Inhibition of Salt Water Survival and Na-K-ATPase Elevation in Steelhead Trout (*Salmo gairdneri*) by Moderate Water Temperatures

B. L. Adams, W. S. Zaugg, and L. R. McLain

Western Fish Nutrition Laboratory
Cook, Washington 98605

ABSTRACT

The steelhead trout metamorphosis from a freshwater parr to a sea water-tolerant smolt possessing the migration tendency was evaluated at six different growth temperatures ranging from 6 to 15 C during January through July. The highest temperature where a transformation was indicated was 11.3 C. By April fish reared at 6 C had elevated ATPase levels typical of smolts or migratory animals and showed 92% survival in sea water. Ten and 11.3 C-reared fish showed a short-lived elevation in ATPase in mid-April alone concurrently with 100% sea water survival at that time. Only in 6 C-reared animals did the salt water survival ability continue into May. High ATPase levels likewise were prolonged into May and June only in the 6 C-reared group. The data indicate that metamorphosis (and therefore successful migration) of juvenile steelhead trout is directly controlled by water temperature.

The transformation of a freshwater salmon (*Oncorhynchus* sp.) or steelhead trout (*Salmo gairdneri*) to a sea water-tolerant smolt involves the activation of a sodium pump (sodium-potassium-dependent ATPase) in the gill (Zaugg and McLain 1969, 1971). The same is true for certain other teleosts (Epstein et al. 1967; Kamiya and Utida 1969). The sodium pump activity increases naturally before spring seaward migration of salmonids (Zaugg and McLain 1970). It has been shown that the smolt-related elevation of the salt pump activity is inhibited in steelhead held at 15 C and above (Zaugg et al. 1972; Adams et al. 1973). Speculation that fish whose Na-K-ATPase elevation was inhibited by the warmer water might not show the normal tendency to migrate to the ocean was later confirmed by experiments in which migration downstream during the normal period was decreased by prior residence in water about 13 C or higher (Zaugg and Wagner 1973). A high correlation was also found between migration and the sodium pump activity.

Inasmuch as salmonids grow faster and utilize food more efficiently near 15 C than at 6 or 10 C, several large salmon and steelhead hatcheries use heated water to rear fish. Increases in stream temperatures also result from flow-through cooling systems of hydroelectric dams and nuclear power plants. These factors indicate the need to know exactly the maximum temperature at which steelhead and other salmonids can be reared and still maintain their urge and capacity to migrate successfully to the ocean from hatcheries or natural spawning grounds. The present experiment reports an evaluation of the temperature tolerance of steelhead smolts as judged by gill Na-K-ATPase and by survival in 35 ‰ sea water.

METHODS

Summer steelhead obtained from Washington State Fish Hatchery at Skamania were reared at 10, 11.3, 12.7, or 14 C; and winter steelhead obtained from Eagle Creek National Fish Hatchery in Oregon were reared at 6 or 15 C. On 16 December 1971, at the beginning of the experiment, all fish were treated with formalin (120 ml/500 liters for 1 hour) for possible external parasites. Oregon Moist Pellet was fed 3 times daily, 6 days a week. Fish were maintained indoors in 524-liter fiberglass tanks with fluorescent lighting controlled to give normal variation in day length. Three fish were taken from each temperature at about 2-week intervals and assayed separately for gill microsome Na-K-stimulated ATPase, as reported previously (Zaugg and McLain 1970). These three values were then averaged. Salt water survival tests were made by placing fish from the various temperatures directly into 130 liters of recirculated 35 ‰ sea water at 10 C pumped through 2 liters of oyster shells 15 cm deep. We used this con-

TABLE 1.—*Chemical constituents of sea water (supplied by Aquarium Systems, Eastlake, Ohio) and of fresh water used in this study. Composition in parts per million.*

Chemical	Sea water	Fresh water
Cl	19,300	0.0
Na	10,700	2.0
SO_4	2,630	8.0
Mg	1,260	2.0
K	390	0.4
Ca	390	3.6
HCO_3	147	0.0
H_3BO_4	26	0.0
Sr	8.5	0.0
PO_4	1.0	0.3
Mn	1.0	0.0
MoO_4	0.7	0.0
S_2O_3	0.4	0.0
Li	0.2	0.0
Zn	0.0	0.006
Cu	0.0	0.0017
Cd	0.0	0.0003
Total	34,854.8	16.3080

centration in testing salt water survival, instead of 28–30 ‰ (sometimes used for survival tests), to intensify the distinction between nonsmolts and true smolts. The transfer resulted in a several-fold reduction in fish load in the test salt water compared to the rearing fresh water. No feed was given during the salt water test. The composition of the salt water and that of the fresh water are given in Table 1. The percent survival was figured as the number of fish tested times the number of days each fish survived times 100%, divided by the total possible survival days until the test was concluded at day 13. Fish transferred to a similar freshwater recirculating tank for several weeks suffered no mortalities.

RESULTS

The average value for gill microsome Na-K-ATPase activity was near 15 units in steelhead parr reared at 6 and 10 C, and lower in fish reared at each of the higher temperatures (Fig. 1). Only two out of ninety fish sampled for the six temperature groups showed ATPase values above 22 before late March. Between early May and mid-June no individuals from 6 C water had ATPase values below 22. The data from this and other studies suggest that at any temperature gill ATPase activities above 20–22 are necessary for and also indicative of the smolt condition including migration tendency, reduced coefficient of condition, and

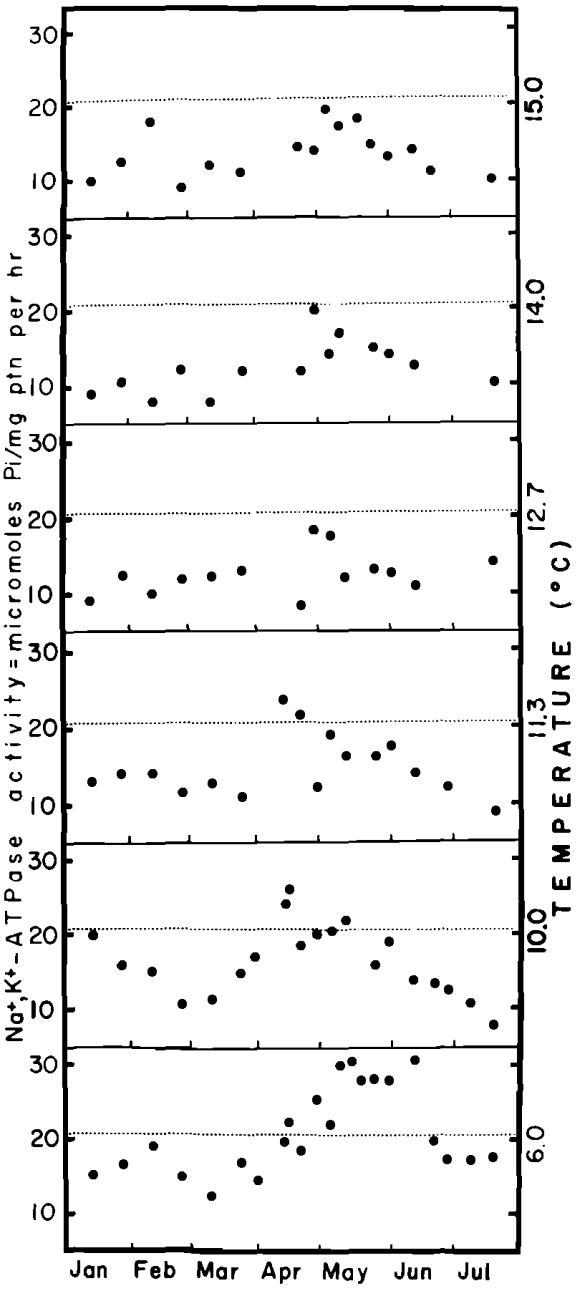

FIGURE 1.—*Sodium-potassium-stimulated, magnesium-dependent adenosine triphosphatase activity from steelhead trout gill microsomes. One unit of activity produces one micromole of inorganic phosphate per milligram of protein in the reaction mixture per hour as the consequence of enzymatic hydrolysis of adenosine triphosphate. Each point is the average value from three fish. Date 1972.*

salt water survival. Thus an ATPase value of 21 ± 2 units might be considered as a general lower threshold level for steelhead smolts when assayed according to our method (Zaugg, Adams, and McLain 1972; Adams, Zaugg, and

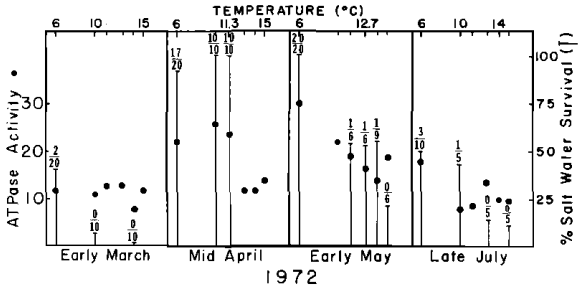

FIGURE 2.—*Percent survival of steelhead after they were transferred directly to 35 ‰ salt water. The survival rate is calculated as the total number of days survived (number of fish times the days each fish survived) divided by the total survival days possible times 100%. The test lasted for 13 days. The fraction of fish alive at the end of the test period and the number used for each group is also shown. ATPase activity (micromoles of inorganic phosphate/mg protein per h) is shown for all six temperatures at the time each sea water survival test was started.*

McLain 1973; Zaugg and Wagner 1973). By mid-April the ATPase in fish held at 6 C was increasing but had not yet attained its maximum. The 10 and 11.3 C had attained maximum ATPase activity by this time, but at a lower level than later found in 6-C-held fish. The groups held at 12.7, 14.0, and 15 C showed no evidence of a smolt-related elevation in ATPase at this time. The maximum ATPase in 6-C fish was attained in early May, when activities in all other groups were declining.

In early March salt water survival, like ATPase, was very low especially at 10 and 14 C (Fig. 2). Survival in mid-April was 92% in 6 C fish and 100% in 10 and 11.3 C fish, being proportional to the sodium pump elevation in these same groups. By early May sea water survival rate, like ATPase activity, had declined in 11.3 C fish to a value intermediate between that in the parr and the smolt. In May survival of fish was less at 15 C (22%) than that for fish held at any of the lower temperatures.

Mechanical problems caused a loss in some salt water survival data. However, in the case of 10 C-reared fish, prior tests have shown essentially 100% survival in early May when ATPase values in the same group were well above 20.

By late July, when gill microsomal Na-K-ATPase in all groups had returned to the presmolt value (Fig. 2), salt water survival was similar to that of the presmolts in March.

Where ATPase was not elevated, the number of fish surviving 13 days in salt water (2/20 etc.—see Fig. 2) was much lower than the percent survival (bar heights), but where ATPase was elevated both methods of calculation gave similar results near 100%. The number surviving 13 days may have been the truer indication of sea water adaptability.

DISCUSSION

The data suggest that steelhead trout undergo the smolt transformation when reared in water at temperatures below 11.3 C but not at higher water temperatures. They further indicate that at temperatures above 10 C the smolt or migration season may be very short. Prior studies have shown a correlation between steelhead migration and gill Na-K-ATPase (Zaugg and Wagner 1973). Since salt water survival and gill microsomal Na-K-ATPase are concomitantly high in the spring, and are both low during the nonsmolt seasons, we believe that the correlation of these factors with the successful migration of steelhead trout and other salmonids is strengthened. The relationship of temperature to successful transformation would explain a lack of consistency in studies relating photoperiod to smolt character (Wagner 1974; Saunders and Henderson 1970). The experimental water temperature in those experiments was about 12 C during the smolt season.

Other studies have shown that there is an elevation of Na-K-ATPase in coho salmon smolts, but, unlike the case with steelhead, the maximum allowable temperature of coho is above 15 C (Zaugg and McLain, in preparation). This demonstrates that the steelhead is a colder water fish than the coho salmon. As in steelhead, however, the period of elevation of the sodium pump in coho salmon was abbreviated in warmer water.

Gill ATPase seems to be a sure and quantitative test to determine the optimum release date of salmonids from hatcheries. It has also been an extremely powerful tool in determining the exact temperature at which the parr-to-smolt transformation is depressed in steelhead trout.

The salt water survival rate in May among fish held at the upper four temperatures,

although not indicative of smolts, was higher than in March or July and corresponds to a slight increase of ATPase in May in the same groups. We noticed also that winter steelhead held at 15 C had slightly higher ATPase and a little less color than the summer steelhead at 14 C. These factors indicate that selective breeding, aided by Na-K-ATPase studies, might produce a more heat-tolerant migrating steelhead trout.

LITERATURE CITED

ADAMS, B. L., W. S. ZAUGG, AND L. R. McLAIN. 1973. Temperature effect on parr-smolt transformation in steelhead trout (*Salmo gairdneri*) as measured by gill sodium-potassium stimulated adenosine triphosphatase. Comp. Biochem. Physiol. 44A:1333–1339.

EPSTEIN, FRANKLIN H., ADRIAN I. KATZ, AND GRACE E. PICKFORD. 1967. Sodium and potassium-activated adenosine triphosphatase of gills: role in adaptation of teleosts to salt water. Science 156: 1245–1247.

KAMIYA, MIE, AND SEITIRO UTIDA. 1969. Sodium-potassium-activated adenosine triphosphatase activity in gills of fresh-water, marine and euryhaline teleosts. Comp. Biochem. Physiol. 31: 671–674.

SAUNDERS, RICHARD L., AND EUGENE B. HENDERSON. 1970. Influence of photoperiod on smolt development and growth of Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 27:1295–1311.

WAGNER, H. H. 1974. Seawater adaptation independent of photoperiod in steelhead trout (*Salmo gairdneri*). Can. J. Zool. 52:805–812.

ZAUGG, W. S., B. L. ADAMS, AND L. R. McLAIN. 1972. Steelhead migration: potential temperature effects as indicated by gill adenosine triphosphatase activities. Science 176:415–416.

ZAUGG, W. S., AND L. R. McLAIN. 1969. Inorganic salt effects on growth, salt water adaptation and gill ATPase of Pacific Salmon. Pages 293–306 *in* O. W. Neuhaus and J. E. Halver, eds. Fish in research. Academic Press, New York, N. Y.

ZAUGG, W. S.,AND L. R. McLAIN. 1970. Adenosine triphosphatase activity in gills of salmonids: seasonal variations and salt water influence in coho salmon (*Oncorhynchus kisutch*). Comp. Biochem. Physiol. 35:587–596.

ZAUGG, W. S., AND L. R. McLAIN. 1971. Gill sampling as a method of following biochemical changes: ATPase activities altered by ouabain injection and salt water adaptation. Comp. Biochem. Physiol. 38B:501–506.

ZAUGG, W. S., AND H. H. WAGNER. 1973. Gill ATPase activity related to parr-smolt transformation and migration in steelhead trout (*Salmo gairdneri*) : influence of photoperiod and temperature. Comp. Biochem. Physiol. 45B:955–965.