INTERLOCAL COOPERATION AGREEMENT
SA 08-186/430-2644
CHANGE ORDER NO. 2

Pursuant to Article IX, the following changes are hereby incorporated into this Interlocal Agreement between Public Utility District No. 1 of Chelan County, WA (“Chelan”) and Public Utility District No. 2 of Grant County, WA (“Grant”).

A. Description of Changes: Extension of the termination date by eighteen (18) months and additional funds as described below. A third year of the pilot study will be conducted in 2010 and 2011 in accordance with the Scope of Work attached as Appendix “A”. Approximately 200,000 summer Chinook from Dryden will be reared in circular ponds to test (1) the efficacy of re-use for future production of Wenatchee summer Chinook by Grant, and (2) to determine the effect of over-inter acclimation on fish reared in a re-use system. Chelan may invoice Grant $90,000 after the fish are transferred out of the circular tanks in 2011 and $10,000 upon completion of the draft 2010-2011 report.

B. Time of Completion: Change Order No. 2 will extend the contract termination date by eighteen (18) months, from May 31, 2010 to December 31, 2011.

C. Contract Price Adjustment: As a result of this Change Order, the not to exceed Contract Price shall be increased by the sum of $100,000. Additionally, the number of PIT-tags provided by Grant will be increased by up to 30,000 to a new total of up to 70,000 PIT-tags. This Change Order shall not provide for any other payments to or claims by Chelan as a result of or arising out of the performance of the work described herein. The new total revised maximum Contract Price is $250,000, which amount, Chelan PUD acknowledges is the maximum Contract amount for the Contract work including changes incorporated by this Change Order.

D. Except as specifically provided herein, all other Contract terms and conditions shall remain unchanged.

PUBLIC UTILITY DISTRICT NO. 2
OF GRANT COUNTY, WASHINGTON

By: ____________________________
    Tom Dresser,
    Acting Director of Natural Resources

Date: ____________

PUBLIC UTILITY DISTRICT NO. 1
OF CHELAN COUNTY, WASHINGTON

By: ____________________________
    Richard Riazzi, General Manager

Date: ____________
APPENDIX A

2010 Dryden Re-use Pilot Study

Purpose:
In 2010 and 2011, Chelan PUD will be conducting a study to determine the efficacy of water re-use for rearing the Dryden stock of summer Chinook through (2) evaluating the effect of exposure to different durations of re-use rearing and (2) describing the health and physiological condition of the different reuse treatments.

Study Element 1: Exposure to different durations of reuse.
The first element of the study will compare the survival and migration timing of summer Chinook raised under three different conditions representing different exposures to re-use rearing, Treatment 1: “Short Term Rearing”, short-term exposure to re-use; Treatment 2: “Overwinter Rearing”, long-term exposure to re-use; and Control: “Raceway Rearing”, no exposure to re-use (Figures 1 & 2).

Treatment 1 will consist of three rearing components: (1) ponding fish at Eastbank re-use aquaculture system (EB PRAS) in June, (2) transfer to and rearing in an Eastbank raceway from middle October through late February, and (3) transfer to and acclimation in Dryden Acclimation Pond from March through release in May.

Treatment 2 will consist of two rearing components: (1) ponding fish at Eastbank re-use aquaculture system (EB PRAS) in June with rearing through late February, and (2) transfer to and acclimation in Dryden Acclimation Pond from March through release in May.

The Control will consist of two rearing components: (1) ponding and rearing fish at an Eastbank raceway in June through Late February, and (2) transfer to and acclimation in Dryden Acclimation Pond from March through release in May.
Figure 1: Conceptual study schedule with two treatments (1) Short Term Rearing and (2) Overwinter and Raceway control. Blue square “EB PRAS” = Eastbank Re-use vessel; black square “Raceway” = Eastbank Raceway; and Red square “Dryden” = Dryden Acclimation Pond.

Figure 2: Diagrammatic representation of temporal locations and splits of fish in Treatments 1 and 2. Green circular shapes represent individual water re-use vessels.
Each experimental group will be monitored by hatchery staff (WDFW) to determine within-hatchery survival rates. Additionally, a subsample of 10,000 individuals from each group will be PIT tagged for comparisons of (1) survival from release to McNary and (2) relative migration timing among groups. Estimates of survival will be obtained using the Cormack-Jolly-Seber model. All quantitative comparisons of survival will be performed using methodologies developed by Columbia Basin Research (CBR), University of Washington. Specifically, survival will be evaluated using the hypotheses:

$$H_0: S_{\text{Treatment 1}} = S_{\text{Treatment 2}} = S_{\text{control}}$$

Versus

$$H_A: S_{\text{Treatment 1}} = S_{\text{Treatment 2}} > S_{\text{control}}$$

On-the-ground participants:

- Washington Department of Fish and Wildlife (WDFW)-conduct hatchery rearing and within hatchery fish health and survival monitoring.
- CBR-(PITpro software)-Quantitative analyses of release to McNary survival and migration timing.
- Chelan PUD-PIT Tagging fish and study coordination

**Study Element 2: Health and physiological condition of experimental groups.**

The second part of the study will focus on describing the health and physiological condition of each experimental group.

**Fish Health:** A licensed veterinarian will assess the performance of the treatments and control groups against a panel of standard fish-health metrics used in aquaculture. The relative performance of the treatments and control will also be compared to one another to generate a comparative assessment.

Fish health metrics:

- Fish pathogen screening
- Histology
- Fin assessment
- Whole blood gas and chemistry assessment
- Whole body proximate analysis

The Conservation Fund’s Freshwater Institute will conduct fish health assessments. Protocols will follow previous work conducted on the pilot re-use system (see Good, C. and B. Vinci. 2010. *Eastbank Hatchery Pilot Project YEAR II: Assessing performance, health, and welfare of juvenile Chinook salmon in water reuse and raceway environments*. The Conservation Fund’s Freshwater Institute Shepherdstown, West Virginia).

**Physiology:** The treatment and control groups will also be assessed for physiological performance related to standard metrics used for evaluating smoltification and early male maturation among salmonids. Specifically, smoltification will be evaluated by measuring profiles of growth, gill Na+/K+-ATPase enzyme activity, and the endocrine factor insulin-like growth factor-I (IGF-1) in fish blood. The prevalence of early male maturation will be evaluated by measuring the steroid 11-Ketotestosterone (11-KT) in fish blood.
Additionally, these metrics will be considered as they interact with fish growth and size. The hypotheses tested will include:

a) **Size**

_Hypothesis_{H0}: There are no significant differences in length and weight between treatment and control fish._

b) **Specific growth rate**

_Hypothesis_{H0}: There are no significant differences in growth rate between treatment and control fish._

c) **Smolt physiology – Gill Na\(^+\)/K\(^+\)-ATPase activity**

_Hypothesis_{H0}: There are no significant differences in seasonal gill Na\(^+\)/K\(^+\)-ATPase activity profiles between treatment and control fish._

d) **Growth physiology – Plasma Insulin-like growth factor-I (IGF-1)**

_Hypothesis_{H0}: There are no significant differences in seasonal plasma IGF-I profiles between treatment and control fish._

All smolt physiology work will be conducted by scientists from NOAA Fisheries in accordance with established protocols (e.g., Larsen, D.A., B. R. Beckman, K. A. Cooper, D. Barrett, M. Johnston, P. Swanson, and W. W. Dickhoff. 2004. Assessment of high rates of precocious male maturation in a spring Chinook salmon supplementation hatchery program. Transactions of the American Fisheries Society 133:98-120.)

On-the-ground participants:

- Washington Department of Fish and Wildlife (WDFW)-conduct hatchery rearing and within hatchery fish health and survival monitoring.
- NOAA Fisheries.
- The Conservation Fund’s Freshwater Institute
CHANGE ORDER FORM
NO. 1

Pursuant to Article IX, the following changes are hereby incorporated into this Contract:

A. Description of Changes: Extension of the termination date by eighteen (18) months and additional funds as described in Section B below. A second year of the pilot study will be conducted during 2009. Approximately 200,000 summer Chinook will be reared in the circular tanks during the 2009 pilot study. All activities and rearing schedules described in Attachment A of the original Agreement will be continued for the 2009 pilot (e.g. fish culture, fish health monitoring and evaluation and data analysis and reporting activities). Chelan may invoice Grant $40,000 after the fish are transferred out of the circular tanks in 2009 and $10,000 upon completion of the draft 2009 report.

B. Time of Completion: Change Order No. 1 will extend the contract termination date by eighteen (18) months, from November 30, 2008 to May 31, 2010.

C. Contract Price Adjustment: As a result of this Change Order, the not to exceed contract amount shall be increased by $50,000.00 to a new not to exceed contract amount of $150,000.00. Additionally, the number of PIT-tags provided by Grant PUD will be increased by up to 20,000 to a new total of up to 40,000 PIT-tags. This Change Order shall not provide any basis for any other payments to or claims by Chelan PUD as a result of or arising out of the performance of the work described herein.

D. Except as specifically provided herein, all other Contract terms and conditions shall remain unchanged.

PUBLIC UTILITY DISTRICT NO. 1
OF CHELAN COUNTY, WASHINGTON

Accepted By: Joe Jarvis
Authorized Signature: [Signature]
Title: [Title]
Date: 1-18-09

PUBLIC UTILITY DISTRICT NO. 2
OF GRANT COUNTY, WASHINGTON

Accepted By: Gerry O'Seeff
Authorized Signature: [Signature]
Title: NR Director
Date: 1-13-09
INTERLOCAL COOPERATION AGREEMENT

THIS AGREEMENT is made by and between PUBLIC UTILITY DISTRICT NO. 1 OF CHelan COUNTY, WASHINGTON ("Chelan"), and PUBLIC UTILITY DISTRICT NO. 2 OF GRANT COUNTY WASHINGTON ("Grant").

Recitals:

Public Utility Districts are authorized pursuant to RCW Chapter 39.34 to enter into Cooperative Agreements.

Chelan and Grant desire to investigate a partial reuse aquaculture system incorporating circular culture tanks with dual drains utilizing partial water reuse. The purpose of the investigation is to determine the viability of this technology which uses less water than conventional raceways. The 2008 pilot project for these purposes will utilize the Eastbank fish hatchery owned by Chelan and operated by the Washington Department of Fish and Wildlife and will use Chelan River (A.K.A. Turtle Rock Island Yearling) summer Chinook. Approximately 100,000 Summer Chinook salmon will be reared for a 5-month period (June 2008-November 2008) in a partial water reuse system constructed at the Eastbank Hatchery while the remainder of this population will be raised for the same period at Eastbank Hatchery in traditional flow-through raceway units. Such technologies have been successfully applied elsewhere to improve rearing volume use, reduce site footprint, improve control over culture conditions, and reduce water consumption and energy costs.

Grant is participating in this pilot project to determine the applicability for use with their hatchery programs. Grant is developing new hatchery sites at which water is a limiting factor for some of the sites. This technology may support the viability and development of Grant's hatchery sites.

Grant is defined as including any employee, contractor or agent of Public Utility District No. 2 of Grant County, Washington.

Chelan is defined as including any employee, contractor or agent of Public Utility District No. 1 of Chelan County, Washington.

NOW THEREFORE; in consideration of the mutual promises and covenant contained herein, the parties agree as follows:

I. OBLIGATIONS OF CHELAN AND GRANT

a. Chelan will utilize the Eastbank Hatchery to rear approximately 100,000 Summer Chinook in two circular ponds (50,000 each).

b. Grant will provide $100,000 in funding to support this pilot program and up to 20,000 passive integrated transponder (PIT) tags to be used to evaluate the biological effectiveness of the program. Chelan will be responsible for tagging the study fish and providing all related tagging supplies.

c. Chelan will be responsible for all design, procurement, and installation aspects of this program.
d. Chelan's contractor, Washington Department of Fish and Wildlife (WDFW) will operate the fish culture system in accordance with the pilot study criteria and monitoring and evaluation protocols and is provided as Attachment A.

e. Upon completion of fish condition and health assessments outlined in Attachment A, Chelan will be responsible for drafting a report comparing the results of the treatment and control groups.

II. TERM

This Agreement shall be effective from the date of execution and shall remain in full force and effect until November 30, 2008. This Agreement may be terminated earlier by written notice issued to the other party at least 30 days in advance of the date of termination. This Agreement shall terminate no later than November 30, 2008. Payments and indemnity waivers of claims provisions shall remain effective after termination of the Agreement.

III. INDEMNITY and WAIVER OF CLAIMS

Chelan shall have no liability to Grant for any loss of fish or incidental or consequential damages arising from or as a result of Chelan’s performance of this Agreement.

With respect to third party claims, damages and causes of action, Grant and Chelan each agree to indemnify, defend and hold harmless the other from and against all suits, actions, proceedings, claims, demands, judgments, or damages arising out of or due to: (a) its own omission, fault, strict liability or negligence in connection with or incident to their respective obligations under this Agreement; (b) any injury to or death of any person or persons or damage to any property in connection with or incident to this Agreement.

Notwithstanding the foregoing, Grant's and Chelan's obligations under the preceding paragraph arising from the concurrent negligence or fault of the parties, shall be enforceable only to the extent of the negligence of the other party and neither party shall be obligated to indemnify the other for the sole negligence of such other party or its agents, contractors or employees.

Grant and Chelan agree to hold each other harmless for injuries suffered by their own employees or contractors/subcontractors. This indemnity obligation specifically includes liability or alleged liability that may arise from injury or loss suffered by an employee of either party regardless of any immunity provided by the Washington Industrial Insurance Act, RCW Title 51, or any other applicable law.

IV. PURPOSE

The purpose of this Agreement is to identify responsibilities of Chelan and Grant associates with the pilot study to investigate and document fish health differences among fish raised in traditional raceways compared to fish raised in a partial reuse system.

V. PAYMENT

Chelan may invoice Grant $90,000 upon completion of the construction phase of the pilot project and $10,000 upon completion of the draft report. Payment will be due and payable within thirty (30) days of the invoice. If payment is delayed, interest will accrue and be payable on the unpaid balance at the rate of twelve (12%) percent per annum.
VI. ADMINISTRATION OF AGREEMENT

This Agreement shall be jointly administered by a representative of Grant and a representative of Chelan. Absent written notice by one party to the other, the administrators shall be:

Chelan County PUD
Attn: Shaun Seaman
PO Box 1231
327 N. Wenatchee Avenue
Wenatchee, WA 98807

Grant County PUD
Attn: Tom Dresser
PO Box 878
30 C Street SW
Ephrata, WA 98823

VII. EFFECT OF OTHER AGREEMENTS

This Agreement shall not change or affect the responsibilities and obligations of Chelan under its Rocky Reach and Rock Island Habitat Conservation Plans. This agreement shall not change or affect the responsibilities and obligations of Grant under its Biological Opinion and settlement agreements.

VIII. JURISDICTION AND ATTORNEY FEES

This Agreement is made, executed under and is to be governed by, construed and enforced in accordance with the laws of the State of Washington. In the event of a suit, the undersigned agree that a visiting judge shall be assigned to the case so that a resident judge, who is also a customer of either Chelan or Grant, will not hear the case. The substantially prevailing party in any legal action herein shall be entitled to reasonable attorney fees and all reasonable costs, including, but not limited to, expert witness fees and travel and lodging expenses.

IX. GENERAL PROVISIONS

Any modification of this Agreement or additional obligations assumed by either party in connection with this Agreement shall be binding only if evidenced in writing signed by each party or any authorized representative of each party. This Agreement constitutes the entire agreement between the parties, and any prior understanding or representation of any kind preceding the date of this Agreement shall not be binding on either party except to the extent incorporated in this Agreement.

This Agreement shall be governed by, construed and enforced in accordance with laws of the State of Washington. Any modification of this Agreement or additional obligation assumed by either party in connection with this Agreement shall be binding only if evidenced in writing signed by each party or authorized representative of each party.

This Agreement constitutes the entire agreement between the Parties, and any prior understanding or representation of any kind preceding the date of the Agreement shall not be binding on either party except to the extent incorporated in the Agreement.

X. AUTHORITY

Each person signing this Agreement has the full authority of the entities on behalf of which they are signing to execute this Agreement and to bind those entities to the terms of this Agreement.

XI. RELATIONSHIP OF THE PARTIES
No agent, employee or representative of Grant shall be deemed to be an agent, employee, or representative of Chelan for any purpose, and the employees of Grant are not entitled to any of benefits Chelan provides to Chelan employees. No agent, employee or representative for Chelan shall be deemed to be an agent, employee or representative of Grant for any purpose, and the employees of Chelan are not entitled to any of the benefits Grant provides to Grant employees.

In performance of the work herein contemplated, the party performing the work is an independent contractor with authority to control and direct the performance of the details of the

XII. FILING

The administrators shall, in compliance with RCW 39.34, upon execution of this Agreement, post and electronic copy of the Agreement on Grant and Chelan’s Websites at the following addresses:
Grant: http://www.gcpud.org
And Chelan: http://www.chelanpud.org/interlocal-agreements.html

IN WITNESS WHEREOF, each party to this Agreement has caused it to be executed on the date indicated below.

PUBLIC UTILITY DISTRICT NO. 2
OF GRANT COUNTY, WASHINGTON

By: 
Title: Interim Natural Resources Director
Date: 8/2/08

PUBLIC UTILITY DISTRICT NO. 1
OF CHELAN COUNTY, WASHINGTON

By: 
Title: Executive Manager
Date: 8/27/06
ATTACHMENT A

Partial Water Reuse Pilot Study
Monitoring and Evaluation

The District will investigate a partial reuse aquaculture system incorporating circular culture tanks with dual drains. Such technologies have been successfully applied elsewhere to improve rearing volume use, reduce site footprint, improve control over culture conditions, and reduce water consumption and energy costs.

The pilot project at the Eastbank Hatchery will use Chelan River (A.K.A. TRI Yearling) Summer Chinook. The following piloting parameters have been defined but may be subject to change:

**Table 1:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per tank volume</td>
<td>3884.6 ft³</td>
</tr>
<tr>
<td>Total tank volume</td>
<td>7769.2 ft³</td>
</tr>
<tr>
<td>Density index</td>
<td>0.125 lb/cf-in</td>
</tr>
<tr>
<td>Max rearing density</td>
<td>9.2 kg/m³ (1.24 lb/gal)</td>
</tr>
<tr>
<td>Minimum Flow Index</td>
<td>0.75 lb/gpm-in</td>
</tr>
<tr>
<td>Tank exchange rate</td>
<td>calculated</td>
</tr>
<tr>
<td>Condition factor</td>
<td>0.0121 g/cm³</td>
</tr>
<tr>
<td>Release length</td>
<td>4.6 in (11.7 cm)</td>
</tr>
<tr>
<td>Release weight</td>
<td>23.5 fish/lb (19.4 g)</td>
</tr>
<tr>
<td>Total number of fish</td>
<td>104,500</td>
</tr>
<tr>
<td>Total fish biomass</td>
<td>4,467 lb (9848.7 kg)</td>
</tr>
<tr>
<td>Maximum temperature</td>
<td>59 °F (15 °C)</td>
</tr>
<tr>
<td>Reuse rate</td>
<td>75%</td>
</tr>
<tr>
<td>Total flow rate (2 tanks, flow index based)²</td>
<td>1295 gpm (4902 lpm)</td>
</tr>
<tr>
<td>Influent flow rate (2 tanks)</td>
<td>323 gpm (1222 lpm)</td>
</tr>
</tbody>
</table>

1 Based upon moving fish in November to acclimation facility
2 Single pass water use provisions will be provided

To facilitate an effective piloting process, the District will use a hatchery consultant to design an aquaculture systems, supply and support equipment, perform training, and assist with data analysis during the pilot.

**Design Concept**

Based on design parameters, the estimated equipment requirements are:

1. **30 ft Diameter Circular Culture Tank System (Qty = 2)**
   a. 30 ft diameter x 6 ft wall height circular culture tank, FRP walls and floor, sectional
   b. Bottom drain sump and screen
   c. Side drain (Cornell style) and screen
   d. Bottom drain standpipe
   e. Spraybar assembly
2. Partial Reuse Aquaculture System  
   a. Drum filter (Qty = 1)  
   b. 89 micron screens  
   c. Pump sump (Qty = 1)  
   d. Reuse pumps (Qty = 2 or 3)  
   e. Oxytower Gas Transfer System (Qty = 2)  
      i. CO2 stripper and Low Head Oxygenator (LHO)  
      ii. Gas transfer media
3. Motor Control Panel (Qty = 1)  
   a. Alarm relays
4. Water Quality Monitoring System  
   a. Analyzers (4-DO, 1-Temperature, 1-pH)  
   b. Flow meter (1 for influent 1 for reuse)  
   c. Multi-channel transmitter unit with local display and alarm relays  
   d. Data logging capabilities  
   e. Software package for PC
5. Effluent Treatment  
   a. Radial Flow Settlers (Qty = 2)
6. Ancillary Equipment
7. Culture tank jump screens or covers
8. Feeding systems will be manual (to make a consistent comparison)
9. Spare parts and materials as needed or related tools.

Scope of Work

The pilot study work is organized into the following tasks:

1. Scoping and concept design  
   a. Site review and layout analysis.  
   b. Identify design constraints and preferences.  
   c. Production parameters.  
   d. Rearing parameters.  
   e. Water quality parameters.  
   f. Calculate mass balance and verify flow and treatment requirements.  
   g. Develop process and layout drawings.  
   h. Check equipment list and performance criteria.  
   i. Calculate influent and effluent water quality.  
   j. Prepare water quality report template.
2. Detailed system design and design coordination  
   a. Aquaculture system process design.  
   b. Layout of aquaculture systems.  
   c. Detailed design analysis and design calculations  
   d. Prepare detailed list of electrical loads, mechanical loads, and other service requirements.  
   e. Integrate aquaculture system to site.  
   f. Develop construction drawing
3. Equipment supply
4. Construction
5. Commissioning
6. Training
   a. Prepare and provide Operation and Maintenance Manual for System and for Components.
   b. Prepare training program for O&M personnel.
   c. Coordinate and conduct O&M personnel training.

Operational support
   d. Provide qualified personnel for operational advice and water quality troubleshooting.

7. Monitoring Parameters
   a. The following parameters will be monitored continuously with analyzers and meters:
      i. Dissolved Oxygen (DO) in each of the tank side drains (2 places).
      ii. Dissolved Oxygen (DO) in the header tank.
      iii. Dissolved Oxygen (DO) in the pump sump.
      iv. Water temperature in the header tank.
      v. Water pH in the header tank.
      vi. Water flow rate on influent water supply (make-up water).
      vii. Water flow rate on the reuse flow directly downstream of the pumps.
   b. The following parameters are to be monitored using a colorimetric test kit or laboratory methods (frequency to be determined):
      i. Dissolved carbon dioxide at the pump sump and the header tank.
      ii. Dissolved total ammonia nitrogen (TAN) at the pump sump.
      iii. Alkalinity (as calcium carbonate) at the pump sump.
      iv. Biological Oxygen Demand (BOD) in the culture tanks and in the effluent.
      v. Total Suspended Solids (TSS) in the culture tanks and in the effluent.
   c. The following additional parameters are examples of additional parameters that will be read and/or recorded manually (frequency to be determined):
      i. Pressure as measured with gauges upstream and downstream of pumps.
      ii. Flow split between bottom and side drain of tank (using portable flow meter)
      iii. Rotational period at perimeter of the culture tanks (using a float)
      iv. Oxygen use rate (at oxygen flow meter on LHO inlet)
      v. Daily feed usage and feeding time of day

8. Data analysis and reporting
   a. Provide analysis and trend development for water quality data. Track dissolved oxygen, dissolved CO₂, pH, temperature, Total Ammonia Nitrogen, BOD, and TSS. Analysis will be performed monthly but may be more frequent if so required for troubleshooting purposes.
   b. Prepare a monthly summary report of system performance and water quality.

The following schedule of milestones is estimated:

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Estimated Completion Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scoping and concept design</td>
<td>2008-01-31</td>
</tr>
<tr>
<td>Detailed design documents</td>
<td>2008-02-29</td>
</tr>
<tr>
<td>Equipment delivery</td>
<td>2008-03-31</td>
</tr>
<tr>
<td>Pilot equipment installation</td>
<td>2008-04-30</td>
</tr>
<tr>
<td>Commissioning</td>
<td>2008-05-15</td>
</tr>
<tr>
<td>O&amp;M training</td>
<td>2008-05-30</td>
</tr>
<tr>
<td>Milestone</td>
<td>Estimated Completion Date</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>Piloting period</td>
<td>2008-06-01 (start) to 2009-05-31 (end)</td>
</tr>
<tr>
<td>Decommissioning or contract renewal</td>
<td>2009-06-01</td>
</tr>
</tbody>
</table>

Note: All dates assume initiation of contract by December 20, 2007.
Partial Water Reuse Fish Health
Monitoring and Evaluation

Background

Modern partial reuse aquaculture systems have the capacity to reuse up to 85% while maintaining water quality parameters (e.g. DO, CO₂, ammonia) within safe limits. The capacity to reuse water makes the technology applicable to those who are investigating methods to reduce source water usage. One of the most important concerns for using any different technology is how fish health might be affected.

The pilot study purpose is to investigate and document fish health differences among fish raised in traditional raceways compared to fish raised in a partial reuse system.

A partial water reuse system will be constructed at the Eastbank Hatchery to rear approximately 100,000 Summer Chinook salmon for a 5-month period (June 2008–November 2008) while the remainder of this population will be raised for the same period at Eastbank Hatchery in traditional flow-through raceway units. Both groups will be differentially marked and transferred to the acclimation pond prior to release. In addition, 10,000 fish of the test and the same number of control fish will be PIT tagged for evaluation of survival and travel time comparison to McNary dam. Fish health and welfare will be evaluated according to the below plan.

Proposed Study Details

Start date: June, 2008
End date: November, 2008

(For a complete time-line of the proposed study, see Appendix A)

Hypothesis – Fish growth and health_{partial reuse} ≥ Fish growth and health_{traditional raceway}

Study design – This study follows a prospective cohort epidemiological design, and will assess specific health and welfare indicators between two cohorts of fish of the same background (genetic strain, early rearing environment, etc.) exposed to two different rearing systems, with other exposures (water source, management, feeding rates, etc.) being equal.

Methodology

1. **Performance** – Fish will be sampled from both cohorts at regular monthly intervals for length and weight, from which growth curves and (with feeding data) feed conversion ratios will be generated. These data will be analyzed for statistically significant differences over time between the two cohorts.

2. **Fish Health** – There will be multiple assessments:
   a. Mortality data will be collected throughout the study period, and a proportional hazards survival analysis will be carried out at the end of the study to determine differences in overall survival between the two cohorts.
   b. Samples of 60 fish from each cohort (120 fish total per sampling event) will be collected at the start, middle, and end of the rearing period. These fish will be euthanized, packed in ice and shipped overnight to an accredited fish disease diagnostic laboratory for screening of listed viral, bacterial, and parasitic pathogens, following Blue Book protocols (see Appendix B). This testing will
reveal the presence or absence of subclinical infections, and will be used to assess changes in subclinical infection over time between the two cohorts.

c. In the event of clinical disease outbreaks during the study period, WDFW fish pathologist will diagnose and treat the study populations with support from a fish pathologist. Diagnostic and treatment records for each cohort will be summarized at the end of the study, and compared statistically.

d. At the end of the study, 50 fish from each cohort will be euthanized, and samples of multiple tissues (gill, heart, liver, spleen, pyloric cecae, intestine, swim bladder, anterior and posterior kidney, skin, and fillet) will be sent to a fish pathologist for histopathological assessment to determine the extent of organ pathology within each cohort.

e. At the end of the study, 50 fish from each cohort will be bled, and frozen plasma samples will be sent to a diagnostic laboratory for biochemistry profiles as agreed upon with WDFW for further comparison of pathological processes, as well as indicators of long-term stress.

3. Fish Welfare – At the end of the study, 50 fish from each cohort will have their fin condition assessed. This will be carried out for all rayed fins, with measurements by digital calipers to calculate the overall fin indices (i.e. length of longest ray of each fin standardized to fork length) for each fish. Differences in fin indices between the two cohorts will be assessed statistically. This work will be coordinated with WDFW to insure proper techniques and methods are used.
Table 1: Estimated Effort and Time

<table>
<thead>
<tr>
<th>Task</th>
<th>Effort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field Work</td>
<td></td>
</tr>
<tr>
<td>Site Visits and Field Work (Principal Investigator)</td>
<td>15 days</td>
</tr>
<tr>
<td>Site Visits and Field Work (Technician)</td>
<td>5 days</td>
</tr>
<tr>
<td><strong>Field Work TOTAL</strong></td>
<td><strong>20 days</strong></td>
</tr>
<tr>
<td>Laboratory Analyses – Allowances</td>
<td></td>
</tr>
<tr>
<td>Pathogen Screening: 6 samples</td>
<td></td>
</tr>
<tr>
<td>Diagnostic pathology (blood chemistry)</td>
<td></td>
</tr>
<tr>
<td>Sample Shipment, Field Work Equipment, etc.</td>
<td></td>
</tr>
<tr>
<td><strong>Laboratory Analyses TOTAL</strong></td>
<td></td>
</tr>
<tr>
<td>Data Analysis and Report Preparation</td>
<td></td>
</tr>
<tr>
<td>Monthly Data Collection and Study Coordination</td>
<td>5 days</td>
</tr>
<tr>
<td>Analysis and Report Writing</td>
<td>15 days</td>
</tr>
<tr>
<td><strong>Data Analysis TOTAL</strong></td>
<td><strong>20 days</strong></td>
</tr>
<tr>
<td><strong>PROJECT TOTAL(^1)</strong></td>
<td><strong>40 Days</strong></td>
</tr>
</tbody>
</table>

\(^1\)Diagnostic veterinary services to address clinical disease are not included in this estimate. These services are assumed to be part of the normal hatchery program.
<table>
<thead>
<tr>
<th>Date</th>
<th>Activity</th>
</tr>
</thead>
</table>
| June 2008           | • Chris Good to travel to Eastbank Hatchery  
                      • First sampling of 60 fish per system for **pathogen screening** (see Appendix B) as cohorts begin early rearing at Eastbank  
                      **Details:**  
                      • Sampling for pathogen screening requires MS-222 for euthanasia, and hard-sided coolers packed with ice for shipment  
                      • Purposive sampling is employed (as opposed to random sampling), in that smaller, unthrifty fish or those exhibiting clinical signs will be targeted  |
| August 2008         | • Chris Good to travel to Eastbank Hatchery  
                      • Second sampling of 60 fish per system for **pathogen screening**  |
| November 2008       | • Chris Good plus technician final visit to Eastbank Hatchery  
                      • Final sampling of 60 fish per system for **pathogen screening**  
                      • Blood sample collection from 50 fish per system for **biochemistry profile analysis**  
                      **Details:**  
                      • Blood collection will require 3ml syringes with 22-gauge needles, blood collection tubes and freezer space to freeze samples prior to shipment (hard-sided cooler plus ice)  
                      • **Tissue collection** from 50 fish per system for histological assessment  
                      **Details:**  
                      • Tissue collection requires dissection kits, histological grade formalin, and plastic jars for specimen fixation  
                      • Once fixation is complete (48 hours), tissues are removed, placed in Whirlpak bags with small amounts of formalin, and shipped to the pathologist in a hard-sided cooler  
                      • **Fin data collection** for 50 fish per system for fin health assessment, and this requires use of a digital micrometer  |
| Throughout study period | • Routine mortality data collection following established facility protocols  
                      • Routine feeding data collection  
                      • Routine performance (length, weight, etc.) data collection  
                      • As needed veterinary sampling and diagnoses for clinical disease conditions as they arise |
Appendix B:
Listed Pathogen Screening

The following fish pathogens will be screened in samples of 60 fish from each system according to Blue Book guidelines. The sample size of 60 fish in populations greater than 100,000 provides a 95% confidence of pathogen detection when pathogen apparent prevalence is at least 5%.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Disease</th>
<th>Detection Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aeromonas salmonicida</em></td>
<td>Furunculosis</td>
<td>Culture</td>
</tr>
<tr>
<td><em>Yersinia ruckeri</em></td>
<td>Enteric redmouth disease</td>
<td>Culture</td>
</tr>
<tr>
<td><em>Renibacterium salmoninarum</em></td>
<td>Bacterial kidney disease</td>
<td>ELISA</td>
</tr>
<tr>
<td>Viruses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IHNV</td>
<td>Infectious hematopoietic necrosis</td>
<td>Cell culture on CHSE, EPC</td>
</tr>
<tr>
<td>IPNV</td>
<td>Infectious pancreatic necrosis</td>
<td>Same</td>
</tr>
<tr>
<td>VHSV</td>
<td>Viral hemorrhagic septicemia</td>
<td>Same</td>
</tr>
<tr>
<td>Parasites</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Myxobolus cerebralis</em></td>
<td>Whirling disease</td>
<td>Tissue digestion / light microscopy</td>
</tr>
<tr>
<td>Additional Screening</td>
<td></td>
<td>As determined by WDFW</td>
</tr>
</tbody>
</table>