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Changes in Plasma Estradiol and Effects of Triiodothyronine on Plasma Estradiol during Smoltification of Coho Salmon, *Oncorhynchus kisutch*

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Plasma estradiol-17β levels were measured by radioimmunoassay in untreated coho salmon of both sexes or in fish fed triiodo-L-thyronine during the smoltification period. Mean plasma estradiol increased between February 25 and April 1 from 94 to 142 pg/ml, and then by May 13, it had decreased to 80 pg/ml. This hormonal cycle was followed by a second significant increase to 219 pg/ml on June 22. Plasma thyroxine level covaried with that of estradiol. Treatment with triiodothyronine had no effect on plasma estradiol or thyroxine levels. Plasma estradiol surges during the smoltification period must be considered along with other significant endocrine changes for a possible role in the developmental phenomena that characterize this period.

Coho salmon *(Oncorhynchus kisutch),* an anadromous species, undergo two migrations during the life cycle. The first occurs in the spring, when the young salmon migrate downstream while undergoing the morphological and physiological changes referred to as smoltification; these changes at least in part, prepare the fish for life in seawater (Hoar, 1976). A surge of plasma thyroxine levels, coinciding with the developmental events of the spring smoltification period, have been well documented in salmon (Dickhoff *et al.,* 1978; Nishikawa *et al.,* 1979). After 18 to 24 months at sea, the fish return as adults to freshwater to complete their final maturation and spawning, after which they die.

During the first migration the salmon are progressing through distinct and defined maturational stages. Prior to smoltification the juvenile coho salmon (parr stage) have quiescent rudimentary gonads, whereas after seawater entry (smolt stage) gonadal development has begun (Fontaine, 1954; Chestnut, 1970). Despite the morphological evidence indicating that coho salmon undergo distinct gonadal maturation before and near the time of smoltification, only two previous studies have addressed the role of reproductive hormones in the smoltification process. These studies have focused on the possible influence of gonadal steroids on migratory behavior in juvenile salmon (Hoar *et al.,* 1952; Baggerman, 1960). Although there appears to be an activation of the thyroid and reproductive systems of salmon at the time of seawater entry, no measurements of gonadal steroids have yet been reported during this period.

Annual cycles in the gonads and thyroid gland and their interactions have been described for numerous species of vertebrates. However, evidence for the interrelatedness of such cycles or for interactions of the thyroid and reproductive hormones in teleosts is limited (Sage, 1973).

Thyroid hormone administration in juvenile salmon has been shown to elicit or accelerate many of the physiological changes which are associated with smolti-

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fication. Among these changes are increased growth rate, alterations of body proximate composition, development of silvery body coloration due to integumentary deposition of purines, and an accelerated development of salinity tolerance (Higgs *et al.*, 1982; McBride *et al.*, 1982). Yet, the reciprocal actions of thyroid hormones and reproductive hormones have not been evaluated in juvenile coho salmon.

Our principal objectives were to measure plasma estradiol-17β concentrations, to recognize any relationship between changes in plasma estradiol and thyroxine, and to determine whether exogenous triiodothyronine administered to raise plasma thyroid hormone levels has any effect on estradiol levels during the presmoltification and smoltification periods of coho salmon.

MATERIALS AND METHODS

The experimental animals used in this study were yearling coho salmon from the 1981 brood. These fish were from a stock which had originated from Southern Baranoff Island, Alaska, and had been reared for two generations at the Northwest and Alaska Fisheries Center, National Marine Fisheries Service, Seattle, Washington. Sixteen hundred fish (mean weight 10 g) were randomly distributed in eight 540-liter circular fiberglass tanks, with each tank receiving 200 salmon. The fish were reared in Lake Washington freshwater at the ambient temperature ranging from 6 to 15° under natural photoperiod. The fish were fed a commercial salmon diet (Omp II, Moore Clarke, Inc., La Conner, Wash.) to satiation twice daily, at approximately 0800 and 1500 hr.

The hormone diet was prepared by dissolving 3, 5, 3'-triiodothyronine (T_3) in alkaline ethanol (33 ml 95% EtOH/12 ml 0.1 *N* NaOH) and spraying this mixture onto the surface of an α-cellulose carrier (Sigma Chemical Co.) in a commercial food mixer. A chromatograph sprayer and nitrogen vehicle were used. The concentration of the hormone solution was adjusted so that each kilogram of food contained 12 ppm of T_3 and 1% α-cellulose. The carrier and diet were homogenized in a food mixer, repelleted, and stored at −20°. The control diet was prepared in an identical fashion except that the hormone was omitted from the preparation. The hormone-containing diet was introduced on 3 February 1982.

The fish were sampled by anesthetizing with ethyl *m*-aminobenzoate methanesulfonate at regular intervals, beginning 2 February 1982 and continuing to September 1982. Fish were not fed during the 24 hr before sampling. Specimens were not separated or identified by sex. Mean length, mean weight, and the degree of smoltification were evaluated and recorded for 10 to 40 fish removed from each treatment group at each sampling. Degree of smoltification of the fish was judged by use of arbitrary morphological criteria modified from Gorbman *et al.* (1982) (Table 1). At the time of sampling, 10 to 40 fish from each treatment group were killed and sampled for blood. Blood was collected immediately after death from the severed caudal blood vessels and directed into ammonium-heparinized capillary tubes; it was centrifuged and the plasma was stored at −20° until analyzed for thyroxine and estradiol-17β.

Thyroxine was determined in 10 μl of plasma by radioimmunoassay as described by Dickhoff *et al.* (1978). The plasma estradiol-17β (E_2) level was measured by a radioimmunoassay procedure described by Sower and Schreck (1982). Briefly, 100 μl of plasma samples was extracted twice with diethyl ether; the extract was evaporated to dryness with nitrogen gas, and assayed directly for estradiol. Antiestradiol-17β antibody (S-244) was obtained from Dr. G. Niswender (Colorado State University, Fort Collins, Colo.) and diluted 1:8500 in phosphate-buffered saline–gelatin (PG). The lower limit of detection was about 6 pg/ml. The antibody binding was 57%. For each treatment type, equal sized plasma samples taken from two or three fish were pooled for estradiol-17β assay determinations.

Data for length, weight, and hormone concentrations were evaluated by analysis of variance. Data expressing the degree of smoltification (expressed as a percentage) were analyzed by the use of a 2 × 2 contingency table (Zar, 1974). In all tests the level of significance for mean data differences among the animal groups $P < 0.05$.

RESULTS

With respect to morphological criteria most of the control and T_3-treated fish were considered to be smolts by May 27 (Table 2). Degree of smoltification was significantly different between hormone-treated and control fish only on April 29 (controls, 0.100, T_3-treated fish, 0.467). However, in T_3-treated fish, weight and length were increased significantly compared to the controls (Table 2).

Treatment with T_3 had no measurable effect on plasma estradiol or thyroxine.

TABLE 1
MORPHOLOGICAL CHARACTERISTICS USED TO DETERMINE SMOLT INDEX

Stage	Parr marks	Body color		Fin color	
		Dorsal	Ventral	Caudal	Anal
Parr (1)	Distinct	Brown	Yellow	Yellow	Yellow
Parr/transitional (2)	Distinct	Brown	Yellow	Clearing with margin darkening	Clearing
Transitional (3)	Fading but evident	Yellow/green	Yellow/green	Clearing with margin darkening	Clearing
Transitional/smolt (4)	Almost absent	Green to blue	Silvery	Clear with margin darkening	Clear
Smolt (5)	Absent	Blue	Silvery	Clear with black posterior margin	Clear

Note. Stages were assigned numerical values, in parentheses. Parr marks consist of dark vertical bars of pigment on the lateral surface which are characteristic of the juvenile (parr) salmon prior to smoltification.

TABLE 2
WEIGHT, FORK LENGTH, AND PERCENTAGE SMOLT OF CONTROL AND TRIIODOTHYRONINE (T_3)-TREATED COHO SALMON SAMPLED FROM FEBRUARY TO SEPTEMBER 1982

Date	Treatment	Weight[a] (g)	Fork length (cm)	Percentage smolt
February 2	Control (40)	10.5 ± 0.4	9.5 ± 0.8	0.00
	T_3 (40)	11.3 ± 0.4	9.7 ± 0.7	0.00
February 25	Control (40)	11.8 ± 0.4	9.7 ± 0.7	0.00
	T_3 (40)	11.7 ± 0.4	9.7 ± 0.7	0.00
March 18	Control (40)	13.7 ± 0.5	10.3 ± 0.1	0.00
	T_3 (40)	15.4 ± 0.5*	10.7 ± 0.1*	0.00
April 1	Control (40)	18.0 ± 0.5	11.2 ± 0.1	0.00
	T_3 (40)	20.8 ± 0.6*	11.9 ± 0.1*	0.00
April 15	Control (40)	18.7 ± 0.6	11.7 ± 0.1	0.00
	T_3 (40)	21.2 ± 0.7*	12.4 ± 0.1*	0.025 ± 0.03
April 29	Control (30)	20.2 ± 0.7	12.3 ± 0.1	0.100 ± 0.06
	T_3 (30)	23.0 ± 0.9*	12.8 ± 0.1*	0.467 ± 0.09*
May 13	Control (30)	22.8 ± 0.6	12.8 ± 0.1	0.200 ± 0.07
	T_3 (30)	25.9 ± 1.2*	13.3 ± 0.1*	0.333 ± 0.09
May 27	Control (20)	27.2 ± 1.2	13.4 ± 0.1	0.850 ± 0.08
	T_3 (20)	26.4 ± 1.3	13.3 ± 0.2	0.900 ± 0.07
June 8	Control (20)	32.1 ± 1.8	14.1 ± 0.2	0.900 ± 0.07
	T_3 (20)	32.9 ± 2.1	14.5 ± 0.3	0.950 ± 0.05
June 22	Control (20)	33.2 ± 2.2	14.9 ± 0.2	0.800 ± 0.09
	T_3 (20)	35.8 ± 1.9	14.7 ± 0.2	0.900 ± 0.07
July 19	Control (10)	35.2 ± 1.4	14.8 ± 0.1	0.900 ± 0.00
	T_3 (10)	40.4 ± 2.5	15.2 ± 0.3	1.000 —
September	Control (10)	50.4 ± 3.9	15.8 ± 0.3	1.000 —
	T_3 (10)	85.6 ± 9.4*	17.3 ± 0.5*	0.900 ± 0.10

[a] Mean ± SE, n in parentheses.
* Significant at $P < 0.05$.

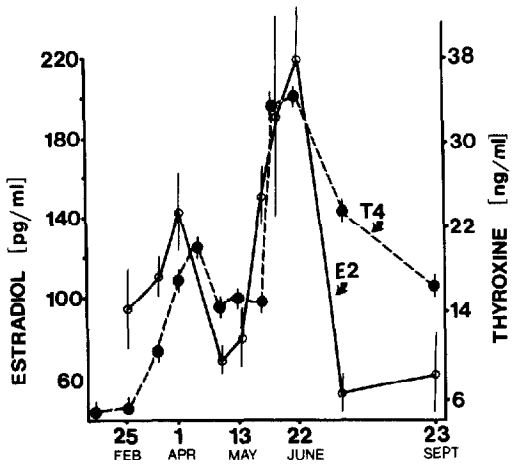

FIG. 1. Plasma estradiol-17β and thyroxine during smoltification of coho salmon in the spring of 1982.

Therefore, with respect to these two measurements, the data from fish treated with T_3 were combined with the data from the controls for each sampling time.

Plasma estradiol varied significantly through time (Fig. 1). Plasma estradiol increased markedly between February 25 and April 1 from 94 ± 23 to 142 ± 22 SE pg/ml. Plasma estradiol then decreased to 80 ± 14 pg/ml on May 13, followed by a second significant increase to 219 ± 27 pg/ml on June 22.

Plasma thyroxine also changed significantly through time and paralleled estradiol changes (Fig. 1). Plasma thyroxine increased between February 2 and April 1 from 5.3 ± 0.2 to 17.0 ± 0.4 ng/ml and increased further to 21.0 ± 1.0 ng/ml on April 15. Plasma thyroxine then decreased to 14.5 ± 0.6 ng/ml on April 29, followed by a second significant increase to 34 ± 1.6 ng/ml on June 22.

DISCUSSION

The changes in physiological properties occurring at smoltification undoubtedly reflect a complex series of processes. Elevated thyroid (Dickhoff *et al.*, 1978, 1982; Nishikawa *et al.*, 1979) and possibly cortisol (Specker and Schreck, 1982) hormonal levels with little evidence of changes in other circulating hormonal levels have been documented in smoltifying salmon. To our knowledge, the data provided here comprise the first evidence for developmental stage-limited cycles of increase in plasma levels of estradiol followed by a decrease during smoltification in coho salmon. The elevations of estradiol coincided with similar elevations of thyroxine. Plasma estradiol peaked in early June before the salmon were morphologically classifiable as smolts, and again in June after the salmon had completely smolted.

Whether estradiol might have a role in development during this period is unclear. Juvenile salmon during their migration to sea are found with quiescent gonads; following the entrance of the salmon into seawater developing gonads have been described (Fontaine, 1954; Chestnut, 1970). There appears to be no difference in the levels of estradiol in the juvenile male and female salmon. Estradiol levels measured later in individual zero-age and yearling coho salmon were not different in males and females (S. Sower and C. V. Sullivan, unpublished). Furthermore, no major differences in [^{3}H]estradiol-17β distribution between sexes were noted in a distribution and clearance study of [^{3}H]estradiol-17β in yearling coho salmon (S. Sower and C. B. Schreck, unpublished). These authors also demonstrated higher radioactive estradiol uptake occurring in the brain, pituitary, and liver in both males and females, with much lower radioactive estradiol uptake occurring in the gonads, kidney, and spleen. Lambert and van Bohemen (1979) suggested from limited observations in previtellogenic rainbow trout *(Salmo gairdneri)* that the brain may be a major source of the estrogens, estradiol, and estrone at this stage; interrenal tissue, liver, fat tissue, blood, and muscle were shown by these authors not to contain aromatizing enzymes. Furthermore, in rainbow trout, plasma levels of estradiol were found to be high in previtellogenic trout during the period of low gonadal estrogen synthesis (Lambert *et*

al., 1978; Lambert and van Bohemen, 1979).

It is possible that in coho salmon estradiol is partly extragonadal in origin, being synthesized in the brain, as suggested by Lambert and Bohemen (1979). The dynamics of estrogen production in the brain indicate a potential physiological or behavioral role in salmon during this period. The earlier experiments of Hoar *et al.* (1952) and Tinbergen (1950) in salmon and stickleback, respectively, showed that changes in gonadal hormones either directly or indirectly activated nervous centers initiating or influencing migratory behavior. Baggerman (1960), from consideration of the studies cited above, and from her own studies, suggested that the gonadal hormones affect sensory perception in a way which may make the fish more sensitive to external stimuli influencing the migration and/or smoltification processes. Coho salmon sex reversed or sterilized with sex steroids were shown to undergo normal smoltification but did not undertake anadromous migration back into freshwater (Hunter *et al.*, 1982); thus, these studies give further evidence of a potential influence of sex steroids on migratory behavior. Thyroid hormones also have been shown to influence behaviors associated with migration (Hoar *et al.*, 1952, 1955). Thus, gonadal and also thyroid hormones may be both involved in modifying migratory behavior associated with smoltification.

Numerous activities are evidently organized in some related manner with respect to smoltification and migration. The time-limited elevations of estradiol are clear events and they occur slightly before the surges of thyroxine. Several studies in other fish species have indicated that sex steroids can influence thyroid activity. Androgen administered to immature male and female rainbow trout caused an increase in thyroid hormone levels (Hunt and Eales, 1979). In male *Poecilia reticulata*, estradiol and estrone stimulated hypophyseal thyrotrophic cells *in vivo* (Sage and Bromage, 1970). In hypophysectomized catfish, estrogen treatment restored thyroidal activity to the level of control fish (Singh, 1969). Thus, estradiol in some way, may have a regulatory influence on the observed thyroid hormone changes or may be independently and coincidently associated with the numerous developmental changes that comprise smoltification.

The fact that estradiol surges correspond in time to the thyroxine surges indicates that these hormones may be responding to the same inciting stimuli. The major environmental cue for the onset of salmon smoltification has been considered to be photoperiod (reviewed by Hoar (1976)). More recently, the lunar cycle also has been proposed as a Zeitgeber that may influence the onset of salmon smoltification; the new moon has been shown to coincide with the thyroxine surge in salmon (Grau *et al.*, 1981). In the present study the surge of thyroxine and estradiol in June coincided with the new moon; however, the surge of these hormones in April was unrelated to the new moon. Because of this lack of correlation with the lunar cycle, it is possible that some other environmental factor, perhaps photoperiod, could be regulating and coordinating plasma thyroid hormonal and estradiol cycles in salmon during smoltification.

Exogenous triiodothyronine in our experiment enhanced growth, but had no influence on plasma estradiol, and did not affect plasma thyroxine levels. Dietary triiodothyronine adminstered over a period of time in similar studies eventually induced many morphological changes, including enhanced growth and in some cases enhanced salinity tolerance in juvenile salmon (reviewed by McBride *et al.* (1982) and Higgs *et al.* (1982)). However, effects from exogeneous triiodothyronine on reproductive activity may not be immediately evident or assessable. This past year several anadromous rainbow trout which had been given 4 ppm triiodothyronine orally as juveniles, and released into seawater, returned to freshwater as fully sexually ma-

tured fish in the spring instead of returning in the fall which is their only known time of entrance into freshwater (Sawyer, personal communication). Thus, exogenous triiodothyronine may have some subtle effect on reproductive activity that is not detectable until the fish have matured. However, it is not clear from the present extended studies whether there is any interaction between exogenous thyroid hormones and the developing reproductive processes of young salmon. More information on other reproductive hormones, plasma estradiol, and gonadal histology correlated with the dynamics of thyroid function would be required to determine the interactions.

The significance and mechanism of the estradiol surges in presmolt coho salmon in April and in coho salmon smolts in June and their relationship to thyroxine surges cannot yet be evaluated. The increased activity in various endocrine tissues suggested by histological studies (Nishioka *et al.*, 1982; Chestnut, 1970) and the surges of thyroxine and estradiol during salmon smoltification strongly suggest the occurrence of a general developmental phenomena.

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