Project 198740100

Assessment of Smolt Condition: Biological and Environmental Interactions

Sponsor: USGS, CRRL
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ISRP: paragraph 1, last sentence; paragraph 3. Separate new proposal from on going tasks.

The proposed tasks are not new but represent a progression of increasingly detailed investigations of environmental and biological factors that influence smolt development and condition. Our primary objective, since 1999, has been to investigate the effects of rearing (hatchery and wild) and river conditions on the physiology and migration success of juvenile salmonids. We have also adapted two methods for mass screening of lysozyme levels to monitor condition and development during rearing. We have applied the methods in both cooperative studies and technical assistance activities, and have found that individual fish in poor condition may be identified using this method. The table outlines the emphases of cooperative research and technical assistance activities for the project since 1997 that have led to the current objectives.

Emphasis	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**
Smolt Condition						
travel time/transportation	*	*	*	*	*	*
immunity - disease incidence/prevalence	*	*	*	*	*	*
in-river survival/estuary	*		*	*	*	*
temperature	*	*				
precocity/residualism—control by growth and temperature, higher incidence in hatchery fish	*		*		*	*
semi-natural rearing – natural bottom pond		*	*	*		
release strategies/acclimation – serial releases, acclimation ponds	*	*	*	*	*	*
enhanced feeds/glucan – effects on growth and immunity	*	*	*		*	*
stress/handling - transportation,		*			*	*
reintroduction/supplementation	*	*	*		*	*
rearing conditions at production facilities – density, temperature, flow, water quality, feed enhancement, release condition, release strategies	*	*	*	*	*	*
genetic differences hatchery v. wild			*	*	*	*

We believe strongly that this is not new research, but is a logical and adaptive extension of our work with hatcheries to determine how environmental factors influence fish development, condition, and long-term survival. The proposed tasks describe a progressive approach to distinguish among environmental and genetic effects on juvenile salmonid development and condition. We originally proposed this approach in 2001 and several new submissions including 35014, 35027, 35039, and 35041 are now proposing related approaches for investigating genetic and phenotypic traits in wild and hatchery populations. Our emphasis on the effects of the aquatic environment, especially factors that are known to affect growth, development, and disease resistance, is the logical result of our extensive analysis of hatchery rearing, environmental, physiological, demographic, in-river, and adult return data. Those analyses determined that (1) smolt growth was correlated to many rearing and environmental conditions, and (2) estimated growth rates were correlated to the first principal component for three water quality variables, which include temperature and changes in temperature, and average monthly proportion of river water to which fish were exposed over the entire rearing period (see Attachment A). The results of our studies of feed enhancers (with the USFWS in 1997, unpublished data), and application of enhanced feeds to improve growth (Dworshak NFH, 1998-99, www.efw.bpa.gov/Environment/EW/EWP/DOCS/REPORTS/DOWNSTRM/D35245-9.pdf) demonstrated that: 1) immunostimulants can enhance fish growth and disease resistance, 2) responses are different at different times of year and life stage, and 3) effects persist after release and during seawater growth. Based on these results, we propose to determine if characteristics of the rearing water contribute to these effects, and if there are stock specific responses to different aquatic environments.

ISRP: paragraph 1, 4th sentence; paragraph 2, 3rd and 4th sentences. "...descriptions of methods and tasks are inadequate."

It appears that the ISRP may not have seen Part 1 of the proposal submission that included specific statements of tasks associated with the five objectives of the proposal. We hope that the following narrative, and listing of tasks and methods will address the ISRP concerns.

The main goal of this proposal is to address the question of how differences between hatchery and wild fish develop and influence survival to adulthood. This question was stated eloquently in the ISRP review comments to proposal 35041: "The critical uncertainty about differences in fitness between wild and hatchery-produced fish lies at the heart of most of the ongoing and proposed research into captive broodstock and supplementation technology, and seemingly at the core of RPA 182 also. Indeed, understanding differences in fitness between the two groups, and whether conservation-oriented hatcheries can produce fish that can integrate into natural populations and lead to long-term sustainability (i.e. the fitness question) is the $64 million question around which much of the present recovery plan hinges."

Our objectives are designed to incrementally address both environmental and genetic influences on smolt condition.

Objective 1. Provide science support and technical assistance to federal, state, and Tribal fishery agencies to determine if juvenile salmonid condition is determined by biological and environmental interactions that are distinguishable from genetic effects.

Task 1.1 Prioritize research stocks and select from at-risk species based on HGMP results (RPA 169).

The selection of a hatchery and wild stock for the experiments will be restricted by production schedules and the necessary ESA permits. We are currently working in the Upper Columbia Spring Run Chinook ESU where naturally spawning populations are listed as endangered and have been designated as the second priority in recovery efforts. We are cooperating with agencies involved in the recovery efforts, and plan to use local stocks for the investigation.

Task 1.2 Verify genetic origin or conduct genetic sampling/analysis in cooperation with regional genetics programs.

Many stocks have already been characterized, or this information may become available through related projects such as 198909600, 199005200, 199202200, 35014, 35041 or from the state programs. It is especially important to determine how the genetic origin of the test group may influence the range of responses we are measuring. If domestication selection is more than a theoretical construct, and the use of non-local broodstock is to be curtailed, then all studies investigating how different stocks interact with their environment could contribute to the effort by providing the pedigree of their test fish so results can be integrated with those from other studies.

Task 1.3 Develop research design based on site specific environmental variables relevant to ecology of local natural or wild spawning populations, and proximate hatchery stocks. See design below.

Task 1.4 Cooperate with regional management agencies to coordinate juvenile rearing investigations with measures of adult reproductive success.

Coordinate with hatchery CWT program. Coordinate with regional PIT-tagging programs to insure that we can detect differences in behavior and survival of test groups.

Task 1.5 Provide analytical support to regional juvenile salmonid projects, complete ongoing analysis and reporting.

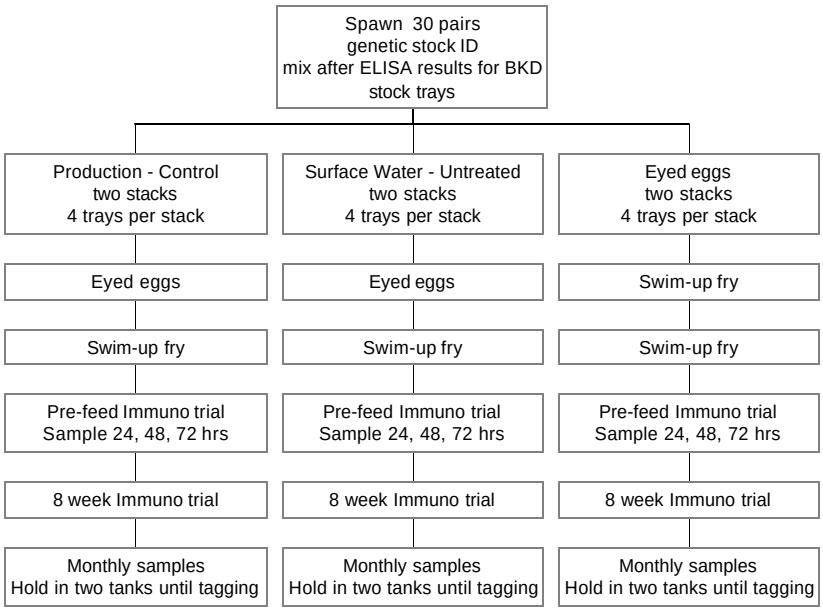
Complete second year of support at Hagerman National Fish Hatchery and Warm Springs National Fish Hatchery.

Task 1.6 Organize annual smolt workshop.

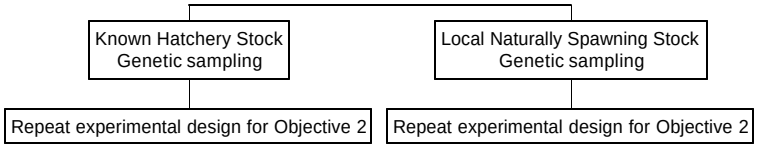
Objectives 2 through 5 were developed as a result of work we supported (i.e., cooperative research or technical assistance) in previous years to determine selective factors at hatcheries that contribute to differences in physiological and immunological development between wild and hatchery fish. Results from 1999 suggested that exposure to river water is a significant contributor to growth and smolt development. Therefore, we ran a pilot study to determine if the water sources available to hatcheries, usually groundwater, might be influencing fish development (see the next response for some of those results). Furthermore, because we were already involved in evaluations of immunostimulants in hatcheries as feed enhancers, we also looked at naturally occurring immunostimulation from the different water sources. This was not new work, but was a part of our investigations to look at differences in the wild and hatchery environment, in this case potential sources of immunostimulants The consistent source of immunostimulants is the water. Great differences exist between surface and ground water based on water chemistry and biotic components—including food. We will conduct initial and monthly water quality screening throughout the study. The water source is the single most important variable that remains to be investigated, and the following objectives address that need.

Proposed design for objectives 2-5.

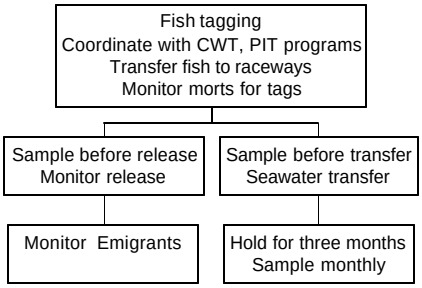
Environmental Exposure Experiments: Single Stock
Early Rearing Phase (Objective 2)



Environmental Exposures: Two Stock Comparison
Early rearing phase (Objective 3)



Behavioral and Survival Testing of Progeny
from Objectives 2 and 3
(Objectives 4 and 5)



Objective 2. Determine if juvenile salmonids of the same genetic origin show differential growth and condition under varied controlled environmental conditions.
Task 2.1 Rear fish of the same genetic origin in different environmental regimes from the time of spawning through the time of release.
Task 2.2 Use controlled immunostimulant exposures to determine differences in response of fish reared in different environmental regimes.
Task 2.3 Analyze physiological and immunological samples from different treatment groups.
Task 2.4 Genetic sampling and analysis.
Task 2.5 Data analysis.
Task 2.6 Report preparation.

Objective 3. Determine if juvenile salmonids of the same species of different genetic origin show differential growth and condition under similar environmental conditions.
Task 3.1 Rear fish of known genetic origin in similar environmental regimes from the time of spawning through the time of release.
Task 3.2 Use controlled immunostimulant exposures to determine differences in response of fish reared in similar environmental regimes.
Task 3.3 Analyze physiological and immunological samples from different treatment groups.
Task 3.4 Genetic sampling and analysis.
Task 3.5 Data analysis.
Task 3.6 Report preparation

Objective 4. Determine if juvenile salmonids of the same genetic origin show differential emigration behavior or seawater survival when reared in different, controlled rearing environments.
Task 4.1. PIT tag fish of the same genetic origin reared in different environmental regimes (coordinate PIT tagging with CWT and other PIT tag programs).
Task 4.2. Monitor PIT tagged fish during outmigration.
Task 4.3. Collect PIT tag/CWT information on adult returns.
Task 4.4. Genetic sampling and analysis of adult returns.
Task 4.5. Data analysis.
Task 4.6. Report preparation.

Objective 5. Determine if juvenile salmonids of the same species of different genetic origin show differential emigration behavior or seawater survival after rearing in similar environmental regimes.
Task 5.1 PIT tag fish of different genetic origin reared in similar environmental regimes (coordinate PIT tagging with CWT and other PIT tag programs).
Task 5.2 Monitor PIT tagged fish during outmigration.
Task 5.3 Collect PIT tag/CWT information on adult returns.
Task 5.4 Genetic sampling and analysis of adult returns.
Task 5.5 Data analysis.
Task 5.6 Report preparation.

ISRP: paragraph 2, 5th sentence. "No design for statistical analysis is presented."

Measurements for each factor will be compared for single treatments among dates to test for differences at different stages of growth, and among treatments on a single date to test for treatment effects by analysis of variance using the GLM procedure of SAS , Version 8.1. Using the same program, least squares analysis will be performed to test for origin effects when stock origins are known or genetic sampling accompany the physiological evaluations (Fevolden et al. 1991, 1994; Fevolden and Roed 1993).

Fevolden, S. E., T. Refstie and K. H. Roed. 1991. Selection for high and low cortisol stress response in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). Aquaculture 95: 53-65.

Fevolden, S. E. and K. H. Røed. 1993. Cortisol and immune characteristics in rainbow trout (*Oncorhynchus mykiss*) selected for high or low tolerance to stress. Journal of Fish Biology 43: 919-930.

Fevolden, S. E., K. H. Røed and B. Gjerde. 1994. Genetic components of post-stress cortisol and lysozyme activity in Atlantic salmon; correlation to disease resistance. Fish & Shellfish Immunology 4: 507-519.

Based on power analyses of our earlier work on juvenile coho salmon and spring chinook salmon (Schrock et al. 2001) a sample size of 80 with a = 0.05, will detect differences in mucous lysozyme of skin, vent and nare of 10, 64 and 103 µg/mL respectively, with 90% power. In the current study, using a new lysozyme assay, differences of 965 U/mL can be detected in kidney tissue. This sample size is well in excess of sample sizes from referenced studies for the other immunological tests we propose. All of these differences represent 10% - 30% of values from controls. Sample sizes will equal 80 eggs, fry, or juveniles per treatment.

Water quality:

- Daily temperature and flow
- Initial and monthly water chemistry
- Heterotrophic plate counts
- Total fungus counts

Immune factors:

- Lysozyme
- Total protein and albumin for calculation of total immunoglobulins
- IgM
- Complement assay

Physiology :

Weight and length, condition factor
Weight of lymphoid tissue to whole body weight
Gill ATPase of parr and smolts

IgM levels have been found not to differ in two lines of fish selected for high and low lysozyme, however they did differ after vaccination (Roed et. Al 2002). This particular measurement will be an important measurement after the immunostimulant trials.

Roed, K H., S. E. Fevolden, and K. T. Fjalestad. 2002. Disease resistance and immune characteristics in rainbow trout (*Oncorhynchus mykiss)* selected for lysozyme activity. Aquaculture 209:91-101.

We refer to Ewing et al 1998 in developing our sampling protocol for hatcheries. We sample 80 fish per tank or raceway, using eight dip net samples from different locations in the raceway with ten fish collected from each dipnet. Excess fish in the net are placed in a tote and returned to the raceway after sampling of all 80 fish is completed.

Ewing, R. D., T. R. Walters, M. A. Lewis and J. E. Sheahan. 1998. Evaluation of inventory procedures for hatchery fish III. Nonrandom distributions of chinook salmon in raceways. Progressive Fish-Culturist : 159-166.

ISRP: paragraph 2, 6th sentence. " The intention and value of the genetic screening are not clear."

Our purpose is to determine if observed physiological and behavioral (or developmental) differences between hatchery and wild fish are driven by differences in the wild and hatchery environments. Genetic screening is required in order to account for, and minimize, genetic differences in our test fish. Furthermore, the genetic screening will document the origin of the fish used in the study for coordination with projects such as 35014. Objectives 2 and 4 require that the fish be of the same stock, however in the subsequent tasks, 3 and 5, comparison of the test stock with the corresponding wild or hatchery stock will be made. Our ability to separate the genetic from environmental basis for differences in response of various stocks, be they hatchery or wild, will not be complete unless we know the stock origin of the fish, and understand how the rearing environment shapes phenotypes from genetically distinct groups, and how these groups respond to the different environments encounter throughout the life history. This is of interest to managers, especially in programs that will use hatcheries to rear fish for supplementation or restoration purposes. Results may also be considered by hatcheries in selecting stocks to replace out-of-basin hatchery stocks with local stocks. The question of the genetic origin of the test fish is not unrelated to the study – levels of immune factors such as lysozyme are know to demonstrate heritability (Fevolden et. al 1994) and are known to change in selective breeding programs. It is a regional priority to characterize all stocks for better management of wild/hatchery interactions.

198740100 Attachment A Analysis of production variables, smolt, condition, travel time and adult returns of Winthrop Hatchery Spring Chinook releases 1987-94.

This analysis of compiled hatchery rearing and environmental data from Winthrop National Fish Hatchery represents the most complete of ten hatcheries reviewed for the investigation. The purpose was to determine what factors influence juvenile growth and size, mortality, and physiological condition at the time of release. There was a significant relationship between smolt condition at the time of release and travel time. The average proportion of river water in the raceways is one of three variables, including temperature and changes in temperature that explain variability in growth rates. It is the single variable that has not been investigated.

Figure 1: Relationships among variable types in the Winthrop Hatchery data set.