Comp. Biochem. Physiol. Vol. 84A, No. 3, pp. 487–493, 1986
Printed in Great Britain

0300-9629/86 $3.00 + 0.00
Pergamon Journals Ltd

CHANGES IN BLOOD LEVELS OF NUCLEOSIDE TRIPHOSPHATES, HEMOGLOBIN AND HEMATOCRITS DURING PARR–SMOLT TRANSFORMATION OF COHO SALMON (*ONCORHYNCHUS KISUTCH*)

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(*Received* 6 *November* 1985)

Abstract—1. Increases and subsequent decreases in gill Na^+-K^+ ATPase activity during parr–smolt transformation in coho salmon were accompanied by changes in blood nucleoside triphosphate (NTP) levels, hemoglobin concentrations and hematocrits.

2. An advanced photoperiod schedule accelerated the parr–smolt transformation and the rate of changes in Na^+–K^+ ATPase activity, NTP and hematocrit levels.

3. Ratios of NTP:hematocrits and of NTP:hemoglobin increased during smoltification.

4. Hematological changes suggest preparation for increased oxygen demand during migration and greater energy requirements by erythrocytes during smoltification and sea-water adaptation.

INTRODUCTION

Numerous morphological and physiological changes occur as juvenile salmon, residing in fresh water, transform to seaward-migrating smolts. Many of these changes have been extensively described in the literature (Hoar, 1976; Folmar and Dickhoff, 1980; *Aquaculture* **28,** 1–270, 1982) and are currently being investigated in many laboratories worldwide. Zaugg (1982a) reported that blood levels of nucleoside triphosphates (NTP) in coho salmon (*Oncorhynchus kisutch*) increased during parr–smolt transformation simultaneously with elevating levels of gill Na^+–K^+ ATPase activity. The increase in NTP is interesting because purine nucleotides, adenosine triphosphate (ATP) and guanosine triphosphate (GTP), have all been implicated as potential regulators of hemoglobin–oxygen affinity in fish (Gillen and Riggs, 1971; Wood and Johansen, 1972; Johansen *et al.*, 1976; Vaccaro Torracca *et al.*, 1977; Weber and Lykkeboe, 1978). Investigators have also observed that erythrocyte ATP concentrations decrease in fish under hypoxic conditions (Weber *et al.*, 1975; Wood *et al.*, 1975; Greany and Powers, 1978). By observing shifts in oxygen dissociation curves, Brunori (1975) showed that ATP and GTP hinder oxygen-binding to trout hemoglobins. Both ATP and GTP occur in widely varying amounts in freshwater and seawater fishes (Leray, 1979 and 1982).

Adenosine triphosphate is also directly involved in the transport of ions across erythrocyte membranes by energy-dependent systems (Caviares, 1977; Sarkadi and Tosteson, 1979) and in many other metabolic activities of the cells. Lane (1984) reported that NTP content of rainbow trout (*Salmo gairdneri*) erythrocytes increases as the cells mature. Therefore, levels of NTP in red cells appear to fluctuate as requirements for these energy sources change, and the evidence of higher concentrations at the time of the parr–smolt transformation suggests an increased demand by some function necessary for the transition of the freshwater-dwelling parr to a seawater-tolerant smolt.

Here, we report changes in NTP, hemoglobin and hematocrit levels that were observed during the parr–smolt transformation of coho salmon, as measured by changes in gill Na^+–K^+ ATPase activity.

MATERIALS AND METHODS

On 16 December 1981, two groups of juvenile coho salmon from the Willard National Fish Hatchery, near Cook, Washington, USA, were placed in 1.5 m diameter tanks with 1 m water depth. Group 1 (280 fish, mean weight 9.9 g) was subjected to a 3-month advance in photoperiod (AP) by extending evening light using a 150 W incandescent flood lamp positioned 1.3 m above the tank. Beginning 21 December 1981, the light was turned on each afternoon at 1500 hr and off at 2030 hr. Artificial day length was extended about 15 min each 9–10 days so that by 8 April 1982 the light was turned off at 2315 hr. On 23 April the advanced photoperiod schedule was terminated, and thereafter the fish experienced natural light until the end of the experiment on 16 August. Group 2 (285 fish, mean weight 9.4 g), the controls, were kept in natural light (NP). Water from the Little White Salmon River, a tributary of the Columbia River, was regulated to a flow of 19 l/min and ranged from 6 to 7.5°C during the experiment. Fish were fed Oregon Moist Pellets to satiation twice daily on weekdays. In addition to the two experimental groups, fish from one of the hatchery ponds (HAT) were sampled on each sampling date from 18 February to 19 May, just prior to release.

Biological samples were obtained approximately every 2 weeks from 10 fish in each group, selected randomly by dip net. Blood was obtained from each fish by severing the caudal peduncle and dripping the blood onto a piece of

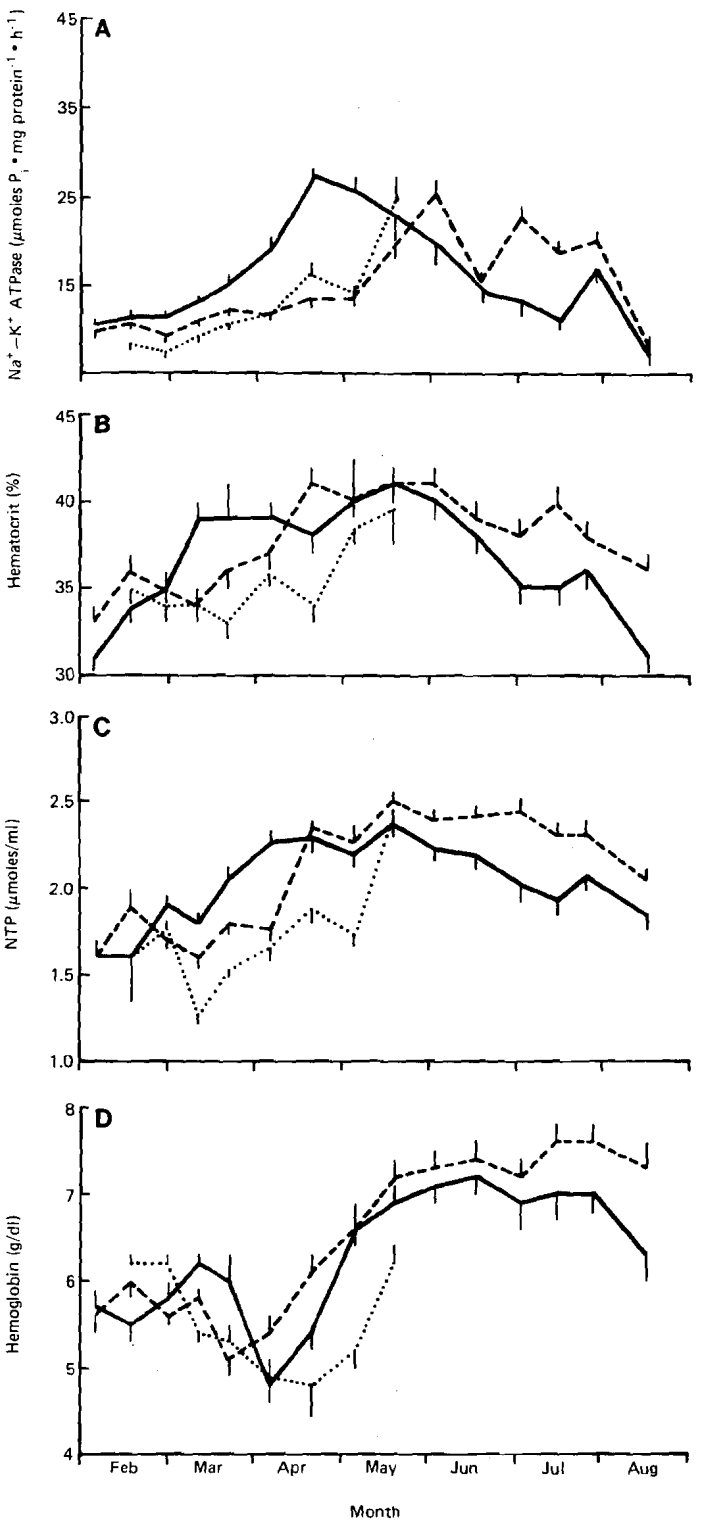

Fig. 1. Gill Na^+-K^+ ATPase activity, hematocrits, NTP and hemoglobin concentrations in coho salmon under advanced or natural photoperiods. Mean (SE) gill Na^+-K^+ ATPase activities (A), hematocrits (B), NTP (C) and hemoglobin (D) levels are shown for experimental groups of coho salmon exposed to a 3-month advance in photoperiod (AP ——) and to natural photoperiods (NP – – –). These same measurements are also shown for hatchery production fish (HAT ···).

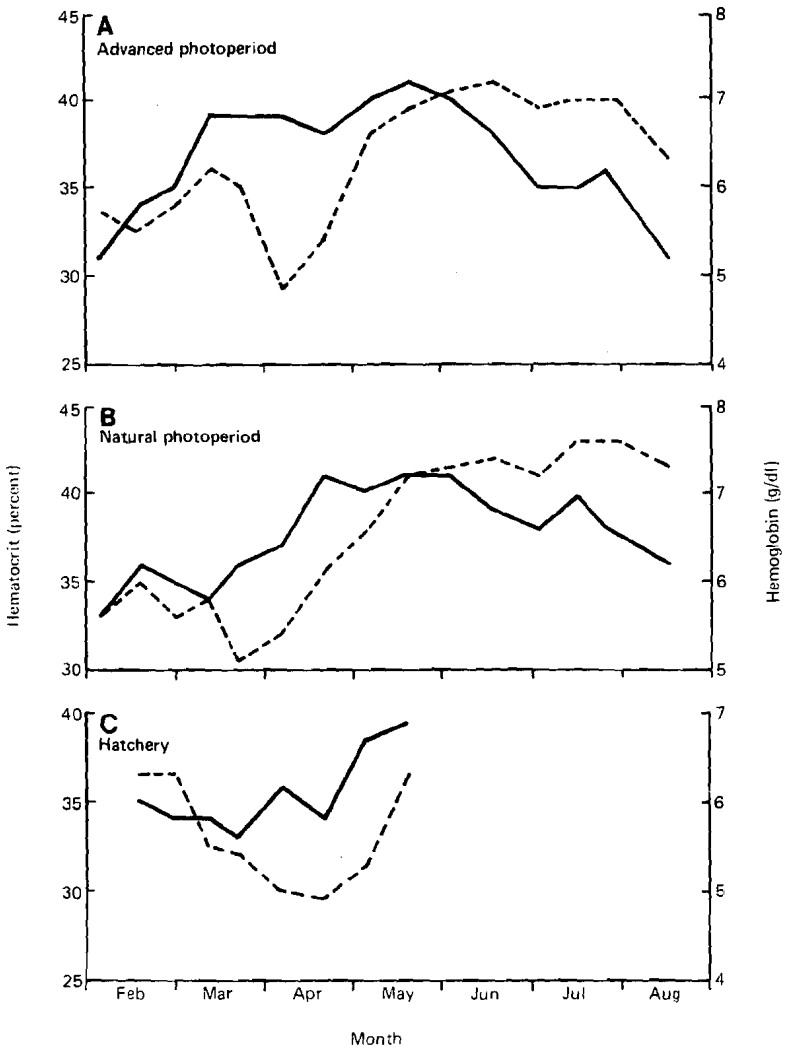

Fig. 2. Comparison of changes in hematocrit and hemoglobin levels during smoltification of coho salmon. Curves from Fig. 1 are replotted to illustrate relationships in the changes of hematocrit (——) and hemoglobin (– – –) values for AP, NP and HAT coho salmon.

Parafilm.* From this pool, 10 μl were taken for hemoglobin and 20 μl for NTP analyses, and a sample for hematocrit determination was collected in a heparinized micro-hematocrit tube and centrifuged for 3 min in a micro-hematocrit centrifuge. Hemoglobin analysis was performed by pipetting the 10 μl of blood into 2.5 ml of cyan-methemoglobin reagent (Hycel), centrifuging briefly in a clinical centrifuge to sediment the debris, then withdrawing the supernatant liquid for examination of absorbance at 540 nm. Blood levels of NTP were determined by slight adjustments of the procedure provided by Sigma (Tech. Bul. 366–UV). The blood was placed into 7.2% trichloroacetic acid solution (150 μl). After mixing thoroughly and standing for at least 3 min (up to 1 hr) in ice water, the material was centrifuged for 5 min at about 3000 r.p.m. in a clinical centrifuge. An aliquot (100 μl) was withdrawn from the supernatant liquid and placed in 0.65 ml of a stock reaction solution consisting of 10 ml Sigma's PGA Buffered Solution, 16 ml H_2O and enough reduced nicotinamide adenine dinucleotide (approximately 6 mg) to give an absorbance of about 1. After thorough mixing, the absorbance at 340 nm was determined; 10 μl of Sigma's GAPD/PGK suspension was introduced, the contents mixed and the reaction allowed to go to completion (about 5 min). The final absorbance at 340 nm was determined and NTP levels calculated from the differences in absorbance. Gill Na^+–K^+ ATPase activities (μmoles P_i·mg/protein/hr) were determined on gill filaments from individual fish, using a simplified, partially purified homogenate described elsewhere (Zaugg, 1982b).

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

RESULTS

For the purpose of these experiments, the time period during which pronounced changes in gill Na^+–K^+ ATPase activities occurred is designated as the time of smoltification. This is a simplification of the entire process, where some aspects begin prior to the elevation of Na^+–K^+ ATPase activity and others extend beyond its return to "pre-smolt" levels.

Gill Na^+–K^+ ATPase activity underwent accelerated development in the advanced photoperiod group

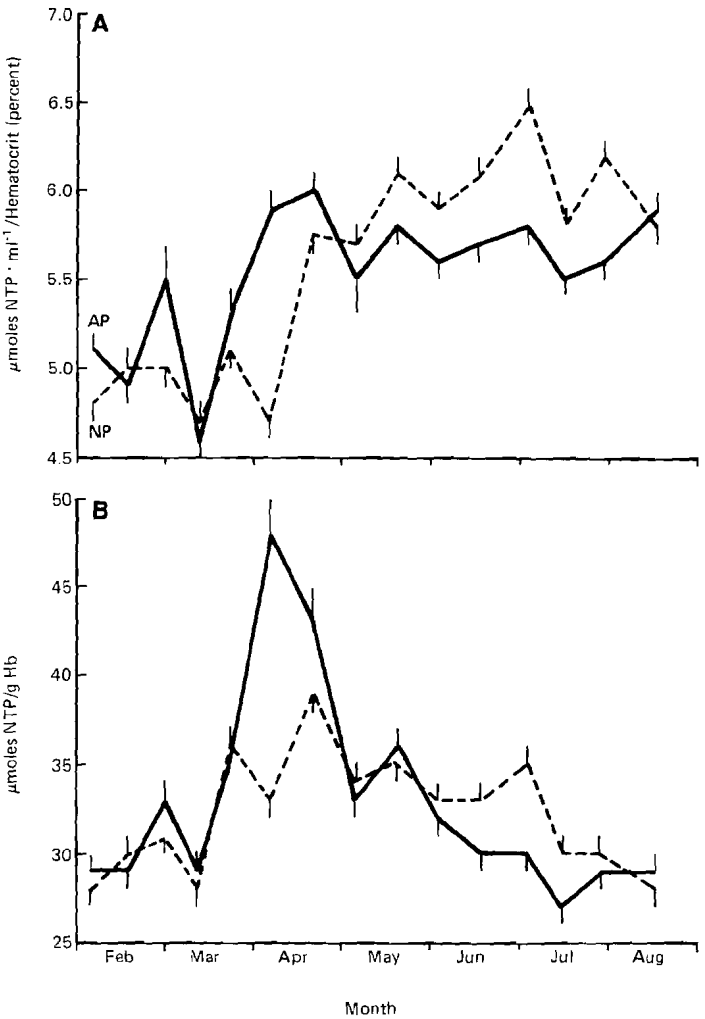

Fig. 3. Relationships of blood NTP concentrations to hematocrits and to hemoglobin levels in coho salmon under advanced or natural photoperiods. Mean values (SE) are plotted for ratios of NTP:hematocrits (3A) for coho salmon under advanced (AP ——) and natural (NP – – –) photoperiods, and for ratios of NTP:hemoglobin (3B) under the same conditions.

compared to the control and hatchery production fish (Fig. 1A). Similar relations were observed in packed red blood cells per volume (hematocrit) (Fig. 1B). Blood concentrations of NTP, including ATP and GTP, are shown in Fig. 1C, while hemoglobin levels are indicated in Fig. 1D. Correlations within each of the experimental groups show a lag in hemoglobin changes compared to changes in hematocrits (Fig. 2).

Comparisons of NTP concentration with hematocrits (Fig. 3A) and with hemoglobin levels (Fig. 3B) indicate an increase in the concentration of these energy sources during the smolting period.

Mean fork lengths (mm) for the groups of fish used in this study are shown in Fig. 4.

DISCUSSION

Parr–smolt transformation in anadromous salmonids is not defined by a single event but by a combination of physiological, biochemical, morphological and behavioural changes that are essential to successful seaward migration and survival in seawater. In this study, we have compared certain hematological changes to changes in gill Na^+–K^+ ATPase activity, one of the events in parr–smolt transformation. We observed high correlation between changes in this enzyme activity and hematocrits in all three studied groups of coho salmon (Fig. 1, Table 1); values tended to increase and decrease synchronously. An advance in the increase–decrease cycle of hematocrits caused by advancing the photoperiod (AP group), compared to the hematocrit cycle in controls (NP), strongly suggests a direct relationship of hematocrit changes to smoltification. In support of this conclusion, elevated hematocrits (45–50%) have been observed in seaward-migrating spring chinook salmon (*O. tshawytscha*) smolts (Dutchuk, personal communication*). Although hematological values have been reported to change

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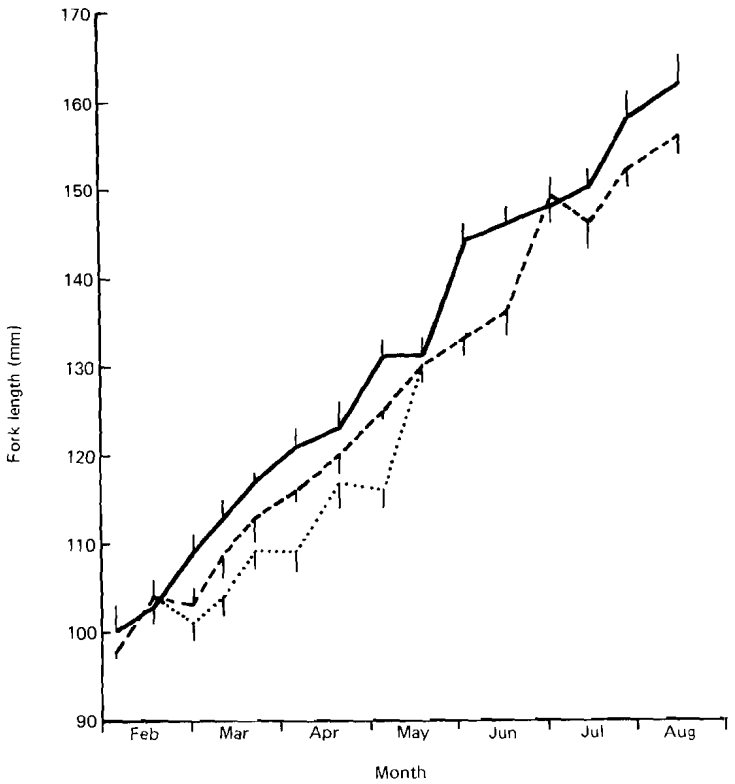

Fig. 4. Fork lengths of fish sampled. Mean fork lengths are plotted for each group of fish used in this study; (AP ——), (NP – – –) and (HAT ···).

with temperature, there appears to be considerable inconsistency (Koss and Houston, 1981). Banks *et al.* (1971) reported increased hematocrits with increased temperature in small (<4.0 g) fall chinook salmon fingerlings, whereas no significant response was observed in larger fish. Miles and Smith (1968) reported increased hematocrits in juvenile coho salmon as water temperature increased in the spring. However, considering the results of the present study, that increase may have been due to smoltification rather than temperature. During our study, water temperatures increased by only about 1.5°C, and by the end of the experiment in August, when the temperature was maximal, hematocrits had returned to February levels.

Hemoglobin levels inexplicably decreased during the initial increase in hematocrits, but later increased in all three groups of fish (Figs. 1 and 2). The lag in hemoglobin increase (Fig. 2) suggests that the newly-produced cells were slow in synthesizing a normal complement of hemoglobin. This is similar to recoveries of normal hematology demonstrated by chinook salmon made anaemic with phenylhydrazine, where there was a greater delay in the return towards normal hemoglobin values than towards normal hematocrits (Smith *et al.*, 1971).

Nucleoside triphosphate content of young red cells is less than that of mature cells in rainbow trout (Lane, 1984). However, we observed an increase in NTP levels as hematocrits increased, apparently because of the production of new cells. Ratios of NTP to hematocrits and to hemoglobin levels increased as hematocrits and hemoglobin values increased (Fig. 3). However, as a result of the delay in hemoglobin synthesis, there was less correlation between hemoglobin values and levels of hematocrits, NTP and Na^+–K^+ ATPase activities than between any of the other pairs of parameters investigated (Table 1). Fish on an accelerated photoperiod again showed earlier changes than those on natural light. Lane (1984) observed that the formation of NTP in young cells came primarily from oxidative phosphorylation and in older cells by glycolysis. Whether increasing amounts of NTP arose through oxidative phosphorylation in younger cells that contain mito-

Table 1. Correlation coefficients (r) calculated from the means of all sampling periods, for advanced photoperiod (AP), natural photoperiod (NP) and hatchery (HAT) coho salmon groups

Group	Hemoglobin	NTP	ATPase
Advanced photoperiod			
Hematocrit	0.17	0.78	0.76
Hemoglobin	—	0.22	0
NTP	—	—	0.80
Natural photoperiod			
Hematocrit	0.61	0.90	0.69
Hemoglobin	—	0.81	0.66
NTP	—	—	0.76
Hatchery			
Hematocrit	0.25	0.72	0.76
Hemoglobin	—	0.32	0
NTP	—	—	0.86

chondria or by glycolysis in older cells that lack mitochondria, or by both processes, was not determined. Regardless, the levels of NTP increased during smoltification in all three groups of coho salmon. Similar increases in chinook salmon and steelhead (*Salmo gairdneri*) have been observed (Zaugg, unpublished data).

The noticeably delayed increases in hematocrits, NTP and hemoglobin in fish from hatchery production ponds, when compared to the experimental group on natural light (Fig. 1B, 1C, 1D), probably resulted from a slightly smaller size and slower growth rate (Fig. 4).

One might speculate that hematocrits and hemoglobin levels should increase during smoltification in anticipation of the need for greater oxygen supplies in the tissues during seaward migration and seawater adaptation. Cameron and Wohlschlag (1969) suggested that anadromous salmonids might be expected to show hemoglobin increases before and during migration to reduce the cardiac energy required over prolonged periods of activity. The rapid depletion of lipid reserves observed in smolting and migrating fish (Hoar, 1939; Vanstone and Markert, 1968; Fessler and Wagner, 1969) occurs through oxygen-requiring metabolic processes that must surely increase oxygen demand and therefore increase red cell production.

The reason for the increases in erythrocyte NTP content must be more speculative. If a primary function of NTP is to decrease hemoglobin–oxygen binding (Gillen and Riggs, 1971; Wood and Johansen, 1972; Brunori, 1975), then elevated NTP levels would not be expected during smoltification, when the demand for oxygen increases. Perhaps NTP synthesized under these conditions is insufficient to significantly affect the *in vivo* hemoglobin–oxygen equilibrium, but is used for other cellular functions. A build-up of NTP may reflect preparation for some increase in osmoregulatory activity upon seawater entry. Synthesis of new hemoglobins that appear during parr–smolt transformation (Giles and Vanstone, 1976; Kock, 1982; Sullivan *et al.*, 1985) would require energy that could be readily supplied by NTP.

In these experimental groups of yearling coho salmon we have observed changes in hematocrits, hemoglobin levels and NTP concentrations that occurred during the period of parr–smolt transformation. We have observed similar changes in other groups of Pacific salmonids. Nevertheless, we hesitate to suggest that hematological changes would occur during all instances of smoltification, for we recognize that not all groups of anadromous salmonids undergo the same degree of transformation, and that factors such as diet, disease and temperature may alter relationships among physiological changes at that time. The extent to which the hematological changes we have reported can be related to other events in parr–smolt transformation must await more exhaustive investigations.

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