Aquaculture, 28 (1982) 143—151
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

SOME CHANGES IN SMOLTIFICATION AND SEAWATER ADAPTABILITY OF SALMONIDS RESULTING FROM ENVIRONMENTAL AND OTHER FACTORS

W.S. ZAUGG

National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Coastal Zone and Estuarine Studies Division, Northwest and Alaska Fisheries Center, 2725 Montlake Boulevard East, Seattle, WA 98112 (U.S.A.)

Present address: National Marine Fisheries Service, Cook Field Station, Cook, WA 98605 (U.S.A.)

(Accepted 15 January 1982)

ABSTRACT

Zaugg, W.S., 1982. Some changes in smoltification and seawater adaptability of salmonids resulting from environmental and other factors. *Aquaculture*, 28: 143—151.

Gill Na^+-K^+ ATPase activities varied from year to year in hatchery-reared fall chinook salmon, suggesting changes in annual patterns of smoltification. Production fall chinook salmon fed diets containing added NaCl for 6 weeks prior to release contributed 49 to 64% more adults than controls. Coho salmon held at a hatchery for delayed releases (June and July) migrated seaward more rapidly and in greater numbers than fish released in May. Although coho in the delayed releases had experienced decreases in gill Na^+-K^+ ATPase activity, they were capable of rapidly regenerating elevated activity when released into the river. Concentrations of blood nucleoside triphosphates increase during smoltification of coho and chinook salmon, and steelhead trout.

INTRODUCTION

Biologists and physiologists have studied the phenomenon of parr—smolt transformation, or smoltification, and seawater adaptation in anadromous salmonids for many years. However, in the last couple of decades attempts to acquire a greater understanding of the physiological changes that occur during these events have greatly intensified. It is now recognized that much of the success of salmonid seawater rearing, sea ranching, freshwater restoration, and enhancement programs depends upon the production of high quality smolts and that environmental factors have important effects on the process of smoltification. In order to consistently produce smolts of highest quality we must eliminate those factors that have detrimental effects while taking advantage of those that enhance the ability of salmonids to develop and maintain a well-smolted condition, to migrate seaward successfully, and to fully adapt to an ocean environment. Wedemeyer et al. (1980) have re-

viewed many of the recent publications concerning this subject, and other recent reviews are also pertinent (Hoar, 1976; Folmar and Dickhoff, 1980).

This communication discusses changes in smoltification and seawater adaptation of chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon resulting from hatchery rearing conditions, dietary salt, temperature, liberation from hatchery environment, and altered photoperiod schedules. Indicators of smolt transformation used in this study were gill Na^+-K^+ ATPase activity and blood nucleoside triphosphate (NTP) concentrations.

METHODS

Gill Na^+-K^+ ATPase activities were determined using samples of gill filaments from either individual fish or combined from groups of three or more. Partially purified enzyme suspensions were prepared and analyses conducted as described elsewhere (Zaugg, 1982). Activities are presented as μmoles ATP hydrolyzed·mg protein^{-1}·h^{-1}.

Fall chinook salmon were netted from production ponds at the Spring Creek National Fish Hatchery at periodic intervals during 1978—1980. Thirty fish in each sampling were separated into ten groups of three fish each from which approximately equal amounts of gill filaments were removed for ATPase assay. ATPase assays in 1981 were conducted on individual fish (20 fish at each sampling). Seawater survivals were determined by transporting representative populations to the Manchester Fisheries Research Laboratory (Washington) where 300 fish were placed into seawater net pens in Clam Bay. Only mortalities occurring up to day 10 in seawater were counted as resulting from osmoregulatory dysfunction.

On each sampling date at the Washington State Washougal Hatchery 30 fish were netted from each production pond being tested. Gill Na^+-K^+ ATPase activities were determined on filaments in pooled samples from three fish. Coded wire tagged migrants were captured at Jones Beach (Oregon) by purse or beach seine and used individually for gill ATPase determinations.

Two groups of approximately 250 yearling coho salmon were taken from a production raceway at the Willard National Fish Hatchery (Washington) in January 1981 and placed in separate 1.5 m circular concrete tanks. One group was placed on a 3-month advanced photoperiod schedule similar to one used previously (Zaugg, 1981) using one 150-watt flood lamp to extend hours of light into the evening. The light was first turned on on 21 January, and the advanced light schedule was terminated on 23 April. On each sampling date gill ATPase activities and blood nucleoside triphosphate (NTP) concentrations were determined on ten individual fish. Blood NTP was determined enzymatically using the assay described by Sigma Technical Bulletin No. 366-UV[1]. The following volumes of reagents were used: 50 μl blood in 0.3 ml 7.2% TCA; 0.5 ml PGA, 0.7 ml H_2O, 0.2 ml TCA supernatant liquid, 0.1 ml NADH (0.15 mg/ml), and 20 μl GAPD/PGK.

[1] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

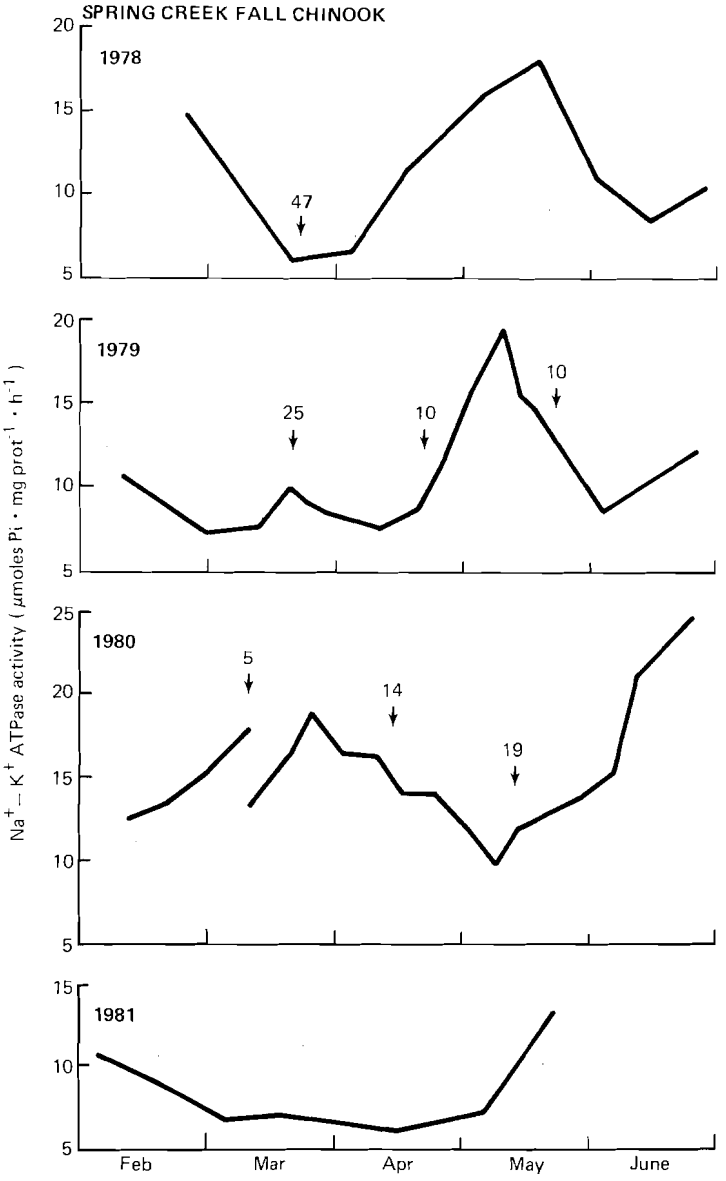

Fig. 1. Gill Na^+-K^+ ATPase activities and seawater mortality in sub-yearling fall chinook salmon.

Gill Na^+-K^+ ATPase activities of fall chinook salmon from production ponds at the Spring Creek NFH were monitored from 1978—1981. At certain indicated release times (arrows) representative samples (300) of the population were tested in seawater (28‰) and percent mortality after 10 days is indicated. Mean fork lengths (mm) at seawater entry were: 73 (22 March 1978), 69 (22 March 1979), 77 (21 April 1979), 91 (22 May 1979), 71 (12 March 1980), 80 (14 April 1980), 90 (13 May 1980). Difference in ATPase activities for 12 March 1980 (at break in curve) resulted from feeding supplemental salt (7%) for 4 weeks to the group released on this date (higher activity). Those not receiving salt (lower activity) remained at the hatchery for later releases.

RESULTS

Changes in gill Na^+-K^+ ATPase activity in coho salmon and steelhead trout (*Salmo gairdneri*) follow a rather consistent pattern from year to year, but in sub-yearling fall chinook salmon this activity may vary considerably (Fig. 1). Profiles of the enzyme activity in production fall chinook salmon at Spring Creek NFH were qualitatively similar in 1978 and 1979, but much different in 1980. Fish released in March, 1980 (Fig. 1, arrow), had higher gill Na^+-K^+ ATPase activities than those scheduled for later releases (lower activity on same date). This increase resulted from feeding a diet containing added NaCl (7% dry weight) for 4 weeks prior to release. Such salt-induced increases in activity have been observed in other studies and generally reach maximum levels after 4—6 weeks on diet. Fish not receiving the salt diet continued to show increasing levels of activity until 25 March. In an earlier experiment salt added to diets of fall chinook salmon at Spring Creek NFH in 1976 resulted in increased ocean survival as reflected in adult returns (Table I).

TABLE I

Salt feeding and adult returns of chinook salmon[a]

Diet	Number released	Total tags recovered[b]	Percent increase
Control	102503	293	—
NaCl—KCl	101080	436	49
NaCl	94137	480	64

[a]Controls were fed Abernathy Dry pellets, NaCl—KCl contained added 5% NaCl, 2% KCl (dry weight), and 7% NaCl was added to NaCl diet. Salt diets were fed for 6 weeks prior to release.
[b]Ocean catch and hatchery returns adjusted for differences in numbers released.

Mortality of fall chinook salmon transferred directly to seawater was high on 22 March 1978 (low gill Na^+-K^+ ATPase activity). Of the three seawater transfers made in 1979, highest mortality was again evident in March (Fig. 1) with lower mortalities in April and May, as would be expected with increasing size and/or higher gill Na^+-K^+ ATPase activity. Seawater survival tests in 1980, however, showed a trend opposite to that of the previous year. Mortality was lowest in March (high Na^+-K^+ ATPase activity and salt feeding) and greatest in May (low Na^+-K^+ ATPase activity) even though fish were larger.

Coho salmon were released in May, June and July 1979, from the Washougal Hatchery (Washington Department of Fisheries). Gill Na^+-K^+ ATPase activities of these three groups are shown in Fig. 2. Numbers of migrants captured at Jones Beach (Oregon), 78 km from the mouth of the Columbia River, are also shown in Fig. 2, along with gill Na^+-K^+ ATPase activities of individual migrants captured at Jones Beach. Although characterized by lower ATPase

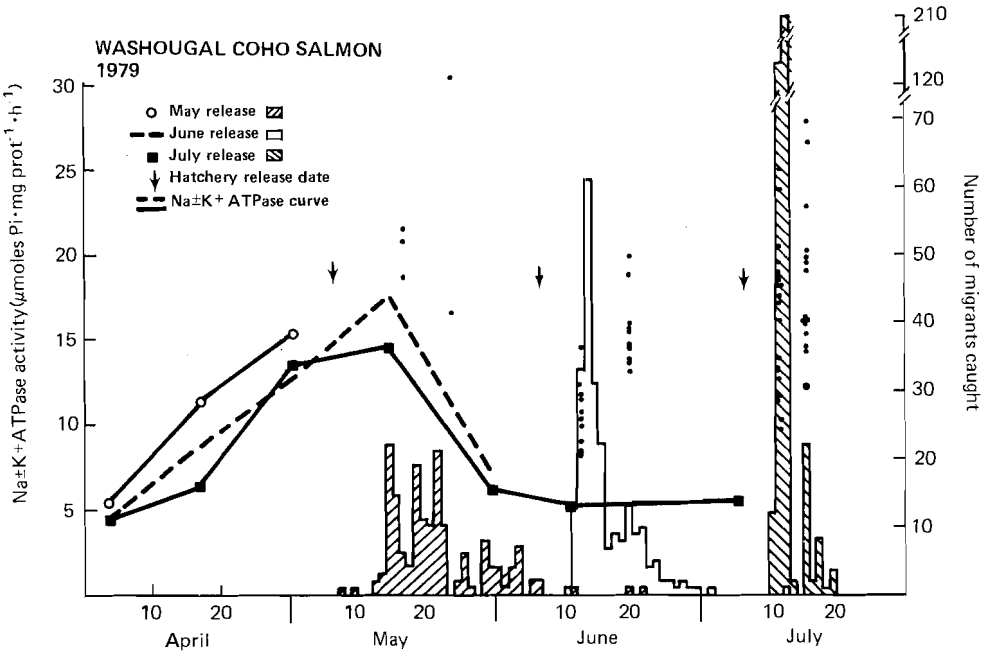

Fig. 2. Gill Na^+-K^+ ATPase activities in coho salmon from the Washougal Hatchery and numbers of migrants caught at Jones Beach (OR), 78 km from mouth of Columbia River.

Arrows indicate releases on 7 May, 7 June, and 7 July. Single points (∘) show gill Na^+-K^+ ATPase activities for individual migrants caught at Jones Beach. Data show actual numbers of migrants captured from releases of 155 030 (May), 155 897 (June), and 163 094 (July) fish. Total numbers captured were 168 (May), 238 (June), and 383 (July).

activities at release, migrants in June and July moved seaward more rapidly than fish released in May (peak ATPase activity). These migrating fish, however, had elevated levels of gill Na^+-K^+ ATPase activity (Fig. 2).

The ability of coho salmon to regenerate rapidly high levels of enzyme activity after having experienced a lowering of previously elevated levels is shown by another experiment illustrated in Fig. 3. In this instance premature decrease in activity was caused by high temperatures, but when palced in cooler water, coho demonstrated a rapid regeneration of elevated Na^+-K^+ ATPase levels (see Zaugg and McLain, 1976). Rates at which this regeneration of elevated activity occurred (6 and 10°C) were much greater than the initial development of activity that accompanied smoltification (Fig. 3, top).

Yearling coho salmon subjected to an advanced photoperiod schedule (AP) showed increased gill Na^+-K^+ ATPase activity on 30 March, about 2 weeks prior to controls and nearly 4 weeks before fish held in hatchery ponds (Fig. 4, top). Peak activity in the AP group occurred earlier and was much higher (36.2 μmoles Pi · mg protein^{-1} · h^{-1}) than in controls where it leveled off at 25—26 from 28 May to 15 June (not shown in figure) then decreased to 10 by 6 July (not shown).

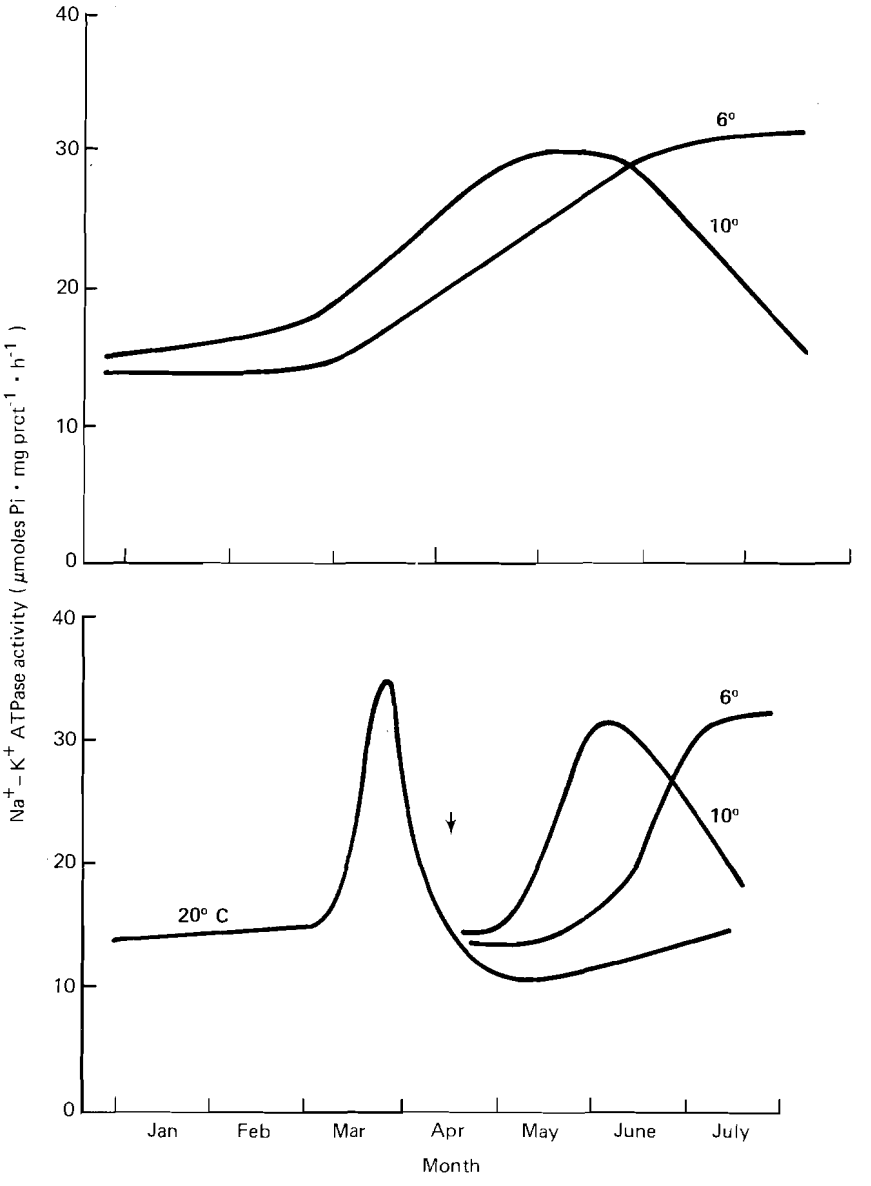

Fig. 3. Gill Na^+-K^+ ATPase activities in coho salmon held at constant temperatures.
Yearling coho salmon were held in Fiberglass tanks at 6 and 10°C during test period (top). At the point shown (arrow, bottom) some fish were transferred to 6 and 10 from 20°C (from Zaugg and McLain, 1976).

Blood nucleoside triphosphate (NTP) levels in controls began increasing at the same time as increases in AP fish, both being earlier than the observed increases in hatchery fish (Fig. 4, bottom). Although NTP concentrations con-

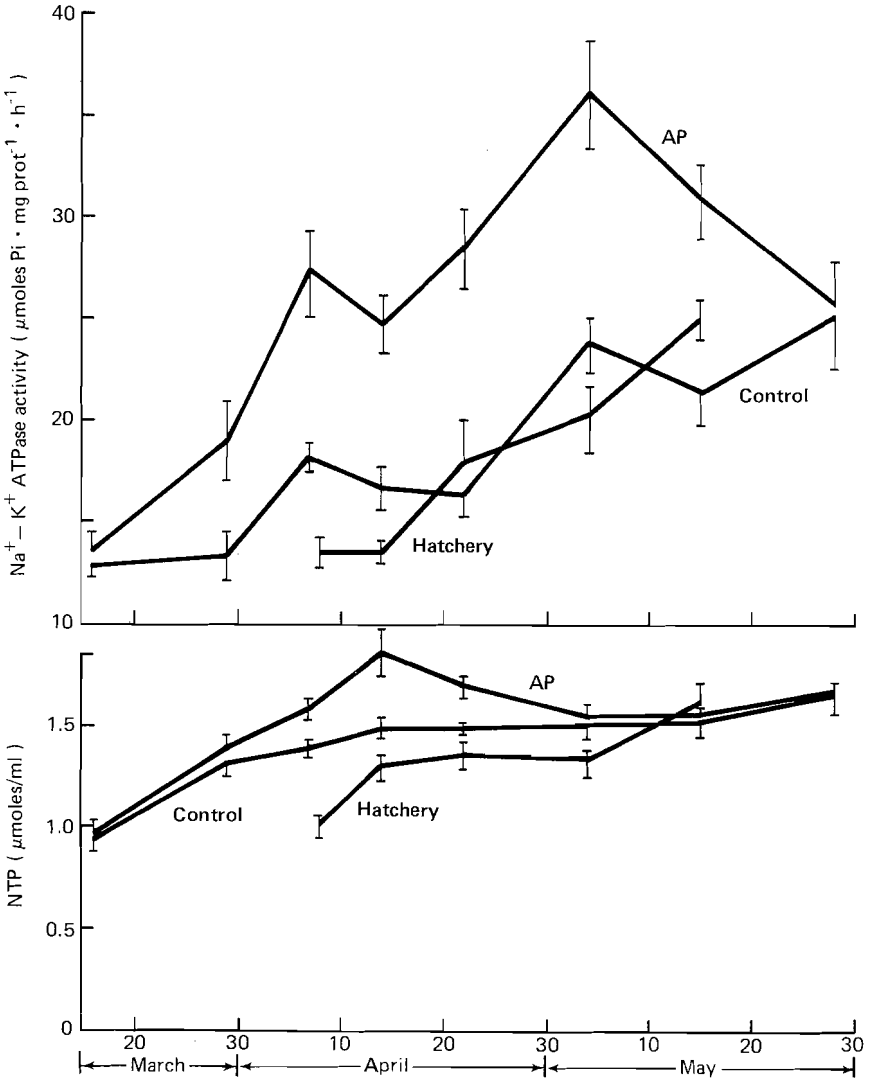

Fig. 4. Gill Na^+-K^+ ATPase activities and blood nucleoside triphosphate concentrations in yearling coho salmon under an advanced photoperiod schedule and under natural light.

Beginning 21 January coho salmon were subjected to a 3-month advance in photoperiod (AP) or were held under natural light as controls. For comparison other fish were taken from the original hatchery pond from which the experimental fish were obtained. Nucleoside triphosphates are assumed to be primarily ATP, and concentrations were determined in whole blood.

tinued to show some decline after 28 May, they had not dropped to presmolt levels by 20 July, whereas gill Na^+-K^+ ATPase activities had decreased to presmolt levels (8 μmoles Pi · mg $protein^{-1}$ · h^{-1}) by this date.

DISCUSSION

Changes in gill Na^+-K^+ ATPase activity may occur rapidly at times in subyearling fall chinook salmon. For example, average levels of activity in fish from Spring Creek NFH increased two-fold in the 19-day period from 20 April to 9 May 1979 (Fig. 1). Yet, in 1978, over twice that length of time was required for animals to experience a similar change (4 April to 18 May). A dramatic change in the 1980 ATPase profile from profiles of the previous 2 years and other changes in 1981 indicate that smoltification in fall chinook salmon can be variable from one year to the next. At the present time we do not know what factors are involved in causing these differences, but such variability in times of smoltification probably has an influence on survival of chinook salmon released from hatcheries on the same date year after year.

Coho salmon fed diets containing supplemental inorganic salts under laboratory conditions experienced increases in gill Na^+-K^+ ATPase activity and survival when transferred directly to salt water (Zaugg and McLain, 1969). Direct transfer of production chinook salmon to ocean net pens by the Washington Department of Fisheries resulted in 16% mortality in controls but only 2% in fish fed a diet containing 7.9% added NaCl for 6 weeks (unpublished). These salt fed salmon also had experienced increased gill Na^+-K^+ ATPase activities. Chinook salmon not directly transferred to salt water, but migrating a distance of 267 km from Spring Creek NFH before entering the ocean also appeared to be beneficially affected by salt feeding (Table I). Although this test was not conducted in replicate, the results suggest that in some cases salt feeding may be a highly effective method of increasing the contribution of hatchery-reared fall chinook salmon.

Coho salmon held at the hatchery beyond a normal May release date experienced a loss of both elevated gill Na^+-K^+ ATPase activity (Fig. 2) and silvery coloration. However, these fish were capable of rapid seaward migration and regeneration of elevated enzyme activity upon liberation in June and July (Fig. 2). The ability of coho to rapidly regain high levels of gill Na^+-K^+ ATPase activity and migrate quickly downstream in greater numbers than fish released in May may partially account for increased survival of late released groups (Novotny, 1980). Regeneration of high enzyme activities in cool water after temperature-induced reversion of previously elevated levels (Fig. 3, bottom) illustrates how much more rapid the rate of regeneration is than the initial development of elevated activity in coho salmon (Fig. 3, top). These results suggest that, at least within the time frame of these observations, loss of elevated gill Na^+-K^+ ATPase activity does not reflect a complete return to parr physiology. Certain elements which limit the rate of development of greater enzyme activity as parr—smolt transformation proceeds either are not involved or have much less effect in the regeneration of high activities.

Increases in blood nucleoside triphosphate concentrations (assumed to be primarily ATP) at the time of smoltification suggest the development of energy-requiring processes in the red blood cell. The nature of these processes

is yet to be determined. Increases in hematocrit values from about 35% in March to 42% in middle to late May (a 20% increase) in the three groups of coho salmon examined account for some of the NTP increase, but not all, as concentrations of NTP rose from about 1 μmole/ml to at least 1.6 μmole/ml (1.9 μmole/ml in coho on advanced photoperiods). Similar increases in NTP concentrations with the onset of elevations in gill Na^+-K^+ ATPase activity have also been observed in steelhead trout and chinook salmon (unpublished). Determination of blood NTP (ATP) concentrations may be a relative simple and convenient method of assessing the status of smoltification.

REFERENCES

Folmar, L.C. and Dickhoff, W.W., 1980. The parr—smolt transformation (smoltification) and seawater adaptation in salmonids. A review of selected literature. Aquaculture, 21: 1—37.

Hoar, W.S., 1976. Smolt transformation: evolution, behavior, and physiology. J. Fish. Res. Board Can., 33: 1234—1252.

Novotny, A.J., 1980. Delayed release of salmon. In: J.G. Thorpe (Editor), Salmon Ranching. Academic Press, London, pp. 325—369.

Wedemeyer, G.A., Saunders, R.I. and Clarke, W.C., 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. Mar. Fish. Rev., (June): 1—14.

Zaugg, W.S., 1981. Advanced photoperiod and water temperature effects on gill Na^+-K^+ adenosine triphosphatase activity and migration of juvenile steelhead (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci., 38: 758—764.

Zaugg, W.S., 1982. A simplified preparation for adenosine triphosphatase determination in gill tissue. Can. J. Fish. Aquat. Sci., 39: 215—217.

Zaugg, W.S. and McLain, L.R., 1969. Inorganic salt effects on growth, saltwater adaptation, and gill ATPase of Pacific salmon. In: O.W. Neuhaus and J.E. Halver (Editors), Fish in Research. Academic Press, New York, pp. 293—306.

Zaugg, W.S. and McLain, L.R., 1976. Influence of water temperature on gill sodium, potassium-stimulated ATPase activity in juvenile coho salmon (*Oncorhynchus kisutch*). Comp. Biochem. Physiol., 54A: 419—421.