PRELIMINARY RESEARCH PROPOSAL
SUBMITTED TO THE U.S. ARMY CORPS OF ENGINEERS UNDER THE ANADROMOUS FISH EVALUATION PROGRAM
2007 PROJECT YEAR

I. BASIC INFORMATION

A. TITLE OF PROJECT

Pressure Acclimation Investigations to Support Biological Index Testing

B. PROJECT LEADERS

Thomas Carlson – Principal Investigator, Pacific Northwest National Laboratory, P.O. Box 999, Richland, WA 99352, Tel: 503/417-7562 or 503/784-6356, Email: Thomas.Carlson@pnl.gov

C. STUDY CODES

TSP-05-01

D. ANTICIPATED DURATION

October 2006-December 2007

E. DATE OF SUBMISSION

August 2006 On-Going from FY05

II. PROJECT SUMMARY

A.1. RESULTS TO DATE

2005 Study Results: In 2005 the response of depth acclimated run of the river spring and fall chinook to exposure to a simulated turbine passage pressure time history with a nadir pressure between 1.5 and 2.5 psi was tested. Study treatment factors were: acclimation depth (surface, 10 ft, 20 ft, and 40 ft), presence or absence of a radio frequency tag, and gastric or surgical tag implantation. During acclimation test fish were permitted access to an air bubble to permit them to fill their air bladders to achieve neutral buoyancy.

For spring chinook, 48 hour mortality was found to increase with depth, and mortality for fish implanted with radio transmitters was higher than that for fish without transmitters. In addition, mortality was higher for fish with gastrically implanted tags than for those with surgically implanted tags.

The 48 hour mortality for fall chinook was similar in pattern to that for spring chinook except mortality rates were generally higher.

Necropsies and pathology examinations of test fish determined the causes for death. These were: bubbles in the gills and heart and hemorrhaging of the vascular system. A high incidence of swim bladder rupture was also observed. Miscellaneous other causes of mortality were also observed.

The principal causes of death, bubble formation in the gills and heart along with caudal vein rupture and hemorrhaging in the heart cause concern for the exposure of run-of-the-river fish to levels of total dissolved gas saturation higher than those present during this test. Total dissolved gas levels in the test chambers were about 102%, considerably less than the 115% present in the forebay of McNary dam.

Time to death was variable with some fish dying at exposure and others within a 48 hour holding period. Necropsies identified injuries that would most likely have been fatal at some indeterminate time following exposure or would likely have rendered the fish more susceptible to predation. These included damage to the spleen or loss of the spleen of surgically implanted fish, and rupture of the caudal vein and swim bladder. In addition to this physical injury, some fish were observed to be temporarily stunned or disoriented following exposure.

Swim bladder rupture did not always result in a test fish being negatively buoyant and unable to achieve neutral buoyancy during holding. In some cases swim bladder rupture resulted in air being trapped in the body of the fish rendering the fish unable to expel the gas, the result being persistent positive buoyancy.

Loss of tags was also observed for both gastric and surgical implants. The mechanism of loss was ejection of the tag from the mouth or surgical scar. In the case of ejection from the surgical scar the tag would be carried by the fish until the transmitter antenna was pulled free of the fish's body by drag forces acting of the body of the tag as the fish swam.

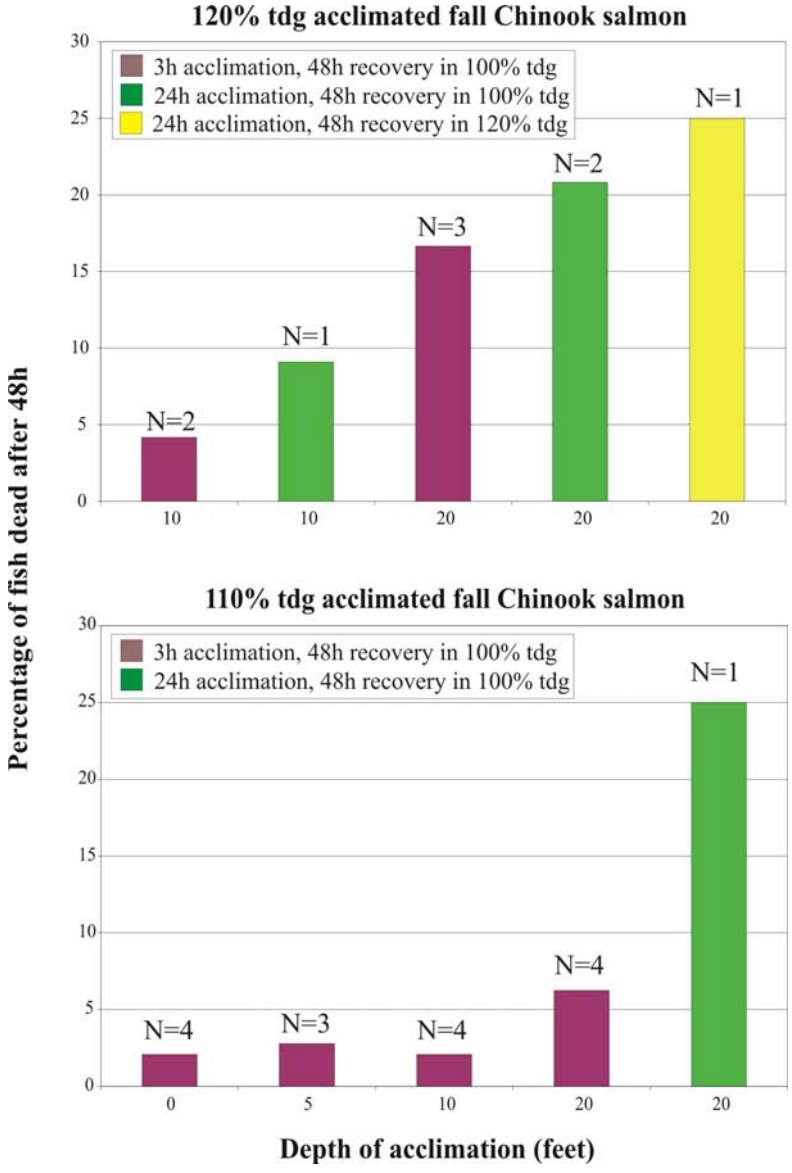
Mortality and injury rates were consistently higher for tagged than untagged fish. The reason for this is not obvious and was not determined during the study. However, results to date indicate that turbine passage survival estimates for spring and fall chinook using the tags tested may be biased. In addition, injuries that would have resulted in delayed mortality of exposed fish or might increase their susceptibility to predation were also observed.

The results of the 2005 study point to several lines of inquiry that might help explain differences between direct turbine passage survival estimates obtained using balloon tags and turbine passage route survival estimates obtained using telemetry.

2006 Study Results: The original goals for FY06 studies focused on identification of the turbine passage pressure nadir threshold for direct mortality of turbine passed juvenile chinook and to investigate nonlethal injuries that might contribute to indirect mortality for turbine passed fish. The information obtained by achieving these goals was to provide design guidance for turbine runner replacement actions during turbine rehabilitation programs and to aid implementation of Biological Index Testing (BIT) for turbine operations.

Delays in delivery of new hyperbaric chambers resulted in modification of the FY06 plan of study, which, in turn, resulted in focus on tests to better define elements of experimental designs that would incorporate Total Dissolved Gas (TDG) thereby more accurately representing the exposure history and physiological condition of juvenile chinook prior to turbine passage. In an associated study of turbine passage conditions using sensor fish, analysis of sensor fish data files resulted in reconsideration of turbine passage pressure time history characteristics needed to adequately represent this aspect of study design.

At the time of this pre-proposal, initial results from 2006 studies are showing that each of the considered experimental factors: (1) TDG acclimation level and duration, (2) depth equivalent pressure acclimation, and (3) TDG exposure during recovery causes an increase in mortality that also varies between sub-yearling and yearling juvenile chinook. In studies completed to date, as acclimation depth increases so does mortality, as acclimation time increases when fish are exposed to higher levels of TDG saturation so does mortality and finally, if fish are recovered in water with elevated TDG saturation, mortality increases (see figure below). Although not isolated as a study variable in 2006 tests using river-run juvenile chinook, it also appears that water temperature is another factor that impacts fish survival in an additive manner, particularly in the presence of TDGS.



Given the number of factors that may affect the survival of turbine passed fish, study designs to isolate the variables and examine the effects of combinations of variables, require larger sample sizes per treatment, a larger number of treatments, better control of test fish condition, and control

of extraneous variables, such as water temperature. These study requirements indicate that much experimental work needs to be done outside of the yearling, and sub-yearling chinook outmigrations. For this reason the surrogacy of hatchery chinook for river-run yearling and sub-yearling chinook is also being investigated and will be included as a study variable in 2007 studies.

Preliminary results from 2006 surrogacy testing indicates that river-run yearling chinook respond to simulated turbine passage pressure exposure the same as hatchery yearling chinook. Testing of sub-yearling river-run fish is still underway, but partial results indicate findings similar to those for yearling chinook, that being hatchery and river-run fish respond the same to simulated turbine passage pressure exposure.

Given the results obtained in 2005 and 2006 the strategy for the 2007 study is to conduct a multi-factor experimental design beginning in October 2006 and ending in March 2007 using hatchery chinook. The assumption of surrogacy of hatchery chinook for river-run yearling and sub-yearling chinook will be reevaluated by exposing river-run chinook to a subset of the 2007 study treatment matrix and testing the hypothesis that the mortality rates for river-run fish are the same as those observed for surrogates.

Treatment exposures in the 2007 study will include the factors given in section II.B. The termination point for treatments will be when test fish are removed from the hyperbaric chambers. Test fish will not be held to evaluate delayed mortality. Upon removal from the chambers all test fish will be euthanized then necropsied to determine cause of death, in the event of mortality prior to that time, or to determine physiological condition at a time roughly equivalent to exit from a turbine's draft tube and powerhouse tailrace entry. A subsample of test fish will be prepared for histological examination and examination for the presence and quantity of a protein biomarker that may indicate the severity of brain trauma resulting from pressure exposure.

A.2. GOALS

1. Determine the turbine passage pressure nadir threshold for mortality of hatchery juvenile salmonids factored by buoyancy acclimation depth and total dissolved gas supersaturation acclimation.
2. Determine the occurrence and severity of sublethal injuries that could cause delayed mortality or increased susceptibility to predation for fish exposed to a turbine passage pressure cycle.
3. Test the assumption of surrogacy of hatchery juvenile chinook for 2007 river-run yearling and sub-yearling chinook.

B. OBJECTIVES

1. Perform a multi-factor study to evaluate the statistical significance on mortality and injury rates for juvenile chinook salmon of the factors and combinations of the factors listed below for an acclimation time of 24 hours and no holding period following treatment exposure:
    a. Yearling and sub-yearling chinook salmon;
    b. Turbine passage pressure nadir;
        i. 3, 6, and 12 and 24 psia
    c. Turbine passage pressure rate of change,

        i. 100, 300, and 500 psi/sec
    d. Neutral buoyancy acclimation depth,
        i. surface, 5 ft, 10 ft, and 20 ft
    e. Total dissolved gas acclimation level
        i. 100%, 110%, and 120%
2. Using necropsy and pathology methods developed during the 2005 and 2006 studies with the addition of protein biomarker analysis, assess the presence and severity of physical injuries to juvenile salmonids exposed to a turbine passage pressure cycle to determine:
    a. The cause of death for fish that die at the time of exposure or during holding following exposure,
    b. The incidence and severity of injuries for fish that survive treatment exposures.
3. Conduct a test of the hypothesis that the response of surrogate hatchery fish to simulated turbine passage pressure exposure under the conditions of tests conducted over the winter of 2006-2007 is the same as that of 2007 river-run chinook juveniles.

C. METHODOLOGY

Laboratory testing of the response of run-of-the-river subyearling and yearling chinook salmon to turbine passage pressure cycles will be conducted using hyperbaric chambers based on those used during the FY05 study that have been designed specifically for this type of testing.

Test fish will be loaded into the chambers in groups of approximately 12 fish. Each fish will be marked so that it can be recognized as an individual in video recordings. Over an acclimation period of 24 hours, test fish will be held at pressures corresponding to combination of acclimation depth and total dissolved gas supersaturation. While acclimating, test fish will be permitted access to air so that they can achieve “neutral buoyancy” at the acclimation pressure. Acclimation to neutral buoyancy will be evaluated by visual assessment of the swimming attitude of the fish in the test chamber; hereafter the term visual neutral buoyancy will be used to distinguish this assessment of fish condition from other means of accessing fish density relative to the water in which they reside.

Following acclimation, test fish will be exposed to a simulated turbine passage pressure time history. The turbine passage pressure time history will be derived from actual turbine passage pressure time histories obtained using sensor fish at mainstem Columbia River dams. The level of the nadir in the pressure time history and the rate of change in pressure during runner passage will be treatment factors in the study.

Following exposure the test fish will be removed from the test chamber. Dead fish will be removed from the sample and immediately sub-sampled and necropsied to determine the cause of death. The remainder will be retained for examination by a pathologist for additional effort to determine the causes of death. This examination will include detection and quantification of brain proteins produced in response to brain trauma experienced during pressure exposure.

The remaining fish will be euthanized and processed as were the fish that died during simulated turbine passage pressure exposure. At the end of the holding period all test fish will be sub sampled and either necropsied or submitted to detailed pathological examination.

The hatchery fish that will be used in over-winter testing will be obtained from excess inventory of chinook juveniles from regional hatcheries. Acquired juvenile chinook will be held at the PNNL hatchery where holding temperature and feeding will be managed to grow the fish out to

desired size on a schedule that accommodates planned needs for yearling and sub-yearling test fish. Over-winter testing will be conducted at the PNNL hatchery.

Tests of hypothesis of surrogacy will be conducted at McNary Dam using river-run fish obtained from the McNary juvenile fish facility.

The sample size of fish required to estimate the mortality rate for each treatment group has not been finalized.

D. RELEVANCE TO THE BIOLOGICAL OPINION

Evaluation of fish passage measures at mainstem dams requires accurate evaluation of the survival of fish passing by dams. Past estimates of direct and total turbine passage survival vary a great deal. It is not clear why direct survival estimates, typically made using balloon tagging methods result in survival estimates that are significantly higher than those obtained using telemetry methods where the behavior of test fish is assumed to mimic that of run-of-the-river fish. This differential mortality has been assumed to be due to predation, however, it may be that other factors related to the presence of telemetry devices may be causing test fish to be killed, injured, or disabled at a higher rate that run-of-the-river migrants, thereby becoming differentially vulnerable to predation. In the final analysis, assessment of potential bias in fish survival assessment methodologies is required to have assurance in the accuracy of fish dam passage route survival estimates.

III. PROJECT DESCRIPTION

A. BACKGROUND

A. 1. Problem Description

Research conducted in 2005 found that fish acclimated to depth with access to air during acclimation the exposed to a turbine pressure cycle showed an increase in mortality with depth. This research also showed that fish with radio telemetry tags had higher mortality than fish without tags. These results present two problems. The first problem is the mortality of fish without tags that are more representative of run-of-the-river fish than those with tags. The second is the indication of bias when radio telemetry tags are used to estimate turbine passage survival.

Studies conducted in 2006 indicated that exposure to TDGS during acclimation, during pressure cycle exposure to worst case pressure nadirs, and during holding each incrementally increase mortality and injury rates. Studies conducted during 2006 did not include the additional effect, if any, of the presence of a telemetry device in test fish. Studies to evaluate the use of hatchery juvenile chinook as surrogates for river-run juvenile chinook were also conducted in 2006. The preliminary results of this work indicated that the hatchery fish tested were surrogates for 2006 river-run yearling and sub-yearling chinook. This result supports use hatchery juvenile chinook as surrogates for river-run fish for the over-winter studies planned for 2006-2007. However, these results are not considered sufficient for application of any results observed in the over-winter studies without additional testing the assumption of surrogacy using 2007 river-run juvenile chinook. Surrogacy of juvenile chinook for other juvenile salmonids is not assumed and any results obtained are expected to apply to juvenile chinook only unless appropriate surrogacy testing shows otherwise.

A. 2. SITE DESCRIPTION

Over-winter pressure cycle testing with hatchery juvenile chinook will take place at the PNNL hatchery. Tests to further evaluate surrogacy will be conducted at McNary Dam using river-run juvenile chinook.

A.3. PRESSURE TESTING EXPERIMENTAL DESIGN

Yearling and subyearling chinook and any other species included in testing will be considered as separate experiments. Within each species/age group the following treatments will be applied:

1. Control – depth acclimation with access to air to visual neutral buoyancy in the pressure chamber at identified pressure equivalents and TDGS conditions, no pressure cycling, all other handling and processing the same as for other test fish.
2. Treatment groups – treatment groups and sample sizes are still in development.

Effects of interest to be examined by the experiments are:

1. Control – The biological impacts (injury characterization and immediate and delayed mortality rates) to test fish of pressure acclimation and handling.
2. Treatment – Treatment factors will include:
    a. Yearling and sub-yearling chinook salmon,
    b. 24 hr acclimation at one of three TDG conditions, 100%, 110%, and 120%,
    c. Acclimation with access to air at pressures equivalent to depths of surface, 5, 10, and 20 feet,
    d. Simulated turbine passage pressure nadirs of 3, 6, 12, and 24 psia,
    e. Simulated turbine passage turbine runner passage pressure rates of change of 100, 300, and 500 psi/sec

The data acquired during research conducted in 2005 and 2006 is being used to aid calculation of sample sizes for treatment groups.

Mortality and injury rate estimates obtained for each treatment group will be tested for significance.

A.4. LIMITATIONS/EXPECTED DIFFICULTIES

We expect few difficulties that might prevent completion of the studies as described. Very similar studies were successfully completed in 2005 and 2006.

New hyperbaric chambers originally scheduled for delivery in the spring of 2006 will be delivered in the fourth quarter of FY06. The new chambers use many elements of the design of the original chambers and the new design will address limitations of the original design.

Test fish for the study elements to be conducted over-winter in 2006-2007 will be acquired from the surplus stock of regional hatcheries. Acquired fish will be held and grown-out at the PNNL hatchery on a schedule that meets test requirements.

For test fish required for surrogacy hypothesis to be conducted during the spring and summer of 2007 we will rely on provision of test fish out of run-of-the-river fish that pass through fish facilities at MCN dam. At this time it is not possible to accurate estimate the availability of test fish. In the event that sufficient numbers of fish are not available to complete all treatments, the treatments will be prioritized prior to initiation of the study and the higher priority treatments will be completed firs.

A.5. EXPECTED RESULTS AND APPLICABILITY

We expect to identify a turbine passage pressure nadir and pressure rate of change that does not result in high injury and mortality rates for turbine passed yearling and sub-yearling chinook under TDGS conditions typical of the federal hydropower system during the spring and summer juvenile fish outmigration periods. This information is critical to future assessment of turbine designs and operations that could be implemented to reduce the loss of fish to pressure effects during turbine passage.

A.6. SCHEDULE

The conduct of pressure cycle testing will be completed in the first three quarters of FY07. Some data analysis and initial results, particularly for the over-winter multi-factor study, will be available in FY07, but completion of data analysis and reporting will take place in the first quarter of FY08.

Pressure cycle testing using hatchery fish will be completed in a six month period of time beginning as soon as possible in FY07. Surrogacy hypothesis testing will take place in two blocks. The first block will be yearling fish collected in the April-May time frame. The second will be subyearling fish collected in the June-July time frame. Letter reports summarizing the findings of each segment of work (over-winter, yearling surrogacy, sub-yearling surrogacy) will be submitted to the CE. The letter report for the over-winter study will be submitted in May 2007. A letter report for surrogacy testing for yearling chinook will be submitted at the completion of exposure testing in mid-summer and that for sub-yearlings in late summer. All other data (necropsy, histology, biomarker) will require additional processing and will not be available prior to completion of a draft final project report. The final draft report will be completed in the first quarter of FY07.

B. FACILITIES AND EQUIPMENT

B.1. REQUIREMENTS

New hyperbaric chambers are scheduled for delivery to PNNL in the fourth quarter of FY06. Acceptance testing, any rework, calibration, and certification are scheduled for completion prior to acceptance and delivery.

Test fish, hatchery and river-run juvenile chinook salmon are needed. Hatchery fish will be obtained from excess stock at regional hatcheries. River-run fish will be collected at MCN Dam. A source for any other test fish identified prior to completion of a final plan of study will have to be identified.

B.2. JUSTIFICATION FOR SPECIAL EQUIPMENT OR SERVICES

No special equipment other than that already in procurement is anticipated.

C. IMPACTS

C.1. OTHER RESEARCH

Impacts on other research are not anticipated.

C.2. PROJECTS

No special assistance from McNary or other project staff different from that of previous years is expected. Most issues with the location of test equipment and staff facilities, electrical power, water, and telephone lines will follow procedures developed in previous years.

C.3. BIOLOGICAL EFFECTS

A number of hatchery and river-run chinook salmon juveniles will be sacrificed for the pressure cycling study.

D. COLLABORATIVE ARRANGEMENTS AND/OR SUB-CONTRACTS

PNNL expects to subcontract with the fish pathologist who assisted with the pathology assessment of test fish in FY05.

IV. LIST OF KEY PERSONNEL AND PROJECT DUTIES

Thomas J. Carlson (Battelle) – Principal Investigator, technical oversight, project tasks as required, report preparation, project management.

C. Scott Abernethy (Battelle) – Staff Scientist, test fish animal care and experimental protocols, test fish experimental exposure, test fish necropsy, test data acquisition, data analysis, report preparation.

Richard S. Brown (Battelle) – Staff Scientist, test fish experimental exposure, videography, test fish necropsy, test data acquisition, data analysis, report preparation

Craig A. McKinstry (Battelle) – Staff Scientist, experimental design, statistical analysis.

V. TECHNOLOGY TRANSFER

The principal means of technology transfer will be reporting.

VI. BUDGET

To be provided under separate cover.