

**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

Development of a Head Trauma Biomarker for Assessing Injury Impacts to Salmon from Hydropower Operations

B. PROJECT LEADERS:

Dr. Ann Miracle, Pacific Northwest National Laboratory, P. O. Box 999, Richland, WA 99352, Tel: 509/372-4327

C. STUDY CODES

SPE-P-02-2
SBE-W-07-01
SBE-W-07-02-new
SPE-W-04-2
SPE-W-07-new
SBE-W-05-1
SBE-W-05-2

D. ANTICIPATED DURATION

January 2007 to December 2008

E. DATE OF SUBMISSION

August 15, 2006

II. PROJECT SUMMARY

The impact of hydropower configurations associated with fish passage on injury and mortality is an ongoing concern in trying to determine and reduce risk to populations of migrating fish. Changes in dam operations or addition of passage structures require testing and optimization to reduce risk of injury. Despite the lack of visible injury, certain passage strategies result in significant delayed mortality which has no clear link to probable cause of death.

In rats and humans suffering from traumatic brain injury, certain protein products released from the damaged brain tissue function as biomarkers for the severity of injury. For example, a human protein antibody biomarker has been developed by Banyan Biomarkers to determine the severity of brain injury for the US Army to use in rapid triage assessments.

In an independent research project (Miracle 2006), we successfully demonstrated the cross-reaction of the human polyclonal antibody with enzymatically-treated salmonid brain tissues to mimic brain injury. We then investigated the use of this antibody in juvenile salmon and found a strong correlation between injury protein expression and brain injury (Figures 1 and 2).

While the human polyclonal antibody has utility for use in salmon, a specific, monoclonal antibody would provide increased specificity and sensitivity and could be used to sample plasma (i.e., non-invasive method) rather than brain tissues. We propose to use a salmonid-specific biomarker assay to investigate short term response of brain injury and associated recovery using samples collected from ongoing field hydropower testing in the Columbia basin.

III. PROJECT DESCRIPTION

A. GOAL

This project will result in the application of a salmon specific protein biomarker that will quantitatively assess the degree of head trauma after migration through hydropower operations and correlate severity of trauma with delayed mortality. This technology will provide a biologically-based assessment of damage to salmon migrating through various hydropower configurations.

B. OBJECTIVES

The objectives of this project will be to:

1. Development of a monoclonal antibody for use in blood biomarker sampling.
2. Application of biomarker with various hydropower field study collections.
3. Correlation of pressure and delayed mortality metrics from field studies with biomarker levels to assess biomarker response for field applications.

C. METHODOLOGY

Objective 1. Develop an antibody that is specific for the salmon protein that is the basis for the mammalian assay. An assay will be developed and validated for rapid assessment from blood samples, negating the need for lethal sampling, and allowing optimization of ongoing field studies.

Objective 2. Collection and analysis of brain tissues and blood samples from ongoing field studies will be performed to assess severity of brain trauma to fish by hydropower configurations for spill, bypass, and turbine passage.

Objective 3. Results from Objective 2 will be compared with metrics collected from the various field studies (sensor fish data, delayed mortality in holding time, etc). These correlations will validate the use of the brain injury biomarker in providing information on biological impacts from migration strategies associated with hydropower operations.

A sample size of 10 to 20 fish would be assessed for a single type of passage treatment in order to provide statistically significant results. Fish blood samples would be collected cooperatively through ongoing or planned direct injury and survival studies such as balloon-tag studies with fish that have a known passage history. Blood samples will be taken from fish within 2-3 hours of collection on project, and returned to collection holding conditions. Blood samples will be transported back to Battelle facilities for assay and results reported as appropriate.

D. RELEVANCE

This project has relevance to studies associated with direct injury and survival such as SPE-P-02-2, SBE-W-07-01, SBE-W-07-02-new, SPE-W-04-2, SPE-W-07-new, SBE-W-05-1, and SBE-W-05-2. Brain injury assessments will reduce uncertainties associated with risk of injury for configurations that mitigate fish passage and can be used in the management of improving migration success.

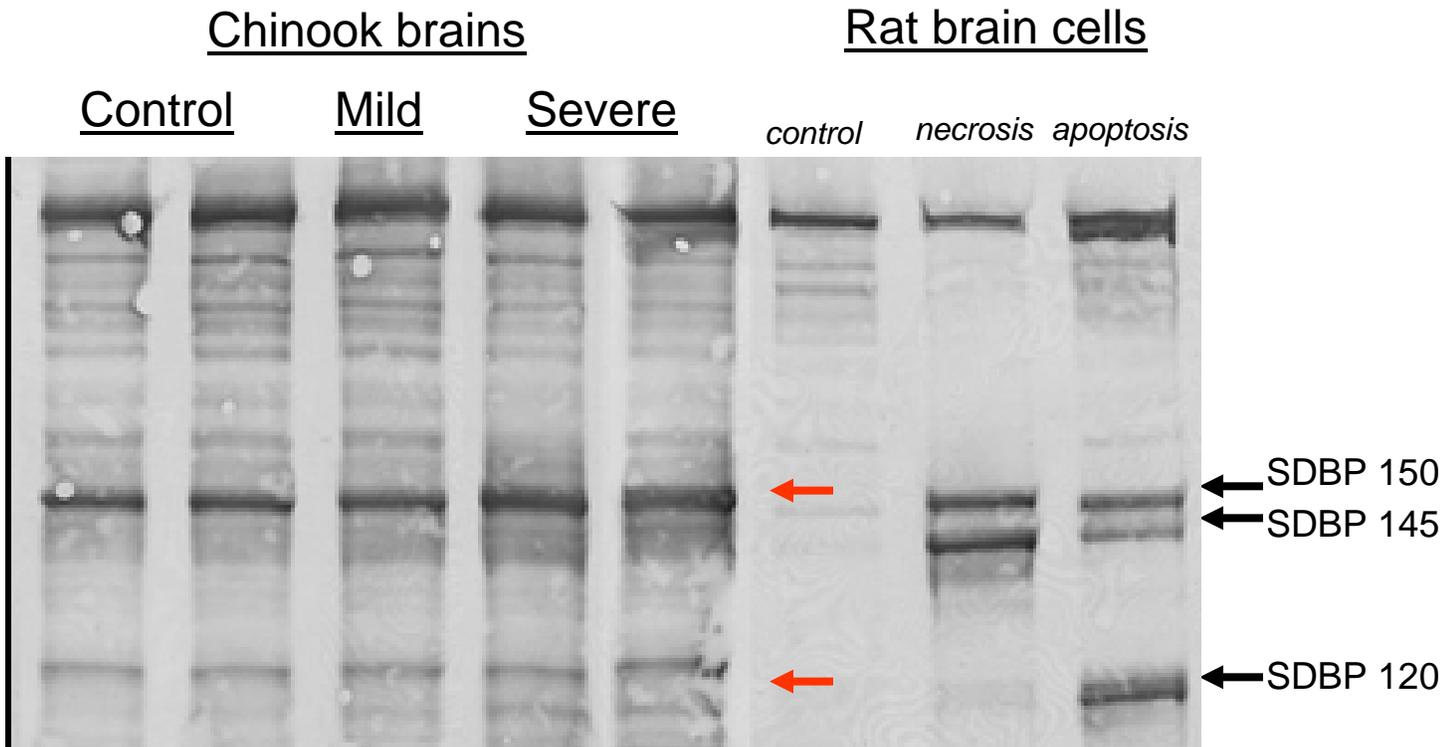


Figure 1. These samples were treated with a human polyclonal antibody that recognizes breakdown products of a protein associated with severity and type of brain injury. The rat samples were enzymatically treated to produce products associated with necrosis or apoptosis injury, and serve as positive controls. The “severe” Chinook samples clearly show an apoptotic reaction which resulted immediately after treatment and indicates a reaction by the brain cells to auto-destruct following the trauma and were assayed immediately following trauma induction. Three samples from each treatment were assayed (not all data shown here) with patterns similar to those shown here for each group.

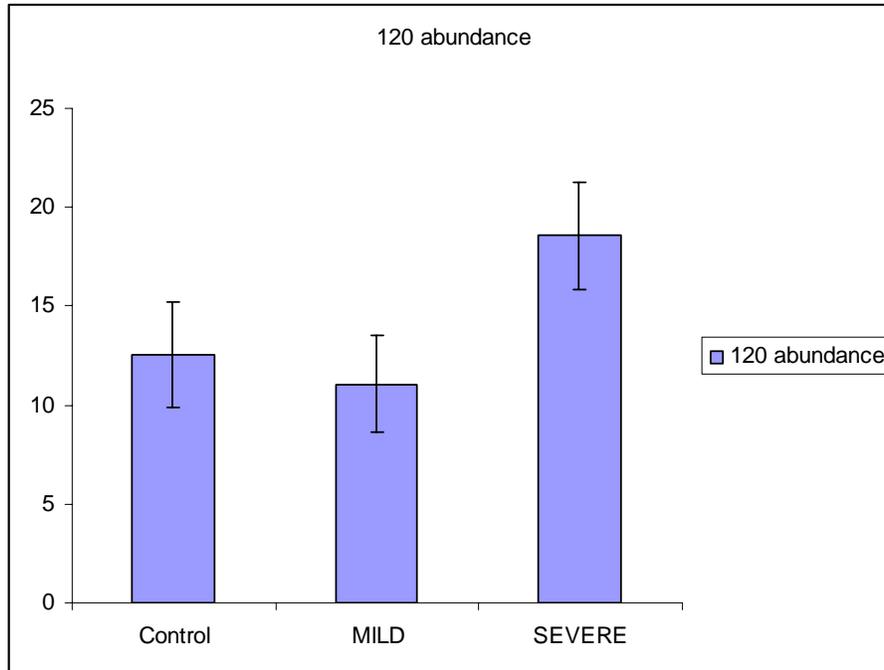


Figure 2. Graphical depiction of protein fragment SDBP 120 abundance following western blot exposure as depicted in Figure 1. The mean abundance is given with standard error associated with N=3 for each group.

IV. LIST OF KEY PERSONNEL AND PROJECT DUTIES

Dr. Ann Miracle (PNNL) will be responsible for oversight of all experimental procedures and reporting. Dr. Nancy Denslow (BB Inc.) will be on subcontract to assist in the brain trauma assays. All laboratory work will be conducted at PNNL's Biomarker Laboratory. Field work will be coordinated with ongoing/planned studies of direct injury/survival at various hydropower projects. Tom Carlson (PNNL) will coordinate field activities.

Some work will be performed under subcontract by Dr. Nancy Denslow, Banyan Biomarkers Inc., Alachua, FL.

V. TECHNOLOGY TRANSFER

Information acquired during the proposed work will be transferred in the form of written and oral research reports and scientific publications. Each year a presentation will be made at the Corps' annual Anadromous Fish Evaluation Program Review. A draft annual report will be provided to the COE by February 28 following each study-year, and after appropriate review final reports will be completed in a timely manner each year. Technology transfer activities may also include presentation of research results at regional or national fisheries symposia.

VI. LIST OF REFERENCES/LITERATURE CITED

Ham, K.D., A.L. Miracle, I. Schultz. 2006. Monitoring the Condition of Migrating Salmonids with DNA MicroArrays. Pacific Northwest National Laboratory, Laboratory Directed Research and Development Project.