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Changes in Brain Gonadotropin-Releasing Hormone, Pituitary and Plasma Gonadotropins, and Plasma Thyroxine during Smoltification in Chinook Salmon (*Oncorhynchus tschawytscha*)

KIM A. LEWIS,* PENNY SWANSON,† AND STACIA A. SOWER*,[1]

*Department of Zoology, University of New Hampshire, Durham, New Hampshire 03824; and †School of Fisheries, University of Washington, Seattle, Washington

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Concentrations of brain salmon gonadotropin-releasing hormone (sGnRH), plasma gonadotropin I (GTH I), and pituitary GTH I and GTH II were determined in yearling chinook salmon (*Oncorhynchus tschawytscha*) during the parr–smolt transformation in two successive seasons. There were significant elevations in brain sGnRH content from February to March in 1988, and from February to April in 1989. Increases in brain sGnRH content coincided with elevations in plasma thyroxine levels that occurred from February to March, 1988 and 1989. Plasma GTH levels were relatively constant (1–2 ng/ml) throughout the period of sampling. However, during 1988, plasma concentrations of GTH I decreased significantly between late March and early April. During 1989, plasma GTH I levels appeared to reach a peak (2 ng/ml) in mid-February, but otherwise remained near 1 ng/ml. Previous studies have shown that GTH II was not detectable in plasma at this stage. During 1989, pituitary GTH I concentrations were 50- to 70-fold higher than that of GTH II, and increased, though not significantly, from February through April. Although GTH II was detected in the pituitary by RIA, it is likely that the measurable levels are due to GTH I cross-reaction in the GTH II RIA. Histological examination of the gonads indicated that throughout smoltification the oocytes remained in the perinucleolar stage of oogenesis and the testes were in the spermatogonial stage of spermatogenesis. Although no observable changes in gametogenesis occurred, the changes in brain sGnRH content, plasma GTH I levels, and pituitary GTH content suggest that some changes in the hypothalamic–pituitary axis may occur during smoltification.

Chinook salmon (*Oncorhynchus tschawytscha*) migrate from freshwater streams to the ocean during the early spring of the first 1 to 2 years of their life. The morphological, physiological, and behavioral changes which occur during this period represent the process of smoltification. The parr-to-smolt transformation is coordinated by increases in endocrine activity in various tissues. Thyroid hormones, growth hormone, prolactin, and corticosteroids are the hormones most thoroughly studied and are considered to be integrally involved in the smoltification process (Fontaine, 1975; Folmar and Dickhoff, 1980; Hoar, 1988; Bjornsson *et al.*, 1989; Prunet *et al.*, 1989; Young *et al.*, 1989; Dickhoff *et al.*, 1990). The reproductive system of salmon during smoltification has not been extensively studied; however, distinct reproductive changes appear to occur during this period.

In salmonids, sexual maturation accelerates after smoltification. Histological analysis of the gonads of coho salmon (*O. kisutch*) (Chestnut, 1970) and Atlantic salmon (*Salmon salar*) parr (Jones, 1940) revealed the presence of immature gonads prior to smoltification. After seawater entry, the gonads of smolts were in more ad-

[1] To whom requests for reprints should be addressed at Department of Zoology, Spaulding Life Science Building, University of New Hampshire, Durham, NH 03824.

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vanced stages of gonadal development (vitellogenesis and spermatogenesis). It could be inferred from these studies that gonadal development and perhaps the hypothalamic–pituitary–gonad axis may be stimulated during smoltification. Consistent with this idea are the changes in plasma estradiol levels that were found in coho salmon during smoltification (Sower *et al.*, 1984, 1992).

Reproduction in teleosts, like other vertebrates, is regulated by pituitary gonadotropins. Studies of Suzuki *et al.* (1988) and Swanson *et al.* (1991) have demonstrated the existence of two pituitary gonadotropins (GTH I and GTH II) in pituitaries of chum (*O. keta*) and coho salmon, respectively. In further studies, Nozaki and colleagues examined ontogenic changes of pituitary GTH I and GTH II in different reproductive stages of salmonids. GTH I, not GTH II, was present in pituitary gonadotrophs prior to the onset of vitellogenesis and spermatogenesis in rainbow trout (Nozaki *et al.*, 1990). In this study, both GTH I and GTH II were found in pituitaries of trout in late stages of vitellogenesis and spermatogenesis, but the number of immunoreactive GTH I cells exceeded that of GTH II. In contrast, the number of immunoreactive GTH II cells exceeded that of GTH I in pituitaries from ovulated or spermiating trout. Similar ontogenetic changes in plasma levels of GTH I and GTH II were observed in coho salmon. In previtellogenic and prespermatogenic coho salmon, GTH I but not GTH II was detected in the plasma, even though both GTHs could stimulate *in vitro* release of steroids by gonads from these immature fish (Swanson *et al.*, 1989). Throughout the period of active vitellogenesis and spermatogenesis, plasma levels of GTH I increased whereas GTH II levels were very low or nondetectable. During the period of final gamete maturation and release, plasma GTH II levels increased dramatically while GTH I levels decreased (Swanson, 1991). These data indicate that GTH I is the pituitary gonadotropin likely to be involved in regulating gonadal development in juvenile salmonids.

In teleosts, gonadotropin-releasing hormone (GnRH) as well as other factors regulate gonadotropin secretion (Peter, 1983; Peter *et al.*, 1991). Multiple forms of GnRH have been identified in the brains of several teleost species (Sherwood, 1987). In salmon, two forms of GnRH have been isolated from the brain; however, the structure has been determined for only one of the two forms, salmon GnRH (sGnRH) (Sherwood *et al.*, 1983). The second form is chromatographically and immunologically similar to chicken GnRH II (cGnRH II) (Sherwood, 1987). In a recent study, Okuzawa *et al.* (1990) demonstrated that in both yearling immature trout and 3-year-old maturing trout, sGnRH but not cGnRH II was detected by radioimmunoassay (RIA) in the pituitary even though both were found in the hypothalamus. In all regions of the brain analyzed in that study, sGnRH concentrations greatly exceeded cGnRH II except in the cerebellum and medulla oblongata. These authors suggested that in trout, sGnRH, not cGnRH II, plays an active role in regulating gonadotropin secretion, and thus gonadal maturation. Many studies have shown that sGnRH and various analogues of sGnRH or mammalian GnRH stimulate the pituitary–gonadal axis in adult salmon (c.f. Donaldson *et al.*, 1981; Crim, 1984; Sherwood, 1987). In immature coho salmon, it was demonstrated that a mammalian GnRH analogue stimulated *in vitro* secretion of pituitary GTH I; GTH II secretion was not detected (Swanson *et al.*, 1989). Together, these data suggest that the hypothalamic (sGnRH)–pituitary (GTH I) axis may be functional in juvenile salmon.

The objective of this study was to determine levels of brain sGnRH, plasma GTH I, and pituitary GTH I and GTH II during the parr-to-smolt transformation to determine whether there were smoltification-associated changes in these reproductive

hormones. Plasma thyroxine levels were also measured as an index of the progress of smoltification. Reproductive development was assessed by histological examination of gonads.

METHODS AND MATERIALS

Yearling chinook salmon were sampled at the New Hampshire Fish and Game Twin Mountain fish hatchery (Twin Mt., NH) during the period of smoltification in two successive years (1988 and 1989). In 1988, gonads, blood, and brains were collected from 15 fish every 14 days from February until the end of April. Total length (l), weight (w), and sex were recorded. Condition factor was calculated as w (g)/l^3 (cm^3) × 100. In 1989, 30 fish were sacrificed every 14 days and the same tissues, in addition to pituitaries, were collected. Following a blow to the head, blood was collected from the caudal vasculature by lithium heparin-treated micropipets after severing the caudal peduncle. Plasma was stored at −80° until assayed for thyroxine and GTH I.

All fish were sexed macroscopically at the time of sampling. Gonads were taken from 5 fish in 1988 and from 30 fish in 1989 at each sampling time for analysis of relative gonadal development. The gonad samples were preserved in Bouin's solution, dehydrated in a series of ethanol solutions, and embedded in paraffin. Sections (10 μm) were stained with Harris's hematoxylin and eosin for subsequent histological analysis of gonadal development.

The brains (excluding olfactory nerve) were rapidly removed, placed on dry ice, and then stored at −80° until extraction. Each brain was weighed and homogenized separately in 2 ml ice-cold 2 *M* acetic acid according to the methods described by Yu *et al.* (1987). Brains were pooled in groups of two or three according to sex (1988), or extracted individually (1989) and assayed by RIA for salmon GnRH using methods described by Powell *et al.* (1985) and Stopa *et al.* (1988) using sGnRH antibody 432. Antibody 432 has a cross-reactivity of 119% with cGnRH II. The sensitivity of the sGnRH RIA was 1.95 pg/tube an antibody binding averaged 30% (1988) or 32% (1989). Brains extracts were initially analyzed by HPLC followed by RIA (Stopa *et al.*, 1988). It was determined that it was not necessary to separate sGnRH from cGnRH-II by HPLC prior to RIA because cGnRH-II was not detectable in these samples. Brain extracts were then assayed directly for total GnRH by RIA. Serial dilutions of chinook brain were parallel to sGnRH standards in sGnRH RIA.

For each of the sampling periods, equal volumes of plasma samples from two to three fish of the same sex were pooled for RIA of thyroxine and GTH I. Plasma thyroxine levels were measured as described by Dickhoff *et al.* (1978). The lower limit of detection was 125 and 23 pg/tube for 1988 and 1989, respectively. The binding efficiencies ranges between 27.7 and 36.9%. GTH I levels were measured by RIA as described by Swanson *et al.* (1989). Pituitaries were extracted as described in Swanson *et al.* (1989) and assayed for both GTH I and GTH II. The cross-reactivities of GTH II, GH, PRL, and a TSH fraction were 14.8, <0.1, 0.04, and 0.38%, respectively, in the GTH I RIA. In the GTH II RIA, the cross-reactivities of GTH I, GH, PRL, and a TSH fraction were 3.6, <0.01, <0.01, and 0.8%, respectively. The lower limit of detection (two standard deviations from binding in absence of standard) were 0.39 and 0.5 ng/ml for GTH I and GTH II, respectively. Serial dilutions of chinook salmon plasma and pituitaries were parallel to coho salmon GTH I and GTH II standards in the respective assays. Protein content of pituitary extracts was determined by the method of Hartree (1971).

Data for hormone concentrations were analyzed by the Students *t* test or by the Student–Newman–Keuls' test after a preliminary analysis of variance. In all tests, the level of significance for differing groups was $P < 0.05$.

RESULTS

1988

The condition factor decreased during February from 0.79 ± 0.01 to 0.69 ± 0.21 (mean ± standard error), and then increased significantly ($P < 0.01$) by April 11 (0.88 ± 0.01) (Table 1). A bimodal variation occurred in growth in fish sampled on April 11, 1988. Five of the 15 fish exhibited a condition factor greater than 1.03 and the remaining 10 fish had condition factors less than 0.84 with an average of 0.73.

Histological examination revealed no change in oogenesis. All ovaries contained oocytes in the late perinucleolar stage of development. Spermatogenesis advanced over the sampling period in only 6 of the 35 male fish examined. One fish observed on February 1 and two fish observed on February 15 have testes with primary spermatocytes. The testis from one male examined on April 11 and one on April 25 had primary and secondary spermatocytes. In addition, testes of one fish sampled on April 25 showed signs of cell proliferation character-

TABLE 1
MEAN WEIGHT (g ± SE), TOTAL LENGTH (cm ± SE), AND CONDITION FACTOR (CALCULATED AS CF = w(g)/l(cm)^3 × 100) OF YEARLING CHINOOK SALMON IN TWO SUCCESSIVE YEARS, 1988 AND 1989 (n = 15 AND n = 30, RESPECTIVELY, AT EACH SAMPLING TIME)

	X ± SE		
	Weight	Length	Condition factor
1988			
Feb 1	33.80 ± 3.47	15.81 ± 0.54	0.79 ± 0.01
Feb 16	36.73 ± 3.80	17.22 ± 0.53	0.79 ± 0.01
Feb 29	30.37 ± 2.99	16.13 ± 0.40	0.69 ± 0.21
Mar 14	27.77 ± 4.52	15.16 ± 0.61	0.79 ± 0.03
Mar 28	33.86 ± 3.73	15.70 ± 0.61	0.79 ± 0.03
Apr 11	34.50 ± 4.83	15.33 ± 0.70	0.88 ± 0.01*
Apr 25	47.88 ± 4.58*	18.16 ± 0.47*	0.76 ± 0.01*
1989			
Jan 16	21.44 ± 1.86	13.54 ± 0.35	0.80 ± 0.01
Feb 2	25.55 ± 1.98	14.57 ± 0.30	0.78 ± 0.28
Feb 16	21.23 ± 1.54	13.68 ± 0.31	0.78 ± 0.01
Mar 1	18.26 ± 1.22	13.02 ± 0.28	0.79 ± 0.01
Mar 15	23.46 ± 1.15	14.26 ± 0.72	0.78 ± 0.01
Mar 29	27.33 ± 1.97	14.80 ± 0.45	0.85 ± 0.03
Apr 12	25.98 ± 2.48	15.68 ± 0.33	0.61 ± 0.03*
Apr 26	37.13 ± 2.19*	16.71 ± 0.25	0.89 ± 0.01*

* A significant difference ($P < 0.05$) in the mean when compared to the previous mean.

istic of initial stages of spermatogenesis. Testicular development in all other fish remained in the spermatogonia stage.

There were fluctuations in plasma thyroxine concentrations during the sampling period (Fig. 1). Plasma thyroxine levels increased from 3.40 ± 0.50 ng/ml on February 29 to 8.50 ± 4.7 ng/ml on March 14 and returned to 3.5 ± 0.50 ng/ml on April 11. These differences were not significantly different ($P < 0.05$). Brain sGnRH content varied significantly and paralleled changes in plasma thyroxine levels (Fig. 1). Brain sGnRH content increased markedly from February 29 (57.77 ± 6.89 pg/mg brain) to March 14 (120.57 ± 12.72 pg/mg brain). Plasma GTH I levels were relatively constant (Fig. 1). However, a significant decrease occurred between March 28 (1.59 ± 0.34 ng/ml) and April 11 (0.62 ± 0.07 ng/ml).

Water temperature ranged from 3.3° to 6.1° (Fig. 3).

1989

Condition factor decreased significantly in the salmon from 0.85 ± 0.03 on March 29 to 0.61 ± 0.03 on April 12. This decrease was followed by a significant increase to 0.89 ± 0.01 on April 26 (Table 1). Histological examination revealed little or no change in gametogenesis in either male or female fish. All ovaries contained oocytes that were in the late perinucleolar stage of oogenesis. All testes had cells in the spermatogonial stage with some evidence of cell proliferation throughout the study period.

Brain sGnRH content varied significantly during the sampling period (Fig. 2). Brain sGnRH content decreased markedly from 61.3 ± 6.3 pg/mg brain on February 2 to 32.7 ± 3.1 pg/mg brain on February 16. This was followed by a significant rise to the highest level (199.8 ± 12.2 pg/mg brain) on April 12. Plasma thyroxine also changed

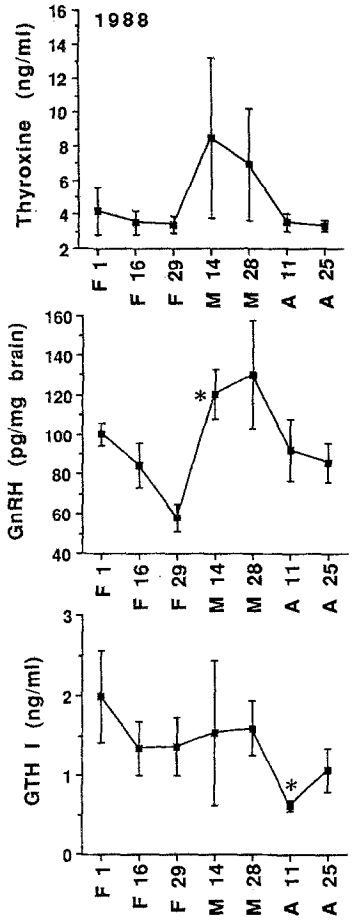

FIG. 1. Plasma levels of thyroxine (ng/ml), salmon GnRH (pg/mg brain), and plasma GTH I (ng/ml) in yearling chinook salmon from Feb to April 1988. (Data are mean ± standard error; $n = 15$.) *Indicates a significant difference ($P < 0.05$) in the mean level when compared to the previous mean.

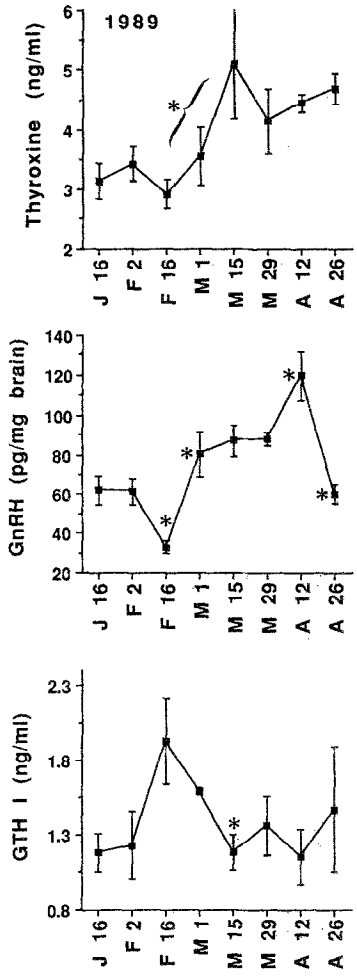

FIG. 2. Plasma levels of thyroxine (ng/ml), salmon GnRH (pg/mg brain), and plasma GTH I (ng/ml) in yearling chinook salmon from Jan to April 1989. (Data are mean ± standard error; $n = 15$.) *Indicates a significant difference ($P < 0.05$) in the mean level when compared to the previous mean.

significantly through time. A marked increase occurred between February 16 and March 15 from 2.91 ± 0.24 to 5.0 ± 0.94 ng/ml (Fig. 2).

Levels of plasma GTH I were relatively constant; however, a significant decrease occurred from February 16 (1.93 ± 0.28 ng/ml) to March 1 (1.59 ± 0.02 ng/ml) and March 15 (1.18 ± 0.12 ng/ml) (Fig. 2). Pituitary GTH I and II content increased through time; however, these changes were not significant (Table 2).

Water temperatures ranged from 3.8° to 5.5° (Fig. 3).

DISCUSSION

Chinook salmon have the most diverse life history of any of the Pacific salmon; however, few studies have examined smoltification in this species. Furthermore, there is little information on the reproduc-

TABLE 2
MEAN PITUITARY GTH I (ng/μg PROTEIN ± SE) AND GTH II (pg/μg PROTEIN ± SE) IN YEARLING CHINOOK SALMON IN 1989

	$\bar{X} \pm SE$	
	GTH I	GTH II
1989		
Feb 16	4.65 ± 0.97	0.077 ± 0.005
Mar 1	4.15 ± 0.84	0.079 ± 0.006
Mar 15	5.81 ± 1.01	0.080 ± 0.008
Mar 29	6.13 ± 1.81	0.107 ± 0.033
Apr 12	7.36 ± 1.94	0.100 ± 0.027
Apr 26	5.14 ± 1.47	0.139 ± 0.060

Note. n = 30 at each sampling date.

tive endocrine system in salmonids during smoltification. Results of the present study demonstrate that brain concentrations of sGnRH, plasma GTH I levels, and plasma thyroxine levels fluctuated during smoltification in juvenile chinook salmon. In successive years, a springtime increase in plasma thyroxine levels occurred at the same time as a noted elevation of sGnRH concentrations in the brain. This was followed by a decrease in plasma GTH I levels. However, there was no significant correlation between changes in brain concentrations of sGnRH and plasma levels of GTH I. These data suggest that there may be smoltification-associated alterations in the hypothalamic–pituitary axis in chinook salmon.

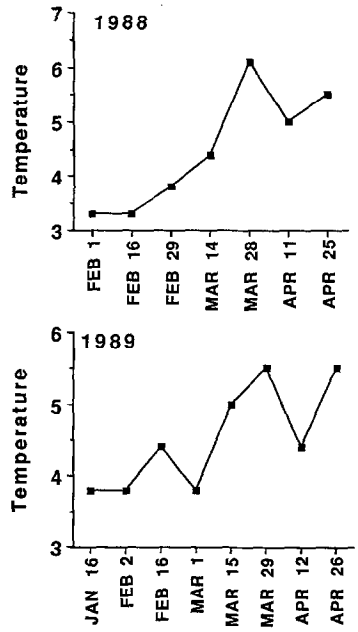

FIG. 3. Water temperatures in 1988 and 1989.

To our knowledge, this is the first investigation documenting the changes in brain concentrations of sGnRH and plasma GTH I levels in chinook salmon during smoltification. In both years, brain concentrations of sGnRH increased from low levels in February to highest levels in late March or early April and decreased by the end of April. The elevations in brain sGnRH content coincided with increases in plasma thyroxine levels from February to March. The concurrent elevations in brain sGnRH content and plasma thyroxine levels during smoltification may indicate that there is a coordination of these events by environmental cues. There is ample evidence that photoperiod is probably the most important environmental factor involved in either synchronizing or regulating the precise timing of smoltification, whereas temperature appears to be a secondary factor (reviewed by Hoar, 1976, 1988; Wedemeyer *et al.*, 1980). In this study, elevations of brain GnRH content and plasma thyroxine levels seemed to be correlated with increases in water temperature. It is not clear whether these hormonal changes are triggered endogenously or by some environmental stimulus. These baseline studies indicate that brain sGnRH content fluctuates during the period of smoltification of chinook salmon. The physiological significance of these changes is not known.

Two distinct gonadotropins, GTH I and GTH II, have been isolated in coho salmon (Swanson *et al.*, 1991). GTH I has been measured in the pituitaries and circulating plasma of salmon parr during early oogenesis and spermatogenesis (Swanson *et al.*, 1989). GTH II was not detectable in the

plasma of coho salmon parr, and remained below 2 ng/ml throughout spermatogenesis and vitellogenesis (Swanson *et al.*, 1989; Swanson, 1991), whereas plasma GTH I levels increased during this period. During final maturation and gamete release, GTH II levels increased and exceeded GTH I levels (Suzuki *et al.*, 1988; Swanson, 1991). Therefore, plasma of juvenile chinook salmon was analyzed only for GTH I. Although plasma levels of GTH I remained relatively constant in both studies, it is interesting to note that in 1989 plasma GTH I levels were highest when plasma thyroxine levels and brain sGnRH content were lowest. Furthermore, the significant decrease in plasma GTH I levels from February to March occurred at the same time as significant elevations in plasma thyroxine levels and brain sGnRH content. During 1989, pituitary GTH I concentrations were 50- to 70-fold higher than that of GTH II, and increased, though not significantly, from February through April. Although GTH II was detected in the pituitary by RIA, it is likely that the measurable levels were due to GTH I cross-reaction in the GTH II RIA because the amount of GTH II detected was roughly 3–4% of the GTH I, similar to the estimate of 3.6% cross-reactivity. These results indicate that during smoltification, the pituitary content of GTH I gradually increased, whereas plasma levels remained relatively constant with the exception of a slight decline near the end of smoltification.

Further research on the role of GnRH in the regulation of GTH release in juvenile salmon is necessary to elucidate any relationship between brain sGnRH and plasma GTH I in salmon. It is possible that decreasing GnRH levels reflect decreased synthesis and/or increased release of the peptide. Breton *et al.* (1986) suggested that a noted increase in brain GnRH content is related to a GnRH system closely involved with maturation and ovulation in brown trout. However, these authors demonstrated that pituitary GnRH levels, but not total brain GnRH levels, were correlated with GTH levels in the pituitary and plasma. Similarly, in the present study, no significant correlation was found between total brain sGnRH and plasma GTH I levels. Unfortunately, pituitary GnRH concentrations were not measured in the present study. It is clear that further studies are necessary to elucidate any possible changes in the hypothalamic–pituitary axis during the smoltification in chinook salmon.

Reproductive activity proceeds in a synchronous manner within the single spawning population of chinook salmon, and thus it would be expected that gonadal development would be coordinated with other developmental changes, such as smoltification. In 1988, 30% of the fish had testes that showed evidence of advanced stages of spermatogenesis. However, in 1989, all male fish possessed testes in the spermatogonial stage suggesting that there was little gonadal activity during this time and that the prior advances in spermatogenesis may have been the result of sexual precocity in a percentage of the population. Precocious male sexual maturation occurs particularly in hatchery-reared fish (Saunders *et al.*, 1982). Unfortunately, since GTH I levels were not measured in individuals, it was not possible to correlate any specific changes in gonadal stages with GTH levels. No change in oogenesis in either year was noted, as all oocytes remained in the early perinucleolar stage of development. These results for chinook salmon are in support of those by Chestnut (1970) in which he reported that prior to smoltification, juvenile coho salmon parr possess quiescent rudimentary gonads. It is not until after smoltification that gametogenesis proceeds further.

The plasma thyroxine surge during smoltification was first documented by Dickhoff *et al.* (1978) in coho salmon. Since then, there have been numerous investigations

which have shown that thyroid hormones affect growth, morphogenesis, skin pigmentation, osmoregulation, and behavior during the parr-to-smolt transformation (reviewed by Hoar, 1988). Elevations of plasma thyroxine in coho salmon occur after hatching and during yolk absorbance (Sullivan *et al.*, 1987), during the first and second spring of freshwater residence (Dickhoff *et al.*, 1978, 1982), and during early stages of sexual maturation (Sower and Schreck, 1982). These times represent periods of critical growth and development of coho salmon in which hormones are likely to play a coordinating role (Dickhoff and Sullivan, 1987). There are also fluctuations in plasma thyroxine levels in spring chinook salmon concurrent with the time of the parr to smolt transformation (Dickhoff *et al.*, 1982). In the study by Dickhoff and colleagues (1982), plasma thyroxine reached peak levels of 25 ng/ml during late April. In the present study, elevations in plasma thyroxine levels as high as 8 ng/ml occurred in March and further increases occurred in late April (1989 only). Dickhoff *et al.* (1978) noted that the plasma thyroxine surge can be 30–60 days in length in coho salmon with plasma thyroxine reaching levels three- to sevenfold higher than presurge levels. However, in chinook salmon, there appears to be a two- to threefold increase in plasma thyroxine during smoltification. Extended sampling times may be necessary to further evaluate any possible seasonal changes in plasma thyroxine.

In summary, these data provide evidence for changes in concentrations of sGnRH in the brain and GTH I in the plasma associated with increases in plasma thyroxine levels during smoltification of chinook salmon. Although no observable changes in gametogenesis occurred, the changes in brain sGnRH content and plasma GTH I levels suggest some changes in the hypothalamic–pituitary axis may occur during smoltification.

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