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Thyroid Hormones in Smoltification of Anadromous Salmonids

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Abstract

Analysis of plasma concentrations of thyroxine (T,) and triiodothyronine (T,) of srnoltifying yearling coho salmon maintained at ten Columbia River hatcheries has revealed a distinct springtime peak in plasma T,. Groups of these hatchery-reared fish were transferred to seawater net-pens at the aquaculture facilities at Manchester, Washington at times corresponding to their hatchery release dates. Data regarding the percentage of surviving and smolted fish were collected throughout six months of seawater residence. These data were compared with various parameters of the freshwater T, peak. One aspect of the T, peak (proportion of the area under the T, curve) showed a significant correlation with survival of fish in seawater after six months. These data suggest that analysis of plasma T, of smolts in freshwater may provide a predictive index of their seawater performance. Similar studies comparing yearling coho salmon with underyearling ("O-age") fish whose growth was accelerated by rearing at elevated water temperature indicated a seawater performance curve correlated with a T, peak which occurred during the early summer. These results suggest that smoltification of yearling and 0-age salmon occurs by different mechanisms. These data suggest that analysis of changes in plasma thyroid hormones provides useful information regarding the optimal time to transfer cultured salmonids from fresh- to seawater facilities.

Introduction

Since the first observations of the activation of the thyroid gland during salmon smoltification (Hoar, 1939), the thyroid has been implicated in a variety of biochemical, physiological and behavioral changes associated with the parr-smolt transformation (see reviews by Dodd and Matty, 1964; Fontaine, 1975; Woodhead, 1975; Hoar, 1976; Bern, 1978, Wedemeyer, et al., 1980; Folmar and Dickhoff, 1980a). Recently, through the use of modern biochemical techniques, principally hormone radioimmunoassay, seasonal cycles of plasma thyroid hormone concentration have been quantified in brook trout, Salvelinus fontinalis (White and Henderson. 1977), rainbow trout, Salmo gairdneri (Osborn, et al., 1978), coho salmon Oncorhynchus kisutch (Dickhoff, et al., 1978) and masu salmon, Oncorhynchus masou (Nishikawa, et al., 1979). Evidence accumulated to date indicates that the plasma concentration of thyroid hormones during smoltification of coho salmon is influenced by a range of genetic and environmental (photoperiod, temperature, diet, xenobiotic, etc.) factors. Thus, measurement of thyroid hormones of salmon appears to be a sensitive biochemical index of those factors which may influence parr-smolt.

Additional studies of the cycles of thyroid hormones in coho salmon with relation to the time of seawater entry of particular stocks of fish have suggested that such measurements may be useful indicators of the extent of smoltification in freshwater (Folmar and Dickhoff, 1980b). An accurate, predictive index of the appropriate time for transfer of salmon to

seawater is of obvious value to salmon aquaculturists. The present report discusses the relationship between thyroid hormones, time of seawater entry and survival of salmon in seawater net-pens in both yearling and 0+ age coho salmon.

Materials and Methods

Yearling coho salmon used in this study were obtained from hatcheries located in the Columbia River basin. Plasma samples were taken from fish in freshwater at two-week intervals from March through June. Samples were stored frozen until they were assayed for thyroid hormone concentration. At times corresponding to the release dates at the various hatcheries, approximately 650 fish were collected from each hatchery and transported by tank truck to the National Marine Fisheries Service Aquaculture Research Station at Manchester, Washington. Upon arrival at. Manchester, the fish were placed in freshwater, weighed and measured, and then divided into two groups: one for destructive subsampling (350 fish) and the other for a six-month growth and survival study (300 fish). Fish designated for the survival study were further divided into two groups of 150 fish each; one group was vaccinated with a bivalent Vibrio vaccine, and the other was sham injected and fin clipped. Vaccination was accomplished by a 0.15 nil intraperitoneal (ip) injection of heat killed Vibrio anguillarum (Biotype I and Biotype II in a 3:1 ratio; Schiewe, et al., 1977) in a vehicle of 0.85 percent sodium chloride solution. At this point, all of the experimental fish were transferred to seawater net-pens (2.1 x 1.2 x 1.2 m nylon mesh with 5 mm aperture, Delta weave). At the time of transfer, the salinity of Clam Bay was 27 parts per thousand (ppt). During their six months in seawater, all fish were weighed to the nearest 0.1 g and measured to the nearest mm at approximately 30-day intervals from May through November. Mortalities were removed from the net-pens twice daily.

For the study comparing 0-age and yearlings, experimental fish were obtained as eggs from the Toutle River hatchery (Washington Department of Fisheries) in November of 1977. The eggs were transported to the National Marine Fisheries Service, Northwest and Alaska Fisheries Center in Seattle, and then divided into two test groups; one group was placed in an accelerated growth regime to enter seawater as 0-age animals (1978), while the other group was reared under normal hatchery conditions to enter seawater as yearling animals (1979). Acceleration of growth was accomplished by increasing the water temperature in 1°C/day increments, from 8 to 12°C at the swim-up stage and then maintaining the temperature at 12 to 13°C until transfer to seawater. The fish reared for transfer to seawater as yearlings were maintained on an ambient temperature regime which ranged from 6°C in the winter months to 20°C in late summer.

While in freshwater, the yearling group experienced epizootics of both myxobacterial disease (Myxobacteriurn sp.) in 1978 and furunculosis (Aeromonas salmonicida) in 1979. The outbreak of furunculosis occurred concurrently with the fifth serial seawater entry of the yearling fish. These disease outbreaks were treated with the antibiotic, chloramphenicol,' which was administered in the diet at 3 g/kg of food for a ten-day period.

Sampling to collect plasma from fish to freshwater was commenced in April 1978 for the 0-age fish and March 1979 for the yearling fish and continued at two-week intervals throughout the freshwater residence period. The 0-age fish were transferred to seawater net-pens on 15 May and then every two weeks thereafter throughout August 1978 (eight entries). The yearling fish were transferred to seawater net-pens at two-week intervals from 15 March through 15 June and then at one-month intervals through 15 September 1979 (nine entries).

For the blood sampling, fish were stunned by a blow to the head and the tail was severed. Blood was collected from the caudal vessels with a heparinized pipette. Blood was centrifuged to separate the plasma. Plasma samples were stored frozen until assayed. Plasma con-

centrations of thyroxine (T,) and triiodothyronine (T_3) were measured using specific radioimmunoassay methods (Uickhoff, et al., 1978). Antisera were purchased from Endocrine Sciences (Tarzanna, Calif.); 1 ^{125}-labeled hormones (specific activity: 750 to 1300 mCi/mmole) were obtained from New England Nuclear (Boston, Mass.). A 10 µl volume of plasma sample (unknown) or standard containing known concentrations of thyroid hormone diluted in hormone-free salmon plasma was dispensed into 12 x 75 mm glass test tubes. Standards were run in triplicate; samples were run in duplicate. To each of the assay tubes, 250µl of the following mixture was added: bovine y-globulin (Cohn fraction II, Sigma), 100 mg; 8-anilino-1-naphthalenesulfonic acid (sodium salt), 60 mg; 8 x 10° cpm radiolabeled hormone; 0.11 M barbital buffer (pH 9.0), 100 int; antiserum. The amount of antiserum added was adjusted to result in 50 percent radiolabeled hormone bound with no added unlabeled hormone. The standard curve ranged from 2.5ng/ml to 40 ng/ml of T. or T_3. Assay tubes were capped and incubated for two hours at 37° followed by 15 minutes at 4°. Antibody was then precipitated by addition of 0.3 ml of cold (4°) 20 percent (wlv) polyethylene glycol followed by thorough mixing. The precipitate was centrifuged at 2000 g for 15 minutes at 4°.The supernatant was decanted by inverting the tubes. The pellet was counted in a Micromedic gamma well counter for three minutes per tube.

The standard curve was constructed and unknowns were calculated by a log-logit plot and weighted regression analysis (Rodbard, 1974) using a Hewlett-Packard 9815A computer.

Results

A typical pattern of change in plasma concentrations of T_4 and T_3 of yearling coho salmon in freshwater is shown in Figure 1. Plasma levels of T. showed a distinct peak while the concentration of T_3 showed either a gradual increase or no change. The peak height and duration of elevated plasma T_4 concentrations were unique for each hatchery stock. However, the time of highest T. concentration was similar for all groups sampled in 1978.

The effect of transferring fish from freshwater to seawater is shown in Figure 2. Within one to four days of seawater residence, the plasma concentration of T_4 fell to a level equivalent to the lowest measured in freshwater. Plasma concentrations of T_4 in fish remaining in freshwater stayed elevated (Figure 2, dotted line). Thus, the cycle of plasma T. was shorter for those fish entering seawater.

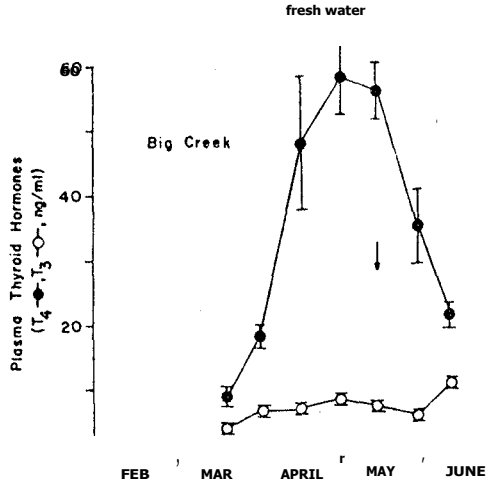

Figure 1. Plasma concentrations *of thyroxine (T_4) and* triiodothyronine (T,) *of yearling coho* salmon *during* smoltification in freshwater at Big Creek hatchery.

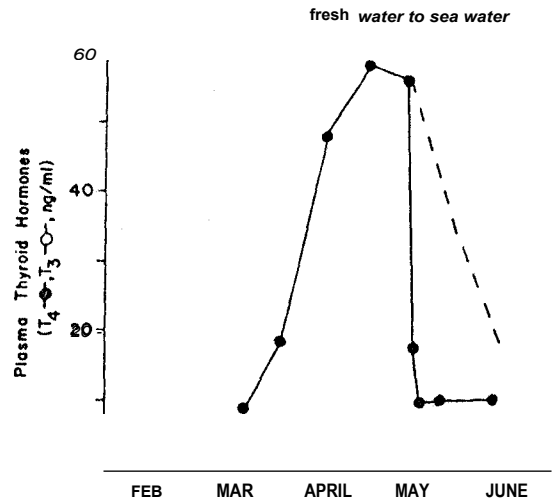

Figure 2. Effect of transferring coho salmon to seawater (arrow) on plasma T, and T, concentrations.

Various aspects of the cycles of T, in fish remaining in freshwater or entering sea water were compared with the survival of each hatchery stock of fish in seawater net-pens after six months. A significant relationship between survival and the proportion of the area under the T, peak at the time of seawater entry was found. This is explained in Figures 3 and 4. The proportion of the T. peak (percent T, peak) was determined by dividing the area under the T,peak for the group of fish entering seawater (area A, Figure 3) by the area under the T. peak for the group remaining in freshwater (area A x area B, Figure 3). A plot of the precent of the T, peak versus percent survival of fish after six months in seawater net-pens is shown in Figure 4. This analysis implies that a greater proportion of fish will survive in seawater net-pens if they are transferred to seawater near the completion of the freshwater cycle of plasma T, concentration.

The patterns of change in plasma thyroid hormone concentrations of 0-age and yearling coho salmon in freshwater are shown in Figure 5. There were peaks of plasma T, concentra-

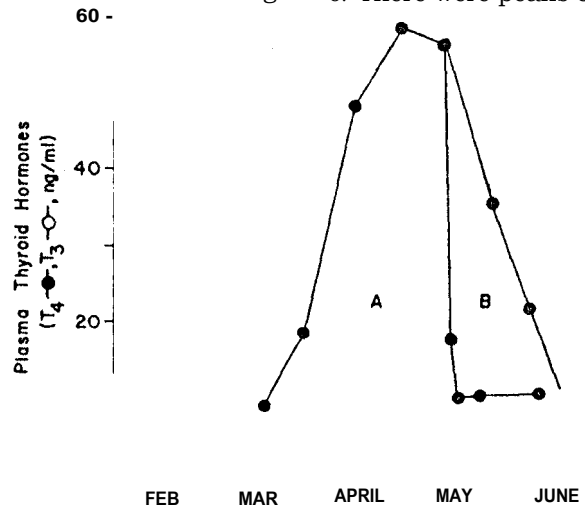

Figure 3. Analysis of patterns of change of plasma T, concentrations of yearling coho salmon remaining in freshwater and those transferred to seawater. The integrated area under the T, curve of fish entering seawater (A) was divided by the area under the curve for fish remaining in freshwater (A + B). This calculation resulted in a proportion of the 'I', curve (A/A + 13) designated "% T, **peak.**"

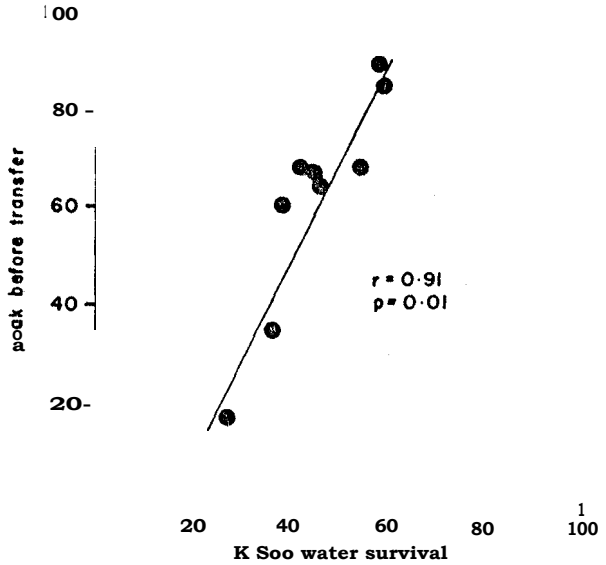

Figure 4. Relationship between percent peak and percent survival of yearling coho after 6 months of residence in seawater net-pens.

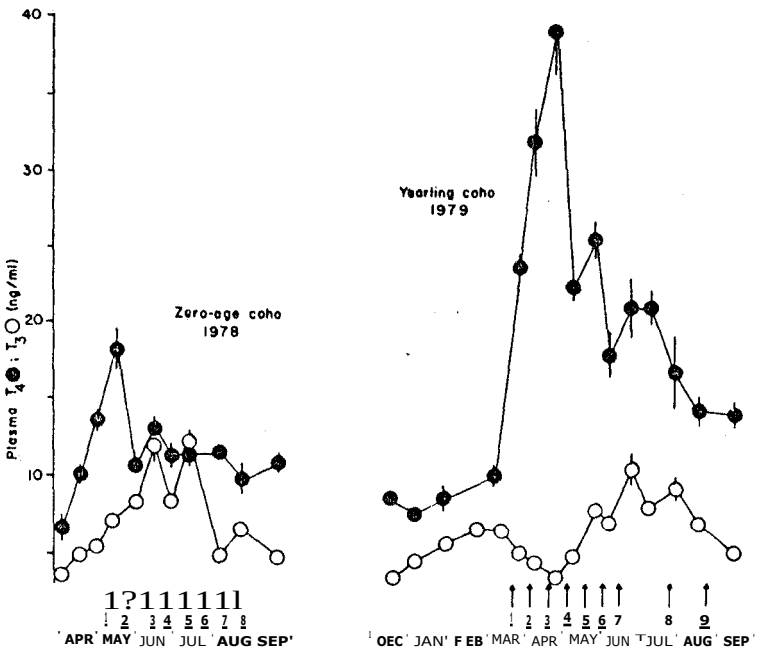

Figure 5. Change in plasma thyroid hormones of coho salmon during the first (1978) and second (1979) year of development in freshwater. Arrows indicate the time of transfer of groups of fish to seawater net-pens.

tion in both age groups. However, the yearlings had a T_4 peak which was greater in magnitude and occurred earlier in the spring. Plasma T, levels were elevated during early summer in both groups of fish. The percentages of surviving and smolted fish in seawater net-pens as of November of 1978 (0-age fish) and November of 1979 (yearling fish) are shown in Table 1. The percent survival, percent smolt, and percent surviving smolts of the groups entering seawater showed progressive increases during the time of the cycle of plasma T_4 in

Table 1. Comparison of seawater performance of groups of 0-age and yearling coho. Proportion of surviving and smolted fish was determined in November of the year of transfer.

Entry Group	Entry Data	% Survival (A)	% Smolt (B)	% Surviving Smolts (A x B)
		1978 Zero Age		
1	5/16	16	12	2
2	5/31	13	15	2
3	6/14	19	32	6
4	6/27	29	36	11
5	7/11	42	33	14
6	7/25	35	28	10
7	8/8	14	33	5
8	8/22	12	6	1
		1979 Yearling		
1	3/19	31	87	27
2	4/2	22	73	16
3	4/16	34	84	29
4	41:30	37	90	33
5	5/14	41	91	37
6	5/29	38	84	32
7	6/11	42	89	37
8	7/23	57	85	48
9	8/20	57	84	48

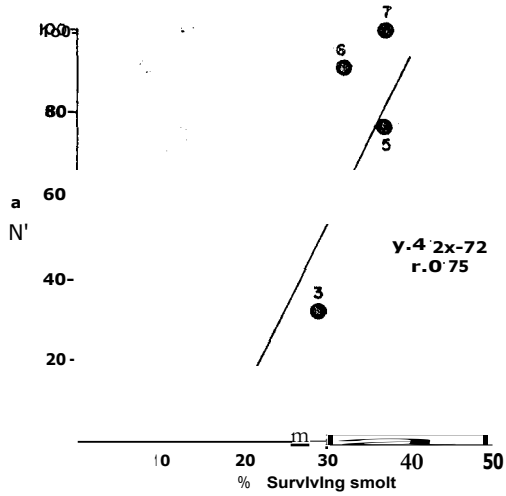

Figure 6. Relationship between percent peak and percent surviving smolt as of November 1979.

fish in freshwater (entry groups 2 through 5). An analysis of the percent of the T, peak (area under the curve) versus seawater performance of these groups of fish is shown in Figure 6. This relationship implies that fish entering seawater near the completion of the T, cycle in freshwater will show better long-term survival smolts of the 0-ago fish transferred to seawater showed progressive increases through the first five entry groups (Table 1). This increase corresponds to the time of elevated plasma T, levels in fish in freshwater (Figure 5). A comparison of the proportion of the area under the T_3 peak at the time of seawater entry and percent surviving smolts is shown in Figure 7. This relationship implies that 0-age fish transferred to seawater near the end of the time of elevated plasma T_3 in freshwater show maximal seawater performance.

Analysis of plasma thyroid hormone concentrations in yearling chinook salmon (On-

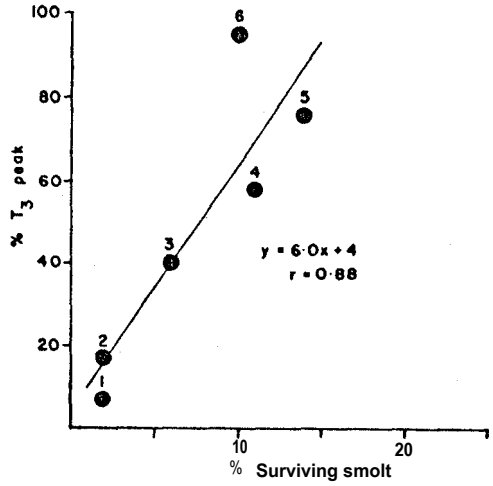

Figure 7. *Relationship between* percent T, *peak and* percent *surviving* smolt *as of* November *1979.*

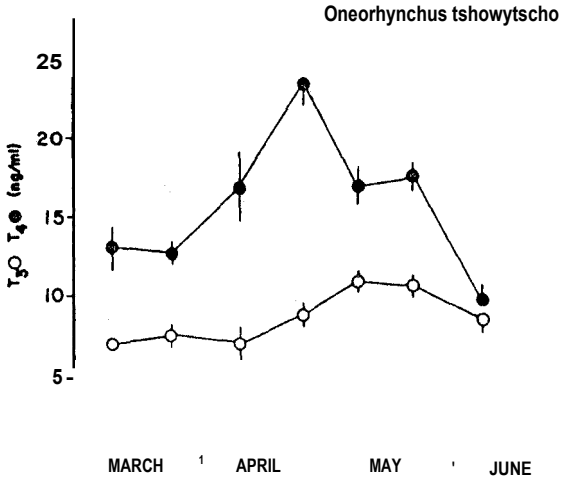

Figure 8. Pattern of change in plasma thyroid *hormone concentration of Chinook salmon during* smoltification *in* freshwater.

corhynchus tshawytscha) and anadromous steelhead trout (Saimo gairdneri) also showed seasonal cycles during smoltification (Figure 8 and 9). This opens the possibility that such analyses may be useful for understanding the biology of smoltification of other salmonid fishes.

Discussion

The cycle of thyroid hormones during smoltification of coho salmon is a consistent, quantifiable phenomenon.' Presumably, the elevated levels of T. and T, function to accelerate physiologic changes associated with the parr-smolt transformation. Premature transfer of fish from fresh- to seawater during the early stages of the thyroid hormone cycle causes a reduction in the plasma concentration of T,. The depressed plasma T_4 of fish in seawater

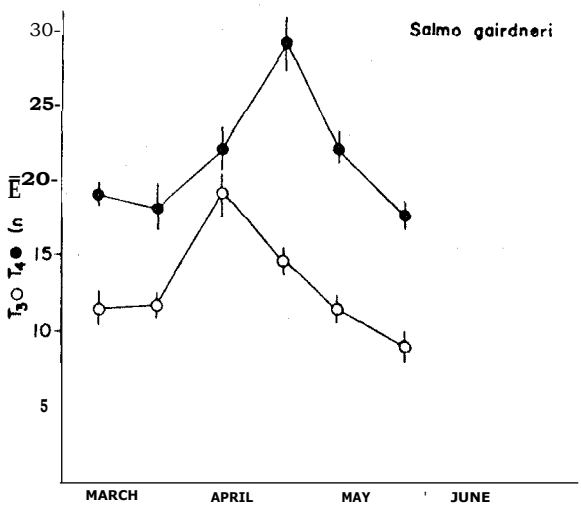

Figure 9. Pattern of change in plasma thyroid hormone concentration of steelhead trout during smoltification in freshwater.

may be due to lowered secretion of the hormone. Milne and Leatherland (1980) have recently presented evidence that the thyroid is less sensitive to thyroid stimulating hormone (TSH) in coho salmon in seawater. Furthermore, premature transfer of salmon to seawater causes stunting of growth associated with depressed thyroid. activity (Clarke and Nagahama, 1977). Thus, the accumulated evidence suggests that the cycle of thyroid hormones in fish in freshwater plays an important role in determining the readiness of fish for seawater entry.

The cycles of T_4 and T_3, during the spring and early summer of both 0-age and yearling coho indicate that these events are seasonal occurrences happening every year regardless of the age or size of the fish. The relationship between seawater survival and time of entry in relation to the peak in yearling fish reared at the Northwest and Alaska Fisheries Center is similar to that observed for yearlings from the Columbia River hatcheries. The correlation of survival and the T_3 cycle of 0-age fish suggests that the physiological changes preparatory for seawater entry in this group occur by a mechanism which is different than that of yearlings. It may be that 0-age fish require a longer time of exposure to thyroid hormone for sufficient development for entry into seawater. Accordingly, their seawater performance would be optimal during early summer after they had experienced elevations of both plasma T_4 and T_3 concentration. The subsequent springtime elevation of T_3 in yearlings might then be sufficient for smoltification of the greatest proportion of the population. The seawater survival of 0-age fish in this study was generally poor. This is similar to the findings of Harache, et al. (1980). The poor performance may have been due to genetic factors since the stock used in this study (Tulle) had not been selected for 0-age maturation and were smaller than 0-age fish used in some aquaculture programs.

These results suggest that analysis of plasma thyroid hormone concentrations in salmon in freshwater may be a useful indication of the extent of smoltification and readiness for seawater entry. Additional confirmatory studies are presently underway. At this time these findings may be applicable only for net-pen culture operations in which fish are transferred directly to seawater. Many of the fish released from Columbia River hatcheries require 15 to 30 days to migrate to the estuary. We do not know how migration of fish may affect their plasma concentration of thyroid hormones or whether this might influence seawater performance.

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