	0.98 ± 0.01
May 22	0.95 ± 0.01

(470 ± 45 and 499 ± 60 pg/ml, respectively) (Fig. 2).

1985: Plasma Thyroxine, Estradiol, and $17\alpha,20\beta$ PROG

Plasma thyroxine levels increased significantly ($P < 0.05$) in May during the smoltification period (Fig. 3) with two peaks. Plasma thyroxine gradually increased from 3.4 ± 0.2 ng/ml on February 13 to 4.7 ± 0.7 ng/ml on April 10 and continued to increase to 5.7 ± 0.5 ng/ml on May 22.

Plasma estradiol levels of coho salmon increased significantly on ($P < 0.05$) February 27, May 8 and May 22. Estradiol increased from 30 ± 5 pg/ml on February 13 to 67 ± 7 pg/ml on February 27, then decreased to 11 ± 2 pg/ml on March 13, and remained low until May 8 with an increase to 65 ± 5 pg/ml on May 8 and 30 ± 6 pg/ml on May 22 (Fig. 3).

Plasma $17\alpha,20\beta$ PROG was not significantly different between male and female coho salmon and did not vary significantly

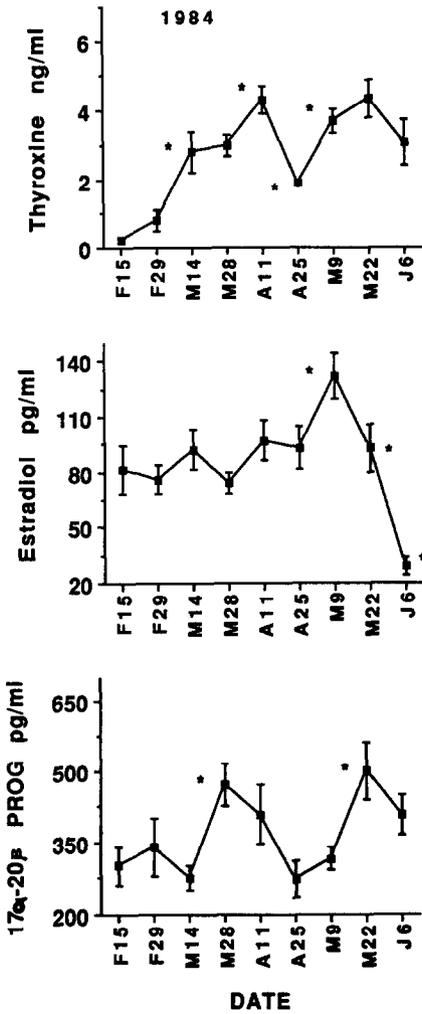


FIG. 2. 1984: Plasma thyroxine, estradiol, and 17 α ,20 β -dihydroxy-4-pregnen-3-one (17 α ,20 β PROG) of coho salmon during smoltification. Each point represents the mean \pm SE ($n = 10-20$). F, February; M, March; A, April; M, May; and J, June. *Represents a significance difference ($P < 0.05$) in the mean level when compared to the previous mean.

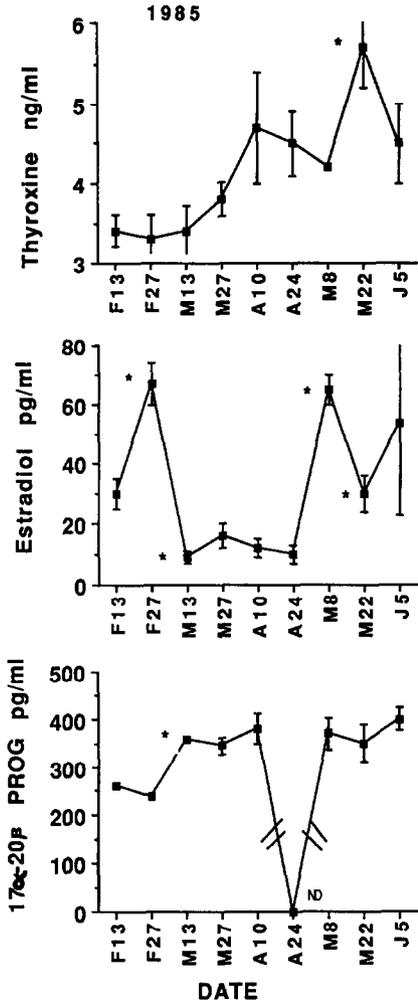


FIG. 3. 1985: Plasma thyroxine, estradiol, and 17 α ,20 β -dihydroxy-4-pregnen-3-one (17 α ,20 β PROG) of coho salmon during smoltification. Each point represents the mean \pm SE ($n = 10-20$). F, February; M, March; A, April; M, May; and J, June. *Represents a significance difference ($P < 0.05$) in the mean level when compared to the previous mean.

through time (Fig. 3). There was a major depression of 17 α ,20 β PROG on April 24, however the levels were nondetectable, in both male and female fish.

DISCUSSION

The results of this study confirm earlier studies demonstrating increases of thyroxine and estradiol activity in coho salmon

undergoing smoltification (Sower *et al.*, 1984a). There were two elevations of thyroxine, the first occurring in April followed by a second peak in May in salmon in successive years. Elevations of estradiol preceded the second elevation of thyroxine in May during the later stages of smoltification as determined by morphological criteria and condition factor (Sower *et al.*, 1984a). The salmon were completely

smolted by the end of June. Estradiol levels were dynamic with variable changes particularly in postsmolts when estradiol levels were either depressed in 1984 or high in 1985. There were no consistent changes of $17\alpha,20\beta$ PROG during smoltification in juvenile salmon in the two experiments, although there were significant elevations and depressions of $17\alpha,20\beta$ PROG in 1984 with an unexplained major depression of $17\alpha,20\beta$ PROG on April 24, 1985.

Plasma thyroxine levels in Sower *et al.*'s (1984) study were higher than those observed in the present study. It may be considered that the different values of thyroxine observed reflect the different rearing conditions, water temperature, or different stock of fish between the two studies.

The present studies indicate seasonal changes of estradiol occurring concurrently with smoltification processes. During their life cycle, the salmon progress through distinct and defined maturational stages. Fontaine (1954) and Chestnut (1970) have demonstrated in morphological studies that coho salmon undergo distinct gonadal maturation before and near the time of smoltification. This information suggests that there may be several coordinated processes which respond to environmental cues such as photoperiod and temperature. In the present study similar to Sower *et al.*'s (1984a) study, elevations of estradiol slightly preceded elevations of thyroxine in May with increasing water temperatures during late April and early May. Various studies have focused on the possible influence of gonadal steroids on migratory behavior in juvenile salmon (Hoar *et al.*, 1952; Baggerman, 1960). Baggerman (1960) concluded from her studies and others that gonadal steroids may influence the fish's ability to sense external stimuli which would influence the migrational processes. Smolt migration has been correlated with rising springtime temperatures (Foerster, 1937; White, 1939). Increases in steroids during smoltification may reflect increases in tem-

perature that cue migratory behavior. Coho salmon sex reversed or sterilized with sex steroids were shown to undergo normal smoltification but failed to undertake anadromous migration back into fresh water (Hunter *et al.*, 1982). Thus one possible role of gonadal steroids may be involved in influencing migratory behavior associated with smoltification. Further studies would be required to determine the possible role of steroids in migratory behavior in juvenile salmon.

Seasonal changes in the gonads and thyroid gland and their interactions have been described in salmonid species (White and Henderson, 1977; Osborn *et al.*, 1978; Leatherland and Sonstegard, 1980, 1981; Pickering and Christie, 1981; Ueda *et al.*, 1984; Cyr *et al.*, 1988a,b). Cyr and Eales (1988) have demonstrated in rainbow trout that thyroid hormones can act directly upon ovarian follicles and amplify the response to salmon gonadotropin on estradiol secretion. Likewise, several studies in fish have indicated sex steroids can influence thyroid activity (Hunt and Eales, 1979; Sage and Bromage, 1970; Singh, 1969). For example, androgen administered to immature male and female trout caused an increase in thyroid hormone levels (Hunt and Eales, 1979). The present study provides further evidence for correlations between thyroid and reproductive changes during seasonal events.

These data and studies of Sower *et al.* (1984a) are in contrast with studies of Patino and Schreck (1986) who found sexual differences but not seasonal changes in circulating levels of plasma estradiol in coho salmon undergoing smoltification. There are several possible explanations for the differences noted in the relationship of estradiol during smoltification including stock of fish, rearing conditions, water temperature, and experimental sampling period. In other studies, high doses of estradiol (2.5, 25 $\mu\text{g/g}$ diet) administered for 4 months inhibited smoltification in masu salmon (*On-*

corhynchus masou) (Ikuta *et al.*, 1987). These doses induced blood levels that were threefold higher compared to control fish. Normal levels of estradiol in juvenile salmon are generally less than 1 ng/ml compared to a range of 1–15 ng/ml for adult salmonids (Sower and Schreck, 1982; Sower *et al.*, 1984a; Ueda *et al.*, 1984; Scott *et al.*, 1983). It has been demonstrated that treatment of salmon with dietary estradiol at 20 µg/g diet has resulted in high mortalities (Ashby, 1957; Goetz *et al.*, 1979; Johnstone *et al.*, 1979; Sower *et al.*, 1984b). These studies indicated as in mammals (Serra *et al.*, 1983) that administered high levels of estradiol are toxic in fish. Therefore it is not surprising that administered high doses of estradiol inhibited smoltification in studies of Ikuta *et al.* (1987). There have been no studies examining the role of administered physiological doses of estradiol in salmon during smoltification. Further studies are needed to examine the possible effects of estradiol on smoltification in salmonids.

In the present study, plasma levels of 17 α ,20 β PROG were similar in males and females during smoltification which is similar to Patino and Schreck's (1986) study that did not show any consistent sexual differences nor any changes occurring during smoltification in salmon. 17 α ,20 β PROG has been associated with maturing adult salmon including *Onchorhynchus nerka* (Schmidt and Idler, 1962), *O. rhodurus* (Ueda *et al.*, 1983), and *Salmo gairdneri* (Scott *et al.*, 1982; Scott and Baynes, 1982). In female salmon, 17 α ,20 β PROG is considered to be the maturational inducing steroid (Nagahama and Yamashita, 1989). In adult male salmon, the exact function of 17 α ,20 β PROG is unknown although Liley *et al.* (1986) suggested that this steroid may stimulate an increase in milt production. A possible function for 17 α ,20 β PROG during development is not clear since there are no distinct patterns of this steroid during smol-

tification and further studies would be required to determine any such function.

In summary, increases of plasma thyroxine and estradiol in juvenile salmon undergoing smoltification were associated with increases in water temperature, suggesting that there are seasonal changes of estradiol coinciding with other developmental changes during smoltification. Further experiments are necessary to determine any possible interactions between thyroid and reproductive processes including the measurement of circulating gonadotropins during smoltification.

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