Skin Reflectance as a Non-Lethal Measure of Smoltification for Juvenile Steelhead at Dworshak National Fish Hatchery

Final Report

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Introduction

At Dworshak National Fish Hatchery, the Idaho Fish Health Center (FHC) has responsibility for conducting smolt assessments of summer steelhead prior to their release in the spring. The assessment includes a wide array of fish health and condition parameters and the use of the Goede Index. However, the Idaho FHC does not have an index or measure to actually assess the smoltification process itself as it develops throughout the spring. An index of smoltification could be very useful in assessing the readiness of summer steelhead for release from the hatchery and as an evaluation tool for recommending changes in rearing and production practices.

Juvenile salmonids go through a series of physiological, morphological, and behavioral changes while they are rearing in freshwater that prepare them for migration to the ocean. These biological changes that occur are collectively referred to as the parr-smolt transformation process, or smoltification (Wedemeyer 1996; Folmar and Dickhoff 1981; Wedemeyer et al. 1980). Some of the changes that occur have been developed into smolt indices to monitor the smolt transformation process. A common index of smoltification is condition factor, the relation between fish length and fish weight (Wedemeyer 1996). Condition factor typically decreases during smoltification and has been used successfully as a criteria of smoltification in steelhead (Wagner 1974). The change is apparently associated with decreases in total body lipids (Fessler and Wagner 1969).

Another change that occurs during smoltification is the development of saltwater tolerance and an increase in hypoosmoregulatory ability. This change requires an increase in gill Na+, K+ -ATPase levels, an enzyme system that provides the energy necessary for the chloride cells in the gills to actively transport ions absorbed from the ocean back into seawater (Wedemeyer 1996). Gill ATPase levels have been used extensively in research to evaluate the success of various rearing programs at many Columbia River hatcheries (Zaugg et al. 1991). However, measuring gill ATPase levels is not practical for routine monitoring at the production level. Cost of sampling and analyzing the tissue is too high. Currently there are no commercial laboratorys available where routine samples can be sent for analysis. Besides, the turn around time for the data after the samples are collected is often so long that the data is not available for making timely management decisions.

Another change that smolting fish exhibit is an increase in “silvering” due to the reflection of light from guanine and other purines deposited in the scales and deep skin tissues (Hoar 1976; Folmar and Dickhoff 1980). Guanine and hypoxanthine are produced as a result of seasonal

thyroid hormone-induced increases in protein catabolism (Wedemeyer 1996). Guanine levels have been used as an assessment tool in the past, but the sampling method is lethal and the process is very tedious and time consuming (Johnson and Eales 1968). Haner et al. (1995) developed a non-lethal method for quantitatively measuring skin "silvering" using a photo reflectance video analysis system (PRVAS). The authors found that mean skin reflectance of steelhead and spring chinook salmon released from Dworshak and Kooskia National Fish Hatcheries was significantly correlated with mean gill ATPase activity and mean skin guanine concentrations. The authors concluded that skin reflectance could be used as a non-lethal and cost effective indicator of smolt development. Haner et al. (1995) monitored skin reflectance immediately prior to release and during the outmigration process but did not collect data for any extended period leading up to release. Using their system as a means of pre-release monitoring and assessement has never been tested.

The goal of this study was to determine if skin reflectance could be used as a non-lethal, low maintenance, low cost method of monitoring juvenile steelhead smoltification at Dworshak NFH during the two to three month period prior to release. The objectives of the study were:

1) To measure the quantitative changes in gill ATPase activity, skin reflectanace, and condition factor for steelhead during the three month period before their release from the hatchery,

2) To quantitatively measure the relationship between gill ATPase activity, skin reflectance, and condition factor for steelhead during the three month period before their release from the hatchery.

Methods

Collecting and Processing Fish

To measure changes in gill ATPase, skin reflectance, and condition factor over time, fish were sampled once monthly during Februrary, March, and April, 1997. Random samples of 20 fish were taken from ten Burrows ponds representing three separate experimental groups and two controls. Fish were anesthetized using tricaine methanesulfonate (MS-222). Data for skin reflectance was obtained by placing each fish into a 24 x 13 x 10 cm plexiglass box equipped with two photo bulbs that produced diffuse light (Haner et al 1995). The fish was placed into a small trough that could be easily inserted and removed from the box. The fish, one black and one gray calibration tab, a label identifying the fish, and enough water to just cover the fish were placed in the trough. An image of the fish was taken using an Epson Photo PC 600 XGA color digital camera . After the photo image was obtained, each fish was measured for fork length (mm), weight (g), and a gill tissue sample was taken to determine gill ATPase activity. Gill ATPase was measured using the microassay technique developed by Schrock et al. (1994). Condition factor was calculated as K (Anderson and Gutreuter 1983).

Converting Photos to Quantitative Values of Skin Reflectance

The images were downloaded from the camera to a computer using Epson Image Expert 1.1.2 software. For each Burrows pond, fish images weres "named" using the fishes identification number and the entire group was then saved in an "album" under the corresponding pond number and date.
Sigma Scan Pro Image Analysis (Version 4.01.003) was used to calculate actual reflectance. Area measurement was used for skin reflectance and was defined as a sum of calibrated pixel units in a defined area. The black and gray tabs were used to calibrate the pixels. The area was chosen by first drawing a line from the posterior end of the pectoral fin up to the lateral line. The second line was drawn along the lateral line just to the anterior portion of the dorsal fin. A line was then drawn directly down to the ventral side of the fish. The final line was drawn back to the initial beginning point. The values could then be saved into Lotus for further evaluation.

Data Analysis and Statistical Comparisons

Data from replicates were combined into a single sample. Mean values for skin reflectance, gill

ATPase activity, and condition factor were calculated monthly. Pearson's correlation coefficients were calculated for comparisons between mean monthly values of skin reflectance, condition, and gill ATPase activity. Statistical analyses were performed using Systat (Wilkinson 1990) and differences were considered significant at $P \leq 0.05$.

Results

Skin Reflectance

Mean skin reflectance increased from February to April for all five experimental groups (Table 1). However, the rate of increase varied considerably. For example, mean skin reflectance for the Growth experimental group increased by 1.3 units from February to March but by only 0.1 units from March to April. In contrast, mean skin reflectance for the Control group in that experiment increased by 0.5 units from February to March and from March to April. The Abernathy experimental group and the Control group for that experiment were the only groups that exhibited increased rates of increasing mean skin reflectance from February to April.

Gill ATPase

Similar to mean skin reflectance, mean gill ATPase activity increased each month for all five experimental groups (Table 1). Unlike skin reflectance, however, mean gill ATPase exhibited a consistent rate of increase from February to April for all five experimental groups, with one exception. There was no increase in mean gill ATPase from February to March in the Control group for the Growth experiment.

Condition Factor

Mean condition factor did not exhibit the classical decreasing trend characteristic of smoltification except in the Control group for the Growth experiment (Table 1). Mean condition factor for this group exhibited an increased rate of decrease from February to April. All four of the other experimental groups exhibited a slight increase in mean condition factor from February to March followed by a decrease from March to April. However, the decrease from March to April did not always result in a mean condition factor that was lower than in February.

Correlations Between Indices

Very strong positive correlations were calculated between mean skin reflectance and mean gill ATPAse activity (Table 2). Strong negative correlations were calculated between gill ATPase and condition factor and between skin reflectance and condition factor for most of the groups examined. However, the results were not consistant. None of the correlations were statistically significant because sample sizes were too small.

Discussion

I guess we shouldn't be surprised to see such a slow rate of increase in skin reflectance since gill ATPase usually does not increase on the hatchery very much either. What is encouraging about this is that there does indeed appear to be a very strong relationship between skin reflectance and gill ATPase. We will need to do some further work with skin reflectance with both spring chinook and steelhead in order to get to the point where we can be sure of its reliability to tell us how our fish are developing. The pre-release smolt assessments on the BY 97 spring chinook salmon in April 1999 provides a good example of how this technique could have been useful. The Fish Health Center examined 60 fish and determined that they were probably not quite ready for release because they did not appear to be as silvery as in previous years. Parr marks were clearly visible. Skin reflectance data from previous months and prior years could have been very useful for comparisons, had it been available, and the Center could have included a quantitative assessment to their evaluations.

Table 1. Statistical summary of skin reflectance, gill ATPase, and condition factors for each of the treatment and control groups by month.

Treatment Group	Month	N	Skin Reflectance		Gill ATPase		Condition Factor	
			Mean	SD	Mean	SD	Mean	SD
Growth	February	39	4.3	1.2	3.4	1.2	0.95	0.12
	March	39	5.6	0.9	3.7	0.9	1.01	0.07
	April	40	5.7	0.8	5.5	1.6	0.95	0.05
Growth Control	February	38	4.5	1.3	3.6	0.7	0.97	0.05
	March	39	5.0	1.3	3.6	1.1	0.95	0.11
	April	40	5.5	1.1	5.0	1.4	0.92	0.04
Abernathy Diet	February	40	4.0	0.9	3.1	0.7	0.94	0.11
	March	38	4.5	1.0	3.4	1.0	0.95	0.09
	April	39	5.1	0.9	4.6	1.3	0.94	0.06
Moore-Clark	February	39	4.1	1.2	3.3	0.6	0.95	0.14
	March	38	5.0	0.9	3.4	0.8	0.97	0.05
	April	40	5.4	0.9	6.8	2.3	0.96	0.04
Diet Control	February	39	4.3	1.3	3.5	0.9	0.98	0.05
	March	40	4.7	1.3	3.7	1.1	0.99	0.09
	April	40	5.3	1.1	5.2	1.7	0.95	0.05

Table 2. Pearson correlation coefficients calculated for comparisons of skin reflectance, condition factor, and gill ATPase for each treatment and control group.

Treatment Group	Reflectance x Condition	Reflectance x ATPase	Condition x ATPase
Growth	0.44	0.66	-0.38
Growth Control	-0.99	0.87	-0.92
Abernathy Diet	-0.05	0.96	-0.33
Moore-Clark Diet	-0.80	0.75	0.03
Diet Control	-0.80	0.96	-0.94

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