# Freshwater Development and Smoltification in Coho Salmon from the Columbia River

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## ABSTRACT

**Changes in gill $Na^+$-$K^+$ ATPase activities and plasma concentrations of thyroxine ($T_4$), triiodothyronine ($T_3$), $Na^+$, $K^+$, and $Cl^-$ were monitored during the period of smoltification in 10 groups of yearling and 1 group of zero-age hatchery-reared coho salmon, *Oncorhynchus kisutch*.**

**During this period, dramatic increases in ATPase activity and plasma $T_4$ concentrations were observed in the yearling groups but not in the zero-age group. Plasma $T_3$ concentrations increased in some, but not all, of the groups. Plasma $T_4$ concentrations were significantly correlated with both gill $Na^+$-$K^+$ ATPase and plasma $T_3$ concentrations. Plasma concentrations of $Na^+$ and $Cl^-$ were found to be significantly related, but unrelated to the other measured parameters.**

**Serial entry experiments with the zero-age fish have suggested that developmental stage was more important than size or age in successful adaptation to seawater. In the yearling fish, there was no relationship between survival and the number of smolted fish, suggesting that smoltification and seawater adaptation are not interdependent events.**

**A component of the $T_4$ curve in freshwater fish was found to be significantly related to seawater survival. The usefulness of this comparison in predicting hatchery releases is discussed.**

## INTRODUCTION

Due to the declining number of natural salmon runs on the Columbia River, it has become increasingly necessary to supplement these populations with hatchery-reared fish. Releases from Columbia River hatcheries now average 110 million fish (2.5 million lb) annually (Wahle et al. 1975). The success of these supplemental releases, in terms of returns and contribution to the fishery, can vary from one hatchery location to another and at the same location from year to year. If oceanic conditions are considered to be relatively uniform, the differential return rates among hatcheries are most likely due to differences in the quality (ability of the fish to migrate successfully and to survive and grow in seawater) of the smolts produced at the hatchery. Variation in the quality of the fish is probably due to different environmental conditions at the various hatcheries or annual environmental changes at a particular hatchery. However, before we can evaluate environmental factors such as temperature, photoperiod, xenobiotic chemicals, feed, or effects of pathogenic organisms, it is essential that we have a thorough understanding of the normal physiological changes which occur during freshwater development, migration, and entry into seawater.

The purpose of this study was to establish patterns of some basic quantifiable physiological changes (i.e., gill sodium, potassium-activated adenosine triphosphatase ($Na^+$-$K^+$ ATPase) activities, plasma thyroxine ($T_4$), triiodothyronine ($T_3$), sodium ($Na^+$), potassium ($K^+$), and chloride ($Cl^-$) ion concentrations) which occur in coho salmon, *Oncorhynchus kisutch*, during the period of smoltification in freshwater. All of the physiological measurements accumulated during the course of this investigation were compared with the number of surviving and smolted fish through 6 mo of seawater residence to: 1) determine whether any statistically significant relationship existed and 2) if such relationships could be used as an index to predict optimal hatchery release dates. Additionally, comparative physiological profiles were established for fish on an accelerated growth regimen to enter seawater as zero-age animals and cohorts reared under normal hatchery conditions to enter seawater as yearling animals.

## MATERIALS AND METHODS

The yearling coho salmon used in the first part of this study were obtained from hatcheries on tributaries to the Columbia River: Klickitat, Kalama Falls, Rocky Reach, Toutle (Washington Department of Fisheries); Big Creek, Sandy (Oregon Department of Fish and Wildlife); and Willard (U.S. Fish and Wildlife Service).

Experimental fish used to determine developmental differences between zero-age and yearling coho salmon were obtained as eggs from the Toutle Hatchery. The eggs were transported to the National Marine Fisheries Service (NMFS) Northwest and Alaska Fisheries Center (NWAFC) in Seattle, Wash., and then divided into two test groups. One group was placed on an accelerated growth regimen to enter seawater as zero-age animals, and the other group was reared under normal hatchery conditions to enter seawater as yearlings. Acceleration of growth was accomplished by raising the water temperature in 1°C/d increments, from 8° to 12°C at the swim-up stage, and then maintaining the temperature at 12°-13°C until transfer to seawater.

Samples of gill filaments and plasma were collected from random samples of fish in the freshwater raceways at the indivdual Columbia River hatcheries, every 2 wk, from February through June 1978. In the developmental study at NWAFC, random samples of gill tissue and plasma were collected biweekly from April through August 1978.

Gill filaments were analyzed for $Na^+$-$K^+$ ATPase activity by the method of Zaugg (1979). Plasma $T_4$ and $T_3$ concentrations were determined by radioimmunoassay using the methods of Dickhoff et al. (1978). Plasma ions were measured with a flame photometer

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($Na^+$, $K^+$) and chloridometer ($Cl^-$). Samples for plasma ion determinations were prepared in accordance with the manufacturer's suggested methods for each instrument. Normal clinical control serum of known composition was prepared and analyzed in parallel with every 20 samples.

Data resulting from these experiments were subjected to computerized statistical analysis. The statistical methods used to calculate linear regressions, correlation coefficients, and analysis of variance (ANOVA) were those of Sokal and Rohlf (1969).

## RESULTS

Although there were some variations in the magnitude and duration of the responses, Figures 1 and 2 represent typical changes in gill $Na^+$-$K^+$ ATPase activities and plasma $T_4$ and $T_3$ concentrations which were observed for each of the Columbia River test groups. Values for both gill $Na^+$-$K^+$ ATPase and plasma $T_4$ measurements began to increase in March, peaked in April, and returned to starting levels by the end of May.

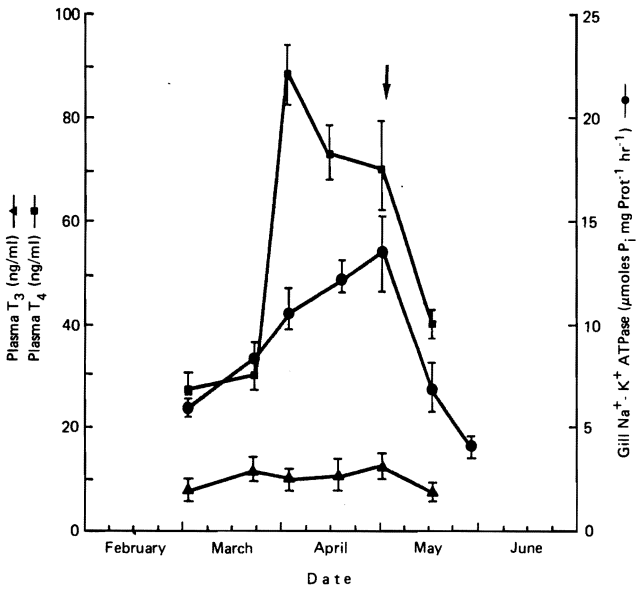


**Figure 1.—Changes in gill $Na^+ - K^+$ ATPase specific activities and plasma $T_3$ and $T_4$ concentrations for coho salmon in freshwater reared at the Sandy Hatchery (hatchery release date shown by arrow).**

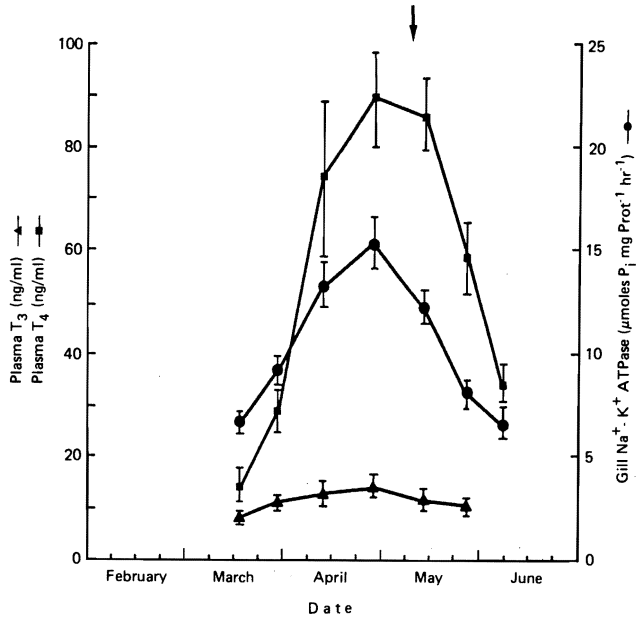


**Figure 2.—Changes in gill $Na^+ - K^+$ ATPase specific activities and plasma $T_4$ and $T_3$ concentrations for coho salmon in freshwater reared at the Big Creek Hatchery ($N = 10$). (Hatchery release date shown by arrow.)**

Typical patterns of plasma electrolyte ($Na^+$, $K^+$, $Cl^-$) fluctuations during the same period are shown in Figure 3. Sodium and chloride ions appear to be regulated by the same mechanism since their levels fluctuated together (Fig. 4, $P \leq 0.001$, $r = 0.76$, $N = 286$). However, potassium ions appeared to be independent of $Na^+$ and $Cl^-$ regulation. Although there was a slight trend for plasma $Na^+$ and $Cl^-$ to decrease with the increase in gill $Na^+$-$K^+$ ATPase activity during the same time period (Figs. 1, 2), the decrease was not significant and there was no statistical correlation between gill $Na^+$-$K^+$ ATPase activity and plasma $Na^+$ concentrations (Fig. 5, $r = 0.09$, $N = 428$).

This study was terminated on 1 November 1978. At that time, each vaccinated test group was evaluated to determine survival (Fig. 6) and the percentage of smolted animals among the survivors (Fig. 7). Smolted animals were determined by visual characteristics which included body and fin coloration as well as the presence or absence of parr marks. Survival ranged from 27% (Kalama Falls) to 80% (Sandy I), whereas the percentage of smolts ranged from 48% (Willard II) to 90% (Sandy II). The results of these final seawater evaluations were compared with the physiological measurements taken

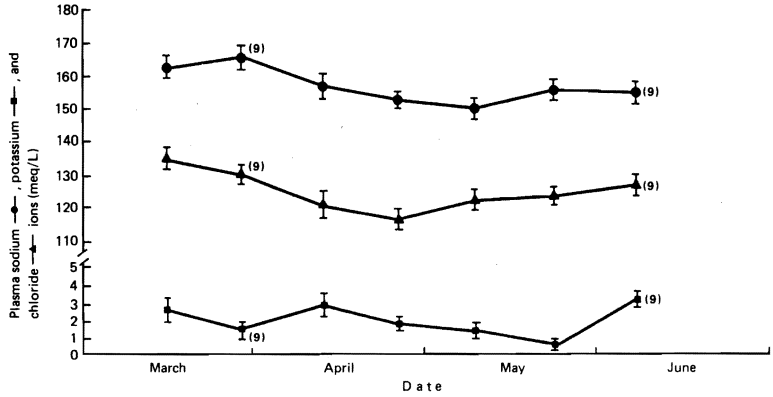


**Figure 3.—Plasma electrolyte ($Na^+$, $K^+$, $Cl^-$) levels from coho salmon in freshwater at the Big Creek Hatchery. ($N = 10$ except where parenthetically noted.)**

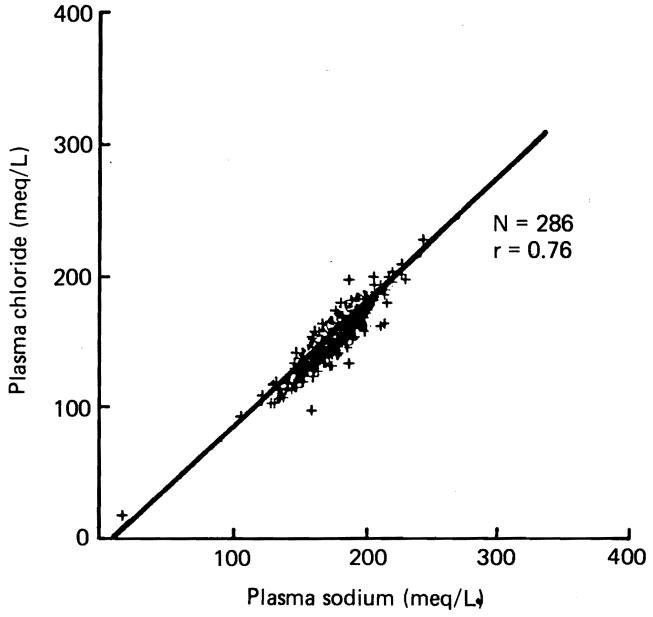


**Figure 4.—A linear regression analysis of the relationship between plasma sodium and plasma chloride in seawater for all of the Columbia River stocks tested.**

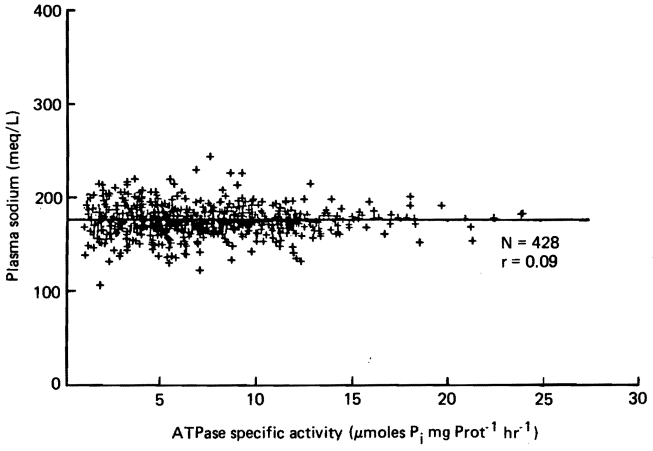


**Figure 5.—A linear regression analysis of the relationship between gill $Na^+ - K^+$ ATPase and plasma sodium concentrations in seawater for all of the Columbia River stocks tested.**

during the period of freshwater residence.

While the fish were in freshwater, gill $Na^+$-$K^+$ ATPase activities and plasma $T_4$ concentrations appeared cyclical and related in time. To evaluate this possible interrelationship statistically and compare each of the individual curves with other events occurring in both freshwater and seawater, it was necessary to separate the measurable components in each curve (Figs. 8, 9). Figure 8 shows a hypothetical representation of plasma $T_4$ concentrations in the coho salmon groups in freshwater, indicating the peak height ($A_1$), duration of the curve ($B_1$), and the integrated area beneath the curve ($C_1$). In Figure 9, $A_2$ represents the proportion of the duration of the peak value at release, $B_2$ represents the proportion of the duration of the peak at seawater transfer, and $C_2$ (shaded area) represents the proportion of the area beneath the curve which had transpired at the time of release. As shown in Figure 10, there was a significant relationship ($P \leq 0.001$, $r = 0.92$, $N = 10$) between the proportion of the area of the $T_4$ curve which had transpired at the time of release ($C_2/C_1$) (Figs. 8, 9) and the

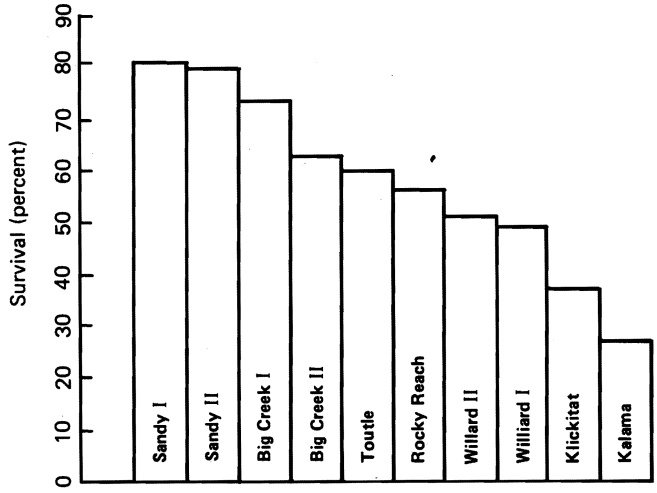


**Figure 6.—Survival levels for each of the Columbia River test stocks after 6 mo in seawater.**

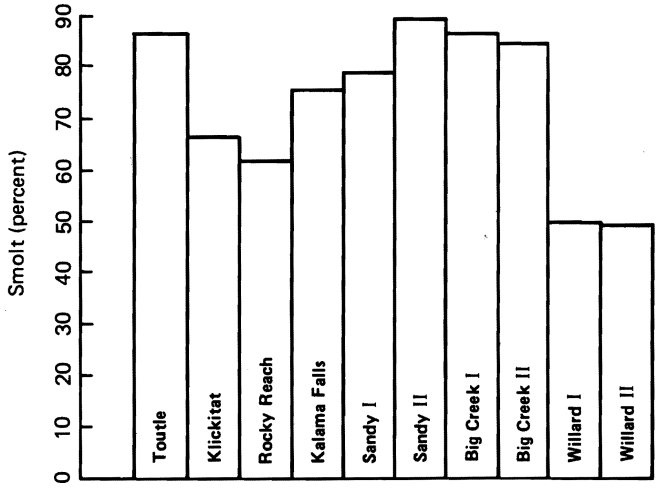


**Figure 7.—Success of smoltification for each of the Columbia River test stocks after 6 mo in seawater.**

percentage of the fish which survived until November in seawater (Fig. 6).

The significance of this correlation has been illustrated in the scheme presented in Figure 11. The numbers 1 to 5 indicate hypothetical successive seawater entry dates along a $T_4$ curve. As the linear regression in Figure 10 shows, the further along the curve (i.e., points 1 through 5, Fig. 11) that the fish were transferred from the hatchery to seawater, the better was their survival. This suggests that the complete cycle of change in $T_4$ must occur in freshwater in order to have maximal survival. There was no relationship between the components of the $T_4$ curve and the number of successful smolts. The number of smolted fish was found to be statistically unrelated to the number of surviving fish. The presence of nonsmolted, but surviving fish has been previously reported for coho salmon reared in net-pen culture systems (Bern 1978; Clarke and Nagahama 1977; Folmar and Dickhoff 1981; Mahnken et al. in press). Since seawater parrs are not observed under natural conditons, this relationship may only be valid for net-pen cultured coho salmon. The gill $Na^+$-$K^+$ ATPase curve was evaluated in a similar manner; however, under our test conditions there were no significant relationships between the components of that curve with either survival or number of successful

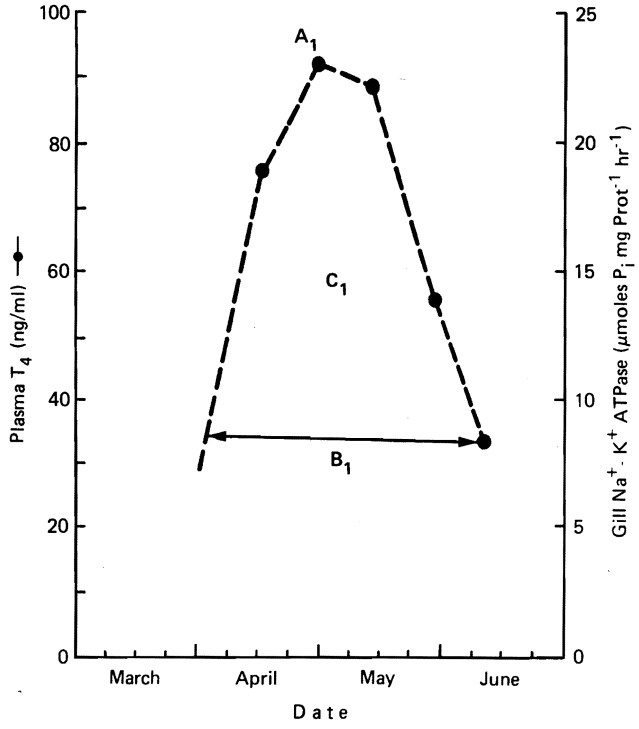


**Figure 8.—A representative graph of plasma $T_4$ concentrations from Columbia River coho salmon in freshwater. Letters indicate aspects (parameters) of the curve which can be measured for analysis.**

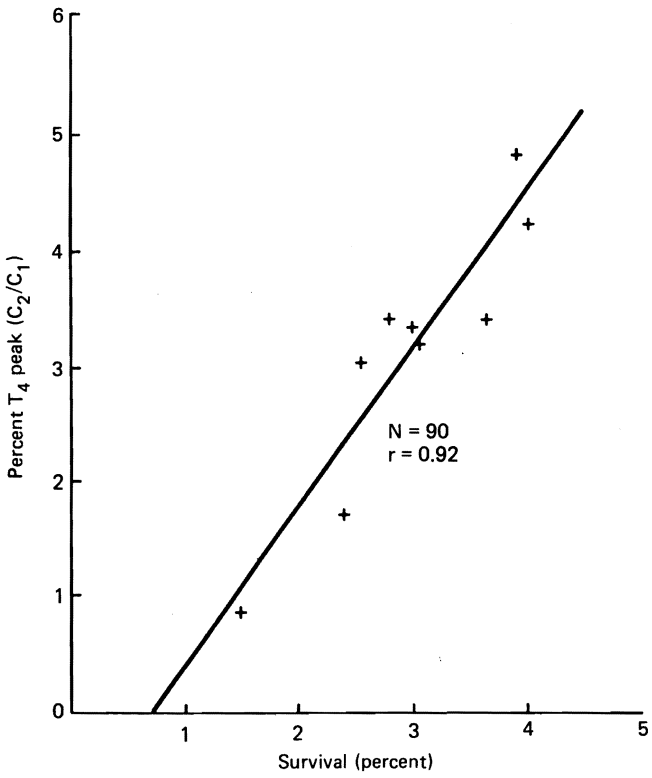


**Figure 10.—A linear regression analysis of the relationship between percent survival and the percent of the integrated area of the $T_4$ curve ($C_2/C_1$) which had transpired at the time of release for each of the 9 Columbia River test stocks.**

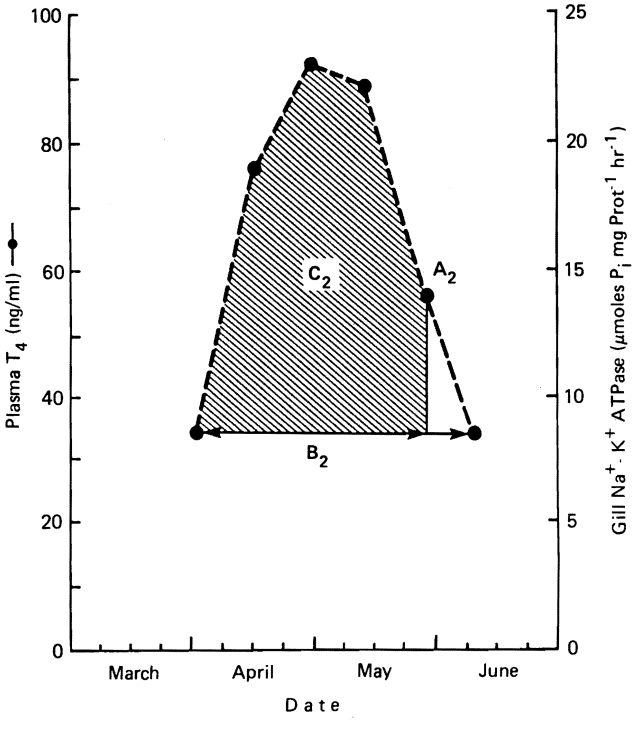


**Figure 9.—A representative graph of plasma $T_4$ concentrations from Columbia River coho salmon in freshwater. This graph shows the effect of the hatchery release date on the measurements shown in Figure 8.**

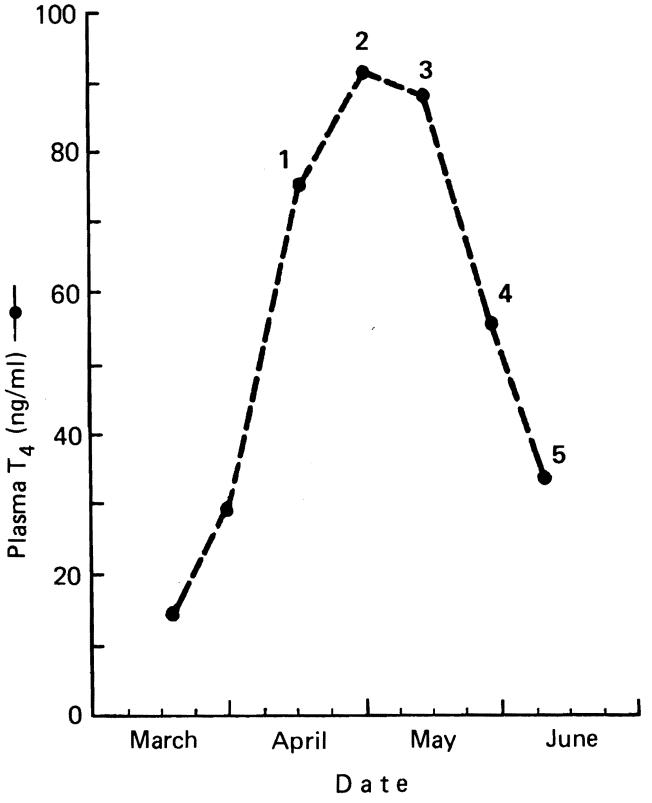


**Figure 11.—A representative graph of plasma $T_4$ concentrations in freshwater showing hypothetical serial release dates.**

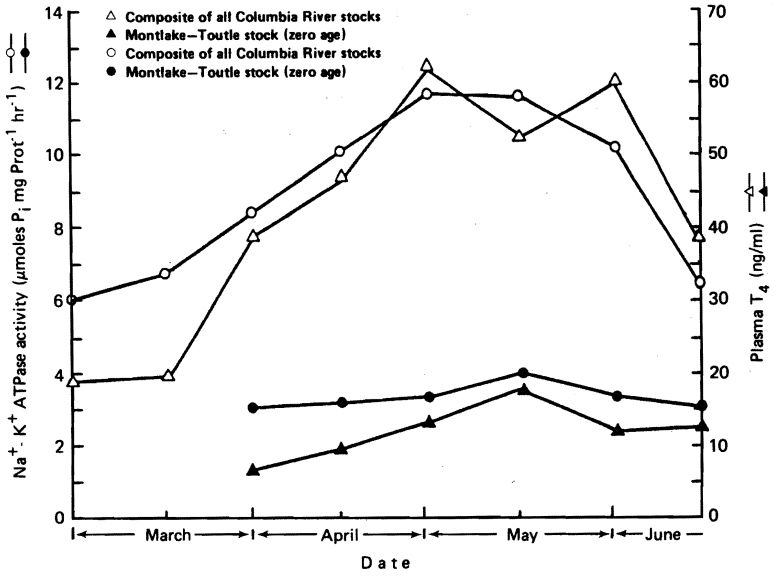


**Figure 12.—A comparison of gill $Na^+ - K^+$ ATPase and plasma $T_4$ concentrations in three groups of coho salmon (Montlake-Toutle 0-age, and a composite of 10 groups of Columbia River yearling fish).**

smolts. There were no other significant relationships between any of the other measurements ($T_3$, $Na^+$, $K^+$, $Cl^-$) obtained and the percentages of surviving or smolted fish.

Figure 12 represents a comparison of the developmental changes (gill $Na^+$-$K^+$ ATPase activities and plasma $T_4$ concentrations) observed for the zero-age fish versus a composite for all yearling fish from the Columbia River hatcheries. Peak values occurred during the first 2 wk of May in both groups; however, both the $Na^+$-$K^+$ ATPase and $T_4$ peaks were significantly lower in the zero-age fish than in yearling fish.

Figure 13 presents the percentage of surviving fish from each of the zero-age seawater serial entries compared with the seawater survival of the Toutle Hatchery yearling fish. The percentage of survival was based upon the number of fish remaining on 1 November 1978. The survival of the Toutle Hatchery yearling fish was greater than all of the zero-age groups, which ranged from 13 to 49%. Survival was greatest for those zero-age fish which entered seawater during July.

Figure 14 shows the percentage of smolted fish from each of the zero-age seawater serial entries compared with the percentage of smolts in yearling fish from the Toutle Hatchery. The percentage of smolts was based on the number of fish surviving on 1 November 1978. The percentage of smolted yearling fish from the Toutle Hatchery was much greater than any of the zero-age groups which ranged from 10 to 47%. The groups with the highest percentage of smolted fish were those which entered seawater from late June through early August.

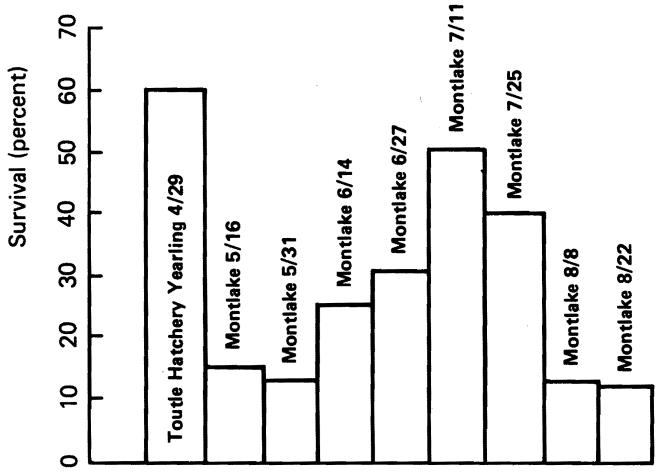


**Figure 13.—A comparison of survival rates between the serial releases of Montlake-Toutle 0-age fish, and Toutle Hatchery yearling fish. (Seawater entry dates are shown within individual bars.)**

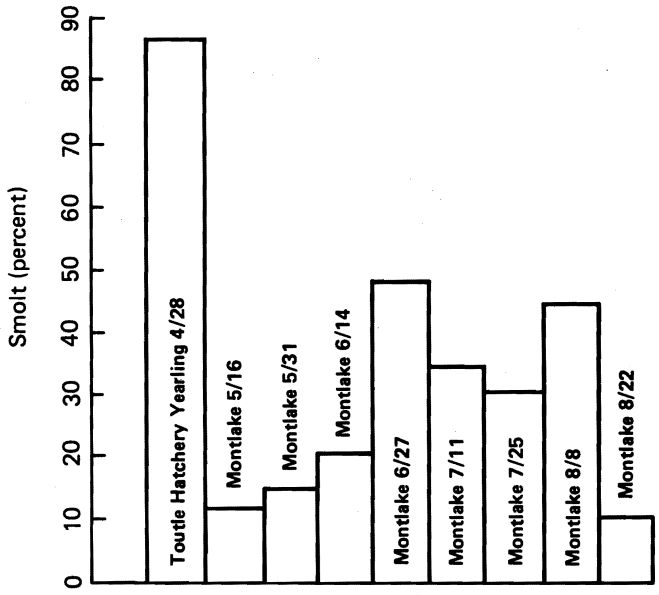


**Figure 14.—A comparison of smolt success between the serial releases of Montlake-Toutle 0-age fish, and Toutle Hatchery yearling fish. (Seawater entry dates are shown within individual bars.)**

## DISCUSSION

Representative time course changes in gill $Na^+$-$K^+$ ATPase activities and plasma $T_4$ concentrations for two groups of yearling coho salmon in freshwater were presented in Figures 1 and 2. The observed increases in gill $Na^+$-$K^+$ ATPase activity are comparable with those reported by Adams et al. (1973) and Zaugg and McLain (1972) for coho salmon and steelhead trout, *Salmo gairdneri*, in freshwater. Also, gradual increases in circulating $T_4$ levels have been reported in brook trout, *Salvelinus fontinalis*, during the spring (White and Henderson 1977); however, prior to this study there had been no previous reports of a surge in circulating $T_4$ levels associated with smoltification in anadromous salmonids (Dickhoff et al. 1978). Both plasma $T_4$ and $T_3$ were found to be significantly related ($P<0.01$, $r = 0.12$, $N = 483$). In both Figures 1 and 2, the peaks of enzyme activities and plasma $T_4$ concentrations occurred withing 2 wk of one another. This relationship was observed in all 10 groups of fish tested and was found to be statistically significant by correlation ($P<0.01$, $r = 0.17$, $N = 534$). Plasma $T_3$ concentrations were also found to be statistically correlated with gill $Na^+$-$K^+$ ATPase activity ($P<0.01$, $r=0.29$, $N=523$). There are several possible explanations for this relationship. Thyroid hormone has been reported to regulate $Na^+$-$K^+$ ATPase activity in a number of mammalian tissues (Ismail-Beigi and Edelman 1970, 1971, 1974; Valcana and Timiras 1969), amphibian epidermis (Kawada et al. 1969), and in nurse shark gill and kidney tissue (Honn and Chavin 1977). Also, there may be involvment of other hormones acting synergistically with thyroid hormone. Production of prolactin and cortisol is regulated by environmental salinity, and both have been demonstrated to affect kidney $Na^+$-$K^+$ ATPase. Prolactin stimulates kidney $Na^+$-$K^+$ ATPase in freshwater, while cortisol stimulates gill $Na^+$-$K^+$ ATPase activity in seawater (Epstein et al. 1971; Pickford et al. 1970). It is also possible that the simultaneous increase in gill $Na^+$-$K^+$ ATPase activities and plasma $T_4$ concentrations are independent but responding to the same stimulus, or that they are not causally related. The observed changes in gill $Na^+$-$K^+$ ATPase activities and plasma $T_4$ concentrations at different hatchery locations appeared unique in both timing and pattern for all the groups tested. This uniqueness may be attributable to the genetic strain, environmental conditons, diet, or a combination of these factors.

Figure 3 represents the changes in three plasma monovalent ions during the freshwater development of the coho salmon test groups. The strong correlation between $Na^+$ and $Cl^-$ concentrations indicates that they may be regulated by the same mechanism. The slight depression in both $Na^+$ and $Cl^-$ concentrations corresponds with the time period of gill $Na^+$-$K^+$ ATPase activity increase shown in Figures 1 and 2; however, this relationship was not statistically significant. These data suggest that monovalent plasma electrolyte levels may not be directly regulated by gill $Na^+$-$K^+$ ATPase, but rather by a complex series of physiological events which may include gill $Na^+$-$K^+$ ATPase as a component. This evidence does not support the theory that the migration and increased salinity preference are due to changes in plasma ion balance in freshwater.

There were no correlations between gill $Na^+$-$K^+$ ATPase activities and plasma $T_4$ and $T_3$ concentrations during the seawater acclimation phase, however, as in freshwater, plasma $T_4$ and $T_3$ concentrations were significantly related. The fact that gill $Na^+$-$K^+$ ATPase activities and plasma $T_4$ and $T_3$ concentrations were significantly correlated in freshwater but not in seawater indicates that the hormone and enzyme relationship may be a freshwater developmental phenomenon which serves to prepare the fish for seawater entry rather than $T_4$ acting as a regulatory mechanism of $Na^+$-$K^+$ ATPase during seawater acclimation.

In addition to obtaining basic information relative to the parr-smolt transformation, we were interested in any statistically significant relationships between these basic physiological measurements and survival (Fig. 4) or smolt success (Fig. 5). Figures 6 and 7 demonstrated the method by which the freshwater curves for gill $Na^+$-$K^+$ ATPase and plasma $T_4$ were evaluated. The curves were analyzed for each of the test groups to determine maximum value, duration of the peak, integrated area beneath the curve, and the proportion of each of those values which existed at the time of release. These values were then compared with the percentage of survival and smolts of each group after 6 mo of seawater residence. As shown in Figure 8, there was a significant relationship between the proportion of the area beneath the $T_4$ curves and the percentage of the fish which survived 6 mo in seawater (Fig. 4). This relationship suggested that the survival of our test fish was related to their respective date of transfer to seawater, and that the further along the curve they were transferred, the greater was their survival. This is depicted graphically in Figure 9, where fish released at point number 5 should have greater survival potential than those fish released at points 1 through 4. There was no relationship between the $T_4$ peaks in freshwater fish and the percentage of smolts in the population. There were no relationships between any of the $T_4$ measurements taken in seawater and survival or smolt success. There was no relationship between the $T_4$ peaks in freshwater fish and the percentage of smolts in the population. There were no relationships between any of the $T_4$ measurements taken in seawater and survival or smolt success. Plasma levels of $T_4$ and $T_3$ were significantly related in both freshwater and seawater. At the present time, we cannot report whether this relationship is due to simultaneous hormone synthesis and release, or a uniform conversion rate of $T_4$ to $T_3$ in peripheral tissue.

Treatment of the gill $Na^+$-$K^+$ ATPase data in a similar manner showed no statistical relationship with either survival or smolt success after 6 mo of seawater residence. Survival and smolt success were also unrelated to any gill $Na^+$-$K^+$ ATPase measurements taken in seawater. Our results suggested that gill $Na^+$-$K^+$ ATPase is important as an integrated component of smoltification, but as a single discrete measurement did not predict the potential success of seawater acclimation in net-pen culture of coho salmon.

There were no statistically significant relationships between any of the plasma electrolyte measurements and 6-mo seawater survival or smolt success. The maintenance of a proper balance of body fluid electrolytes is essential to seawater survival; however, as with gill $Na^+$-$K^+$ ATPase, this is a process with many components and discrete measurements of $Na^+$, $K^+$, or $Cl^-$ are not adequate to predict seawater success.

The use of warmed water (12°-13°C) to accelerate the growth of zero-age fish to yearling smolt length as 6-mo-old fish was quite successful. However, a comparison of the time course changes in gill $Na^+$-$K^+$ ATPase activities and plasma $T_4$ concentrations for the zero-age fish and the composite of all yearling Columbia River groups (Fig. 10) suggested that the length of the fish was not necessarily a reflection of successful development. Although all of the fish were approximately the same length at seawater entry, the zero-age fish had markedly lower gill $Na^+$-$K^+$ ATPase activities and plasma $T_4$ concentrations during their freshwater development than did yearling fish. If the occurrence of a distinct thyroid hormone surge, as seen in yearling fish, is a requirement for successful seawater adaptation, this may partially explain the poor performance of the zero-age fish in terms of survival (Fig. 11) and smolt success (Fig. 12).

The relationship between gill $Na^+$-$K^+$ ATPase and plasma $T_4$ in the zero-age fish was similar to the yearling fish in that there was a

**Table 1.—A comparison of gill $Na^+ - K^+$ ATPase activities and plasma $T_4$ concentrations in 0-age and yearling coho salmon after 8 d in seawater. All values expressed as $\bar{X} \pm SE$.**

| | 0-age | | Yearling (ranges for 10 Columbia River stocks) | |
|---|---|---|---|---|
| Seawater entry date (1978) | Gill $Na^+ - K^+$ ATPase ($\mu$moles $P_i$/mg protein per h) | Plasma $T_4$ (ng/ml) | Gill $Na^+ - K^+$ ATPase ($\mu$moles $P_i$/mg protein per h) | Plasma $T_4$ (ng/ml) |
| 5/16 | 8.9±2.3 | 4.1±0.2 | 14.6±1.7-27.2±5.4 | 12.5±2.2-38.2±2.7 |
| 5/31 | 14.0±2.2 | 13.5±1.1 | | |
| 6/14 | 14.3±1.1 | 23.0±2.5 | | |
| 6/27 | 9.1±0.9 | 15.2±1.9 | | |
| 7/11 | 14.1±1.6 | 23.9±1.7 | | |
| 7/25 | 20.1±3.8 | 19.9±1.7 | | |
| 8/08 | 13.8±2.5 | 6.6±1.0 | | |
| 8/22 | no data | no data | | |

significant correlation in freshwater but not in seawater. Although gill $Na^+$-$K^+$ ATPase activities were lower in the zero-age fish than in the yearling fish in freshwater, five of the seven zero-age serial seawater entries had gill $Na^+$-$K^+$ ATPase activities comparable with those of yearling fish after the period of seawater acclimation (Table 1). Likewise, five of the zero-age serial seawater entries had plasma $T_4$ concentrations comparable with those of the yearling fish after seawater acclimation. These results suggest that there are qualitative differences between the increases in gill $Na^+$-$K^+$ ATPase activities and plasma $T_4$ concentrations of fish in freshwater and those observed in seawater. The increased enzyme activities and hormonal concentrations in seawater adapted fish appear to be a reflection of the marine environment, and it is possible to induce these changes in fish prematurely transferred to seawater. Our measurements in seawater acclimated fish indicated that the zero-age fish were as well developed as normal yearling fish; however, our measurements in freshwater acclimated fish clearly indicated that the zero-age fish were not as well developed as the yearling fish. Although we cannot make a valid comparison without the data from the yearling cohorts of the zero-age fish, we have tentatively concluded that the poor seawater survival and the high incidence of seawater parr in the zero-age group were related to incomplete development in freshwater, rather than the inability of the fish to adapt to seawater.

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