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Influence of Ration Level and Salinity on Circulating Thyroid Hormones in Juvenile Atlantic Salmon (*Salmo salar*)

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Following acute exposure to seawater (30 ppt), plasma thyroxine (T_4) of Atlantic salmon (*Salmo salar*) smolts increased 80% in the first 6 hr, declined to initial levels after 24 hr, and remained stable for 18 days thereafter. In nonsmolts, plasma T_4 did not rise immediately after exposure to seawater, fell slightly after 2 days, and remained low for 18 days. Plasma triiodothyronine (T_3) of smolts and nonsmolts was not affected by acute exposure to seawater. To examine the effect of long-term adaptation to ration and salinity, Atlantic salmon smolts were acclimated to three salinities (0, 10, and 30 ppt) and four ration levels (0, 0.2, 0.8, and 1.6% wet weight per day) for 6 weeks. Plasma T_4 increased with increasing ration level ($P < 0.001$) but was not significantly affected by salinity ($P = 0.4$). Plasma T_3 also increased with increasing ration ($P < 0.001$) and was more strongly correlated with ration level ($r = 0.85$) and growth rate ($r = 0.88$) than was plasma T_4 ($r = 0.73$ and 0.75, respectively). At low ration (0 and 0.2% per day), fish in 10 ppt had slightly but significantly lower plasma T_3 than fish in 0 ppt. There was no effect of salinity on plasma T_3 at the higher rations, nor did plasma T_3 levels differ significantly in fish in 0 and 30 ppt at any ration. The results indicate that ration level is a more important influence on circulating levels of plasma thyroid hormones than is salinity.

Circulating levels of thyroxine (T_4) increase during the parr–smolt transformation of anadromous salmonids, and exogenous thyroid hormone can induce several (but not all) aspects of the transformation, including silvering and salinity preference (for review see Dickhoff and Sullivan, 1987). Less clear is the role of thyroid hormones in osmoregulatory changes that accompany the parr–smolt transformation, and the process of seawater adaptation. McCormick *et al.* (1987) found that photoperiod manipulation of juvenile Atlantic salmon could alter the timing of increases in salinity tolerance and gill Na^+,K^+-ATPase activity, but did not alter the timing of increases in plasma (T_4). Several studies have indicated that seawater adaptation of coho salmon (*Oncorhynchus kisutch*) results in decreased thyroid activity (Clarke and Nagahama, 1977; Dickhoff *et al.*, 1982a), although more recent studies suggest that this depression is dependent on developmental stage in both coho and Atlantic salmon (Specker and Kobuke, 1987; Specker *et al.*, 1989).

It is clear from these studies that there is only a partial understanding of the influence of salinity on changes in plasma thyroid hormones in anadromous salmonids, and even less is known of the possible interaction of salinity with other environmental factors. Starvation, which is known to affect plasma T_4 and T_3 levels in freshwater rainbow trout (*O. mykiss;* see Leatherland, 1982), has not been examined previously in Atlantic salmon, nor has the effect of varying ration level been examined in any tele-

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ost. In the present study we examine the effect of acute exposure to seawater, and long-term adaptation to several salinities and feeding levels, on plasma concentrations of T_4 and T_3 in juvenile Atlantic salmon (*Salmo salar*).

MATERIALS AND METHODS

Atlantic salmon alevins of Saint John River stock were transported to St. Andrews Biological Station, New Brunswick, Canada, just after hatching in April and reared in 1-m^2, 400-liter capacity tanks supplied with fresh water at 12 liter · min^{-1}. Fish were fed with automatic feeders at a rate adjusted for changes in body weight and temperature. Overhead lighting to all tanks was by a single standard fluorescent bulbs (40 W) which provided light intensities at the water surface of 430–540 lx.

Exposure to seawater. All fish were initially exposed to simulated natural photoperiod which provided a seasonal change in daylength. On September 15 one group of fish was exposed to continuous light (24 h/d) and held under this regime for the remainder of the study (L24; nonsmolts), while the other group remained on simulated natural photoperiod (SNP; smolts). The former treatment has previously been shown to inhibit the increases in salinity tolerance and gill Na^+,K^+-ATPase activity which normally occur during the parr–smolt transformation (Saunders *et al.*, 1985; McCormick *et al.*, 1987). Water temperature fluctuated seasonally (6–18°) during early rearing (April through December) and was then kept constant (6–8°) from January through June (including the time of exposure to seawater). All fish were fed during the daylight hours of the SNP group.

In early May, SNP fish were judged to be smolts by virtue of increased gill Na^+,K^+-ATPase activity and hypoosmoregulatory ability (see McCormick *et al.*, 1989a). On May 13 the SNP and L24 tanks were partially drained and the incoming water was switched from fresh water to seawater. Within 2 hr each tank reached the salinity of the incoming seawater (30 ± 1 ppt). Five fish from each group were sampled just prior to, and after 6 hr, 1, 2, 4, 8, and 18 days following the change from fresh water to seawater. Fish were starved overnight and during the morning prior to sampling. After being stunned by a blow to the head, fish were measured for length and weight, and blood was collected from the caudal blood vessels with a heparinized syringe, centrifuged at 5000*g* for 2 min, and plasma stored at −20°.

Salinity and ration. Rearing conditions for this experiment have been described in detail (McCormick *et al.*, 1989c). Experimental tanks were 1-m^2, 400-liter capacity with a nylon mesh divider to obtain two ration groups per tank. Opaque blinds were placed around the tanks to minimize visual disturbance. Fish were fed through plastic tubes which deposited food at the water surface, which allowed us to ensure that all food presented was eaten. The low ration (0.2% per day) was fed every other day (0.4% per feeding) so as to reduce competition for the small amount of food; other groups were fed daily. Corey No. 4 dry pellet was used throughout the experiment.

On May 1, 144 smolts (23–70 g) previously reared under simulated natural photoperiod were randomly separated into 12 groups of 12 fish each. Eight of these were gradually acclimated to either 10 or 30 (±1) ppt, over a 2-week period. Ten ppt was achieved by mixing preheated or precooled 30 ppt and 0 ppt water in a 2:1 ratio in an insulated header tank. Salinity and temperature of rearing tanks were checked daily. Water was maintained at 13° (±0.2). Dissolved oxygen was 8.3–9.3 mg O_2/liter and was approximately 5 and 10% lower at 10 and 30 ppt, respectively, than at 0 ppt. This difference is less than the inherent differences in oxygen solubility due to increased salt concentration. With this exception, all other rearing conditions were identical among the salinity groups.

For each salinity (0, 10, and 30 ppt), four ration levels of 0.0 (starvation), 0.2, 0.8, and 1.6% (percentage wet weight per day) were begun on May 15. Fish were weighed every 2 weeks, and the ration was adjusted after each weighing. After the third 2-week interval, the experiment was terminated and blood was sampled from each fish as described above. Instantaneous growth rate (expressed as percentage change per day) was calculated as $((\ln W_{t2} - \ln W_{t1})/(t_2 - t_1)) \cdot 100$, where W = average weight of a group at a particular time interval (t_1 or the later t_2).

Radioimmunoassay. T_4 and T_3 concentrations were measured by radioimmunoassay (Dickhoff *et al.*, 1978; McCormick *et al.*, 1987). Duplicate 10 µl plasma samples were measured for each individual. Charcoal-stripped Atlantic salmon plasma was used to make all standards. The lower limit of detection of each radioimmunoassay was 0.1 ng/ml. Intra- and interassay coefficients of variation for these assays were 5–13% and 9–12%, respectively. Samples for each experiment were run in the same assay.

Statistics. The statistical significance of experimental treatments was tested with one- and two-way analysis of variance. If differences due to treatment were deemed significant ($P < 0.05$), individual treatments were compared using the Student–Newman–Keuls test (SNK; $P < 0.05$). All analyses were performed with the CRISP statistical program (CRUNCH, Berkeley, CA).

RESULTS

Exposure to Seawater

Initial levels of plasma thyroxine of

smolts (SNP) and nonsmolts (L24) in fresh water were similar (4.5 ± 0.7 and 3.6 ± 0.2, respectively). After 6 hr in seawater, plasma T_4 of smolts increased from 4.5 to 8.4 ng/ml ($P < 0.05$, SNK test; Fig. 1). These elevated values subsequently declined within 1 day and were not significantly different from initial levels through the 18 days of sampling following exposure to seawater. Plasma T_4 of nonsmolts did not increase following exposure to seawater, and significant declines were observed between 2 and 18 days (Fig. 1).

Initial levels of plasma triiodo-L-thyronine in smolts and nonsmolts were similar (1.6 ± 0.2 and 2.2 ± 0.4, respectively). There was no significant change from initial levels of plasma T_3 in either group in the 18 days following exposure to seawater ($P > 0.05$; Fig. 1).

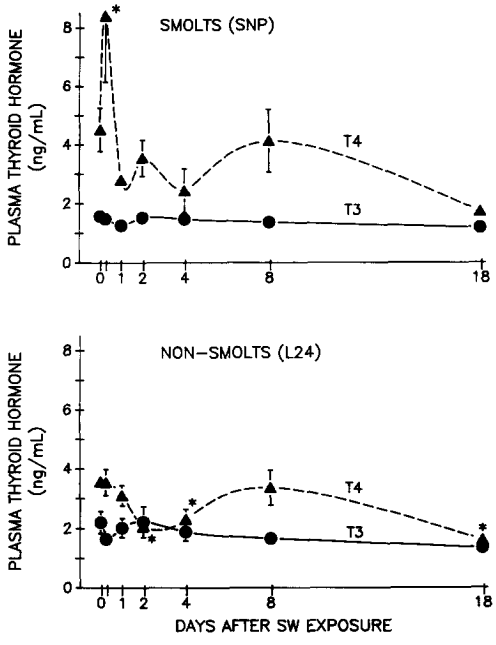

FIG. 1. Plasma thyroxine (T_4) and triiodo-L-thyronine (T_3) of Atlantic salmon exposed to simulated natural photoperiod (smolts; upper) or continuous light (nonsmolts; lower) and subsequently exposed to seawater. Values are means ± SE of five fish per group at each sampling date. Standard errors not shown are less than the value taken up by the symbol of their respective mean. An asterisk signifies a significant difference from the initial (Time 0) sampling period ($P < 0.05$, two-way ANOVA followed by Student–Newman–Keuls test).

Salinity and Ration Level

Atlantic salmon smolts were adapted to three salinities (0, 10, and 30 ppt) and four ration levels (0, 0.2, 0.8, and 1.6% wet weight per day) for 6 weeks. Plasma T_3 was significantly affected by both ration ($P < 0.001$) and salinity ($P = 0.008$, two-way ANOVA; Fig. 2), with a significant interaction between the two ($P = 0.04$). At each salinity, plasma T_3 increased with ration

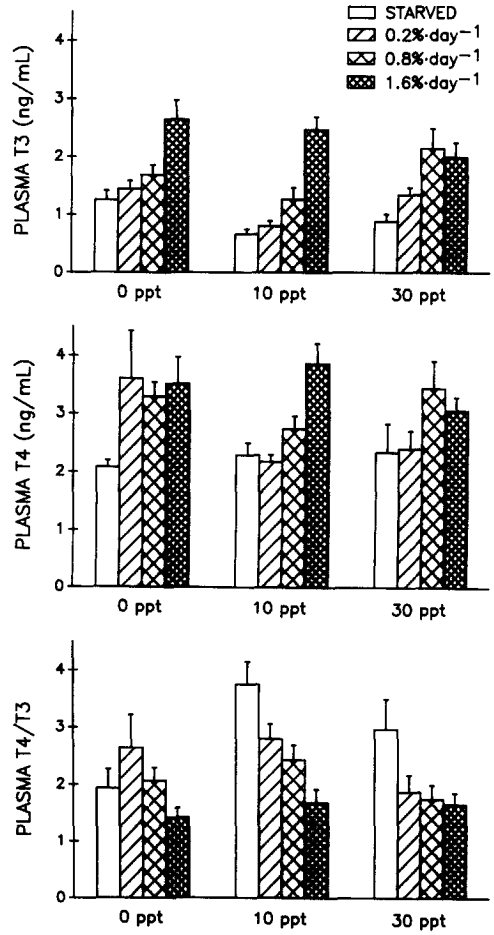

FIG. 2. Plasma triiodo-L-thyronine (T_3), thyroxine (T_4), and T_4/T_3 ratio of Atlantic salmon adapted to three salinities (0, 10, and 30 ppt) and four ration levels (0, 0.2, 0.8, and 1.6% body weight per day) for 6 weeks. Values are means ± SE of 10 to 12 fish per group.

(Fig. 2), although in 30 ppt plasma T_3 was similar at the 0.8 and 1.6% per day ration levels. There was a strong positive correlation between plasma T_3 and growth rate (r = 0.88; Fig. 3). The proportional increase in T_3 due to ration (percentage increase from 0 to 1.6% per day) was 110% in 0 ppt, 270% in 10 ppt, and 130% in 30 ppt. The statistical significance of salinity was due to a difference in plasma T_3 of fish in 10 ppt relative to those in 0 ppt at the lower two rations (0 and 0.2% per day, $P < 0.05$ SNK test). There was no effect of salinity on plasma T_3 at the higher rations, nor did plasma T_3 levels differ significantly in fish in 0 and 30 ppt at any ration.

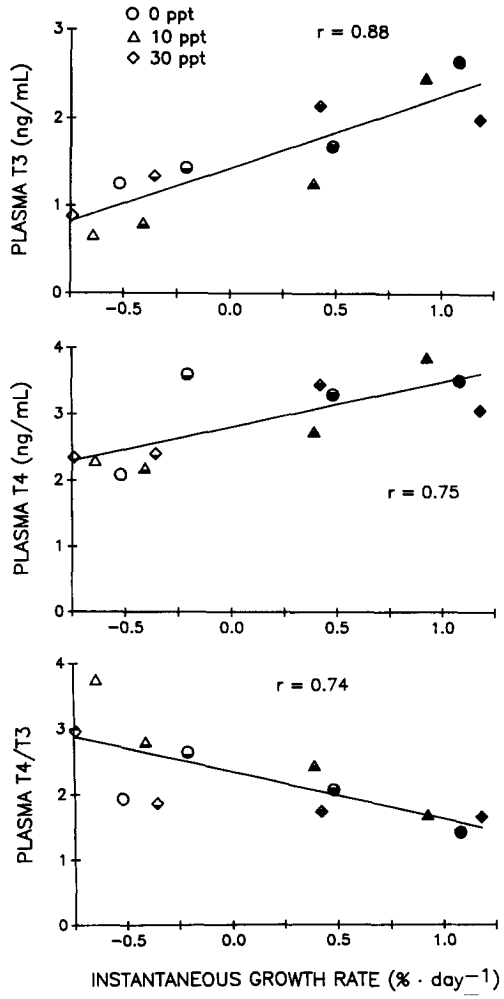

FIG. 3. Correlation of plasma triiodo-L-thyronine (T_3), thyroxine (T_4), and T_4/T_3 ratio with instantaneous growth rate of Atlantic salmon adapted to three salinities: 0, 10, and 30 ppt, and four ration levels: 0 (open symbols), 0.2% (one-third filled symbols), 0.8% (two-third filled symbols), and 1.6% (closed symbols) body weight per day for 6 weeks. Values are means of 10 to 12 fish per group.

Plasma T_4 was significantly affected by ration (P = 0.001), but not by salinity (P = 0.4). Plasma T_4 increased with increasing ration in each salinity, although the response at intermediate rations was variable (Fig. 2). The proportional increase in plasma T_4 due to ration (percentage increase from 0 to 1.6% per day) ranged between 30 and 70%, less than that exhibited by T_3. The positive correlation between growth rate and plasma T_4 (r = 0.75; Fig. 3) was also lower than that between growth rate and plasma T_3.

The ratio of plasma T_4 to T_3 (T_4/T_3) was significantly affected by ration ($P < 0.001$) and salinity (P = 0.015), with no statistically significant interaction between the two (P = 0.077). In general, plasma T_4/T_3 decreased with increasing ration, although this was less apparent in the 0 ppt group (Fig. 2). Plasma T_4/T_3 was negatively correlated with instantaneous growth rate (r = 0.74; Fig. 3). The statistical significance of salinity on plasma T_4/T_3 was primarily due to a significantly lower plasma T_4/T_3 in fish adapted to 0 ppt relative to those in 10 ppt at the lowest ration (0% per day; P = 0.05, SNK test).

DISCUSSION

Previous studies have demonstrated that exposure of juvenile Atlantic salmon to continuous light early in development and through the spring prevents the increases in salinity tolerance, hypoosmoregulatory ability, and gill Na^+,K^+-ATPase activity that normally accompany the parr–smolt transformation (Saunders *et al.*, 1985; McCormick *et al.*, 1987; McCormick *et al.*, 1989a, b). Because their size and growth are

similar to those of smolts, these nonsmolts serve as good controls for examining physiological differences that arise from the parr–smolt transformation. In the present study, plasma thyroxine of smolts increased markedly following 6 hr of exposure to seawater; such an increase did not occur in nonsmolts. Specker *et al.* (1989) found a similar increase in plasma T_4 after 8 hr of exposure to seawater in saline-injected smolts, but not in nonsmolts (exposed to continuous light) or parr (juvenile Atlantic salmon too small to undergo the parr–smolt transformation). Injection of bovine thyrotropin (TSH) had at least an additive affect on the ability of seawater to elevate plasma T_4 in smolts, but not in nonsmolts or parr. Specker and Schreck (1984) and Swanson and Dickhoff (1987) have previously reported that the efficacy of bovine TSH injections to increase plasma T_4 of coho salmon increases during the parr–smolt transformation. Seasonal changes in sensitivity to TSH have been found in several teleosts (see Brown, 1988). The difference in circulating plasma T_4 following seawater exposure of smolts and nonsmolts in the present study may be explained by a developmental, photoperiod-responsive increase in the sensitivity of the thyroid to trophic stimulation which may be induced by seawater.

Exposure of Atlantic salmon smolts and nonsmolts to seawater had no impact on plasma T_3 levels for the 18 days following initial exposure, nor was there a significant difference in plasma T_3 following long-term adaptation of smolts to 0 or 30 ppt (Fig. 2). Similarly, Specker *et al.* (1989) found little change in saline-injected Atlantic salmon smolts and nonsmolts following the first 48 hr of exposure to seawater. Young *et al.* (1989) report a 50% decline in plasma T_3 24 hr after exposure of coho salmon to seawater, which subsequently returns to initial levels within 96 hr. The results of the latter study may represent a difference between Atlantic and coho salmon, which show other variations in their endocrine response to seawater acclimation (Young *et al.*, 1989; Prunet *et al.*, 1989).

The transient nature of the change in plasma T_4 and the absence of changes in plasma T_3 following exposure to seawater do not allow us to conclude that *changes* in circulating thyroid hormones are an important part of the seawater adaptation process of juvenile Atlantic salmon. Specker *et al.* (1984) found little effect of short-term seawater exposure (1 hr) on T_4 kinetics of coho salmon, although these kinetics change substantially during the parr–smolt transformation. However, Specker *et al.* (1989) suggest that a seawater challenge and a resulting increase in T_4 provide a useful predictor of marine growth of Atlantic salmon.

The physiological significance of the transient increase in plasma T_4 following exposure to seawater remains unclear. Recent studies have found that exposure of salmonids to a novel water source results in increased plasma T_4 (Dickhoff *et al.*, 1982b), which may in turn be associated with olfaction, imprinting, and migration (Morin *et al.*, 1989). We are tempted to speculate that the transient increase in T_4 following exposure to seawater may play a role in imprinting and the development of homing; the entrance into seawater perhaps being a critical reference point for return migration.

Long-term adaptation to various ration levels had a consistent effect on thyroid hormones, particularly plasma T_3. In each salinity, plasma T_3 increased with increasing ration level (although at 30 ppt, plasma T_3 was the same at the two highest ration levels). Plasma T_4 also increased with increasing ration, but there was much greater variability in the response at intermediate rations. This resulted in a lower correlation of growth rate with plasma T_4 than with plasma T_3 ($r = 0.75$ and 0.88, respectively). Starvation results in decreased plasma T_4 and T_3 in several teleosts (see Leatherland, 1982); the magnitude of changes in plasma

T_4 and T_3 in response to starvation in the present study are similar to those reported for rainbow trout (Milne *et al.*, 1979; Flood and Eales, 1983).

To our knowledge, effects of intermediate rations (those between maximum and starvation rations) on plasma thyroid hormones have not been previously investigated. The near-linear response of plasma T_3 to increasing ration and the fact that differences in feeding rate can account for 80% of the variation in plasma T_3 underscores the strong relationship between thyroid hormones and metabolism in this species. Although long-term exogenous thyroid hormone treatment can result in significant increases in growth in Atlantic salmon (Saunders *et al.*, 1985), it is generally agreed that the primary effects of thyroid hormones are through its interaction with other hormones and its effects on intermediary metabolism (Leatherland, 1982).

The plasma T_4/T_3 ratio decreased with increasing ration level of Atlantic salmon, although these differences were least apparent in the 0 ppt group (Fig. 2). Flood and Eales (1983) report no significant effect of starvation on plasma T_4/T_3 in rainbow trout starved for up to 20 days, whereas Milne *et al.* (1979) found a decrease in plasma T_4/T_3 in rainbow trout starved for 45–60 days. Although these may represent species differences, our results are consistent with decreased hepatic deiodination found in starved rainbow trout (Shields and Eales, 1986). Clearly, an examination of changes in T_4 and T_3 kinetics will be necessary before we can fully understand at what levels (e.g., secretion, deiodination, and excretion) changes in ration level are influencing circulating thyroid hormones.

Long-term adaptation to various salinities had little effect on circulating thyroid hormones (Fig. 2); there was no significant difference between fish in 0 and 30 ppt; and differences between fish in 0 and 10 ppt occurred only at the two lowest ration levels. These findings are consistent with results from exposure to seawater experiments in which plasma T_3 was not affected and only transient changes were found in plasma T_4. We conclude that feeding rate is a more important determinant of circulating thyroid hormones of Atlantic salmon than is salinity.

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