RESEARCH SECTION

Oregon Department of Fish and Wildlife

Parr-smolt Transformation in Chinook Salmon (*Oncorhynchus tshawytscha*) W.
1. Gill (Na + K)-activated Adenosinetriphosphatase Activity Under Various Temperature and Photoperiod Regimes
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activity under various temperature and photo-
period regimes.

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ABSTRACT

Juvenile spring chinook salmon reared at two temperatures and photoperiods were examined for changes in gill (Na+K)-activated adenosinetriphosphatase activity. In the course of two years, three peaks in gill (Na+K)-ATPase activity were observed: a peak in October of the first year, a second higher peak the following May, and a peak in October of the second year. Advanced photoperiods during the first year suppressed the October peak. Changes in rearing temperatures did not directly influence the enzyme activity, but did cause changes in growth rates of the fish which resulted in differences in gill (Na+K)-ATPase activity in the two temperature groups. A threshold of 8-9 cm was suggested as the minimal size at which the fish could respond to the appropriate photoperiod and enter the gill (Na+K)-ATPase cycle.

INTRODUCTION

Parr-smolt transformation in anadromous salmonids is one of the most important periods in their life history. At this time juveniles undergo physiological changes which initiate migration and adapt them for oceanic life (Hoar 1976). They also are thought to imprint on the home stream at this time. Thus, changes in environmental parameters which influence the onset of parr-smolt transformation, such as water temperature and food supply, could have significant effects on future salmonid populations.

The physiological basis for parr-smolt transformation is not well understood. Its most reliable indicators are the changes in physiological processes common for survival of fish in fresh water to those common for sea water. These include changes in pigmentation (Johnston and Eales 1967, 1968), lipids (Ota and Yamada 1974), and ionic constituents (Houston and Threadgold 1963) and increased levels of gill (Na+K)-activated ATPase (Zaugg and McLain 1972; Zaugg and Wagner 1973; Giles and Van Stone 1976).

A relationship between gill (Na+K)-activated ATPase and parr-smolt transformation was established by studies of downstream migration in steelhead (Zaugg and Wagner 1973) and coho (Lorz and McPherson 1976). The present study was undertaken in an effort to determine whether gill (Na+K)-activated ATPase is also a suitable indicator of parr-smolt transformation in chinook salmon and how environmental effects influence changes in levels of this enzyme.

MATERIALS AND METHODS

Rearing Conditions

Green eggs of spring chinook salmon (1974 brood) from the Rogue River, Oregon, were transported to the Oregon Department of Fish and Wildlife Research Laboratory at Corvallis, disinfected with Wescodyne, and incubated at 10 C until their yolk sac was absorbed. The resultant fry were then transferred into circular, hooded tanks, 1.5 m in diameter, supplied with running well water with an exchange rate of 3 l/min. Rearing temperatures were maintained at 8 ± 1 C or 12 ± 1 C for all tanks except two which were regulated to simulate the annual temperature profile of the Rogue River.
Lighting in the hooded tanks was provided by four 40-watt fluorescent bulbs controlled by Sangamo astral timers. Photoperiod lengths were adjusted to normal seasonal day-lengths (± 10 min) for Medford, Oregon, as determined by the U. S. Naval Observatory there. Two 15-watt incandescent bulbs regulated by astral timers were utilized to provide a 30-minute twilight condition in each tank. During the first year of rearing, each of the environmental conditions was duplicated in a second tank. Duplicate tanks were discontinued during the second year. All fish were fed to repletion daily on Oregon Moist Pellet in graded sizes.

Analyses

Analyses of gill (Na+K)-activated ATPase were begun in May of the first year after the fish reached 5 cm in length. A random sample of 30 fish was collected monthly from each experimental tank. Each fish was stunned by a blow to the head, measured and weighed, and decapitated with a razor blade. The gill arches were dissected free from the branchial cavity and blotted with kimwipes. Gill filaments were dissected from branchial arches, weighed on a Cahn microgram electro-balance, and homogenized at 12.5 mg gill wet weight per ml of a homogenizing medium. (Na+K)-activated ATPase activity was determined by the whole homogenate method of Johnson et al. (1977). Protein was measured by a modification of the method of Lowry et al. (1941).

RESULTS

Relationship Between Gill (Na+K)-Activity and Parr-Smolt Transformation

Under simulated river temperatures and normal lighting, specific activity of gill (Na+K)-activated ATPase reached a shoulder in August and a peak in October (Fig. 1). This profile was reproduced in all tanks under normal photoperiods. The peak activity in October corresponds with the time of maximum entry of the Rogue River juvenile chinook into the ocean (Lichatowich, 1977). Scales of returning adult chinook salmon also show that the majority entered the ocean as juveniles in September and October (Schluchter and Lichatowich 1977). This support from independent observations provides evidence that gill (Na+K)-activated ATPase activity in Rogue River spring chinook salmon is an indicator of parr-smolt transformation and may be used to examine the effects of environmental changes on the smolting process.

Effects of Photoperiod on Gill (Na+K)-ATPase Activity

Juveniles reared under advanced and normal photoperiods at 8 C had different seasonal patterns of gill (Na+K)-activated ATPase activity during the first year (Fig. 2). Under normal photoperiods, gill (Na+K)-ATPase activity peaked in October, as shown in Fig. 1, while under advanced photoperiods there was only a slight upward trend in specific activity. Lengths of fish exposed to each photoperiod remained comparable, although fish under normal photoperiod grew slightly faster (Table 1).

Similar results were found with juveniles held at 12 C. Under normal photoperiods there was a peak in enzyme activity in October (Fig. 3), while fish under advanced photoperiods showed only a slight rise in specific activity. Fish reared at 12 C were nearly 5 cm larger in October 1976, than those reared at 8 C (Table 1).
Fig. 1. Changes in gill (Na+K)-ATPase activity with time. Juveniles were maintained at temperatures simulating average temperatures in the Rogue River, Oregon.
Fig. 2. Comparison of seasonal changes in gill (Na+K)-ATPase activity in fish under normal and advanced photoperiods at 8°C.
<table>
<thead>
<tr>
<th>Date</th>
<th>Normal photoperiod</th>
<th>Advanced photoperiod</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 C</td>
<td>12 C</td>
</tr>
<tr>
<td>June 1975</td>
<td>7.75 ± 0.09</td>
<td>8.92 ± 0.13</td>
</tr>
<tr>
<td>August 1975</td>
<td>9.99 ± 0.13</td>
<td>12.11 ± 0.08</td>
</tr>
<tr>
<td>October 1975</td>
<td>12.09 ± 0.15</td>
<td>15.52 ± 0.24</td>
</tr>
<tr>
<td>December 1975</td>
<td>13.94 ± 0.27</td>
<td>17.49 ± 0.25</td>
</tr>
<tr>
<td>Feb. 17, 1976</td>
<td>14.61 ± 0.48</td>
<td>20.19 ± 0.55</td>
</tr>
<tr>
<td>Apr. 7, 1976</td>
<td>16.88 ± 0.38</td>
<td>22.45 ± 0.43</td>
</tr>
<tr>
<td>July 6, 1976</td>
<td>18.11 ± 0.37</td>
<td>24.57 ± 0.25</td>
</tr>
<tr>
<td>Aug. 24, 1976</td>
<td>19.20 ± 0.23</td>
<td>26.56 ± 0.48</td>
</tr>
<tr>
<td>Oct. 7, 1976</td>
<td>20.15 ± 0.41</td>
<td>27.65 ± 0.32</td>
</tr>
</tbody>
</table>

1/ Lengths are given as means ± standard errors for a sample of 30-60 fish. Values for 1975 are averages of two 30-fish samples from replicate tanks. Photoperiods and temperatures are as described in Materials and Methods.

2/ Significantly different at 95% confidence level from corresponding values under normal photoperiods.
Fig. 3. Comparison of seasonal changes in gill (Na+K)-ATPase activity in fish under normal and advanced photoperiods at 12 C.
Effect of Fish Size on Gill (Na+K)-ATPase Activity

Fork lengths of fish and specific activities of gill (Na+K)-activated ATPase were correlated throughout the year in all experimental conditions. This relationship is demonstrated in Fig. 4 for juveniles reared under advanced photoperiods at 8 C in which the effects of parr-smolt transformation on gill (Na+K)-ATPase activity is blocked. Under these conditions, it can be seen that larger fish had higher levels of gill (Na+K)-ATPase activity. This relationship resulted from changes in gill (Na+K)-ATPase activity rather than changes in gill protein levels. There was no correlation between gill protein and fork length of fish.

Gill (Na+K)-activated ATPase activity of fish reared under normal photoperiods at 8 C were examined by size class during September, October and November. Activities in each size class were corrected for changes in enzyme activity due to the growth of the fish during the 3 month period. In 9-10 and 12-13 cm fish there were only small changes in (Na+K)-ATPase activity from September to October (Fig. 5 and Table 2). In 10-12 cm fish, however, there was a large increase in gill (Na+K)-ATPase activity from September to October (Fig. 5). This increase was followed by a decrease in activity in November (Table 2). These results suggest that (Na+K)-ATPase activity increased in response to photoperiod and that the magnitude of the response depended both on the length of fish and on the initial levels of activity.

Effect of Temperature on Fish Growth and Gill (Na+K)-ATPase Activity

Under repletion feeding, growth of fish in all tanks was linear with time. The rates of growth, however, differed (Table 3). Maximal growth rates occurred at 12 C under normal photoperiod. The growth rates under advanced photoperiod were similar for 10, 12 and 14 C but depressed at 8 C. Growth under advanced photoperiod was somewhat slower than under normal photoperiods (Tables 1 and 3).

The effect of water temperature on (Na+K)-activated ATPase activity of the gills was exerted chiefly through growth rates. The relationship between enzyme activity and size shown in Fig. 4 was used to correct changes in activity due to differences in length at the two temperatures. When this correction was made, there was no significant difference in enzyme activity between fish reared at 8 C and 12 C except when influenced by photoperiod. When rates of increase of (Na+K)-ATPase activity were compared between 8 C and 12 C under advanced photoperiods (to prevent increases due to parr-smolt transformation), the activity increased at a rate similar to the growth rate of the fish (Table 4). There was an increase in activity of approximately 0.33 umoles Pi/hr/mg protein for every cm/month change in growth rate of the fish, regardless of the temperature at which the fish was reared. Ratios of growth rate (cm/month) to gill (Na+K)-ATPase activity were constant at approximately 3.0 (Table 4).

Gill (Na+K)-Activated ATPase in Yearling Chinook

Since many river systems contain juvenile chinook which migrate to sea as yearlings, juveniles were carried over on normal photoperiods for a second year to examine changes in gill (Na+K)-activated ATPase. Specific activity reached a peak during April-May which was even higher than that in October (Fig. 6). A third peak in specific activity was seen during October of the second year.
Fig. 4. Changes in gill (Na+K)-ATPase activity with fork length of fish. Juveniles reared at 8 C under advanced photoperiod.
Fig. 5. The change in gill (Na+K)-ATPase activity in spring chinook from September to October by size class intervals.
Table 2. Increases in gill (Na+K)-ATPase activity with time in various size subclasses.

<table>
<thead>
<tr>
<th>Size in September (cm)</th>
<th>(Na+K)-ATPase activity (µmoles/hr/mg protein) 1/</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>September</td>
<td>October</td>
<td>November</td>
</tr>
<tr>
<td>9-10</td>
<td>2.5</td>
<td>4.0</td>
<td>3.9</td>
</tr>
<tr>
<td>10-11</td>
<td>2.9</td>
<td>6.3</td>
<td>4.1</td>
</tr>
<tr>
<td>11-12</td>
<td>4.0</td>
<td>6.7</td>
<td>5.8</td>
</tr>
<tr>
<td>12-13</td>
<td>6.3</td>
<td>6.8</td>
<td>6.0</td>
</tr>
</tbody>
</table>

1/ Values are means of size subclasses from a sample of 60 fish reared under normal photoperiods at 8°C. Values are corrected for growth of the fish during the three month period.

Table 3. Growth rates of spring chinook juveniles under advanced and normal photoperiods at different water temperatures. 1/

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Growth rate (cm/month)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Advanced photoperiod</td>
</tr>
<tr>
<td>8</td>
<td>0.53</td>
</tr>
<tr>
<td>10 2/</td>
<td>1.53</td>
</tr>
<tr>
<td>12</td>
<td>1.50</td>
</tr>
<tr>
<td>14 2/</td>
<td>1.58</td>
</tr>
</tbody>
</table>

1/ Values are slopes of growth curves plotted as length vs month, of which each point represents the mean length of 60 fish.

2/ No (Na+K)-ATPase analysis was made in 1974.
Table 4. Comparison of growth rates and rates of increase in (Na+K)-ATPase specific activity at variable temperatures.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Growth rate (cm/month)</th>
<th>Increased (Na+K)-ATPase activity (specific activity/month)</th>
<th>G. R. ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0.53</td>
<td>0.18</td>
<td>2.9</td>
</tr>
<tr>
<td>12</td>
<td>1.50</td>
<td>0.50</td>
<td>3.0</td>
</tr>
</tbody>
</table>

1/ Growth rates and specific activities were calculated from samples of 60 fish reared under advanced photoperiods. Rates of increase in gill (Na+K)-ATPase activity were estimated from the slopes of plots of specific activity vs time, similar to that shown in Fig. 4.
Fig. 6. Gill (Na+K)-ATPase activity with time through a two-year time period.
DISCUSSION

Research on parr-smolt transformation in chinook salmon has been seriously hampered by the lack of a rigorous definition of a "smolt." Equating "smolting" with migration is not satisfactory in chinook as it is in steelhead and coho salmon, since a number of different types of migrants have been identified (Lichatowich 1977; Schluchter and Lichatowich 1977). In this and subsequent papers, we have adopted a definition which reflects the management position of the smolting process. We have defined a chinook smolt as a juvenile in fresh water which undergoes certain physiological changes, undertakes migration to the sea and successfully survives in the sea to return as a spawning adult. While this definition seems rather extensive, it does include the components required for use in a hatchery or management context. A successful smolt must be able to survive and grow in the ocean to provide harvestable fish for the ocean and fresh water fisheries and to produce the spawning adult.

Since this definition does not permit the identification of an individual "smolt" until its capture in a fishery or its return as an adult, the study of parr-smolt transformation in juveniles relies heavily on collaborative evidence from scale analyses of returning adults. In other salmonids, downstream migration and parr-smolt transformation are highly correlated. Zaugg and Wagner (1973) found that high levels of gill (Na+K)-activated ATPase activity were correlated with downstream movement in steelhead trout. Kerstetter and Keeler (1976) confirmed this in hatchery-reared steelhead on the Trinity River. Zaugg and Lorz (unpublished) and Lorz and McPherson (1976) found similar correlations in coho salmon.

Other studies have demonstrated cyclic changes in gill (Na+K)-ATPase activity but have not related these changes to other measurements of the smolting process (Zaugg and McLain 1972; Zaugg et al. 1972; Giles and Vanstone 1976). Without external reference to the smolting process (downstream migration or scales of returned adults) it is difficult to rule out cyclic changes unrelated to parr-smolt transformation as the agents responsible for these increased levels of activity (Beatty 1966).

Present data indicate that there are two peaks in gill (Na+K)-activated ATPase activity in Rogue River spring chinook salmon. The peak in September-October occurs at a time when approximately 85% of the juveniles which return as adults first enter the ocean (Schluchter and Lichatowich 1977). During the second peak the following April and May, this percentage drops to 8%. Thus, the gill (Na+K)-activated ATPase cycle in these spring chinook differs in its timing from those reported for other anadromous salmonids in that it possesses at least two cycles in specific activity with the most important cycle occurring in September and October.

The influence of fish size on gill (Na+K)-activated ATPase activity has not been reported in other studies, possibly because most of these have dealt with yearlings. In our yearling populations, we have found that there is little relationship between size and specific activity. In smaller, younger fish, however, this relationship is quite strong (Fig. 4) and persists until the fish reach sizes greater than 15-16 cm. This relationship appears to depend upon changes in the ATPase activity with size rather than changes in protein content of the gills. The size of the fish regulates the extent to which the gill (Na+K)-ATPase activity is increased in response to photoperiod. In the range of 10-12 cm, there is a large increase in specific activity from September to October (Fig. 4); however, in both larger and
smaller size classes, there is little change in specific activity. It has been shown from the analysis of adult scales that approximately one-half of the returning spring chinook adults in the Rogue River entered the ocean at 10-12 cm.

Examination of the curve in Fig. 5 suggests that there is a minimum length which a juvenile must attain before it can enter the gill (Na+K)-activated ATPase cycle. The length which must be attained by September-October is greater than 8-9 cm. This hypothesis is strengthened by the results shown in Fig. 2. Under advanced photoperiods, early juveniles (7-8 cm) did not show a peak in July, when the day-lengths were those of October. Critical size for smolting has been reported for steelhead (Wagner 1974), although the size threshold was approximately 16 cm long, almost twice the hypothesized critical size for spring chinook. Results from experiments designed to test this minimum size hypothesis are currently being analyzed and will be reported in this series at a later time.

The relationship between water temperature and gill (Na+K)-ATPase activity appears to be different for chinook from that reported by Zaugg and McLain (1976) for coho salmon and by Adams et al. (1973) for steelhead trout. There was no relationship between gill (Na+K)-ATPase activity in either the 8 or 12 C temperatures examined (Table 4). Increased activity of gill (Na+K)-ATPase in fish reared at 12 C could be accounted for on the basis of increased growth rates at the higher temperature and the relationship between size and gill (Na+K)-ATPase levels (Fig. 4). In addition, we found no indication of inhibition of gill (Na+K)-ATPase activity at 12 C, although this is somewhat below the critical temperature of 15 C reported for steelhead and coho (Zaugg et al. 1972; Zaugg and McLain 1976). Fish residing in the Rogue River might be expected to exhibit higher tolerances through adaptation to the high temperatures often present during the summer months.
LITERATURE CITED


