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Changes in thyroid hormone levels in eggs and larvae and in iodide uptake by eggs of coho and chinook salmon, *Oncorhynchus kisutsch* and *O. tschawytscha*

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Abstract

Developmental profiles of thyroxin (T_4), triiodothyronine (T_3) and radioactive iodide uptake were established for eggs and T_4 and T_3 profiles were established for larvae (whole-body, yolk-only and body-only) of coho and chinook salmon. T_4 and T_3 were consistently present in all samples. In eggs, hormone levels remained fairly constant in all cohorts for at least the first three weeks of incubation, but then fluctuated in both directions in some sample groups. Large increases in T_4 (from 9 ng/g to 245 ng/g) were seen in 1985 chinook eggs 28 days after fertilization. Radioactive iodide uptake (which was used as a possible indicator of thyroxinogenesis) increased at least 10-fold in both 1986 coho and chinook eggs from 23–30 days after fertilization. T_4 (62 ng/g) and T_3 (393 ng/g) were found in the bodies of 28-day-old 1986 chinook embryos. In whole larvae, hormone levels varied depending upon the cohort studied. In general, initial body-only concentrations of both T_4 and T_3 decreased as body weight increased, but before yolksac resorption was completed, both thyroid hormone content and concentration increased (except for chinook T_3). T_4 and T_3 content in larval yolk stayed constant as yolksac size decreased, resulting in increased thyroid hormone concentration in the yolksac. All of these data suggest that the initial source of thyroid hormones in coho and chinook salmon eggs is maternal, but that by approximately 3–4 weeks after fertilization, the developing embryos begin to produce their own thyroid hormones. After hatching, increases in tissue T_4 and T_3 concentration coupled with constant T_4 and T_3 content in diminishing yolksacs suggest that larvae also produce their own thyroid hormones; yolksac content then may reflect both the original maternal hormones and the larva-produced hormones.

Introduction

The thyroid and its hormones, thyroxin (T_4) and triiodothyronine (T_3), have been implicated as important regulators of early development in teleosts (Hoar 1939; Leatherland and Lin 1975; Wabuke-Bunoti and Firling 1983; Brown *et al.* 1987; Kobuke *et al.*1987; Tagawa and Hirano 1987). Hoar (1939) reported that in Atlantic salmon (*Salmo salar*) fully-formed thyroid follicles were present in the

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subpharyngeal area at the time of hatching. Wabuke-Bunoti and Firling (1983) established that in fathead minnow (*Pimephales promelas*) embryos (stage 30) there was follicular organization of iodide-concentrating thyroid tissue. At this time the follicles had already begun to empty. In coho salmon (*Oncorhynchus kisutch*) embryos one week before hatching, Leatherland and Lin (1975) described 'active-looking' thyroid follicles and existence of a modest number of cells in the pituitary that were most likely thyrotropes. Conversely, studies on the tilapia *Oreochromis niloticus* showed that thyroid follicles were absent in yolksac larvae and present in fry (Nacario 1983).

T_4 and T_3 are present in various developing teleost eggs and larvae. Kobuke *et al.* (1987) showed that in coho salmon, the mean level of T_4 in eggs 10 days before hatching was 28 ng/g. After hatching, as the yolksac was resorbed, T_4 fell to around 5 ng/g fish. Measurements of T_4 in the eggs of chum salmon (*O. keta*) by Tagawa and Hirano (1987) showed levels just after fertilization to be 4–5 ng/g. These levels remained constant throughout the incubation period, but there was a gradual decrease in T_4 in whole-body chum salmon larvae after hatching. At yolksac resorption, T_4 concentration increased from 2 to 10 ng/g. Brown *et al.* (1987) reported that T_4 levels in unfertilized striped bass (*Morone saxatilis*) eggs were 5 ng/g and and T_3 levels were 4.5 ng/g. Sullivan *et al.* (1987) measured changes in T_4 and T_3 plasma levels in the larvae of pink salmon (*O. gorbuscha*), chum salmon (*O. keta*), coho salmon (*O. kisutch*), chinook salmon (*O. tschawytscha*), and Atlantic salmon (*Salmo salar*). For all species, plasma T_4 concentrations generally increased from the time of 50% through total yolksac resorption, while plasma T_3 decreased to either low (pink salmon) or non-detectable (all other species) levels.

In addition, numerous experiments have shown that exogenous thyroid hormone supplementation can have effects on the development of teleostean larvae. In tilapia (*Oreochromis mossambicus*), Lam (1980) showed that larvae immersed in T_4 (0.1 ppm) had accelerated growth as well as an improved rate of survival. Nacario (1983), in his study of the tilapia *Oreochromis niloticus*, found that T_4 immersion (0.5 ppm) of yolksac larvae caused accelerated yolksac resorption. In contrast, in chum salmon, Dales and Hoar (1954) found decreased yolksac resorption (*O. keta*) when the larvae were immersed in T_4 (1:12.5 million thyroxine sodium). The combination of T_4 with varying salinities promoted larval survival, growth and development in the carp (*Cyprinus carpio*) (Lam and Sharma 1985). Inui and Miwa (1985) and Miwa and Inui (1987) have shown that T_4 and T_3 treatment can induce metamorphosis of flounder larvae (*Paralichthys olivaceus*).

Although the source of thyroid hormones and the roles that they play during the developmental processes may vary greatly with species, it seems that T_4 is generally present. Whole-body T_3 levels of eggs and larvae, on the other hand, have not been rigorously studied in the development of teleosts. These measurements are especially vital since T_3 is widely considered to be the actual physiological mediator, whereas T_4 may be only the prohormone (Eales 1985).

Our present data provide developmental profiles of both T_4 and T_3 in the eggs, whole larvae, larval yolk-only and larval body-only of coho and chinook salmon. We have attempted to determine at which point in the development of these species the embryo's own thyroid hormone synthesis begins, and we have also measured the compartmentalization of the hormones between the yolk and body of the larva.

Materials and methods

Procurement, maintenance and handling of fish

Eggs from both coho (*O. kisutch*) and chinook (*O. tschawytscha*) salmon were obtained from Iron Gate Hatchery, Hornbrook CA. At Iron Gate, spawning females and males were sacrificed and the mature eggs were fertilized. The 1985 chinook eggs (group A) were all from the same female, and they were fertilized with the milt of one male. 1985 coho eggs (group B) were collected from two females, and were fertilized with the milt of two males. 1986 chinook eggs (group C) were taken from two fe-

males and fertilized with the milt from two males. 1986 coho eggs (group D) were from one female and fertilized with the milt from three males.

While at the hatchery, 20 unfertilized (except group D) and 20 fertilized eggs were sampled from each group. At the same time, immature eggs were collected from one 1985 coho and one 1986 chinook female. These eggs would have needed approximately an additional week of gestation in the female to have been considered fertile (Marge Rightmeier, Hornbrook, CA, personal communication). All eggs were frozen on dry ice until arrival at Berkeley. The samples were stored at −80°C until the time of extraction.

About one half-hour after fertilization, eggs (1900–4700) were transported back to Berkeley and kept at approximately 12°C on a 12-hour light/12-hour dark photoperiod. Eggs from groups A and B were randomly sampled (n = 8) every four days, frozen immediately on dry ice, and stored at −80°C. Eggs from groups C and D were sampled (n = 8) every 2–4 days until the appearance of eyes 18 days after fertilization. After this time, samples were collected every day until hatching. Twenty-eight days after fertilization, embryos (n = 8) were dissected out of eggs from group C, and the yolk was separated from the body in order to measure T_4 and T_3 in the separate compartments.

The majority of 1985 eggs hatched approximately 40 days after fertilization, while most 1986 eggs hatched 36 days after fertilization. Every four days, larvae from groups A and B were randomly sampled (n = 8), frozen immediately on dry ice, and stored at −80°C for later extraction of thyroid hormones. In addition, an extra set of larvae (n = 8) was taken every four days, and the yolk was separated from the body in order to measure the compartmentalization of the thyroid hormones in these two regions. The separated yolk is referred to as 'yolk-only' and the body is 'body-only'. Larvae from group D were sampled every 4–8 days until the time of yolksac resorption. No larvae were sampled from group C. Thirty-six days after hatching, the chinook larvae had resorbed most of the yolksac, while the coho had resorbed their yolksacs by 32 days after hatching. The larvae were not fed.

Thyroid hormone extraction

All eggs and larvae were defrosted, blotted with tissue and weighed. For compartmentalization measurements, the yolk was removed from the larval body. The remaining body was weighed and the difference between whole larval weight and body weight was taken as yolk weight. Larval bodies were minced into small cubes. All samples were put in 17 × 60 mm cylindrical glass vials containing 2 ml 95% ethanol with 1 mM 6-N-propylthiouracil (PTU, Sigma, St. Louis, MO). PTU stops the conversion of T_4 to T_3 by 5′-monodeiodinase. Samples were then homogenized with a Brinkman Polytron (PT-10) at force 5.5 and sonicated for 30 sec. with a Heat Systems Microson sonicator at 50% output. The homogenates were transferred into 12 × 75 mm borosilicate glass tubes. The cylindrical glass vials were cleansed with 1 ml ethanol containing PTU, and the rinse was added to the test tubes. The tubes were capped, vortexed vigorously twice, and centrifuged (Sorvall RC-3B) at 4°C, 3000 rpm for 10 min. The supernatants were decanted into new 12 × 75 mm tubes. Two ml ethanol/PTU were added to the pellets; the tubes were capped and vortexed vigorously twice and recentrifuged. The new supernatant was added to the original supernatant.

In 1985 (groups A and B), two 1-ml aliquots (duplicates of all samples) were taken from the supernatant pools, put in 10 × 75 mm borosilicate glass tubes, and vacuum-dried overnight at 60°C. The desiccated samples were then stored in a refrigerator at 4°C.

The supernatant pools from groups C and D were kept in the 12 × 75 tubes and vacuum-dried overnight. There were no duplicates of these samples. The desiccated samples were stored at −20°C.

Solubilization of thyroid hormone extracts

For the eggs of groups A and B, the solubilization method used was a modification of inorganic-organic phase separation described by Tagawa and Hirano (1987). The desiccated samples were resolubilized with 1 ml chloroform and 1 ml barbital buffer (Specker and Richman 1984) (containing

2 mg/ml 8-anilino-1-naphthalene sulfonic acid (ANS)). The samples were capped, shaken until all material had dissolved, and then centrifuged at 4°C, 3000 rpm for 10 minutes. The top, aqueous layer was removed for assaying.

Unfortunately, using this method, the concentration of T_3 in the eggs was always at the lower limits of assay; therefore the T_3 assay could not be validated for parallelism. As a consequence, there are no T_3 values for the eggs of group A and group B.

$^{125}I-T_4$ tracer (Sp. Act. 750 mCi/mg; NEN, Boston, MA) was added to the eggs prior to homogenization. After the desiccated extracts were solubilized, the percent of the label that was recovered in the barbital buffer was 32.4 ± 0.6% (n = 16) and 28.1 ± 1.1% (n = 8) for coho and chinook eggs, respectively.

For the 1985 larval samples and all 1986 samples (groups C and D), a different solubilization procedure was used, so that both T_4 and T_3 could be validly measured. For groups A and B, 0.25 ml barbital buffer (with 2.5 mg/ml ANS), 0.25 ml ethanol, and 1.0 ml chloroform were used. The ethanol moved into the organic and inorganic phases at a 4:1 ratio. After capping, shaking and spinning, the aqueous phase had a volume of 0.3 ml. For groups C and D, the volumes used were 0.5 ml, 0.5 ml and 2.0 ml of barbital buffer (with 2.5 mg/ml ANS), ethanol, and chloroform, respectively. The final aqueous layer had a volume of 0.6 ml. This solubilization method improved recoveries.

Traces of ^{125}I-T_4 and ^{125}I-T_3 (Sp. Act. 750 mCi/mg, 100 mCi/mg, respectively; New England Nuclear, Boston, MA) were added to samples prior to homogenization and recoveries of the traces were counted in the aqueous layer of the solubilized samples. T_4 recoveries for chinook and coho eggs were 49.6 ± 0.9% (n = 18) and 49.8 ± 0.8% (n = 19), respectively and 79.0 ± 1.4% (n = 24) and 84.4 ± 1.0% (n = 32) for chinook and coho larvae, respectively. T_3 recoveries were 56.2 ± 1.1% (n = 16) and 52.0 ± 1.5% (n = 16) for chinook and coho eggs and 76.1 ± 1.1% (n = 32) and 75.1 ± 0.9% (n = 23) for chinook and coho larvae, respectively.

Four samples from different stages in egg development and sample pools (n = 8, combined into one large sample) of whole-body, body-only and yolk-only from both coho and chinook cohorts were measured for parallelism in the T_4 and T_3 assays. The results from all eight egg samples and all the sample pools showed that the extraction method and radioimmunoassays were valid for both T_4 and T_3. An example of the results follows: T_4 was measured in chinook egg (nine days after fertilization) extracts at 4.25, 9.03, and 17.67 ng/ml for 10, 20, and 40 μl volumes, respectively. T_3 was measured in coho egg (10 days after fertilization) extracts at 4.31, 9.26, and 12.78 ng/ml for 25, 50 and 75 μl volumes, respectively. T_4 in chinook yolk-only (20 days after hatching) extracts were 1.42, 2.44, and 4.26 ng/ml for 10, 20, and 40 μl volumes, respectively. T_3 in chinook body-only (20 days after hatching) extracts were 2.76, 5.26, 7.97 ng/ml for 25, 50 and 75 μl volumes, respectively. T_3 in whole coho larvae (28 days after hatching) extracts were 1.66, 3.28, 4.42 ng/ml for 25, 50 and 75 μl volumes, respectively.

T_4 and T_3 measurement by radioimmunoassay (RIA)

T_4 and T_3 in the solubilized samples were measured by RIA. The T_4 RIA was a barbital buffer system as previously described by Specker and Richman (1984) and required a 20-μl sample volume. For T_3, a borate buffer system was used. The method followed was that of Endocrine Sciences (Tarzana, CA), and a 50-μl sample volume was used. Samples were measured in duplicate in both assays.

Radioactive iodide uptake studies

Radioactive iodide uptake of 1986 eggs was measured as a possible indicator of endogenous thyroid activity. Eggs from groups C and D were sampled every four or five days until hatching. Approximately 10.2 μCi (7.24×10^{-4} μg NaI) $Na^{125}I$ (Amersham) were added to 500 ml water at 12°C, which was then divided into two beakers. To account for decay of ^{125}I, the counts per minute (cpm) of the water for each sampling were stan-

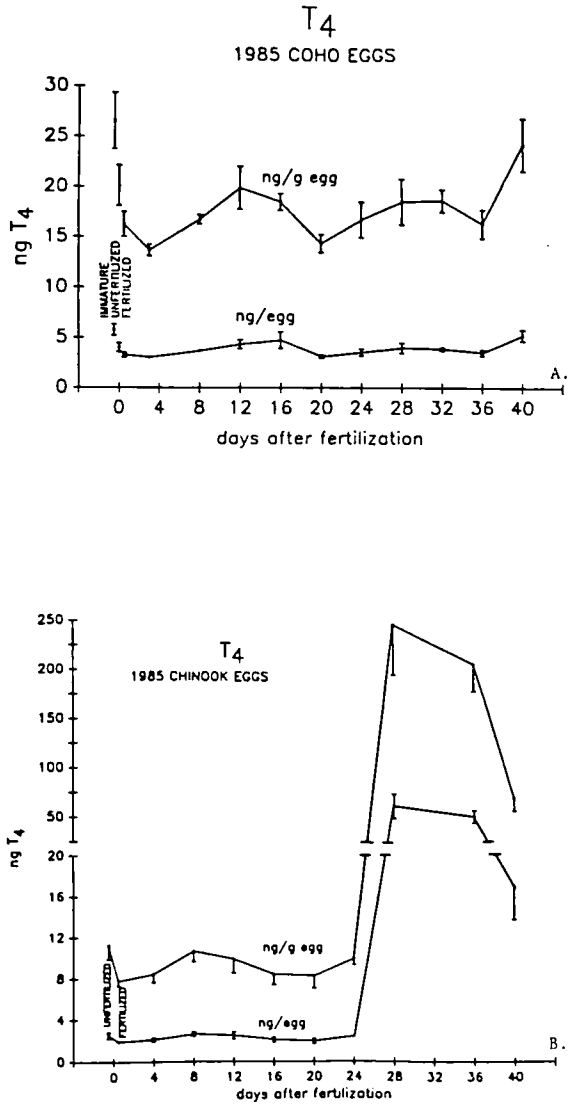


Fig. 1. Changes in T_4 in 1985 coho and chinook eggs. *A*. Coho eggs; *B*. Chinook eggs.

dardized at 50,000 cpm/0.5 ml water. The non-specific ^{125}I uptake was measured in the presence of a 100-fold (chinook eggs, group C) or 1000-fold (coho eggs, group D) excess of cold NaI. The higher concentration of non-radioactive iodide proved to be better at eliminating ^{125}I uptake by the eggs.

Eggs (n = 25 for group C, n = 12–14 for group D) were placed in each beaker and left for 16 hours. The eggs were then rinsed, blotted with tissue, and put in test tubes for gamma counting. Radioactive uptake was measured as (% dose taken up/dose in 1 ml water)/g tissue. This measurement allowed for the standardization of different amounts of ^{125}I in the different beakers.

Statistics

All statistical calculations were done using CRISP software (Crunch, Oakland, CA). All tests for significant differences in thyroid hormone levels or iodide uptakes were performed using one-way ANOVA followed by the Tukey-A test. Significant values using this method are shown at either $p < 0.05$ or $p < 0.01$. Simple regression was employed on some data, as there were many data points, and the overall trend of change in the hormone levels is perhaps more relevant to examine than any specific peak.

Results

All values given here and elsewhere are mean ± standard error of the mean, with n = 7–8 unless otherwise noted.

Eggs

Coho salmon

Weights

In 1985, the weights of immature, unfertilized and fertilized eggs were 218 ± 2 mg, 200 ± 2 mg and 202 ± 3 mg, respectively. Three days after fertilization, the weights of the eggs were 223 ± 3 mg and remained constant at approximately 220 mg until hatching. In 1986, the weights of the eggs remained relatively constant at approximately 240 mg throughout the incubation period.

T_4 and T_3

In 1985, T_4 was present in all samples. Initial T_4 levels varied among immature (26.5 ± 2.8 ng/g, 5.8 ± 0.6 ng/egg), unfertilized (20.1 ± 2.0 ng/g, 4.0 ± 0.4 ng/egg) and fertilized (16.3 ± 1.2 ng/g, 3.3 ± 0.3 ng/egg) eggs (Fig. 1A). The levels remained

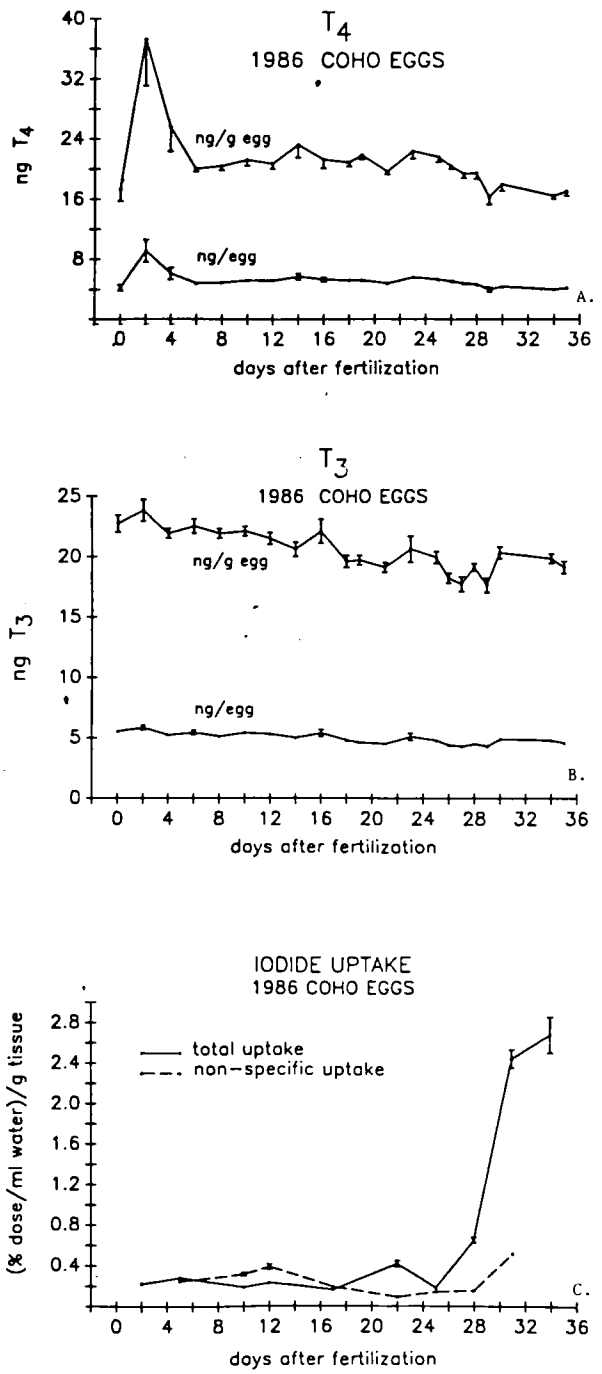


Fig. 2. Changes in T_4, T_3 and iodide uptake in 1986 coho eggs. *A*. Egg T_4; *B*. Egg T_3; *C*. Iodide uptake.

relatively constant (approximately 16–18 ng/g, 3–4 ng/egg) until there was an increase in T_4 from 36 days (16.4 ± 1.4 ng/g, 3.54 ± 0.3 ng/egg) to 40 days (24.3 ± 2.6 ng/g, 5.3 ± 0.6 ng/egg) after fertilization (ANOVA-Tukey-A, $p < 0.05$, $p < 0.01$, respectively).

In 1986, T_4 and T_3 were present in all samples. T_4 levels (Fig. 2A) remained relatively constant throughout most of the incubation period at approximately 20 ng/g, 5 ng/egg. There was one increase in thyroxin, however, from the day of fertilization (17.3 ± 1.6 ng/g, 4.2 ± 0.4 ng/egg) to two days (37.2 ± 6.2 ng/g, 9.1 ± 1.5 ng/egg) after fertilization (ANOVA-Tukey-A, $p < 0.01$, for both values). The T_4 levels dropped from these high levels to 20.0 ± 0.4 ng/g, 4.8 ± 0.1 ng/egg, six days after fertilization (ANOVA-Tukey-A, $p < 0.01$ for both values). T_3 levels (Fig. 2B) in the eggs stayed relatively constant from fertilization through hatching at 18–22 ng/g, 4–6 ng/egg.

1986 iodide uptake

The levels of total iodide (^{125}I-only) uptake and non-specific (^{125}I + 1000-fold NaI) uptake are shown in Fig. 2C. Specific uptake rose manifold from 25–31 days after fertilization. The levels of non-specific uptake were low during this time (no data are available for 34 days after fertilization) and did not show the large magnitude of increase as did total uptake.

Chinook salmon

Weights

In 1985, the weights of unfertilized and fertilized eggs were 225 ± 2 mg and 248 ± 2 mg, respectively. Four days after fertilization, the weights of the eggs were 254 ± 3 mg. The weights of the eggs remained constant at approximately 250 mg until hatching. In 1986, the weights of immature, unfertilized and fertilized eggs were 276 ± 3 mg, 323 ± 3 mg and 239 ± 10 mg, respectively. The fertilized eggs from the two females that were sampled throughout the incubation period were noticeably different. One female was large and her eggs weighed approximately 345 mg each. The other female was relatively small, and her eggs weighed approximately 240 mg each. This difference was noted upon return to Berkeley and from that point onward, whenever possible, only the large eggs were sampled (except for the iodide uptake study which used both large

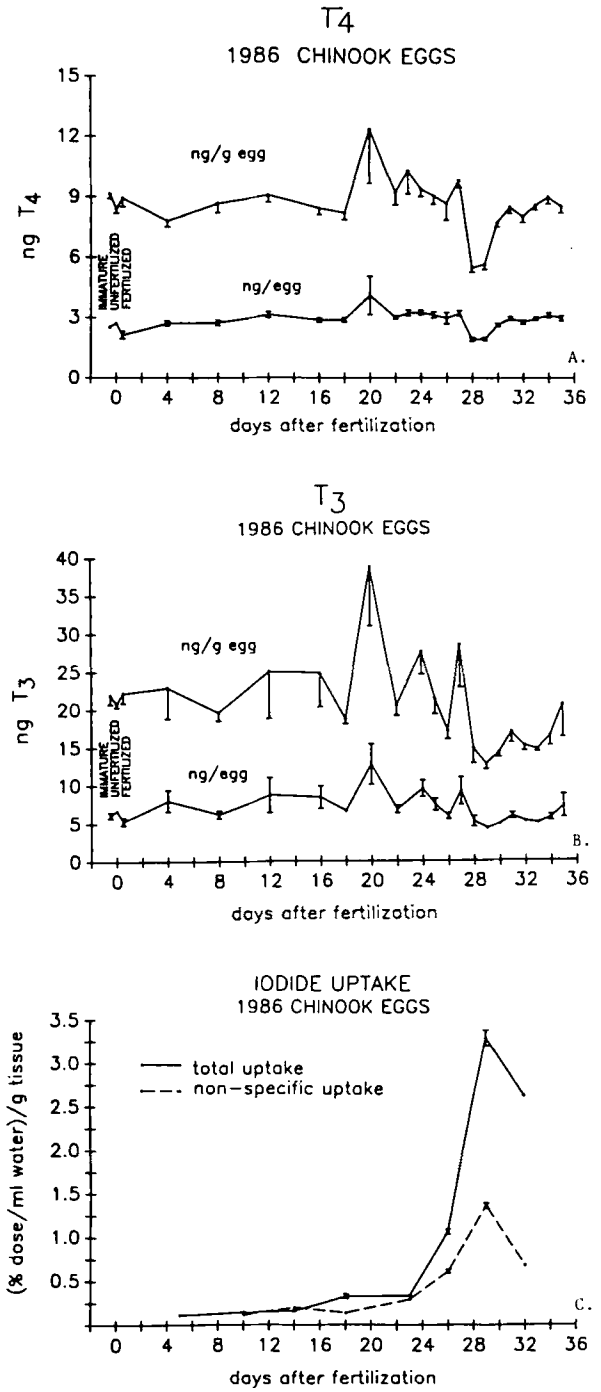


Fig. 3. Changes in T_4, T_3 and iodide uptake in 1986 chinook eggs. *A*. Egg T_4; *B*. Egg T_3; *C*. Iodide uptake.

and small eggs). Occasionally, it was necessary to include smaller eggs in the sample groups (*e.g.*, the fertilized eggs were small eggs only). From four days after fertilization through hatching, the weight of the eggs sampled remained relatively constant at approximately 345 mg.

T_4 and T_3

In 1985, T_4 was present in all samples. Mean initial T_4 levels differed between unfertilized (11.2 ± 1.4 ng/g, 2.25 ± 0.3 ng/egg) and fertilized (7.8 ± 0.5 ng/g, 1.9 ± 0.1 ng/egg) eggs (Fig. 1B). The levels remained relatively constant (approximately 8–10 ng/g, 2–3 ng/egg) through 24 days after fertilization when T_4 was 10.1 ± 0.6 ng/g, 2.5 ± 0.2 ng/egg. There was a large increase from 24 to 28 days after fertilization to 245.1 ± 51.0 ng/g, 60.9 ± 12.6 ng/egg (ANOVA-Tukey-A, $p < 0.01$ for both values). Similarly high levels of T_4 were also seen 36 days after fertilization (203.9 ± 27.3 ng/g, 50.5 ± 6.4 ng/egg), but four days later (40 days after fertilization) they fell to 68.1 ± 12.7 ng/g, 17.1 ± 3.3 ng/egg (ANOVA-Tukey-A, $p < 0.01$ for both values).

In 1986, T_4 and T_3 were present in all samples. T_4 levels (Fig. 3A) remained relatively constant in the eggs for the first 27 days after fertilization at approximately 8–10 ng/g, 3 ng/egg. There was a decrease from 20 days (12.3 ± 2.7 ng/g, 4.1 ± 1.0 ng/egg) to 28 days (5.5 ± 0.2 ng/g, 1.9 ± 0.1 ng/egg) after fertilization (ANOVA-Tukey-A, $p < 0.01$ for both values). On the last day of incubation (35 days after fertilization), the levels were 8.5 ± 0.3 ng/g, 3.0 ± 0.1 ng/egg.

T_3 levels (Fig. 3B) remained constant for the first 18 days of incubation at approximately 18–25 ng/g, 6–8 ng/egg. There was an increase from 18 days (18.7 ± 0.7 ng/g, 6.6 ± 0.2 ng/egg) to 20 days (38.7 ± 7.8 ng/g, 12.7 ± 2.7 ng/egg) after fertilization (ANOVA-Tukey-A, $p < 0.01$, $p < 0.05$, respectively). From 20 to 22 days after fertilization, T_3 concentration and content dropped to 20.5 ± 1.4 ng/g, 6.8 ± 0.5 ng/egg (ANOVA-Tukey-A, $p < 0.05$ for both values). Twenty-nine days after fertilization T_3 levels were 12.7 ± 0.7 ng/g, 4.3 ± 0.2 ng/egg. On the last day of incubation (35 days after fertilization), the levels were 20.5 ± 4.3 ng/g, 7.2 ± 1.5 ng/egg.

T_4 and T_3 in 1986 28-day old embryonic body and yolk

1. Concentration and content. Embryonic T_4 levels

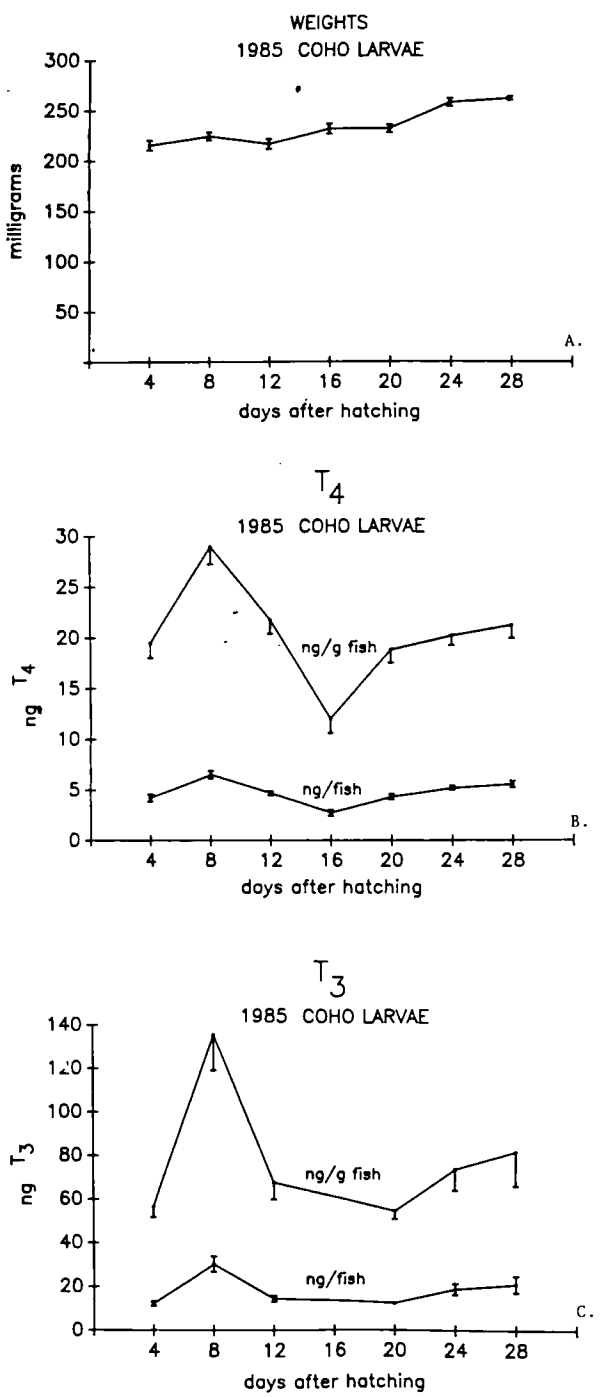


Fig. 4. Changes in weight, T_4, and T_3 in 1985 coho salmon larvae. *A.* Weight weight; *B.* Whole-body T_4; *C.* Whole-body T_3.

28 days after fertilization were 62.0 ± 13.3 ng/g, 1.0 ± 0.2 ng/body for the body and 10.4 ± 0.8 ng/g, 1.6 ± 0.1 ng/yolk for the yolk. Embryonic body T_3 levels 28 days after fertilization were 393.2 ± 117.1 ng/g, 6.4 ± 2.0 ng/body. This T_3 concentration was the highest measured throughout the study for any sample, but these bodies also weighed less than any other samples measured. T_3 in the yolk at this time was 24.9 ± 2.7 ng/g, 3.8 ± 0.1 ng/yolk.

2. Compartmentalization. In 28-day-old embryos, the body accounted for 9% of the total weight, with the yolk accounting for 91%. T_4 in the body accounted for 38% of total T_4, with the yolk accounting for 62%. In contrast, 63% of total T_3 was found in the body, with 37% of the T_3 in the yolk.

1986 iodide uptake

The levels of total iodide (^{125}I-only) uptake and non-specific (^{125}I + 100-fold NaI) uptake are shown in Fig. 3C. It is evident that specific uptake rose manifold from 23–29 days after fertilization. Hundred-fold NaI was probably not sufficient to inhibit ^{125}I fully, but the levels of non-specific uptake were still relatively low during this time and did not show the large magnitude of increase as did total uptake.

Larvae

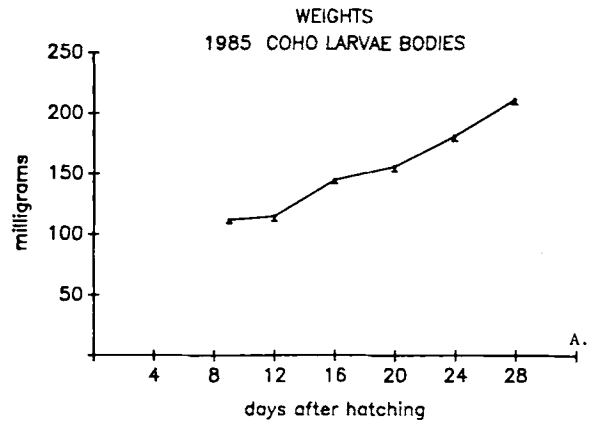
Coho salmon

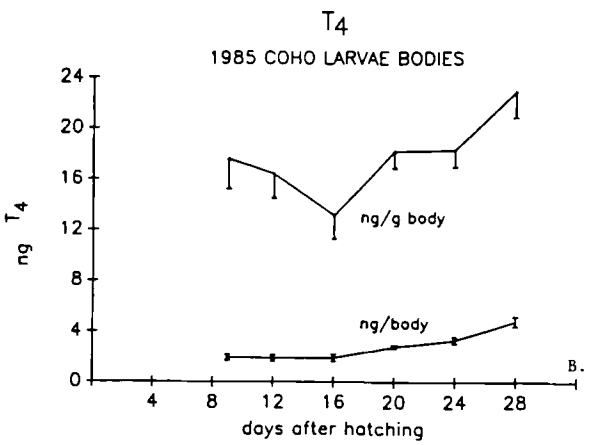
Weights

1985 larval weights (Fig. 4A) increased steadily from four days after hatching (215 ± 4 mg) through 28 days after hatching (261 ± 2 mg). Body-only weights (Fig. 5A) increased steadily from nine days after hatching (112 ± 3 mg) through 28 days after hatching (211 ± 4 mg). Yolk-only weights (Fig. 6A) decreased from 127 ± 2 mg at 12 days after hatching to 56 ± 3 mg 28 days after hatching. 1986 larval weights (Fig. 7A) increased steadily from the day of hatching (234 ± 5 mg) through 32 days after hatching (335 ± 8 mg).

Whole-body T_4 and T_3

In 1985, larval T_4 levels (Fig. 4B) increased from four days (19.4 ± 1.5 ng/g, 4.2 ± 0.4 ng/fish) after hatching to eight days (29.0 ± 1.7 ng/g, 6.5 ± 0.4





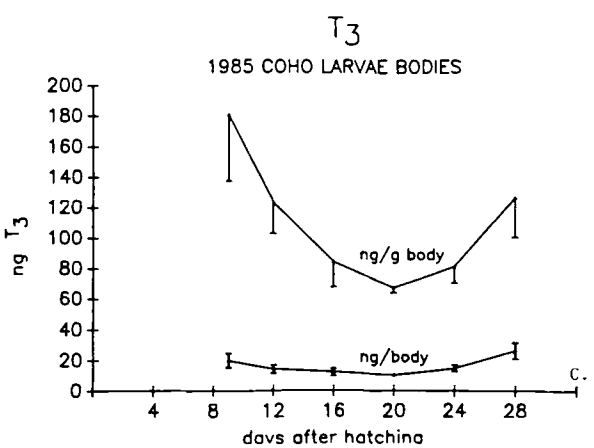
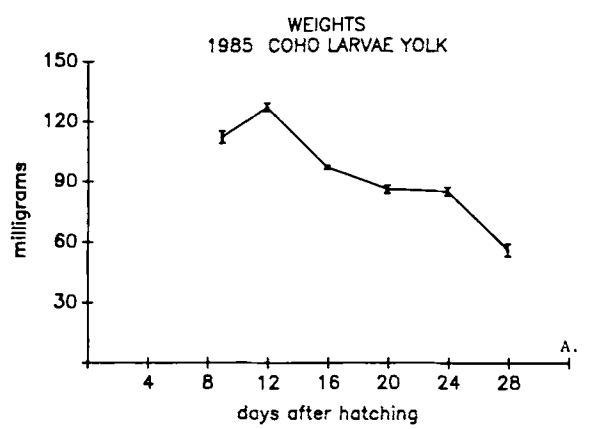
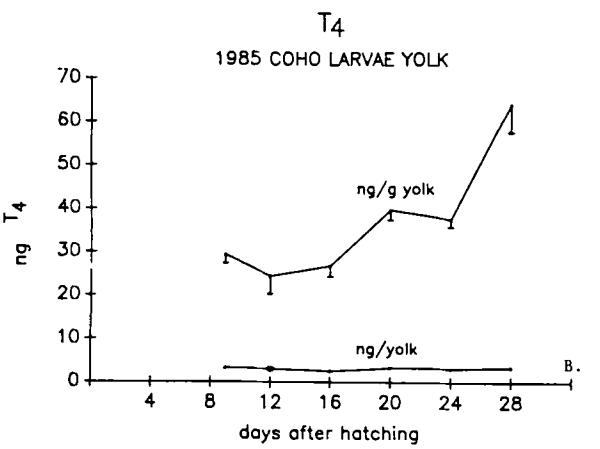


Fig. 5. Changes in weight, T_4, and T_3 in the bodies only of 1985 coho salmon larvae. *A*. Wet weight; *B*. Body-only T_4; *C*. Body-only T_3.





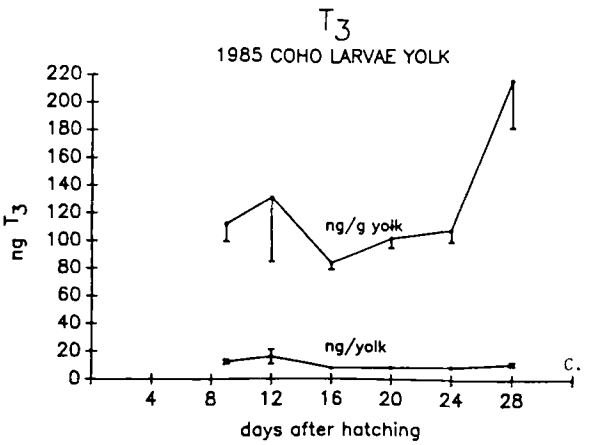


Fig. 6. Changes in weight, T_4, and T_3 in the yolk only of 1985 coho salmon larvae. *A*. Wet weight; *B*. Yolk-only T_4. *C*. Yolk-only T_3.

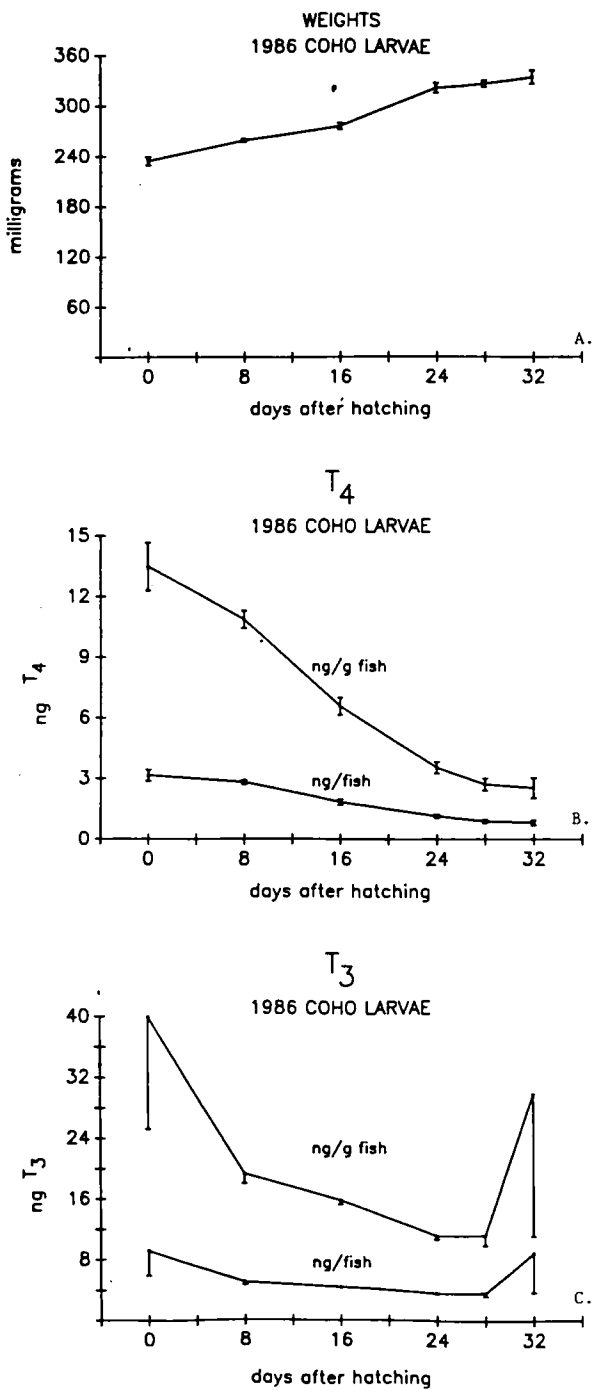


Fig. 7. Changes in weight, T_4, and T_3 in 1986 coho salmon larvae. *A.* Wet weight; *B.* Whole-body T_4; *C.* Whole-body T_3.

ng/fish) after hatching (ANOVA-Tukey-A, $p < 0.01$ for both values). From eight to 16 days after hatching, T_4 fell to 13.1 ± 1.9 ng/g, 2.7 ± 0.3 ng/fish (ANOVA-Tukey-A, $p < 0.01$ for both values). From 16 to 28 days after hatching, T_4 levels increased to 21.2 ± 1.3 ng/g, 5.5 ± 0.3 ng/fish (ANOVA-Tukey-A, $p < 0.01$ for both values).

T_3 levels (Fig. 4C) more than doubled from four days (56.6 ± 4.8 ng/g, 12.2 ± 1.1 ng/fish) to eight days (135.6 ± 16.4 ng/g, 30.2 ± 3.5 ng/fish) after hatching (ANOVA-Tukey-A, $p < 0.01$ for both values). T_3 then dropped from eight to 20 days after hatching to 55.0 ± 3.7 ng/g, 12.6 ± 0.7 ng/fish (ANOVA-Tukey-A, $p < 0.01$ for both values).

In 1986, larval T_4 levels (Fig. 7B) were highest at the time of hatching (13.5 ± 1.2 ng/g, 3.2 ± 0.3 ng/fish). These levels decreased steadily through 32 days after hatching, at which time they were at their lowest levels of 2.5 ± 0.5 ng/g, 0.8 ± 0.1 ng/fish (ANOVA-Tukey-A, $p < 0.01$ for both values).

1986 larval T_3 levels (Fig. 7C) eight days after hatching were 19.4 ± 1.3 ng/g, 5.0 ± 0.3 ng/fish. Twenty-four days after hatching T_3 levels were 11.2 ± 0.5 ng/g, 3.6 ± 0.1 ng/fish. No statistical significance was established for this drop or any other changes in 1986 coho whole-body T_3 levels.

T_4 and T_3 in body and yolk (1985)

1. Concentration and content. Larval body T_4 concentrations (Fig. 5B) were lowest 16 days after hatching (13.1 ± 1.9 ng/g), but these levels were not significantly different from the levels 9 and 12 days after hatching. Total T_4 was approximately 1.9 ng/body for all three sample groups. The levels rose significantly from 16 to 28 days after hatching to 23.0 ± 2.1 ng/g, 4.8 ± 0.4 ng/body (ANOVA-Tukey-A, $p < 0.05$, $p < 0.01$, respectively).

Larval body T_3 concentrations (Fig. 5C) nine days after hatching were 181.3 ± 43.5 ng/g. This level dropped to 68.1 ± 3.4 ng/g 20 days after hatching (ANOVA-Tukey-A, $p < 0.05$). Total T_3 content (10–20 ng/body) did not significantly change from nine to 20 days after hatching. There was an increase in total T_3 from 20 days (10.6 ± 0.4 ng/body) to 28 days (26.4 ± 5.3 ng/body) after hatching (ANOVA-Tukey-A, $p < 0.05$). However, T_3 concentration did not rise significantly during this period.

Larval yolk T_4 concentrations (Fig. 6B) in-

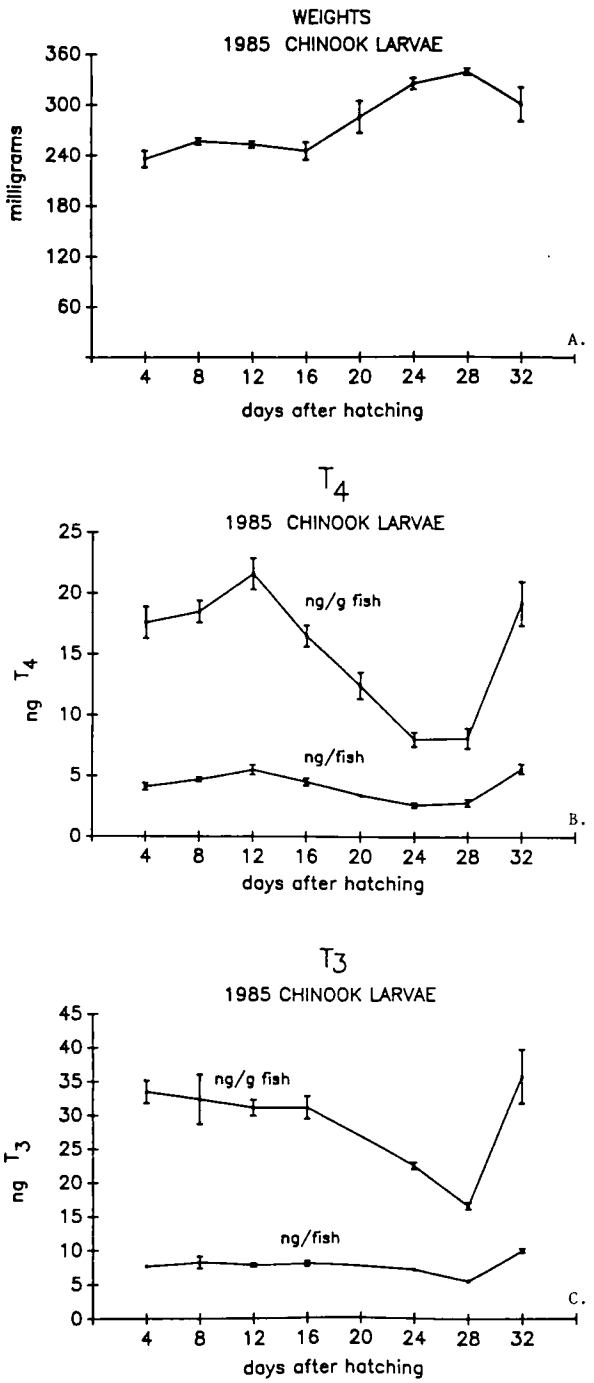


Fig. 8. Changes in weight, T_4, and T_3 in 1985 chinook salmon larvae. *A.* Wet weight; *B.* Whole-body T_4; *C.* Whole-body T_3.

creased from nine to 28 days after hatching (simple regression, $p < 0.0001$). The yolk T_4 content (ng/yolk) did not change throughout the larval period sampled.

Larval yolk T_3 concentrations (Fig. 6C) also rose from nine to 28 days after hatching (simple regression, $p < 0.05$). The T_3 yolk content stayed constant throughout the larval period.

2. Compartmentalization. Nine days after hatching, body and yolk each accounted for 50% of the total weight of the fish. At this time, the total T_4 was compartmentalized between body and yolk at 37% and 63%, respectively. In contrast, 61% of total T_3 was in the body and 39% of the T_3 was in the yolk.

Sixteen days after hatching, the body was 60% of total weight, while the yolk was 40%. Forty-two percent of total T_4 was in the body, while 57% of the T_4 was in the yolk. At this time, T_3 compartmentalization was not noticeably different from nine days after hatching (60% in body, 40% in yolk).

Twenty-eight days after hatching, the body accounted for 79% of the total weight and the yolk only 21%. T_4 in the body increased to 58% of total T_4, while the relatively small quantity of yolk still contained 42% of the T_4. At this time, 70% of total T_3 was in the body, while 30% of the T_3 was in the yolk.

Chinook salmon

Weights

In 1985, larval weights (Fig. 8A) stayed constant (approximately 250 mg) from four through 16 days after hatching, then increased steadily through 28 days after hatching (340 ± 4 mg), and were 300 ± 20 mg 32 days after hatching. Larval body weights (Fig. 9A) stayed constant from 4–12 days after hatching (approximately 80–90 mg). The body weights then increased steadily through 28 days after hatching (248 ± 13 mg). Larval yolk weights (Fig. 10A) decreased from 182 ± 14 mg eight days after hatching to 79 ± 4 mg 32 days after hatching.

Whole-body T_4 and T_3 (1985)

Larval T_4 levels (Fig. 8B) decreased from four days (17.6 ± 1.3 ng/g, 4.1 ± 0.3 ng/fish) through 24 days (8.0 ± 0.6 ng/g, 2.6 ± 0.2 ng/fish) after hatching (ANOVA-Tukey-A, $p < 0.01$, $p < 0.05$, respectively) The decrease in total T_4 was not so

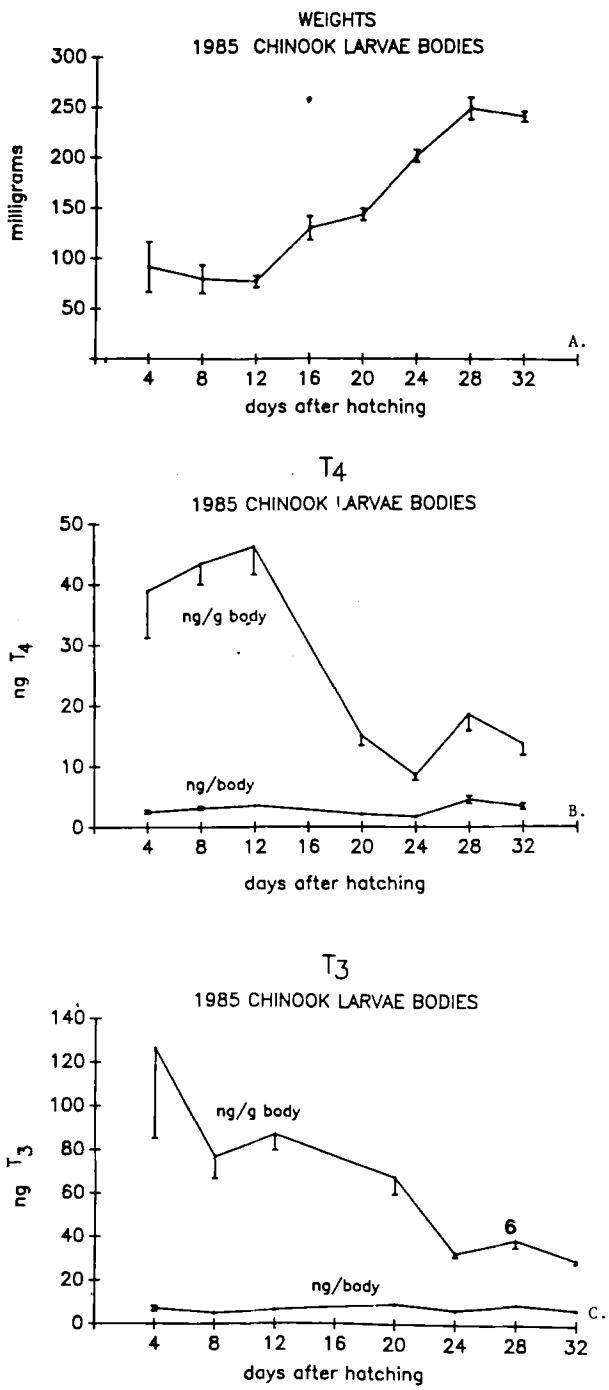


Fig. 9. Changes in weight, T_4, and T_3 in the bodies only of 1985 chinook salmon larvae. *A.* Weight weight; *B.* Body-only T_4; *C.* Body-only T_3; n = 7–8 except for day 28 in C, when n = 6.

great as the fall in T_4 concentration because the larvae gained weight at the same time. From 24 to 32 days after hatching, larval T_4 levels increased to 19.2 ± 1.8 ng/g, 5.6 ± 0.4 ng/fish (ANOVA-Tukey-A, $p < 0.01$ for both values).

Larval T_3 levels (Fig. 8C) decreased from four days (33.5 ± 1.7 ng/g, 7.7 ± 0.1 ng/fish) to 28 days (16.7 ± 0.5 ng/g, 5.6 ± 0.1 ng/fish) after hatching (ANOVA-Tukey-A, $p < 0.01$, $p < 0.05$, respectively). As with the T_4 levels, the T_3 levels increased from 28 to 32 days (35.8 ± 4.0 ng/g, 10.1 ± 0.3 ng/fish) after hatching (ANOVA-Tukey-A, $p < 0.01$ for both values).

T_4 and T_3 in body and yolk (1985)

1. Concentration and content. Larval body T_4 concentrations (Fig. 9B) remained fairly constant from four to 12 days after hatching (approximately 39–46 ng/g); however, as the body weight increased, the concentrations began to fall. From four days (38.9 ± 7.7 ng/g) to 32 days (13.6 ± 1.9 ng/g) after fertilization, there was a drop in T_4 concentration (ANOVA, $p < 0.01$). The larval T_4 content (ng/body) stayed relatively constant from four through 32 days after hatching at approximately 2–4 ng/body, although there was a rise from 24 days (1.7 ± 0.2 ng/body) to 28 days (4.5 ± 0.6 ng/body) after hatching (ANOVA-Tukey-A, $p < 0.01$).

Larval body T_3 (Fig. 9C) concentrations decreased from four through 32 days after hatching (simple regression, $p < 0.0001$). The concentration decreased as body weight increased. T_3 body content increased during this same time period (simple regression, $p < 0.05$).

Larval yolk T_4 (Fig. 10B) levels did not vary much from four to 24 days after hatching, although yolk content dropped from four days (3.5 ± 0.2 ng/yolk) to 16 days (1.9 ± 0.1 ng/yolk) after hatching (ANOVA-Tukey-A, $p < 0.05$). A rise occurred from 24 days (21.2 ± 2.8 ng/g, 1.2 ± 0.1 ng/yolk) to 28 days (54.5 ± 11.4 ng/g, 4.4 ± 0.7 ng/yolk) after hatching (ANOVA-Tukey-A, $p < 0.01$ for both values). There was a decrease in yolk T_4 content from 28 to 32 days (2.5 ± 0.2 ng/yolk) after hatching (ANOVA, $p < 0.01$), but T_4 concentration did not change during this period.

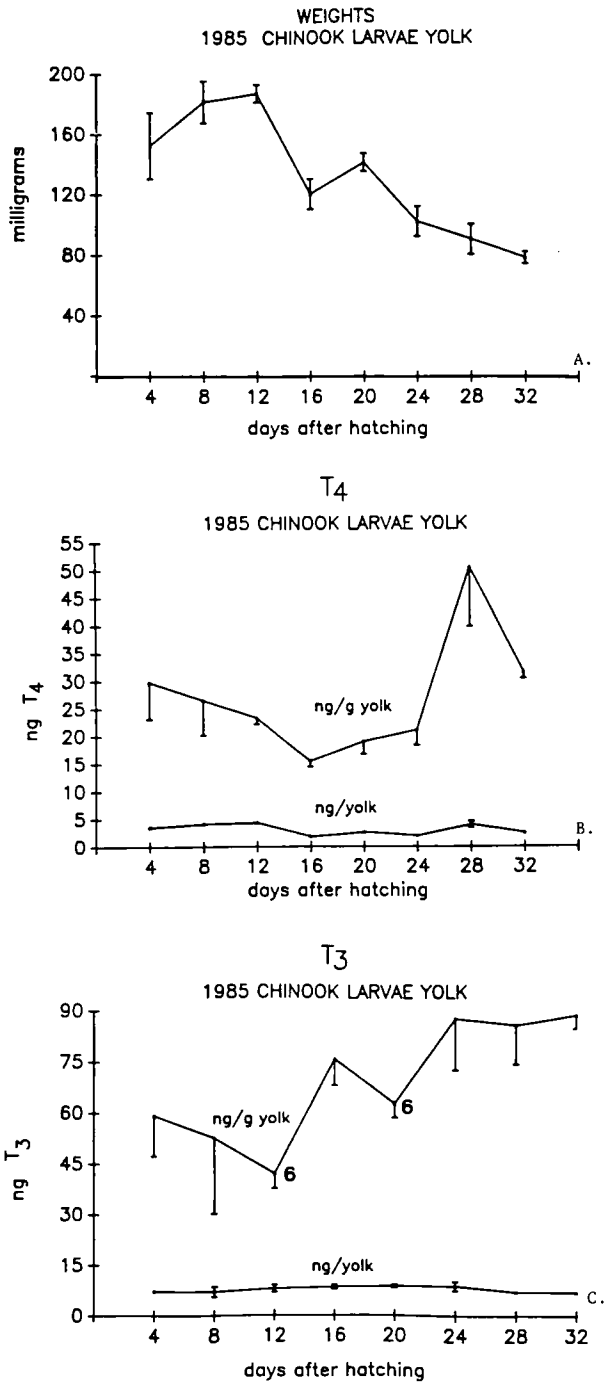


Fig. 10. Changes in weight, T_4, and T_3 in yolk only of 1985 chinook salmon larvae. *A.* Wet weight; *B.* Yolk-only T_4; *C.* Yolk-only T_3; n = 7–8, except for days 12 and 20 in C, where n = 6.

Larval yolk T_3 (Fig. 10C) concentrations rose from four to 32 days after hatching (simple regression, $p < 0.01$). The T_3 yolk content stayed constant throughout the larval period.

2. Compartmentalization. Four days after hatching, the body was 43% of total weight, while the yolk was 57%. At this time, total T_4 was compartmentalized between body and yolk at 41% and 59%, respectively. In contrast, 49% of total T_3 was in the body and 51% was in the yolk.

Twenty days after hatching, bodies and yolks each accounted for 50% of the total weight of the fish. Forty-four percent of total T_4 was in the body, while 56% of the thyroxin was in the yolk. T_3 compartmentalization did not change noticeably from nine days after hatching (52% of T_3 in body, 48% of T_3 in yolk).

Twenty-eight days after hatching, the body accounted for 76% of the total weight; yolk was only 24% of the fish. Both T_4 and T_3 in the body increased to 57% of total hormone quantity, while the yolk contained 43% of T_4 and T_3.

Discussion

Thyroid hormones may be important factors in the development of teleosts. The results from this study show that both T_4 and T_3 are present throughout all stages of early development of coho and chinook salmon. These findings supplement previous data showing that T_4 is present in coho salmon eggs (Kobuke *et al.* 1987), that both T_4 and T_3 are present in unfertilized and maturing striped bass eggs (Brown *et al.* 1987) and that T_4 occurs in unfertilized and maturing chum salmon eggs (Tagawa and Hirano 1987). The presence of the hormones in immature eggs of both coho and chinook salmon in concentrations similar to that seen in mature eggs suggests that thyroid hormones are transferred to the eggs before final maturation.

This idea is supported by other work. Lam and Loy's (1985) findings that the immersion of female guppies in thyroxin accelerated the rate of vitellogenesis and Sullivan, Hara, Bernard and Dickhoff's unpublished work showing that both T_3 and T_4 specifically bind to vitellogenin, suggest that

thyroid hormones may be incorporated into the oocytes before they mature. In addition, Sower and Shreck (1982), Biddiscombe and Idler (1983) and Udea *et al.* (1984) have all reported that plasma levels of T_4 (and T_3 in Biddiscombe and Idler's study) decreased during the spawning season after reentry into fresh water in coho, sockeye (*Oncorhynchus nerka*) and chum salmon, respectively. Sower and Shreck also reported that the plasma thyroid hormones of coho held in seawater (and thus unable to spawn) did not decrease to the same degree as in migrating coho. It is possible that thyroid hormones are necessary for maintenance of maturing sex structures in these salmon. Since both the ovary and testis become enlarged at this time, while other organs tend to degenerate, it may be that thyroid hormones are transferred into both gonads during spawning. In some cases (Sower and Shreck 1982; Biddiscombe and Idler 1983; Dickhoff *et al.* in press), a greater decline is seen in female salmonids than in males. In the female, the bulk of the mature ovary is composed of eggs. On the other hand, Leatherland and Sonstegard (1980) reported that in 'landlocked' coho salmon, immature jacks experienced a drop in plasma T_4 and T_3 similar to that seen in sexually mature adults around the time of spawning. The drop in thyroid hormone levels may be related as well to seasonal changes. However, it is also possible that in the case of these landlocked salmon, thyroid physiology may be entirely different than in anadromous salmon.

In the egg stage, differences in thyroid hormone levels along with changes in radioactive iodide uptake levels can be used to estimate when thyroid follicles begin to contribute hormones to the stores of T_4 and T_3 in the developing salmon. T_4 levels in both chinook and coho in 1985 stayed relatively constant for at least the first four weeks of development (Figs 1A, B). Twentyeight days after fertilization, the chinook T_4 levels increased 20-fold. Forty days after fertilization, coho T_4 levels also increased. Similar increases did not occur in the 1986 T_4 measurements. Twenty-eight days after fertilization, when the 1985 chinook T_4 levels surged, 1986 chinook T_4 and T_3 levels were relatively low (Figs 3A, B). The most prominent increase seen in 1986 chinook egg thyroid hormone levels was 20 days after fertilization. In 1986 coho eggs, T_3 levels (Fig. 2B) did not change at all, while the only conspicuous change in T_4 was two days after fertilization (Fig. 2A). All of these data suggest that there is not only large variation in thyroid hormone levels between species, but also among individual cohorts. A change in T_4 or T_3 levels that occurred in any given coho or chinook group may be characteristic of only that particular cohort. Peaks seen in 1985 coho and chinook profiles were not repeated in the 1986 profiles. There was some variation in the extraction technique between 1985 and 1986, which may account for differences in the profiles, but it is also likely that the variation between cohorts was biologically based.

Since the members of each individual cohort came from the eggs of only one or two females fertilized with the milt of one to three males, the disparity in T_4 and T_3 levels in the same species from different years may actually have been a consequence of variation of the thyroid function of the individual progenitors. Occasionally, individual eggs or larvae from a cohort showed extremely low or high T_4 and/or T_3 levels relative to other individuals in the same sample group. If there is large variation in the thyroid activity of the offspring from 1–2 females fertilized by 1–3 males, then the variation in thyroid activity among individuals in the general population of Klamath River salmon could also be large. The results from any one cohort of coho or chinook salmon cannot, therefore, be considered representative of all coho or chinook, but instead of only one set of offspring of that particular species. If T_4 and T_3 change within a specific cohort, the given fluctuation shows us that members of the species are capable of this activity, but this change may not occur in other members of the same species.

Iodide uptake levels were approximately the same for coho and chinook eggs. There was a large increase in total uptake after about 20 days of incubation (Figs 2C and 3C). If this increase is interpreted as indicative of thyroid activity, then thyroxinogenesis occurs in the developing embryo. The 1986 iodide uptake studies correlate fairly well with the 1985 T_4 data for coho and chinook eggs (Figs

1A, B) and even with the peaks in T_4 and T_3 at 20 days in the 1986 chinook eggs (Figs 3A, B), but they do not correlate at all with 1986 coho egg T_4 and T_3 data (Figs 2A, B) and the relatively low levels of these hormones 28 days after fertilization in the 1986 chinook eggs (Figs 3A, B). It is also possible that the iodide uptake is not indicative of thyroxinogenesis, as has been suggested by Barrington and Rawdon (1971).

A measure of the rate of decay of the hormones in the eggs would provide important information. If hormone production is greatly increased four weeks after fertilization (as indicated by increased specific iodide uptake), but hormone concentrations have remained constant or have decreased, then degradation of the hormones may be an important factor. Taking degradation into account and assuming that the thyroid follicles are active in the embryos (which is further substantiated by Leatherland and Lin's (1975) active-looking coho embryo thyroid follicles), then the reported egg data can be interpreted as follows: After about 18 days of development, there was a fairly well-defined embryo inside the egg. In general, neither T_4 nor T_3 levels varied much before this time. Iodide uptake levels were relatively low, so that not much thyroxinogenesis could have yet occurred. Nevertheless, it is possible that there was some thyroid biosynthetic activity earlier than this time (as indicated by small changes in iodide uptake (Figs 2C and 3C), 1986 coho T_4 levels (Fig. 2A), and the possibility that the thyroid anlage had some activity soon after formation, as was reported by Wabuke-Bunoti and Firling in 1983 for the fathead minnow). Thus, until approximately 3–4 weeks after fertilization, the main source of thyroid hormones in the eggs seems to be maternal. As embryonic development progressed, endogenous production became increasingly important.

It may be that in the 1985 chinook eggs, the embryonic thyroid produced T_4 at a rate much greater than the rate of decay of T_4 in the liver and elsewhere. The 1986 data suggest that the embryos degraded thyroid hormones at a rate similar to endogenous thyroid production, so that no large increases in thyroid hormones were seen in those eggs.

The levels of 5′-monodeiodinase, which influence the rate of change of the relatively inactive precursor hormone T_4 into the active T_3, were not determined in this study. The relatively large amount of T_3 compared with T_4 in the 28-day-old chinook embryo body could reflect the presence of 5′-monodeiodinase in the developing organism.

The sizable quantities of tissue T_3 generated by the peripheral conversion of T_4 to T_3 suggest that thyroid hormone action may occur on a fairly large scale starting at least 28 days after fertilization. T_3 has been implicated as an important factor in the development of the nervous system of mammals. Neonatal hypothyroidism causes reduction in cellular proliferation and differentiation, and impairs growth of neuronal processes and the formation and organization of synapses of rats (Geel 1977). Interestingly, 1986 coho embryos removed from the eggs showed almost no motor function 20 days after fertilization, but by 30 days after fertilization they engaged in active movement. It may be more than coincidence that high thyroid activity (iodide uptake) and onset of motor function occurred at the same time in coho embryos.

Previous measurements of whole-body thyroxin changes during larval development in coho and chum salmon (Kobuke *et al.* 1987; Tagawa and Hirano 1987) established the occurrence of a steady decrease in thyroxin content from the time of hatching to yolksac resorption. 1986 coho larvae showed the same tendency as described above: T_4 concentration and content fell to their lowest levels at the time of yolksac resorption (Fig. 7B). In addition, whole-body T_3 generally decreased at this time, although the decreases were not statistically significant (Fig. 7C). In 1985 chinook larvae, T_4 and T_3 dropped from hatching toward the time of yolksac resorption, but there was an increase in both hormones (content and concentration) before all of the yolksac had been resorbed 32 days after hatching (Figs 8B, C). At this time, both T_4 and T_3 concentrations were similar to those seen four days after hatching. On the other hand, there was a greater content of the hormones 32 days after hatching than at the beginning of the larval period because the fish were much bigger at this later time. The data from 1985 coho larvae complicate the pic-

ture, as levels immediately increased rather than decreased (Figs 4B, C). Both T_4 and T_3 levels were at their highest eight days after hatching. The levels then dropped as expected, but T_4 and T_3 concentration and content rose again before yolksac resorption.

Previously, it had been thought that the decrease in thyroxin in developing larvae represented depletion of the maternal source of the hormone, and that once the yolksac had been resorbed, thyroxinogenesis occurred (Kobuke *et al.* 1987). As endogenous thyroid activity most likely begins in the embryonic stage, it seems likely that in 1985 both coho and chinook actively produced thyroid hormones during the larval period. Although larvae may generally produce their own thyroid hormones, it is possible that in some cases (such as those of Kobuke *et al.* (1987) and Tagawa and Hirano (1987) and in the 1986 coho of the present study (Fig. 7B)), the rate of hormone turnover was greater than that of production, leading to steady decreases.

The measurements of the compartmentalization of T_4 and T_3 between the body and yolk of the larvae give more information about thyroid hormones during the larval period. In younger coho larvae, there was a greater percentage of T_3 in the relatively small developing body (Fig. 5C) than there was in the larger yolk mass (Fig. 6C), while more T_4 tended to be present in the yolk (Fig. 6B) than in the body (Fig. 5B). If it is assumed that the yolksac and its contents represent storage, then the presence of the major portions of T_3 in the developing body and T_4 in the degenerating yolksac suggests that T_3 is utilized more than T_4 for the body's developmental processes, supporting the hypothesis that T_4 may only be the precursor to the physiologically active T_3 (Eales 1985).

As the body grew and the yolk mass diminished, there was a tendency for more of both hormones to be in the body. While the amount of yolk decreased, the amounts of T_4 and T_3 stored therein stayed relatively constant (Figs 6 and 10). As a result, there was a large increase in the yolk concentration of both thyroid hormones toward the end of the larval stage.

In the body, general decreases in T_4 and T_3 concentrations seen at the beginning of the larval period mainly resulted from an increase in body weight. At this time, the actual contents of the hormones did not change appreciably. In coho bodies, toward the end of the larval phase, there were marked increases in both T_4 and T_3 content and concentration (Fig. 5B). Chinook bodies also showed a rise in T_4 content at this time (Fig. 6B). These elevations further substantiate the notion that endogenous thyroxinogenesis occurs in the larval stage.

The results from this study can be correlated with the data of Sullivan *et al.* (1987) on T_4 and T_3 concentrations in plasma of chinook and coho larvae. They reported that after 50% resorption of the yolksac, there was an elevation in plasma T_4 and a decrease in plasma T_3 concentrations. Perhaps the general fall in larval whole-body T_3 concentration in this study, owing to its interaction with newly-forming tissue, signaled the larval thyroid to produce T_4. This signal may have been be transmitted through the hypothalamo-hypophysio-thyroid axis, as Leatherland and Lin (1975) have described thyrotropes in the pituitary of coho embryos one week before hatching. The T_4 would then have been synthesized, released into the blood and localized in tissue for ultimate conversion to T_3; hence, there were increases in T_4 in both the body (from thyroid follicle activity) and the plasma (from release). The newly-formed tissue, on the other hand, presumably required T_3 for growth and maintenance; so plasma T_3 was transferred into the tissues and its plasma concentration decreased. In the 1985 coho, T_4 seems to have converted to T_3 in a large enough quantity to increase T_3 levels (Figs 5B, C). In 1985 chinook, T_3 content in the body remained constant and T_4 content increased as the fish grew. These changes resulted in overall lower T_3 but increased T_4 concentrations in the body of the larvae (Figs 9B, C).

It appears that the two sources of thyroid hormones, maternal and endogenous, are both available during the development of salmon larvae. Since the initial source of the thyroid hormones in the yolk is probably maternal, it is likely that the spawning females deposit excess hormones into the eggs. This idea may apply to other hormones as G. Feist *et al.* (personal communication) found high levels of sex steroids in unfertilized coho eggs.

Larval yolk T_4 and T_3 contents remained fairly constant while the concentrations increased. Perhaps the hormones stored in the yolk are less needed by the body tissue once endogenous production of the hormones begins, and indeed endogenous hormones could be transported to and stored in the slowly-resorbing yolksac. The increase in T_4 content in chinook yolk 28 days after hatching supports this notion (Fig. 10B).

After resorption of the yolksac, the stored yolk thyroid hormones enter the body to be used or degraded. It is possible but not established that maternally-derived hormones play an important role in larval development, but in the larva, the contribution of the yolk cannot be distinguished from that of the thyroid. Certainly exogenous thyroid hormones can increase survival and speed up development of larvae of various teleost species (Lam 1980; Nacario 1983; Inui and Miwa 1985; Lam *et al.* 1985; Lam and Sharma 1985; Miwa and Inui 1987).

We conclude from this study that T_4 and T_3 are present in eggs and larvae of developing coho and chinook salmon. Initially, the hormones are maternal in origin, but at some point in development the salmonid embryos become capable of producing their own thyroid hormones. Future work should aim to elucidate the physiological roles of both maternal and embryonic T_4 and T_3 in developmental processes.

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