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# Steelhead Kelt Reconditioning and Reproductive Success 2010 Annual Report

**Doug Hatch, Ryan Branstetter, Jeff Stephenson, and Andrew Pierce**

**June 29, 2011**

## 2010 Annual Report

# Steelhead Kelt Reconditioning and Reproductive Success


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## ABSTRACT

Iteroparity, the ability to repeat spawn, is a natural life history strategy expressed by some species from the family Salmonidae. Observed iteroparity rates for steelhead *Oncorhynchus mykiss* in the Columbia River Basin are currently depressed due to anthropogenic development including operation of the hydropower system and other habitat degradations. Reconditioning post-spawned fish (kelts) in a captive environment to encourage reinitiating feeding, growth, and redevelopment of gonads is evaluated in this study as an approach to restore depressed steelhead populations. To test the efficacy of utilizing steelhead kelt as a management and recovery tool, different scenarios were investigated ranging from little intervention (collect and return fish to river) to high intensity (collect and feed fish in captivity until rematuration). Transport of Yakima and Snake River steelhead resumed with an attempt to move kelts further downriver, initial results are mixed and additional study should provide better results. Examination of gamete and progeny viability continued on first-time spawners and reconditioned kelt steelhead with results continuing to suggest that egg quantity/quality and juvenile factors are similar. Successful reproduction has been confirmed for 3 of 4 reconditioned kelts detected in Omak Creek. Genetic analysis confirmed that Naches and Toppenish kelts had more frequent post release PIT tag detections at Prosser Dam. The Columbia Inter-Tribal Fish Commission along with the Nez Perce Tribe and the University of Idaho are continuing with research in the Snake River Basin to determine which kelt reconditioning methods may be helpful in improving kelt survival in that basin and working to innovate new approaches that may benefit the entire Columbia River Basin. The University of Idaho (Idaho Cooperative Fish and Wildlife Research Unit) is testing transportation options and evaluating plasma factors in relation to life history stage of steelhead to optimize kelt survival and reproductive contribution. Sex hormone analysis appears to be able to give a predictive capability to determine if kelts will spawn within the year. The Nez Perce Tribe is continuing to develop a steelhead kelt master management plan for the Snake River Basin.

## Acknowledgements

We would like to thank the following individuals and organizations for providing time and expertise toward this project: The Bonneville Power Administration for providing funding for this research project and to Tracy Hauser for project assistance. Hatchery staff for the care provided to this important species: Parkdale Fish Facility (Jim Gidley, Albert Santos and Chuck Gehling), Prosser Fish Hatchery (Bill Fiander, Mark Johnston, Michael Fiander, Carrie Skahan, and OJ Davis), Cassimer Bar Fish Facility (Tony), and Warm Springs National Fish Hatchery. The trapping crews for the long hours worked carefully capturing steelhead: Chandler Juvenile Fish Facility, Shitike Creek, Omak and Bonaparte Creeks, and Parkdale Dam Trap (Megan Heinrich and Crew). Thanks to the vigilant work of the virology staff at Oregon Department of Fish and Wildlife (Tony Amandi, John Kauffman, Jerry Jones, Sharon Vendshus), Washington Department of Fish and Wildlife (Joan Thomas), Lower Columbia Fish Health Center (Ken Lujan), and Eagle Creek Fish Health Center (Doug Monson). We would also like to thank the following individuals for providing valuable expertise, information and supplies: Marla Chaney and Colleen Weiss at the Bonneville Captive Brood Program, Brian Tarabochia and Gordy Haglund, Steve Meshke, Allen Dietrichs, and Chris Ketchem at CEDC. Dr. Robert Flecker (Hillsdale Veterinary Group), Bob Rodgers (WDFW), Rod French (ODFW) and Greg Davis (ODFW). Thanks to the following Columbia River Inter-Tribal Fish Staff for their technical expertise, suggestions, and assistance: John Whiteaker, Bobby Begay, Shawn Narum, Phil Roger, Denise Kelsey, David Graves, Rob Lothrop, Christine Golightly, Peter Galbreath, Jeff Fryer, Gabe Sheoships, David Liberty, Winfred Perez, Charles Torbeck, Jacinda Mainord, Richard McConville, Neil Graham, Hardo Lopez, and Katie Cobell.

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# Introduction

*Oncorhynchus mykiss* are considered to have one of the most diverse life histories in *Salmonidae* (Behnke 1992) with variants that include resident, estuarine, and anadromous ecotypes, widely ranging ages of maturity, timing of juvenile and adult migrations, and various reproductive strategies including precocity, semelparity, and iteroparity. This complex array of life history variation is possibly a compensating or bet hedging device for life in stochastic environments (Taborsky 2001). Overlapping generations provide resources especially for small populations in the event of failure of any brood year due to brief catastrophic events (Seamons and Quinn 2010). While fluctuating populations and overlapping generations may reduce the effective population size (Ne; Waples 2002), retention of genetic diversity and persistence of the species may be favored due to these compensating life histories (Seamons and Quinn 2010; Narum et al. 2008). Lifetime reproductive success of steelhead spawning multiple times will average twice the reproductive success of steelhead spawning a single time (Seamons and Quinn 2010).

Populations of wild steelhead *O. mykiss* have declined dramatically from historical levels in the Columbia and Snake rivers (Nehlsen et al. 1991; NRC 1996; US v. Oregon 1997; ISRP 1999). In 1997, steelhead from the upper Columbia River were listed as endangered and those in the Snake River as threatened under the Endangered Species Act (ESA) (NMFS 1997). Stocks originating in the mid-Columbia were listed as threatened in 1999 (NMFS 1999). The causes of the species decline are numerous and well known. The two biggest impacts are hydropower operations and habitat loss (TRP 1995; NPPC 1986; NRC 1996; ISRP 1999; Keefer et al. 2008). Regional conservation plans recognize the need to protect and enhance weak upriver steelhead populations while maintaining the genetic integrity of those stocks (NPPC 1995).

Iteroparity, the ability to repeat spawn, is a natural life history strategy expressed by *0. mykis;*, at rates estimated to be as high as 79% for populations in the Utkholok River of Kamchatka, Russia (Savvaitova et al. 1996), and as high as 30% for British Columbia (Withler 1966). Historical rates for the Columbia River are not well documented but adult emigrating steelhead averaged 58% of the total upstream runs in the Clackamas River from 1956 to 1964 (Gunsolus and Eicher 1970). Current iteroparity rates for Columbia River Basin steelhead are considerably lower, due largely to high mortality of downstream migrating kelts (post-spawn steelhead) at hydropower dams (Evans and Beaty 2001), and potentially inherent differences in iteroparity rate based on latitudinal and inland distance effects (Withler 1966; Bell 1980; Fleming 1998). The highest recent estimates of repeat spawners from the Columbia River Basin were in the Kalama River (tributary of the unimpounded lower Columbia River), which exceeded 17% (NMFS 1996). %. A total of 8.3% of the adult steelhead from Snow Creek, WA were identified as repeat spawners based on scale samples (Seamons and Quinn 2010). In Hood River, repeat spawning

summer run steelhead comprise on average 5.7% of the run based on scale pattern analysis (Olsen 2008). Iteroparity rates for Klickitat River steelhead were reported at 3.3% from 1979 to 1981 (Howell et al. 1984). Summer steelhead in the South Fork Walla Walla River have expressed 2% to 9% iteroparity rates (J. Gourmand, ODFW, pers. comm.). Hockersmith et al. (1995) reported that repeat spawners composed 1.6% of the Yakima River wild run and recent tagging data shows average return rates to Bonneville Dam of 3.77

# Rationale

Post-spawn steelhead represent a portion of the population that have successfully survived through an entire life cycle culminating with spawning. Reconditioning these kelts may counter the negative selective forces against iteroparity associated with the hydrosystem, thereby helping to preserve the evolutionary legacy of the species. Kelt reconditioning starts with the introduction of feed, thereby enabling kelts to survive and rebuild energy reserves required for gonadal development and repeat spawning. Techniques used in kelt reconditioning were initially developed for Atlantic salmon *Salmo salar* and sea-trout *S. trutta*. A review of these studies and those applicable to steelhead kelts are summarized in Evans et al. (2001). Additional reviews of this subject (Hatch et al. 2002 and 2003b) provide support of the benefits of kelt reconditioning to address population demographic and genetic issues in steelhead recovery. We are estimating survival and return rates of artificially reconditioned kelt steelhead subjected to various management treatments ranging from low to high intensity efforts.. Although it is difficult to observe individual fish spawning in the wild, and even more difficult to assess the viability and quality of gametes produced in the wild, we are conducting experiments (gamete/progeny viability and reproductive success) to determine the extent to which reconditioned kelts are contributing to subsequent generations. The success of kelt reconditioning should be assessed based on the number of individuals that successfully spawn in the wild following reconditioning and release

This report is divided into 3 chapters:

- **Chapter 1:** Management Scenario Evaluation: Describes the evaluation of various management strategies that could be used as tactics for steelhead restoration programs.
    - Section A: Steelhead Kelt Collection and In-River Release
        - Yakima River
        - Snake River
        - Okanogan River basin
    - Section B: Transport of Unfed kelts
        - Yakima River
        - Snake River
    - Section C: Long-term reconditioning

        - Yakima River (Prosser, WA)
        - Omak Creek (Omak, WA)
        - Hood River (Parkdale, OR)
        - Clearwater River (Dworshak, ID)
        - Young's Bay Net Pens (Warrenton, OR
        - Mill Creek (Warm Springs, OR)
    - Section D: Management Scenario Analysis and Evaluation.

- **Chapter 2:** Progeny and Gamete Evaluation
    - Section A: Includes an evaluation of progeny and gamete viability of Skamania and Winter stock steelhead in the Hood River.
        - 2008 (kelt spawning and progeny status)
        - 2009 (Kelt spawning and progeny status)
        - 2010 (Maiden Spawn Status)(Skamania and Winter)
    - Section B: Field study of reproductive success of reconditioned kelt steelhead in the following basins:
        - Omak Creek
        - Yakima River

- **Chapter 3:** Snake River Basin kelt steelhead evaluations. The Nez Perce Tribe and two University of Idaho groups are conducting studies on kelt steelhead.

    - Idaho Cooperative Fish and Wildlife Research unit
        - Developing Strategies to Improve Survival and Return Recruitment of Steelhead Kelts from Snake River Stocks
    - Dr. James Nagler's lab
        - Reproductive Development and Migration Behavior of Reconditioned Steelhead (Oncorhynchus mykiss) Kelts in the Yakima River, Washington
        - Effects of energy restriction on metabolic factors and reproductive development in post-spawning female rainbow trout
        - Effects of long-term administration of ghrelin and growth hormone on feed intake and growth in juvenile rainbow trout
    - Kelt Master Plan Development Update

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# Chapter 1: Management Scenario Evaluation

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## Goals

The goal of this group of studies is to develop and evaluate potential strategies that fishery managers could use for steelhead restoration. The studies attempt to include measures that span from low intensity and associated costs through relatively high intensity and associated costs.

## Objectives

1. Investigate and develop approaches to utilize the steelhead kelt life stage to increase steelhead populations.

Rationale: Providing assistance to post-spawn steelhead in the forms of transportation, feed, and prophylactic measures will increase the probability that individual steelhead repeat spawn and contribute to population growth. In this objective we measure the variation in steelhead response to intervention method. We are attempting to estimate survival and migration characteristics of kelt steelhead in the Lower Columbia River by utilizing acoustic transmitter technology.

2. Apply kelt steelhead reconditioning techniques at selected streams to post-spawners then release.

Rationale: This objective will test the following hypothesizes:

Ho: Kelt steelhead reconditioning rates are similar spatially and temporally.

Ho: Kelt steelhead rematuration rates are similar spatially and temporally.

### Management Scenario Evaluation

An evaluation of reconditioning is based on two fundamental hypotheses aimed at comparing the relative survival and rematuration rates of program fish.

$H_o$: Iteroparity rates are similar among all treatments including: in-river release, transport and release, short-term recondition and transport, and long-term recondition and release.

$H_o$: Rematuration rates are similar among all treatments including: in-river release, transport and release, short-term recondition and transport, and long-term recondition and release.

Management scenarios include four styles (in-river control, transport unfed kelts, transport fed kelts long-term reconditioning) described below, with the long-term treatment replicated geographically.

#### *In-River Release (Yakima and Snake Rivers)*

A systematically selected portion of the kelts that would have been suitable for reconditioning were PIT-tagged and released immediately back to the Yakima and Snake Rivers to act as a control group. These

PIT-tagged kelts provide baseline data and an opportunity to compare current repeat spawner rates to those reported by Hockersmith et al. (1995) which were ascertained from scale pattern analysis from the Yakima River.

### *Transport Unfed (No-term) Treatment (Yakima and Snake Rivers)*

In this treatment we directly transport steelhead kelts around the hydro-system and evaluate success by measuring survival to the ocean and presumed kelt spawning migration return to acoustic arrays and PIT-tag detectors. Given the high mortality rates of seaward migrating kelts observed during radio telemetry experiments in the Snake and Columbia Rivers (Evans et al. 2001; Evans 2002; Hatch et al. 2003a) iteroparity may be augmented by simply transporting kelts around the hydro system, thereby improving access to the marine environment. The Transport Unfed kelts (No-term) release was reinitiated in 2010 to compare transport treatments from the Snake River (Lower Granite Dam) and the Yakima River (Chandler Fish Facility).

The purpose of this objective is to evaluate the lowest cost alternative aimed at increasing steelhead iteroparity. Prior to implementation of a large-scale kelt steelhead transportation program it is important to consider potential effects on non-target fish. If kelts maintain residence in the estuary rather than migrating to the ocean, It is also important to assess whether transportation impacts the survival and homing capability of these fish. To address these concerns, all steelhead kelts were PIT-tagged with a smaller portion implanted with hydro-acoustic tags. This technology will provide us with the necessary information regarding fish survival (based on detection or lack of), movement, distribution, travel time, velocity, residence time in the estuary, and return rates.

### *Long-term Reconditioning Treatment*

We define long-term reconditioning as holding and feeding post-spawn steelhead until the steelhead upstream migrating runs appear locally, typically in middle to late Fall the same year. The fish are released to over-winter and return to the spawning sites volitionally. The long-term steelhead reconditioning diet and treatments which were established from the studies conducted in 2001 and 2002 (krill and Moore-Clark pellets) (Hatch et al. 2002 and Hatch et al. 2003b) continued to be followed by the kelt reconditioning facilities at Prosser, WA, Cassimer Bar Hatchery, WA, Young's Bay, OR, and Dworshak, ID.

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# Section A: Kelt Collection and In-River Release (Yakima and Snake Rivers)

## Study Area

### Yakima River Basin

The Yakima River is approximately 344 km (214mi) in length and enters the Columbia River at Rkm 539. The basin is 6,150 sq mi (15,928 km²) and average discharge is 99 $m^3/s$. Summer steelhead populations primarily spawn upstream from Prosser Dam in Satus Creek, Toppenish Creek, Naches River, and other tributaries of the Yakima River (TRP 1995) (Figure 1).

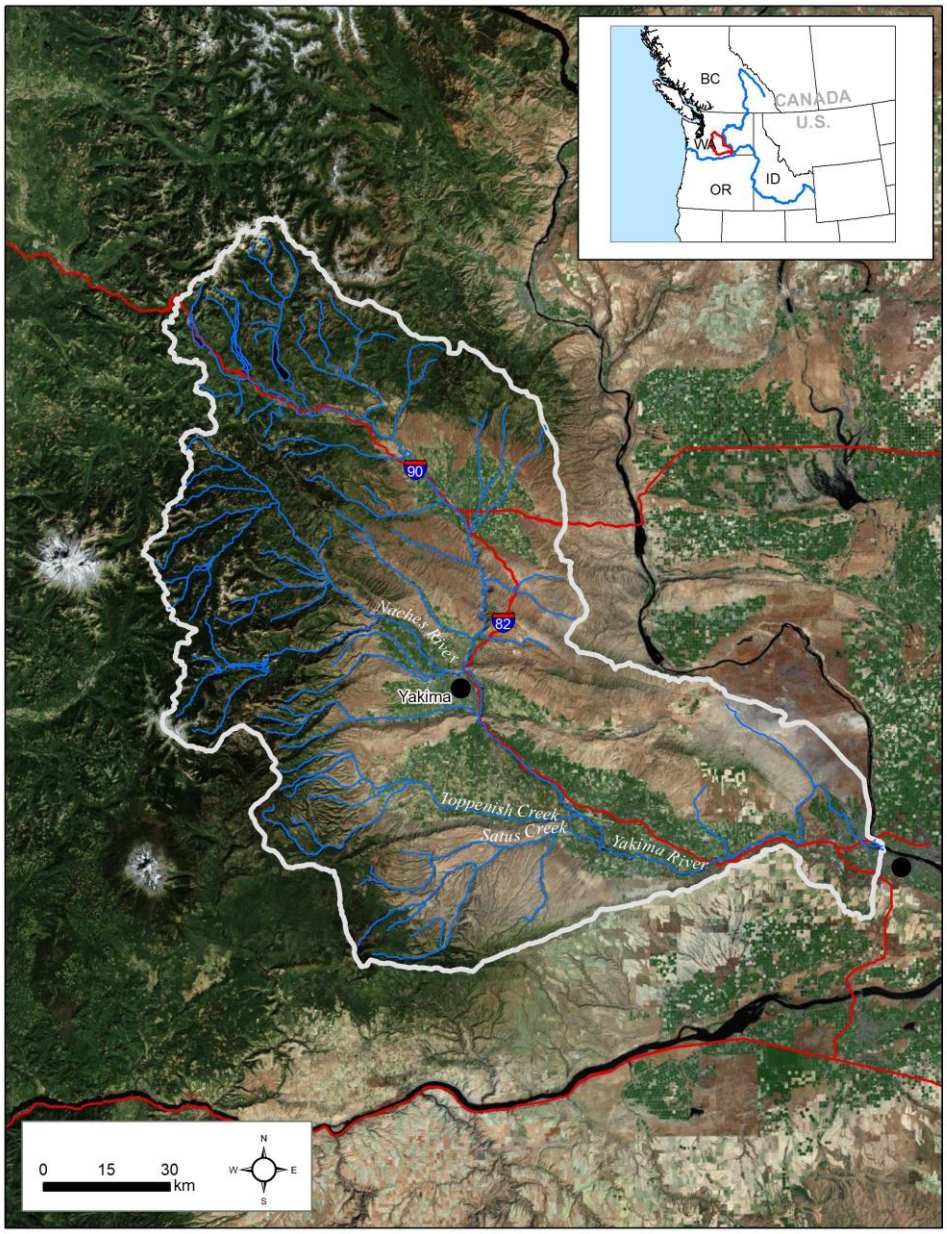


**Figure 1: Map of the Yakima River Subbasin.**

## Snake River Basin

The Snake River watershed is the tenth largest among North American rivers, and covers almost 108,000 square miles (280,000 $km^2$) in portions of six U.S. states: Wyoming, Idaho, Nevada, Utah, Oregon, and Washington, with the largest portion in Idaho. Most of the Snake River watershed lies between the Rocky Mountains on the east and the Columbia Plateau on the northwest. The largest tributary of the Columbia River, the Snake River watershed makes up about 41 % of the entire Columbia River Basin. The Snake River enters the Columbia at Rkm 523. Its average discharge at the mouth constitutes 31 % of the Columbia's flow at that point. The Snake River's average flow is 54,830 cubic feet

per second (1,553 $m^3/s$). At Anatone, Washington, downstream of the confluences with the Salmon, Clearwater and others of the Snake's largest tributaries, the mean discharge is 34,560 cubic feet per second (979 $m^3/s$) (Figure 2).

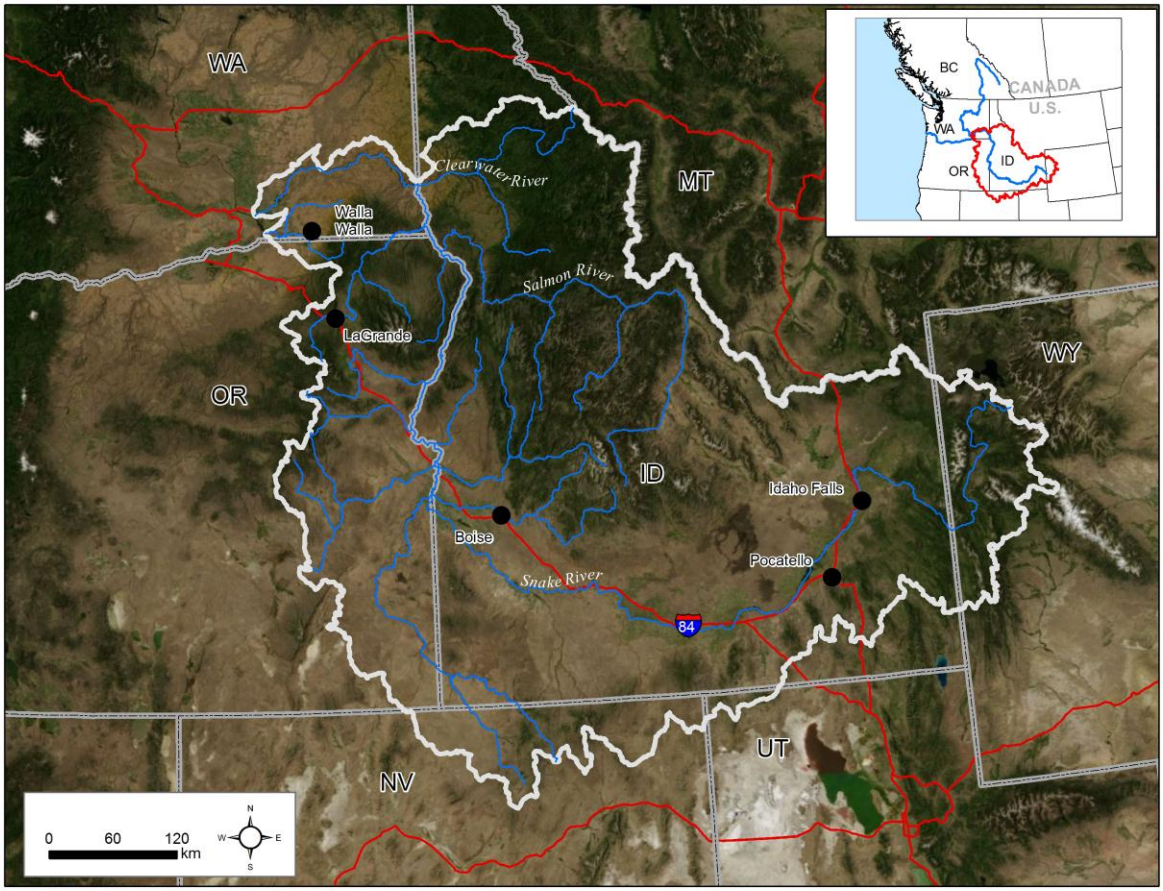


**Figure2:Map of the Snake River Basin.**

## Okanogan River Subbasin Kelts (Upper Columbia River Steelhead)

The Okanogan River is a tributary of the Columbia River and the confluence is located at Rkm 858 of the Columbia River. The Okanogan drainage area is 8,200 sq mi (21,238 $km^2$) with an average discharge rate of (86 $m^3/s$). Omak Creek, a tributary to the Okanogan River, is located in Okanogan County in North Central Washington, the confluences of Omak Creek is located at RKM of the Okanogan River. Omak Creek is approximately 35.4 km in length (Figure 3) running entirely within the Colville Confederated Tribes (CCT) reservation boundaries. Bonaparte Creek which runs for one mile is a tributary to the Okanogan River, which closely parallels State Route 20 east of Tonasket. Lower Salmon Creek (4.3 miles of Salmon Creek) is a tributary of the Okanogan River that has a diversion dam which prevents upstream fish passage. Steelhead spawn in Omak and Bonaparte Creeks with limited spawning possibly in Salmon Creek.

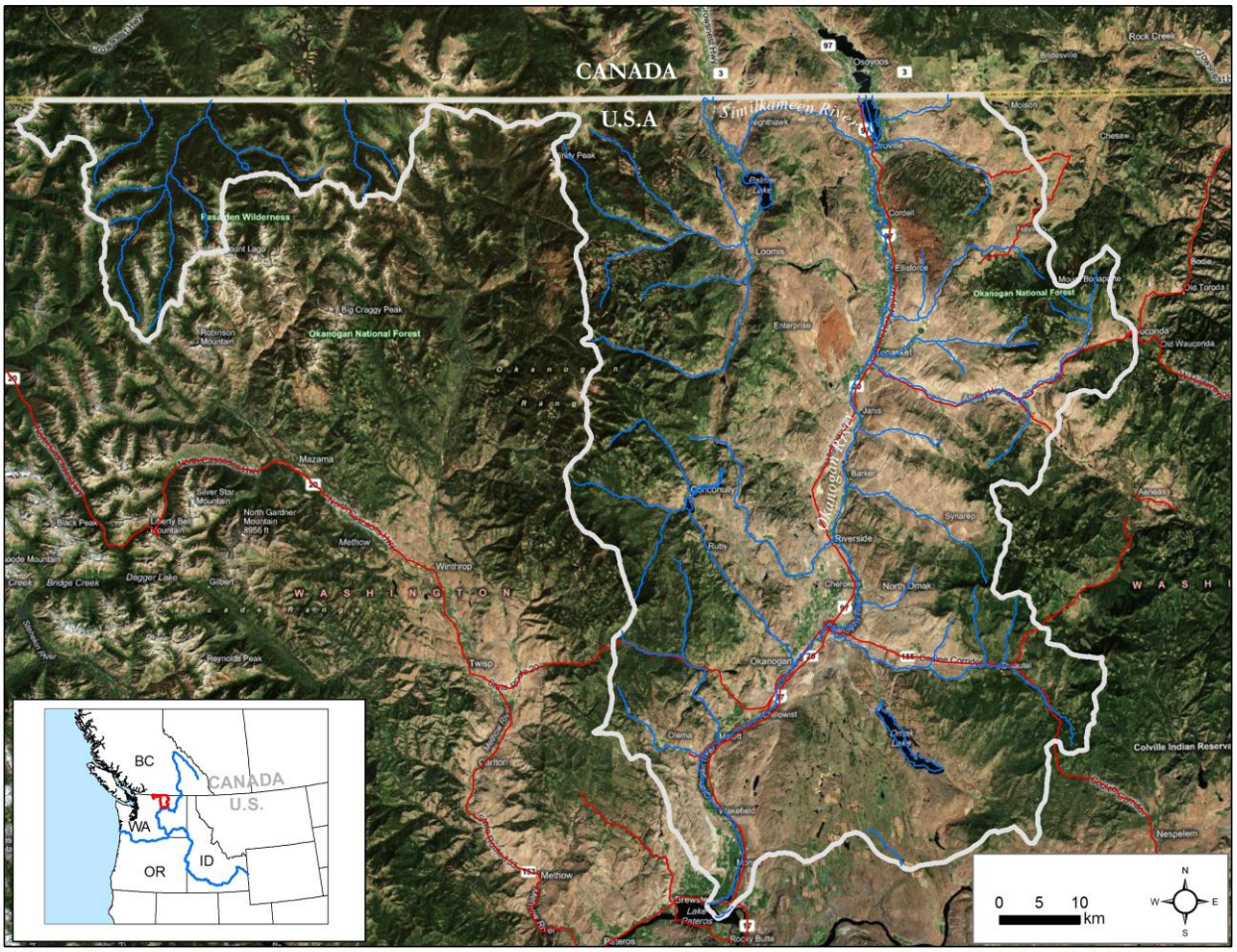


**Figure 3: Map of the Okanogan River Subbasin.**

## Hood River

The Hood River is a tributary of the Columbia River (confluence at Rkm 272) in northwestern Oregon. Approximately 40 km long from its mouth to its farthest headwaters, the river descends from wilderness areas on Mount Hood and flows through the agricultural Hood River Valley to join the Columbia River in the Columbia River Gorge. The Drainage area is 723$^2$ km with an average discharge of 28 m$^3$/s (Figure 4).

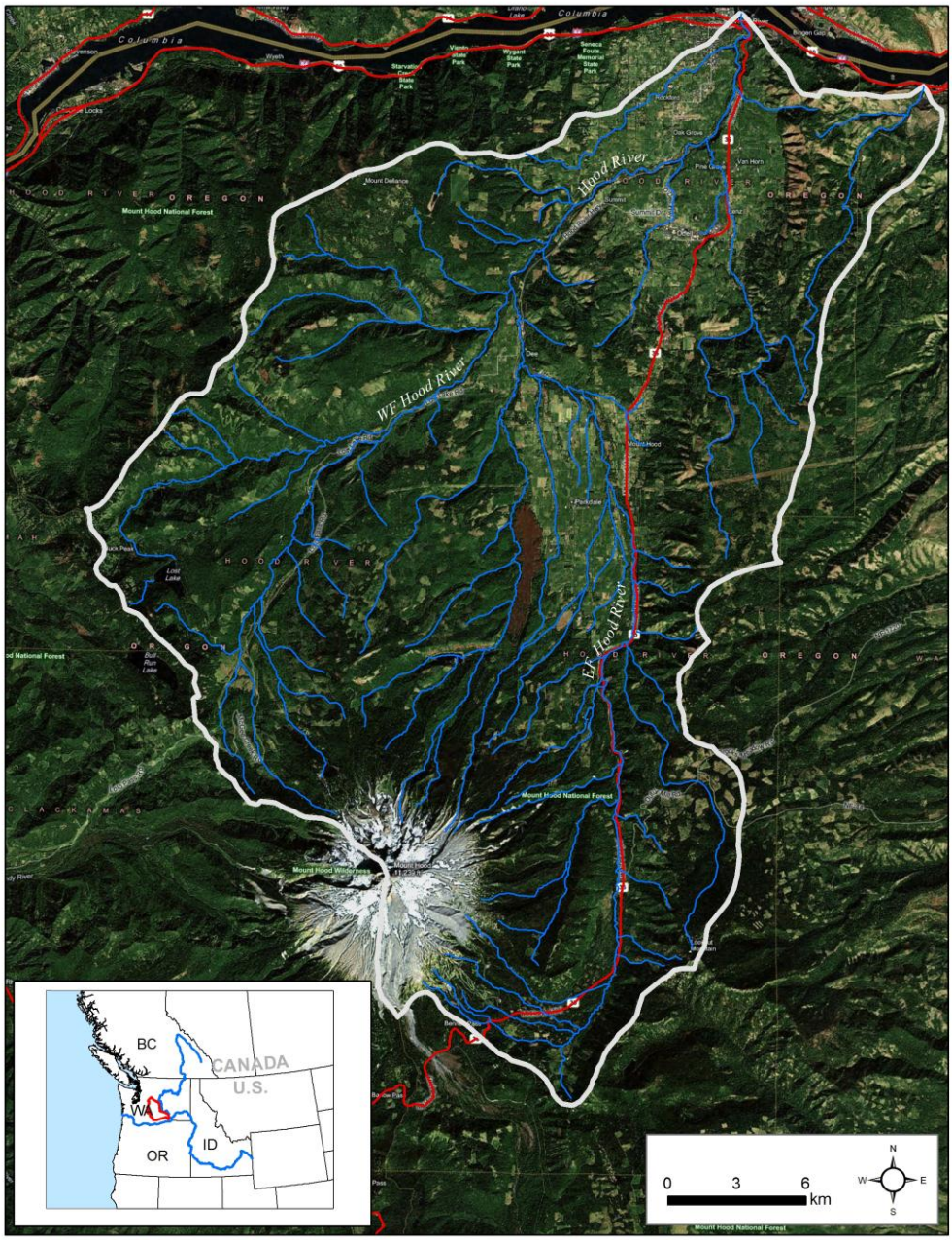


**Figure 4: Map of the Hood River Subbasin.**

# Methods

## *Kelt Collection and In-Processing*

**Chandler Juvenile Evaluation Facility (Yakima River)**

Post spawn steelhead migrating downriver are collected by way of the irrigation canal which diverts these kelts with fish screens divert migratory fishes away from irrigation canal into the Chandler Juvenile Evaluation Facility (CJEF). Once diverted into the CJEF, emigrating kelts are manually collected from a fish separation device (a device which allows smaller juvenile salmonids to "fall through" for processing in the juvenile facility while larger fish can be dipnetted off the separator rack for processing (Figure 5). Yakama Nation staff monitored the Chandler bypass separator 24 hours a day from March 19 to June 23, 2010.

**Figure 5. Inside view of the Chandler Evaluation Facility showing the separator rack where kelt steelhead are collected.**

All kelts are dipnetted and are placed into a water-lubricated PVC pipe slide that is directly connected to a temporary holding tank 20' (l) x 6' (w) x 4'(h) containing oxygenated well water ($57^0$F or $13.8^0$C) (Figure 6). Post-spawned steelhead kelts and transferred to a 190-L sampling tank containing fresh river water, and anesthetized in a buffered solution of tricaine methanesulfonate (MS-222) at 60 ppm. Steelhead

kelts are designated for an in-river release, transport, or long-term reconditioning. All specimens visually determined to be prespawn individuals were immediately returned to the Yakima River.

**Figure 6. Chandler Juvenile Evaluation Facility PVC slide and holding tanks.**

Following kelt identification, we collected data on weight (collected in pounds but converted to kg for this report), condition (good- lack of any wounds or descaling, fair- lack of any major wounds and/or descaling, poor- major wounds and/or descaling), coloration (bright, medium, dark), and presence or absence of physical anomalies (e.g., head burn, eye damage). Passive Integrated Transponder (PIT) tags (if not already present) were implanted in the fish's pelvic girdle for later individual identification

**The Lower Granite Juvenile Evaluation Facility (Snake River)**

Steelhead kelts migrating from tributaries of the Snake River above Lower Granite Dam that do not emigrate via the Removable Spillway Weir (RSW) are directed towards a large collector to the Juvenile Bypass Facility where they are collected by Army Corps of Engineer Staff and moved to a chute which leads to two holding tanks (Figure 7). Both B-run and A-run fish were selected. Staff from the Nez Perce Tribe, University of Idaho (UI), and CRITFC assisted US Army Corps of Engineers (COE) personnel at the LGR fish separator.

**Figure 7: Juvenile bypass seporator screen at Lower Granite Dam located on the lower Snake River. The photo on the right is the with kelt chute entrance where kelts are placed after being netted off of the seporator screeen.**

The holding tanks manufactured for the steelhead kelt program by the University of Idaho Engineering department are 6 'wide by 25 ' long and 6 'deep and have built in crowders to collect steelhead for sampling or allow for easy return to the river via an exit chute built at the rear of the holding tank closest to the river (Figure 8).

**Figure 8: Tanks designed by the UI for holding and sorting kelts at Lower Granite Dam.**

Fish were held for a minimum of 24 hours at the Lower Granite Juvenile Fish Facility. This allowed any potential ingested invasive species (mud snails) to pass through the gut prior to transport. However, to minimize stress, fish were not held more than two days. After this temporary holding, fish were anesthetized in tricaine methanesulfonate (MS-222) buffered with standard stock solution of sodium bicarbonate to decrease stress and mortality (McCann et al. 1994). The condition of the fish was assessed by taking length, weight, color, condition factor, and presence absence of injuries. Fish also had blood and tissue samples collected for physiological measures and genetic profiling. All fish that were not moribund received a PIT-tag before being assigned to a treatment or released back to the river.

**Omak and Bonaparte Creeks**

The Omak Creek weir (Rkm 0.8) is utilized to collect broodstock and steelhead kelts for reconditioning (Figure 9). This stock is being used by the Cassimer Bar Hatchery to develop a naturalized steelhead broodstock for the Okanogan River and Omak Creek. To increase the total number of kelts available for reconditioning, kelts were also collected from the Bonaparte Creek weir (0.4 Rkm) which is a tributary of the Okanogan River (Figure 10).

**Figure 9: Resistance board weir located on Omak Creek.**


**Figure 10: Bonaparte Creek capture weir.**

All anadromous *O. mykiss,* regardless of up or downstream movement including those selected for broodstock or reconditioning, were sampled for length, condition factor, inspected for tags (PIT or other), sampled for DNA and marked with a fin clip. PIT tags were applied if not already present. Steelhead kelts at Cassimer Bar Hatchery received salt treatments on a regular basis to help prevent against fungus and copepod infestation.

**Powerdale Trap (The Hood River)**

Oregon Department of Fish and Wildlife Employees capture anadromous summer-run (Skamania origin) and winter run (endemic) spawners at the Powerdale trap (Figure 11). Approximately 80 total (20 Summer and: 20 winter) spawning females and males (20 summer and: 20 winter) were trucked to the Parkdale Fish Facility where they were held until fully ripened. These fish are typically recycled through the fisheries three times before they are terminated and donated to the Oregon state Food Bank Program. We retained fish that visually appeared to be in good condition to maximize the success of spawning and reconditioning. Fish are sexed, weighed, and measured at collection to evaluate the impact of reconditioning. Trapping begins in June and ends in early March for the Skamania project fish and begins in February through early June for hatchery reared winter steelhead. Collection for this study ended when we obtained first time spawning steelhead for both groups.

**Figure 11: Powerdale trap on the Hood River.**

## Tagging

## PIT Tags

All fish in this study received a PIT tag in the pelvic girdle at the time of capture. Each tag is unique and identifies an individual fish to assess performance throughout the reconditioning process and to determine the fate of kelts after release by measuring movement, timing, and survival. Automatic adult PIT-tag detectors are present in all ladders at Bonneville Dam, McNary Dam, Prosser Dam, and weirs on smaller systems.

### *In River Release*

#### Yakima River

A systematic sample (1 of 10) of kelts suitable for reconditioning, were PIT-tagged and immediately released back into the Yakima River (Prosser, WA Rkm 75.6) to monitor the rate of natural iteroparity. These data will be compared to iteroparity rates from other treatments and inferred from scale pattern analysis in the Yakima River (Hockersmith et al. 1995). In-river release specimens were selected systematically throughout the duration of the steelhead kelt run.

**Snake River**

We detected 2 of the 2009 Snake River in-river fish returning in 2010. The first fish passed Bonneville Dam in mid-July and the second one passed in early August. The first fish was detected moving upriver in October and passed Lower Granite Dam in less than a week. The second fish managed to avoid detection up to Lower Granite Dam where it was detected in mid-September. No kelts from the 2010 releases were detected returning from the ocean that year.

## Results and Discussion

### General Population Characteristics

#### *Yakima River*

A total of 1,659 live kelts were captured between March 19 and June 23, 2010 at the CJEF. Of the total captures, 2 were mortalities in the bypass, 89 were immediately returned to the Yakima due to poor or prespawn condition, 155 were used in the Yakima River in-river Collection was continuous throughout the outward migration, with peak collection occurring on April 22, 2010 (Figure 12). The total number of kelts captured represented 24.4% (1,659 of 6,793) of the Yakima River spawning migration based on fish ladder counts obtained from Prosser Dam for the period July 1, 2009 through June 30, 2010. The exact steelhead kelt numbers in the Yakima River are likely higher with our collection only representing a portion of that total number.

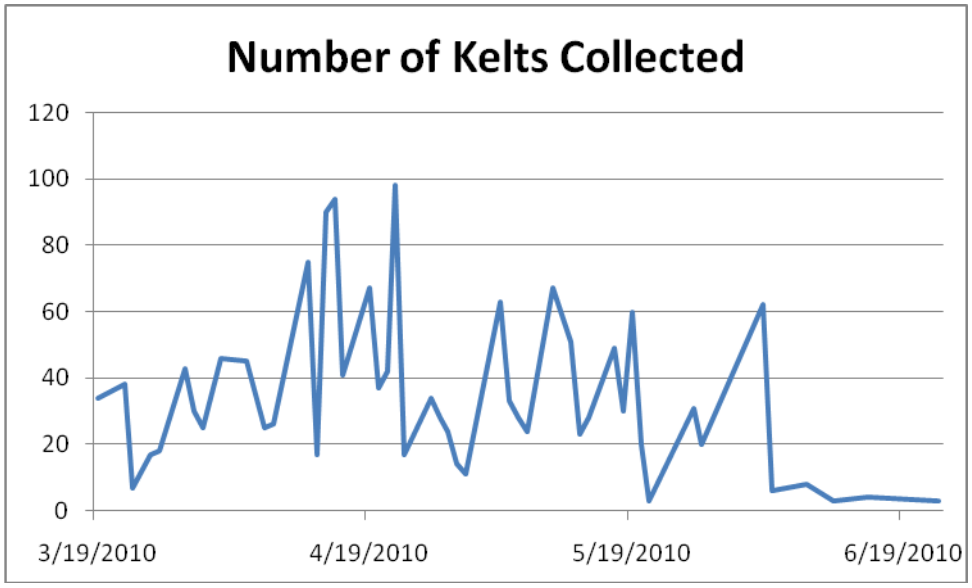


**Figure 12: Steelhead Collection at Prosser, WA 2010.**

The overwhelming majority of kelts captured were female which is consistent with previous findings (Branstetter et al. 2010, Branstetter et al. 2009, Branstetter et al. 2008, Branstetter et al. 2007a, Branstetter et al. 2007b, Branstetter et al. 2006, Branstetter et al. 2005, Hatch et al. 2003a, Hatch et al. 2003b, Hatch et al. 2004, Evans et al. 2002). Based on visual observations, in 2010 1438 (87%) of the kelts were female, 218 (13%) were male, with 3 fish not Identifiable. Most Yakima River kelts collected

during 2010 were classified as being in good (n=583, 35%) or fair (n=1003, 60%) condition, with the remaining fish classified as poor (n=73, 4%). Coloration was predominately intermediate (n=841, 51%) or bright (n=741, 45%) with a small percentage that were dark (n=77, 4%).

## *Snake River*

The majority of fish collected from the Snake River at Lower Granite juvenile bypass in 2010 were considered in good shape. This means that most of the fish were without any major wounds (scraps, cuts, fungal infections) with the majority of them caught in the month of May (Table 1). The majority of these fish in good shape were female and smaller than 70 cm or a-run fish (Table 2). The majority of our larger fish or b-run fish were also in good shape but by less of a margin than our a-run fish (Table 2).

**Table 1: Condition of Snake River Kelts collected at the Lower Granite juvenile bypass in 2010.**

| | April | May | June | July |
|---|---|---|---|---|
| **Good** | 289 | 1028 | 319 | 2 |
| **Fair** | 148 | 438 | 147 | 1 |
| **Poor** | 80 | 183 | 47 | 0 |

**Table 2: Condition of steelhead kelts by sex and size at the Lower Granite juvenile bypass in 2010.**

| **Female (79%)** | **Good (61%)** | **Fair (27%)** | **Poor (12%)** | **% of run** |
|---|---|---|---|---|
| **< 70cm** | 1202 | 514 | 180 | 70.6% |
| **≥ 70 cm** | 158 | 39 | 19 | 8.0% |
| | | | **total** | 78.6% |
| **Male (21%)** | | | | |
| **< 70cm** | 272 | 172 | 106 | 20.5% |
| **≥ 70 cm** | 4 | 8 | 4 | 0.6% |
| | | | **total** | 21.1% |
| **Total** | 1636 | 733 | 309 | 2682 |

It was observed that the fish that were injured had head wounds that appeared to have multiple points of contact. These head wounds look very similar in nature (deep tissue wounds) which concerns us that there is something repeatedly or mechanistically causing serious injury to fish on their journey to or through the bypass (Figure 13). This type of wounds are not typically seen in our other reconditioning sites and if present, not observed as high a frequency as is found in the Snake River. Overall, the proportion of head injuries was just over 10% (Table 3). The majority of these injuries occurred in May but proportionally a higher percentage occurred in June when spring flows started to move beyond the average of 50kcfs in May to the prevailing 100 kcfs a day in June with some days above 200 kcfs. Fungus was prevalent on steelhead kelts migrating in the Snake River. Generally, 27% of the kelts

collected at the bypass had greater than 6% of the fish's body area of this group 10% had greater than 15% percent coverage of fungus collected at the separator (Figure 14)

**Figure 13: Head wounds on steelhead kelts collected at the Lower Granite juvenile bypass.**

**Table 3: Proportion of headwounds on steelhead kelts at the Lower Granite juvenile bypass.**

| | April | May | June | July | Total |
|---|---|---|---|---|---|
| No | 0.91 (N=471) | .87 (N=1439) | .82 (N=424) | .66 (N=2) | .87 (N=2336) |
| Yes | 0.08 (N=46) | .12 (N=210) | .17 (N=89) | .33 (N=1) | .12 (N=346) |
| Total | .19 (N=517) | .61 (N=1649) | .19 (N=513) | .001 n(N=3) | 1.0 N=2682 |

**Figure 14: Fungal infection (>15%) on kelt collected from the Lower Granite juvenile bypass.**

## *Omak Creek*

The trap was operation led from February 20 through July 19, 2010. During the season 212 summer steelhead were passed above the weir. The 2010 season had the highest number of fish enumerated since trapping started in 2001. The natural-origin ratio was also the highest level seen, with over 80% of the fish returning being of natrual origin. There were 140 males and 72 females processed at the trap (Table 4). In addition to the large numbers of fish 2010 had the earliest returning group of fish. Weather conditions maintained good water throughout the trapping season but numbers of kelts seen at the trap was minimal. In the downstream trap a total of 37 kelts were collected. There were 13 good condition kelts and 20 dead-on-arrival mortalities and 4 which were passed downstream due to poor condition. A total of 17 fish (7 females and 10 males) were captured and transported to Cassimer Bar Hatchery for the Colville captive broodstock program.

**Table 4: Percentage and totals of male, female and wild summer steelhead passed above the Omak Creek weir, 2010.**

| | **Total (N)** | **Natural (N)** | **Percent-Natural (%)** |
|---|---|---|---|
| **Total** | 212 | 171 | 80.7 |
| **Males** | 140 | 113 | 80.7 |
| **Females** | 72 | 58 | 80.6 |

## *Bonaparte Creek*

An adult weir trap was installed in Bonaparte Creek on March 22, 2010 and fished until removed on July, 1, 2010 due to low water. Most migrating kelts were trapped between March 22 and April 30$^{th}$. There were a total of 28 natural males and 11 natural females with 39 hatchery males and 10 hatchery females sampled (Table 5). Sixty-seven summer steelhead (46 males; 21 females) were collected. The table below shows the breakout of wild and hatchery fish. One female fish was captured and transported to Cassimer Bar Hatchery for the Colville captive broodstock program.

**Table 5: Proportions and totals of male, female, and wild summer steelhead passed above the Bonaparte Creek weir, 2010.**

| | Total | Wild | Percent Wild |
|---|---|---|---|
| | (N) | (N) | (%) |
| Males | 67 | 28 | 41.8% |
| Females | 21 | 11 | 52.4% |
| **Total** | **88** | **39** | **44.3%** |

The downstream trap at Bonaparte Creek collected only 5 live kelts and 7 kelt mortalities. All live kelts were in poor condition and passed downriver.

### *Hood River*

2010 was the last year for Skamania kelt capture. Winter run hatchery fish were captured this year to measure their reproductive capability. The first fish was captured on June 24, 2009 and ended June 14, 2010. A total of 43 Skamania steelhead first-time spawners were captured (13 males and 30 females). There were 26 male and 33 females captured as candidates for this portion of the program. All of these fish upon initial inspection appeared to be in good to excellent shape with little to no tissue scrapping or damage.

## In-River Release and Detection Results

### *Yakima River*

There was a total of 155 kelts released as in-river fish into the Yakima River in 2010.
In River return PIT-tag detection return results in 2010 were extremely low. In the Yakima River there was a single returning fish from the 2009 in-river release. It was first detected passing Bonneville Dam in late July 2010 and passed McNary Dam in October that year.

### *Snake River*

There were a total of 1,399 kelts released as in-river fish into the Snake River in 2010.

We detected 2 of the 2009 Snake River in-river fish returning in 2010. The first fish passed Bonneville Dam in mid-July and the second one passed in early August. The first fish was detected moving upriver in October and passed Lower Granite Dam in less than a week. The second fish managed to avoid detection up to Lower Granite Dam where it was detected in mid-September. No kelts from the 2010 releases were detected returning from the ocean that year.

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# Section B. Transport Unfed (No-term) Treatment (Yakima and Snake Rivers)

## Study Area

Steelhead for the transport treatments were trucked and released at two sites. The first one is Hamilton Island Boat Ramp (Rkm 231) located downriver from Bonneville Dam on the Washington shore of the Columbia River in 2010 (Figure 1 ). The second release site at Aldrich Point is located in the upper portion of the Columbia Estuary at rkm 75 (Figure 1). The lower Columbia River habitat from approximately Rkm 75-0 is typified as an estuarine environment, and is influenced by tidal oscillations from the Pacific Ocean. Acoustic telemetry technology (Figure 2) (Rkm 231 to 0.) (Appendix A) was utilized to monitor this release and prior years' releases.

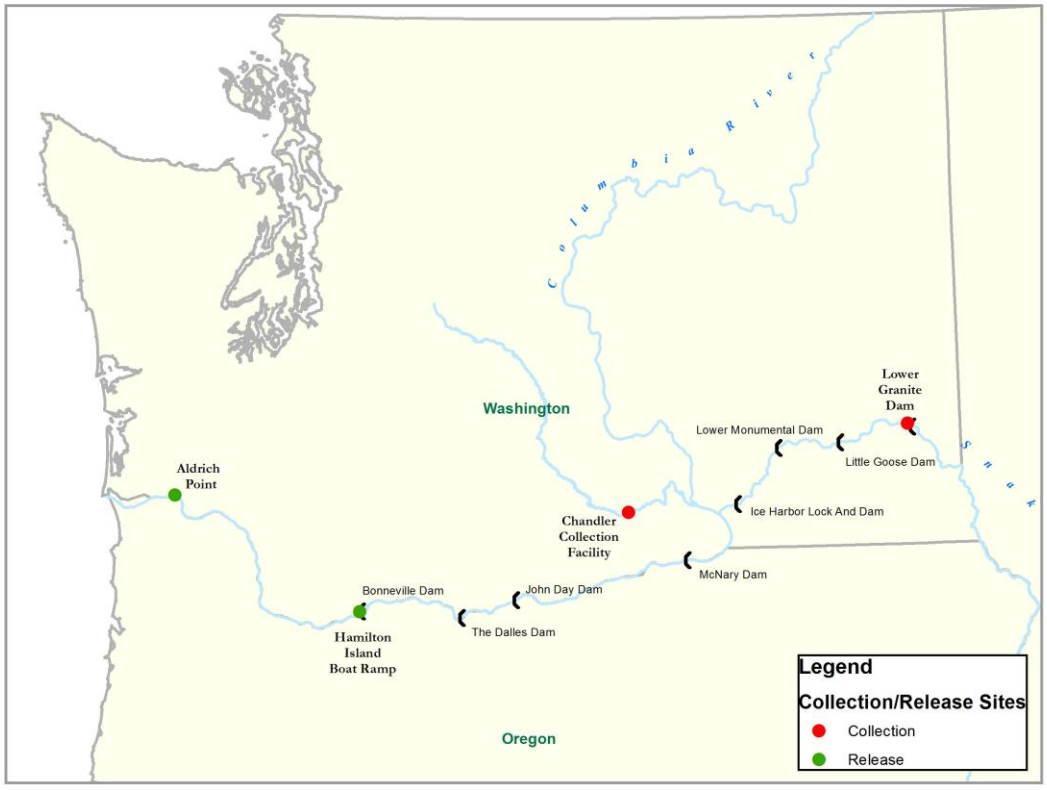


**Figure 1. Map of the Columbia River showing kelt collection and release sites for transported steelhead kelts.**

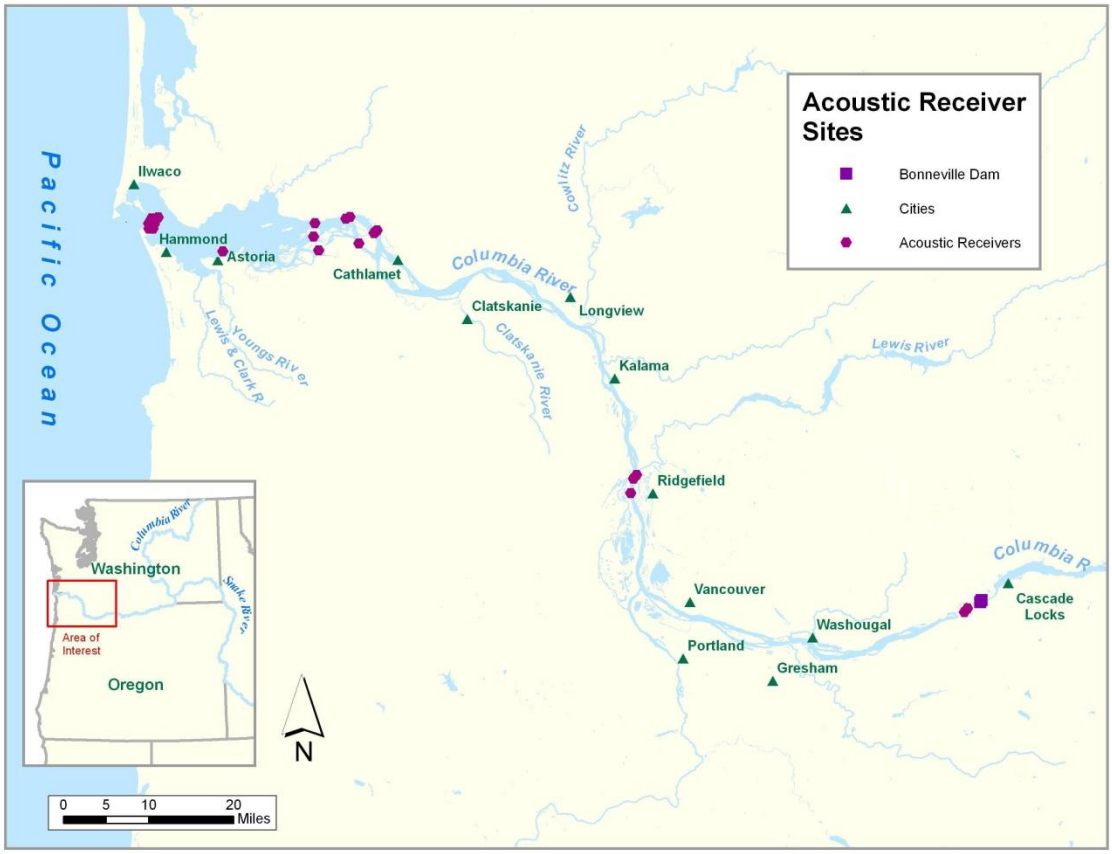


**Figure 2 Map of the lower Columbia River showing locations of acoustic receiver arrays in 2010.**

# Methods

## PIT-tags

Every steelhead captured for this experiment was injected in the pelvic girdle with a PIT-tag, including the acoustic tagged fish. At the time of release all fish were scanned for the presence of PIT-tags, if a tag was shed we retagged that individual. A portion of the releases were solely tagged with PIT-tags to evaluate any potential acoustic tagging effect.

## Acoustic Tags

A portion of the steelhead kelts captured at the CJEF and Lower Granite juvenile bypass had a coded Vemco© V16-4H acoustic transmitter surgically implanted intraperitoneally (body cavity) using standard surgical procedures in 2010 that we have used previously (Branstetter et al. 2010, Branstetter et al. 2009, Branstetter et. al. 2008, and Branstetter et. al. 2007). Each acoustic tag has a unique bandwidth pulse that provides individual identification codes. The weight impact of the tag on adult fish was nominal, with its length at 65 mm and weight in water at 10g, constitutes on average 0.25% of the fishes

total body weight. In an internal implantation, an incision just smaller than the transmitter diameter is made into the body cavity, usually on the midline of the ventral surface halfway between the pectoral and pelvic fins (Langford et al. 1977). The incision is spread open utilizing a sterilized gloved finger as a dilator. The use of dilation splits the muscle which causes less damage and speeds healing than cutting the muscle tissue all the way through. Transmitters were disinfected before placement into the body cavity. Once the transmitter is securely inside the fish the original incision is closed with several interrupted sutures. Sterile, non-reabsorbing suture was used due to concerns of seawater prematurely causing the suture to split. General anesthetics (MS-222) were used during surgery, and fish were returned to freshwater immediately following surgeries to recover. A biologist trained by a licensed veterinarian performed surgeries to minimize adverse effects associated with handling and surgery and to ensure a high tag retention rate.

## Release

The unfed transport treatment groups were released at either the Hamilton Island Boat Ramp (Rkm 231) below Bonneville Dam or at Aldrich Point (Rkm 75) in 2010. After release, migration to the Pacific Ocean was tracked using acoustic telemetry arrays that spanned sections of the Columbia River and estuary below Bonneville Dam (Appendix A). The complete array was deployed in early March 2010 and was retrieved late October the same year. This year's array placement remained nearly identical to previous year's (Branstetter et al. 2009, Branstetter et. al. 2008, and Branstetter et. al. 2007) (Appendix A). This arrangement provides data on survival and timing in-river, to the estuary, and to the ocean. Using acoustic telemetry data we can compare the two different lower release strategies with and between how fish from two different capture areas. We will assess the releases by fish return rates, movement, distribution, travel time, velocity, as well as residence time in the estuary (Appendix A). Acoustic arrays will be deployed in 2011 and again in 2012 to detect skip-year spawning fish.

## Detection History

Detection is based on the presence or absence of detection history obtained from acoustic receivers. Vemco receivers need to obtain a minimum amount of detections (#) before the software considers the detection legitimate. The detection history is used to derive our survival and detection probability values. Emigration timing is also derived from our first and last detections within an array line. We assume that fish that are never detected post release and fish which have detections that remain stationary (over a month) are likely mortalities. Detections that are made in July-August after having had a final detection at the mouth (usually a week to 3 weeks after release) are likely returning fish from the ocean. Automatic adult PIT-tag detectors are present in all ladders at Bonneville Dam, McNary Dam, Prosser Dam, and weirs on smaller systems to provide additional survival and movement information.

## Estimating Survival

The lower Columbia River was divided into 3 reaches (St. Helens reach Rkm 231 to Rkm 150; Estuary Reach Rkm 150 to Rkm 75; River mouth Rkm 75 to Rkm 0). Survival and detection probability was calculated for each release reach independently, except for the River mouth reach where survival and detection probability cannot be separated. Survival and detection probably were calculated to array using a maximum likelihood function.

We describe estimation of parameters in a generalized setting where two detection locations are considered. Even when there are more than two detection locations, the estimation principal remains the same. In case of the experiment design, numbers of fish released at stage 1 and then detected at stage 2 or 3 become multinomial random variables.

$$(n_{g12}, n_{g13}) \sim Multinomial(R_{g1}, \theta) \quad (1)$$

Where parameter vector $\theta = (s_{g1}p_1, s_{g1}(1-p_1)s_{g2}p_2)$. First element of vector $\theta$ (i.e., $\theta_{(1)} = s_{g1}p_1$) means the probability that a fish from stage 1 survives to next stage, and also is detected at the next stage. The second element $\theta_{(2)}$ (= $s_{g1}(1-p_1)s_{g2}p_2$) indicates the probability that a fish from stage 1 survives to stage 2, is not detected at stage 2, survives from stage 2 to stage 3 and finally is detected at stage 3.

Also, when considering that the number of fish detected at stage 2 is the new release number, the number of fish detected at stage 2 being detected at stage 3 again becomes a binomial random variable.

$$n_{g23} \sim Binomial(n_{g12}, s_{g2}p_2) \quad (2)$$

where $s_{g2}p_2$ means the probability that a fish from stage 2 survives to stage 3 and then is detected at stage 3. However, so-called success/failure probability in the binomial mass function in eq. $s_{g2}$ and $p_2$ consists of two parameters of $s_{g2}$ and $p_2$, and such two parameters cause an over-parameterization problem because a success/failure parameter in a binomial mass function is only one. That is, we cannot separately estimate $s_{g2}$ and $p_2$ and thus express the product as one parameter, say $\lambda_g$. The expression of $\lambda_g$ is not problematic in this study, because our ultimate goals are to compare two fish groups (control vs. treatment) not to estimate receivers' detection rates. A difference in $\lambda_g$ between two fish groups is due to only fish survival $s_{g2}$ not receivers' detection rate $p_2$. So, comparing two fish groups based on estimates of $\lambda_g$ is justifiable.

Further these multinomial and binomial events do not affect each other, so they are independent. That is, the probability of those two events is the product of the respective probabilities.

$$\begin{aligned} p(n_{g12}, n_{g13}, n_{g23} \mid \theta, l_g) &= p(n_{g12}, n_{g13} \mid \theta) \times p(n_{g23} \mid l_g) \\ &= Multinomial(R_{g1}, \theta) \times Binomial(n_{g12}, l_g) \end{aligned} \quad (3)$$

By definition, the likelihood function of parameters, $L(\theta, l_g \mid n_{g12}, n_{g13}, n_{g23})$ is eq. 3. Ignoring constants with respect to parameters, the likelihood function of parameters is

$$L(\theta, l_g) \propto \theta_{(1)}^{n_{g12}} \times \theta_{(2)}^{n_{g13}} \times (1 - \theta_{(1)} - \theta_{(2)})^{R_{g1} - n_{g12} - n_{g13}} \times l_g^{n_{g23}} \times (1 - l_g)^{n_{g12} - n_{g23}} \quad (4)$$

Note that this likelihood function has three parameters as variables: $s_{g1}$, $p_1$, and $\lambda_g$. For convenience of the calculation of maximum likelihood estimates (MLEs) of those three parameters and the variances,

we take the natural logarithm for the likelihood function of eq. 4. The conversion to the log likelihood form is straightforward so we don't show it here. Finally, implementing the log likelihood function to software, Automatic Differentiation Model Builder (ADMB) (Fournier 2000), we differentiate the log likelihood function with respect to parameters to obtain the MLEs, and further calculate the Hessian matrix for calculation of the variances.

## Results and Discussion

### 2010 Release Detection and Survival Estimates

Transportation benefits were compared between steelhead stocks (Snake and Yakima river) and between release locations (Hamilton Island Rkm 231 and Aldrich Point Rkm 75) using detections of acoustic tags.

Our array lines held up well with minimal disturbances due to either human caused interference or weather related disturbances. Receivers were replaced quickly in such cases. We opportunistically included detections of an array near Bonneville, which was part of another study. The inclusion of the Bonneville detection array is meant to determine possible immediate release mortality after transport, but some Snake River and the Yakima releases had low numbers at Bonneville due to release times coinciding with high spill events from Bonneville Dam and rapid movement from the area.
A total of 237 fish were acoustic tagged, transported and released. One hundred eighteen fish were from the Snake River and 119 from the Yakima River. Releases occurred between April 14 and June 14, 2010 (Table 1 and 2). Fish were trucked and released at Aldrich Point from mid- to late April. Some transport groups from the Snake River were trucked but the majority was barged. Detections declined primarily from the upper estuary to the mouth of the river (Tables 1 and 2). The Snake River release groups had two barge and two truck releases that had no detections to the ocean. The detection pattern of kelts from the Yakima River released at Hamilton Island was consistent with observations in previous years (Branstetter et al. 2009, Branstetter et al. 2008, Branstetter et al. 2007).

**Table 1: Acoustic detections of kelt steelhead from the Yakima River transported and released below Bonneville Dam in 2010.**

| Origin | Release Location | Release Date | Type | Release # | # of Acoustic tags released | Detection at Bonneville Line | Detection at St. Helens line | Detections at Upper Estuary line | Mouth of Ocean |
|---|---|---|---|---|---|---|---|---|---|
| Yakima River | Aldrich Pt. | 4/14/2010 | Truck | 1 | 60 | ND | ND | 58 | 13 |
| Yakima River | Hamilton Is. | 5/12/2010 | Truck | 2 | 59 | 33 | 55 | 53 | 27 |
| Total | | | | | 119 | 33 | 55 | 111 | 40 |

ND= means no detection due to a release occurring downstream of these lines.

**Table 2: Acoustic detections of kelt steelhead from the Snake River transported and released below Bonneville Dam in 2010.**

| Origin | Release Location | Release Date | Type | Release # | # of Acoustic tags released | Detection at Bonneville Line | Detection at St. Helens line | Detections at Upper Estuary line | Mouth of Ocean |
|---|---|---|---|---|---|---|---|---|---|
| Snake River | Hamilton Is. | 4/21/2010 | Barge | 1 | 6 | 4 | 3 | 3 | 0 |
| Snake River | Hamilton Is. | 4/28/2010 | Barge | 2 | 10 | 8 | 6 | 0 | 0 |
| Snake River | Aldrich Pt. | 4/28/2010 | Truck | 3 | 10 | ND | ND | 7 | 4 |
| Snake River | Hamilton Is. | 5/5/2010 | Barge | 4 | 9 | 0 | 5 | 4 | 1 |
| Snake River | Hamilton Is. | 5/5/2010 | Truck | 5 | 10 | 1 | 6 | 1 | 0 |
| Snake | Hamilton Is. | 5/12/2010 | Barge | 6 | 10 | 10 | 4 | 1 | 1 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| River | | | | | | | | | |
| Snake River | Hamilton Is. | 5/12/2010 | Truck | 7 | 8 | 2 | 5 | 4 | 0 |
| Snake River | Hamilton Is. | 5/18/2010 | Barge | 8 | 17 | 16 | 8 | 6 | 3 |
| Snake River | Hamilton Is. | 5/25/2010 | Barge | 9 | 8 | 7 | 6 | 7 | 4 |
| Snake River | Hamilton Is. | 5/25/2010 | Truck | 10 | 9 | 6 | 6 | 5 | 1 |
| Snake River | Hamilton Is. | 6/2/2010 | Barge | 11 | 13 | 11 | 3 | 5 | 2 |
| Snake River | Hamilton Is. | 6/14/2010 | Barge | 12 | 8 | 7 | 3 | 6 | 1 |
| Total | | | | | 118 | 72 | 55 | 49 | 17 |

ND= means no detection due to a release occurring downstream of these lines.

Survival for Aldrich Point releases to ocean detections were lower than Hamilton Island releases for the Yakima and Snake River groups (Tables 3 and 4).

Snake River kelt releases at Hamilton Island t had lower reach survivals than Yakima River releases at the same location (Tables 3). The lowest reach survival was in the estuary for both Snake River and Yakima River origin fish. Assuming 100% detection by the array at the mouth of the river, survival for Snake and Yakima River fish in the estuary was 50% and 35%, respectively.

**Table 3. Survival and standard deviation (SD) by reach for Yakima River and Snake River kelts transported and released near Hamilton Island in 2010.**

| | Yakima River | | Snake River | |
|---|---|---|---|---|
| **Array** | **Survival** | **SD** | **Survival** | **SD** |
| Survival to St. Helens | 0.93 | 0.03 | 0.64 | 0.06 |
| Survival to Estuary | 1.00 | 0.03 | 0.64 | 0.07 |
| Detection Probability to St. Helens | 0.98 | 0.02 | 0.79 | 0.06 |
| Detection Probability to Estuary | 0.96 | 0.04 | 0.94 | 0.06 |
| (Survival x Probability) River Mouth | 0.50 | 0.07 | 0.35 | 0.08 |

Kelt steelhead from the Snake River were transported to Hamilton Island by barge (n=81) or by truck (n=27). We compared the reach survival and detection probabilities for these two transport methods in Table (4). Survival from release to St. Helens and through the estuary reach was nearly the same for barged and trucked groups. Survival through the river mouth favors barged over trucked fish though with the estimates are within 2 standard deviations (Table 4).

**Table 4. Survival and standard deviation (SD) by reach for Snake River kelts transported by barge and truck and released near Hamilton Island in 2010.**

| | Barged | | Trucked | |
|---|---|---|---|---|
| **Array** | **Survival** | **SD** | **Survival** | **SD** |
| Survival to St. Helens | 0.64 | 0.06 | 0.63 | 0.09 |
| Survival to Estuary | 0.67 | 0.08 | 0.59 | 0.12 |
| Detection Probability to St. Helens | 0.76 | 0.07 | 1.00 | 0.0 |
| Detection Probability to Estuary | 0.94 | 0.06 | 1.00 | 0.0 |
| (Survival x Probability) River Mouth | 0.46 | 0.10 | 0.10 | 0.09 |

Seventy acoustic tagged fish were trucked and released at Aldrich Point (Rkm 75). Ten fish were from the Snake River and 60 from the Yakima River. Survival estimates from release to the estuary array was 93% for the Snake River fish and 95% for the Yakima River fish (Table 5). Survival estimates to the ocean for Snake River fish was 43% though this estimates was within two standard deviations of the Yakima River survival estimate of 23%.

**Table 5. Survival and standard deviation (SD) by reach for Yakima and Snake River kelts transported and Released at Aldrich Point in April 2010.**

| | Yakima River (n=60) | | | Snake River (n=10) | |
|---|---|---|---|---|---|
| **Array** | **Survival** | **SD** | | **Survival** | **SD** |
| Survival to Estuary | 0.95 | 0.03 | | 0.93 | 0.22 |
| Probability | 1.00 | 0.0 | | 0.75 | 0.22 |
| (Survival x Probability) Mouth of Ocean | 0.23 | 0.06 | | 0.43 | 0.19 |

Steelhead kelt condition data suggests that kelts from the Yakima River basin generally appear in better shape than fish from the Snake River (Table 6). The quality of fish coinciding with other possible issues like increased migrational distance for Snake River fish, a negative long-distance transport effect, possible issues with bypass design, or combination of these issues may help to explain the lower survival rate of Snake River fish. We plan to improve the low sample size for the Snake River groups and plan to build upon this data to determine which release strategies are the most beneficial option.

**Table 6. Condition ratings of kelt steelhead collected at Lower Granite Dam (Snake River) and at Prosser Dam.**

| | Location | | |
|---|---|---|---|
| **Condition** | **Snake River** | **Yakima River** | **Total** |
| **Good** | 94 | 119 | 213 |
| **Fair** | 24 | 0 | 24 |
| **Poor** | 0 | 0 | 0 |
| **Total** | 118 | 119 | 237 |

## Kelt Migration Rates

For fish that migrated to the ocean, Snake River kelts migrated very quickly in some instances in less than half the time of the Yakima River kelts (Figure 3). This held true for even the Aldrich Point release with the Snake River release migrating at half the time of the Yakima River kelts(Figure 3). Snake River origin kelts released at Hamilton Island traveled approximately 120-150 hours quicker to the ocean than Yakima River origin kelts that were released in the estuary, even though the Snake River fish traveled some 192 km further.

When comparing Yakima River kelt migration timing against previous years it is within normal measures of previous kelt releases (Branstetter et al. 2009, Branstetter et al. 2008 and Branstetter et al. 2007). The one exception to the quick migration times was Snake River release 8 which took the longest and was greater than 50 hours longer than the Yakima Hamilton Island release (Appendix B). The portions of the river which took Snake River fish the longest to navigate was from Bonneville to St. Helens (Figure 3). The Yakima releases resided at the estuary for a longer period of time than Snake River Fish before moving to the mouth of the ocean (Figure 4). The Aldrich Point release residence is almost twice as long as the Hamilton Island release (Figure 4). Overall both Yakima groups took the longest time total to migrate with the Hamilton Release group (Hamilton Island truck) taking almost 3 times longer than the

fastest Snake River release group (Hamilton Island truck). Of the Snake River groups the longest to migrate to the ocean was the barged group.

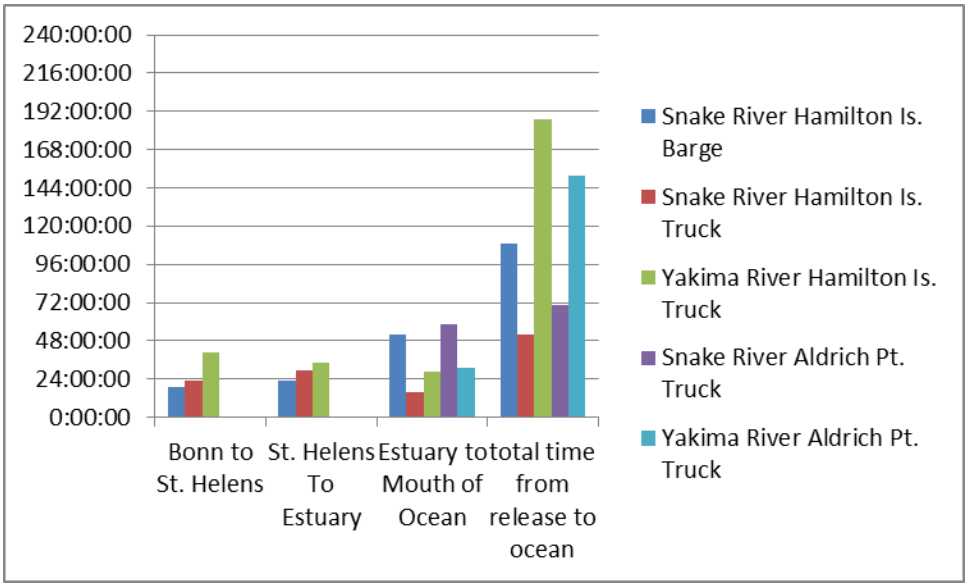


**Figure 3: Travel times to each reach for ocean migrators (hr:min:sec).**

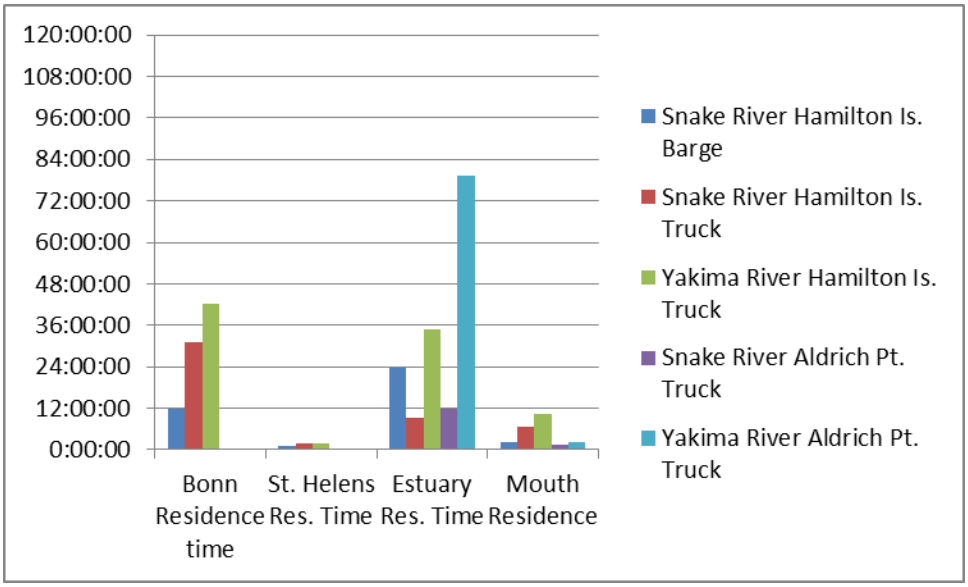


**Figure 4: Residence times at each reach for the different releases for ocean migrators (hr:min:sec).**

There does not appear to be a large amount of time difference between the fish that successfully migrate to the ocean and non-ocean migrators from Bonneville to the Estuary (Figure 5). The biggest time difference was found in the estuary residence times, with non-ocean migrating kelts residing

almost twice as long as their ocean going cohorts  with the exception of the Snake River Truck release at Hamilton Island (Figure 6).

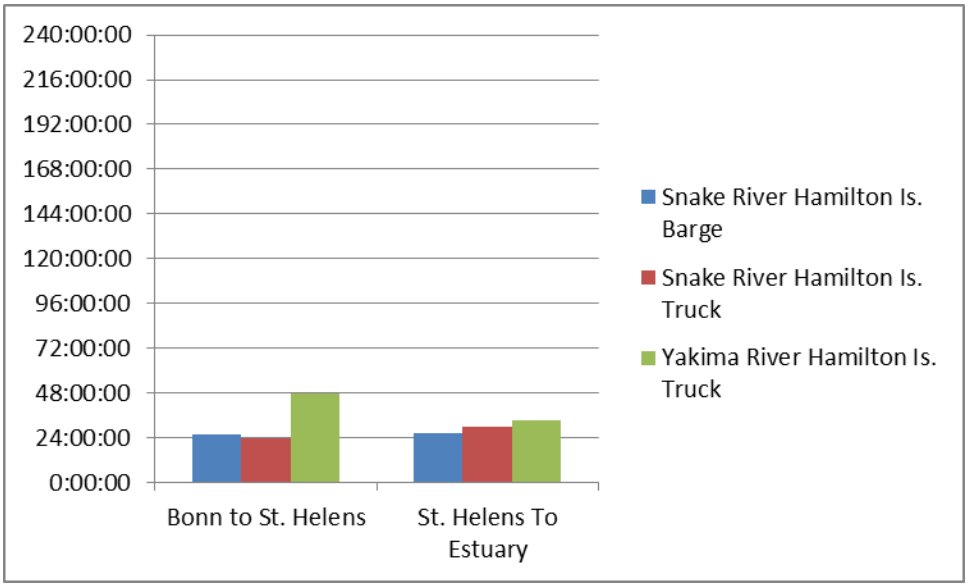


**Figure 5:  Travel times to each reach for non-ocean migrators (hr:min:sec).**

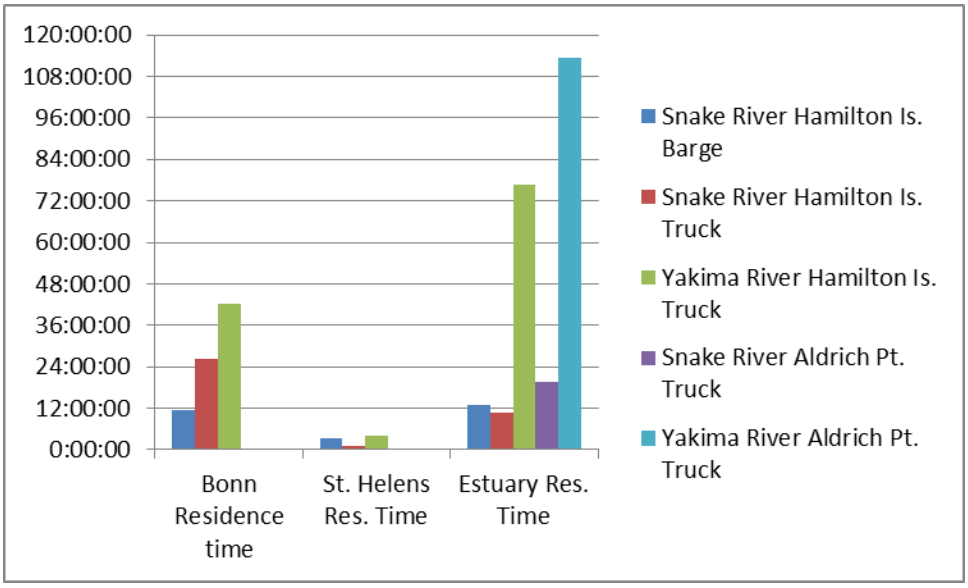


**Figure 6:  Residence times for non-ocean migrating kelts (hr:min:sec).**

It is unknown what the cause of this migration timing difference is. Additional Snake River detection points should allow us to determine if this difference is typical, if this is was a one year effect, or result of low sample size from the Snake River.

### Acoustic Tag Return Detection of the Hamilton Island and Aldrich Point Releases

#### *2008 Acoustic Release Return Detection*

We did not detect any steelhead kelts that were released in 2008 attempting to return in 2010.

#### *2010 Acoustic Release Return Detection*

We detected one acoustic tagged kelt from the Yakima origin Hamilton Island release group returning from the ocean to the Estuary array line in mid-August. This kelt passed Bonneville Dam in less than a week. It was detected moving again past McNary Dam in early October and passing the Prosser Dam in early November.

#### *PIT-tag Detections*

One PIT-tagged only Yakima origin steelhead kelt from the Aldrich Point release was detected passing Bonneville Dam in late August. This fish continued to migrate past McNary Dam in early September and was detected moving past the Prosser Dam in early November.

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# Section C. Long-term Reconditioning Treatment

## Study Area

### Prosser Hatchery

The Prosser Hatchery is located on the Yakima River just downstream of Prosser Dam at (Rkm) 75.6. The Prosser Hatchery is operated by the Yakama Nation, with a primary function of rearing, acclimating, and release of fall chinook salmon (*Oncorhynchus tshawytscha)*, and is also used for coho salmon (*Oncorhynchus kisutch)* rearing prior to acclimation and release in the upper Yakima River Basin (Figure 1).

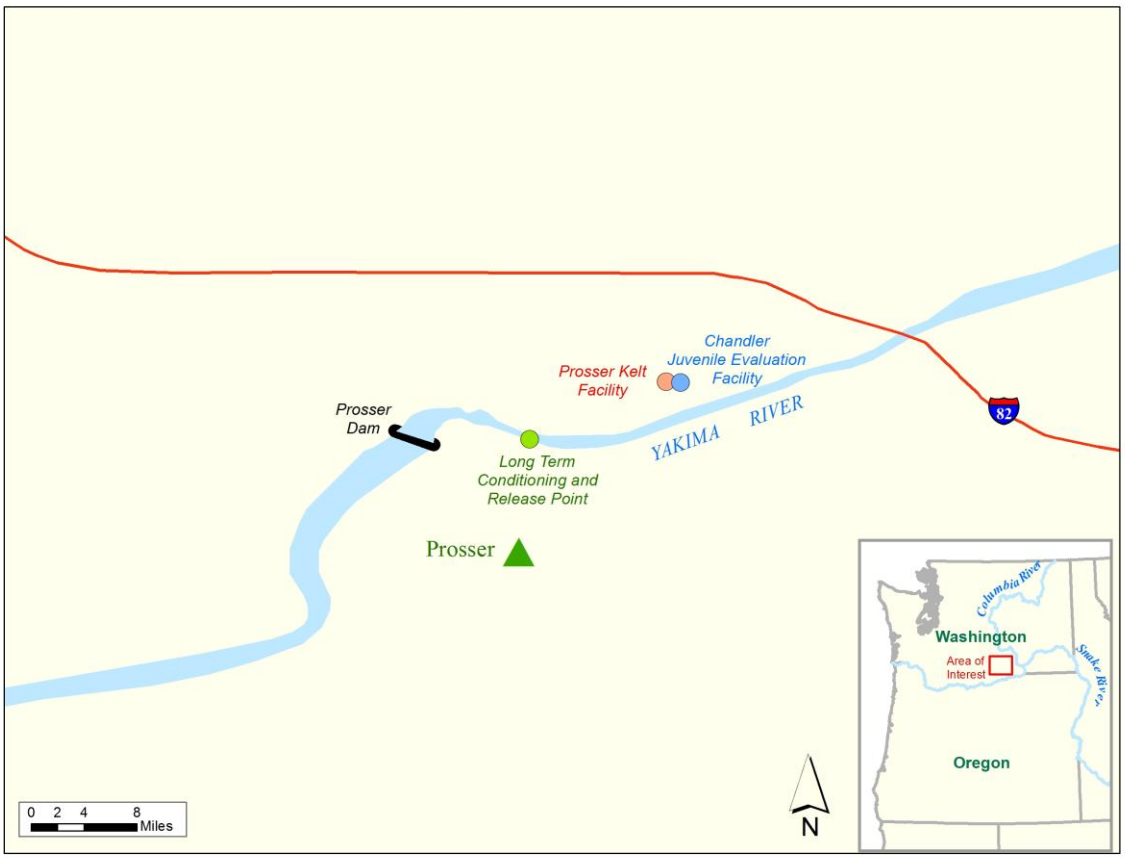


**Figure 1: Map showing the location of Prosser Dam and the kelt reconditioning facility at Prosser, WA.**

## Dworshak Fish Hatchery

Kelt reconditioning facilities are located at Dworshak National Fish Hatchery (DNFH) in Ahsahka, Idaho (Figure 2). DNFH is located at the confluence of the North Fork of the Clearwater River (river kilometer 65). Dworshak National Fish Hatchery is a "mitigation" hatchery located within the Nez Perce Reservation near Orofino, in north-central Idaho. It was constructed in 1969 by the Army Corps of Engineers, and is presently co-managed by the U.S. Fish and Wildlife Service and the Nez Perce Tribe. Steelhead, Chinook , and Coho salmon are spawned and reared at the facility. The primary goal of the steelhead program at DNFH is to “Conserve and perpetuate the unique North Fork Clearwater River ‘B-run’ summer steelhead population.” DNFH production aims to release 2.11 – 2.21 million B-run steelhead smolts per year (USFWS 2009).

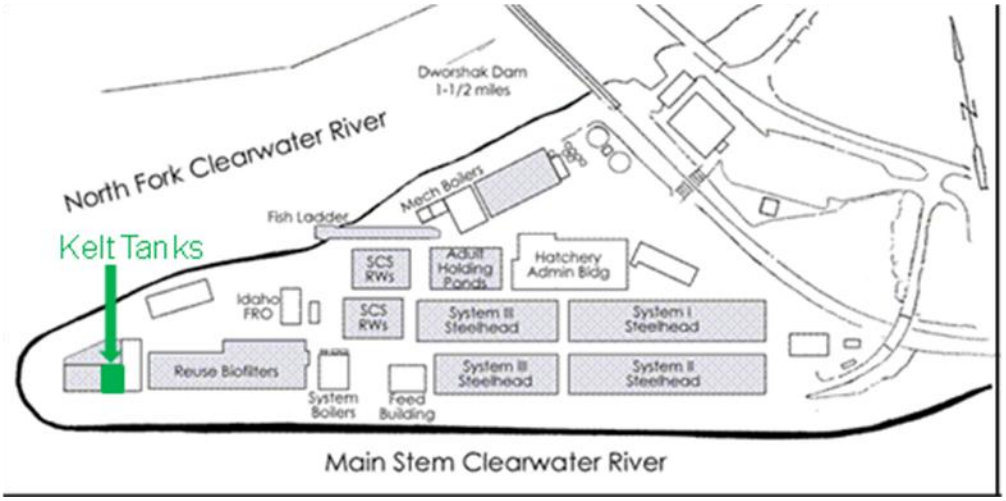


**Figure 2: Map showing the location of experimental kelt reconditioning tanks at Dworshak National Fish Hatchery. Figure modified from USFWS 2009.**

## Cassimer Bar Hatchery

Omak Creek and Bonaparte kelt steelhead were reconditioned at the Cassimer Bar Hatchery located at the confluence of the Okanogan River (Figure 3). Currently the Colville Confederated Tribes operate the Cassimer Bar Hatchery. The facility was originally constructed in 1994, as a sockeye salmon *Oncorhynchus nerka* production facility in an attempt to supplement Lake Osoyoos and is currently utilized for the development of locally-adapted stock to supplement natural production of steelhead in Omak Creek.

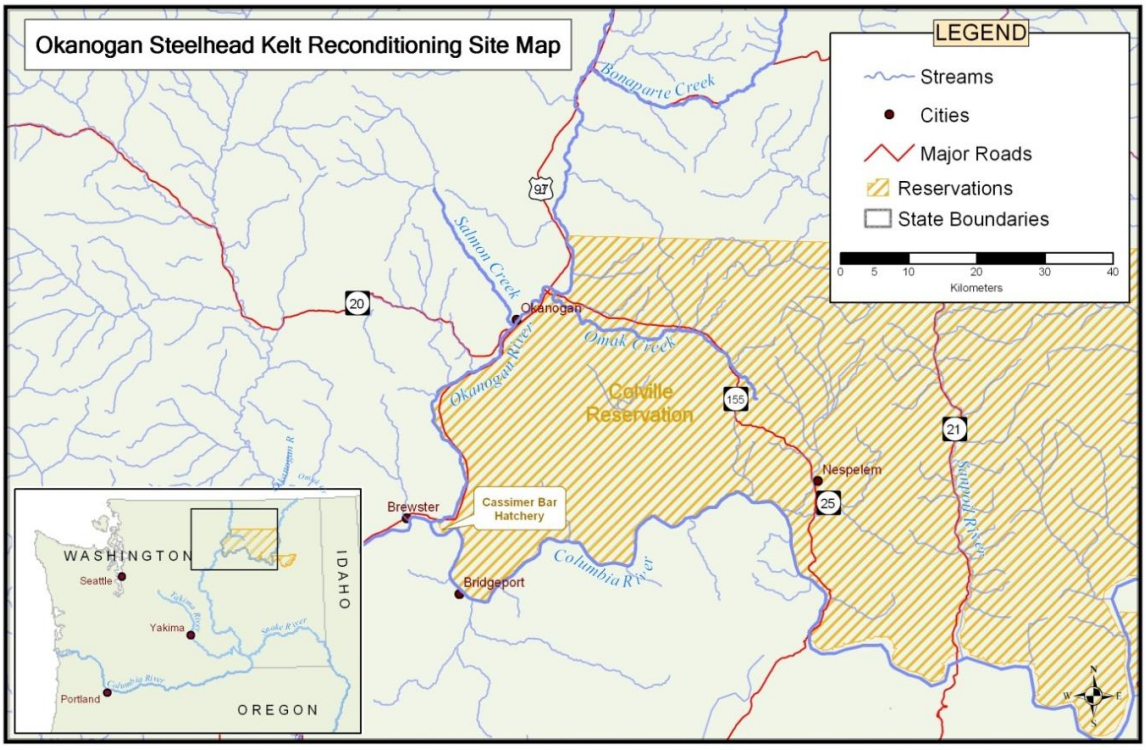


**Figure 3: Map showing the locations of Omak Creek as well as the Confederated Tribes of the Colville Reservation.**

## Parkdale Fish Facility

Steelhead kelt reconditioning for the Hood River was performed at the Parkdale Fish Facility located at Rkm 5.6 on the Middle Fork of the Hood River (Figure 4). Adult steelhead collection for the Parkdale Fish Facility was conducted at the Powerdale Dam located on at Rkm 6.4 North of the city of Hood River, Oregon and operated by Oregon Department of Fish and Wildlife (ODFW).

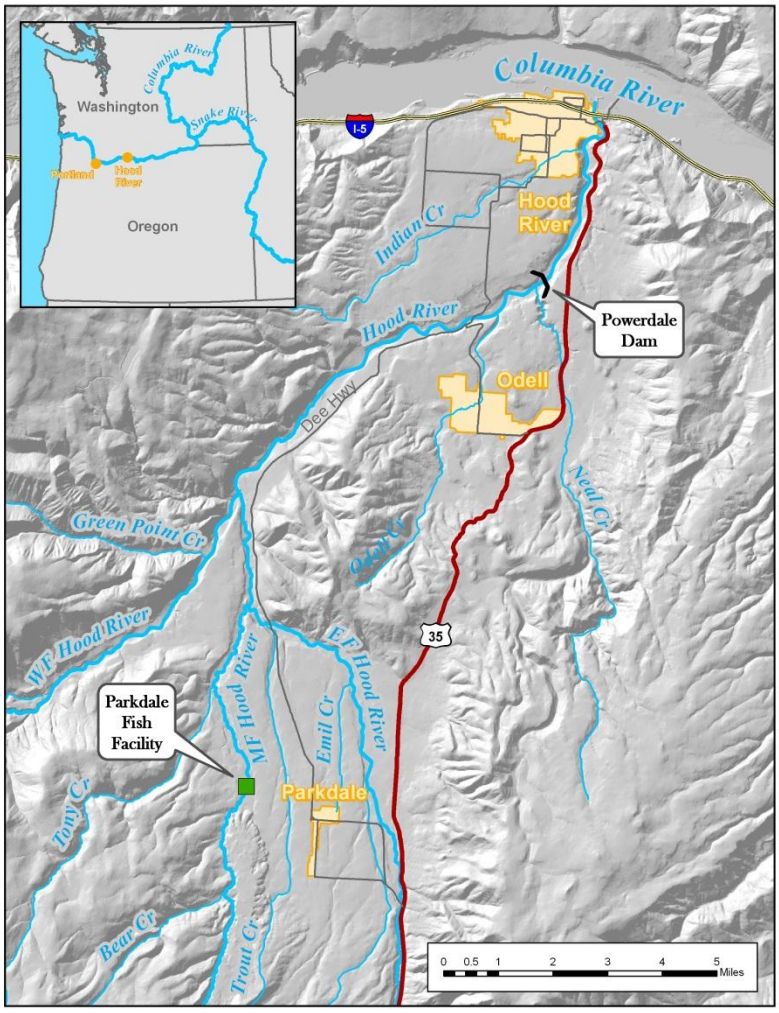


**Figure 4: Map showing the location of Parkdale Fish Facility and Powerdale Dam/ Fish Trap.**

## Young's Bay

A small group of steelhead kelts (20 fish) from the Yakima River were captured and truck transported to the Young's Bay net pens just outside of Astoria, Oregon (Figure 5). The Young's River, from its headwaters to the entrance of the Bay, is approximately 17 miles long. The lower reaches of the Lewis and Clark River and Young's River are components of the Columbia River Estuary. The net pens are located at the Rkm 19 of the Young's Bay River. These facilities are managed by the Clatsop Economic Development Council.

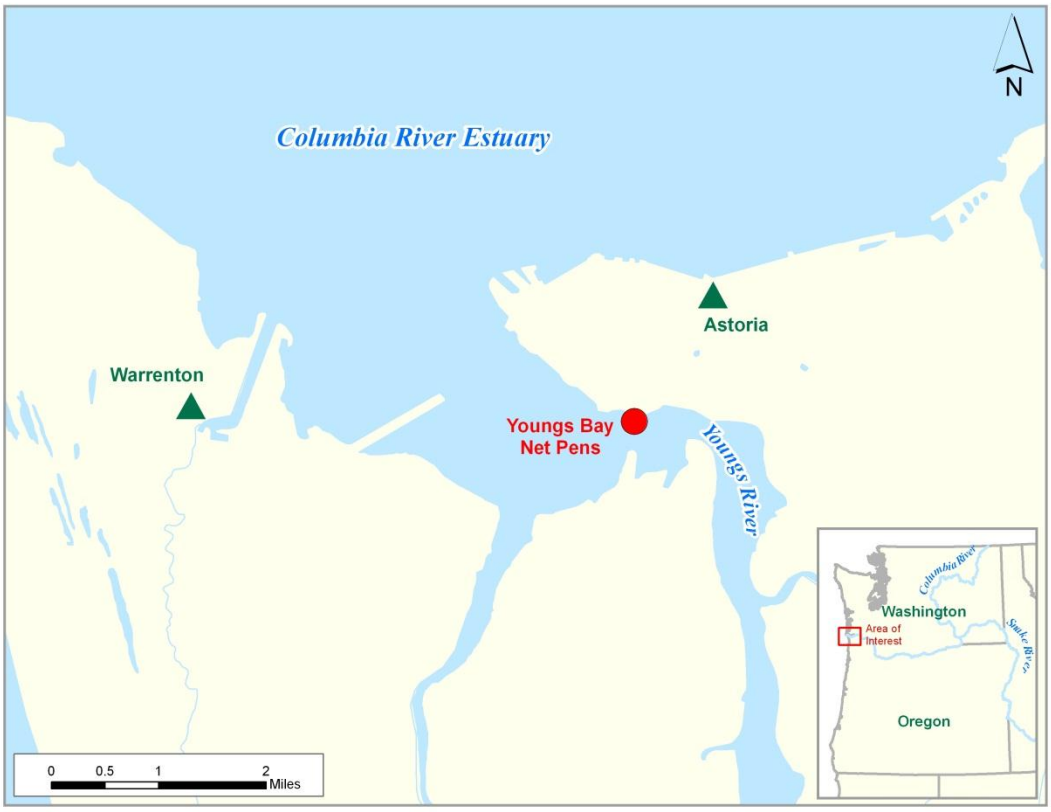


**Figure 5: Map showing the location of Youngs Bay net pens in 2010.**

## Mill Creek

This year we were wrapping up the kelt project with the Warm Springs Tribe at Mill Creek (Figure 6 ) a tributary of the Warm Springs River (44 51 29.79 latitude, -121 04 0.62 longitude.) which is the largest river system within the Warm Springs Reservation. The river flows for 85 kilometers and draining 54,394 hectares before entering the Deschutes River at Rkm 135. Mill Creek is a major tributary to the Warm Springs River at RKM 32. Two weirs were placed in Mill Creek (Rkm 25.2 and 26.5) to inhibit immigration and emigration to or from the reintroduction site.

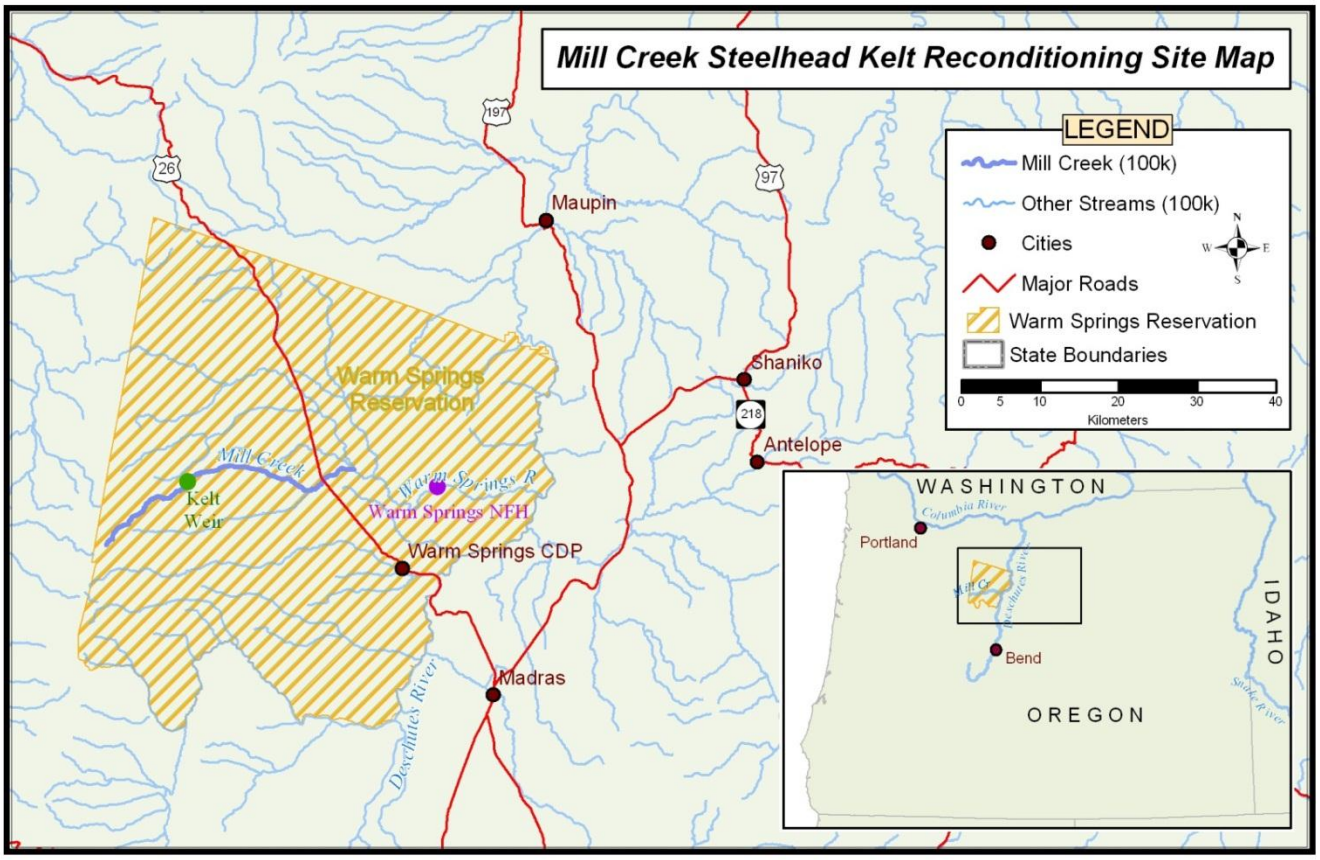


**Figure 6: Map of the Warm Springs kelt project area showing Mill Creek and Warm Springs National Fish Hatchery.**

## Methods

### Long-term Reconditioning Facilities

#### *Prosser Hatchery*

Steelhead kelts retained for the long-term reconditioning treatments at Prosser Hatchery were held in one of four 20' (d) x 4' (h) feet circular tanks (Figure 7). Loading densities were below 300 fish carrying capacities of these tanks. Tanks were fed oxygenated 13.8$^{0}$C (57.$^{0}$F) well water at 200 gallons/minute.

**Figure 7: Steelhead kelt reconditioning tanks Prosser, WA.**

The steelhead kelts deemed to be in "good" to "fair" condition were retained for reconditioning while steelhead kelts found to be in "poor" condition and dark in color were released back to the river. A portion of collected steelhead kelts that were found to be in good condition were released back to the river as an in-river treatment to establish baseline data on the natural iteroparity rate in the Yakima River (In-river release group).

All kelts held for an extended period of time in reconditioning tanks, are susceptible to severe infestation of parasites which can be lethal to cultured fishes. Formalin is administered approximately five times a week (depending on fungal growth) at 1:6,000 for 1 hour in all reconditioning tanks to prevent fungal outbreaks. Another concern with holding wild steelhead was susceptibility to *Salmincola* in such environments. *Salmincola* is a genus of parasitic copepod that can inhibit oxygen uptake and gas exchange at the gill lamellae/water surface interface by attachment to the lamellae. Recent research by Johnson and Heindel (2000), suggested that Ivermectin™ – a treatment often used to control parasites in swine and cattle – increases the survivorship of cultured fish by killing the adult morph of the parasite. Due to its successful use in treating *Salminicola* in this project's kelt reconditioning experiments during 2000 (Evans and Beaty 2001), Ivermectin™ was diluted with saline (1:30) and injected into the fish's esophagus using a small (1cc) plastic syringe. Antibiotic treatments were resumed after we suffered an IHN outbreak in 2009.

## *Dworshak National Fish Hatchery*

### Transport to Dworshak from Lower Granite Dam

Fish destined for DNFH were dipped netted from the adult holding tank at Lower Granite Dam and placed in a transport truck. Nets were large enough to handle active adult steelhead and consisted of a soft cotton or natural fiber mesh. The transport truck had a 400 gallon tank fitted with supplemental regulated, compressed oxygen fed air stones, and a 12 volt powered tank aeration pump. Stress Coat® or PolyAqua® was used to replace the natural protective slime coating that may have been compromised by handling. In addition, salt was added to reduce osmoregulatory stress. Temperature and dissolved oxygen levels were monitored during transport. Loading densities were kept to a minimum; no more than 20 kelts were transported at one time.

### Reconditioning Facility and Treatment

Four 15 foot diameter tanks are located at DNFH (Figure 8). River water was provided from a fire suppression line at with an in line valve and flow meter at a rate of 50 gpm per tank. Tank outflows are plumbed to the DNFH settling pond. Tanks are provided with both an internal standpipe and an external vented vertical loop to control tanks level. A four-bucket Koch ring packed column degassing system supported by external posts is installed on the inflow to each kelt tank. An aeration system is installed. Flow, temperature, and dissolved gas levels were constantly monitored.

A prophylactic treatment of Ivermectin and oxy-tetracycline was administered to LGR transfers during their initial survey. Feeding began after initial sampling. Formalin treatments (baths) were applied routinely to control fungus.

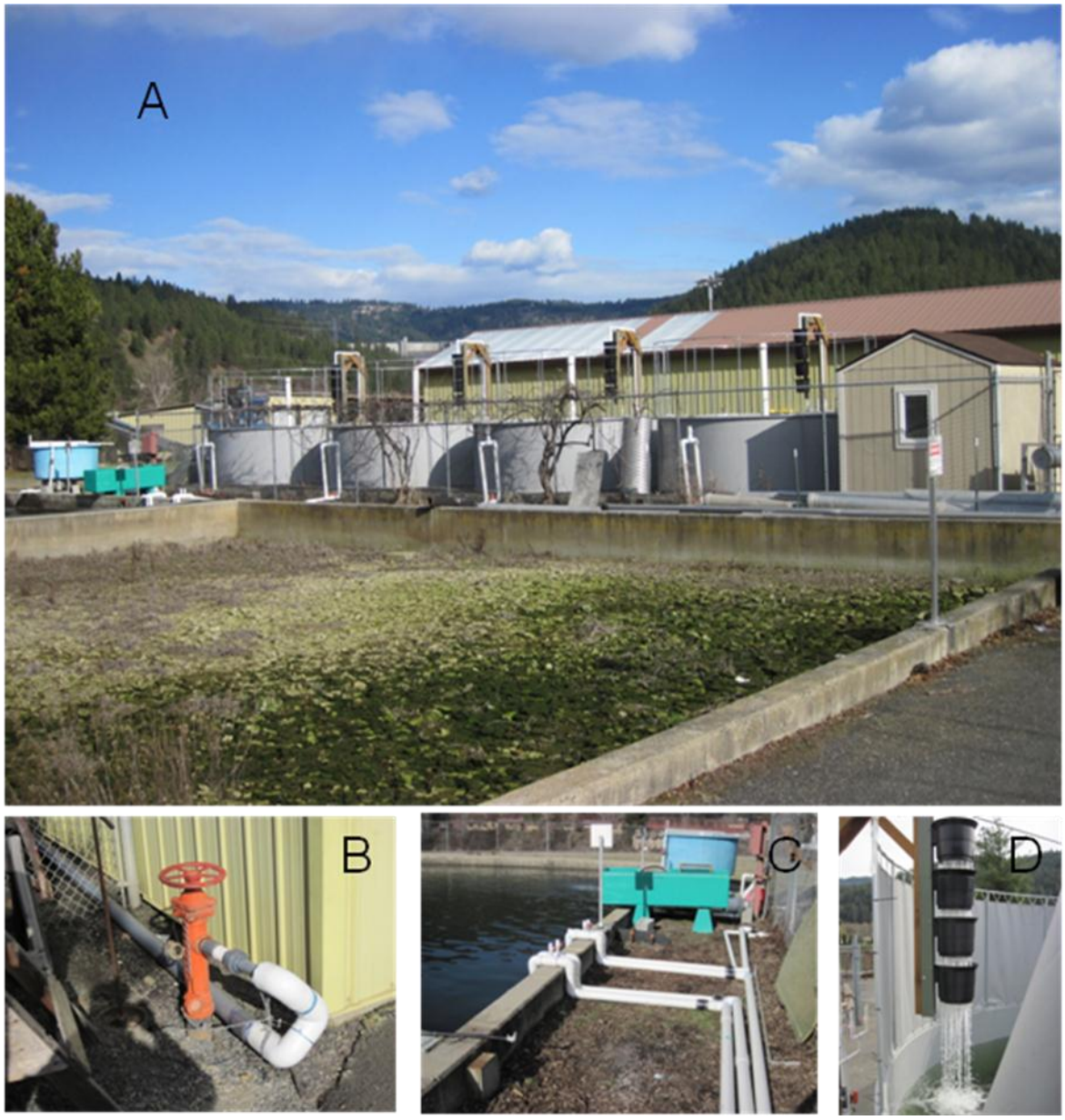


**Figure 8: Experimental kelt reconditioning tanks at Dworshak National Fish Hatchery. (A) Kelt tank overview. (B) Fire hydrant water supply. (C) Outflow to settling pond. (D) Four-bucket Koch ring degassing system in operation.**

## *Cassimer Bar Hatchery*

Kelts are collected for reconditioning in either of two ways at Cassimer Bar: 1) broodstock that survive spawning are put into the kelt tank for reconditioning. 2) kelts exiting Omak Creek or Bonaparte Creek are collected at or above the trap site of the respective creek and transported to the Cassimer Bar hatchery. Fish were transported by truck to Cassimer Bar from capture sites.

One 22'foot circular tank was used to recondition Omak Creek steelhead kelts (Figure 9). Water was circulated at 120 gallons/minute at an average temperature of 13.3$^0$C (56.0$^0$F). Kelts were separated by sex into circulars at Cassimer Bar. The fish were separated because hormone levels were still elevated enough to cause territorial behavior when both sexes are kept in the same circular.

**Figure 9: Steelhead kelt at Cassimer Bar Hatchery. Reconditioning tanks to left and right w/ sampling area in center.**

## *Parkdale Fish Facility*

Skamania and Winter run steelhead kelts were segregated by run in 40'l x 8'w x 4'd feet raceways at 400 gal/min until ripened and ready for spawning (Figure 10). All incoming fish were inspected for copepods and received a 1-2cc dosage of diluted Ivermectin solution as a parasitic preventative and florfenicol (2ml) as a preventative against cold water disease. Formalin treatments were administered at 1:6000, 3 times weekly for one hour to prevent against fungal infections. After air spawning steelhead were moved to round tanks (4'h x 10'd), segregated by run, with water flow at 60 gal/min for reconditioning and held until late September when they were placed into the raceway for the duration of the winter season until the following year's spawning (Figure 11). Post spawn females were administered another dosage of ivermectin after completion of air spawning. Fish were checked again in late spring (May) for the presence of copepods and administered additional ivermectin treatment if copepods were present.

**Figure 10: Parkdale Fish Facility raceways where kelts are held from late fall to early spring.**

**Figure 11: : Circular tanks at Parkdale Fish Facility used seasonally (late spring to early fall) for reconditioning kelts.**

### *Young's Bay Net Pens*

Twenty kelts captured at the CJEF were trucked to the Young's Bay Net Pens. Temperatures at the reconditioning facilities at Prosser 9.4$^0$C (49$^0$F) and the net pens 9.4$^0$C (49$^0$F) in Astoria were the same. Fish are transferred from the fish hauling truck to a small powered float that has two oxygenated totes (4' x 4' x 4' ft) then netted from the totes and released to the net pens. Kelts were held in a single 10 x 10 x 20 foot net pen with a tightly woven pattern at the Clatsop Economic Development Council net pen docks in Astoria, Oregon (Figure 12).

**Figure 12: Example of Net Pen set-up at Young's Bay.**

## Feeding

Modified versions of the feeding and holding protocols developed at Prosser Hatchery are utilized for long term reconditioning at Dworshak Hatchery, Cassimer Bar Hatchery, Warm Springs National Fish Hatchery, Young's Bay and the Parkdale Fish Facility (Hatch et al. 2004). Hatchery managers and project staff are allowed to modify protocols as needed to improve survival.

#### *Prosser*

Long-term reconditioned fish at Prosser Hatchery were initially fed frozen krill for 2.5-8 weeks and then slowly switched over to a modified Moore-Clarke Trout Broodstock pellet until release. Feeding occurs 2-3 times a day to satiation, and is monitored to prevent overfeeding which causes pollution in the holding. Krill is utilized as a starter feed due to the readiness of kelts to consume this specific feed.

### *Dworshak National Fish Hatchery*

DNFH steelhead kelts were fed frozen krill in 2010. Fish were offered both the smaller North Pacific *(Euphausia pacifica*) and larger Antarctic krill *(E. superba)*, and appeared to feed more readily on the larger variety. Fish were fed 1 to 2 times daily.

### *Cassimer Bar*

Food is introduced to the new kelts after an initial holding period of 24 hours. Initially, krill coated with cod liver oil are offered to the kelts. Kelts are observed closely during feeding periods to assess feeding response. Kelts are initially fed easily digested natural foods including krill coated with cod liver oil, and squid to provide a rich source of nutrients that the fish would naturally feed on once in the estuary or ocean. In addition, the food appears easier to digest than pelletized food. After the last kelt arrives on station, kelts are fed natural food for an additional 2 weeks, to ensure that their digestive systems are functioning properly before introducing any pelletized food. During the pelletized food introduction, natural food continues to be offered. Eventually, fish are fed a rotating diet of natural and hand extruded food to ensure they are receiving the most complete array of nutrients available. Fish are fed to satiation multiple times throughout the day. Fish are also observed during feeding to check for any possible signs of pathogens or change in feeding response.

### *Parkdale Fish Facility*

Upon entering the Parkdale facility, fish were initially fed krill 3 times daily to satiation and provided pellet feed (Bio-oregon Brood pellets) from automatic feeders which are tuned by hatchery staff to meet feeding needs. Towards the end of December fish naturally discontinued eating to prepare for spawning. Fish resumed the usual feeding schedule after spawning and entering the round tanks where they could begin to recondition.

### *Young's Bay*

The CEDC determined that they had a permit to allow for raw food to be feed to the kelts. We began feeding fish krill with a top dressing of Menahudan oil, cyclopeez, and spirulina three times daily until satiation.

## Kelt Mortalities

On discovery of a mortality, fish were collected and examined externally by hatchery personnel to record the suspected time of death, general condition (good, fair, poor), fish color (bright, intermediate, dark), color of the gill arches (red, pink, white), size of the abdomen (fat, thin), presence of any scars or obvious lesions, and any other anomalies. Once the external exam was completed, an internal examination was conducted to record color of muscle tissue (red, pink, white), type of gonads (ovaries, testes), size of gametes (small, large), and presence of any internal anomalies. Internal acoustic and PIT tags were removed from mortalities and identification numbers recorded onto computer databases along with growth measurement data. We reused viable acoustic tags whenever possible. The Lower Columbia Fish Health Center, Washington Department of Fish and Wildlife Pathology, and Oregon

Department of Fish and Wildlife Pathology provided disease monitoring services to insure the health of reconditioned steelhead kelts.

## Steelhead Kelt Status and Release

Prior to release, all steelhead kelts were scanned for PIT tags, weighed, and measured for fork-length Reconditioning success was based on the proportion of fish that survived the reconditioning process to the point of release (some reconditioning sites did not release fish). Reconditioned kelts were classified as either feeding or non-feeding at the time of release based on weight change. Prior to release or release date, growth measurement data and rematuration status were recorded on all individuals. Reconditioning success was based on the proportion of fish that survived the reconditioning process and the number of fish that were detected actively to natal spawning areas.

### *Prosser*

Long-term reconditioned fish located in Prosser are released just below Prosser Dam so that we can utilize PIT-tag detectors in the dam's fish ladders to determine the number of steelhead kelts that are actively migrating to spawning grounds.

### *Snake River*

The Fish at the Snake River are currently being lethally sampled to obtain important physiological data for future indices to rate maturation status (See Chapter 3, Section B for further details).

### *Cassimer Bar*

Fish in the long-term experiments were released in late September 2010 when river water temperatures matched well water temperatures at the hatchery facilities and the spawning run is peaking in the river. They were released immediately downstream into the Okanagon River (Rkm 1). These long term reconditioned kelts over-winter within the systems they are released to, and are able to volitionally return to the spawning grounds in late winter and spring. PIT-tag scanners (crump weirs) and detection at adult weirs are used to determine steelhead kelt return to natal spawning streams in the Okanagon basin.

### *Parkdale*

Parkdale Fish are not released into the Hood River due to their hatchery fish designation they are retained for additional reconditioning or are terminated.

### *Young's Bay*

Any surviving fish from the Young's Bay net pen experiment are hauled in a tote to the mainstem of the Columbia River and released. These fish have inserted PIT-tags to determine if they attempt to return from the ocean.

# Results

## Long-Term Reconditioning and Survival to Release or Spawning

### *Prosser Fish Hatchery*

A total of 38.7% (n=426) of the fish collected for long-term reconditioning survived to October 13, 2010 when they were released into the Yakima River (Table 1) (Figure 13). Fish were released below Prosser Dam (75.6 Rkm).

**Table 1: Long-term reconditioning results by tank 2010 at Prosser Hatchery.**

| Tank: | C1 | C2 | C3 | C4 | Total |
|---|---|---|---|---|---|
| | | | | | |
| Held for Reconditioning | 278 | 274 | 272 | 276 | 1100 |
| Released | 90 | 123 | 111 | 102 | 426 |
| Survival Rate | 32.4% | 44.9% | 40.8% | 37.0% | 38.7% |
| Pct with wt gain | | | | | 89.7% |
| Avg wt gain/loss (lbs) | | | | | 1.87 |

To date, 145 (34%) fish from the long-term release were detected by PIT tag presence migrating past Prosser Dam. Most migratory movements occurred just after release in October but there were two additional groups detected migrating upriver in November and in December of 2010.

**Figure 13. Long term reconditioned kelt steelhead from the Yakima River just prior to release.**

## *Dworshak National Fish Hatchery.*

### Water Quality

In 2010, water quality in DNFH kelt tanks was monitored with a Hach Hydrolab MS-5 Minisonde fitted with probes for total dissolved gas, dissolved oxygen, pH, conductivity, and temperature. Water quality data were recorded hourly on a Hach Surveyor 4 attached to the sonde (Figure 14).

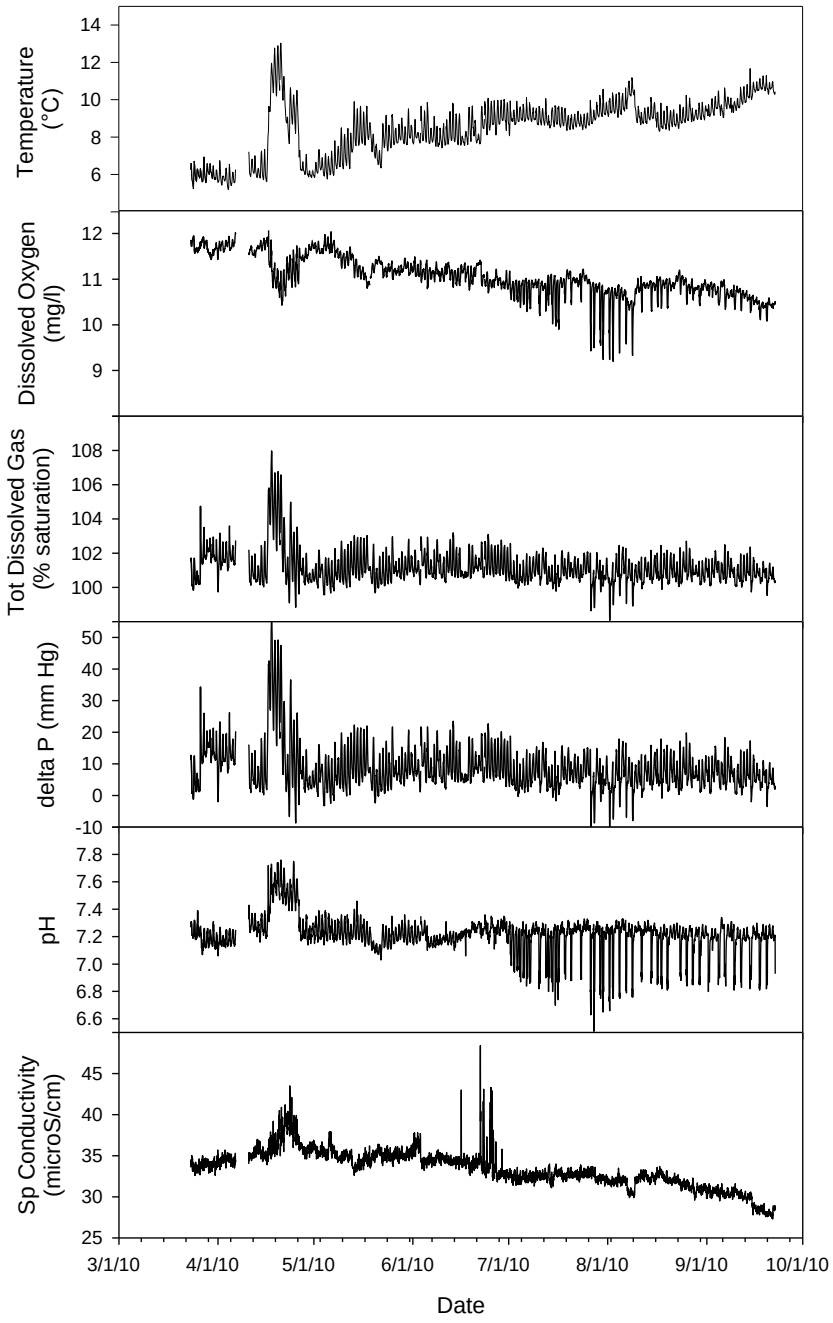


**Figure 14: Water quality parameters measured in DNFH kelt tanks. Degassing equipment was bypassed from 3/27/11 to 4/6/11, and the Hydrolab probe was removed from the tank for servicing 4/6/10-4/10/10. At the request of DNFH, flows were reduced to minimal (~10 GPM) from 4/16/10-4/26/10. Fish had not yet been stocked into tanks during any of these operations.**

Water quality records indicate that water quality was good during the entire period of reconditioning. Temperature and total dissolved oxygen were well within recommended ranges for rainbow trout (Colt 2000a; Stickney 2000). The rather cold water temperature at DNFH would be expected to reduce metabolic rate in kelts, which may enhance survival but could inhibit growth. DNFH water is largely drawn from the North Fork of the Clearwater River, and may contain supersaturated levels of dissolved gas due to spill from Dworshak Dam. Nevertheless, in our kelt tanks with degassing columns in operation, gas supersaturation levels were controlled to a delta P (water dissolved gas – barometric pressure) less than 20 mm Hg at all times when fish were in the tanks. This meets recognized standards for sensitive animals (Colt 2000b). The pH of kelt tank water was within acceptable limits, although greater buffering capacity and alkalinity would be desirable (Wedemeyer 2000). An interesting pattern of reductions in pH during the afternoon occurred during July-Sept. This may relate to the growth of phytoplankton in Dworshak reservoir during hours of peak insolation. The specific conductivity of kelt tank water was quite low, indicative of low mineral content. Changes in several parameters during minimal flows during a DNFH water shutdown 4/16/10-4/26/10 suggest that water quality monitoring equipment was working properly. No fish were in the tanks during this period. During August of 2010, the Nez Perce Tribe stocked juvenile Coho salmon into one of the kelt tanks as a biological test of water quality. The juvenile Coho were observed to feed actively, and very little mortality occurred, providing biological evidence that water quality was good.

In August, 2010, the fitting attaching the inflow water line to the fire hydrant blew apart, resulting in loss of water to the kelt tanks. Fortunately, Scott Everett, the Nez Perce Tribe Kelt Coordinator, was on site and was able to repair the plumbing. This incident illustrates the need for a more secure and reliable water source for the DNFH kelt tanks.

**Reconditioning**

Difficulties were encountered in obtaining fish for reconditioning in 2010. Strategies for obtaining B-run steelhead kelts for the pilot scale reconditioning program were discussed with collaborators. We believe that the establishment of a hatchery model for kelt studies is a critical step to enable further studies on the reconditioning and reproductive success of Columbia Basin steelhead kelts. In order to establish this model, we initially planned to non-lethally spawn female hatchery origin fish returning to the ladder at DNFH, transfer them to our tanks, and attempt reconditioning. However, DNFH production was unable to include eggs harvested by non-lethally spawning female steelhead into hatchery production, due to concerns regarding egg quality. Nez Perce Tribe policy requires that a conservation or enhancement use be made of all viable gametes harvested. Due to these issues, we were not able to obtain hatchery fish at DNFH for our studies. As an alternative, we collected hatchery origin kelts at Lower Granite Dam. However, B-run hatchery kelts were not available in anticipated numbers at LGR, and many fish had head injuries at the time of collection (Table 2, Fig 15). The cause of the head injuries is not known; however, many injuries were fresh at the time of collection and are likely to have occurred during passage through the Lower Granite Juvenile Bypass System. Twenty B-run and

50 A-run hatchery origin kelts were collected From April 1 to July 2, 2010 personnel from the UI collected 1,492 kelts from the LGR JFF (Table 2).

One A-run female survived reconditioning in 2010. This fish was lethally sampled on Sept 22. Blood vitellogenin levels were low in this fish, and she had immature ovaries, indicating that she was on a skip-spawning life history trajectory.

**Table 2: Hatchery origin Snake River steelhead kelts collected at Lower Granite Dam and transported to DNFH for reconditioning in 2010.**

| Type | N | Dates | M | F | B-run | A-run | Head wound |
|---|---|---|---|---|---|---|---|
| Survivor | 1 | Sampled 9/22 | 0 | 1 | 0 | 1 | 1 |
| Post-Tag Mort | 19 | 5/22-7/2 | 8 | 11 | 3 | 16 | 12 |
| Pre-Tag Mort | 45 | 4/29-7/14 | 6 | 39 | 12 | 33 | 25 |
| Transport Mort | 5 | | 0 | 5 | 5 | 0 | 4 |
| | | | | | | | |
| Sum | 70 | | 14 | 56 | 20 | 50 | 42 |

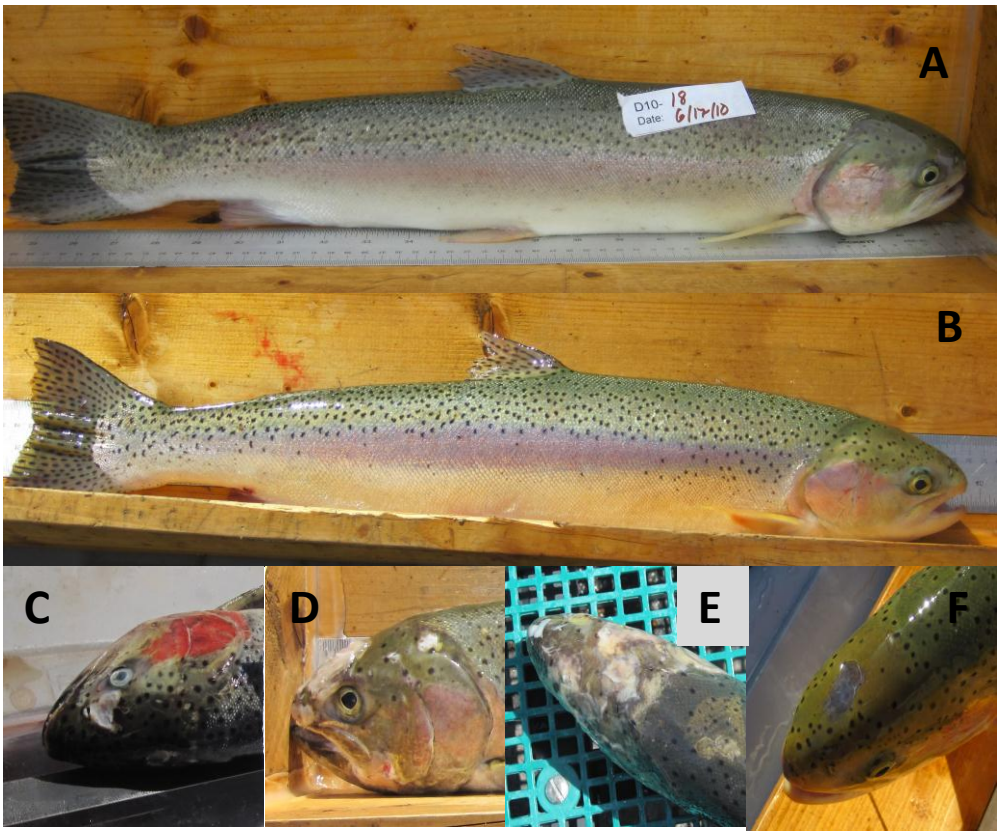


**Figure 15: Snake river kelts. (A-B) Survivor at initial sampling and release. (C-E) Examples of head injuries on mortalities. (F) Healed head injury on survivor at final sampling.**

Post Reconditioning Issues at Dworshak

**RELIABLE WATER SOURCE AT DNFH**

On August 13, 2010, the fitting attaching the inflow water line to the fire hydrant blew apart, resulting in loss of water to the kelt tanks. Fortunately, staff was on site and was able to repair the plumbing. This incident illustrates the need for a more secure and reliable water source for the DNFH kelt tanks.

**OFF-SEASON (WINTER) MAINTENANCE**

Considerable over-winter rain water collected in each of the four kelt tanks. The out flow lines slowly froze resulting in a build-up of water and ice. The out flow lines were located in the center of each tank, unreachable under the several feet of water and ice. The ice had to be carefully removed by hand and the out flow lines cleared (Figure 16). In order to avoid this in the future, a durable cover that is sturdy enough to handle rain and snow will need to be outfitted for each tank.

**Figure 16. Ice was carefully removed by hand to avoid damaging the tanks. Several days of effort were necessary to clear the tanks and the out flow lines of all the ice.**

## *Cassimer Bar Hatchery*

There were 13 post spawn kelts that were shipped to Cassimer Bar of which 3 were hatchery fish and (10) were wild. There were 14 captive brood fish retained for reconditioning as well. In September we released 6 steelhead kelts into the Okanagon River (1male:5 females). Most of these fish were in good to excellent condition (Figure 17).

**Figure 17: Long-term reconditioned Omak Creek Kelt just prior to release.**

## *Parkdale Hatchery*

### Skamania Steelhead

2010 Brood

The 2010 brood year was the final year that Skamania steelhead were collected for this portion of the study. We successfully spawned 22 fish which 7 of these had to be culled due to the presence of IHN in the ovarian fluid. There were 15 of the Skamania female steelhead by the beginning of summer 2010. We lost 12 fish that fall and winter. We are still in the process of attempting to spawn the remaining kelts. We have 3 surviving kelts that will be candidates for kelt spawning in 2011 or 2012.

2009 Brood

We had 6 remaining steelhead kelts that were reconditioned, 3 of which died early in the beginning of the year. The remaining 3 did not ripen that year and were likely skip spawner candidates according to hatchery staff. This brood year has not performed well possibly due to either rearing conditions at the hatchery, poor migration conditions (poor water quality, parasites, communicable diseases), and/or bad ocean conditions.

### 2008 Brood

There were 5 kelts remaining at the beginning of 2010 all of which were successfully spawned. One fish was terminated due to the presence of IHN in the ovarian fluid and four died toward the end of the year, one which likely died from a *Ceratomyxa shasta* infection (table 3). At the end of 2010 2 fish were still being reconditioned. This group of fish have been extraordinary survivors and done well being reconditioned. This group of fish though small in numbers, may give us an idea of the reproductive viability of 3rd time kelt spawners which we have detected moving through the hydrosystem (table 3).

**Table 3: Skamania Kelt Reconditioning 2006-2007. TBD=To Be Determined. IHN= Infectious hematopoietic necrosis.**

| Brood Year | 2006 | 2007 | 2008 | 2009 | 2010 |
|---|---|---|---|---|---|
| **Aive as of 4/2011** | 0 | 0 | 2 | 3 | 3 |
| **Maiden spawn** | 1 | 15 (2 culled IHN) | 14 | 12 (3 culled IHN) | 22 (7 culled IHN) |
| **Spawned 1st Kelt yr** | 1 | 1 | 4 | 0 | 3 max |
| **Succ. Recon Rate %** | 100% | 8% | 50% | TBD (33% max) | TBD (20% max) |
| **Skip Spawner kelt** | 0 | 1 | 3 | 3 max | TBD |
| **% kelt skip spawner of reconditioned fish** | 0% | 8% | 21% | TBD (33% max) | TBD |
| **2nd kelt Spawn** | 0 | 0 | 2 | TBD | TBD |
| **3rd kelt spawn** | 0 | 0 | 2 max | TBD | TBD |

## Winter Steelhead

### 2010 Brood

We began the collection of winter steelhead in 2010. A total of 22 females were spawned. Three of these fish had to be culled due to the presence of IHN in the ovarian fluid. By the end of the 2010 we had 12 remaining fish (table 4).

**Table 4: Winter Kelt Reconditioning 2010. TBD=To Be Determined. IHN= Infectious hematopoietic necrosis.**

| Brood Year | 2010 |
|---|---|
| **Aive as of 4/2011** | 12 |
| **Maiden spawn** | 22 (3 culled IHN) |
| **Spawned 1st Kelt yr** | TBD (12 max) |
| **Succ. Recon Rate %** | TBD (63% max) |

### *Young's Bay Net Pens*

Water temperatures in Young's Bay were cool at 9.4C at the point of induction due to the above average precipitation in spring of 2010. Fish appeared to be eating well according to the caretaker but, by that weekend the caretaker had noticed that the fish discontinued eating and that a number of them had large patches of fungus. Our technician checked on them by the end of week 2 and found that all but one had perished. Fish had rotted quickly in the brackish water so suitable samples were not obtained for pathology analysis. The remaining fish was promptly released to the mainstem of the Columbia. In 2009 water temperatures in conjunction with IHN seemed like the likely cause of mortality (Branstetter et al. 2010). We believed that in 2010 reconditioning would be improved due to the prevailing good conditions (low water temperature, good quality fish, and reintroduction of antibiotics to treat fish), but this turned out not to be the case. One possible problem is that we may have had too much fresh water input into Young's Bay and without the prevalence of salt water fungus may have spread and been lethal to the kelts. Another possibility is that there may have been a transport effect from the long-distance that we had to haul fish which may have caused lethal elevations of stress and the fungus was only a symptom. Finally, fish may have been exposed to a pathogen in the Young's Bay but due to the fast rate of decay we could not get a viable disease screen. We have since discontinued sending kelts to the lower river for reconditioning at the moment as the environmental factors that may be contributing towards kelt mortality are difficult to isolate. The three fish that were released, 2 in 2009, and 1 in 2010, have not been detected at any of the mainstem dams. Saltwater reconditioning may still have benefits to steelhead kelts but travel distance and insuring adequate salinity are important factors to be considered.

### *Mill Creek (Warm Springs)*

The lone steelhead kelt which was released from WSNFH into Mill Creek managed to overwinter successfully. Warm Springs Tribal Fisheries staff captured 2 males and placed them in the wiered section in the hopes that they may pair and spawn with her. Warm Springs staff later attempted to find and capture any survivors and could not locate the three fish, redd surveys were conducted but none were found (Appendix D). This fish either escaped past the weir or was predated on. As far as the lack of redd construction it is also possible that this fish would have been a skip spawner as this is a trait that we are beginning to recognize in steelhead kelt populations throughout the basin.

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# Section D. Management Scenario Evaluation

## Introduction

Management scenarios have consisted of collecting and transporting unfed or fed kelt steelhead downstream and releasing them below Bonneville Dam and rejuvenating kelts by holding them in large tanks and feeding them until the next season's upstream run occurs when the kelts are liberated. We present 8 years of data from Prosser Hatchery, 4 years from Lower Granite, and 1 year from John Day Dam (Evans et al. 2008). To evaluate success of various management strategies we compared kelt return rates (for transported treatments) and survival rates (for long-term reconditioned kelts) with several "control" groups. Control groups included returns of in-river treatments (fish that were tagged and released back in the river) when available, composition of repeat spawners in the run at large sampled at Bonneville Dam, and values from the literature (Hockersmith et al. 1995). In last year's report (Branstetter et al. 2010) we compared all treatments and locations across all years, so this year we primarily compare results for just 2010 but generally also compare means across years.

## Methods

We calculated transportation benefits for each group by dividing the return rate to Bonneville Dam for the group by each control group. This calculation yields a number that represents the relative positive or negative benefit of the treatment. For example if your treatment return rate to Bonneville Dam was 4% and the control rate was 2%, the treatment would benefit kelt 2x ( 4/2=2 ) versus leaving the kelts in the river. Comparisons were made within each year and across years using weighted means to account for different sample sizes among years.

We calculated reconditioning benefits for long-term reconditioned kelts from Prosser Hatchery, Shitike Creek, Omak Creek, and Parkdale Hatchery in a similar manner. The reconditioning benefits calculation was the survival rate of long-term reconditioned kelts from each location divided by three different control groups. The control groups were: 1. Survival rates of in-river release groups to Bonneville Dam. 2. Literature values (Hockersmith et al. 1995). 3. The composition of repeat spawners in the run at large sampled at Bonneville Dam. None of these control groups are perfect comparisons, for example survival of the in-river release groups is to Bonneville Dam not the river of origin so these are biased high due to mortality that likely occurs between Bonneville Dam and the river of interest. However, the in-river groups are paired by year with the treatment groups reducing annual variation.

## Results and Discussion

In the following paragraphs we attempt to summarize data from a variety of locations that provides insight into evaluating kelt management scenarios. Comparisons are complicated by data being collected at different locations in different years so in Appendix (E) we provide a comprehensive table of return rates and survival for all groups.

## Comparison groups

Our comparison or control groups consisted of 1. The proportion of repeat spawners in the run at large at Bonneville Dam; 2. The return rate to Bonneville Dam of fish PIT tagged and released at Prosser Hatchery; 3. The return rate to Bonneville Dam of fish PIT tagged and released at John Day Dam; The return rate to Bonneville Dam of fish PIT tagged and released at Lower Granite; and, the reported proportion of repeat spawners in the run at Prosser Dam based on scale pattern interpretation (Hockersmith et al. 1995) (Table 1). The proportion of repeat spawners in the run at large at Bonneville Dam is based on scale pattern interpretation of 7 years of data collected from over 10,000 fish sampled in the adult trap (Miranda et al., 2004,(Miranda et al., 2005, Whiteaker et al., 2006, Whiteaker and Fryer 2007, Whiteaker and Fryer 2008, Torbek et al., 2009). The weighted mean composition of repeat spawners in the run at large at Bonneville Dam is 0.53%. This indicates that iteroparity is very low in steelhead populations above Bonneville Dam and in 2010 the return rate was less than the mean. The return rate to Bonneville Dam of kelts tagged and released in-river at Prosser Hatchery in 2010 was 0.00 but the 6 year average of 2.96% is much higher than the run at large at Bonneville Dam suggesting the Yakima River fish may exhibit higher than average iteroparity rates relative to other tributaries. Repeat spawner composition in the Yakima River run based on scale pattern analysis (Hockersmith et al. 1995) was reported at 1.66%. This estimate differs from the other control groups in that it is measured at Prosser Hatchery not at Bonneville Dam but further supports the notion that Yakima River steelhead exhibit higher iteroparity rates relative to the run at large measured at Bonneville Dam. The Bonneville Dam return rate of kelt steelhead tagged and released at John Day Dam was 9.76%. This is very high relative to other sites and includes only a single year (2002). Kelt returns in 2002 were the highest ever recorded for transported fish collected at Prosser Hatchery and Lower Granite Dam as well suggesting that the return rate measured at John Day Dam is likely at the high end of the range. It also indicates that when environmental conditions are conducive, high iteroparity rates can be achieved in upriver stocks. The comparison group tagged and released at Lower Granite Dam returned to Bonneville Dam in 2010 at a rate of 0.0%. This is consistent with low return rates of in-river kelts from other locations in 2010. The 5 year mean return rate to Bonneville Dam for kelts tagged and released at Lower Granite Dam is 0.68. This is quite low and not statistically different (p=0.55) from the run at large at Bonneville Dam.

Table 1. The return rate in 2010 and the mean from available years to Bonneville Dam of repeat spawners from various locations used as “controls” or comparison groups. Note that Hockersmith is a return rate to Prosser Hatchery not Bonneville Dam. Starred groups are based on scale pattern analysis; the remaining groups are based on returns of PIT tagged fish.

| Return Rate timeframe | Bonneville* | Prosser | John Day | Lower Granite | Hockersmith* |
|---|---|---|---|---|---|
| 2010 | 0.45 | 0.00 | - | 0.00 | - |
| mean | 0.53 | 2.96 | 9.76 | 0.68 | 1.66 |

## Treatment Groups

Transported treatment groups in 2010 included kelts collected at Lower Granite Dam and Prosser Dam. For each of these treatment collection locations we used two different release locations: Hamilton Island (below Bonneville Dam where previous transport groups were released) and Aldrich Point, located at approximately river mile 30.

No kelts were detected returning to Bonneville Dam from fish collected at Lower Granite Dam and transported to Hamilton Island or to Aldrich Point. The 5 year mean return rate to Bonneville Dam for fish collected at Lower Granite Dam and transported is 1.17. Two kelts (1 fish from each release location) were detected returning to Bonneville Dam from fish collected at Prosser Dam and transported to Hamilton Island and Aldrich Point. Return rates of Prosser collected fish to Bonneville Dam were 0.81 for the Hamilton Island release and 0.88 for the Aldrich Point release. Both of these return rates are lower than the 8 year mean return rate of 4.38.

Only limited transport benefits can be calculated for the 2010 returns because of the low or zero return rates for transport and in-river groups. The kelts collected at Prosser Hatchery and transported to Hamilton Island had treatment benefits of 0.49 and 1.80 relative to the Hockersmith value of 1.66 and to the steelhead run at large at Bonneville Dam, respectively. The Prosser kelts released at Aldrich Point showed similar treatment benefits of 0.53 and 1.98 relative to the Hockersmith value of 1.66 and to the steelhead run at large at Bonneville Dam, respectively. Remember that any number greater than 1 is a positive benefit and any number less than 1 is a negative benefit. Neither release location resulted in returns to Bonneville Dam substantial enough to yield benefits over control/comparison groups.

Survival from release to the ocean was estimated for from both collection areas Lower Granite and Prosser dams and both release sites, in 2010 using sequential detections of acoustic tags. For the Lower Granite Dam collected kelts survival to the ocean was 12.04% and 40.0% for the Hamilton Island and Aldrich Point release sites, respectively. For the Prosser Dam collected kelts survival to the ocean was 45.76% and 21.67% for the Hamilton Island and Aldrich Point release sites, respectively. The 6 year mean survival from release at Hamilton Island to the ocean is 45.14%. Interpretation of these data after this one is difficult because fish different sources show different survival trends across release sites. The 12.04% survival to the ocean for the Lower Granite group released at Hamilton is the second lowest value that we have recorded. The lowest Hamilton Island release to ocean survival that we have recorded was 10.71% in 2005. These low survival rates could be a result of transportation stress on the fish or river environment impacts. We are repeating the use of the two release sites in 2011 to determine if survival can be boasted by releasing kelts nearer to the ocean.

Survival of long-term reconditioned groups was 34.92% for Prosser, 46.15% for Omak, and 26.67% for Parkdale. Survival for the Prosser and Parkdale fish was slightly below average, but survival for Omak fish was more than double the mean survival for that site (20.14%). Overall this convincingly indicates that steelhead kelts can be successfully reconditioned at a variety of locations.

We calculated the benefits of long-term reconditioning in the same manner as we did the transport benefits but instead of return rate to Bonneville we used survival to release for the long-term treated fish. Fish reconditioned at Prosser Hatchery had an 11.80 times survival advantage over the 6 year average return rate to Bonneville Dam for fish left in the river (Figure 1). We used the 6 year mean for comparison since the 2010 group had 0 returns to Bonneville Dam, therefore, the within year comparison is a minimum estimate. Compared to the proportion of repeat spawners in the run at large at Bonneville Dam, long-term reconditioned kelts at Prosser Hatchery had a 78.10 times survival advantage, those from Omak Creek had a 103.23 times advantage, and steelhead from Parkdale had a 59.64 times advantage (Figure 2). Long-term reconditioning shows great promise as a tool for restoration based on these data.

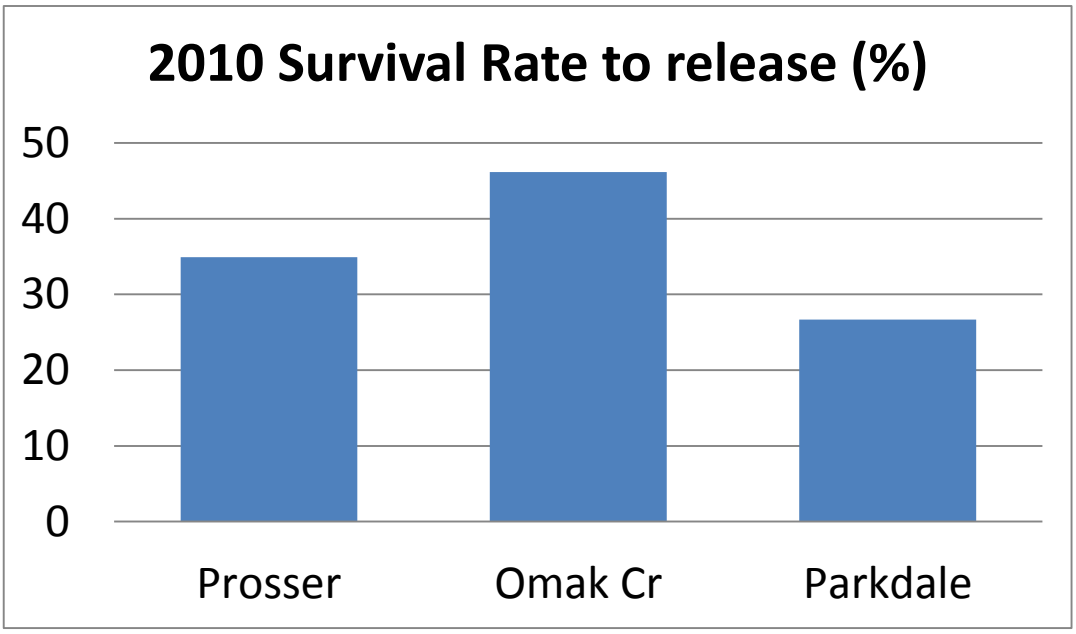


**Figure 1: Survival rate of long-term reconditioned kelt steelhead at 3 locations in 2010.**

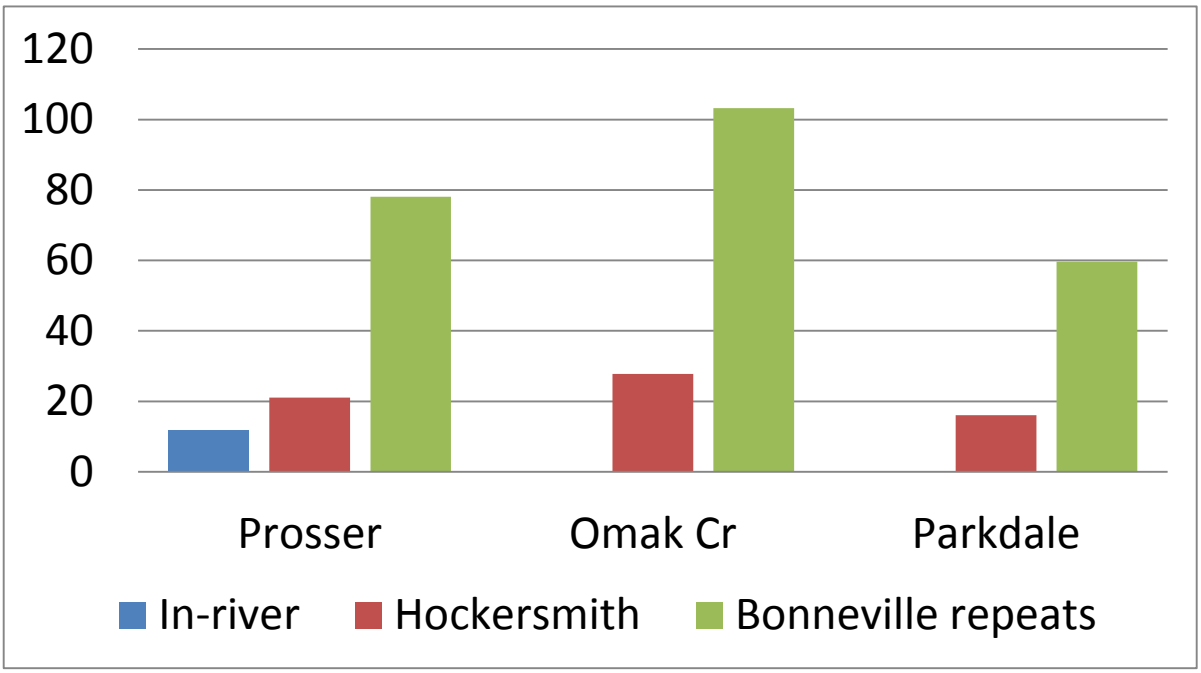


**Figure 2: Long-term reconditioning benefits for 2010, calculated by dividing long-term survival rates by control group metrics.**

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# Chapter 2: Steelhead Kelt Reproductive Success And Genetic Analysis

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# Section A: Steelhead Kelt Gamete and Progeny Viability

## Introduction

Reproductive success is difficult to observe in the field. Steelhead in particular are problematic as migration and spawn timing is associated with high flow events in the Spring. This limits the operation of weirs and traps and makes direct observation of spawning difficult. In addition to poor sampling of migratory adults due to spring flow regimes, unsampled resident fish may contribute to the gene pool. Consequentially, we are investigating gamete and progeny viability of reconditioned kelt steelhead in a hatchery setting where variables can be controlled. The design is to collect hatchery-origin prespawn adults and transport them to the hatchery for controlled studies. We initially began this experiment utilizing Skamania stock steelhead which is a highly aggregated commercial stock, and have begun collecting locally adapted winter run steelhead for comparative purposes while phasing out the Skamania portion of the experiment. After the female fish are ripe they are air spawned, eggs are fertilized with cryopreserved milt, and the offspring are raised for several weeks while recording various measures of quality. After air spawning, females are placed in tanks and reconditioned in a manner similar to the other long-term reconditioning treatments (Prosser, Omak, and Dworshak). This experiment utilizes a replicated, repeated measures experimental design to assess and compare egg and progeny viability of maiden versus reconditioned spawners. Long-term reconditioning and subsequent captive spawning provides valuable quantitative data on gonad processes, maturation rates and juvenile survival. Data resulting from this research will greatly contribute to the evaluation of reconditioning as a conservation tool. The hypothesis we are testing is:

Ho: Measures of gamete and progeny viability and quality are similar between maiden spawning and second spawning following artificial reconditioning.

## Study Area

Work was performed at the Parkdale Fish Facility located at Rkm 5.6 on the Middle Fork of the Hood River (Figure 1). This facility is co-managed by The Confederated Tribes of Warm Springs and the Oregon Department of Fish and Wildlife. The hatchery is fed water from the middle fork of the Hood River and Rogers Creek which is a spring fed system. This facility currently operates as a supplementation hatchery for winter steelhead and spring Chinook but has been used for supplementing summer steelhead and also sport hatchery fish (Skamania summer steelhead and Big Creek winter steelhead).

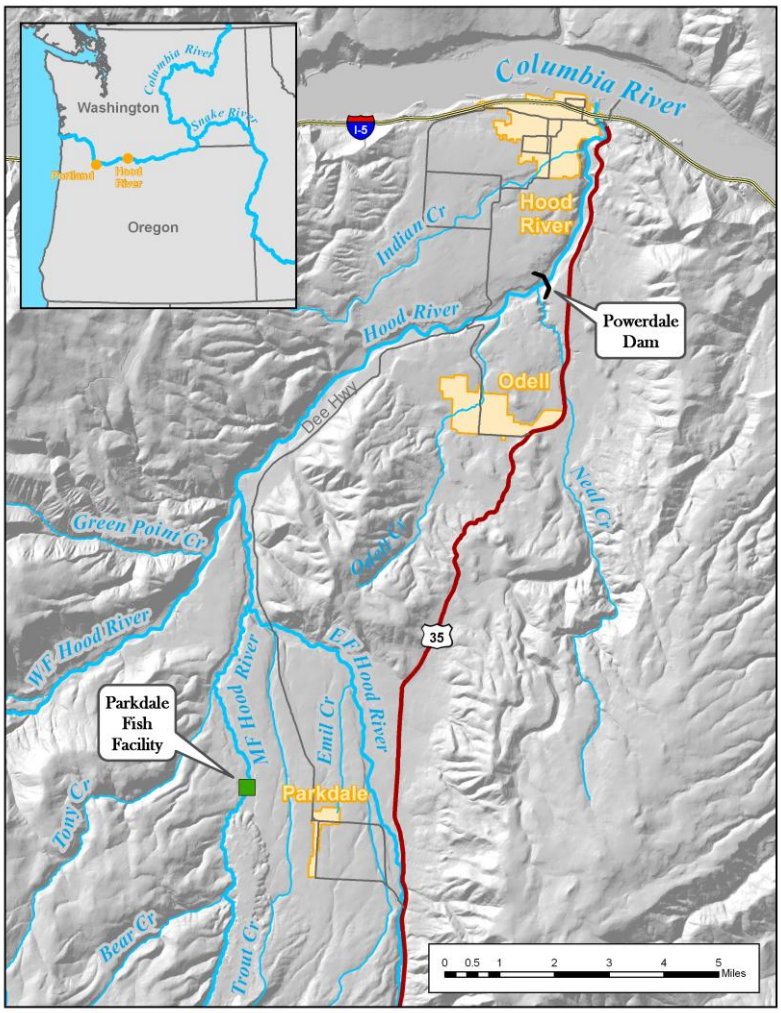


**Figure 1. Location of Parkdale Fish Facility and Powerdale Dam/ Fish Trap.**

## Methods

For collection and reconditioning methods see Chapter A Sections 1 and 2. Staff sorted fish biweekly from February through June checking for ripeness. Male gametes were collected manually and cryogenically stored (Cloud & Osborne 1997) prior to egg fertilization. This allowed us to use the same males for both maiden and reconditioned spawnings, thus controlling any variable male effect. Female gametes were collected by air-spawning (Leitritz and Lewis 1980) (Figure 2 and 3).

**Figure 2: The hatch house in the background, where fish are spawned and eggs are incubated.**

**Figure 3: Airspawning female steelhead at Parkdale Fish Facility Pictured left to right Ryan Branstetter, Jim Gidley, and Albert Santos.**

Organ tissue and gamete samples were collected from post spawn males and a sample of ovarian fluid was obtained and then submitted to the ODFW pathology lab to screen for infectious diseases including Infectious

Hematopoietic Necrosis virus (IHNV) and Bacterial Kidney Disease (BKD). Eggs and surviving fish were immediately euthanized and disposed of If disease screens were determined to be positive for any of the parents. After air spawning, the total number of eggs was estimated utilizing the Von Bayer method (Wedemeyer 2002). A total of 1500 eggs from each female were spawned and subdivided into three groups. Each egg group was mixed with thawed, cryopreserved milt (ODFW 2008) assigning two individual males that were pooled per egg group (up to 6 individual male contributions per female) to guard against any disease positive males destroying an entire batch of eggs (Figure 4). Surviving females were reconditioned at the Parkdale Fish Facility and spawned a second time with cryopreserved milt from the same male combinations. The use of cryopreserved milt allows us to spawn the same female with the same male for both the initial and post reconditioning crosses, thereby minimizing the male variable and emphasizing the reconditioning effect on a female kelt's eggs and juvenile development.

**Figure 4: Utilizing cryopreserved milt to fertilize steelhead eggs.**

Each egg group was held in isolation baskets. Water hardened eggs were treated with a diluted solution of iodaphor Povadine (Argentyne) to ensure disinfection of the eggs prior to placement into vertical stack incubators. Eggs were incubated at 5.5$^{0}$C water and treated with formalin 3 times weekly at 1:600 for 15 minutes. Eggs were subsampled (N=20) on day 15 (average of 120 Temperature Units put the eggs at the

epiboly stage of development) and fixed in Stockard's solution to estimate initial fertilization by counting the number of keels present. The proportion of eggs that were successfully fertilized post cold shock (Pennel and Barton 1996) and alevin that died post hatch was also recorded.

The fry subgroups were transferred to a picking trough (there are 5, 14'l x 16.5" w x 4.5"d troughs with water flowing at 15gal/min) and subdivided into single female groups within the troughs (34" x 16.5" or 55.5" x 16.5 depending on stocking density) for isolation purposes (Figure 5). They are started on Biovita starter feed #0 every hour during daylight hours for the first 4 weeks to satiation then gradually moved to Biovita #1 and #2 at 4 times daily to satiation for the remaining 10 weeks. Water temperatures remained a constant $5.5^{0}$C. Fry were sampled by collecting two 8" random quick netted subsamples of juveniles every week for 10 weeks. Wet weights were collected and a subsample of 20 individuals was collected for average length. All fish were anesthetized utilizing MS-222. At the end of the 10- week period all juvenile fry were euthanized by administering a fatal dosage of MS-222.

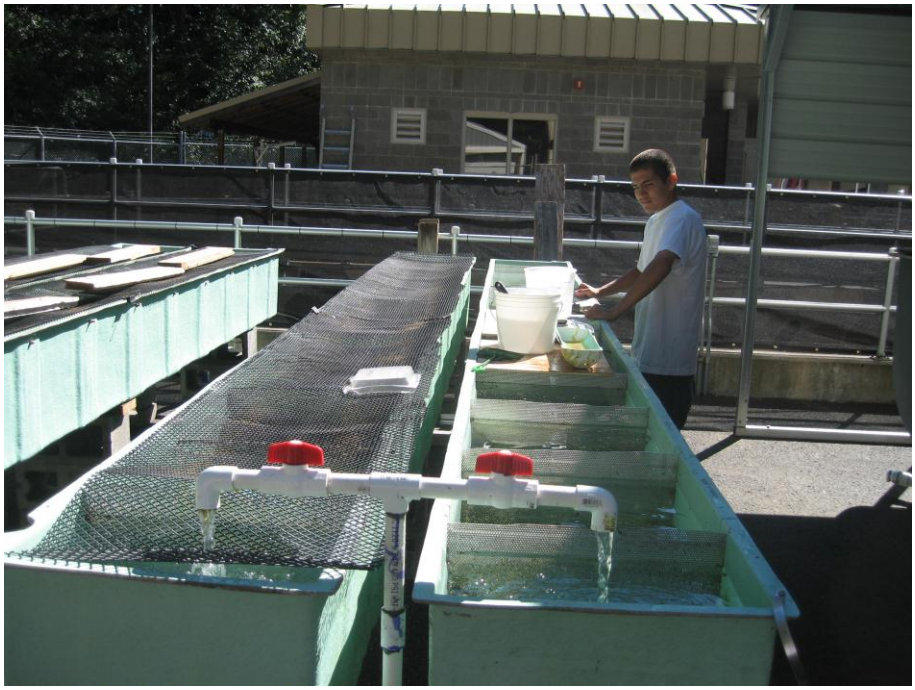

**Figure 5: CRITFC-intern (Hardo Lopez) sampling juvenile fish from picking trough to collect weight and length measures.**

# Results and Discussion

## 2010 Skamania and Winter Steelhead Broodstock Maiden Spawning

### *Skamania Steelhead*

#### Eggs

The annual fecundity of the 22 females that we spawned averaged 3,707 eggs per female with a minimum of 2,376 eggs and a maximum egg production of 5,524. On average 57% of eggs were successfully fertilized based on eyed egg counts. This number was comparable to the average keel estimates of 51% egg fertilization.

#### Juveniles

The average starting weigh for juveniles was 0.25g per fish and the average increase in weight for juveniles was 0.81grams with the average ending weight for individuals at 1.07 grams per fish. The average starting length for fish was 3.2 cm which increased on average 1.8 cm with an average ending weight of 5.1 cm. Juveniles born in late May tended to increase in size and length more than juveniles hatched in mid to late June.

Survival for juvenile fish was on average greater than 95% with over half of all juvenile mortalities occurring immediately post-hatch with an average of 1 or 2 mortalities per group, a week, thereafter. There did not appear to be any mortality specific to any group. Most of the latter mortality was likely attributable to trough cleaning with juvenile fish getting caught in the brush or tank vacuum.

### *Winter Steelhead*

#### Eggs

The estimated average fecundity of the 22 female 2010 brood maidens was 4,567 eggs per spawner with a minimum of 2,640 eggs and maximum of 6,318 eggs produced. Average fertilization success based on eyed egg survival was 62%. Keel samples taken at day 15 demonstrate that there was little to no egg loss with samples showing a 58% fertilization success rate.

#### Juveniles

The average starting weight was at 0.22 g per fish with an average increase of 0.81 grams for an average ending weight of 1.07 grams. The average starting length of fish was 3.16 cm which on average increased 1.4 cm with an ending length of 4.60 cm. The juvenile population showed little difference in both growth and length.

There was no unusual mortality patterns observed, the majority occurred just after ponding with an average of 1-2 a week afterwards. Survival was greater than 98% for kelt progeny.

## Kelt Spawning 2010 Details

### *2009 Skamania Broodstock Kelt Spawning*

There was no 2009 brood spawned in 2010.

### *2008 Skamania Broodstock Kelt Spawning*

At the end of 2010 we had 2 females remaining which for these 2 fish this was the 3rd time spawning for them. We have seen kelt multiple spawners from PIT-tag data so it was good to be able to test the reproductive capabilities of a 3rd time spawner.

## Egg

The egg production from the 5, 2008 broodstock, in 2010 averaged 5,698 per female spawner with a maximum 7,202 eggs and a minimum of 4,320 eggs. This represented an average increase per spawner of 989 eggs from maiden spawning in 2008 (Figure 6). All the kelts increased in egg clutch with the exception of individual 2701 a 3rd time spawner which had a small decline in the number of eggs (Figure 6).

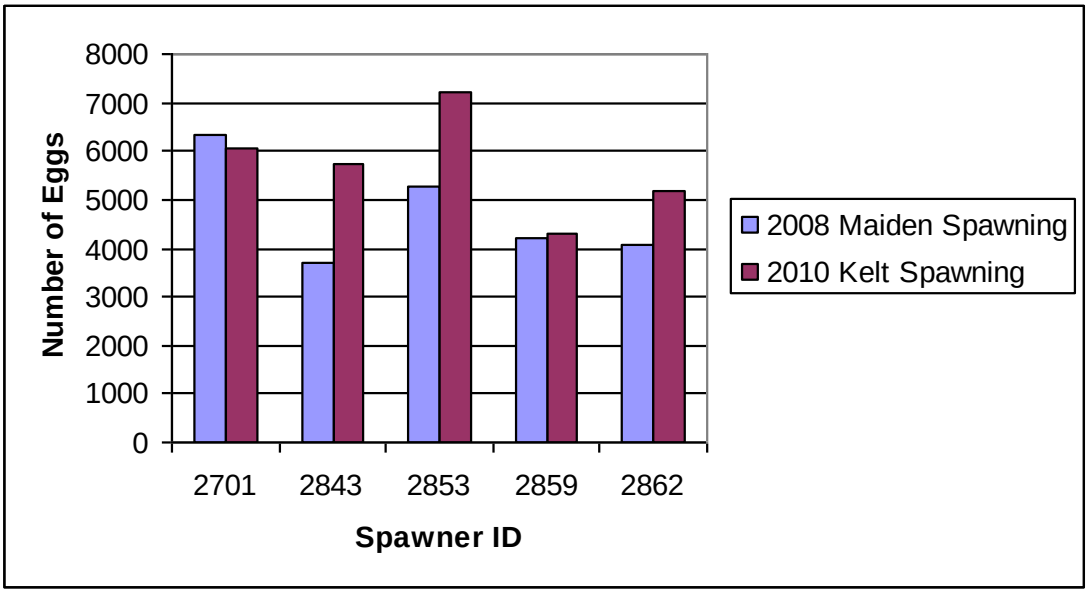


**Figure 6: 2008 Brood Egg Production: 2008 maiden spawners versus 2010 kelt spawners.**

Post shock survival was 50% with keel samples at the same value. When comparing the 2008 maiden versus the 2010 kelt spawners survival of progeny on average 7% lower than the 2010 maiden spawners which is not a large difference (difference of 300 eggs)(Figure 7). Most spawners increased eyed egg survival but 2 of the 5 spawners had a large decrease in egg survival. Oddly one of the 3rd time spawners decreased in egg survival while the other improved (figure). Spawner 2843 a 3rd time spawner increased eyed egg survival by more than 30% while 2862 a second time spawner had the largest decline with average eyed egg survival percentage in the single digits. Kelt 2862 soon perished after spawning and was determined by fish pathology to have IHN, data was collected up to the eyed egg stage and eggs were disposed of. This could have been a reason for the marked decline in eyed egg survival but 2701 also had a large decline and tested negative for IHN.

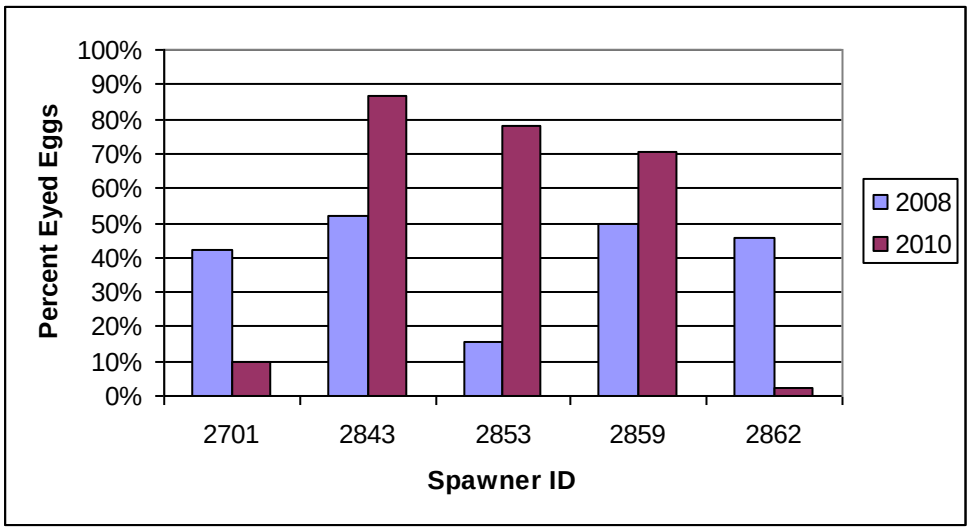


**Figure 7: 2008 Broodstock: Percentage of eyed egg survival 2008 maiden spawner versus 2010 kelt spawner.**

## Juvenile

The average increase in weight for 2008 brood juveniles was .84 grams while the average length increase was 2.3cm (Figures 8 and 9). This did not represent much of a weight difference (.03g) when comparing against 2010 fish but there was a larger difference in length of .5 cm. When comparing the 2010 juveniles versus the 2008 progeny the difference is mostly positive in weight averaging +.20g and length averaging +.3 cm (figure # and #). Post hatch mortality remained low for the kelt progeny at Parkdale with an average of 3% mortality for all groups.

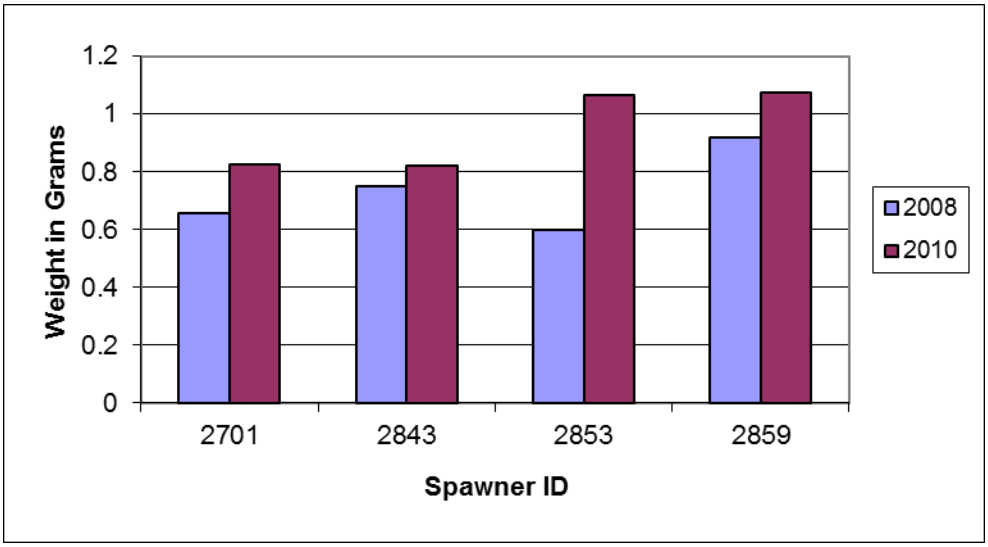


**Figure 8: 2008 Broodstock:: Change in juvenile weight 2008 maiden spawner versus 2010 kelt spawner over a 10 week period.**

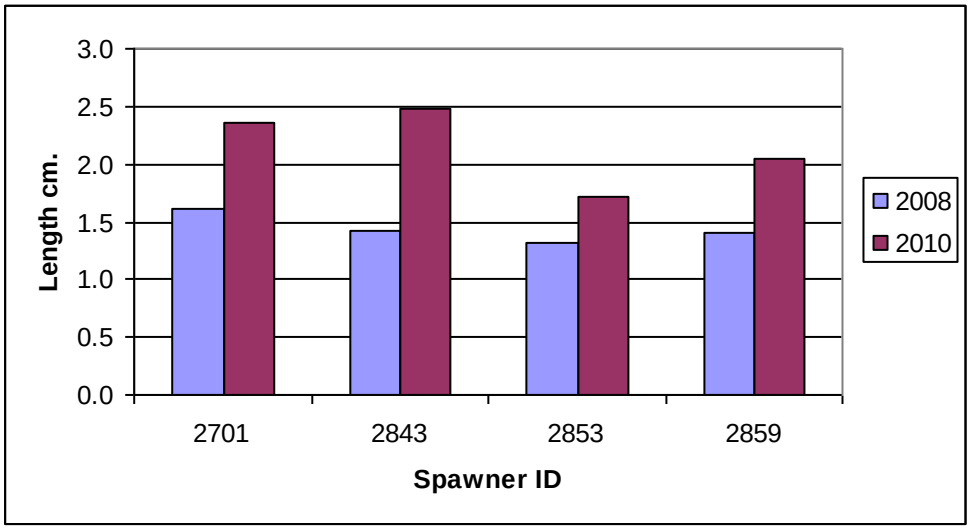


**Figure 9: 2008 Broodstock: Change in length for 2008 maiden spawners and 2010 kelt spawners.**

## *Cumulative Skamania Maiden Spawners versus Kelt Spawners all years.*

In this section we compare maiden and kelt spawners using two approaches. The first approach is a general comparison of maiden fish verse kelt spawners in terms of fecundity, fertilization rates, fry weight and length gain. In all of these comparisons kelts perform significantly better or similar to maiden steelhead. The second approach is a repeated measures design where we compare maiden and kelt spawnings using the same metrics between the 8 fish that we data from both spawn events. In this analysis we find no significant difference in performance between maiden and kelt spawners.

### General Comparison

Comparing the long-term reconditioning kelts against the incoming maiden brood, the kelts perform as well as the best spawners. Kelt spawners on average produced 900+ more eggs than maiden spawners (Figure 10). A two sample t tests with unequal sample sizes suggest that kelt mothers have a significantly higher annual fecundity than maiden fish p=0.031. In Quinn et. al. (2010) kelts were also observed to produce more eggs than 3 year old maiden spawners which they suggest is a result of the increased size of the female fish from the maiden spawning.

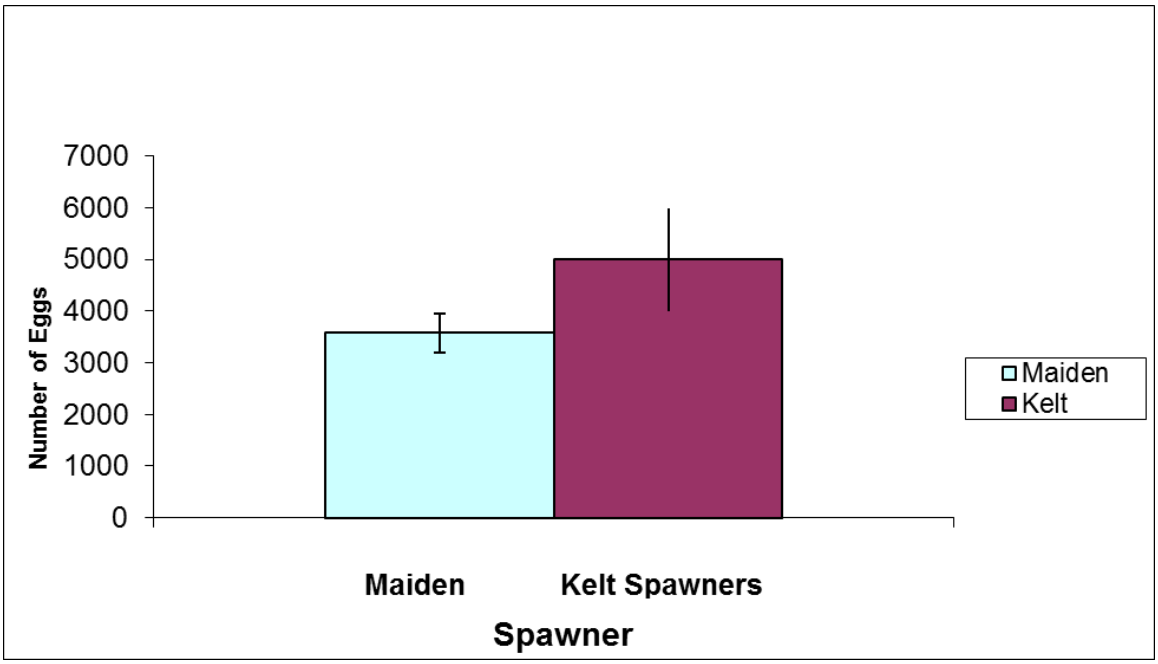


**Figure 10: Average annual fecundity of Maiden vs. Kelt Spawner for the years 2006-2010. The error bars represent the 95% Confidence Interval.**

Average fertilization rate for the maiden spawning event (56%) was slightly higher than average fertilization rates following reconditioning (53%) (Figure 11). Fertilization success rates for maiden and kelt spawning events were slightly better for maiden spawners (Figure 11 ). In Seamons and Quinn (2010) kelts were observed to produce slightly more adult offspring than maiden spawning fish. This could mean that even though initial fertilization is lower, positive kelt juvenile growth factors may give kelt progeny a slight survival advantage.

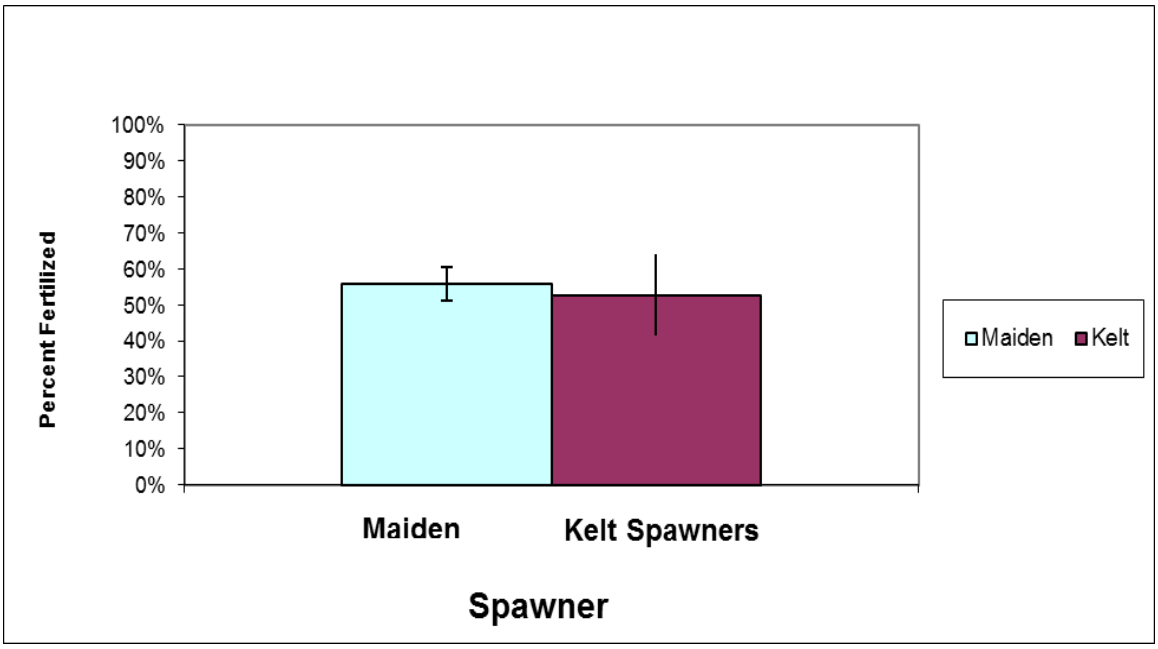


**Figure 11 : Average fertilization success (successful eyed eggs) of Maiden vs. Kelt spawner 2006-2010. The Y-bars represent the 95% confidence interval.**

Kelt progeny on average put on wieght better than maiden progeny in a 10 week period (Figure 12). Though our t-test suggests that these differences are not significant, p = 0.220.

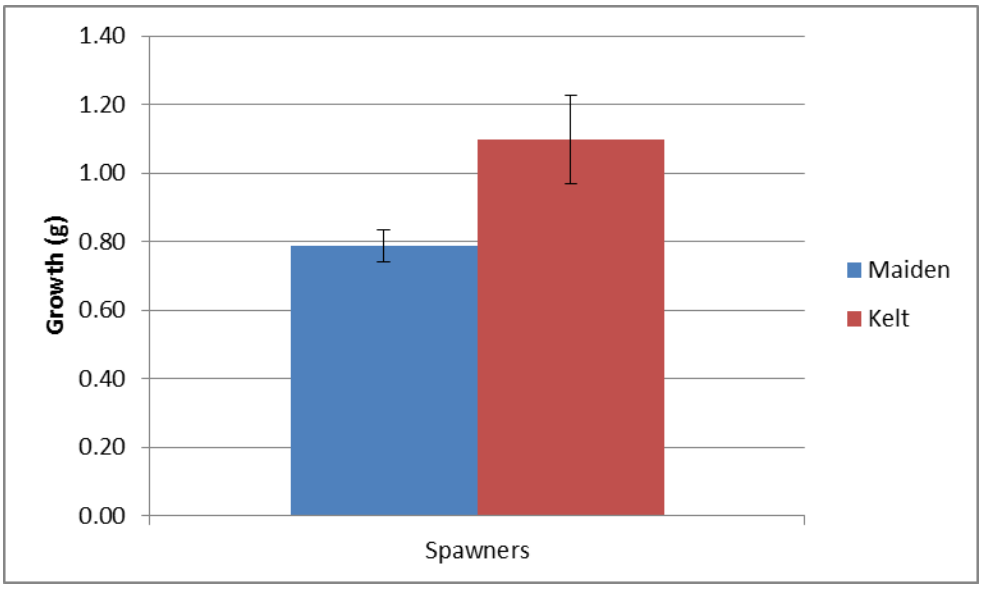


**Figure 12: The average change in wieght of (g) Maiden vs. Kelt spawner progeny for 2007-2010. Y-bars represent the 95% confidence interval.**

Kelt progeny on average grew bigger than the maiden spawning progeny. This weight gain by kelt progeny are significantly longer than maiden progeny p= 0.040 (Figure 13).

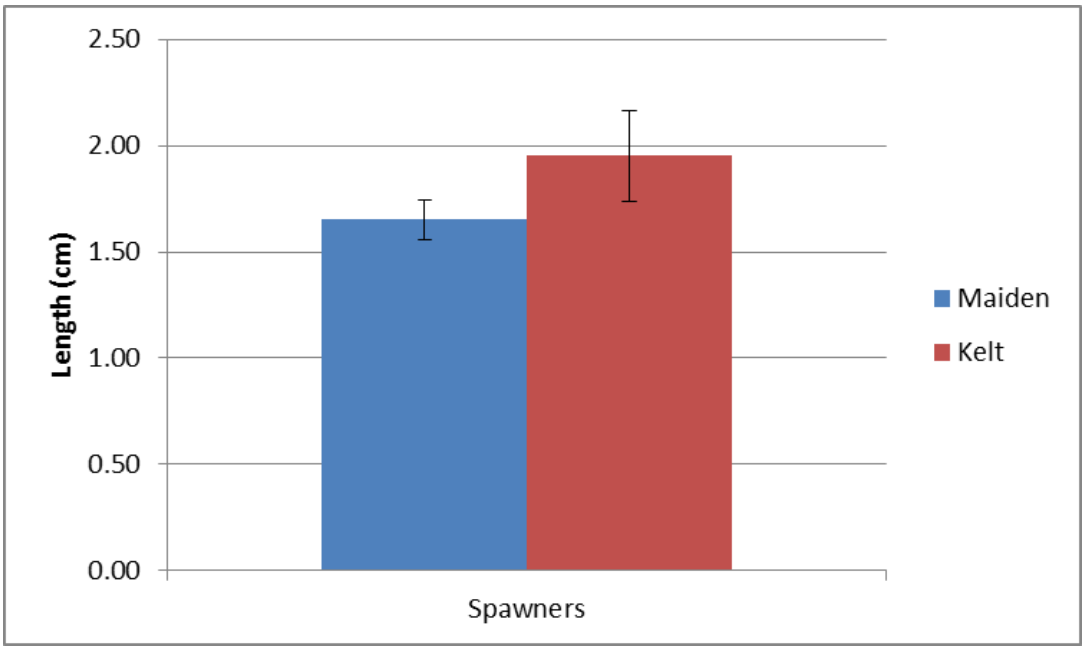


**Figure 13: The average change in length (cm) of Maiden vs. Kelt spawners from 2007-2010.**

Quinn et al. 2010, suggest that steelhead kelts may be foregoing active growth and instead directing the majority of energy into egg production.. The larger size of the maternal fish along with the increased number of eggs and improved growth factors of the progeny (weight and length) should confer an advantage over fisrt time spawners. The increased growth rate of kelt progeny influence the timing of smolting and survival to adulthood (Beckman 1998; Quinn 2005).

Holding space at Parkdale is low which limits the number of fish that we can effectively recondition on site. This limited statistical power (kelt sample size 10) but we should have some additional kelt data to add to our samples in 2011 and 2012.

**Repeated Measures Comparison**

Performance measures between maiden and kelt steelhead are given in Table (1). We find that kelt spawners outperform maiden fish in three of the four metrics measured, however, none of these differences are statistically significant (Table 2). These results may change as our sample sizes increase, but at this point kelt steelhead performance is comparable to maiden steelhead for the metrics we measured.

**Table 1. Mean fecundity, fertilization, fry weight and length for maiden and kelt spawnings of 8 different individuals.**

| Stage | Maiden | Kelt |
|---|---|---|
| Egg fecundity | 4693 | 5229 |
| Egg Fertilization | 0.445 | 0.559 |
| Fry Weight | 0.973 | 0.778 |
| Fry Length | 1.480 | 2.004 |

**Table 2. Statistical comparison of maiden and kelt steelhead spawnings from 8 individuals using 4 reproductive success metrics.**

| Variable | Mean Difference | 95% C.I. Lower limit | 95% C.I. Upper limit | Standard deviation of difference | t | df | P value |
|---|---|---|---|---|---|---|---|
| Fecundity | -366.125 | -1,911.259 | 1,179.009 | 1,848.201 | -0.560 | 7.000 | 0.593 |
| Fertilization | -0.048 | -0.354 | 0.258 | 0.366 | -0.369 | 7.000 | 0.723 |
| Fry wt | -0.198 | -1.449 | 1.053 | 1.353 | -0.388 | 6.000 | 0.712 |
| Fry length | -0.524 | -1.049 | 0.001 | 0.423 | -2.772 | 4.000 | 0.050 |

## References:


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# Section B: Omak Creek Steelhead Kelt Genetic Analysis

## Introduction

The reproductive success of long-term reconditioned kelts needs to be explored to assess the net benefit of this program. Specific questions regarding the success of artificially reconditioning kelt steelhead include: do reconditioned kelts produce viable offspring that contribute to recruitment, how does kelt reproductive success compare with natural first time spawners, and how does kelt reproductive success compare with hatchery origin spawners? We will utilize microsatellite DNA markers and pedigree analysis to help us answer these questions. The answers to these questions will be important in determining if kelt reconditioning is a viable restoration tool that will aid in the recovery of ESA listed steelhead populations in the Columbia River Basin.

## Methods-Sample Collection

Anadromous adults were collected via an adult trap at a semi-permanent weir on Omak Creek, and a temporary weir in Bonaparte Creek. A PIT tag antennae array was also operated upstream of the Omak Creek confluence with the Okanogan River. Downstream juvenile migrants were collected with a screwtrap locate downstream of the weir during the spring. Electrofishing techniques during the fall were used to target resident populations in multiple areas, although it was expected that progeny of the anadromous adults would also be sampled. Collection sites included both below and above Mission Falls, a partial barrier to migration. In 2008 samples above Mission Falls were collected at locations near Haley creek, and Lobe Road. In 2010, fall sampling using electrofishing techniques expanded to Bonaparte and Salmon creeks. Both of these collections targeted juvenile fish that were expected to include progeny of anadromous adults.

Reconditioning efforts and subsequent detections of returning adults are quantified in Table 1. Juvenile sampling and genotyping was designed to preferentially sample fish of appropriate age to the post-reconditioning spawning event. Details for detections at each year can be found in the following text.

**Table 1. Reconditioning efforts. The number of fish released and later detected is reported for each year. Age classes for juveniles resulting from the post-reconditioning spawning event are also listed**

| Capture | Release | | Detection | | Potential contribution to age classes | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | Year | n | Year | n | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 |
| 2005 | 2005 | 3 | 2006 | 1 | Age 0 | Age 1 | Age 2 | Age 3 | | | | | |
| 2006 | 2006 | 1 | 2007 | 0 | | | | | | | | | |
| 2007 | 2007 | 8 | 2008 | 3 | | | Age 0 | Age 1 | Age 2 | Age 3 | | | |
| 2008 | 2008 | 9 | 2009 | 0 | | | | | | | | | |
| 2009 | 2009 | <5 | 2010 | 0 | | | | | | | | | |
| 2010 | 2010 | 6 | 2011 | 5 | | | | | | Age 0 | Age 1 | Age 2 | Age 3 |

Three reconditioned kelts were released in October 2005, one male and two female. The male was processed on 30 March 2006 at the adult trap, having passed the PIT tag antennae undetected. One of the females was detected at the PIT tag antennae on 23 March 2006 and again on 17 April 2006, but was not processed at the

adult trap. The second female was not detected after the October release. While two of the three kelts were not detected above the picket weir, their return and contribution to spawning in Omak Creek cannot be ruled out as weir operations in 2006 were subject to disturbances from high water flows. Additionally, spawning takes place below the picket weir and even below the screwtrap (Arterburn 2008), without an adult being sampled. Genetic sampling of juveniles in Omak Creek was preferentially targeted at age-1 fish in 2007 and age-2 fish in 2008 to increase the chance of detecting the progeny of the reconditioned kelts that returned to spawn in 2006.

Eight reconditioned kelts were released into the Okanogan River in October 2007, four from Bonaparte Creek, and four from Omak Creek. Three of the eight fish were detected following release from reconditioning. The first was captured in Omak Creek on April 10$^{th}$, passed upstream and was captured again on May 3$^{rd}$ after spawning. After the second capture, it was taken to the hatchery for reconditioning. The second was captured April 26$^{th}$, having already spawned below the weir it was also taken to the hatchery for reconditioning. Prior to their capture, both fish were observed directly below the weir, where up to 15 heavily superimposed redds were detected. Fry from these redds were sampled as they emerged after hatching. An additional male was detected by the PIT tag antennae on April 9$^{th}$ 2008, but was not observed at the adult trap.

None of the four reconditioned kelts from Bonaparte Creek were detected again. Both Bonaparte and Omak creeks had low water flows in March of 2008 that limited migration. In particular for Bonaparte Creek, fish that returned early were unable to migrate through the lower reaches of the stream and were thought to have subsequently spawned in the mainstem Okanogan River.

## Methods-Genetic Analysis

Samples were collected and stored in ethanol for preservation of DNA. Genetic analysis was conducted at the Hagerman Fish Culture Experiment Station in Hagerman, ID. DNA was extracted from tissue samples using standard manufacturer's protocols from Qiagen® DNeasy™ extraction kit. The polymerase chain reaction (PCR) was used to amplify 16 microsatellite loci including 13 standardized markers (Stephenson et al. 2008) and 3 others: Omm 1036 (GenBank Accession #AF346686), Omm 1046 (GenBank Accession #AF346693), and One 102 (Olsen et al. 2000). PCR products were genotyped using manufacturer's protocols with an Applied Biosystems® model 3730 genetic analyzer and scored using Genemapper v3.7 Software.

Juvenile samples collected from Bonaparte and Salmon creeks in 2010 were also genotyped for a suite of 192 SNPs (single nucleotide polymorphisms) and are intended for inclusion in a genetic baseline used for Genetic Stock Identification. Further analysis of these samples will be done as part of the 2011 baseline expansion effort.

Prior to statistical analysis, confirmed duplicate samples, samples with incomplete genotypes, and non-target species samples were omitted and are not included in the results.

In order to evaluate genetic diversity, expected and observed heterozygosity were calculated using Excel Microsatellite Toolkit (Park 2001). Number of alleles, allelic richness and private allelic richness for the 16 microsatellites were calculated using HP-Rare (Kalinowski 2005). For rarefaction estimates, gene number was set at 21. Deviation from Hardy-Weinberg equilibrium was evaluated using exact tests (Haldane 1954, Weir 1990, Guo and Thompson 1992) implemented in GENEPOP v3.4 (Raymond and Rousset 1995). The number of loci showing heterozygote excess or deficiency was also quantified (Rousset and Raymond 1995). Linkage disequilibrium was tested using exact tests (Haldane 1954, Weir 1990, Guo and Thompson 1992) implemented in GENEPOP v3.4 (Raymond and Rousset 1995). Corrections to the significant value were made using the

Bonferroni method (Rice, 1989). Parentage analysis was performed using CERVUS v 3.0 (Marshall et al. 1998, Kalinowski et al. 2007). Information on fish gender was not included in the analysis.

To demonstrate inter-population relationships, pairwise genetic distances (Cavalli-Sforza and Edwards 1967) were calculated between all sites using POPULATIONS software (Langella 2001). Genetic chord distances with 1000 iterations of bootstrap replicates were used to construct a neighbor joining tree. The program TREEVIEW (Page 1996) was then used to display the tree.

To help infer population structure in Omak Creek, the program STRUCTURE v.2.0 (Pritchard et al. 2000, Falush et al. 2003) was used. Aside from the known adult anadromous steelhead, samples in Omak Creek were expected to be mixed collections of anadromous steelhead juveniles and resident populations. Potential population numbers (K) from two to ten were tested using four iterations. Lacking strong support for K greater than two, assignment results are reported for two putative populations, as generated and averaged over 10 iterations. The group containing the majority of anadromous adult steelhead was labeled the anadromous population, and the alternative group the resident population. Results are reported for assignment probabilities of both 0.70 and 0.90 or greater.

To supplement the STRUCTURE data, individual assignment tests were performed using methods reported in Anderson et al. (2008) as implemented in the software program ONCOR. Because the baseline (Blankenship et al., in press) included samples from Omak Creek, only samples from Bonaparte and Salmon creeks were analyzed here. Fourteen reporting groups were used for genetic stock identification of the Omak samples. This baseline consists of a total of 147 collections, including ten collections that represent an outgroup (i.e. out-of-basin steelhead from Puget Sound, WA). The fourteen Columbia River Basin reporting groups include the following: lower Columbia R. (# collections; n=24), lower Columbia R. summer-run (n=2), Willamette R. (n=9), Big White Salmon R. (n=1), Klickitat R. (n=10), middle Columbia R./lower Snake R. (n=30), Yakima R. (n=6), upper Columbia R. (n=5), Grand Ronde R. (n=1), Imnaha R. (n=4), upper Clearwater R. (n=17), lower Salmon R. (n=5), Middle Fork/South Fork Salmon R. (n=13), upper Salmon R. (n=10). For a complete list of baseline collections and their locations refer to Narum et al. 2009 (2009 BPA report, available at http://maps.critfc.org/tech/10_12report.html).

Parentage data, when successful, was used to assign ages to juveniles. Length of known age juveniles captured at the screwtrap was then plotted in length histograms. To eliminate variation between years, separate histograms were created for 2007, 2008, and 2009 sampling years. To discriminate between first time and reconditioned kelt spawning events, juveniles assigning to reconditioned kelts were compared to the length histogram of the known age fish.

## Results-Genetic Analysis

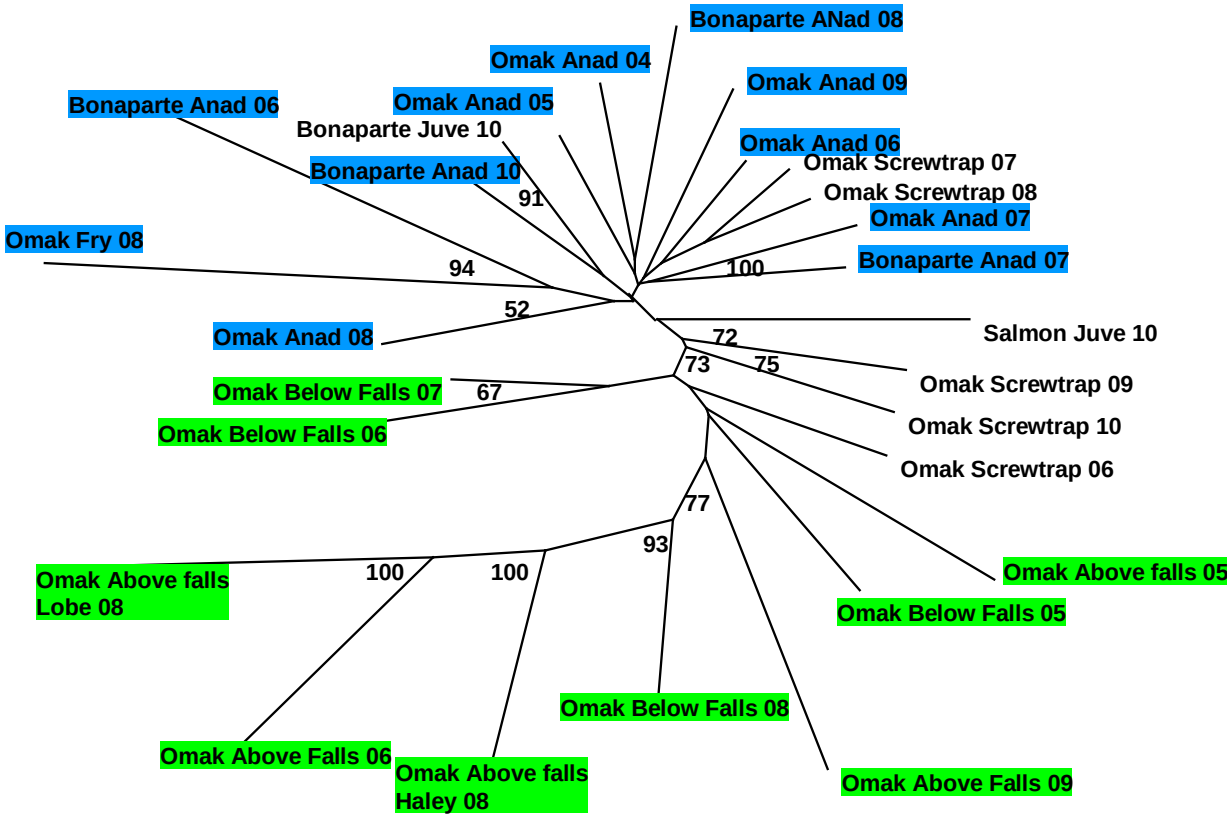
A total of 2149 samples were successfully genotyped. Numbers for each collection, by location and year, can be seen in table 2. Departures from Hardy-Weinberg equilibrium (critical level =0.05 /16 loci = 0.00313) and or linkage disequilibrium (critical level =0.05 /120 pairwise comparisons = 0.00042) were seen in most population collections, commonly as a heterozygote deficit due to Wahlund Effect. Additional statistical analysis proceeded as normal as population mixture in these collections was expected, and the additional analyses do not require Hardy-Weinberg equilibrium to be informative.

**Table 2. Population Statistics. Each collection is reported in terms of sample size (n), expected heterozygosity ($H_E$), observed heterozygosity ($H_O$), average number of alleles per locus (A), allelic richness (AR), number of loci out of Hardy-Weinberg equilibrium (HW), and number of pairwise loci comparisons showing linkage disequilibrium (LD).**

| Collection | Year | n | $H_E$ | $H_O$ | A | AR | H-W | LD |
|---|---|---|---|---|---|---|---|---|
| Omak Anadromous | 2004 | 89 | 0.8117 | 0.8110 | 12.1 | 5.7 | 11 | 64 |
| Omak Anadromous | 2005 | 101 | 0.8153 | 0.7922 | 13.5 | 5.8 | 0 | 23 |
| Omak Anadromous | 2006 | 83 | 0.8169 | 0.8149 | 13.4 | 5.9 | 0 | 7 |
| Omak Anadromous | 2007 | 69 | 0.8274 | 0.8098 | 13.3 | 6.0 | 2 | 15 |
| Omak Anadromous | 2008 | 50 | 0.8147 | 0.8075 | 13.4 | 5.9 | 0 | 2 |
| Omak Anadromous | 2009 | 50 | 0.8197 | 0.8098 | 12.4 | 5.9 | 0 | 4 |
| Omak above falls | 2005 | 21 | 0.8328 | 0.7796 | 10.0 | 5.9 | 0 | 1 |
| Omak above falls | 2006 | 45 | 0.7676 | 0.7904 | 9.7 | 5.1 | 1 | 19 |
| Omak above falls | 2009 | 23 | 0.7761 | 0.7446 | 8.6 | 5.2 | 4 | 27 |
| Omak above falls-Haley | 2008 | 25 | 0.8079 | 0.7660 | 9.7 | 5.5 | 1 | 2 |
| Omak above falls-Lobe | 2008 | 67 | 0.7260 | 0.7148 | 10.1 | 4.7 | 4 | 16 |
| Omak Below falls | 2005 | 76 | 0.8259 | 0.7974 | 12.2 | 5.8 | 7 | 45 |
| Omak Below falls | 2006 | 91 | 0.8449 | 0.8104 | 13.8 | 6.2 | 3 | 17 |
| Omak Below falls | 2007 | 93 | 0.8437 | 0.8322 | 14.4 | 6.2 | 1 | 7 |
| Omak Below falls | 2008 | 43 | 0.8300 | 0.8196 | 12.1 | 5.9 | 2 | 9 |
| Omak Screw trap | 2006 | 94 | 0.8380 | 0.7984 | 13.8 | 6.0 | 6 | 25 |
| Omak Screw trap | 2007 | 278 | 0.8263 | 0.8115 | 15.7 | 6.0 | 10 | 32 |
| Omak Screw trap | 2008 | 322 | 0.8288 | 0.8206 | 15.8 | 6.0 | 8 | 33 |
| Omak Screw trap | 2009 | 68 | 0.8334 | 0.8425 | 13.9 | 6.0 | 1 | 9 |
| Omak Screw trap | 2010 | 87 | 0.8454 | 0.8134 | 14.0 | 6.2 | 2 | 10 |
| Omak Fry | 2008 | 28 | 0.7476 | 0.7716 | 8.1 | 5.0 | 0 | 26 |
| Bonaparte Anadromous | 2006 | 11 | 0.8135 | 0.8443 | 7.9 | 5.8 | 0 | 1 |
| Bonaparte Anadromous | 2007 | 59 | 0.8233 | 0.8167 | 12.8 | 6.0 | 0 | 2 |
| Bonaparte Anadromous | 2008 | 27 | 0.8171 | 0.8009 | 10.8 | 5.8 | 0 | 3 |
| Bonaparte Anadromous | 2010 | 55 | 0.8176 | 0.8091 | 13.1 | 5.9 | 0 | 4 |
| Bonaparte Juveniles | 2010 | 96 | 0.8108 | 0.8161 | 13.3 | 5.8 | 5 | 29 |
| Salmon Cr. Juveniles | 2010 | 98 | 0.8458 | 0.8212 | 14.9 | 6.3 | 1 | 10 |

The relationship of tributary collections is shown in the neighbor joining dendrogram in Figure 1. All known anadromous collections clustered together with a bootstrap value of 72 percent. This cluster also contained the Bonaparte Creek and Salmon Creek juvenile collections as well as the Omak Creek screwtrap collections from 2007 and 2008. All Omak collections sampled by electrofishing clustered together with a bootstrap value of 73 percent. This cluster also contained Omak Screwtrap 2006. The remaining two collections, Omak Screwtrap 2009 and 2010, clustered intermediate to the two primary groups.

**Figure 1. Neigbor joining dendrogram of Cavalli-Sforza Edwards genetic distance among studied populations. Numbers at nodes represent bootstrap percentage from 1000 replicates (only those greater than 50 percent shown). Known anadromous populations are highlighted in Blue. Electrofish samples from Omak Creek are highlighted in Green.**




Pairwise Fst values are shown in Table 3 with corresponding P values shown in Table 4. The majority of comparisons had critical values lower than 0.00192 (corrected for multiple test at 0.05/26). Of the 34 comparisons that were not significant 28 included at least one of three Bonaparte Anadromous collections that had low sample sizes (2006 n=11, 2007 n=59, and 2008 n=27). Comparisons between Omak Anadromous Adults accounted for 4 additional insignificant values. The remaining two insignificant values were seen in Omak Screwtrap to Omak Anadromous adults and Bonaparte Juveniles to Bonaparte Anadromous adults. Fst over all collections averaged 0.031. When collections from above Mission Falls were compared to other collections, Fst was higher with an average of 0.067. Fst within all collections taken above Mission Falls averaged 0

**Table 3. Pairwise Fst values between populations. Fst values greater than 0.05 are highlighted.**

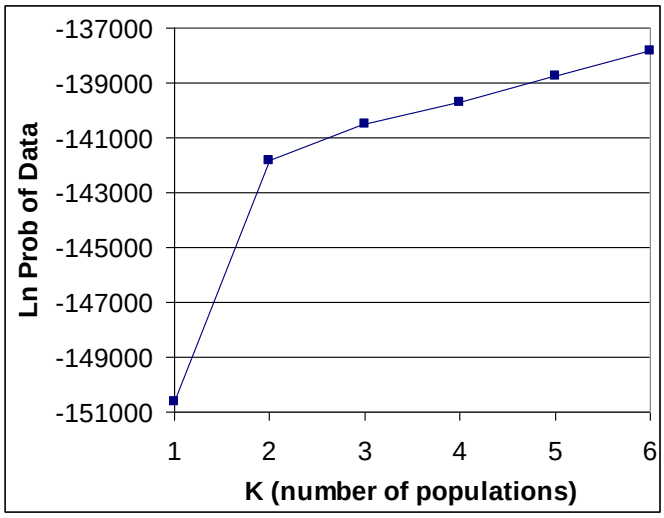
| | | | Omak Anad 04 | Omak Anad 05 | Omak Anad 06 | Omak Anad 07 | Omak Anad 08 | Omak Anad 09 | Omak above 05 | Omak above 06 | Omak above 09 | Omak Haley 08 | Omak Lobe 08 | Omak Below | Below | Below | Below | Omak Screw | Omak Screw | Omak Screw | Omak Screw | Omak Screw | Omak Fry 08 | Bona Anad 06 | Bona Anad 07 | Bona Anad 08 | Bona Anad 10 | Bona Elec 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2005 | Omak Anadromous | Omak Anad 05 | 0.01 | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2006 | Omak Anadromous | Omak Anad 06 | 0.01 | 0.00 | | | | | | | | | | | | | | | | | | | | | | | | |
| 2007 | Omak Anadromous | Omak Anad 07 | 0.00 | 0.00 | 0.00 | | | | | | | | | | | | | | | | | | | | | | | |
| 2008 | Omak Anadromous | Omak Anad 08 | 0.01 | 0.00 | 0.00 | 0.00 | | | | | | | | | | | | | | | | | | | | | | |
| 2009 | Omak Anadromous | Omak Anad 09 | 0.01 | 0.01 | 0.00 | 0.00 | 0.00 | | | | | | | | | | | | | | | | | | | | | |
| 2005 | Omak above falls | Omak above 05 | 0.03 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | | | | | | | | | | | | | | | | | | | | |
| 2006 | Omak above falls | Omak above 06 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.06 | | | | | | | | | | | | | | | | | | | |
| 2009 | Omak above falls | Omak above 09 | 0.05 | 0.05 | 0.05 | 0.04 | 0.05 | 0.05 | 0.03 | 0.06 | | | | | | | | | | | | | | | | | | |
| 2008 | Omak above falls-Ha | Omak Haley 08 | 0.07 | 0.08 | 0.07 | 0.07 | 0.07 | 0.07 | 0.02 | 0.02 | 0.03 | | | | | | | | | | | | | | | | | |
| 2008 | Omak above falls-Lo | Omak Lobe 08 | 0.13 | 0.13 | 0.13 | 0.12 | 0.13 | 0.13 | 0.06 | 0.02 | 0.05 | 0.02 | | | | | | | | | | | | | | | | |
| 2005 | Omak Below falls | Omak Below 05 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.01 | 0.05 | 0.03 | 0.03 | 0.06 | | | | | | | | | | | | | | | |
| 2006 | Omak Below falls | Omak Below 06 | 0.03 | 0.03 | 0.03 | 0.02 | 0.02 | 0.02 | 0.01 | 0.04 | 0.02 | 0.02 | 0.06 | 0.01 | | | | | | | | | | | | | | |
| 2007 | Omak Below falls | Omak Below 07 | 0.02 | 0.02 | 0.01 | 0.01 | 0.02 | 0.01 | 0.01 | 0.06 | 0.03 | 0.03 | 0.08 | 0.01 | 0.00 | | | | | | | | | | | | | |
| 2008 | Omak Below falls | Omak Below 08 | 0.05 | 0.05 | 0.04 | 0.04 | 0.04 | 0.05 | 0.01 | 0.03 | 0.02 | 0.01 | 0.04 | 0.02 | 0.01 | 0.02 | | | | | | | | | | | | |
| 2006 | Omak Screw trap | Omak Screw 06 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.01 | 0.06 | 0.02 | 0.03 | 0.07 | 0.01 | 0.01 | 0.01 | 0.01 | | | | | | | | | | | |
| 2007 | Omak Screw trap | Omak Screw 07 | 0.01 | 0.01 | 0.00 | 0.01 | 0.01 | 0.01 | 0.02 | 0.09 | 0.04 | 0.06 | 0.11 | 0.02 | 0.02 | 0.01 | 0.03 | 0.02 | | | | | | | | | | |
| 2008 | Omak Screw trap | Omak Screw 08 | 0.01 | 0.01 | 0.00 | 0.00 | 0.01 | 0.01 | 0.02 | 0.10 | 0.04 | 0.06 | 0.11 | 0.02 | 0.02 | 0.01 | 0.03 | 0.02 | 0.00 | | | | | | | | | |
| 2009 | Omak Screw trap | Omak Screw 09 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.08 | 0.04 | 0.04 | 0.09 | 0.02 | 0.01 | 0.00 | 0.02 | 0.01 | 0.01 | 0.01 | | | | | | | | |
| 2010 | Omak Screw trap | Omak Screw 10 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.07 | 0.03 | 0.04 | 0.08 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0.01 | 0.01 | 0.00 | | | | | | | |
| 2008 | Omak Fry | Omak Fry 08 | 0.04 | 0.04 | 0.03 | 0.03 | 0.02 | 0.04 | 0.06 | 0.16 | 0.09 | 0.11 | 0.17 | 0.06 | 0.06 | 0.05 | 0.08 | 0.05 | 0.04 | 0.04 | 0.04 | 0.04 | | | | | | |
| 2006 | Bonaparte Anadrom | Bona Anad 06 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.02 | 0.11 | 0.04 | 0.07 | 0.13 | 0.02 | 0.02 | 0.01 | 0.04 | 0.02 | 0.00 | 0.01 | 0.01 | 0.01 | 0.03 | | | | | |
| 2007 | Bonaparte Anadrom | Bona Anad 07 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.11 | 0.04 | 0.07 | 0.13 | 0.03 | 0.02 | 0.01 | 0.04 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.04 | 0.00 | | | | |
| 2008 | Bonaparte Anadrom | Bona Anad 08 | 0.00 | 0.00 | 0.01 | 0.00 | 0.01 | 0.00 | 0.02 | 0.11 | 0.05 | 0.07 | 0.13 | 0.03 | 0.02 | 0.01 | 0.05 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.03 | 0.00 | 0.00 | | | |
| 2010 | Bonaparte Anadrom | Bona Anad 10 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.03 | 0.11 | 0.05 | 0.07 | 0.13 | 0.03 | 0.03 | 0.02 | 0.04 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.03 | 0.01 | 0.00 | 0.00 | | |
| 2010 | Bonaparte Electrofis | Bona Elec 10 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.11 | 0.05 | 0.07 | 0.13 | 0.03 | 0.03 | 0.01 | 0.04 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.03 | 0.00 | 0.00 | 0.01 | 0.00 | |
| 2010 | Salmon Creek | Salmon 10 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.09 | 0.05 | 0.06 | 0.11 | 0.02 | 0.02 | 0.01 | 0.03 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.05 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |

**Table 4. P-Values for population differentiation. Insignificant P-Values (greater than .00192) are highlighted.**

| | Omak Anad 04 | Omak Anad 05 | Omak Anad 06 | Omak Anad 07 | Omak Anad 08 | Omak Anad 09 | Omak above 05 | Omak above 06 | Omak above 09 | Omak Haley 08 | Omak Lobe 08 | Omak Below 05 | Omak Below 06 | Omak Below 07 | Omak Below 08 | Omak Screw 06 | Omak Screw 07 | Omak Screw 08 | Omak Screw 09 | Omak Screw 10 | Omak Fry 08 | Bona Anad 06 | Bona Anad 07 | Bona Anad 08 | Bona Anad 10 | Bona Elec 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Om** | 0.000 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Om** | 0.000 | 0.024 | | | | | | | | | | | | | | | | | | | | | | | | |
| **Om** | 0.000 | 0.000 | 0.009 | | | | | | | | | | | | | | | | | | | | | | | |
| **Om** | 0.000 | 0.000 | 0.014 | 0.001 | | | | | | | | | | | | | | | | | | | | | | |
| **Om** | 0.000 | 0.000 | 0.023 | 0.000 | 0.000 | | | | | | | | | | | | | | | | | | | | | |
| **Om** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | | | | | | | | | | | | | | | | |
| **Om** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | | | | | | | | | | | | | | | |
| **Om** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | | | | | | | | | | | | | | |
| **Om** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | | | | | | | | | | | | | |
| **Om** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | | | | | | | | | | | | |
| **Om** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | | | | | | | | | | | |
| **Om** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | | | | | | | | | | |
| **Om** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | | | | | | | | | | | | | |
| **Om** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | | | | | | | | |
| **Om** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | | | | | | | |
| **Om** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | | | | | | |
| **Om** | 0.000 | 0.000 | 0.006 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | | | | | |
| **Om** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | | | | |
| **Om** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | | | |
| **Om** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | | |
| **Bon** | 0.001 | 0.013 | 0.129 | 0.150 | 0.360 | 0.023 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.009 | 0.000 | 0.000 | 0.004 | 0.002 | 0.003 | 0.003 | 0.000 | | | | | |
| **Bon** | 0.000 | 0.022 | 0.296 | 0.288 | 0.041 | 0.007 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.317 | | | | |
| **Bon** | 0.003 | 0.014 | 0.000 | 0.040 | 0.046 | 0.201 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.124 | 0.021 | | | |
| **Bon** | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.810 | 0.191 | 0.017 | | |
| **Bon** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.179 | 0.000 | 0.000 | 0.012 | |
| **Sal** | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.041 | 0.000 | 0.000 | 0.000 | 0.000 |

Structure results supported multiple populations with the largest change in Ln probability of data between K values of 1 and 2 (Figure 2). While the Ln probability continued to increase with K values greater than 2, the relative increases were minor and do not provide compelling evidence of population substructure beyond 2 (Evanno et al 2005).

**Figure 2. Ln Probability of Data. For each value of K =1-6, the average of three ln probability values is graphed. The increase between K=1 and K=2 is considered to be the only change of significance.**




Structure results for two populations (K=2) are shown in Figure 3. To further quantify the relationships between collections, the percentage assigning to each group are listed in Table 5. Results are reported for both a cutoff value of 0.70 and 0.90. Using the 0.90 cutoff, all anadromous adults assigned to either the anadromous or mixed group.

**Figure 3. Graphical representations of structure results with K=2. The inferred ancestry of each individual is shown as blue (Anadromous), yellow (Resident), or as a portion of both. Results for each site are condensed across all years.**

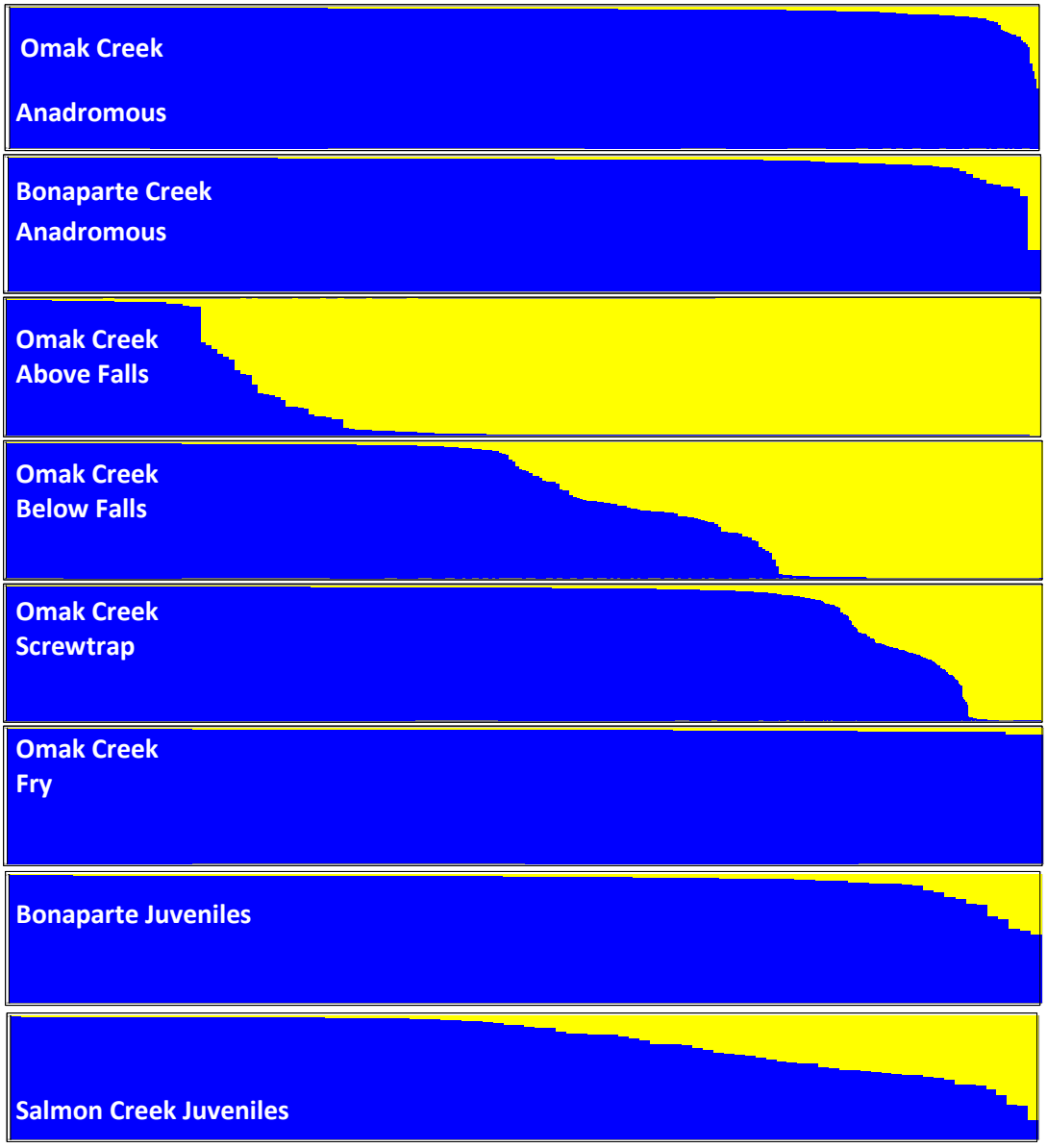


**Table 5. Population assignments by STRUCTURE. Percentage of each collection assigning to the group labeled as Anadromous (Anad), Resident (Res), or Mix. Mix is defined as having intermediate values of less than 0.70 or 0.90 for both the anadromous and resident groups.**

| | | 0.7 Cutoff | | | 0.9 Cutoff | | |
|---|---|---|---|---|---|---|---|
| Location | Year | Anad | Res | Mix | Anad | Res | Mix |
| Omak Anadromous | 2004 | 0.99 | 0.00 | 0.01 | 0.98 | 0.00 | 0.02 |
| Omak Anadromous | 2005 | 1.00 | 0.00 | 0.00 | 0.97 | 0.00 | 0.03 |
| Omak Anadromous | 2006 | 1.00 | 0.00 | 0.00 | 0.99 | 0.00 | 0.01 |
| Omak Anadromous | 2007 | 1.00 | 0.00 | 0.00 | 0.97 | 0.00 | 0.03 |
| Omak Anadromous | 2008 | 0.96 | 0.00 | 0.04 | 0.88 | 0.00 | 0.12 |
| Omak Anadromous | 2009 | 1.00 | 0.00 | 0.00 | 0.92 | 0.00 | 0.08 |
| Omak above falls | 2005 | 0.52 | 0.38 | 0.10 | 0.52 | 0.33 | 0.14 |
| Omak above falls | 2006 | 0.00 | 0.87 | 0.13 | 0.00 | 0.87 | 0.13 |
| Omak above falls | 2009 | 0.24 | 0.72 | 0.04 | 0.24 | 0.64 | 0.12 |
| Omak above falls-Haley | 2008 | 0.06 | 0.90 | 0.04 | 0.06 | 0.82 | 0.12 |
| Omak above falls-Lobe | 2008 | 0.57 | 0.39 | 0.04 | 0.57 | 0.22 | 0.22 |
| Omak Below falls | 2005 | 0.57 | 0.30 | 0.13 | 0.53 | 0.26 | 0.21 |
| Omak Below falls | 2006 | 0.55 | 0.32 | 0.13 | 0.47 | 0.31 | 0.22 |
| Omak Below falls | 2007 | 0.63 | 0.18 | 0.18 | 0.60 | 0.14 | 0.26 |
| Omak Below falls | 2008 | 0.23 | 0.37 | 0.40 | 0.19 | 0.35 | 0.47 |
| Omak Screw trap | 2006 | 1.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 |
| Omak Screw trap | 2007 | 0.68 | 0.20 | 0.12 | 0.61 | 0.19 | 0.20 |
| Omak Screw trap | 2008 | 0.86 | 0.09 | 0.05 | 0.83 | 0.09 | 0.08 |
| Omak Screw trap | 2009 | 0.90 | 0.03 | 0.07 | 0.84 | 0.02 | 0.14 |
| Omak Screw trap | 2010 | 0.72 | 0.06 | 0.22 | 0.72 | 0.01 | 0.26 |
| Omak Fry | 2008 | 0.66 | 0.14 | 0.21 | 0.61 | 0.10 | 0.29 |
| Bonaparte Anadromous | 2006 | 1.00 | 0.00 | 0.00 | 0.91 | 0.00 | 0.09 |
| Bonaparte Anadromous | 2007 | 1.00 | 0.00 | 0.00 | 0.95 | 0.00 | 0.05 |
| Bonaparte Anadromous | 2008 | 1.00 | 0.00 | 0.00 | 0.93 | 0.00 | 0.07 |
| Bonaparte Anadromous | 2010 | 0.98 | 0.00 | 0.02 | 0.89 | 0.00 | 0.11 |
| Bonaparte Electrofish | 2010 | 0.95 | 0.00 | 0.05 | 0.89 | 0.00 | 0.11 |
| Salmon Creek | 2010 | 0.68 | 0.03 | 0.29 | 0.53 | 0.00 | 0.47 |

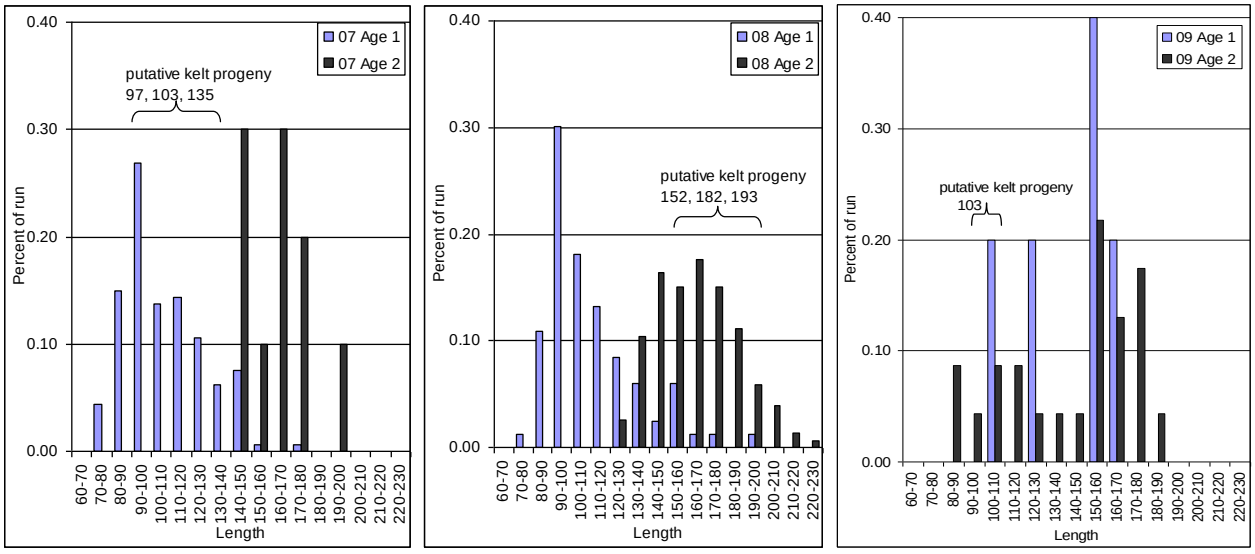
Table (6) shows the individual assignment back to reporting group for Bonaparte Creek and Salmon Creek samples. The majority of samples assigned to either the Upper Columbia reporting group, or did not assign. In Salmon Creek 10% assigned to the Big White Salmon, but this is consistent with resident assignment when there are no resident collections in the baseline.

**Table 6. Individual assignment. The number of fish sampled and ratio of fish either not assigned or assigned to one of seven reporting groups seen. Upper Columbia (Upper col) baseline samples included Omak samples, and is the expected assignment for all individuals.**

| | N | Not assigned | Upper Col | Big White Salmon | Yakima | Mid Col Lower Snake | Klickitat | Upper Salmon | Lower Salmon |
|---|---|---|---|---|---|---|---|---|---|
| Bonaparte Anad | 152 | 0.30 | 0.66 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.00 |
| Bonaparte Juve | 96 | 0.38 | 0.58 | 0.01 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 |
| Salmon Juve | 98 | 0.41 | 0.40 | 0.10 | 0.02 | 0.03 | 0.02 | 0.01 | 0.01 |

Figure (4) shows histogram data for age-1 and age-2 fish collected at the screwtrap in spring of 2007, 2008, and 2009. While there was length overlap between age classes, it occurred at a low frequency in 2007 and 2008. Three juveniles collected at the screwtrap in 2007 were progeny of the male first spawning in 2005 and again as a reconditioned kelt in 2006. At sizes of 97, 103 and 135 mm, these juveniles were consistent with the age-1 size class, therefore the result of the second (kelt) spawning event. In 2008 two more juveniles were assigned to the reconditioned male at age-2 sizes of 152 and 193. In 2008, an age-2 juvenile (182 mm) was also assigned to a female detected in 2008 at the pit tag antenna. This juvenile was previously not reported as parentage assignment had shown a female by female cross. However, subsequent analysis using a gender determining marker, showed the other parent to be a male.

**Figure 4. Length frequency histograms for fish sampled in 2007, 2008, and 2009. Percent of run for each length class is reported for known age juveniles(via parentage) captured in the screwtrap. Range of putative kelt progeny is displayed to demonstrate relationship with known age fish.**



At least one parent was assigned to 26 of the 28 samples collected as emergent fry. Of these, one was assigned to a female reconditioned kelt. After exhibiting spawning behavior below the weir, this fish was captured and released above the weir. It was captured again moving downstream and taken to the hatchery for reconditioning. Four additional progeny from this female were detected in samples collected at the screwtrap in 2009, however, assignment to the kelt spawning event was possible for only one using cross data and male return year. The minimal length data in 2009 (age-1 n=5, age-2 n=23), failed to show distinct age classes (Figure 3), precluding further age assignments.

No emergent fry were assigned to the second female that was captured after observation below the weir. The remaining samples assigned to a first time female (n=20) or to only the male parent (n=5). Fourteen of the twenty fish assigned to a first time female were assigned to a stray female with Chewuch Hatchery PIT tag records.

Table (7) shows a summary of reproductive success attributed to fish that went through the reconditioning process and returned to spawn again. Successful reproduction has been confirmed for three of the four reconditioned kelts that were detected returning to Omak Creek.

**Table 7. Summary of reproductive success. Each detection of reproductive success reported below**

| Progeny | Stage | Length | Sample year | Brood Year | Kelt ID | Kelt Gender |
|---|---|---|---|---|---|---|
| OMRST-216 | Smolt | 103 | 2007 | 2006 | OCKELT-2 | Male |
| OMRST-171 | Smolt | 97 | 2007 | 2006 | OCKELT-2 | Male |
| OMRST-575 | Smolt | 135 | 2007 | 2006 | OCKELT-2 | Male |
| OMRST263 | Smolt | 152 | 2008 | 2006 | OCKELT-2 | Male |
| OMRST109 | Smolt | 193 | 2008 | 2006 | OCKELT-2 | Male |
| OMRST75 | Smolt | 182 | 2008 | 2006 | OCKELT-1 | Female |
| Redd-A1 | Fry | | 2008 | 2008 | OMCT5 | Female |
| OMRST-45 | Smolt | 163 | 2009 | 2008 | OMCT5 | Female |

Of the 96 Bonaparte Creek juveniles genotyped from 2010, 74 were less than 100 mm and considered to be of age-0 representing progeny from the 55 Bonaparte Creek adults genotyped in 2010. Of these 74, 48 assigned back to at least one parent and 16 assigned back to two parents. None of the 98 Salmon Creek juveniles genotyped from 2010 assigned to a parent. This was expected as no adults were sampled from Salmon Creek.

**DISCUSSION**

Departures from Hardy-Weinberg equilibrium and linkage disequilibrium were common in Omak Creek collections. In juvenile collections this is easily explained by the presence of both the anadromous and resident component of *O. mykiss*. While no reference collection of adult residents is available, results from population assignment tests support the presence of multiple populations, with the majority of samples upstream of Mission Falls being assigned to the putative resident collection.

In anadromous adult collections, departures from Hardy-Weinberg equilibrium, and evidence of linkage disequilibrium is also seen. This can be partially explained by the recent re-introduction of steelhead into Omak Creek, and high rates of straying into Omak Creek as evidenced by PIT tag detections. Anadromous stocks in Omak Creek were almost non-existent since the early 1900's. Large scale habitat improvements and barrier removals now allow access to Omak Creek, with only a partial barrier at Mission Falls. Still, the majority of fish that have returned to Omak Creek are probably mixtures of hatchery origin adults. In 2003 it was estimated that two thirds of the fish were of hatchery origin (Fisher and Arterburn 2004), and in 2005 only five individuals (112 fish trapped) were observed to have an intact adipose fin (Arterburn et al. 2005). Additionally the system as a whole has seen major disturbances in the form of fish kills following fire retardant drops in both 2001 and 2003. These disturbances may have led to recent interbreeding between the anadromous and resident forms after disruption of natural breeding systems. The pattern of intermediate assignment values commonly seen in samples collected below Mission Falls would be consistent with gene flow or interbreeding.

Population self-assignment rates using the program STRUCTURE were high for anadromous adults with over 99% of samples assigning to the anadromous group. Although resident samples were only collected as unknowns, the high consistency with which anadromous adults were assigned to a single group supports divergence of the anadromous and resident populations. As Mission Falls is a potential barrier to upstream migration of anadromous adults, fish collected above the falls are more likely to be derived from the resident component. Prior to 2005 when 12 redds were detected above the falls, redds were recorded in only one year (Arterburn et al. 2005). While both parentage and population assignments show anadromous juveniles above the falls, this may be the result of hatchery stocking. Stocking in Stapaloop Creek, a tributary to Omak Creek, was done as early as 1999 (Fisher and Arterburn 2004) and more recently in 2003- 2006, 2008 and 2009.

GSI assignments identified the Upper Columbia as the origin of the majority of fish. The majority of those not assigned to the Upper Columbia were not assigned to any population. While 10% of the Salmon Creek juveniles were assigned to the Big White Salmon, it is likely that this is due to the influence of the resident population which is not represented in the baseline. Alternatively, these results may be attributed to out of basin straying or genetic similarity of these stocks. Within Bonaparte Creek, an adult that was assigned to the Big White Salmon reporting group had four offspring identified by parentage. Of these four, one assigned to the Big White Salmon reporting group, and three assigned to the Yakima reporting group. It is still unclear if this resulted from an out of basin stray, or from a hybridization event between the resident and anadromous collections.

Two primary clusters are seen in the neighbor joining dendrogram, separating anadromous adults from juvenile collections that likely include residents. Consistent with STRUCTURE results, samples from above Mission Falls are especially distinct. Samples collected at the screwtrap which may represent progeny of both the anadromous adults and adfluvial residents, are more intermediate. While there may be additional sub-structure within each drainage, hybridization between anadromous and resident fish, along with high stray rates may confound the ability to detect sub-structure.

Tests for parentage identification were performed for both Bonaparte and Salmon creeks. Salmon Creek did not have any successful parentage assignments, which was expected as no adults from Salmon Creek were genotyped. Parentage assignment for Bonaparte Creek was successful for 55 of the 76 fish identified as Age-0 fish. As reconditioning may be expanded to include Bonaparte Creek, it is informative to know that a high percentage of juveniles sampled in Bonaparte Creek were assigned back to at least one parent.

Reproduction by reconditioned kelts has now been confirmed for three individuals. The male reconditioned kelt that passed above the Omak Creek picket weir in 2006 successfully spawned with progeny detected as both age-1 in 2007 and age-2 in 2008. One of the females returning in 2006 was also shown to reproduce with the detection of an age-2 progeny in 2008. Progeny from the female observed digging below the weir in 2008 were detected as an age-0 emergent fry in 2008 and age-1 in 2009.

Determination of kelt reproductive success is dependant upon separation of first and second time spawning events. During the 2007 and 2008 sampling years length histograms were used to identify brood year. Length histograms in 2009 did not provide a clear relationship between size and age, and the wide ranging sizes of age-2 fish precluded age assignment by length. This unclear relationship may continue in future years, so other options should be considered to determine age of juvenile samples. Three potential options are sampling at age-0, full parental sampling, and scale analysis. Full parental sampling has been unattainable so far, and scale analysis has not been shown as accurate at aging juvenile steelhead. Therefore, sampling of age-0 may be the only realistic option. Age-0 fish would have to be sampled in the fall after they reach an adequate size to target using electrofishing methods.

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# Section C: Yakima River Steelhead Kelt Genetic Analysis

## Introduction

The reproductive success of post reconditioned kelts has not yet been measured in the Yakima River. We genotyped populations of *Oncorhynchus mykiss* within the Yakima River to expand on previous studies (Campton and Johnston 1985, Busack et al. 2006) in an attempt to identify sites favorable to kelt reproductive success studies. Tributaries to the Yakima River that produce a proportionally larger number of kelts will be targeted as sites for reproductive success studies. Parentage analysis of juveniles within these streams will be conducted to determine its feasibility.

## Methods

Anadromous adults were sampled as upstream migrants at Prosser Dam or as kelts migrating downstream at the Chandler Juvenile Evaluation Facility. Adult collections were analyzed separately by year of collection, collection time (fall vs. spring) and collection type (putative first time spawners at Prosser Dam or kelts at Chandler trap. Juveniles were collected with screw traps during the spring and electrofishing techniques during the fall. Juvenile samples were collected for five primary tributaries of the Yakima River: Ahtanum Creek, Teanaway Creek, Toppenish Creek, Satus Creek and Naches River. Within the Naches River, additional samples were collected at North Fork Little Naches River, Nile Creek, Pileup Creek, Little Rattlesnake Creek, and Quartz Creek. All primary tributary collections except Teanaway Creek were conducted for at least a two year time period.

Samples were collected and stored in ethanol for preservation of DNA. Genetic analysis was conducted at the Hagerman Fish Culture Experiment Station in Hagerman, ID. DNA was extracted from tissue samples using standard manufacturer's protocols from Qiagen® DNeasy™ extraction kit. The polymerase chain reaction (PCR) was used to amplify 16 microsatellite loci including 13 standardized markers (Stephenson et al. 2008) and 3 others: Omm 1036 (GenBank Accession #AF346686), Omm 1046 (GenBank Accession #AF346693), and One 102 (Olsen et al. 2000). PCR products were genotyped using manufacturer's protocols with an Applied Biosystems® model 3730 genetic analyzer and scored using Genemapper v3.7 Software.

Ninety six SNP (single nucleotide polymorphism) loci were genotyped for the 2008, 2009 and 2010 efforts. Table 1 shows the 71 SNPs that were genotyped during all three years. SNPs not genotyped over all years are not included in this report. Genotypes were collected using a Fluidigm EP1 instrument in conjunction with Fluidigm SNP Genotyping Analysis software.

Samples from Toppenish and Satus creeks in 2010 were genotyped using the same 16 microsatellites, but a different set of SNPs. A panel of 192 SNPs was used instead with the intent of adding these to the Columbia River Basin genetic baseline used for Genetic Stock Identification. Further analysis of the SNP data generated for these samples will be done as part of the 2011 baseline expansion effort. Sample numbers are included here, and the microsatellite data for these samples were used as part of the test parentage study. Descriptive statistics, however, are limited as they are not directly comparable to the effort of previous years because of the use of different loci.

**Table 1. SNP loci used over all years.**

| Marker Name | Reference | Marker Name | Reference |
|---|---|---|---|
| Omy_113490-159 | Abadia-Cardoso et al. 2011 | Omy_gh-334 | Campbell et al. 2009 |
| Omy_114315-438 | Abadia-Cardoso et al. 2011 | Omy_gh-475 | Campbell et al. 2009 |
| Omy_121006-131 | Abadia-Cardoso et al. 2011 | Omy_hsc715-80 | Campbell & Narum 2009b |
| Omy_121713-115 | Abadia-Cardoso et al. 2011 | Omy_hsf1b-241 | Campbell & Narum 2009b |
| Omy_123044-128 | Abadia-Cardoso et al. 2011 | Omy_hsp47-86 | Campbell & Narum 2009b |
| Omy_123048-119 | Abadia-Cardoso et al. 2011 | Omy_hsp70aPro-329 | Campbell & Narum 2009b |
| Omy_128693-455 | Abadia-Cardoso et al. 2011 | Omy_hsp90BA-193 | Campbell & Narum 2009b |
| Omy_130295-98 | Abadia-Cardoso et al. 2011 | Omy_IL17-185 | Unpublished J. DeKoning |
| Omy_130524-160 | Abadia-Cardoso et al. 2011 | Omy_IL1b-163 | Unpublished J. DeKoning |
| Omy_187760-385 | Abadia-Cardoso et al. 2011 | Omy_IL6-320 | Unpublished J. DeKoning |
| Omy_95489-239 | Abadia-Cardoso et al. 2011 | Omy_inos-97 | Unpublished J. DeKoning |
| Omy_96222-125 | Abadia-Cardoso et al. 2011 | Omy_LDHB-1_i2 | Aguilar & Garza 2008 |
| Omy_97077-73 | Abadia-Cardoso et al. 2011 | Omy_LDHB-2_e5 | Aguilar & Garza 2008 |
| Omy_97660-230 | Abadia-Cardoso et al. 2011 | Omy_LDHB-2_i6 | Aguilar & Garza 2008 |
| Omy_97865-196 | Abadia-Cardoso et al. 2011 | Omy_mapK3-103 | Unpublished N. Campbell |
| Omy_97954-618 | Abadia-Cardoso et al. 2011 | Omy_mcsf-268 | Unpublished J. DeKoning |
| Omy_aldB-165 | Campbell et al. 2009 | Omy_mcsf-371 | Unpublished J. DeKoning |
| Omy_ALDOA_1 | Aguilar and Garza 2008 | Omy_myclarp404-111 | Unpublished N. Campbell |
| Omy_aromat-280 | Unpublished J. DeKoning | Omy_myoD-178 | Campbell et al. 2009 |
| Omy_arp-630 | Campbell et al. 2009 | Omy_NaKATPa3-50 | Campbell et al. 2009 |
| Omy_aspAT-123 | Campbell et al. 2009 | Omy_nkef-241 | Campbell et al. 2009 |
| Omy_aspAT-413 | Campbell et al. 2009 | Omy_nramp-146 | Campbell et al. 2009 |
| Omy_b1-266 | Sprowles et al. 2006 | Omy_Omyclmk436-96 | Unpublished N. Campbell |
| Omy_b9-164 | Sprowles et al. 2006 | Omy_Ots249-227 | Campbell et al. 2009 |
| Omy_BAC-B4-324 | Unpublished S. Young | Omy_oxct-85 | Unpublished J. DeKoning |
| Omy_cd28-130 | Unpublished J. DeKoning | Omy_PEPA-i6 | Aguilar & Garza 2008 |
| Omy_cd59-206 | Unpublished J. DeKoning | Omy_rapd-167 | Sprowles et al. 2006 |
| Omy_cd59b-112 | Unpublished J. DeKoning | Omy_SEXY1 | |
| Omy_colla1-525 | Unpublished J. DeKoning | Omy_sSOD-1 | Brunelli et al. 2008 |
| Omy_cox1-221 | Campbell et al. 2009 | Omy_star-206 | Unpublished J. DeKoning |
| Omy_cox2-335 | Unpublished J. DeKoning | Omy_stat3-273 | Unpublished J. DeKoning |
| Omy_cxcr-169 | Unpublished J. DeKoning | Omy_tgfb-207 | Unpublished J. DeKoning |
| Omy_e1-147 | Sprowles et al. 2006 | Omy_tlr3-377 | Unpublished J. DeKoning |
| Omy_g1-103 | Sprowles et al. 2006 | Omy_tlr5-205 | Unpublished J. DeKoning |
| Omy_g12-82 | Unpublished J. DeKoning | Omy_u07-79-166 | Unpublished S. Young |
| Omy_gdh-271 | Campbell et al. 2009 | | |

Prior to any statistical analysis, two loci used for detection of cutthroat trout hybrids (Omy_myclarp404-111, Omy_Omyclmk436-96), and one developed for sex determination (Omy_SEXY1) were dropped. The remaining loci were tested for linkage disequilibrium using exact tests (Haldane 1954, Weir 1990, Guo and Thompson 1992) implemented in GENEPOP v3.4 (Raymond and Rousset 1995) as part of the 2009 effort (Branstetter et al. 2010). Because loci out of equilibrium were discontinued, and not ran as part of the 2010 effort, results are not reported here.

Of the 3264, initial genotyped samples in the study, 311were removed due to duplicate genotypes, missing data, or hybridization with cutthroat trout. Samples removed by category include the following: duplicate samples (n=4), samples with greater than four incomplete genotypes for 16 of the microsatellites or 10 incomplete genotypes for 96 of the SNPs (n=264), and samples with evidence of cutthroat hybridization (n=43). Data for these fish are not included in the statistical analysis. Fish sampled at both Prosser Dam and again at the Chandler facility were included in the analysis for each collection. This left 2,953 remaining samples for further statistical analyses.

Tributary locations with multiple collections were tested for population differentiation (Weir and Cockerham 1984) using GENEPOP. Collections were pooled when there was no evidence for genetically distinct separation. Otherwise separate collections were treated and reported independently.

In order to evaluate genetic diversity, expected and observed heterozygosity were calculated using Excel Microsatellite Toolkit (Park 2001). Number of alleles, allelic richness and private allelic richness for the 16 microsatellites were calculated using HP-Rare (Kalinowski 2005). For rarefaction estimates, gene number was set at 21, the lowest number of samples in any collection. With this dataset of reduced loci and sample size, deviation from Hardy-Weinberg equilibrium was evaluated using exact tests (Haldane 1954, Weir 1990, Guo and Thompson 1992) implemented in GENEPOP v3.4 (Raymond and Rousset 1995). The number of loci showing heterozygote excess or deficiency was also quantified (Rousset and Raymond 1995). Corrections for multiple tests were not made as the large number of comparisons made corrections difficult to apply. Results are, however, reported as both the number and proportion of significant findings to help demonstrate true significance.

To help infer population structure, the program STRUCTURE v.2.0 (Pritchard et al. 2000, Falush et al. 2003) was completed as part of the 2009 effort. Aside from the known adult anadromous steelhead, collections may include mixed collections of the anadromous steelhead juveniles and resident populations. The number of potential distinct populations (K) was evaluated from a range of 1-10, with four iterations each.

To demonstrate inter-population relationships, pairwise genetic distances (Cavalli-Sforza and Edwards 1967) were calculated between all sites using POPULATIONS software (Langella 2001). Genetic chord distances with 1000 iterations of bootstrap replicates were used to construct a neighbor joining tree. The program TREEVIEW (Page 1996) was then used to display the tree.

Parentage assignments using CERVUS v 3.0 (Marshall et al. 1998, Kalinowski et al. 2007) was performed on samples collected in 2010 at Satus and Toppenish creeks. Because length and age relationships are not currently available, no attempt was made to differentiate between the first and post reconditioning event. Results were interpreted only as a feasibility study.

To determine stock proportions of unknown fish, genetic mixture analysis and individual assignment tests were performed using methods reported in Anderson et al. (2008) as implemented in the software program ONCOR. To test the performance of the baseline samples for accurate stock assignment, known individuals and collections were re-sampled from the baseline, treated as unknowns, and assigned to stocks. Results are reported for both 100% proportion simulations and individual assignment success. After estimates of baseline accuracy were determined, true unknown samples from mixed stock collections at Prosser Dam and the Chandler Trap were then assigned to baseline stocks.

Mixed collections were stratified by additional factors when high sample numbers allowed. Kelts captured at the Chandler Trap were evenly split into early and late groups for both 2009 and 2010. Further division was done in

2010 with seven temporal groups. These groups were formed by sequentially selecting sampling days until the cumulative number of fish in each group was between 77 and 101. Groups were then labeled with the average day of sampling across all fish within each group.

In addition to temporal stratification, mixed collections are also analyzed by gender and status. Status was determined by hatchery records. Status is reported based upon three groups (Mortality, Long Term, and PIT detect). The “mortality” group is comprised of fish arriving as mortalities or suffering mortality early in the reconditioning process. The “long term” group consisted of all fish that survived reconditioning until release in the fall. Samples that both survived reconditioning and were detected by a PIT tag reader following release were also included in the “PIT detect” group.

## Results

Statistical analysis was performed on 2,094 samples. Basic population statistics are reported in Table 2 The number of samples per population ranged from 21 to 305, with a minimum of 81 samples for each of the five primary tributaries (Satus Creek, Teanaway Creek, Toppenish Creek, Ahtanum Creek, Naches River). Mixture collections (Prosser Dam upstream adults, Chandler facility kelts) had the highest levels of diversity with average number of alleles ranging from 14.13 to 18.06 and allelic richness ranging from 7.70 to 8.16. Within the tributaries, the highest average number of alleles was 14.5 as seen in the Naches River. The highest allelic richness in the tributaries was 8.08 as seen in Ahtanum 06.

**Table 2. Population Statistics. Each collection is reported in terms of sample size (N), number of microsatellite alleles ($A_{msat}$), microsatellite allelic richness ($AR_{msat}$), expected heterozygosity ($H_E$), and observed heterozygosity ($H_O$). Expected and observed heterozygosity are reported as combined estimates of both microsatellite and SNP markers. A dashed line separates the tributary collections from the mixture collections.**

| Collection | N | $A_{msat}$ | $AR_{msat}$ | $H_{E\text{-}msat}$ | $H_{O\text{-}msat}$ |
|---|---|---|---|---|---|
| Ahtanum 2001 | 76 | 12.8 | 7.3 | 0.829 | 0.820 |
| Ahtanum 2006 | 82 | 13.9 | 7.5 | 0.830 | 0.796 |
| Ahtanum 07-10 | 26 | 11.1 | 7.5 | 0.835 | 0.827 |
| LR Snake 05, 08 | 46 | 11.3 | 7.0 | 0.813 | 0.821 |
| Naches 04, 06 | 135 | 14.4 | 7.2 | 0.807 | 0.801 |
| NFL Naches 08 | 21 | 9.3 | 6.9 | 0.785 | 0.780 |
| Nile 05, 08 | 58 | 11.6 | 7.0 | 0.814 | 0.823 |
| Pilup 05, 08 | 17 | 8.5 | 6.5 | 0.771 | 0.827 |
| Quartz 08, 08 | 26 | 9.5 | 6.8 | 0.798 | 0.790 |
| Satus 06-09 | 201 | 13.9 | 6.7 | 0.796 | 0.797 |
| Satus 2010* | 28 | 9.8 | 6.4 | 0.791 | 0.782 |
| Teanaway 05 | 79 | 12.4 | 7.1 | 0.789 | 0.778 |
| Toppenish 01-09 | 229 | 13.4 | 6.1 | 0.752 | 0.748 |
| Toppenish 09 fall* | 48 | 10.3 | 6.2 | 0.753 | 0.741 |
| Toppenish 09 fall | 87 | 9.5 | 5.5 | 0.723 | 0.733 |
| Toppenish 10* | 37 | 9.9 | 6.0 | 0.735 | 0.729 |
| Prosser 2007 | 158 | 16.3 | 7.6 | 0.822 | 0.794 |
| Prosser 2008 | 81 | 13.8 | 7.1 | 0.804 | 0.788 |
| Prosser 2009 | 86 | 14.4 | 7.3 | 0.811 | 0.786 |
| Prosser 2010 | 158 | 16.4 | 7.4 | 0.814 | 0.792 |
| Chandler 2006 | 89 | 14.1 | 7.2 | 0.808 | 0.806 |
| Chandler 2008 | 139 | 15.7 | 7.4 | 0.818 | 0.805 |
| Chandler 2008** | 161 | 16.8 | 7.4 | 0.820 | 0.800 |
| Chandler 2009 | 173 | 15.7 | 7.1 | 0.803 | 0.761 |
| Chandler 2009** | 104 | 14.8 | 7.2 | 0.806 | 0.798 |
| Chandler 2010 | 608 | 18.4 | 7.3 | 0.807 | 0.784 |

*** Genotyped for a set of 192 SNP loci**

**** Genotyped for Microsatellites only.**

Results for Hardy-Weinberg equilibrium are reported in Table 3. Results are reported for each collection as both the number and proportion of loci with p values less than 0.05. Mixture collections with individuals from multiple populations were expected to have heterozygote deficits due to Wahlund effect, and a high proportion of heterozygote deficits were observed in mixture samples from Chandler (up to 20% in 2009) and Prosser (15-24%). Deviations from Hardy-Weinberg equilibrium were also found in tributary samples as high as 15% (Toppenish 06-09). Of the 18 collections, 16 had higher incidences of heterozygote deficits than heterozygote excess. Only Pileup Creek had a lower number of deficits (2) than excesses (n=3). While up to 5% of comparisons are expected to be significant due to random chance, many tributary samples had higher than 5% deviations and may indicate Wahlund effects.

**Table 3. Hardy-Weinberg equilibrium. Each collection is reported in terms of the number of comparison (comp), the number and proportion of loci showing departures from Hardy-Weinberg (H-W), and the number and proportion showing evidence of heterozygote deficit (Deficit) or excess (Excess).**

| Collection | Comps | H-W | | Excess | | Deficit 1.1 | |
|---|---|---|---|---|---|---|---|
| Ahtanum 2001 | 82 | 9 | 0.11 | 2 | 0.11 | 7 | 0.024 |
| Ahtanum 2006 | 83 | 13 | 0.157 | 2 | 0.157 | 10 | 0.024 |
| Ahtanum 07-10 | 78 | 4 | 0.051 | 1 | 0.051 | 4 | 0.013 |
| LR Snake 05, 08 | 78 | 5 | 0.064 | 1 | 0.064 | 7 | 0.013 |
| Naches 04, 06 | 81 | 3 | 0.037 | 0 | 0.037 | 6 | 0 |
| NFL Naches 08 | 59 | 2 | 0.034 | 1 | 0.034 | 2 | 0.017 |
| Nile 05, 08 | 78 | 9 | 0.115 | 2 | 0.115 | 4 | 0.026 |
| Pilup 05, 08 | 60 | 3 | 0.05 | 3 | 0.05 | 0 | 0.05 |
| Quartz 08, 08 | 70 | 5 | 0.071 | 0 | 0.071 | 2 | 0 |
| Satus 06-09 | 83 | 9 | 0.108 | 3 | 0.108 | 8 | 0.036 |
| Satus 2010 | 56 | 4 | 0.071 | 0 | 0.071 | 4 | 0 |
| Teanaway 05 | 82 | 5 | 0.061 | 2 | 0.061 | 5 | 0.024 |
| Toppenish 01-09 | 82 | 12 | 0.146 | 2 | 0.146 | 8 | 0.024 |
| Toppenish 09 fall | 59 | 4 | 0.068 | 2 | 0.068 | 2 | 0.034 |
| Toppenish 09 fall | 70 | 10 | 0.143 | 3 | 0.143 | 6 | 0.043 |
| Toppenish 10 | 57 | 0 | 0 | 2 | 0 | 2 | 0.035 |
| Prosser 2007 | 84 | 14 | 0.167 | 1 | 0.167 | 16 | 0.012 |
| Prosser 2008 | 83 | 9 | 0.108 | 1 | 0.108 | 12 | 0.012 |
| Prosser 2009 | 82 | 9 | 0.11 | 2 | 0.11 | 11 | 0.024 |
| Prosser 2010 | 84 | 13 | 0.155 | 0 | 0.155 | 16 | 0 |
| Chandler 2006 | 82 | 5 | 0.061 | 1 | 0.061 | 6 | 0.012 |
| Chandler 2008 | 84 | 12 | 0.143 | 2 | 0.143 | 14 | 0.024 |
| Chandler 2008 | 16 | 5 | 0.313 | 0 | 0.313 | 4 | 0 |
| Chandler 2009 | 84 | 18 | 0.214 | 1 | 0.214 | 14 | 0.012 |
| Chandler 2009 | 16 | 2 | 0.125 | 0 | 0.125 | 2 | 0 |
| Chandler 2010 | 84 | 15 | 0.179 | 1 | 0.179 | 16 | 0.012 |
| Average | | 7.7 | | 1.3 | | 7.2 | |
| Sum | | 184 | | 34 | | 172 | |

Pairwise Fst values are shown in Table 4. Number of loci with p≤ 0.05 for each pairwise comparison are shown in Table 5. At p=0.05 it is expected that 4.45 of 89 loci will be counted as significant by chance alone (0.05 * 89 = 4.45). The majority of pairwise comparisons have greater than 4.45 loci out of equilibrium demonstrating statistically significant population differentiation. The average Fst and number of loci with p≤ 0.05 is 0.017 and 26.9 respectively. Measurements between the five primary tributaries had an average Fst of 0.022 with 45.8 loci. Within Naches these values are lower at Fst=0.016 and 14.5 loci. The lowest values are seen when comparing all adult collections with average Fst of 0.003 and 11.0 loci.

**Table 4. Pairwise Fst values between populations. Fst values greater than 0.020 are highlighted. Comparisons within Naches River tributaries and Ahtanum Creek collection years are shown as bordered blocks.**

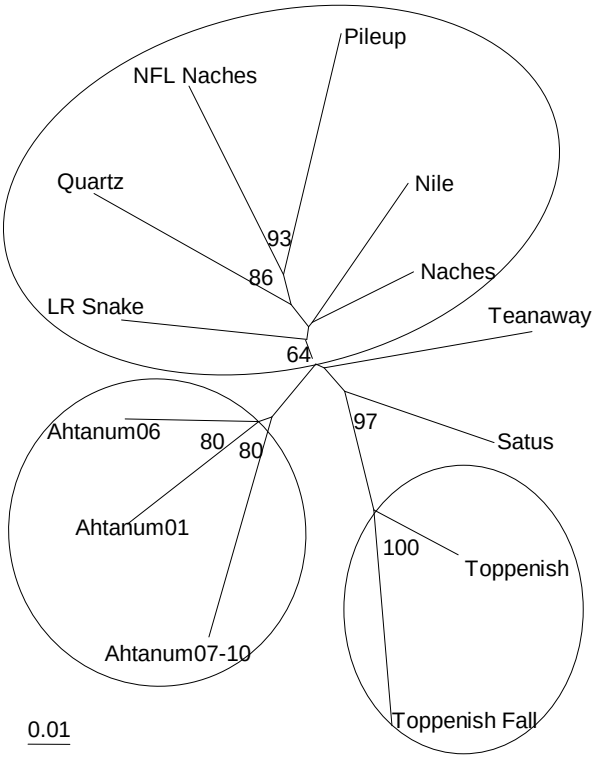
| | Satus 06-09 | Teanaway 05 | Toppenish 06-09 | Toppenish 09Fall | Ahtanum 01 | Ahtanum 06 | Ahtanum 07-10 | LR Snake 05,08 | Naches 04, 06 | NFL Naches 08 | Nile 05,08 | Pileup |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Teanaway 05 | 0.021 | | | | | | | | | | | |
| Toppenish 06-09 | 0.027 | 0.038 | | | | | | | | | | |
| Toppenish 09Fall | 0.051 | 0.062 | 0.017 | | | | | | | | | |
| Ahtanum 01 | 0.028 | 0.025 | 0.041 | 0.064 | | | | | | | | |
| Ahtanum 06 | 0.025 | 0.017 | 0.038 | 0.063 | 0.006 | | | | | | | |
| Ahtanum 07-10 | 0.032 | 0.026 | 0.044 | 0.069 | 0.012 | 0.008 | | | | | | |
| LR Snake 05,08 | 0.020 | 0.020 | 0.036 | 0.064 | 0.026 | 0.018 | 0.023 | | | | | |
| Naches 04, 06 | 0.017 | 0.016 | 0.032 | 0.053 | 0.018 | 0.014 | 0.021 | 0.008 | | | | |
| NFL Naches 08 | 0.016 | 0.019 | 0.036 | 0.058 | 0.033 | 0.030 | 0.037 | 0.014 | 0.006 | | | |
| Nile 05,08 | 0.024 | 0.024 | 0.035 | 0.053 | 0.018 | 0.013 | 0.024 | 0.013 | 0.007 | 0.016 | | |
| Pileup | 0.034 | 0.036 | 0.052 | 0.078 | 0.035 | 0.036 | 0.041 | 0.025 | 0.014 | 0.016 | 0.027 | |
| Quartz 05,08 | 0.029 | 0.026 | 0.042 | 0.066 | 0.026 | 0.023 | 0.032 | 0.019 | 0.011 | 0.016 | 0.015 | 0.025 |

**Table 5. Number of loci with P<0.05. For each population pairwise comparison, the number of loci showing evidence of population differentiation at P<0.05 is shown. Numbers greater than 25 are highlighted. Comparisons within Naches River tributaries and Ahtanum Creek collection years are shown as bordered blocks.**

| | Satus 06-09 | Teanaway 05 | Toppenish 06-09 | Toppenish 09Fall | Ahtanum 01 | Ahtanum 06 | Ahtanum 07-10 | LR Snake 05,08 | Naches 04, 06 | NFL Naches 08 | Nile 05,08 | Pileup |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Teanaway 05 | 51 | | | | | | | | | | | |
| Toppenish 06-09 | 56 | 51 | | | | | | | | | | |
| Toppenish 09Fall | 51 | 54 | 27 | | | | | | | | | |
| Ahtanum 01 | 56 | 47 | 65 | 64 | | | | | | | | |
| Ahtanum 06 | 59 | 41 | 59 | 62 | 20 | | | | | | | |
| Ahtanum 07-10 | 44 | 33 | 45 | 48 | 20 | 20 | | | | | | |
| LR Snake 05,08 | 44 | 37 | 45 | 47 | 38 | 35 | 29 | | | | | |
| Naches 04, 06 | 54 | 44 | 57 | 58 | 49 | 42 | 30 | 24 | | | | |
| NFL Naches 08 | 23 | 26 | 27 | 35 | 36 | 32 | 29 | 14 | 10 | | | |
| Nile 05,08 | 46 | 44 | 47 | 50 | 37 | 34 | 32 | 23 | 20 | 13 | | |
| Pileup | 34 | 29 | 38 | 40 | 33 | 31 | 28 | 20 | 11 | 7 | 21 | |
| Quartz 05,08 | 34 | 33 | 41 | 45 | 30 | 29 | 30 | 19 | 17 | 12 | 18 | 14 |

The relationship of tributary collections is shown in the neighbor joining dendrogram in Figure 1. All Naches River tributaries were separated into a single group with a bootstrap value of 64. Further clustering in the Naches River is seen in Quartz Creek, Pileup Creek and North Fork Little Naches River. All Ahtanum Creek collections clustered with a bootstrap value of 80. Both Toppenish Creek collections grouped together with a bootstrap value of 80. Additionally, Satus Creek and Toppenish Creek collections grouped together with a bootstrap value of 97.

**Figure 1. Neighbor joining dendrogram of Cavalli-Sforza Edwards genetic distance among studied populations. Numbers at nodes represent bootstrap percentage from 1000 replicates (only those greater than 50 percent shown).**




At least one parent was assigned to 7 of the 28 (0.25) Satus Creek juveniles, and 7 of 37 (0.19) of the Toppenish Creek juveniles. Three of the Satus Creek juveniles assigned to both a male and female, otherwise all single assignments were to a female fish.

The ln probablitiy for number of populations (K) within each collection is shown in Figures 2a, and 2b. Only Satus Creek shows evidence of a single population. Collections from Ahtanum Creek, Naches River, Teanaway Creek and Toppenish Creek all show peak or leveling values when K is set at 4 or greater. Similarly, when all tributaries are ran together, the peak values do not begin to level off until K of 5 to 6 is reached. This contrasts adults from either Prosser Dam or Chandler Trap that have peaks that begin to level off around K of 2 to 3. This lower estimation of the number of populations present may indicate

that not all populations represented in the juvenile collections are collected as adult anadromous steelhead.

**Figure 2a. Ln probability of data for tributary collections. For each value of K from 1-10, the average of four iterations of ln probability values is graphed. Standard deviation is graphed for each K value.**

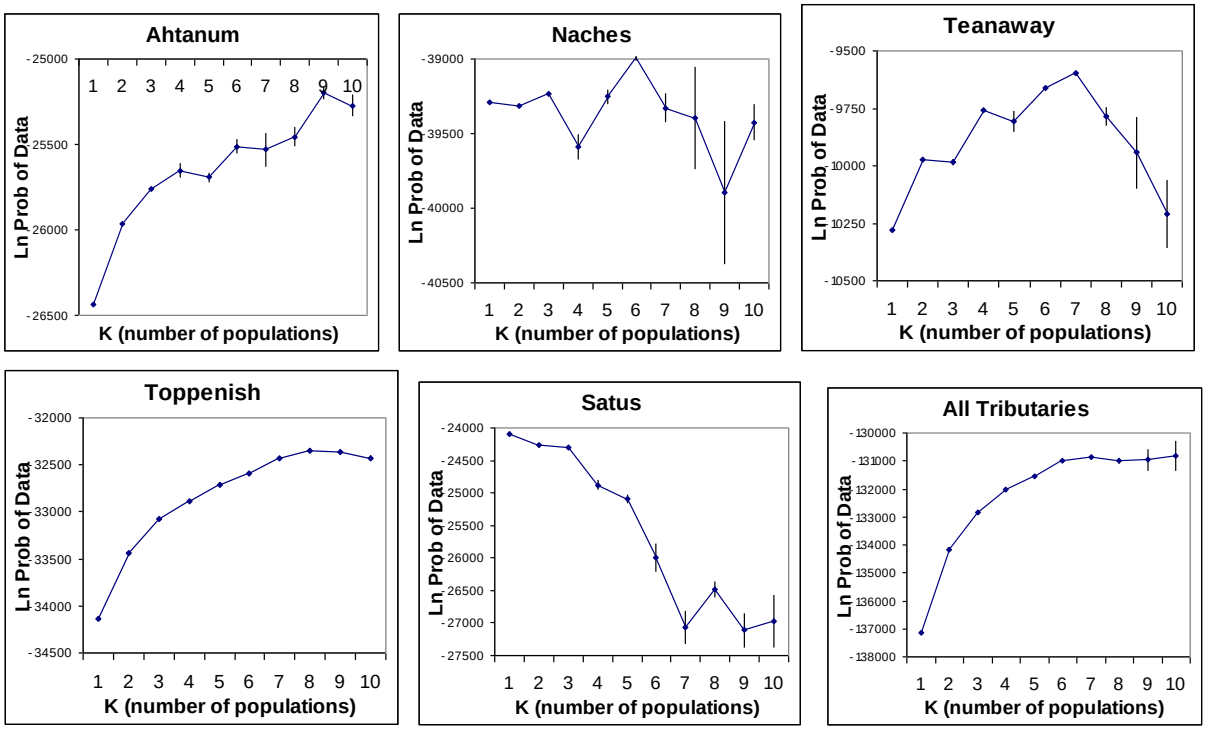
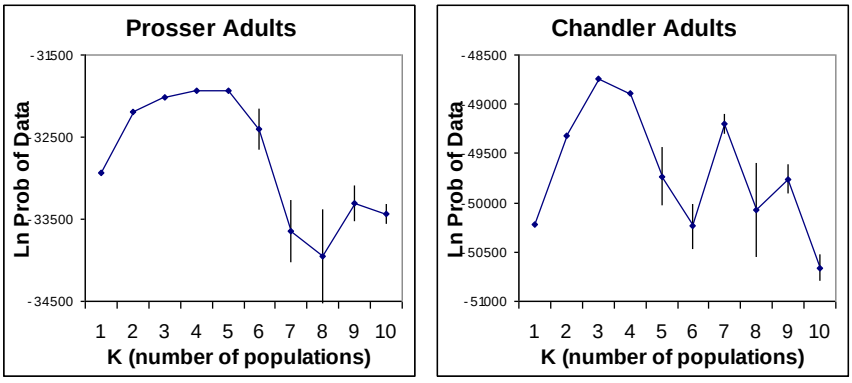


**Figure 2b. Ln probability of data for mixed adult collections. For each value of K from 1-10, the average of four iterations of ln probability values is graphed. Standard deviation is graphed for each K value.**




Results for 100% simulations are reported in Table 6 for both the population of origin, and the assigned reporting group. While assignment results to the population of origin varied from 0.2144 to 0.9966, assignment to reporting groups (major tributaries) were consistently high, ranging from 0.9747 in Ahtanum Creek (2006) to 0.9999 in Toppenish Creek (2009 fall).

**Table 6. 100% simulations to both the population of origin and reporting groups. Populations included in reporting group assignments are shown as bordered blocks.**

| | Population Group | Reporting Group |
|---|---|---|
| Satus 06-09 | 0.9944 | 0.9944 |
| Teanaway 05 | 0.9885 | 0.9885 |
| Toppenish 06-09 | 0.9966 | 0.9985 |
| Toppenish 09Fall | 0.9871 | 0.9999 |
| Ahtanum 01 | 0.9154 | 0.9954 |
| Ahtanum 06 | 0.9103 | 0.9747 |
| Ahtanum 07-10 | 0.4872 | 0.9938 |
| LR Snake 05,08 | 0.6609 | 0.9881 |
| Naches 04, 06 | 0.9455 | 0.9893 |
| NFL Naches 08 | 0.2144 | 0.9777 |
| Nile 05,08 | 0.7583 | 0.9914 |
| Pileup | 0.3321 | 0.9992 |
| Quartz 05,08 | 0.4293 | 0.9989 |
| Average | 0.7400 | 0.9915 |

Individual self-assignments to reference populations are shown in Table 7. Corresponding assignments to reporting groups are in Table 8. Assignment rates back to population of origin averaged only 60.1%, but increased to 90.3% for assignment to reporting groups.

**Table 7. Individual assignments to reference populations. The number of fish assigning to each baseline collection is listed. Each row lists where samples are from. Columns list where individuals were assigned to. Bold numbers indicate assignment values to their population of collection. Populations included in reporting group assignments are shown as bordered blocks. Fish that assigned to an origin different than their collection site are shown in red.**

| | Satus 06-09 | Teanaway 05 | Toppenish 06-09 | Toppenish 09Fall | Ahtanum 01 | Ahtanum 06 | Ahtanum 07-10 | LR Snake 05,08 | Naches 04, 06 | NFL Naches 08 | Nile 05,08 | Pileup | Quartz 05,08 | % Correct |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Satus 06-09 | **131** | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 6 | 1 | 0 | 0 | 0 | 92.3% |
| Teanaway 05 | 1 | **43** | 0 | 0 | 1 | 4 | 0 | 0 | 3 | 0 | 1 | 0 | 0 | 81.1% |
| Toppenish 06-09 | 3 | 1 | **117** | 9 | 3 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 85.4% |
| Toppenish 09Fall | 0 | 0 | 10 | **54** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 84.4% |
| Ahtanum 01 | 0 | 0 | 2 | 0 | **27** | 8 | 2 | 1 | 0 | 0 | 1 | 0 | 0 | 65.9% |
| Ahtanum 06 | 0 | 1 | 4 | 0 | 10 | **34** | 2 | 0 | 4 | 0 | 0 | 0 | 0 | 61.8% |
| Ahtanum 07-10 | 0 | 0 | 0 | 0 | 4 | 6 | **7** | 0 | 0 | 0 | 0 | 0 | 0 | 41.2% |
| LR Snake 05,08 | 2 | 0 | 1 | 0 | 0 | 1 | 0 | **14** | 10 | 0 | 2 | 0 | 1 | 45.2% |
| Naches 04, 06 | 4 | 0 | 0 | 0 | 1 | 1 | 0 | 6 | **55** | 3 | 4 | 2 | 3 | 69.6% |
| NFL Naches 08 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | **5** | 1 | 0 | 0 | 33.3% |
| Nile 05,08 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 11 | 0 | **19** | 0 | 1 | 55.9% |
| Pileup | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 1 | **3** | 0 | 37.5% |
| Quartz 05,08 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | **3** | 27.3% |
| Average | | | | | | | | | | | | | | 60.1% |

**Table 8. Individual assignments to reporting groups. The number of fish assigning to each reporting group is listed. Each row lists where samples are from. Columns list where individuals were assigned to. Bold numbers indicate assignment values to their population of collection. Populations included in group assignments are shown as bordered blocks. Fish that assigned to an origin different than their collection site are shown in red.**

| | Satus | Teanaway | Toppenish | Ahtanum | Naches | % Correct |
|---|---|---|---|---|---|---|
| Satus 06-09 | **131** | 3 | 1 | 0 | 7 | 92.30% |
| Teanaway 05 | 1 | **43** | 0 | 5 | 4 | 81.10% |
| Toppenish 06-09 | 3 | 1 | **126** | 7 | 0 | 92.00% |
| Toppenish 09Fall | 0 | 0 | **64** | 0 | 0 | 100.00% |
| Ahtanum 01 | 0 | 0 | 2 | **37** | 2 | 90.20% |
| Ahtanum 06 | 0 | 1 | 4 | **46** | 4 | 83.60% |
| Ahtanum 07-10 | 0 | 0 | 0 | **17** | 0 | 100.00% |
| LR Snake 05,08 | 2 | 0 | 1 | 1 | **27** | 87.10% |
| Naches 04, 06 | 4 | 0 | 0 | 2 | **73** | 92.40% |
| NFL Naches 08 | 3 | 0 | 0 | 0 | **12** | 80.00% |
| Nile 05,08 | 1 | 0 | 0 | 2 | **31** | 91.20% |
| Pileup | 0 | 0 | 0 | 0 | **8** | 100.00% |
| Quartz 05,08 | 0 | 1 | 0 | 0 | **10** | 90.90% |
| Average | | | | | | 90.83% |

Proportional mixed stock assignments of unknown fish collections are reported back to reporting units consisting of the five primary tributaries. Table 9 shows assignments of upstream migrants collected at Prosser Dam. Data is reported for three spawning years. Samples collected in both fall of 2009 and spring of 2010 represent the same spawning class that was expected to spawn in spring of 2010. Variation between spawn classes years was lower than variation between the two collections (2009, 2010 spring) representing a single spawn class, but collected at different time periods (fall vs. spring). Proportions assigned to Satus Creek in particular differed, with a value of 0.296 in the fall 2009 compared to 0.449 in spring 2010.

**Table 9. Proportional mixed stock assignments. The proportion of fish sampled at Prosser Dam as assigned to the five reporting units. Reporting groups are listed on the left and consist of the five tributary collections. Estimates are reported as both point and 95% C.I. in brackets**

| Prosser Dam | 2007 | | 2008 | | 2009 | | 2010 Spring | |
|---|---|---|---|---|---|---|---|---|
| sample size | 158 | | 81 | | 86 | | 158 | |
| Ahtanum | 0.266 | (0.1657, 0.3512) | 0.205 | (0.1107, 0.2976) | 0.220 | (0.1058, 0.3470) | 0.173 | (0.1153, 0.2509) |
| Naches | 0.344 | (0.2512, 0.4342) | 0.291 | (0.2064, 0.4153) | 0.214 | (0.1398, 0.3473) | 0.188 | (0.1416, 0.2949) |
| Satus | 0.122 | (0.0577, 0.1740) | 0.142 | (0.0418, 0.2108) | 0.296 | (0.1777, 0.3985) | 0.449 | (0.3026, 0.5057) |
| Teanaway | 0.068 | (0.0387, 0.1354) | 0.036 | (0.0000, 0.0940) | 0.058 | (0.0117, 0.1217) | 0.030 | (0.0000, 0.0929) |
| Toppenish | 0.201 | (0.1437, 0.2482) | 0.325 | (0.2223, 0.4048) | 0.212 | (0.1335, 0.3047) | 0.160 | (0.0997, 0.2413) |

Table 10 Shows assignments of downstream migrating kelts captured at the Chandler collection facility. The largest variation between years was again seen in Satus Creek. Approximately 60% of samples genotyped in 2006 were assigned to the Satus Creek reporting group. Subsequent years had values of 27% (2008), 30% (2009), and 39% (2010).

**Table 10. Proportional mixed stock assignments. The proportion fish sampled at the Chandler collection facility as assigned to the five reporting units.**

| Chandler Kelt | 2006 | | 2008 | | 2009 | | 2010 | |
|---|---|---|---|---|---|---|---|---|
| sample size | 89 | | 139 | | 173 | | 608 | |
| Ahtanum | 0.094 | (0.0387, 0.1772) | 0.314 | (0.1780, 0.3583) | 0.143 | (0.0723, 0.2041) | 0.121 | (0.0932, 0.1510) |
| Naches | 0.089 | (0.0373, 0.1867) | 0.220 | (0.1811, 0.3671) | 0.280 | (0.1844, 0.3618) | 0.250 | (0.2350, 0.3261) |
| Satus | 0.603 | (0.4762, 0.6690) | 0.268 | (0.1567, 0.3322) | 0.301 | (0.2291, 0.3528) | 0.391 | (0.3238, 0.4055) |
| Teanaway | 0.031 | (0.0000, 0.0866) | 0.016 | (0.0000, 0.0805) | 0.015 | (0.0039, 0.0714) | 0.030 | (0.0127, 0.0472) |
| Toppenish | 0.183 | (0.0825, 0.2715) | 0.182 | (0.1161, 0.2544) | 0.262 | (0.2068, 0.3325) | 0.208 | (0.1716, 0.2294) |

Table 11 shows the effect of gender on assignments. Males kelts were genotyped at a lower number than females (104 vs. 502). Males were more likely to assign to the Satus Creek reporting groups (0.523) than were females (0.033).

**Table 11. Proportional mixed stock assignments by kelt gender of 2010 captures.**

| Kelt Gender | Male | | Female | |
|---|---|---|---|---|
| sample size | 104 | | 502 | |
| Ahtanum | 0.098 | (0.0284, 0.1835) | 0.126 | (0.0904, 0.1591) |
| Naches | 0.206 | (0.1392, 0.3062) | 0.261 | (0.2387, 0.3416) |
| Satus | 0.523 | (0.3946, 0.5874) | 0.362 | (0.2797, 0.3849) |
| Teanaway | 0.011 | (0.0000, 0.0722) | 0.033 | (0.0223, 0.0592) |
| Toppenish | 0.162 | (0.0850, 0.2212) | 0.218 | (0.1731, 0.2393) |

Assignment by kelt status is shown in Table 12. Status as a mortality or long term reconditioned fish, and PIT tag detection affected the proportional assignments. Assignments to Satus Creek were as follows: 47% of mortality samples, 30% of the Long term samples, and 25% of the PIT detection samples. Within samples that were detected by PIT tag readers, proportional assignments were higher to the Naches River and Toppenish Creek reporting groups.

**Table 12. Proportional mixed stock assignments by kelt status of 2010 captures.**

| Kelt Status | Mortality | | Long Term | | PIT detection | |
|---|---|---|---|---|---|---|
| sample size | 225 | | 212 | | 96 | |
| Ahtanum | 0.126 | (0.0613, 0.1684) | 0.120 | (0.0623, 0.1924) | 0.113 | (0.0382, 0.1943) |
| Naches | 0.219 | (0.1919, 0.3159) | 0.277 | (0.2220, 0.3896) | 0.302 | (0.2201, 0.4307) |
| Satus | 0.474 | (0.3829, 0.5060) | 0.300 | (0.2046, 0.3355) | 0.247 | (0.1453, 0.3115) |
| Teanaway | 0.037 | (0.0070, 0.0775) | 0.031 | (0.0061, 0.0718) | 0.037 | (0.0000, 0.1168) |
| Toppenish | 0.144 | (0.1005, 0.1836) | 0.272 | (0.2169, 0.3245) | 0.301 | (0.2172, 0.3789) |

Table 13 demonstrates the effect of sampling time on proportional assignments. Samples collected early were most likely to assign to the Satus Creek reporting group for both 2009 (0.47) and 2010 (0.619). Samples collected late were more likely to assign to the Naches River reporting group for both 2009 (0.414) and 2010 (0.464).

**Table 13. Proportional mixed stock assignments by kelt arrival at the Chandler Collection Facility. Results are reported by year and classification as early or late.**

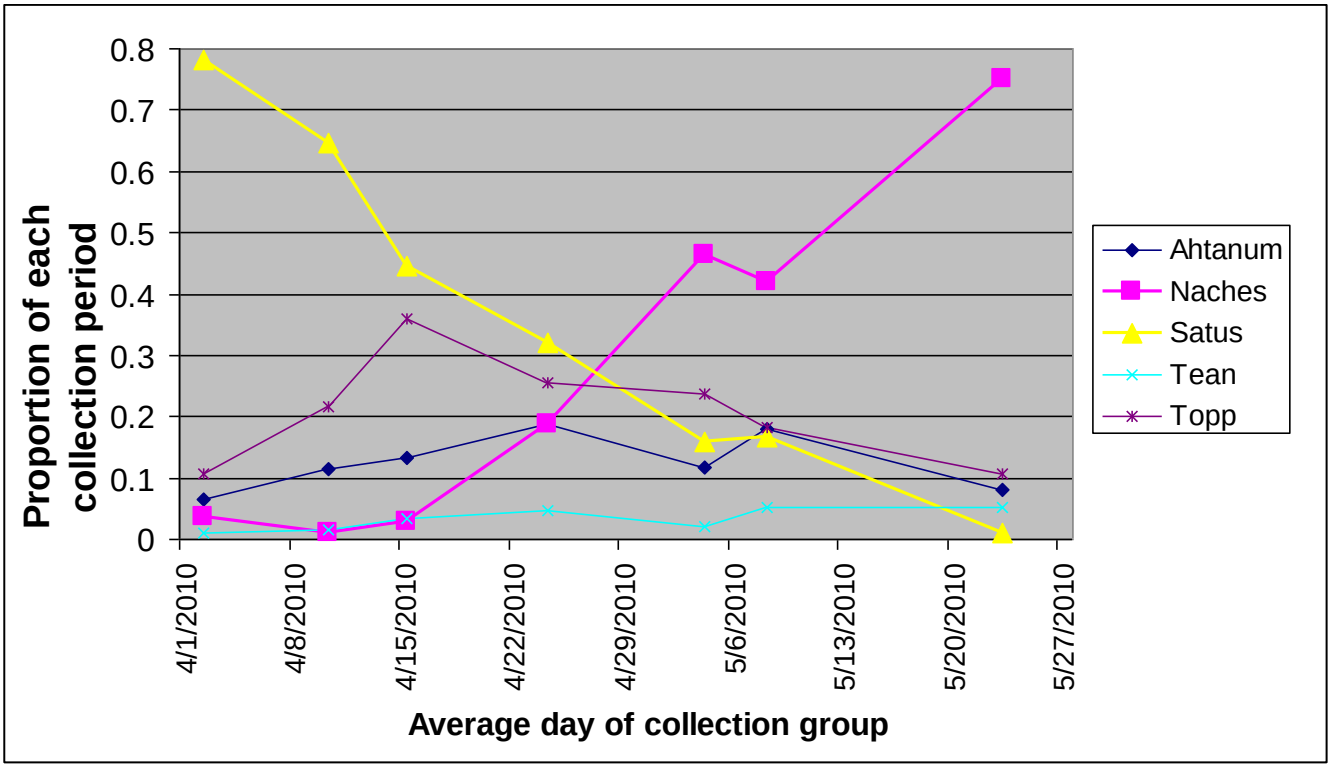
| Kelt Arrival | 2009 Early | | 2009 Late | |
|---|---|---|---|---|
| sample size | 86 | | 87 | |
| Ahtanum | 0.131 | (0.0473, 0.2008) | 0.147 | (0.0642, 0.2948) |
| Naches | 0.146 | (0.0713, 0.2361) | 0.414 | (0.2842, 0.5310) |
| Satus | 0.470 | (0.3338, 0.5708) | 0.136 | (0.0616, 0.2337) |
| Teanaway | 0.021 | (0.0000, 0.0810) | 0.012 | (0.0000, 0.0714) |
| Toppenish | 0.232 | (0.1565, 0.3073) | 0.293 | (0.1856, 0.3773) |
| **Kelt Arrival** | **2010 Early** | | **2010 late** | |
| sample size | 303 | | 305 | |
| Ahtanum | 0.114 | (0.0643, 0.1580) | 0.133 | (0.0750, 0.1981) |
| Naches | 0.028 | (0.0295, 0.1155) | 0.464 | (0.3851, 0.5266) |
| Satus | 0.619 | (0.5160, 0.6546) | 0.163 | (0.0986, 0.2087) |
| Teanaway | 0.018 | (0.0000, 0.0415) | 0.044 | (0.0190, 0.0779) |
| Toppenish | 0.222 | (0.1675, 0.2726) | 0.196 | (0.1480, 0.2276) |

The large sample size of kelts collected in 2010 allowed additional temporal stratification. Proportional assignments across seven sampling periods are shown in Table 14 and Figure 3. The temporal effect on assignment to the Naches River and Satus Creek reporting groups is more pronounced than was reported when the collection was divided evenly between early and late classifications. While 78% of the earliest group assigned to Satus Creek, only 1% of the latest group assigned to Satus Creek. In contrast, 4% of the earliest group and 75% of the latest group assigned to Naches River, respectively. Peak assignment values for Ahtanum Creek and Toppenish Creek occurred intermediately and were not as dramatic as those seen in either Satus Creek or Naches River.

**Table 14. Proportional mixed stock assignments by return date for kelts captured at Chandler Trap in 2010. Groups are reported by average return date.**

| Chandler 2010 | 4/2/2010 | 4/10/2010 | 4/15/2010 | 4/24/2010 | 5/4/2010 | 5/8/2010 | 5/23/2010 |
|---|---|---|---|---|---|---|---|
| **sample size** | **101** | **94** | **98** | **77** | **82** | **77** | **79** |
| **Ahtanum** | 0.066 | 0.113 | 0.134 | 0.189 | 0.118 | 0.181 | 0.080 |
| **Naches** | 0.037 | 0.009 | 0.028 | 0.188 | 0.464 | 0.421 | 0.752 |
| **Satus** | 0.781 | 0.647 | 0.447 | 0.320 | 0.160 | 0.166 | 0.010 |
| **Teanaway** | 0.011 | 0.014 | 0.033 | 0.047 | 0.022 | 0.052 | 0.051 |
| **Toppenish** | 0.106 | 0.215 | 0.359 | 0.256 | 0.236 | 0.181 | 0.108 |

**Figure 3. Proportional mixed stock assignments of 2010 kelt captures by arrival time.**



## Discussion

Analyses clearly show that there are multiple distinct populations of *O. mykiss* in the Yakima River basin and that most of the collections contain multiple breeding groups. The differential results when analyzing either all adults or all tributaries with the program STRUCTURE shows that all populations present in the tributaries may not be represented in the adult anadromous collections. Alternatively, STRUCTURE may be identifying sibling groups within the juvenile collections, which are more likely to be sampled in juvenile rather than adult collections. While there was evidence for only 2-3 populations in the adult samples, the results showed 4-5 likely populations among the tributaries. Separation of the five tributaries as distinct populations is further supported by the significantly different Fst values between them. Further separation within Ahtanum Creek, Naches River and Toppenish Creek is also indicated. While the program STRUCTURE failed to show evidence of multiple populations within Naches River, sample numbers within each tributary of Naches River are small, and may not provide adequate power.

Other studies have shown that resident *O. mykiss* are present and that introgression with anadromous steelhead may have occurred (Campton and Johnston 1985, Busack et al. 2006). While introgression may have occurred, STRUCTURE results still support multiple populations within all tributaries except Satus Creek. Most of the collections are likely demonstrating the Wahlund effect of a heterozygote deficit, with multiple populations present in a single collection. This structure may include distinct populations of both resident and anadromous fish.

Assignment of known origin fish to the proper reporting group was high for both 100% simulations (0.995 average) individual assignments (0.908 average) indicating a powerful genetic baseline for Genetic Stock Identification. Genotyping of a larger number of samples across multiple years and collection types has allowed additional conclusions to be made.

Proportional mixed stock assignment of unknown fish to each of the five primary tributaries varied widely depending on the stratification used. Evidence reported here supports differential migration timing in the tributaries for both the upstream and downstream migrations. This was the first year to include samples collected at Prosser Dam during the spring migration. While the majority of fish are thought to pass Prosser Dam during the fall, a significant portion also migrate during the spring, and these fish are comprised of different stock proportions.

Kelt collections and sampling has previously been designed to target fish that would survive and potentially contribute to subsequent generations. While this may be ideal for reconditioning efforts and future parentage studies, it also introduces a sample bias into the dataset when analyzing proportional mixed stock assignments. Analysis of kelts by gender, status, or temporal factors shows a strong influence on results. Although the results themselves may be biased by our sampling and genotyping regimes, the trends are likely to be true.

As part of kelt reconditioning, we are attempting to quantify the relative reproductive success of reconditioned kelts. This is inherently difficult to do as spawning behavior in steelhead is difficult to observe, and most populations contain relatively few iteroparous individuals. The kelt reconditioning program in the Yakima River Basin should increase the relative proportion of kelts in the spawning population. Identification of sites such as with high proportions of kelts, would make parentage analysis more powerful and lead to better estimates of relative reproductive success. However the previous

suggestion that Satus Creek would have a higher proportion of kelts in the population, may no longer be supported. Multiple factors including migration timing, gender, and status appear to have a confounding effect and will need to be parsed out.

Parentage assignments were low for both Satus Creek and Toppenish Creek juveniles. However, parental sampling was incomplete. Additional fish will be genotyped in the future as additional fish are reconditioned and released.

Results from the 2010 analyses highlight potential demographic factors and relationships with kelt collections. Satus Creek appears to be comprised of fish that migrate upstream later than other populations, but are detected earlier as downstream migrating kelts. The opposite is seen for the downstream migration of kelts from the Naches River drainage, with the most fish migrating in the later half of the season. Satus Creek also appears to contribute a proportionally higher number of males to the kelts collected at the Chandler facility. Because male survival during reconditioning is expected to be lower than female, the increased proportion of males from Satus Creek may be reflected in the higher proportion of mortalities assigned to Satus Creek.

Temporal migration, gender and kelt status patterns within Satus Creek is likely to be at least partially related to the proximity of the collection facilities (Prosser Dam and Chandler Trap). Fish destined for Satus Creek may be predisposed to over-winter lower in the system (below Prosser Dam), or the spring collection may include fallback of fish due to the proximity to the mouth of Satus Creek. Collection of kelts may be biased towards fish from Satus Creek that may not have otherwise been collected if the migration distance was greater. Males in particular may be exhibiting a within basin straying pattern in search of additional mating opportunities. Proximity would also increase the number of weak (future mortalities) fish from Satus Creek that would otherwise have perished prior to sampling with additional distance of migration.

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# Chapter 3: Snake River Steelhead Kelt Research and Steelhead Kelt Master Plan Development

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# Section A: Developing Strategies to Improve Survival and Return Recruitment of Steelhead Kelts from Snake River Stocks

Contract No C10-24
Purchase Order No C1002440

Quarterly Report

Prepared by

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Submitted to
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25 April 2011

## Table of Contents

## Executive Summary

This quarter we continued laboratory analysis and interpretation of samples, summarization and statistical analyses of all data. All graduate students prepared and presented oral papers on their research at the Idaho Chapter American Fisheries Society meeting in March. Graduate student Jessica Buelow prepared a draft thesis, and successfully completed her oral presentation on 6 April, and will complete revisions to her thesis during the spring and summer. We continued analyses of energy and proximate constituents of tissue samples from field and hatchery collections in 2010 and 2011. We held meetings with Idaho Department of Fish and Game staff to plan for acoustical tagging of kelts at three tributaries of the Clearwater River. We have begun tagging of kelts in the Potlatch River system, but river flows have caused delays in schedules and access to fish. We completed plans for a special session on iteroparity in steelhead trout at the 141st annual American Fisheries Society meeting in Seattle, 4-8 September.

## Objective 1. Obtain and synthesize physiological metrics into models that describe the changes observed in hatchery and natural origin steelhead stocks from fall upriver migration through spawning and early kelt migration.

We have begun to develop a simulation model the bioenergetics of two sizes of steelhead migrating upstream during average river conditions, and travel rates. The model has been developed to estimate energy expenditures of steelhead under conditions of no feeding. We have developed a preliminary assessment of the costs of upstream migration and plan to add compartments to consider energy use during freshwater residence during the winter and reproductive costs of spawning. These simulations will be compared with empirical estimates of energy content in tissues from steelhead collected at several stages of migration, at the Celilo fishing area, samples from Idaho tributaries in the late fall, and samples collected from hatchery origin Clearwater River steelhead over several months at Dworshak National Fish Hatchery. Using these energy relations, we will also develop correlations of total body energy with non-lethal physiological assessment from analysis of nutritional factors in the plasma of steelhead sampled at intervals including samples from migrating steelhead at Bonneville Dam and Lower Granite Dam in the fall, and any paired samples of blood taken at the time of tissue samples.

## Objective 2. Obtain a complete profile of the condition and physiology of downstream migrating natural origin stocks captured at Lower Granite Dam bypass facility, and compare and contrast these profiles with fish examined at upriver sites.

*Migrating kelts at Lower Granite Dam* - In 2010, we counted and examined all fish (2,682 kelts) that were diverted into the juvenile fish bypass system for condition, and size (Table 1). For fish that were sampled for blood, we examined a suite of plasma metrics and their association with fish external condition (good, fair, or poor), sex, fish length (< 60 cm, 60-69 cm, or > 70 cm), likely hatchery or natural origin (adipose clipped, adipose intact with dorsal fin erosion, and adipose intact with no dorsal fin erosion), and migration time by month and year.

**Table 1. Number of steelhead kelts collected and number of blood samples analyzed (in parentheses) April – 2 July at daily collections at Lower Granite Dam, 2010. The number of kelts include fish that were anesthetized and sampled and fish visually assessed and released quickly into the river. Kelts are categorized by sex; condition (good, fair, or poor). Fish numbers are separated by fork length into one of three groups: < 60 cm, 60 - 69 cm, or > 70 cm. Fish are separated by kelts observed with and without an adipose fin, and the fish with adipose fins were observed for signs of dorsal fin erosion. We sampled blood from 851 kelts in a stratified random sequence each day. Numbers in parentheses are the total number for each category sampled for blood.**

| | Female | | | Male | | |
|---|---|---|---|---|---|---|
| | | With adipose fin | | | With adipose fin | |
| Fork length (cm) | Adipose clip | Dorsal erosion | No dorsal erosion | Adipose clip | Dorsal erosion | No dorsal erosion |
| | Good condition | | | | | |
| < 60 | 423 (9) | 328 (60) | 451 (53) | 45 (3) | 71 (27) | 156 (26) |
| ≥ 60 - 70 | (6) | (25) | (91) | (2) | (9) | (33) |
| > 70 | 21 (2) | 5 (3) | 132 (102) | 1 (0) | 1 (1) | 2 (2) |
| | Fair condition | | | | | |
| < 60 | 199 (8) | 196 (42) | 119 (28) | 36 (7) | 69 (23) | 67 (14) |
| ≥ 60 - 70 | 0 | (27) | (38) | (1) | (15) | (23) |
| > 70 | 12 (1) | 6 (5) | 21 (20) | 1 (0) | 0 | 7 (6) |
| | Poor condition | | | | | |
| < 60 | 69 (3) | 71 (25) | 40 (10) | 12 (0) | 57 (14) | 37 (7) |
| ≥ 60 - 70 | (2) | (16) | (15) | (1) | (17) | (14) |
| > 70 | 6 (2) | 6 (5) | 7 (5) | 0 | 0 | 4 (3) |

When we analyzed fish visually for condition, we also recorded the state of fish activity, feel of the flesh, amount of silvering, and extent of pale coloration. We validated that our assessment of visual condition was discernable through handling and observation of behavior (Table 2). We found that 100% of all good condition fish ere active, and 98% had firm flesh. We observed 65 and 88% were silvery and bright in coloration. We quantified head wounds in for all steelhead kelts diverted from the juvenile bypass system. We identified 346 (15%) kelts with some head wound, but did not find a higher proportion on the larger sized fish > 70 cm fork length. We found the majority of head wounds occurred on fish that were 60-69 cm fork length, and only 8% of the head wounds were on fish > 70 cm.

**Table 2. Summary of visually and tacitly determined characteristics of steelhead kelts separated by summary assessment of condition at Lower Granite Dam, 2010. The number and percent of each condition are further classified for their labeled with each characteristic is counted and percent total is evaluated.**

| Condition | Active/Listless | Firm / Flaccid | Silver/Dark | Bright/Pale |
|---|---|---|---|---|
| Good | 1,166 / 0 | 1,137 / 24 | 754 / 413 | 1,023 / 142 |
| % | 100 / 0 | 98 / 2 | 65 / 35 | 88 / 12 |
| Fair | 489 / 12 | 443 / 56 | 189 / 314 | 334 / 158 |
| % | 98 / 2 | 89 / 11 | 38 / 62 | 68 / 32 |
| Poor | 143 / 80 | 130 / 93 | 37 / 187 | 87 / 132 |
| % | 64 / 36 | 58 / 42 | 17 / 83 | 40 / 60 |

When we examined the plasma metrics by fish condition, we found good condition kelts had higher nutritional and electrolytes (Table 3). Poor condition kelts had higher levels of tissue damage and stress (Table 3). We examined good condition kelts to explore difference between male and female kelts (Table 4). We found few differences between males and females except but alkaline phosphatase, and cortisol were higher in females. Surprisingly, we found few differences in plasma samples from good condition kelts based on fork length. We separated good condition kelts with adipose fins through examination of the dorsal fin for signs of crooked and deformed fin rays or reduced fin size. These criteria are indicative of hatchery origin fish that were likely released without an adipose fin clip. We found that kelts with no dorsal fin erosion had some higher nutritional factors over those fish with eroded dorsal fins. In addition, some tissue damage factors were higher for kelts with eroded dorsal fins than without.

We also examined trends is metrics across the three months of sampling for good condition kelts. In April, calcium and glucose were higher. In May, we collected 62% of the kelts, and these fish had the highest tissue damage and stress factors. Almost all blood parameters were higher in 2009 for good condition adipose intact < 70 cm kelts than in 2010. This includes nutritional and lipid metabolism factors as well as tissue damage and stress factors.

**Table 3. Median and range in parentheses, and sample size for continuously distributed blood parameters separated by condition (good, fair, poor) for female steelhead kelts <70 cm fork length sampled at Lower Granite Dam 2010. Kelts with no dorsal erosion are summarized.**

| | Good | | Fair | | Poor | |
|---|---|---|---|---|---|---|
| Parameter | N | Median (Range) | N | Median (Range) | N | Median (Range) |
| Sodium | 140 | 157.0 (128-207) | 63 | 149.0 (112-192) | 23 | 140.0 (103-197) |
| Potassium | 129 | 1.9 (0.8-3.7) | 58 | 2.3 (0.8-6.9) | 22 | 2.5 (0.8-5.7) |
| Chloride | 141 | 144 (107-182) | 63 | 136.0 (97-169) | 23 | 117.0 (87-176) |
| Glucose | 144 | 92.0 (42-265) | 66 | 75.5 (21-192) | 25 | 55.0 (9-113) |
| Calcium | 141 | 9.1 (5.5-14.5) | 65 | 7.9 (3.2-10.8) | 25 | 7.2 (4.9-10.7) |
| Magnesium | 143 | 2.0 (1-3.64) | 64 | 1.9 (0.9-3.3) | 24 | 1.8 (1.0-2.4) |
| Phosphorus | 144 | 9.2 (4.2-19.9) | 65 | 10.0 (4.4-19.2) | 25 | 10.7 (6.8-26.5) |
| AP | 144 | 24.0 (3.0-88.0) | 64 | 12.5 (2.0-125) | 24 | 4.5 (2.0-88) |
| Cholesterol | 144 | 84.5 (14-246) | 66 | 44.5 (3.0-135) | 25 | 21.0 (7-126) |
| LDH | 144 | 413.5 (130-2228) | 65 | 770.0 (162-4848) | 25 | 1758.0 (321-9324) |
| AST | 143 | 483.0 (71-2932) | 66 | 587.5 (11-3426) | 25 | 779.0 (147-4428) |
| Cortisol | 38 | 170.6 (28.5-389) | 13 | 200.8 (107-1129) | 18 | 229.4 (74.1-652) |
| ALT | 91 | 38.0 (9-268) | 33 | 104.0 (10-584) | 12 | 120.0 (33-654) |
| Amylase | 53 | 136.0 (46-775) | 32 | 145.5 (43-315) | 13 | 135.0 (66-315) |
| Lipase | 52 | 7.0 (1-13) | 30 | 6.0 (0-13) | 11 | 5.0 (0-14) |

**Table 4. Median, range (in parentheses), number (N), and *P* values for plasma metrics by sex for good condition steelhead kelts collected at Lower Granite Dam,2010, separated by sex of fish. Kelts with adipose fins and no visible dorsal fin erosion < 70 cm fork length were analyzed. The response variables, triglycerides and T4 were numerically coded categorical variables with increasing numbers related to higher values. All others measures were continuous variables. Statistical comparisons were obtained with Proc NPAR1WAY Wilcoxon tests with Monte Carlo estimates for the exact *P*.**

| | Female | | Male | | |
|---|---|---|---|---|---|
| Parameter | N | Median (Range) | N | Median (Range) | Comparison |
| Sodium | 140 | 157 (128-207) | 59 | 154 (114-207) | 0.034 |
| Potassium | 129 | 1.9 (0.8-3.7) | 56 | 1.6 (0.8-3.7) | 0.003 |
| Chloride | 141 | 144 (107-182) | 59 | 143 (95-179) | 0.273 |
| Glucose | 144 | 92 (42-265) | 59 | 93 (61-321) | 0.816 |
| Calcium | 141 | 9.1 (5.5-14.5) | 59 | 8.9 (7.3-11.4) | 0.663 |
| Magnesium | 143 | 2 (1.0-3.6) | 59 | 2 (1.4-3.3) | 0.327 |
| Phosphorus | 144 | 9.2 (4.2-19.9) | 59 | 9.8 (4.6-20.4) | 0.173 |
| AP | 144 | 24 (3-88) | 59 | 20 (6-65) | 0.070 |
| Cholesterol | 144 | 84.5 (14-246) | 59 | 74 (23-251) | 0.935 |
| LDH | 144 | 413.5 (130-2228) | 59 | 398 (167-2598) | 0.151 |
| AST | 143 | 483 (71-2932) | 59 | 534 (5-3216) | 0.420 |
| Cortisol | 38 | 170.6 (28.5-389.3) | 41 | 106.7 (51.0-274.0) | 0.001 |
| TG category | 144 | 3 (1-5) | 59 | 2 (1-5) | 0.003 |
| T4 category | 144 | 3 (1-4) | 59 | 3 (1-4) | 0.525 |
| ALT | 91 | 38 (9-268) | 51 | 40 (9-333) | 0.342 |
| Amylase | 53 | 136 (46-775) | 8 | 155 (94-204) | 0.867 |
| Lipase | 52 | 7 (1-13) | 8 | 8 (2-14) | 0.511 |

We collected 80 prespawning steelhead diverted from the juvenile fish bypass facility during the 2010 sampling season, likely from fall backs. All pre-spawning fish were released back into the river. The majority of prespawning steelhead were collected in April. We considered the steelhead collected on June 28th, likely from spawning year 2011.

*Kelts leaving Clearwater River tributaries*

Selected plasma metrics of kelts from the three tributaries and the Lower Granite juvenile fish bypass in 2010 were separated by condition (good, fair, and poor). Similar trends were seen across all sites for both cholesterol (Figure 1) and triglycerides (Figure 2) with good condition fish showing higher levels of plasma metrics than both fair and poor condition fish.

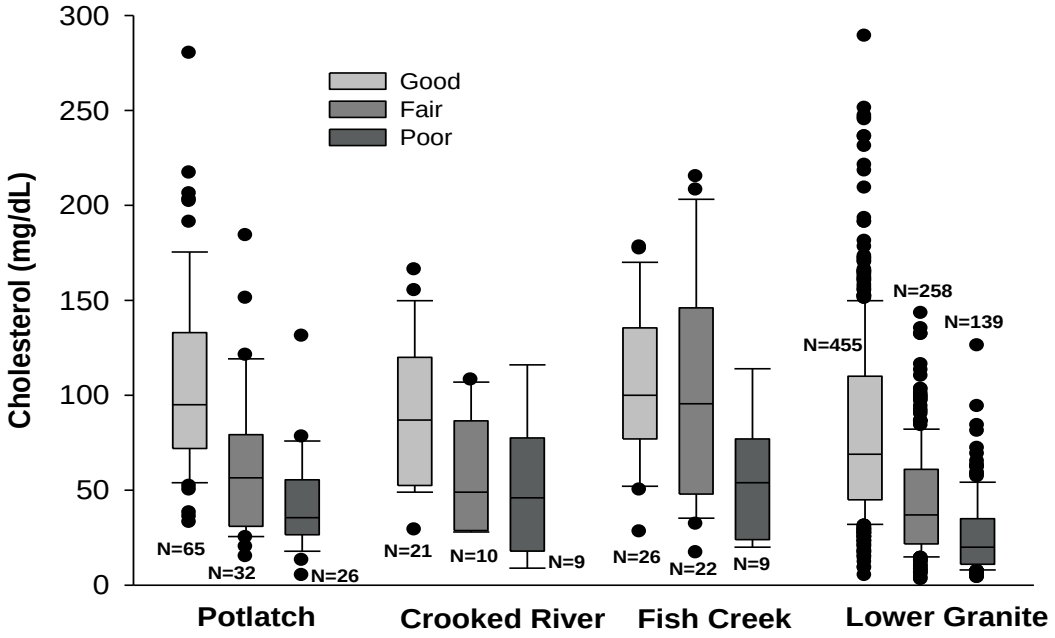


**Figure 1 . Cholesterol in plasma of steelhead kelts captured at tributary weirs and Lower Granite Dam separated by condition, 2010.**

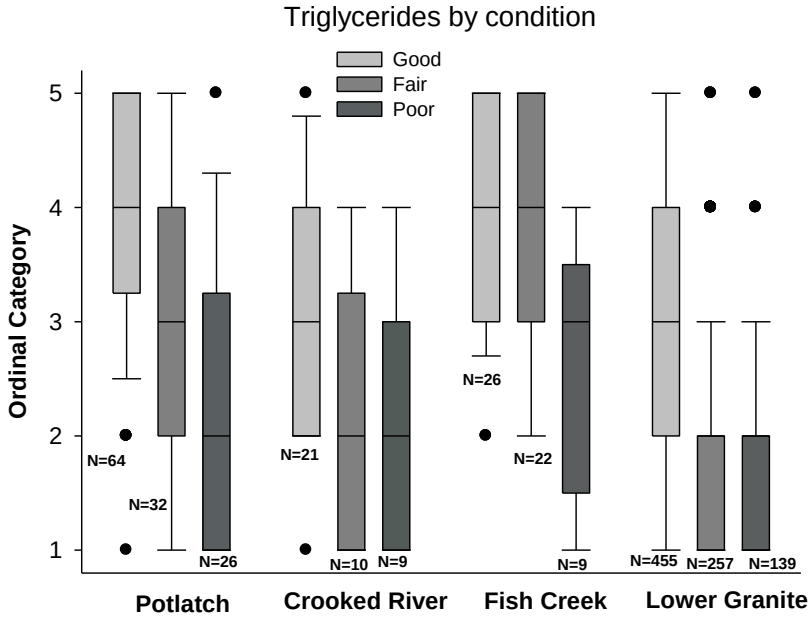


**Figure 2. Triglyceride levels (by ordinal category of increasing values) in plasma sampled from steelhead kelts captured at tributary weirs and Lower Granite Dam in 2010, separated by condition. Triglycerides were grouped into the following ordinal categories: <10 = 1, 10-25 = 2, 26-50 = 3, 51-100 = 4, >100 = 5.**

## Objective 3a. Evaluate the survival and migration behavior of natural origin steelhead kelts collected from the bypass facility at Lower Granite Dam, tagged with acoustic tags and transported via barge or truck to locations below Bonneville Dam.

In 2010, we successfully implanted acoustic transmitters in 119 kelts that were transported from Lower Granite Dam juvenile fish bypass to release sites in the lower Columbia River. Of these fish, 82 were transported via U.S. Corps of Engineers juvenile transport barge and released downstream of Bonneville Dam. The remaining 37 fish were transported via truck and released below Bonneville dam to the Bradford Island release site, or trucked to a release site at Aldrich Point, Oregon, near the estuary. Of those fish released by barge, 61 (74%) were detected by at least one of four groups of acoustic receivers throughout the lower Columbia River (Figure 3). We detected 18 kelts (15%) reaching arrays located at

the mouth of the Columbia River. We found 17 fish were detected by receiver at Bonneville close to the barge and most truck release sites but not detected again elsewhere. We have not completed analyses the plasma metrics of fish that were tagged and released to migrate below Bonneville Dam in 2010. We anticipate completing that during the next quarter and comparing the rate of migration or success in migration with nutritional parameters.

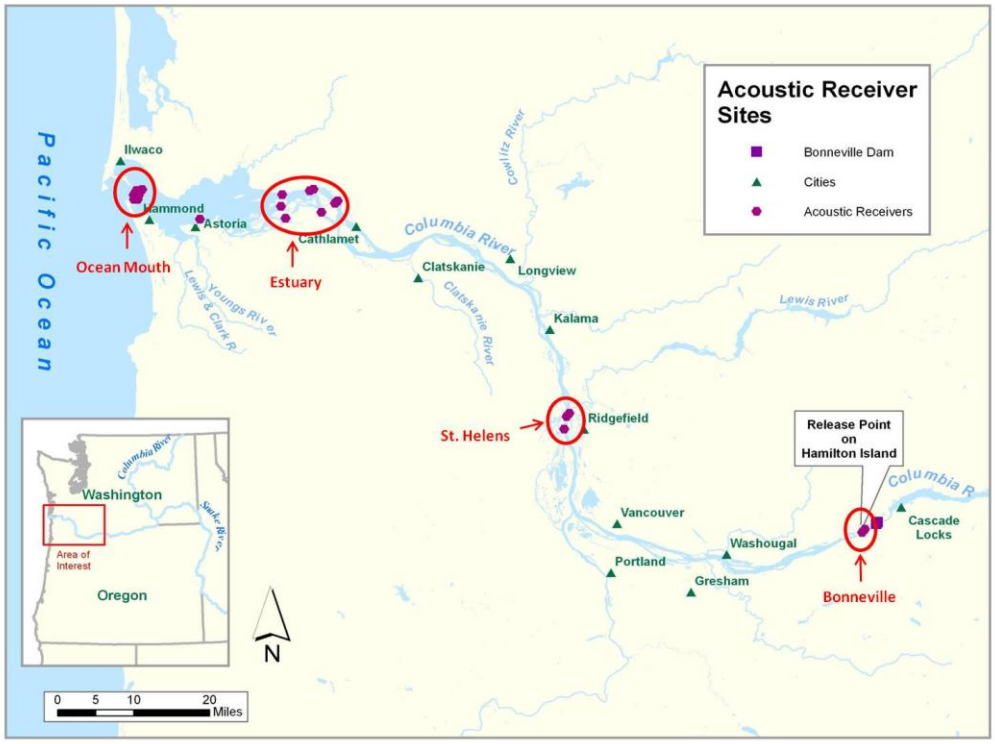


**Figure 3. Map of the lower Columbia River with location and name of acoustic receiver arrays. Most kelts transported from Lower Granite Dam by truck were released at the boat launch at Hamilton Island; however, one tank truck of 10 fish was released at Aldrich Point on 29 April within the Estuary array. Barge releases were generally located near the Hamilton Island location.**

## Objective 3b. Evaluate the behavior and downstream migration success to Lower Granite Dam of natural origin steelhead kelts from the Clearwater River tributaries.

We have analyzed the migration patterns of kelts using data from the weirs in the Clearwater River. We plotted the number of kelts captured per day at the Little Bear weir on the Potlatch River in 2010 against the average daily temperature of the Little Bear weir (Figure 4). We found that kelt emigration from the spawning tributary was somewhat related to river temperatures.

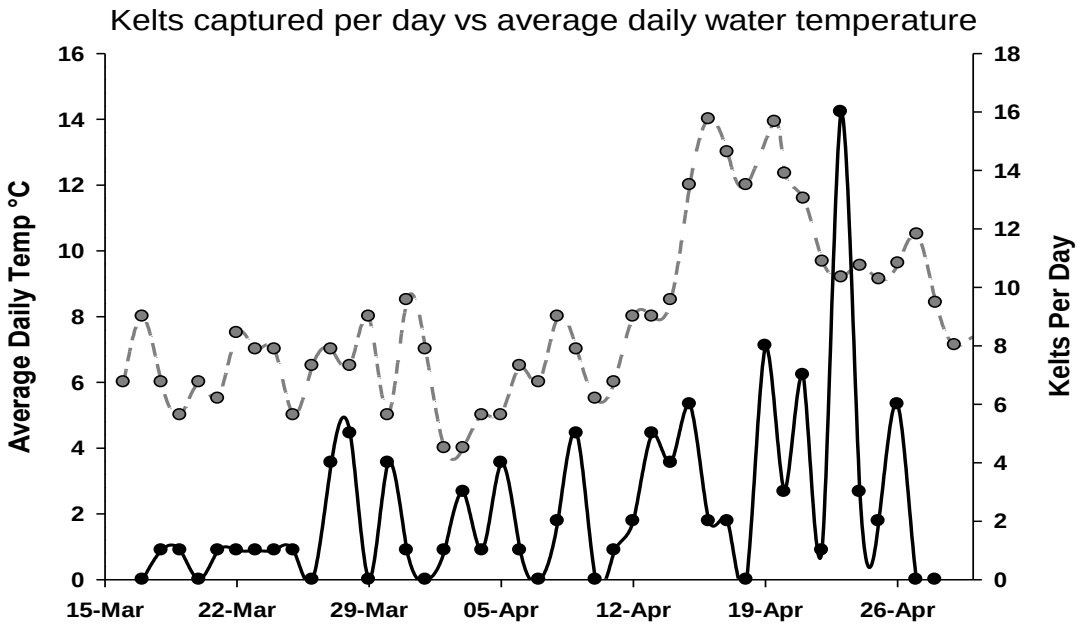


**Figure 4. Number of kelts captured at the Little Bear weir per day (solid black line) compared to the average daily water temperature (dotted grey line). Other weirs not shown due to smaller sample sizes.**

The timing of the kelt migrations in 2010 varied among sites (Figure 5). Kelts from the lower elevation tributaries migrated earlier in the season than did kelts from higher elevation tributaries. Kelts from Little Bear Creek were first to emigrate, beginning in March. Fish Creek kelts were the last to migrate with kelts captures at the weir until the end of June.

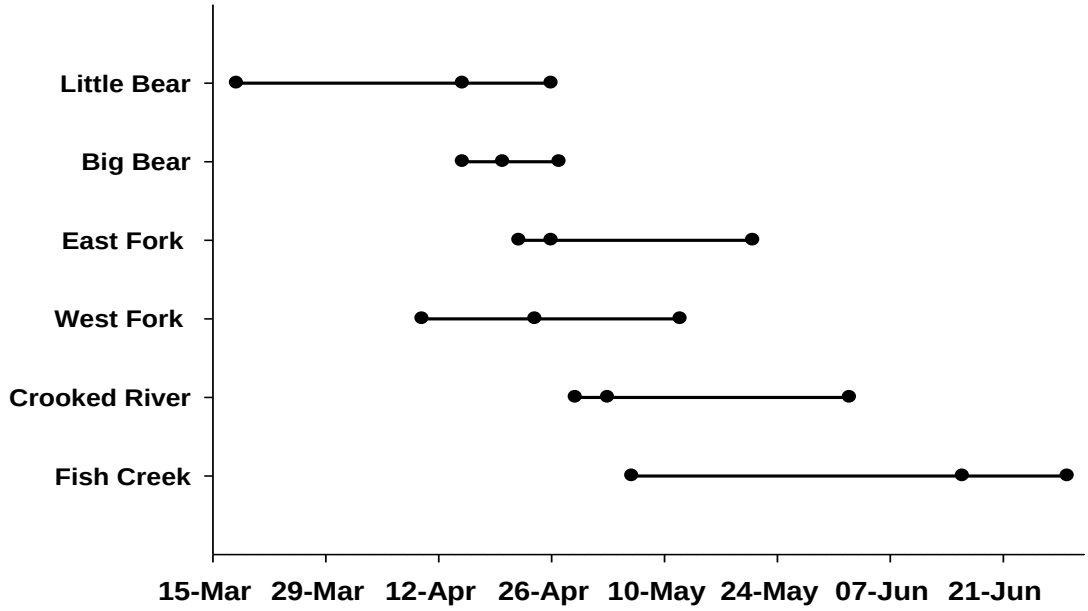


**Figure 5. Timing of kelt emigration from each tributary in 2010. The black dot at the left of each line indicates the date of the first kelt captured, the middle dot is the date by which 50% of the run had passed the weir, and the right dot indicated the last kelt captured.**

In preparation for the acoustic tagging of kelts from the Potlatch, Fish Creek, and Crooked River we obtained permission and permits from various agencies and organizations to deploy acoustic receivers at locations within the Clearwater River and Lower Granite Reservoir (Figure 6). The receivers within the reservoir are suspended under buoys. To anchor the receivers in the Clearwater River, we used a pulley system strung with steel cable and anchored to the bottom at one end and to various shore or bridge based structures at the other end. Data will be downloaded from each receiver on a weekly basis once tagging of kelts ensues. At the time of this report (25 April), we have successfully tagged and released 6 kelts from the Potlatch River.

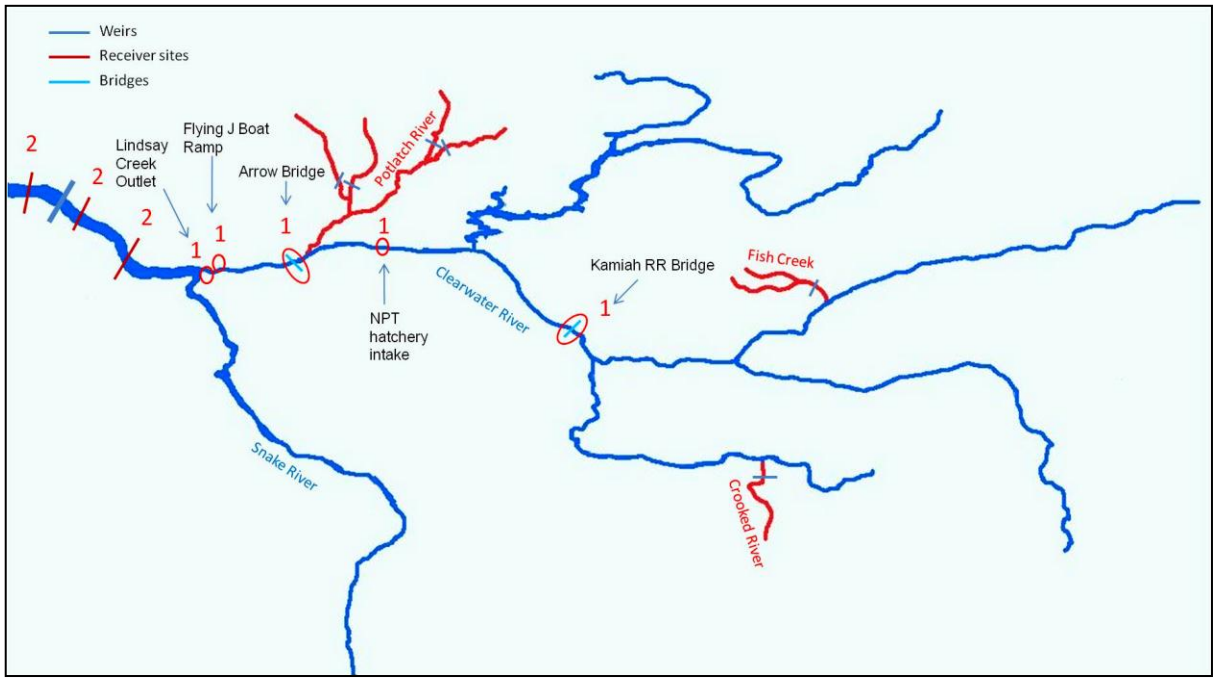


**Figure 6. Map of weir and receiver sites in the Clearwater River and lower Snake River reservoirs (Lower Granite Reservoir and Lake Bryan).**

## Objective 4. Evaluate the emigration of natural origin steelhead kelts PIT tagged and released below Lower Granite Dam to migrate through the Snake and Columbia River hydrosystem.

In 2010 at the Lower Granite Dam juvenile fish bypass, we captured and PIT-tagged a total of 1,398 kelts that were released below the dam to migrate downstream. We detected 252 (18%) kelts at other downstream sites. We found the fish with the best detection rate were good condition males (36%), and poor conditioned females had the lowest detection rate (<1%).

The travel rate of the kelts increased the farther downstream the detection occurred. Of all fish he highest proportion detected occurred at Little Goose Dam, just downstream from Lower Granite Dam. We observed the lowest number of detections at the bypass system at John Day dam. As in previous years, the corner collector at Bonneville Dam detected more than 25% of all detected fish. Travel rates to locations in the Columbia River were faster than those in the Snake River, a characteristic that we observed in 2009, and in previous studies. We observed too few poor or fair condition fish at locations in the Columbia River to compare them or comment on travel rates. However, we did observe a limited

number of PIT-tag detections of fair or poor fish at dams in the Snake River system and their median travel rate was actually faster than that for good condition fish. However, sample sizes for these groups of fish were limited.

We have not received any notification of returned PIT-tags from sites with pelican or other predatory bird roosting. We plan to contact appropriate parties engaged in predation studies to determine if these surveys have been made.

## Problems or Special Needs

We worked with the Idaho Department of Transportation, Washington Department of Ecology, and the U.S. Corps of Engineers to gain permission for placement of anchored acoustic receivers. Excessive flows in the Snake and Clearwater River caused disruption of the weirs, and of the receivers. We recovered one anchored receiver in the Lower Granite Dam that was moved by large woody debris, and we have redeployed one in the permitted location. We have communicated with Michelle Rup (NOAA) but they have not detected any of our tags below Lower Granite as of 15 April. We had one receiver break away from the Arrow Bridge site, and have not yet recovered it. We plan to drag the river when flows recede. High river flows have precluded access to fish several times in the Potlatch River system, but we are continuing to work to tag fish in that system. We will concentrate efforts in May at the higher altitude tributaries at Fish Creek and Crooked Fork weirs.

# Section B: Kelt Reconditioning Physiology Studies

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## Introduction

In 2009 and 2010, studies were initiated to apply tools from fish physiology and endocrinology to issues in kelt reconditioning. By developing and applying indices based on the endocrinology and physiology of reproduction, growth, stress, and osmoregulation in fish, we aim to achieve a detailed understanding of the physiology of reconditioning in kelt steelhead. This knowledge will provide a scientific basis for maximizing the success of kelt reconditioning programs. This research project has goals of establishing a hatchery model of Snake River B-run kelt steelhead, establishing post-spawning rainbow trout as a model for studying reconditioning in kelt steelhead, establishing and validating assays for plasma and tissue level bioindicators of reproductive status, growth, and stress in steelhead kelts and post-spawning rainbow trout, comparing reconditioning profiles of kelt steelhead at different locations in the Columbia basin and rainbow trout using non-lethal sampling, and testing specific interventions such as ghrelin administration to stimulate appetite and growth in the rainbow trout model.

## Steelhead Kelt Physiology Studies

Columbia basin steelhead vary greatly in life history, migration distance, and genetic stock (Brannon, et al. 2004). CRITFC and our collaborators are implementing kelt reconditioning projects at Omak Creek on the upper Columbia, on the Hood River at Parkdale, on the Yakima River at Prosser, and in the Snake River Basin at Dworshak. One of the objectives in the CRITFC kelt project under the Columbia Basin Accords is to compare kelt reconditioning at different locations. We are collecting blood samples to compare kelt reconditioning endocrinology and physiology across the Columbia Basin. Our goals are to develop methods for monitoring reproductive development of kelts, selecting fish for reconditioning, and enhancing the survival, growth, and rematuration of kelts in reconditioning programs. These studies become much more powerful and informative when repeated samples from the same individual fish can be taken, and the survival and reproductive outcome for the individual are known. Because this is often difficult with wild endangered fish, we believe that the establishment of a hatchery model for kelt reconditioning is of critical importance in kelt research. We took steps toward establishing such a this model in 2011 using artificially spawned Dworshak hatchery B-run kelts. For 2010, we are in the process of completing laboratory and data analysis of samples taken from fish in the kelt reconditioning program at Prosser, WA, which is presented in Section A.

## Rainbow Trout Physiology Studies

Very little is known about post-spawning physiology in kelts or in salmonids in general. Lethal sampling and experimental manipulations are difficult with kelts due to the endangered status of fish in most reconditioning programs. Therefore, we have begun studies on post-spawning physiology in rainbow trout. Our initial goal is to construct a profile of growth and reproductive endocrine physiology in post-spawning female rainbow trout. This can then be compared to profiles from kelts, and treatments to stimulate feeding, enhance survival, and increase reproductive maturation can be tested in rainbow

trout. In 2010, we complete an initial study on the physiology of post-spawning rainbow trout, which is presented in Section B. In 2010, we also completed a study testing the effects of administration of the stomach hormone ghrelin to stimulate appetite in rainbow trout, which is presented in Section C.

## References:

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## Section B.I: Reproductive Development and Migration Behavior of Reconditioned Steelhead (Oncorhynchus mykiss) Kelts in the Yakima River, Washington

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## Introduction

Information about historic or current levels of steelhead iteroparity in the Columbia River basin is extremely limited, however estimates range from 0.5-17%, depending on the population (Long & Griffin 1937; Whitt 1954; Leider *et al.* 1986; Meehan & Bjornn 1991; Branstetter *et al.* 2007; Keefer *et al.* 2008). Various anthropogenic effects including high adult mortality associated with the hydroelectric system has led to Endangered Species Act listing of most Columbia River steelhead stocks. Starting in 1999, efforts to restore wild steelhead in the Yakima River have included long term captive reconditioning of post-spawn fish (kelts). Downstream migrating kelts are collected in late spring, tagged with passive integrated transponders (PIT tags), placed in tanks, treated for fungus and other diseases, and fed over the summer. During the fall upstream migration period the kelts are released back into the river. The objective of this program is to increase the rate of iteroparity by releasing reconditioned and sexually rematuring kelts into the river during the normal upstream migration period. However, not all fish that survive and grow during reconditioning initiate a reproductive cycle to spawn the following spring. Here we present data from the two most recent years (2009/2010) of the kelt reconditioning program on the Yakima River in which we sought to identify the proportion of reconditioned female kelts that initiated ovarian development. Furthermore we compared kelt reproductive status and muscle lipid levels to observed post-release migration behavior to estimate the potential contribution of reconditioned kelts to the spawning population.

## Methods

### Fish Husbandry

Wild endangered Yakima River steelhead kelts were captured and reconditioned at Prosser, WA, during the 2009 and 2010 seasons, following protocols established by CRITFC and Yakama Nation Fisheries (Evans *et al.* 2001; Branstetter *et al.* 2007). Fish in the general population of kelts were housed in four 20' diameter tanks. Survival to release in the general population was 27.5% in 2009 and 38.7% in 2010.

### Fish Sampling

In 2009 and 2010, fish from the general population of kelts captured during downstream migration at Prosser dam and stocked into reconditioning tanks were sampled at intake at intake (March-June) and release (October). In addition, in 2009, after the intake plasma samples were collected, a random sub-set of the captive kelts in the general population was sampled in June (n=29) and August (n=31). In 2010, a small (12' diameter) tank was set aside for repeated sampling of individual kelts in order to gain a more comprehensive understanding of the reconditioning and maturation process. Downstream migrating kelts captured at Prosser were stocked into the small tank from June 3-12, near the end of the kelt outmigration season. Small tank kelts (n=42) were sampled again in July and August and at the release sample. Due to conditions encountered in sampling under field conditions, some individual fish were missed at some sampling points in both the general population studies and the small tank study.

### Plasma Collection

Blood samples (3 mL) were collected from anesthetized fish by ventrally inserting a 20-gauge needle into the caudal vein. Each syringe had been internally coated with a heparin solution (3mg/mL in water) to prevent coagulation. After collection, blood was either briefly stored on ice or immediately centrifuged

for 5 minutes at 1000 g to separate plasma from red blood cells. Plasma was then isolated and frozen on dry ice in the field and was stored at -80°C until time of analysis.

Vitellogenin and Muscle Lipid Measurement

Plasma vitellogenin (VTG) concentrations were measured with a rainbow trout enzyme-linked immunosorbent assay (ELISA) kit (Biosense, Cayman Chemical Ann Arbor, MI). All VTG measurements were obtained from freshly thawed plasma samples that had only been frozen one time. Plasma samples were appropriately diluted and triplicate technical replicates assayed in the ELISA according to the manufacturer's instruction manual provided with the kit.
The Distell Fish Fatmeter is an instrument that uses a very low power microwave signal to measure body lipid levels in fish. Fatmeter measurements are rapid, do not harm fish, do not affect the operation of PIT or radio tags, and do not affect egg quality (Colt & Shearer 2001). The Fatmeter averages a number of readings from an individual fish to come up with an overall estimate of lipid levels. Fatmeter readings in our studies were taken at the 2 most anterior measurements sites recommended by the manufacturer on one side of the fish: ~ 1 cm above the lateral line immediately posterior to the operculum, and ~ 1 cm above the lateral line below the anterior half of the dorsal fin, as recommended by researchers working on live adult salmonids (Colt & Shearer 2001; Crossin & Hinch 2005).

Statistical Analysis

All statistical analyses were performed with GraphPad Prism 5 for Mac OSX, GraphPad Software, San Diego California USA, www.graphpad.com". All Fat Meter data were assumed to be non-normal and were analyzed by non-parametric one-way ANOVA (Kruskall-Wallis test) and t test (Mann-Whitney U). Overall significance effects for more than two groups were then tested with Dunn's post-test ($p<0.05$).

**Results**

**Prosser Kelt Vitellogenin**

Plasma VTG measurements were obtained from 249 kelts over two years (2009/2010) of the long term reconditioning program at Prosser Washington. We compared VTG and ovary necropsy data from five mortalities at the time of release in 2010 to categorize release fish from both years into maturing (≥1 mg VTG/mL; ovaries with large follicles) and non-maturing (<1 mg VTG/mL; ovaries with small follicles) groups at the time of release (Fig. 1, Fig. 2)[*]. In both years, plasma VTG levels at release were bi-modally distributed with median values of 14.2 $x10^{-6}$ mg VTG/mL and 2.9 mg VTG/mL for the low and high modes, respectively. In 2009, 53% (45 of 85) of sampled kelts had maturing levels of VTG at the time of release (Fig. 1). In 2010, 25% (26 of 106) of sampled kelts had maturing levels of VTG at the time of release (Fig. 2). Over the two years, 37% of sampled kelts had maturing levels of VTG at the time of release.

In the random sub-samples of kelts from the general population taken in 2009, although the VTG levels observed in the June sample varied widely (0-1.72 mg/mL) there was no separation of the kelts into

[*] This is probably a conservative estimate of maturation; fish with intermediate values of VTG (0.1-0.5mg/mL; n=7 for 2009/2010) may have been able to produce viable gametes by the time of spawning several months after release.

clearly identifiable maturing and non-maturing groups. By August however, the kelt maturation trajectories appeared to separate into two obvious clusters of maturing and non-maturing fish (Fig. 1).

In the small tank of kelts serially sampled in 2010, the observed pattern of VTG production over the summer reconditioning period was similar to that seen in 2009. Starting in July, there was a gradual increase in the level of VTG and by October there was a clear separation of the kelts into maturing and non-maturing groups (Fig. 2). Kelts in the small tank followed three maturation trajectories. Representative maturation trajectories are shown in Figure 3. Non-maturing fish exhibited two different patterns, in the first pattern there was a consistent drop in VTG production over time; in the second pattern there was an initial drop in VTG after intake followed by a temporary increase observed in August and finally another drop in VTG observed in October. All maturing fish initially decreased VTG production after intake and then starting in July, gradually began increasing VTG production to levels greater than 1mg/mL at release in October.

**Prosser Kelt Muscle Lipid Levels**

Muscle lipid levels were measured in 222 kelts over two years (2009/2010) of the long term reconditioning program at Prosser Washington.

In 2009, Fatmeter readings were only obtained from kelts (n=99) at the time of release and the values ranged from 0.6% to 9.3%. There was a significant difference in percent fat between maturing (>1mg/mL VTG) and non-maturing (<1mg/mL VTG) fish (Fig. 4) suggesting that increased lipid energy stores are associated with rematuration in reconditioning programs.
In 2010, Fatmeter readings were obtained at intake and release from the general population of kelts, and at intermediate time points for the small tank group of fish. The mean percent fat increased significantly through the reconditioning period in the general population (Fig. 5). In the small tank fish, muscle lipid levels decreased significantly from intake to 21 July, and then increased significantly through the rest of the reconditioning period (Fig. 6). At the time of release, readings were taken from 108 kelts and the values ranged from 0.6% to 7.5%. Although the pattern of percent fat between maturing and non-maturing fish at release was the same as that observed in 2009, the difference was not significant (Fig. 4; p=0.062).

**Post-Release Migration Behavior and Physiological Indices**

Post-release migration behavior of kelts from 2009 and 2010 was monitored via PIT-tag detections at Prosser dam to estimate the potential contribution of reconditioned kelts to the spawning population. These data were then compared to reproductive status as assessed by plasma VTG, and energy stores as assessed by muscle lipid levels.
In 2009, 53% (45 of 85) of sampled kelts had maturing levels of VTG, and of these 33% (15) were detected moving upstream. No non-maturing fish were detected moving upstream (Fig. 7). In 2010, 26% (25 of 106) of sampled kelts had maturing levels of VTG at the end of the reconditioning period (Fig. 8). Of the 106 sampled fish, 37 were transferred to a spawning channel at the Prosser kelt facility and not released, which makes comparing the migration data from 2009 and 2010 difficult. Of the sampled and released kelts, 16% (17 of 69) were detected moving upstream and of these 17 fish, three had maturing levels of VTG (Fig. 8).

Muscle lipid levels measured at the time of release in 2009 were higher in both female (4.9 ± 0.4%; n=29) and male (6.6 ± 0.79%; n=4) kelts detected moving upriver than in female (3.4 ± 0.2%; n=96) and male (3.2 ± 0.62%; n=8) kelts not detected. Furthermore, there were four females released in 2009 that were recaptured in the spring of 2010 that had the highest muscle lipid levels (7.7 ± 0.9%; n=4) measured at the 2009 release (Fig. 9a). In 2010, the same pattern was observed, female (2.7 ± 0.3%; n=24) and male (2.9%; n=1) kelts detected moving upriver had higher muscle lipid levels than female (1.3 ± 0.1%; n=55) and male (0.9 ± 0.3%; n=2) kelts not detected. As of this writing no data are available on recapture rates for fish released in 2010. These data suggest that percent body fat is a strong predictor of upstream migratory behavior after release.

**Discussion**

Measurement of plasma VTG levels in Prosser kelts showed that there are potentially two life histories in the fish: sequential spawners, which have initiated ovarian development at the time of release, and skip spawners, which have not initiated ovarian development and will not be able to spawn the following spring. The percentage of sequential spawners ranged from approximately 25 to 50 percent over the two years of the study. The skip spawning life history has been documented in naturally repeat spawning fish in the Snake River and British Columbia, where approximately 50% of repeat spawning fish are of this type (Keefer *et al.* 2008). It is reasonable to hypothesize that many surviving fish that were not maturing at the time of release will mature the following year if properly cared for. Therefore, a management strategy to handle the skip spawning life history type will need to be developed. If successful, such as strategy could more than double the success of the Prosser kelt reconditioning project.

Data from the 2009 release suggested that only maturing fish migrated upriver. However, not all maturing fish from the 2009 release were detected at Prosser dam. The fate of the maturing fish not detected is not known at present. It is possible that an improved release strategy could result in a larger number of these potential spawners migrating upriver. Data from the 2010 release are not comparable to 2009 due to the fact that the majority of maturing fish were kept for the spawning channel. Nevertheless, in 2010, non-maturing fish were detected moving upriver through Prosser dam. The reason for the difference between years is not known. Additional years of data are required to determine the migration behavior of maturing and non-maturing kelts released into the river in the fall. Muscle lipid level at release as measured with the Distell Fish Fatmeter was a good predictor of post-release upstream migration, and was also positively associated with maturation. These findings are consistent with energetic constraints limiting maturation and spawning migration in reconditioned steelhead kelts. Fish recaptured in the spring of 2010 had very high muscle lipid levels at release in 2009. It is difficult to explain the behavior pattern of these fish without concluding that they must have spawned. This suggests that, to produce fish that are likely to spawn successfully, the Prosser kelt reconditioning project can aim to produce fish with high muscle lipid levels measured non-lethally with the Fatmeter at release. This is an issue in fish nutrition.

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Figure Legends

Figure 1. Vitellogenin (VTG) levels in kelts in the general population through the 2009 reconditioning period. All fish were sampled at intake and release, and a random sub-set of fish were sampled on 22 June and 18 Aug. VTG is displayed on log10 scale. The empirically derived maturation threshold (1mg/mL) is designated by dotted line.
Figure 2. Vitellogenin (VTG) levels in kelts in the general population (black closed circles) and small tank (green closed circles) through the 2010 reconditioning period. VTG is displayed on log10 scale. The empirically derived maturation threshold (1mg/mL) is designated by dotted line.
Figure 3. Representative vitellogenin (VTG) profiles of maturing (open circles and squares) and non-maturing (closed circles and squares) serially sampled kelts in the small tank through the 2010 reconditioning period. VTG is displayed on log10 scale. The empirically derived maturation threshold (1mg/mL) is designated by dotted line.
Figure 4. Percent body fat at release in Non-maturing and Maturing kelts in 2009 and 2010 (Mann-Whitney U test; *$p<0.05$). Error bars represent standard error of the mean (± SEM).
Figure 5. Percent body fat of kelts in the general population at intake and release in 2010 (Mann-Whitney U test; *$p<0.05$). Error bars represent standard error of the mean (± SEM).
Figure 6. Percent body fat of serially sampled small tank kelts through the 2010 reconditioning period. Letters represent statistical difference between sampling dates according to Dunn's post-test (*$p<0.05$). Error bars represent standard error of the mean (± SEM).
Figure 7. Vitellogenin (VTG) levels at release in 2009 of all sampled kelts, showing upstream migrants detected at Prosser dam (open squares) and fish that were not detected (closed circles).
Figure 8. Vitellogenin (VTG) levels at release in 2010 of all sampled kelts showing upstream migrants detected at Prosser dam (open squares), released fish that were not detected (closed circles), fish selected for the spawning channel (blue diamonds), and known maturing (closed squares) and non-maturing (closed triangles) post sampling mortalities.
Figure 9. Percent body fat of a) female and b) male kelts released in October of 2009 that were Not Detected, Detected and Recaptured the following spring at Prosser Dam on the Yakima River. Letters represent statistical difference between the groups according to Dunn's post-test (Mann-Whitney U test; *$p<0.05$).
Figure10. Percent body fat of a) female and b) male kelts released in 2010 that were Not Detected and Detected at Prosser Dam on the Yakima River. (Mann-Whitney U test; *$p<0.05$).

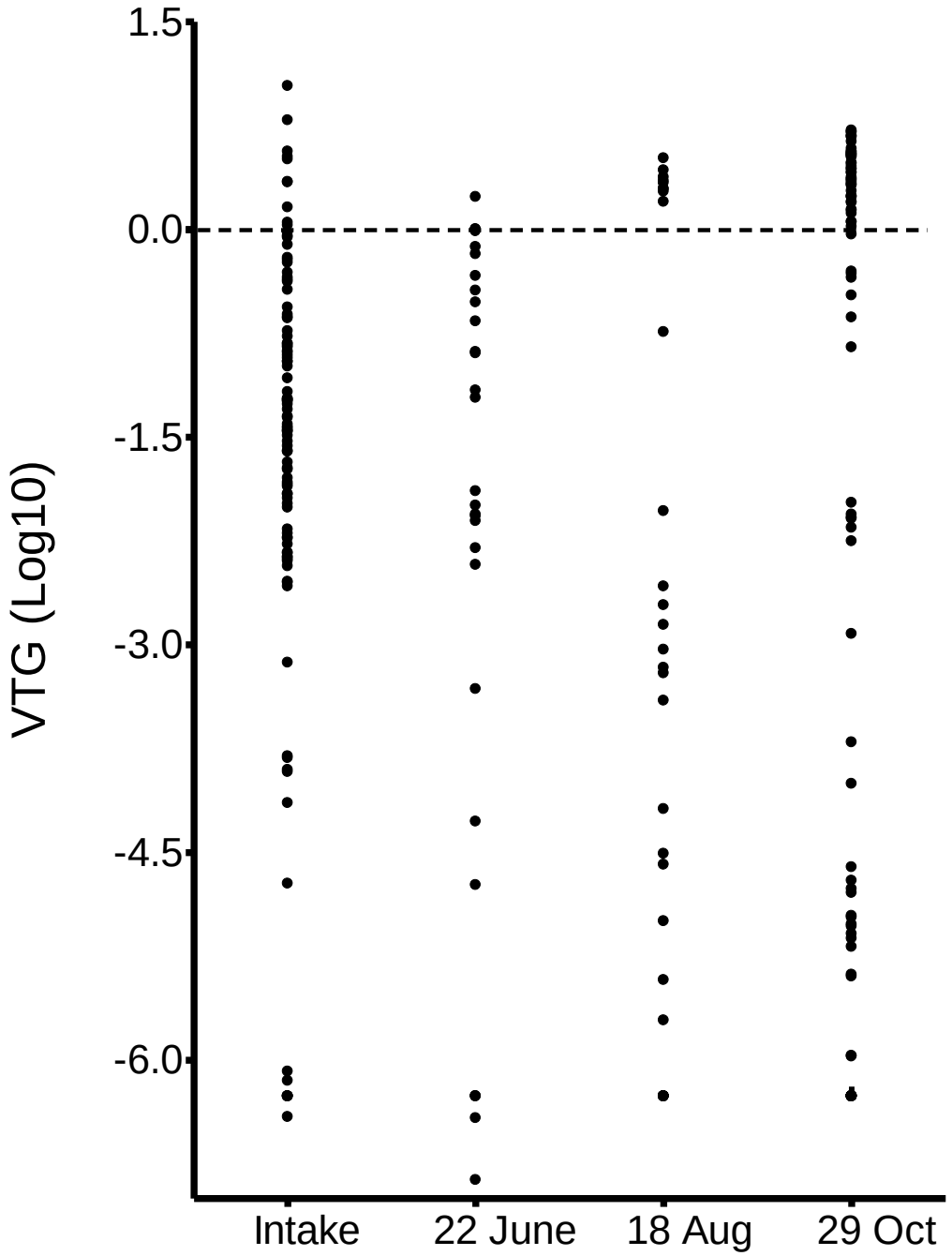



Figure 1

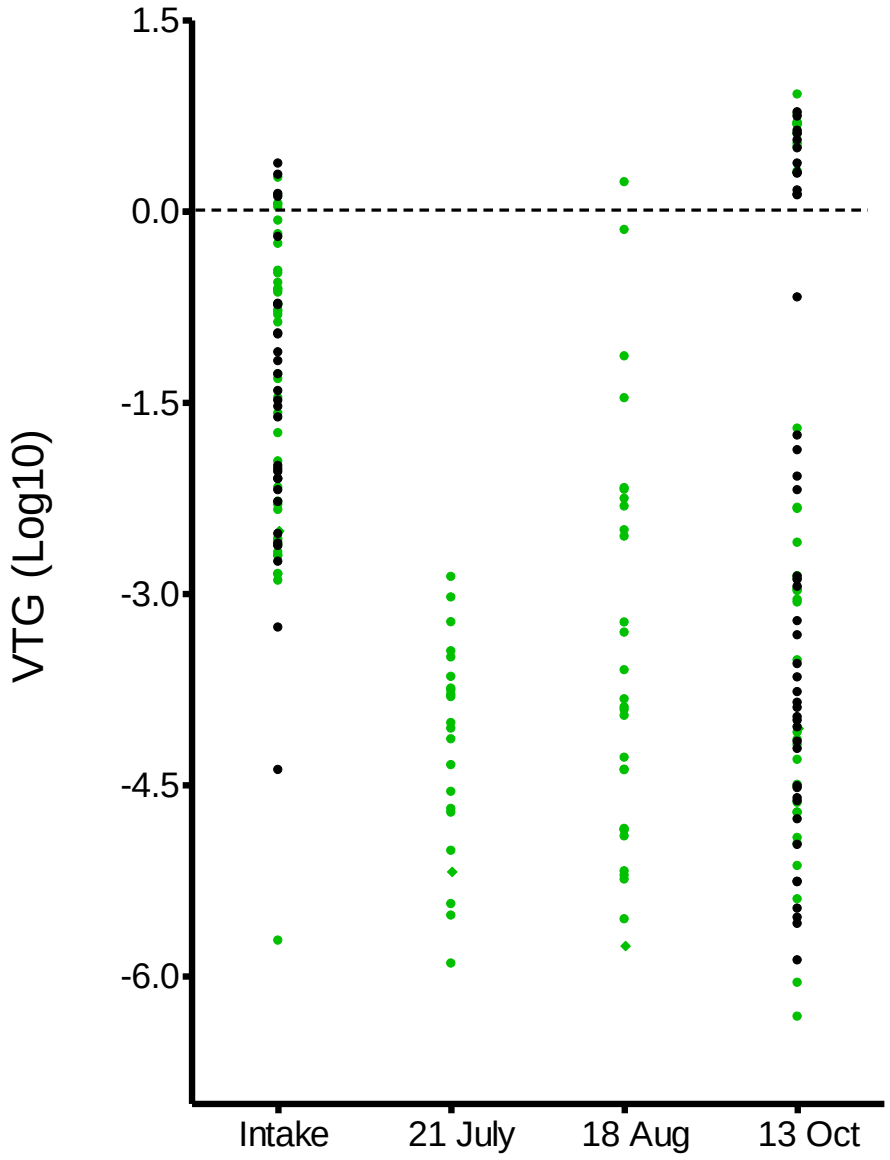



Figure 2

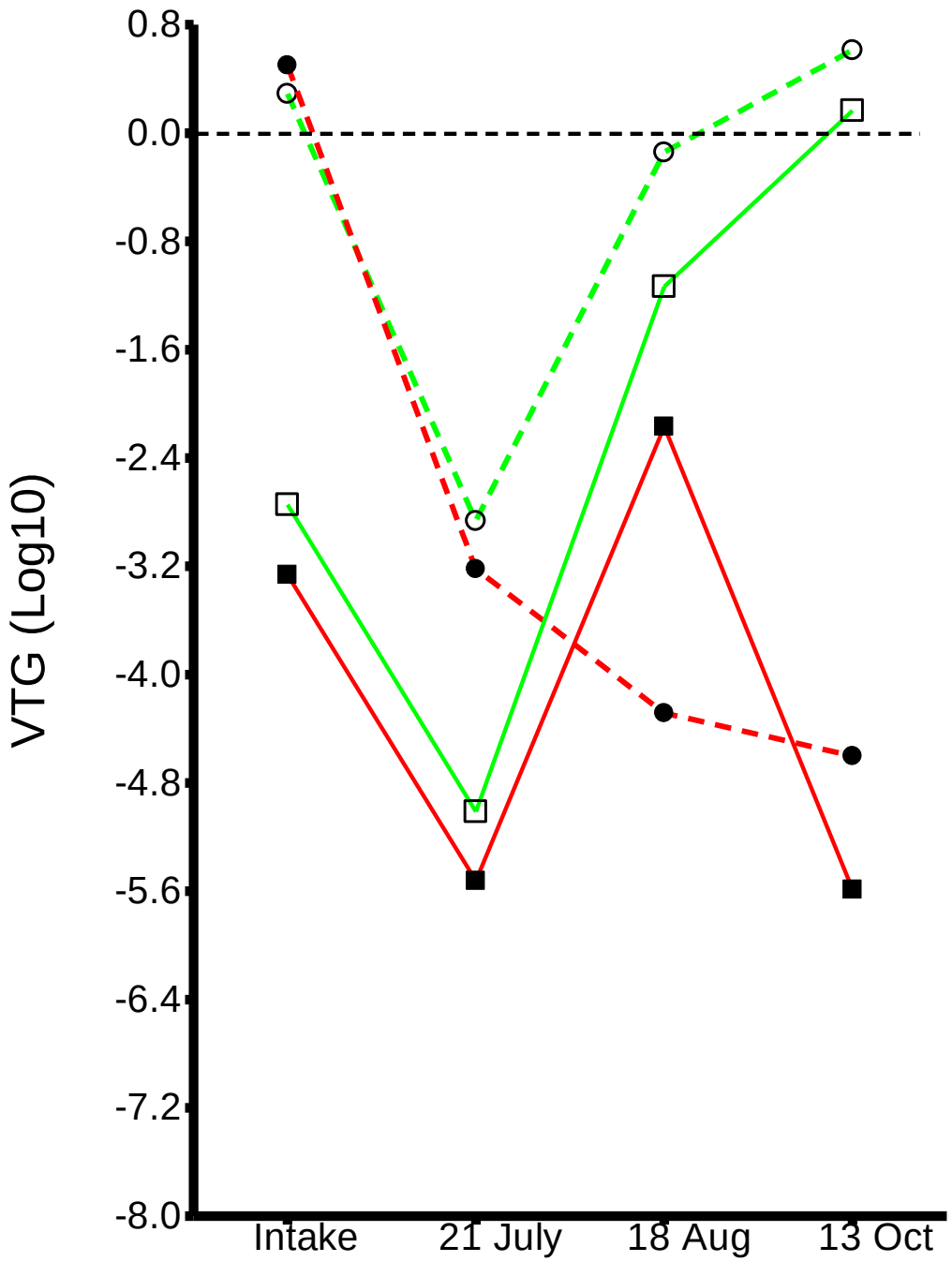
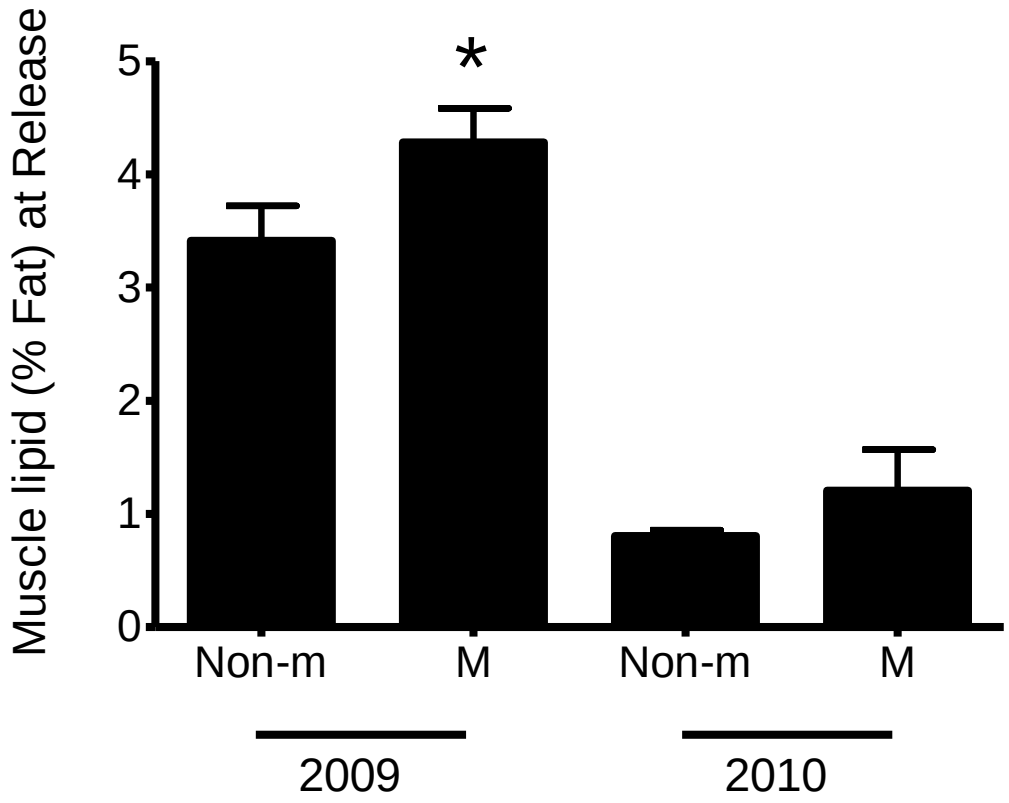

0.8
0.0
-0.8
-1.6
-2.4
-3.2
-4.0
-4.8
-5.6
-6.4
-7.2
-8.0
VTG (Log10)
Intake
21 July
18 Aug
13 Oct

Figure 3

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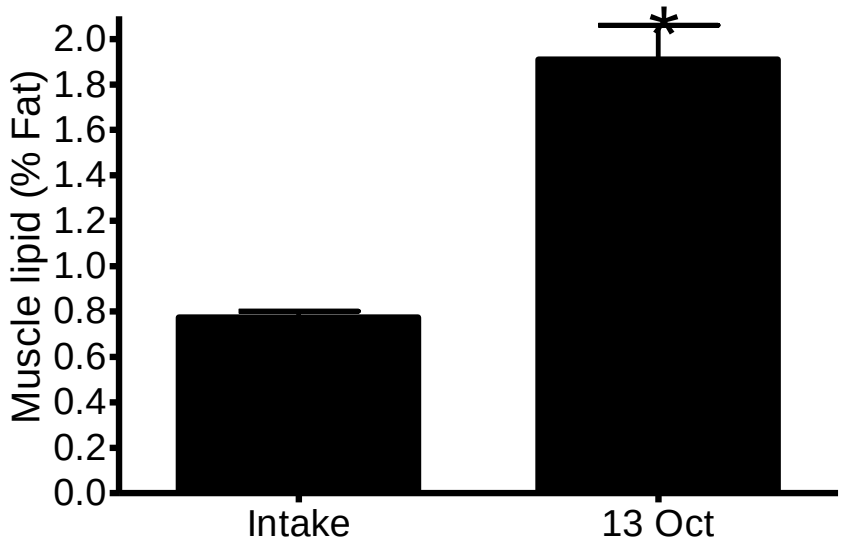


Figure 5

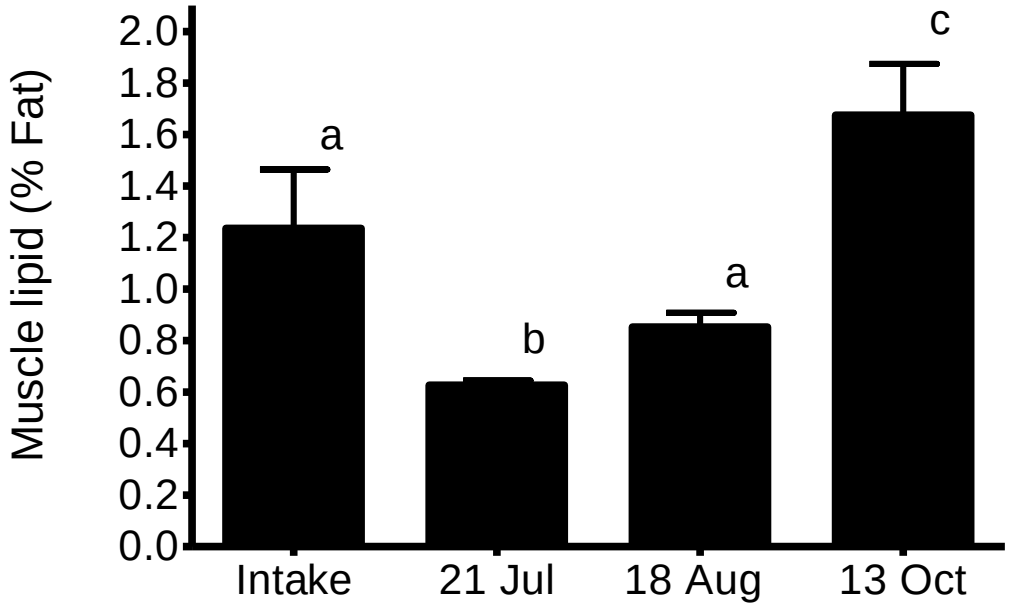


Figure 6

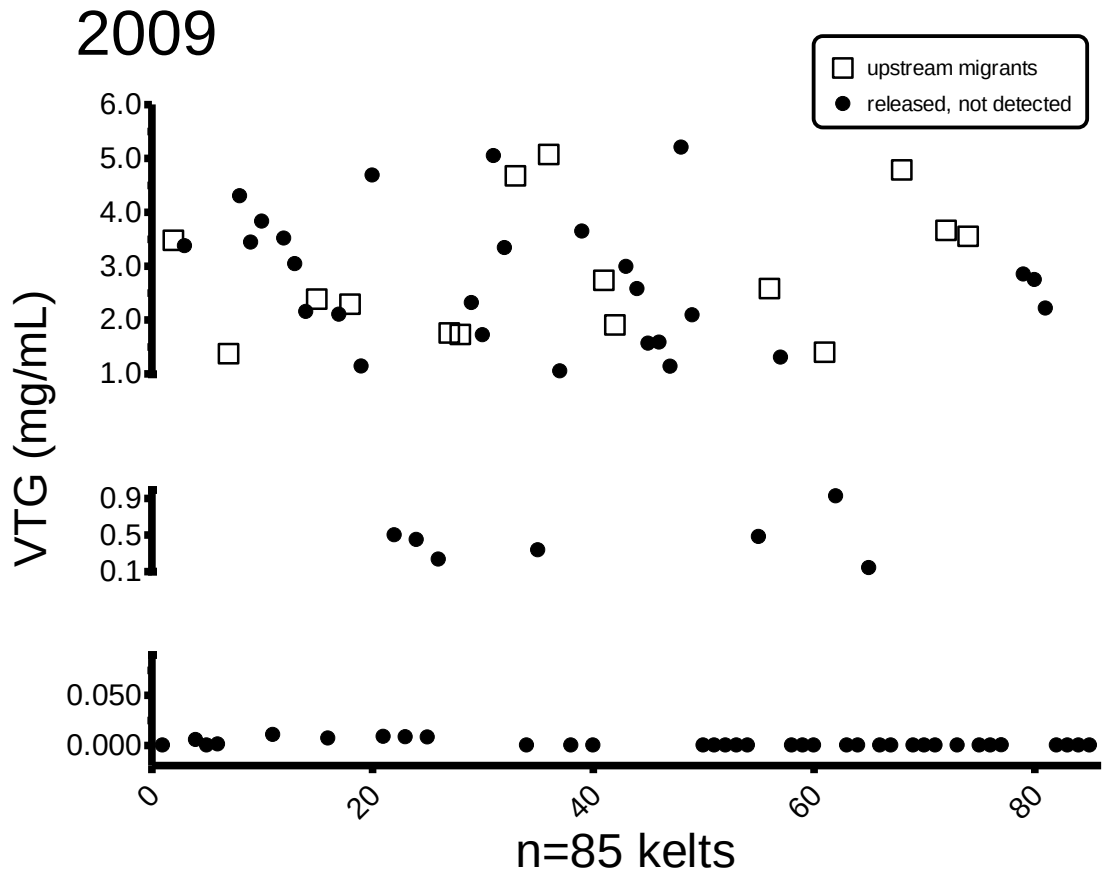
2009
upstream migrants
released, not detected
VTG (mg/mL)
6.0
5.0
4.0
3.0
2.0
1.0
0.9
0.5
0.1
0.050
0.000
0
20
40
60
80
n=85 kelts

Figure 7

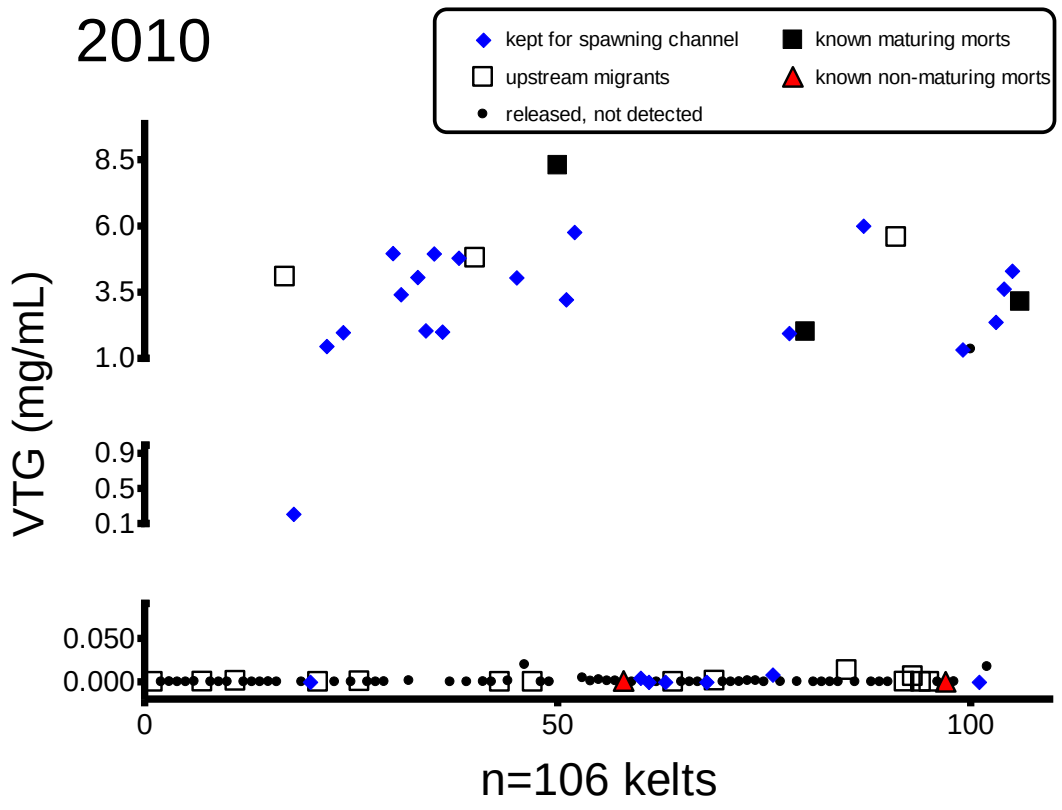
2010
kept for spawning channel
known maturing morts
upstream migrants
known non-maturing morts
released, not detected
VTG (mg/mL)
8.5
6.0
3.5
1.0
0.9
0.5
0.1
0.050
0.000
0
50
100
n=106 kelts

Figure 8

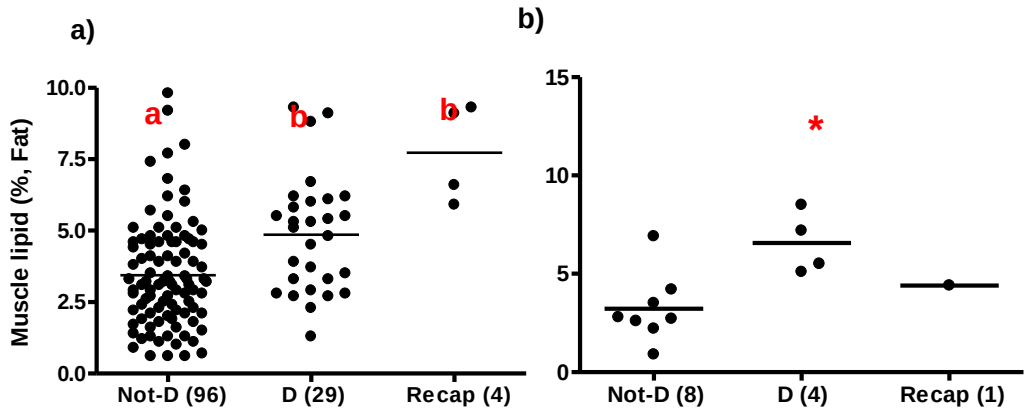


Figure 9

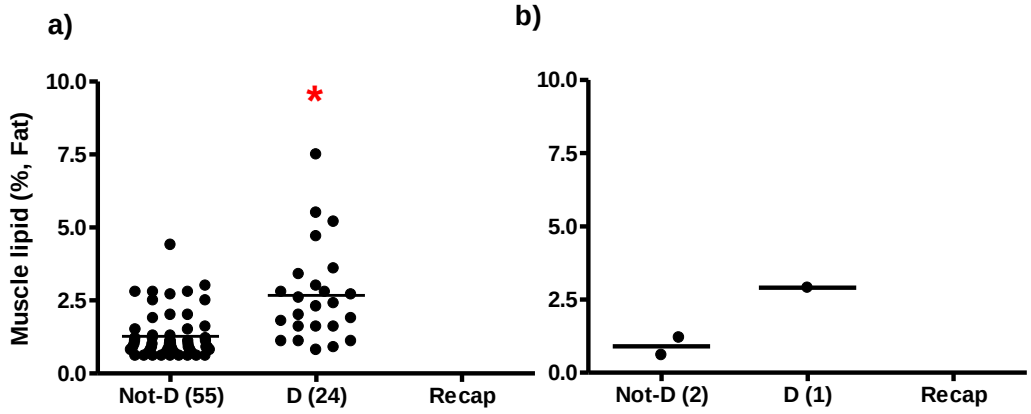


Figure 10

## Section B.II: Effects of energy restriction on metabolic factors and reproductive development in post-spawning female rainbow trout

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**Introduction**

Migration and reproduction in salmonids are energetically costly processes; energy reserves during hypothesized critical periods are an important consideration in decisions to initiate and continue sexual maturation. Steelhead (*Oncorhynchus mykiss*) reconditioning projects are ongoing throughout the Columbia Basin, in an attempt to increase iteroparity within populations of these fish. By clarifying the role that energetic availability plays in gating entry to the reproductive cycle, success of these projects could be improved. However, manipulative experiments and lethal sampling are difficult with kelts. Therefore, we aimed to establish post-spawning rainbow trout as a model system for studying the biology of reconditioning and reproductive development in steelhead kelts. The purpose of this study was to determine how energy balance affects the onset of a new reproductive cycle in the iteroparous rainbow trout life history form of *O. mykiss*.

**Methods**

150 three-year-old female rainbow trout were obtained from a commercial broodstock operation immediately after their second spawning, on 25 February 2010. Fish were held at University of Idaho (Moscow, ID) in six re-circulating tanks (volume approximately 1000L per tank) that share a common head-tank. The group of fish was split into two treatment groups, a control group and a food-restricted group. The control group received the equivalent of 0.5% of body weight in food per day, administered over the course of five days per week (*i.e.,* 0.7% of body weight on Thursday–Monday). The food-restricted group received the equivalent of 0.1% of body weight in food per day, administered on one day per week (*i.e.*, 0.7% of body weight on Monday). This feeding schedule for the food-restricted fish was chosen to minimize dominance effects within the tank that may otherwise have prevented all fish in a tank having access to the limited amount of food. At the first sampling date, all fish were implanted with PIT tags, to enable tracking individuals through the course of the experiment. At four-week intervals beginning on 4 March 2010, all fish were measured for length, weight, and muscle lipid content (using a Distell fish fatmeter). Fatmeter readings were highly correlated ($r^2$>0.6) with proximate composition analysis measuring lipid content of muscle fillets (data not shown). Specific growth rate for weight (SGR-W) was calculated as $\frac{\ln(weight_2/weight_1)}{days\ between\ measurements} \times 100$. Fulton's condition factor (*k*) was calculated as $(\frac{weight}{length^3}) \times 1000$. Liver and pituitary were collected from all terminally sampled fish for q-RT-PCR analysis of tissue mRNA level for genes encoding insulin-like growth Factor-1 (IGF-1), IGF-binding protein-1 (IGFBP-1), and follicle-stimulating hormone (FSH). q-RT-PCR data were analyzed by absolute quantification, based on a standard-curve regression, and mRNA levels were relativized to levels of a reference gene specific to each tissue ($\beta$-actin in the pituitary, elongation factor-1$\alpha$ in the liver). A Grubbs outlier test was used to remove obviously anomalous outliers from q-RT-PCR results. All data were analyzed using one- and two-way ANOVA to detect effects of treatment, time, and treatment by time. If effects were detected, Tukey's multiple comparisons test or one-tailed Student's t-test was used to detect specific differences and assign significance. All analyses were performed using the JMP statistical package (JMP, Version 8; SAS Institute Inc., Cary, NC, 1989-2011).

**Results**

Food restriction significantly (*p*<0.05) reduced SGR-W, *k*, and muscle lipid content in female rainbow trout from two months after spawning onward (Fig 1-3). In the liver, IGF-1 gene expression was significantly (*p*<0.05) reduced in food-restricted fish within two months (Fig 4), whereas IGFBP-1 gene expression significantly (*p*<0.05) decreased over time in both groups but did not differ between the two

treatment groups (Fig 5). These results show that this feed restriction treatment affected factors associated with energy balance within two to four months of restricting feed.

In the pituitary, follicle-stimulating hormone (FSH) β subunit gene expression was significantly ($p$<0.05) lower in food-restricted fish at four months (Fig 6). These results show that this feed restriction treatment affected the reproductive endocrine axis within four months of restricting feed.

**Discussion**

While mean weight of fish in the two treatments groups did not notably diverge through the course of the experiment (data not shown), specific growth rate for weight revealed that the rate of change of weight was different among the two groups (Fig 1). This indicated that the restricting feed after spawning did affect growth of female rainbow trout. Body condition (Fig 2) and muscle lipid content (Fig 3) were similarly affected by restricting food availability, and the two measurements likely assess similar aspects of fish physiology. This would suggest that the weight to length ratio (representing a crude approximation of fish girth) reliably indicates energy stores. The difference in muscle lipid content for fish in the two groups suggests that, in addition to absolute differences in weight, body composition was affected by restricting food availability. In light of these observations, it appears that during the four months of this experiment rainbow trout mobilize energy stored as lipid (in both muscle and liver tissue), to fuel critical metabolic processes and attempt to prepare for the next reproductive cycle.

IGF-1 gene expression in the liver was affected by restricting food; IGF-1 interacts with GnRH signaling in the brain and pituitary to regulate gonadotropin gene expression in salmonids (Ando, Luo et al. 2006; Furukuma, Onuma et al. 2008), possibly by inducing competence of pituitary gonadotropes to respond to GnRH (Luckenbach, Dickey et al. 2010). In this manner, IGF-1 may act as a peripheral metabolic signal, indicating energy stores and capacity for undergoing reproductive development to the reproductive endocrine (*i.e.,* hypothalamus-pituitary-gonad) axis. IGFBP-1 provides a means by which organisms regulate availability of IGFs–and thus control IGF signaling activity–by binding free IGFs (Mohan and Baylink 2002). IGFBP-1 appears to be rapidly upregulated during periods of energy debt or otherwise stressful conditions, and may represent a mechanism by which fish and other animals divert energy away from growth toward more immediately vital processes (Kajimura and Duan 2007). The decrease in liver IGFBP-1 gene expression after spawning suggests that plasma IGFBP-1 may be useful as an indicator of spawning stress and post-spawning recovery in rainbow trout and steelhead kelts.

Follicle stimulating hormone (FSH) is a peptide hormone with reproductive signaling actions that appear highly conserved across vertebrate groups (Kah 2009). FSH appears to be important during early stages of reproductive development in most fishes (Taranger, Carrillo et al. 2010), activating steroidogenic enzymes, and stimulating the accumulation of oocyte cortical alveoli in salmonids (Campbell, Dickey et al. 2003; Campbell, Dickey et al. 2006). Additionally, FSH may have steroid-independent gametogenic activity (Schulz, de Franca et al. 2010). Restricting food intake affected expression of the FSH-β subunit in the pituitary, and this likely would lead to inhibition of reproduction, by suppression of steroidogenesis and gametogenesis. This suggests that measurement of plasma FSH may provide an early non-lethal indicator of reproductive development in post-spawning rainbow trout and steelhead kelts.

While it is unknown whether feed restriction would have resulted in failure to complete ovarian development, this study shows that energy availability in the immediate months after spawning affects metabolic endocrinology and slows reproductive development. Further experimentation will be necessary to determine how energy restriction could arrest, or prevent initiation of, ovarian development, and how these results relate to the reconditioning/recrudescence process in steelhead kelts.

**Figures**

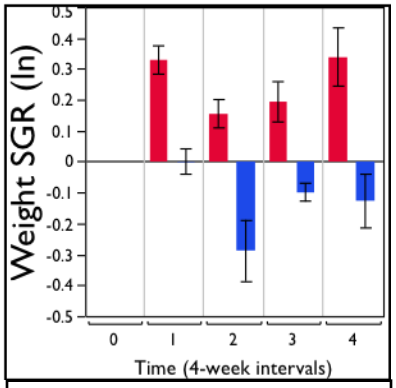


FIG 1: SPECIFIC GROWTH RATE FOR WEIGHT. RED BARS INDICATE THE CONTROL GROUP, BLUE BARS THE FEED-RESTRICTED GROUP. ERROR BARS = 1 SEM.

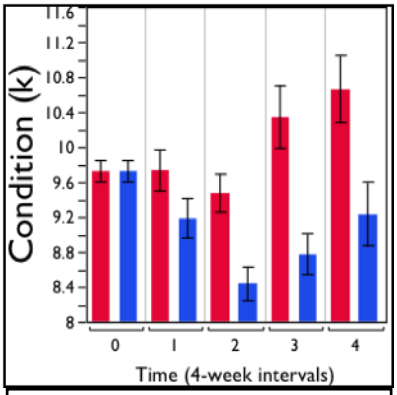


FIG 2: EFFECT OF FOOD RESTRICTION ON FULTON'S CONDITION FACTOR (*K*). RED BARS INDICATE THE CONTROL GROUP, BLUE BARS THE FEED-RESTRICTED GROUP. ERROR BARS = 1 SEM.

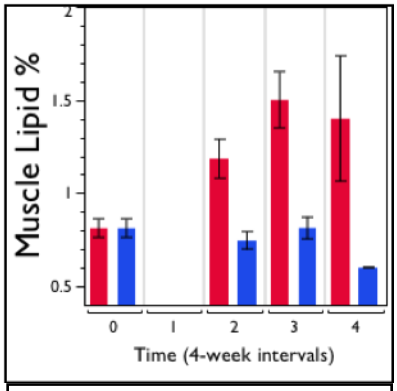


FIG 3: EFFECT OF FOOD RESTRICTION ON MUSCLE LIPID CONTENT (AS MEASURED BY DISTELL FATMETER). RED BARS INDICATE THE CONTROL GROUP, BLUE BARS THE FEED-RESTRICTED GROUP. ERROR BARS

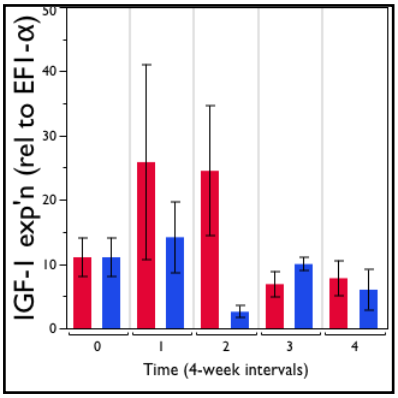


FIG 4: EFFECT OF FOOD RESTRICTION ON LIVER IGF-1 MRNA LEVELS. RED BARS INDICATE THE CONTROL GROUP, BLUE BARS THE FEED-RESTRICTED GROUP. ERROR BARS = 1 SEM.

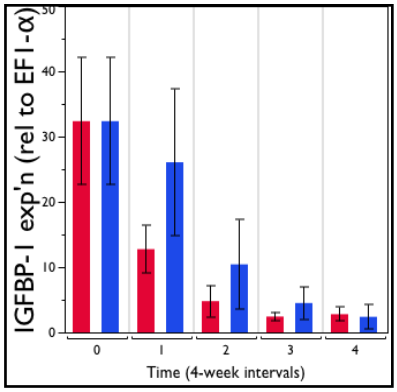


FIG 5: EFFECT OF FOOD RESTRICTION ON LIVER IGFBP-1 MRNA LEVELS. RED BARS INDICATE THE CONTROL GROUP, BLUE BARS THE FEED-RESTRICTED GROUP. ERROR BARS = 1 SEM.

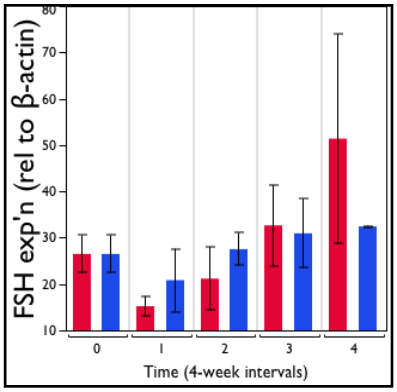


FIG 6: EFFECT OF FOOD RESTRICTION ON PITUITARY FSH MRNA LEVELS. RED BARS INDICATE THE CONTROL GROUP, BLUE BARS THE FEED-RESTRICTED GROUP. ERROR BARS = 1 SEM.

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## Section B.III: Effects of long-term administration of ghrelin and growth hormone on feed intake and growth in juvenile rainbow trout

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**Introduction**

Steelhead fast during their spawning migration. Snake River steelhead kelts show significant atrophy of the digestive tract (C. Moffitt, unpublished results). Getting kelts to initiate feeding appears to be a critical step in reconditioning (Evans *et al.* 2001). In addition, post-spawning fish are highly susceptible to opportunistic pathogens such as fungal infections. Therefore, we propose to explore treatments that may stimulate feeding and immune system function in kelt steelhead. Ghrelin is a peptide hormone produced by the stomach, which is the only peripheral appetite stimulant yet discovered (Kojima & Kangawa 2005). Unlike other appetite stimulating factors, ghrelin crosses the blood-brain barrier. In addition, ghrelin strongly stimulates secretion of growth hormone (GH) by the pituitary. GH stimulates appetite and growth in fishes, and stimulates immune system function (Devlin *et al.* 1994; Bjornsson 1997; Yada 2007). In humans, ghrelin is reduced in stomach atrophy (Osawa *et al.* 2005). Thus, we hypothesize that steelhead kelts may not feed due to reduced plasma ghrelin caused by stomach atrophy. Ghrelin is highly conserved, and commercially available mammalian ghrelins are effective in fish species. In most trials in fish, ghrelin administration increased food intake, growth, and plasma GH levels (Unniappan & Peter 2004; Riley *et al.* 2005; Unniappan & Peter 2005; Shepherd *et al.* 2007). However, in several studies using homologous trout ghrelin, appetite has not been stimulated (Jonsson *et al.* 2007), or has even been suppressed (Jonsson *et al.* 2010). To determine if long term ghrelin or GH administration may stimulate appetite in kelt steelhead, we tested whether these treatments were effective in rainbow trout.

**Methods**

Juvenile rainbow trout (120, 50-100g) were obtained and housed in the ARI lab at UI. On day zero of the experiment (10/25/2010), fish were anesthetized, PIT tagged, length and weight were recorded, and fish were intraperitoneally implanted with Alzet micro-osmotic pumps (model 1007D). Pumps administered n-octanoyl rat ghrelin (20 nanomoles ghrelin $kg^{-1}$ bodyweight $day^{-1}$; PI Proteomics, Huntsville, AL), native bovine GH (10 nanomoles GH $kg^{-1}$ bodyweight $day^{-1}$; United States Biological, Swampscott, MA), or vehicle (non-lactated Ringer's solution, supplemented with 2% Tween 80 and 2% cell culture grade BSA). The detergent and BSA were be included to increase solubility of hormones and reduce dimerization of GH. Under the temperature and salinity conditions used, pumps were calculated to dispense test substances for approximately 25 days. Fish from each treatment were held in separate tanks, 3 tanks per treatment and initially 10 fish per tank. Fish were fed Rangen 4 mm pellets at 9:30 AM and 3:00 PM for 30 minutes daily, uneaten food was collected at the end of the feeding period, uneaten pellets were counted and converted to weight, and the amount of food consumed by each tank of fish was calculated. Fish were nonlethally sampled 10 days after pump implanting, and terminally sampled 20 days after pump implanting. During nonlethal sampling, fish were anesthetized, PIT tag number, length and weight recorded, and returned to the tank. During terminal sampling, fish were anesthetized, PIT tag number, length and weight recorded, and blood drawn for plasma hormone assays. Fish were then killed, the weight of the liver and digestive system (stomach, pyloric caecae, and intestines, excluding the liver) were recorded, and pump operation confirmed. Feed intake of treatment groups was quantified as cumulative percentage body weight of the fish in the tank. Organo-somatic indices were calculated as 100 * [(organ weight) / (body weight of intact fish)]. Individual fish growth rates in length and weight were calculated by the method of Ricker (Ricker 1979).

**Results**

Seventy two fish were implanted with pumps. However, after implanting, descaling and skin lesions were observed on some experimental fish. As a result, 12 fish were culled during the experiment. Culled fish did not have food in the gut on dissection. Consequently, culled fish were excluded from tank biomass in calculation of feed intake rates by treatment, and analysis of organosomatic indices and specific growth rates.

Fish in the ghrelin treated tanks exhibited unusual behavior. During feeding fish were observed to be at the top of the tank, with their backs out of the water, and were highly active. Ghrelin treated fish did not go to the bottom of the tank when tank covers were lifted. Ghrelin treated fish appeared to pursue pellets, but often did not ingest them. Fish in the other two treatments did not show this behavior, but instead would rise to the pellets and then return to the bottom of the tank.

Feed intake in GH treated fish was slightly higher than in the control treatment, whereas ghrelin treated tanks consumed less feed than the other two treatments (Fig. 1).

The hepato-somatic index (HIS) was significantly lower in both ghrelin and GH treated fish versus controls. No significant differences were found between treatments in the viscera-somatic index (Fig. 2).

Ghrelin treated fish had lower growth rates in both weight and length versus the other two treatments Fig. 3). However, these differences were not statistically significant (SGRW ghrelin versus control, p=0.1105; SGRL ghrelin versus control, p= 0.1072).

**Discussion**

This experiment was compromised by poor fish quality. Fish were uneven in size, and excessive mortality (17%) occurred during the 20 days of the experiment. Nevertheless, a few conclusions and directions for future research can be discussed.

Ghrelin appeared to inhibit appetite and growth in rainbow trout at the dosage employed in this study. We used a high dosage of ghrelin to maximize the probability of finding an effect. Another recent study in rainbow trout reported similar findings; however, this study used a cholesterol pellet implant to administer ghrelin (Jonsson *et al.* 2010). The time course and total amount of ghrelin released from the cholesterol pellet are not clear. In other fish species, ghrelin administration stimulates appetite (Unniappan & Peter 2004; Riley *et al.* 2005; Unniappan & Peter 2005; Shepherd *et al.* 2007; Kaiya *et al.* 2008). These findings may be reconciled if the effect of ghrelin depends on dosage. As well as stimulating appetite and growth, ghrelin stimulates corticotrophin-releasing factor (CRF) neural circuits in the brain (Jonsson *et al.* 2010). Stimulation of the CRF system produces increases in activity level and suppresses feed intake (Lowry & Moore 2006), consistent with our observations on the behavior of ghrelin treated fish. Therefore, it is possible that ghrelin administration at a lower dose may increase feed intake and growth in rainbow trout. Further studies are required to test this hypothesis.

GH and ghrelin administration reduced the hepato-somatic index (HSI). High levels of GH are lipolytic, causing release and metabolism of stored lipids (Bjornsson 1997). Ghrelin stimulates GH release by the pituitary. Therefore, the effect of both GH and ghrelin on HSI may be explained by the lipolytic action of GH causing a reduction in liver fat levels.

In this experiment, we demonstrated that administration of test substances using osmotic minipumps is an effective technique for experiments on rainbow trout. Further studies on ghrelin using lower doses are warranted, and the administration of other drugs that may be beneficial in kelt reconditioning can be tested using the techniques established in this study.

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## Figure Legends

Figure 1. Cumulative feed intake as a percentage of the total body weight (% BW) of fish in the experimental treatments.
Figure 2. (A) Hepato-somatic (HSI) and (B) viscero-Somatic (VSI) indices of fish at the final sampling. Lower case letters indicate differences between treatments.
Figure 3. Specific growth rates in (A) weight (SGRW) and (B) length (SGRL), expressed as percent change in body weight (BW) or body length (BL) per day, respectively. Lower case letters indicate differences between treatments.

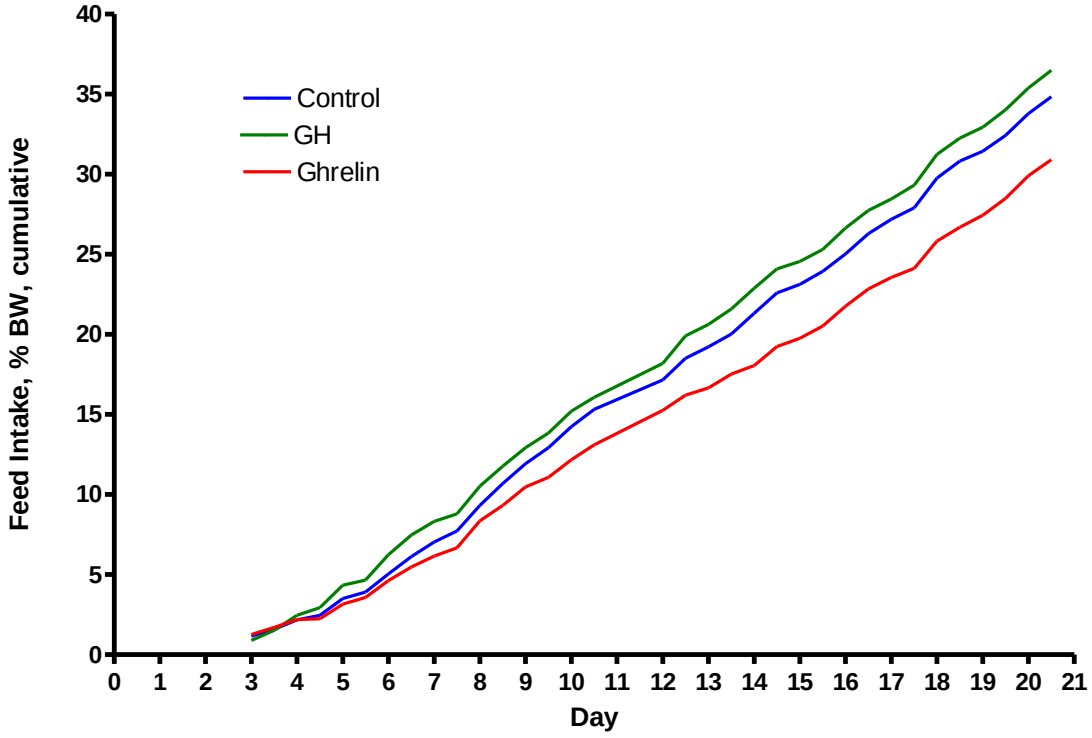
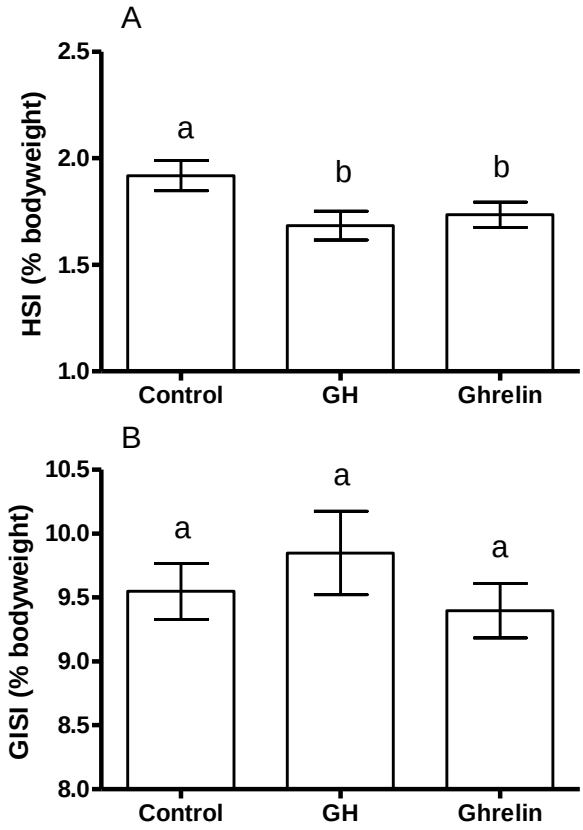

Feed Intake, % BW, cumulative
Day
Control
GH
Ghrelin
0
5
10
15
20
25
30
35
40
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

Figure 1


A
HSI (% bodyweight)
2.5
2.0
1.5
1.0
a
b
b
Control
GH
Ghrelin
B
GISI (% bodyweight)
10.5
10.0
9.5
9.0
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8.0
a
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a
Control
GH
Ghrelin

Figure 2

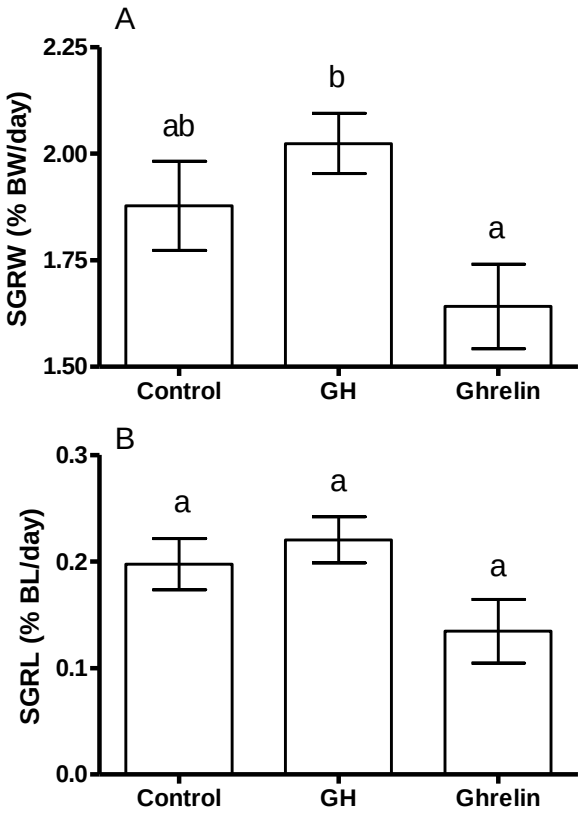
A
2.25
2.00
1.75
1.50
SGRW (% BW/day)
ab
b
a
Control
GH
Ghrelin
B
0.3
0.2
0.1
0.0
SGRL (% BL/day)
a
a
a
Control
GH
Ghrelin

Figure 3

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# Section C: Snake River Master Plan Development Update

## Master Plan Progress

The Master Plan is approximately 50% complete.

## Purpose of the Master Plan

The Northwest Power and Conservation Council (NPCC; formerly the Northwest Power Planning Council - NWPPC) requires Master Plans for new artificial production programs and facilities proposed to restore salmon populations throughout the Columbia River Basin. The purpose of a Master Plan is to provide the NPCC, program proponents, and others with the information they need to make sound decisions about whether the proposed program should move forward to design, construction, and operation.

## Chapters Addressed in 2010:

### CHAPTER 3: INFORMATION USED TO GUIDE MANAGEMENT ACTION SELECTION

- Management Context
- Preliminary Results
    - COE (and others) Operation and Facilities Research
    - Nez Perce Tribe, Yakima (and others) Kelt Reconditioning Research
- Guidance from Water Temperature, Habitat Preference, and Life History Data
- Summary of Life History Characteristics
- Integration of Data Sources

### CHAPTER 6: RESEARCH, MONITORING AND EVALUATION

- Monitoring and Evaluation Goals and Objectives
- Assumptions Associated with Management Action Implementation
- Adaptive Management Approach
- Status Monitoring
- Region Specific Research Activities
- Monitoring and Evaluation of Implemented Actions

## ISSUES OF CONCERN

### *Availability of Data for the Master Plan*

The master plan will need to address baseline data availability. Building on the efforts from the section above, additional information may be necessary to guide management action. Developing ranking criteria will be based on established objectives. However, evaluating actions using these criteria will require knowledge of some vital data associated with each management action. Inherent to the kelt artificial production program are:

**Kelt production levels**

- Collection Numbers, these have varied over the years at current sites
- Collection Sites, pursuing options such are tributary weirs and other mainstem dams
- Collection Type, in river post-spawner versus hatchery air-spawner
- Transport, minimize distance travelled and pre-transport stresses
- Reconditioning, reducing tank densities, increase water quality and feed quality

**Stocking**

- Site selection, mainstem or estuary
- Release Dates, short term, long term, and skip-spawners
- Release Numbers

**Post-release performance**

- Reproductive success and gamete and progeny viability monitoring

The Master Plan will provide an adaptive management platform to address anticipated changes to vital rates. Having good baseline data will narrowing the field of possible contingencies associated with this uncertainty.

## COORDINATION AND MEETINGS

- ISAB meeting, Portland – April 2, 2010
- DNFH Steelhead Kelt Operations Meeting, DNFH – April 12, 2010
- On-site visit for potential kelt collection and holding, Little Goose Dam – June 3, 2010
- Follow-up meeting with Little Goose staff, Little Goose Dam - August 3, 2010
- Lyons Ferry AOP meeting, Dayton - August 31, 2010
- Kelt Research post–season summary meeting, University of Idaho - November 3, 2010
- Hatchery Assessment and Evaluation Team Meeting, DNFH – November 9, 2010
- Air-Spawn training, KNFH – November 16, 2010
- Steelhead Air-Spawn Collection Coordination, DNFH – November 17, 2010
- Kelt Research Field Operations Coordination, University of Idaho – December 15, 2010
- DNFH Steelhead Pre-Spawning Coordination, DNFH – January 4, 2011
- Clearwater Pre-AOP Meeting, Clearwater Hatchery – January 20, 2011
- Clearwater AOP Meeting, DNFH – February 16, 2011
- Pre-season LGR Kelt Activities Coordination, University of Idaho UI – March, 9, 2011
- Corps of Engineers Staff Kelt Operations Meeting, LGR – March 15, 2011
- Kelt Acoustic tag training, University of Idaho UI – March 18, 2011

In addition to the above meetings, we have conducted numerous coordination conference calls and meetings.

## FUTURE PLANNED ACTIVITIES

### *Coordination*

Continue to facilitate coordination meetings with co-managers, researchers and collaborators. Anticipate continuing monthly coordination meetings throughout the year.

## *Kelt Reconditioning*

**Develop strategies to bolster collection numbers.**

- Continue to experiment with air-spawning to augment specimens.
- Pursue other collection sites such as tributary weirs and other mainstem dams.

**Enhance transport systems.**

**Improve environment during collection and holding.**

- Increase primary water supply quality and reliability.
- Develop feed quality.
- Minimize handling stresses.

## *Master Plan Development*

Continue to incorporate information from on-going kelt research activities as data becomes available.

Continue to compile information and conduct appropriate analyses to address concerns and questions raised by the ISRP and other vested interest groups during the master plan review process.

Convene taskforce to develop kelt feed and water quality needs.

# Appendix A. Acoustic line maps

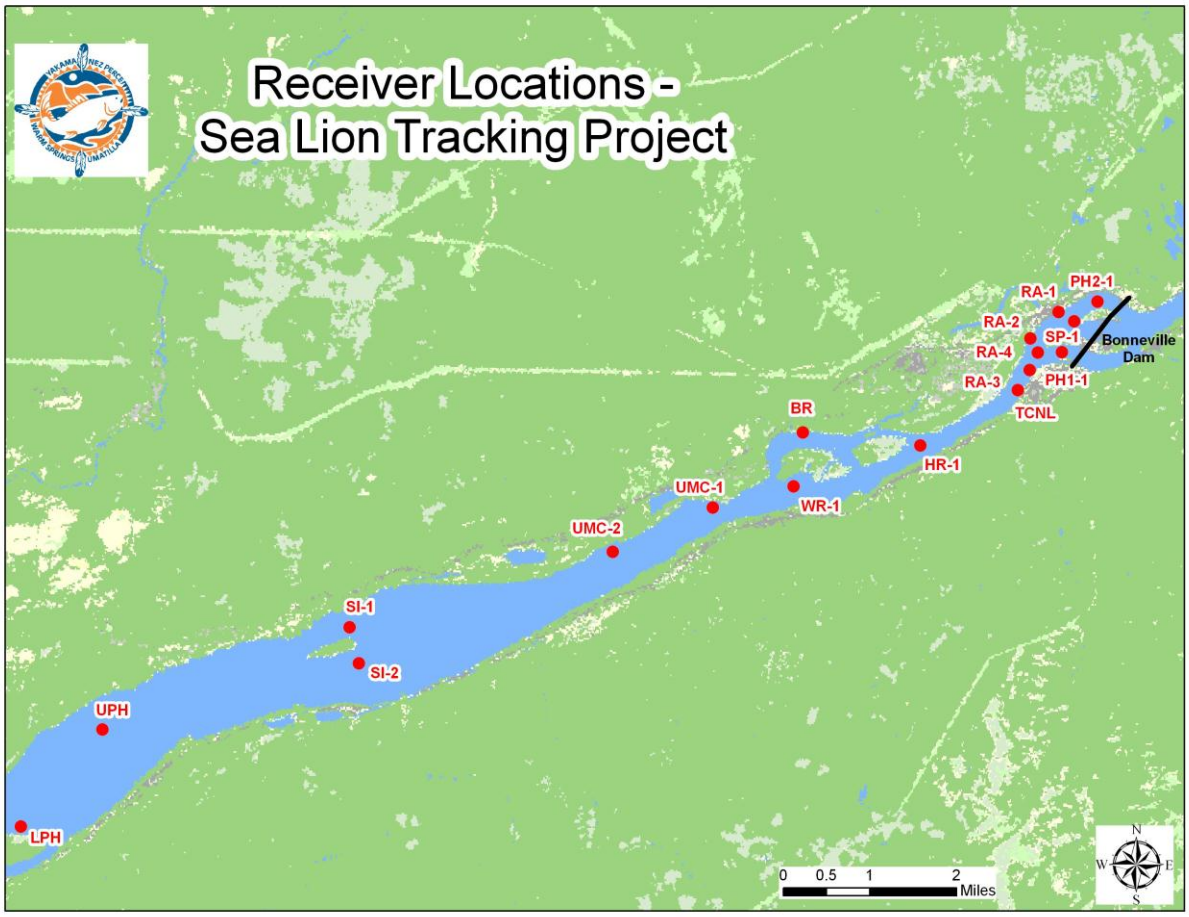



**Figure 1: Bonneville Line (Rkm 233) 2010:**

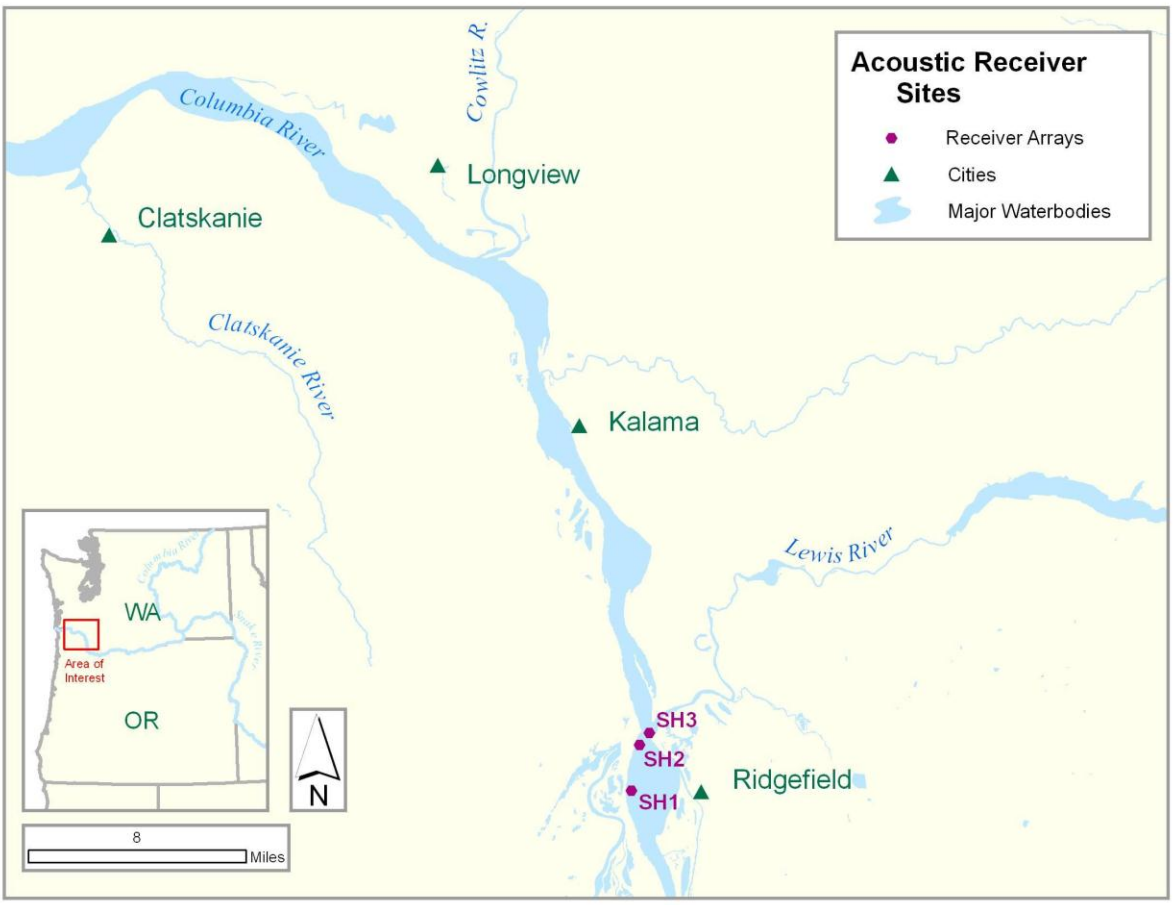



**Figure 2: St. Helens Array (RKM 138) 2010.**

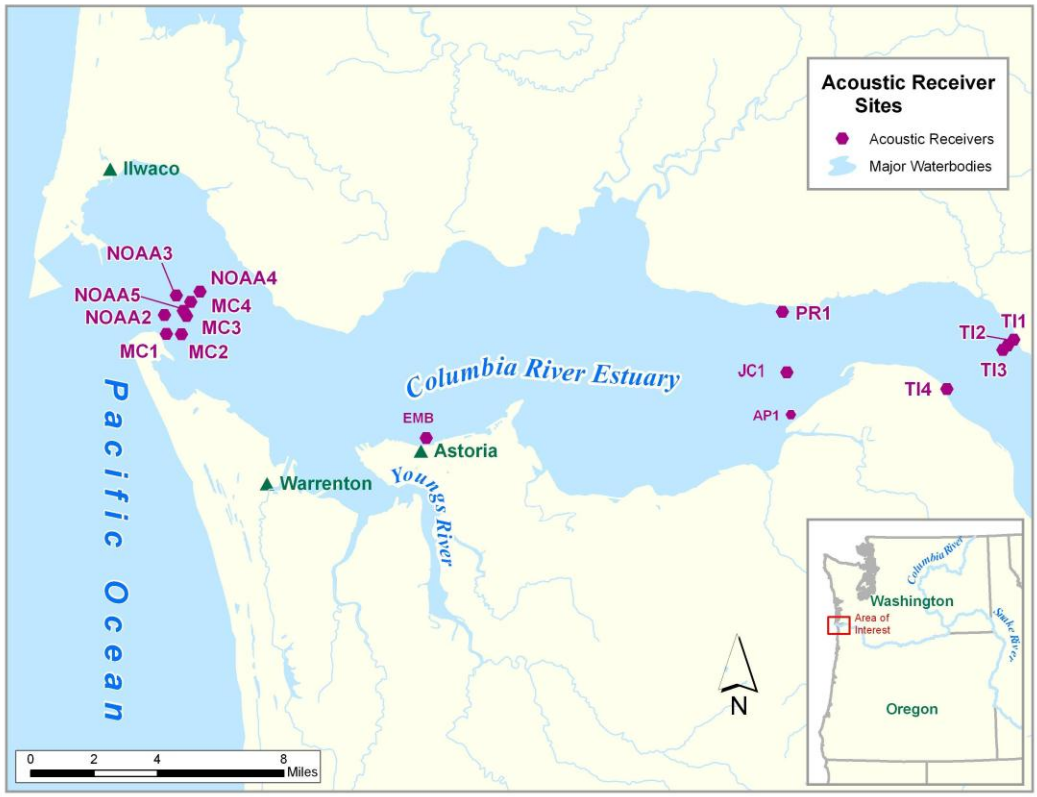



**Figure 3: Estuary Acoustic Array Sets (Mouth of the Columbia Rkm 0 and Estuary Rkm 45).**

C

## Appendix B: Migration Timing of Transport and Release Steelhead Kelts from the Yakima and Snake Rivers.

**Table: Migration timing based on detection data. *Note: Times are in hr:mm:sec**

| Release Group | Ocean or Non-Ocean Migrant | Release to Bonn Det. | Bonn Residence time | Bonn to St. Helens | Release to St. Helens | St. Helens Res. Time | St. Helens to Estuary | Bonn to estuary | Release to Estuary | Estuary Res. Time | Estuary to Ocean | Mouth Residence | total time from release to ocean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SNR1 | non ocean avg | NA | 5:59:33 | 34:01:45 | 40:01:18 | 1:23:52 | 37:18:21 | 72:43:58 | 77:51:47 | 16:43:00 | ND | ND | ND |
| SNR2 | non ocean avg | NA | 6:39:19 | 32:41:42 | 26:46:24 | 16:00:26 | ND | ND | ND | ND | ND | ND | ND |
| SNR 3 | ocean avg | NA | NA | NA | NA | NA | NA | NA | NA | 11:42:22 | 58:22:26 | 1:20:26 | 70:45:01 |
| SNR 3 | non ocean avg | NA | NA | NA | NA | NA | NA | NA | NA | 19:47:47 | ND | ND | ND |
| SNR4 | ocean avg | NA | NA | NA | 23:08:36 | 1:25:36 | 27:31:28 | NA | 52:05:40 | 10:32:32 | 15:09:35 | 2:54:46 | 80:42:33 |
| SNR4 | non ocean avg | NA | NA | NA | 22:19:58 | 1:20:12 | 23:24:37 | NA | 48:01:18 | 5:06:49 | ND | ND | ND |
| SNR5 | non ocean avg | NA | NA | NA | 36:13:41 | 1:29:55 | 24:28:10 | NA | NA | 4:35:05 | ND | ND | ND |
| SNR6 | ocean avg | 23:33:31 | 25:55:43 | ND | ND | ND | ND | ND | ND | ND | ND | ND | 99:05:04 |
| SNR6 | non ocean avg | 23:41:16 | 28:08:16 | 30:00:32 | 58:53:29 | 0:49:26 | 32:41:14 | ND | ND | 45:27:54 | ND | ND | ND |
| SNR7 | non ocean avg | 24:41:30 | 28:17:28 | 20:27:35 | 38:21:20 | 1:05:31 | 34:26:22 | 46:36:27 | 66:25:05 | 2:41:11 | ND | ND | ND |
| SNR8 | ocean avg | 2:29:44 | 18:29:25 | 20:59:08 | NA | 0:54:32 | 24:40:13 | 43:50:32 | 46:20:15 | 2:32:43 | 196:18:36 | 0:03:43 | 245:13:26 |
| SNR8 | non ocean avg | 3:54:08 | 23:33:57 | 27:17:08 | NA | 0:49:36 | 17:52:37 | 38:08:27 | 41:10:52 | 3:54:23 | ND | ND | ND |
| SNR9 | ocean avg | NA | NA | NA | 19:18:17 | 1:45:42 | 26:02:16 | NA | 91:26:34 | 81:47:39 | 13:19:29 | 7:06:45 | 119:48:49 |
| SNR9 | non ocean avg | NA | 10:29:01 | 20:41:20 | 25:33:22 | 0:28:33 | 27:49:24 | 48:25:59 | 53:22:46 | 6:55:01 | ND | ND | ND |
| SNR10 | ocean avg | 26:16:35 | 31:17:29 | 23:19:42 | 80:53:46 | 1:55:56 | 29:19:24 | 54:35:02 | 112:09:06 | 9:21:10 | 16:04:48 | 6:41:35 | 118:00:04 |
| SNR10 | non ocean avg | 18:45:06 | 24:36:02 | 26:07:41 | 69:28:50 | 1:55:57 | 31:27:44 | 60:30:08 | 99:37:43 | 24:28:32 | ND | ND | ND |
| SNR11 | ocean avg | NA | 2:19:20 | 18:00:23 | 20:05:49 | 0:02:28 | 18:00:23 | 35:34:40 | 38:10:27 | 0:06:18 | 19:39:40 | 0:15:58 | 56:48:27 |
| SNR11 | non ocean avg | 1:14:43 | 4:43:17 | 17:27:57 | 23:31:28 | 0:25:55 | 25:39:20 | 36:01:18 | 56:10:31 | 11:41:24 | ND | ND | ND |
| SNR12 | ocean avg | NA | 1:13:30 | 16:47:25 | 18:00:55 | 0:33:00 | 17:27:25 | 34:47:50 | 36:01:20 | NA | 14:56:13 | 1:02:02 | 51:59:35 |
| SNR12 | non ocean avg | 0:28:22 | 0:54:56 | 17:36:14 | 18:41:01 | NA | 20:54:16 | 39:23:11 | 39:36:59 | 1:17:02 | ND | ND | ND |
| Yak1 | ocean avg | NA | NA | NA | NA | NA | NA | NA | NA | 79:09:10 | 31:09:48 | 2:06:15 | 151:20:28 |
| Yak1 | non ocean avg | NA | NA | NA | NA | NA | NA | NA | NA | 113:15:03 | ND | ND | ND |
| Yak2 | ocean avg | 20:52:07 | 42:08:03 | 40:34:52 | 82:27:44 | 1:42:15 | 34:03:23 | 76:17:10 | 114:16:50 | 34:39:21 | 28:40:08 | 10:13:01 | 186:55:04 |
| Yak2 | non ocean avg | 19:06:45 | 42:24:18 | 47:56:59 | 82:21:43 | 3:54:20 | 33:27:11 | 83:49:35 | 113:44:53 | 76:35:02 | ND | ND | ND |
| SNR 3 and Yak 1 Released at Aldrich Point. | | | | | | | | | | | | | |
| NA= Not Available | | | | | | | | | | | | | |
| ND= No Detection | | | | | | | | | | | | | |

*Note: Times are in hr:mm:sec

# Appendix C: Release Survival Estimates for Steelhead Kelts Transported and Released from the Yakima and Snake Rivers.

Key
S1= Reach 1 Survival Estimate (St. Helens for 3 and Estuary for 2)
S2=Reach 2 Survival Estimate
P1= detection probability @ reach 1
P2= detection probability @ reach 2
lamda = Reach 2 or 3 Survival Estimate and Probability (cannot be seperated)

SNR 1
The logarithm of the determinant of the hessian 67.2917

| index | name | value | std dev | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|---|
| 1 | S1 | 0.50 | 0.20 | 1.00 | | | | |
| 2 | S2 | 1.00 | 0.00 | 0.00 | 1.00 | | | |
| 3 | p1 | 1.00 | 0.00 | 0.00 | 0.00 | 1.00 | | |
| 4 | p2 | 1.00 | 0.00 | 0.00 | 0.00 | 0 | 1.00 | |
| 5 | lamda | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0.00 | 1.00 |

SNR 2
Hessian Matrix does not appear to be positive not enough data

SNR 3
The logarithm of the determinant of the hessian 10.3942

| index | name | value | std dev | 1 | 2 | 3 |
|---|---|---|---|---|---|---|
| 1 | S1 | 0.93 | 0.22 | 1.00 | | |
| 2 | p1 | 0.75 | 0.22 | -0.70 | 1.00 | |
| 3 | lamda | 0.43 | 0.19 | -0.47 | 0.38 | 1.00 |

SNR 4
The logarithm of the determinant of the hessian 26.6738

| index | name | value | std dev | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|---|
| 1 | S1 | 0.74 | 0.20 | 1.00 | | | | |
| 2 | S2 | 0.60 | 0.22 | -0.34 | 1.00 | | | |
| 3 | p1 | 0.75 | 0.22 | -0.43 | 0.32 | 1.00 | | |
| 4 | p2 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | |
| 5 | lamda | 0.33 | 0.27 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |

SNR 5

| The logarithm of the determinant of the hessian | | | | 24.9991 | | | | |
|---|---|---|---|---|---|---|---|---|
| index | name | value | std dev | 1 | 2 | 3 | 4 | 5 |
| 1 | S1 | 0.60 | 0.15 | 1.00 | | | | |
| 2 | S2 | 0.33 | 103.87 | 0.00 | 1.00 | | | |
| 3 | p1 | 1.00 | 0.00 | 0.00 | 0.00 | 1.00 | | |
| 4 | p2 | 0.50 | 155.80 | 0.00 | -1.00 | 0.00 | 1.00 | |
| 5 | lamd | 0.00 | 0.00 | 0.00 | -0.30 | 0.00 | 0.30 | 1.00 |

SNR 6

| The logarithm of the determinant of the hessian | | | | 23.4454 | | | | |
|---|---|---|---|---|---|---|---|---|
| index | name | value | std dev | 1 | 2 | 3 | 4 | 5 |
| 1 | S1 | 0.85 | 0.58 | 1.00 | | | | |
| 2 | S2 | 1.00 | 0.01 | 0.00 | 1.00 | | | |
| 3 | p1 | 0.47 | 0.35 | -0.86 | 0.00 | 1.00 | | |
| 4 | p2 | 0.12 | 0.13 | -0.56 | 0.00 | 0.50 | 1.00 | |
| 5 | lamda | 0.12 | 0.13 | -0.56 | 0.00 | 0.50 | 0.33 | 1.00 |

SNR 7

| The logarithm of the determinant of the hessian | | | | 36.6371 | | | | |
|---|---|---|---|---|---|---|---|---|
| index | name | value | std dev | 1 | 2 | 3 | 4 | 5 |
| 1 | S1 | 0.63 | 0.17 | 1.00 | | | | |
| 2 | S2 | 0.99 | 7.15 | 0.00 | 1.00 | | | |
| 3 | p1 | 1.00 | 0.00 | 0.00 | 0.00 | 1.00 | | |
| 4 | p2 | 0.80 | 5.79 | 0.00 | -1.00 | 0.00 | 1.00 | |
| 5 | lamda | 0.00 | 0.00 | 0.00 | -0.01 | 0.00 | 0.01 | 1 |

SNR 8

| The logarithm of the determinant of the hessian | | | | 30.8269 | | | | |
|---|---|---|---|---|---|---|---|---|
| index | name | value | std dev | 1 | 2 | 3 | 4 | 5 |
| 1 | S1 | 0.56 | 0.14 | 1.00 | | | | |
| 2 | S2 | 0.63 | 0.17 | -0.19 | 1.00 | | | |
| 3 | p1 | 0.83 | 0.15 | -0.28 | 0.25 | 1.00 | | |
| 4 | p2 | 1.00 | 0.00 | 0.00 | 0.00 | 0 | 1.00 | |
| 5 | lamda | 0.60 | 0.22 | 0.00 | 0.00 | 0 | 0.00 | 1.00 |

SNR 9

| The logarithm of the determinant of the hessian | | | | 46.6472 | | | | |
|---|---|---|---|---|---|---|---|---|
| index | name | value | std dev | 1 | 2 | 3 | 4 | 5 |
| 1 | S1 | 0.88 | 0.12 | 1.00 | | | | |
| 2 | S2 | 1.00 | 0.00 | 0.00 | 1.00 | | | |
| 3 | p1 | 0.86 | 0.13 | 0.00 | 0.00 | 1.00 | | |
| 4 | p2 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | |
| 5 | lamda | 0.67 | 0.19 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |

SNR 10

| The logarithm of the determinant of the hessian | | | | 41.7974 | | | | |
|---|---|---|---|---|---|---|---|---|
| index | name | value | std dev | 1 | 2 | 3 | 4 | 5 |
| 1 | S1 | 0.67 | 0.16 | 1.00 | | | | |
| 2 | S2 | 0.83 | 0.15 | 0.00 | 1.00 | | | |
| 3 | p1 | 1.00 | 0.00 | 0.00 | 0.00 | 1.00 | | |
| 4 | p2 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | |
| 5 | lamda | 0.20 | 0.18 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |

SNR 11

| The logarithm of the determinant of the hessian | | | | 42.3643 | | | | |
|---|---|---|---|---|---|---|---|---|
| index | name | value | std dev | 1 | 2 | 3 | 4 | 5 |
| 1 | S1 | 0.38 | 0.13 | 1.00 | | | | |
| 2 | S2 | 1.00 | 0.00 | 0.00 | 1.00 | | | |
| 3 | p1 | 0.60 | 0.22 | 0.00 | 0.00 | 1.00 | | |
| 4 | p2 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | |
| 5 | lamda | 0.67 | 0.27 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |

SNR 12

| The logarithm of the determinant of the hessian | | | | 42.1235 | | | | |
|---|---|---|---|---|---|---|---|---|
| index | name | value | std dev | 1 | 2 | 3 | 4 | 5 |
| 1 | S1 | 0.75 | 0.15 | 1.00 | | | | |
| 2 | S2 | 1.00 | 0.00 | 0.00 | 1.00 | | | |
| 3 | p1 | 0.50 | 0.20 | 0.00 | 0.00 | 1.00 | | |
| 4 | p2 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | |
| 5 | lamda | 0.33 | 0.27 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |

Yak 1

| The logarithm of the determinant of the hessian | | | | 31.8719 | | |
|---|---|---|---|---|---|---|
| index | name | value | std dev | 1 | 2 | 3 |
| 1 | S1 | 0.95 | 0.03 | 1.00 | | |
| 2 | p1 | 1.00 | 0.00 | 0.00 | 1.00 | |
| 3 | lamda | 0.23 | 0.06 | 0.00 | 0.00 | 1.00 |

Yak 2

| The logarithm of the determinant of the hessian | | | | 34.8224 | | | | |
|---|---|---|---|---|---|---|---|---|
| index | name | value | std dev | 1 | 2 | 3 | 4 | 5 |
| 1 | S1 | 0.93 | 0.03 | 1.00 | | | | |
| 2 | S2 | 1.00 | 0.03 | -0.01 | 1.00 | | | |
| 3 | p1 | 0.98 | 0.02 | -0.01 | 0.01 | 1.00 | | |
| 4 | p2 | 0.96 | 0.04 | 0.00 | -0.70 | 0.00 | 1.00 | |
| 5 | lamda | 0.50 | 0.07 | 0.00 | -0.19 | 0.00 | 0.14 | 1.00 |

## Appendix D: Warm Springs Steelhead Kelt Reconditioning and Reproductive Success Report

CONFEDERATED TRIBES OF THE WARM SPRINGS RESERVATION OF OREGON

# Steelhead Kelt Reconditioning and Reproductive Success

## Shitike Creek and Warm Springs River

**CTWSRO**

**4/1/2011**

## Contents

## Tables



## Figures

## Acknowledgements

The Confederated Tribes of Warm Springs Reservation of Oregon (CTWSRO), Natural Resources Branch, Fish Production Department conducted the long-term kelt reconditioning experiment from 2005 through 2008. Biologists included: Geoff Fitzgerald (2005), Devin Best (2005), Jens Lovtang (2006-2008), and Lisa Hewlett-Dubisar (2006-2008). Technicians included: Arthur Mitchell, August Scott, Ernel Scott, Jordan Holliday, and Michael Meninick. The CTWSRO Fisheries Research Department conducted the reconditioning experiment in 2009 and included Jen Graham, Lyman Jim, and Larry Holliday with assistance from Joel Santos and Jaym'e Schricker. This experiment was largely conducted at the Warm Springs National Fish Hatchery. We thank the U.S. Fish and Wildlife Service, Michael Piya, Roger Sorenson and the WSNFH staff for assistance. Additionally we'd like to thank CTWSRO Parkdale Hatchery staff, Jim Gidley and Albert Santos, for the assistance and training in airpsawning and helpful project guidance in 2009. Doug Hatch, Ryan Branstetter and Jeff Stephenson with Columbia River Inter-tribal Fish Commission for providing funding, project design assistance, insight and background. Cyndi Baker at CTWSRO for summarizing and writing the final report.

# Introduction

In response to declining abundance of steelhead (*Onchorhynchus mykiss*) populations in the Columbia Basin (Nehlsen et al. 1991; Williams et al. 1996) and federal listing under the Endangered Species Act (ESA, (National Marine Fisheries Service 1997), an experiment to bolster depleted populations of wild steelhead throughout the Columbia River Basin by increasing the number of repeat spawners began in 1999. Yakama Nation Fisheries Program (YN) began reconditioning wild steelhead kelts during spring 1999. Post-spawn steelhead were captured at Prosser Dam on the Yakima River and were held in a controlled environment at Prosser Hatchery (at river kilometer, RKM 75.6), and fed with the purpose of increasing survival and rematuration rates. After this project demonstrated success in survival of captive kelts (17/34) and rematuration rates (7/34), Columbia River Inter Tribal Fish Commission (CRITFC) began partnering with YN and developed a broader framework for hypothesis testing of the use of wild steelhead kelt reconditioning in the Columbia Basin (Evans et al. 2000). In 2005, objectives of the kelt reconditioning project expanded to replicate studies of relative reproductive success of reconditioned wild steelhead kelts at Yakima River to include Omak/Bonapart creeks in Washington and Shitike Creek in Oregon (Branstetter and coauthors 2006). In 2006, Skamania stock steelhead in lower Hood River were added as a long-term reconditioning site, in which steelhead were caught at the trap at Powerdale Dam (RKM 6.9) air spawned and held at Parkdale Fish Facility. The Confederated Tribes of Warm Springs Reservation of Oregon (CTWSRO), in conjunction with the Warm Springs National Fish Hatchery (WSNFH), was selected to evaluate long-term reconditioning (one of four treatments of the larger study, (Branstetter and Coauathors 2007) and reproductive success of artificially-reconditioned wild steelhead kelts in Shitike Creek, a tributary of the Deschutes River.

Artificial reconditioning is a restoration technique intended to take advantage of a particular and somewhat unique life-history strategy among anadromous salmonids, as partial iteroparity is only known in steelhead and Atlantic salmon (*Salmo salar*).

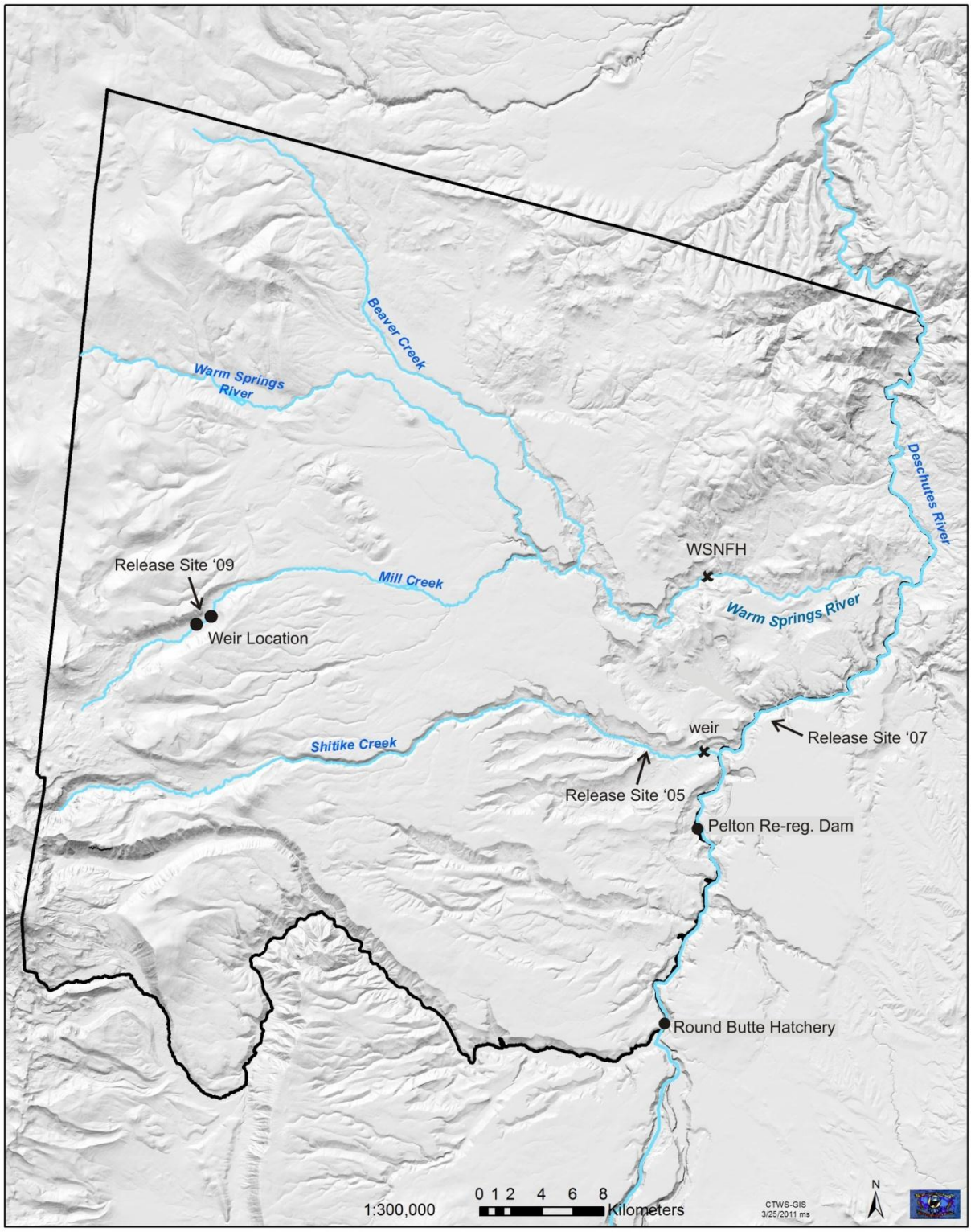
Iteroparity is rare among summer steelhead populations, which predominates in the interior (*e.g.* Columbia Basin) but is more commonly a trait of winter steelhead found in lower elevation coastal streams (Quinn 2005). For example, repeat spawning rates of summer steelhead in Snake River in the mid-Columbia Basin may be less than 2% (Evans and Beaty 2000). In addition to the relative rarity of repeat spawning in Columbia River steelhead, results of a radio-telemetry study suggest successful navigation of downstream migrating kelts through the hydropower system was poor, particularly in low water years (<5% success compared with 15% in a typical flow year) and with increasing distance upstream and numbers of dams to pass (Wertheimer and Evans 2005). Kelt reconditioning may help offset selective forces against iteroparity associated with the hydrosystem.

Survival and rematuration rates of wild steelhead kelts taken from Shitike Creek and Warm Springs River and reconditioned in WSNFH and genotypes of tissue sampled from adult steelhead, adult resident *O. mykiss*, and juvenile *O. mykiss* in these streams are summarized in this report. These data will contribute to a program wide analysis of reconditioning and reproductive success in the Columbia Basin (Branstetter and Coauathors 2007). An evaluation of reconditioning will be based on comparing relative survival and rematuration rates of program fish among four treatments (control, immediate release below Bonneville Dam, short-term and long-term release) in three subbasins (Yakima, Okanogan and Deschutes) . Reproductive success of reconditioned kelts will be evaluated by use of microsatellite DNA markers and pedigree analysis to document that reconditioned kelts produced viable offspring, and kelt reproductive success will be compared with natural first time spawners and hatchery-origin spawners (Branstetter and Coauathors 2007). The answers to these questions will be important in determining if kelt reconditioning is a viable restoration tool that will aid in the recovery of ESA listed steelhead populations in the Columbia River Basin and preservation of the life-history expression of iteroparity in Columbia River steelhead.

## Study Area

The lower Deschutes River Subbasin (HUC 17070306, approximately 5945 $km^2$; Oregon Geospatial Enterprises shape file, 1:24,000 $4^{th}$ field HUC) is located in central Oregon and drains the east slope of the Cascade mountain range. The Deschutes River has a unique flow regime among other eastern Oregon Rivers as seasonal and inter-annual flow is relatively stable due to groundwater flow through porous volcanic soils and lava formations (Gannett et al. 2003; Northwest Power and Conservation Council 2004; O'Connor et al. 2003). A series of hydroelectric dams begins at river kilometer (RKM) 161 on the Deschutes River, where Pelton Re-regulating Dam is located, and ends with Round Butte Dam at RKM 177. Round Butte Hatchery, operated by Oregon Department of Fish and Wildlife, is located at the base of Round Butte Dam and produces steelhead.

The majority of perennial tributaries within the lower Deschutes River Subbasin originate within the boundaries of the CTWSRO. The Reservation covers approximately 240,000 ha on the eastern slopes of the Cascade Mountains (Northwest Power and Conservation Council 2004). Reservation boundaries are the crest of the Cascades to the north and west, the Deschutes River to the east, and the Metolius River to the south (Figure 1). The Warm Springs River (RKM 136) is the largest watershed within the Reservation, flowing 85 km and draining 54,394 ha into the lower Deschutes River. The WSNFH is located on the Warm Springs River at RKM 16.4 and produces spring Chinook (*O. tshawytcha*). Mill Creek (RKM 34.9) is a tributary of Warm Springs River. Shitike Creek (RKM 157) is the third largest tributary to the lower Deschutes River, flowing for 48 RKM and draining 36,000 ha.


Beaver Creek
Warm Springs River
Deschutes River
WSNFH
Release Site '09
Mill Creek
Weir Location
Warm Springs River
weir
Release Site '07
Shitike Creek
Release Site '05
Pelton Re-reg. Dam
Round Butte Hatchery
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0 1 2 4 6 8
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**Figure 1. Vicinity map of the long-term kelt reconditioning sample streams, reconditioning site, and release sites on CTWSRO, 2005-2009 .**

# Methods

## Fish Collection

From 2005 through 2008, steelhead were trapped in the downstream box at a picket weir on Shitike Creek (RKM 1.1) near the confluence with the Deschutes River (Figure 1). The design of the weir incorporated two trap boxes (3 m long x 1.2 m wide x 1 m high), one each for both upstream and downstream migration (Figure 2). To reduce potential impacts on the fish due to high flows, a catch pen was constructed next to the downstream box whereby fish would enter the trap and find an opening on the side to access the catch pen.

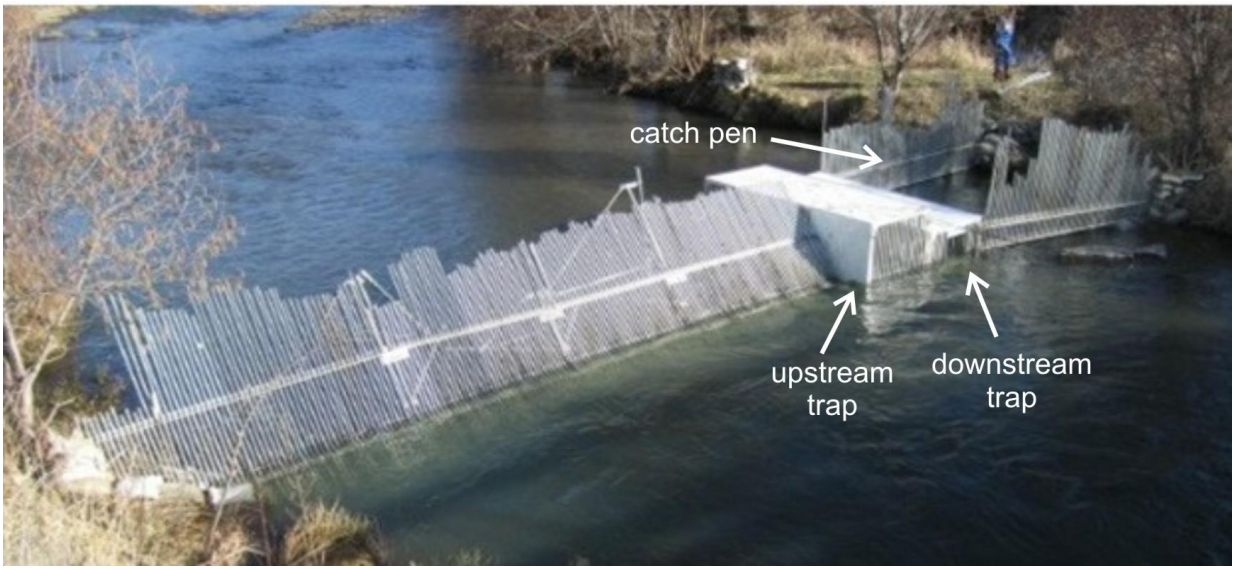


**Figure 2. Weir and fish traps on Shitike Creek, 2005 – 2008.**

The picket fence weir on Shitike Creek was generally installed in March or early April and operated through October. The weir was operated Sunday through Friday and checked in the mornings and evenings. During live-handling in the field, sex, fork length (nearest mm) and direction of travel (upstream/downstream) of all fishes were recorded. On weekends and during periods when water temperatures exceeded 17$^{o}$ C to, the ends of the live boxes were removed allowing unimpeded passage. Fishes were not handled during periods of high water temperatures (>17$^{o}$ C) to avoid excessive handling stress. An underwater video camera system was installed to enumerate immigrating fishes during weekends and periods of warm water temperatures. The camera system consisted of a SplashCam® Deep Blue Pro Color underwater camera that was connected to a Sanyo® Digital Video Recorder DSR-3000. Video images captured on the video recorder were reviewed in the office. The number and species of fishes observed passing upstream and downstream were recorded. River flows were

monitored daily and water temperatures were continuously recorded with a thermograph during weir operation.

Wild steelhead caught in the downstream box were presumed kelts. Those in suitable condition (<50% body covered with fungus, bright or intermediate in coloration) were prepared for transport to WSNFH. Kelts were transferred with a dipnet from the downstream box at the weir to a nearby 190-L sampling tank containing fresh river water, and anesthetized in a buffered solution of tricaine methanesulfonate (MS-222) at 60 ppm.

Information collected on each fish included origin, sex, physical condition, coloration (bright, medium, dark), Floy tag, full-duplex PIT tag number, fork length, and weight. Physical condition was recorded as good (<25% fungus), fair (25-50% fungus), or poor (50-100% fungus). All adipose clipped fish were released downstream. Kelts were transported to WSNFH via 300 gallon oxygenated transport tank.

In 2009, wild, upstream-migrating steelhead were collected at the WSNFH adult trap, air spawned (Leitritz and Lewis 1980), and were reconditioned. The study design for 2009 was to collect 5 pairs of steelhead from WSNFH, every two weeks from mid-March through late-May, if wild steelhead escapement followed the projected goals throughout the run (Table 1) and totaled 400 adult returns by late May, 2009 (Hewlett-Dubisar et al. 2009) . Information recorded from steelhead selected from the adult trap for reconditioning included sex, physical condition, coloration and fork length. Fish were also inspected marks (*e.g.*, tags and secondary marks). Fish condition was based on the presence and amount of fungus (good < 25%, fair =25 - 50%, poor=50 - 100% fungus). Fish in poor condition were not selected for reconditioning.

**Table 2. Summary of Steelhead (STS) collection goals at the WSNFH 2009, based on historic run data.**

| Percent of STS Run to WSNFH | Escapement Target | Fish to be collected for Kelt Project | Female:Male Ratio | Period |
|---|---|---|---|---|
| 20% | 80 | 10 | 1:1 | Mid March |
| 40% | 160 | 10 | 1:1 | Late March |
| 60% | 240 | 10 | 1:1 | Early April |
| 80% | 320 | 10 | 1:1 | Mid April |
| 100% | 400 | 10 | 1:1 | Late May |
| 50%* | 200 | 12 | 3:1 | Early April |

* 50% was a secondary target if the primary targets could not be met.

## Long-term Reconditioning

Upon receiving steelhead for reconditioning, initial processing included treatment for bacterial, parasitic and fungal infections. Both Ivermectin® (an antiparasitic) and Oxytetracycline (an antibiotic) were administered at 1cc per fish before release into the reconditioning tank at WSNFH. Ivermectin® was placed in a pipette down the esophagel passage and Oxytetracycline was injected in the abdominal cavity of each fish. From 2005 to 2008, a formalin treatment of 1:6000 was administered three times a week for 45 minutes. In 2009, water in the reconditioning tank was treated with formalin once per day at a rate of 45 mL per minute to prevent infestations.

A circular, fiberglass tank (4.6m diameter, 1.5m high) was constructed at WSNFH Isolation Rearing Facility. The tank was filled with water from the Warm Springs River that was filtered, ultraviolet light-treated to reduce pathogens. From 2005 to 2008, water was circulated at 380 liters/minute and temperatures were maintained by mixing in chilled water to maintain a steady 15°C through the critical high temperature months (June-September). In 2009, water was continuously pumped rate near 227 liters/minute throughout the reconditioning period. From May thru mid-August 2009, water temperatures in the reconditioning tank were maintained at 10.1$^{o}$C by mixing river water with chilled water produced at WSNFH. After 18 August 2009, the water supply was entirely river water. From 2005 through 2007, artificial lighting was regulated to replicate the natural photoperiod. In 2008, the tank was moved outside and an overhead cover was erected to provide shade.

During the course of the study, the tank was modified to mimic more natural conditions to reduce stress on reconditioning kelts. In 2007, a camouflage pattern was painted (non-toxic paint, TNEMEC Inc., Kansas City, MO) on the inside of the tank (Figure 3). In 2009, a small log, rocks, a small cedar tree, and lumber (plywood, 2x6s) were added to the tank to provide cover (Figure 3).

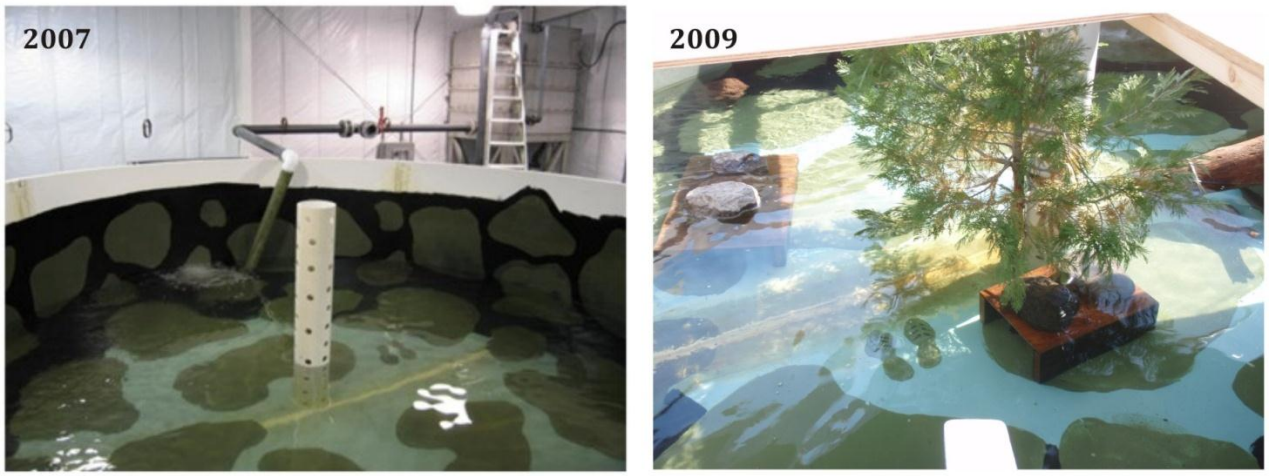


**Figure 3. Reconditioning tank in WSNFH Isolation Rearing Facility after camouflage applied, 2007, and structure added, 2009.**

Feeding regimes were modified through the study period but in all years feeding was initiated with krill and the diet was gradually changed to pellets. In 2005, kelts were fed twice per day, initially with a diet of krill then the diet gradually included Skretting Pellets that were dyed red and combined with krill. In 2006, the feeding regime was limited to krill due to mortalities during the first week. From 2007 to 2009, modified feeding protocols developed at Prosser were used, which included a diet of freeze-dried krill for 2.5 months then kelts were fed Moore-Clarke pellets until they were released (Branstetter and coauthors 2006). Kelts were fed to satiation 2-3 times a day, and were monitored to prevent overfeeding and resultant excessive pollution in the holding tanks. Each feeding event was observed to determine if fish were eating. In 2009, kelts were fed krill or krill coated with Extra Strength Glo Scent Shrimp Oil (Atlas-Mike's Bait, Inc., Fort Atkinson, WI). Further modifications (*e.g.,* squid, pellets) were made throughout the 2009 season to improve feeding and survival.

Mortalities were recorded and examined for cause of death. External and internal examinations were performed on all mortalities. Information collected from each mortality included: suspected time of death, general condition (good, fair, poor), fish color (bright, intermediate, dark), color of the gill arches (red, pink, white), size of the abdomen (fat, thin), presence of any scars or obvious lesions, and any other anomalies. United States Fish and Wildlife Service Fish Health conducted tests on mortalities to determine cause of death.

## Kelt Release

Release sites for reconditioned kelts varied throughout the study, and included Shitike and Mill creeks and the Deschutes River (Figure 1). In 2005, the release site was 5.4km upstream of the mouth of Shitike Creek. In 2007, the release site was the Deschutes River near Dry Creek (RKM 152.4). The release site in 2009 was RKM 27.9 on Mill Creek.

Handling of kelts prior to release varied through the study but included, an examination for maturation, additional or initial PIT tagging, and/or additional dosages of antibiotics. To verify sexual maturation, an ultrasound was performed on released kelts in 2005 and 2007. A half-duplex PIT tag was inserted into the kelt released in 2007 (in addition to the full-duplex tag inserted upon capture in Shitike Creek) and a full-duplex PIT tag was inserted into the reconditioned kelt in 2009 upon release. Prior to release in 2007, the kelt was given a dose of Ivermectin® (5cc 1:30 diluted) and Oxytetracycline (5cc). Similarly, in 2009 the released kelt was treated with 0.5 ml of Ivermectin® and 0.2 ml of Oxytetracycline.

In 2009, steelhead were acclimated prior to release because the water temperature in Mill Creek was colder than that of the transport tank. Upon arrival to the release site water was pumped from Mill Creek into the transport tank until the water temperature cooled 0.5°C. Steelhead were allowed to adjust for 20 minutes before more water was pumped into the transport tank to drop the temperature another 0.5°C. This process was repeated until water temperature in the transport tank was the same as the river. Steelhead were then released into Mill Creek.

During September 2009, two weirs, made of tubular, galvanized pipe with aluminum rails and 1-inch PVC pickets, were placed in Mill Creek prior to kelt release. The downstream weir was at RKM 27.9 and was 11.5 m in length (Figure 4). The upstream weir was at RKM 30.2 and was 12.3 m in length (Figure 5). Weirs were checked regularly (at least 3 times per week unless road conditions prevented access) during fall and winter for debris removal and to determine if immigration, emigration, or mortality had occurred.

**Figure 4. Mill Creek downstream weir RKM 27.9.**

**Figure 5. Mill Creek upstream weir RKM 30.2.**

Prior to release of the reconditioned kelt between weirs in Mill Creek in 2009, historical redd count data for the area were reviewed to determine spawn timing and three single pass redd surveys were completed. Three study reaches in Mill Creek were surveyed June 18 and 19, 2009 by two person teams starting at the top of the reach and walking downstream. For each survey, the date, time, and water temperature was recorded. Three survey reaches included: Reach 1 from RKM 34.3 to RKM 27.9 which includes the weir area, Reach 2 from RKM 27.9 to RKM 24.6, and Reach 3 from RKM 24.6 to RKM 17.9.

The following spring after releasing successfully the reconditioned female kelt in Mill Creek, two males were transported from WSNFH and released between weirs to spawn with the reconditioned female. Six redd surveys were subsequently completed between weirs (RKM 30.2 to 27.9) from April 15 to June 29, 2010 to document whether spawning occurred.

## Reproductive Success

In order to determine relative genetic contribution of reconditioned kelt steelhead to the juvenile progeny in study streams, microsatellite DNA markers and pedigree analyses were used to document whether and what relative proportion juvenile *O. mykiss* in study streams were progeny of reconditioned steelhead (Branstetter and Coauathors 2007). The approach was to sample upstream migrating adults and downstream migrating kelts, as well as resident *O. mykiss* and downstream migrating juvenile *O. mykiss*. Ideally, 100% of the upstream migrating adults would have been genotyped for a complete collection for which to compare juvenile genotypes for parentage assignments. Tissue samples were collected from adult steelhead (> 508mm fork length), adult resident *O. mykiss* (<508mm), and juvenile *O. mykiss* and was stored in 95% non-denatured ethanol. Samples were sent to CRITFC's Hagerman Genetics Laboratory (Hagerman, ID) for genotyping and pedigree analyses (see Branstetter and Coauthors 2007 for description of methods and data analyses).

From 2005 through 2008, tissue samples were collected by caudle fin punch from all *O. mykiss* (steelhead and rainbow trout) trapped at the weir on Shitike Creek (RKM 1.1). Sampling was conducted regardless of status (prespawner or kelt) or condition. After tissue samples were collected and preserved, adult steelhead were released upstream of the weir to spawn naturally.

Tissue samples from juvenile *O. mykiss* were acquired by clipping a small (<15mm$^2$) portion of the caudal fin. From 2005 through 2008, juvenile *O. mykiss* in Shitike Creek were collected using a screw trap located downstream of the adult trap at RKM 0.9. The screw trap was operated in spring from March through June and fall in October and November. In 2009, juvenile *O. mykiss* were collected using a Smith-Root LR-24 backpack electro-fisher and at least one netter to capture stunned fish. Five to ten fish were captured per site from multiple locations in pool-riffle habitats in Mill Creek ranging from RKM 10.3 up to RKM 30.2; above, within and below the enclosed study area (Figure 1). At each site, captured

fish were sedated with MS-222 (80 mg per liter), fork length (nearest mm) was recorded, and a small clip from the caudal fin was stored in an individually numbered vials filled with 95% non-denatured ethanol. Fish were allowed to fully recover prior to release back to original capture location.

## Results

### Adult steelhead catch at Shitike Creek Weir 2005-2008

Adult steelhead were caught in the weir near the mouth of Shitike Creek from March through May or June, 2005 through 2008 (Table 2). The number of live, wild, post-spawned steelhead in acceptable condition for reconditioning was 17% to 31% of the total number of steelhead caught in the downstream trap (Table 3).

**Table 3. Period of Shitike Creek weir operations, 2005-2008.**

| Year | Beginning Operation Date | Last Handling Date* |
|---|---|---|
| 2005 | March 18 | May 27 |
| 2006 | March 6 | June 20 |
| 2007 | March 1 | June 21 |
| 2008 | March 11 | June 24 |

*Trap operations were switched to video only due to high (>17°C) water temperatures.

**Table 4. Numbers of adult steelhead caught in Shitike Creek weir and the number of wild kelts for reconditioning at WSNFH, 2005-2008.**

| Year | Upstream (%wild) | Downstream Total | Downstream Live (%wild) | WSNFH (% female) |
|---|---|---|---|---|
| 2005 | 58 (74%) | 34 | 21 (71%) | 9 (89%) |
| 2006 | 21 (76%) | 24 | 12 (75%) | 4 (100%) |
| 2007 | 93 (67%) | 45 | 37 (81%) | 14 (86%) |
| 2008 | 39 (51%) | 23 | 15 (87%) | 7 (71%) |

Kelts taken for reconditioning ranged in length (fork) from 54 to 71 cm and were from 1.2 to 2.7 kg (Table 4). The earliest date a kelt was retained was March 30 (2007) and the latest was June 7 (2006). Daily average water temperatures ranged from 4.9°C (in 2008) to 13.6°C (in 2007).

**Table 5. Date and condition of kelts captured for reconditioning and release dates, 2005-2008.**

| Capture Date | Day Avg. W. Temp. °C | Stage m | Sex | Len. cm | Wt. kg | PIT | Fish Condition | Color | Date Released |
|---|---|---|---|---|---|---|---|---|---|
| 4/18/2005 | 7.3 | 1.1 | F | 65 | 2.7 | 3D9.1BF1F7F20C | GOOD | - | - |
| 4/19/2005 | 8.1 | 1.1 | F | 54 | 1.4 | 3D9.1BF1F8012E | GOOD | BRIGHT | - |
| 4/19/2005 | | | F | 54 | 1.6 | 3D9.1BF1F7F78D | GOOD | BRIGHT | - |
| 4/19/2005 | | | F | 54 | 2.1 | 3D9.1BF1F719EB | GOOD | BRIGHT | 2/16/2006 |
| 4/19/2005 | | | F | 56 | 1.8 | 3D9.1BF1F70EE7 | GOOD | BRIGHT | - |
| 5/2/2005 | 11.6 | 1.1 | F | 68 | 2.3 | 3D9.1BF1F87981 | - | - | - |
| 5/16/2005 | 11.4 | 1.2 | F | 62 | - | 3D9.1BF1F7AEAF | - | - | - |
| 5/18/2005 | 11.9 | 1.2 | F | 64 | - | 3D9.1BF1A76DC7 | - | - | - |
| 5/23/2005 | 12.2 | 1.1 | M | 62 | 1.9 | 3D9.1BF1F7FB8F | GOOD | - | - |
| 5/1/2006 | 8.8 | 1.4 | F | 74 | 2.9 | 3D9.1BF1F70D07 | GOOD | MEDIUM | - |
| 5/28/2006 | 10.0 | - | F | 64 | 2.1 | 3D9.1BF1F756B0 | POOR | MEDIUM | - |
| 5/30/2006 | 12.5 | 1.3 | F | 59 | 1.7 | 3D9.1BF1F76320 | FAIR | DARK | - |
| 6/7/2006 | 12.9 | | F | 70 | 2.7 | 3D9.1BF1F83DF5 | - | - | - |
| 3/30/2007 | 7.0 | - | F | 58 | - | 3D9.257C5E6FE1 | - | - | - |
| 4/24/2007 | 10.9 | - | F | 72 | - | 3D9.1BF1F7F904 | - | - | - |
| 4/25/2007 | 10.7 | - | F | 71 | - | 3D9.1BF1F83F14 | - | - | - |
| 4/25/2007 | | | M | 58 | 2.2 | 3D9.1BF1F87E75 | GOOD | MEDIUM | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 4/29/2007 | 11.5 | - | F | 70 | - | 3D9.1BF1F727A9 | - | - | - |
| 5/1/2007 | 9.9 | 1.3 | F | 52 | 1.2 | 3D9.1BF1A75AB8 | FAIR | DARK | - |
| 5/4/2007 | 8.5 | 1.3 | F | 57 | 1.4 | 3D9.1BF253D847 | GOOD | MEDIUM | - |
| 5/6/2007 | 11.6 | 1.3 | F | - | 2.1 | 3D9.1BF25361A4 | GOOD | BRIGHT | - |
| 5/6/2007 | | | F | 59 | 1.5 | 3D9.1BF253CE05 | GOOD | BRIGHT | - |
| 5/19/2007 | 11.3 | 1.3 | F | 56 | 1.5 | 3D9.1BF25311A3 | GOOD | BRIGHT | 10/12/2007 |
| 5/19/2007 | | | M | 71 | 2.8 | 3D9.1BF2538296 | GOOD | MEDIUM | - |
| 5/21/2007 | 10.1 | 1.3 | F | 58 | 1.5 | 3D9.1BF1253C654 | GOOD | MEDIUM | - |
| 5/22/2007 | 11.3 | 1.3 | F | 59 | 1.3 | 3D9.1BF2534179 | GOOD | MEDIUM | - |
| 5/26/2007 | 13.6 | 1.3 | F | 64 | 2.1 | 3D9.1BF25390B5 | GOOD | MEDIUM | - |
| 3/25/2008 | 4.9 | 1.3 | F | 67 | 2.3 | 3D9.1BF2535C3C | GOOD | MEDIUM | - |
| 3/25/2008 | | | M | 56 | 1.6 | 3D9.1BF25330E3 | POOR | DARK | - |
| 4/4/2008 | 6.4 | 1.3 | F | 70 | 2.8 | 3D9.1BF253ABFA | FAIR | DARK | - |
| 4/8/2008 | 6.4 | 1.3 | M | 61 | 2.2 | 3D9.1BF2533BF1 | GOOD | DARK | - |
| 4/11/2008 | 8.0 | 1.3 | F | 70 | 3.5 | 3D9.1BF2535DE4 | GOOD | MEDIUM | - |
| 4/29/2008 | 8.8 | 1.4 | M | 76 | 2.9 | 3D9.1BF253C463 | POOR | DARK | - |
| 4/29/2008 | | | M | 62 | 1.0 | 3D9.1BF25336AA | POOR | DARK | - |
| 4/30/2008 | 7.4 | 1.4 | F | 55 | 1.3 | 3D9.1BF2531976 | GOOD | MEDIUM | - |
| 5/8/2008 | 8.5 | 1.4 | F | 62 | 1.8 | 3D9.1BF2531111 | FAIR | MEDIUM | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 5/8/2008 | | | M | 60 | 2.1 | 3D9.1BF25358FC | POOR | DARK | - |
| 5/15/2008 | 10.9 | 1.6 | F | 58.5 | 1.5 | 3D9.1BF253C19C | GOOD | BRIGHT | - |

## Adult steelhead catch at Warm Springs River Weir 2009

Total steelhead counted at Warm Springs River weir in 2009 was 188. Seventy percent (132) were wild steelhead. The first hatchery fish was recorded November 2008 and the first wild fish was recorded on February 2, 2009. The peak of the run was on April 27, when 39 steelhead entered the facility. No hatchery steelhead were passed upstream of the WSNFH.

Eight wild steelhead were captured at the WSNFH weir for air spawning and reconditioning (Table 5). Three-fourths of the steelhead captured (6) were female. Condition of steelhead retained for spawning and reconditioning were categorized as fair (37.5%) or poor (62.5%). Two-thirds of the females were spawned. Those that were not spawned had died in the kelt tank prior to the spawning effort. Four female steelhead were returned to the tank after spawning for reconditioning.

**Table 6. Date and condition of kelts captured for air spawning and reconditioning 2009.**

| Capture Date | Day Avg. W. Temp. °C | Sex | Len. cm | Condition | Air Spawn Date |
|---|---|---|---|---|---|
| 5/11/2009 | 9.3 | M | 55 | POOR | 5/19/2009 |
| | | F | 54 | POOR | 6/17/2009 |
| 5/12/2009 | 8.2 | F | 54 | FAIR | 5/19/2009 |
| 5/19/2009 | 10 | M | 58 | POOR | 5/19 and 5/29/09 |
| 5/22/2009 | 10.6 | F | 57 | FAIR | 5/29/2009 |
| | | F | 60 | POOR | Did not Spawn |
| 5/29/2009 | 12.4 | F | 66 | POOR | Did not Spawn |
| | | F | 69 | FAIR | 6/8/2009 |

## Reconditioning

In 2005, nine wild steelhead kelts held at WSNFH for reconditioning (Table 4) were given a krill-only diet to initiate feeding. The majority (56%) of mortalities occurred within the first week after induction into the reconditioning tank. By the first part of the third week, three more steelhead had perished. Only one female survived for release. The surviving female was given a krill only diet from April 21 to July 7, 2005. On July 8, 2005 a mix of supplement brood diet (Skretting Pellets) dyed red and combined with krill was administered twice per day for seven days a week. In early December 2005, the remaining kelt quit feeding but survived until release, February 2006.

In 2006, four steelhead held in the reconditioning tank at WSNFH (Table 4) were given a diet of krill to initiate feeding. However, no kelts survived beyond the first week.

In 2007, 14 steelhead kelts were transported WSNFH (Table 4). Fish were generally in good condition and contained zero to less than 25% fungus. One female kelt survived 193 days and was released October, 2007. Two females survived five months (150 and 176 days) but died. It was presumed that the cause of mortality was that one quit eating and the other succumbed to copepods (Table 6). Three of the mortalities were attributed to jumping out of the reconditioning tank. Fungal infections were possible threats that adversely affected kelt survival.

**Table 7. Summary of steelhead kelts transported to the WSNFH, 2007.**

| Sex | Len. cm | Date Transported to WSNFH | Mortality Date | Possible Cause of Mortality | Days Alive |
|---|---|---|---|---|---|
| F | 58 | 3/30/2007 | 8/25/2007 | EMPTY STOMACH | 150 |
| F | 72 | 4/24/2007 | N/A | EMPTY STOMACH | N/A |
| F | 71 | 4/25/2007 | 5/14/2007 | FUNGUS/EMPTY STOMACH | 20 |
| M | 58 | 4/25/2007 | N/A | JUMP OUT | N/A |
| F | 70 | 4/29/2007 | N/A | EMPTY STOMACH | N/A |
| F | 52 | 5/1/2007 | 5/9/2007 | FUNGUS/EMPTY STOMACH | 9 |
| F | 57 | 5/4/2007 | 9/26/2007 | COPEPODS | 176 |
| F | - | 5/6/2007 | 5/19/2007 | EMPTY STOMACH | 11 |
| F | 59 | 5/6/2007 | N/A | EMPTY STOMACH | N/A |
| F | 56 | 5/19/07 | N/A | N/A | 193* |
| M | 71 | 5/19/2007 | 7/26/2007 | EMPTY STOMACH | 63 |
| F | 58 | 5/21/2007 | 6/7/2007 | FUNGUS | 17 |

| | | | | | |
|---|---|---|---|---|---|
| F | 59 | 5/22/2007 | 5/25/2007 | JUMP OUT | 4 |
| F | 64 | 5/26/2007 | 5/30/2007 | JUMP OUT | 5 |

*survived for release

In 2008, eleven wild steelhead kelts were transported to WSNFH for reconditioning (Table 4). Four died the first day (Table 7). Two more kelts died within the first week and the remaining kelts did not survive the month. The cause of mortality was unknown. Fish mortality was thought to be attributed to starvation, infestations of copepods and fungal attack.

**Table 8. Summary of steelhead kelts transported to WSNFH, 2008.**

| Sex | Len. cm | Wt. kg | Date Transported to WSNFH | Mortality Date | Days Alive |
|---|---|---|---|---|---|
| F | 67 | 2.32 | 3/25/2008 | 4/2/2008 | 8 |
| M | 56 | 1.64 | 3/25/2008 | 4/9/2008 | 15 |
| F | 70 | 2.8 | 4/4/2008 | 4/9/2008 | 5 |
| M | 61 | 2.17 | 4/8/2008 | 5/3/2008 | 25 |
| F | 70 | 3.52 | 4/11/2008 | 4/15/2008 | 4 |
| M | 76 | 2.88 | 4/29/2008 | 4/29/2008 | 1 |
| M | 62 | 2.01 | 4/29/2008 | 4/29/2008 | 1 |
| F | 55 | 1.31 | 4/30/2008 | 4/8/2008 | 8 |
| F | 62 | 1.75 | 5/8/2008 | 4/9/2008 | 1 |
| M | 60 | 2.05 | 5/8/2008 | 4/9/2008 | 1 |
| F | 58.5 | 1.54 | 5/15/2008 | UNKNOWN | UNKNOWN |

In 2009, 8 steelhead were collected at WSNFH weir and air spawned in order to provide kelts for the reconditioning experiment. Two females died during the first week, presumable due to stress and also fungal growth (Table 8). One male steelhead died during the spawning process at the end of the first week after being held in the reconditioning tank. The second male was spawned twice but died after the second week. It was also plagued with fungal growth. Only 3 of the 8 kelts held for reconditioning began feeding. One survived for 46 days and was fed krill and pellets (Table 8). The other two were fed krill, pellets and squid; one survived 107 days and the other was PIT tagged (3D9.1BF2536826) and released in September 2009 at 129 days of reconditioning. The reconditioned kelt was initially measured for length (57 cm) but not weighed. Upon release, the kelt was 62cm and weighed 2.1kg (an increase in length of 9%).

**Table 9. Summary of steelhead kelts reconditioned at WSNFH, 2009.**

| Sex | Len. cm | Date at WSNFH | Mortality Date | Possible Cause of Mortality | Days Alive |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| M | 55 | 5/11/2009 | 5/19/2009 | Died of severe fungal growth | 8 |
| F | 54 | 5/11/2009 | 6/26/2009 | Died of severe fungal growth | 46[1] |
| F | 54 | 5/12/2009 | 5/19/2009 | Died during spawning process | 7 |
| M | 58 | 5/19/2009 | 6/4/2009 | Spawned twice, died of severe fungal growth | 16 |
| F | 57 | 5/22/2009 | - | Released in Mill Creek | 129+[2] |
| F | 60 | 5/22/2009 | 5/26/2009 | Died of severe fungal growth | 4 |
| F | 66 | 5/29/2009 | 5/30/2009 | Died of stress | 1 |
| F | 69 | 5/29/2009 | 9/13/2009 | Unknown cause of death – fish appeared in good health - no fungus, bright coloration | 107[2] |

[1] Fed krill and pellets

[2] Fed krill, pellets and squid

## Kelt Releases

Out of 46 steelhead experimentally reconditioned from 2005 to 2009, only 3 survived for release (Table 9). The survival rate of kelts reconditioned during this period averaged 6.5%.

**Table 10. Number of kelts transported for reconditioning and survival rate, 2005 – 2009.**

| Year | Kelts Experimentally Reconditioned | Kelts that Survived Reconditioning | % Survival |
|---|---|---|---|
| 2005 | 9 | 1 | 11.1% |
| 2006 | 4 | 0 | 0% |
| 2007 | 14 | 1 | 7.1% |
| 2008 | 11 | 0 | 0% |
| 2009[1] | 8 | 1 | 12.5% |
| **Total** | **46** | **3** | **6.5%** |

[1]In 2009, pre-spawned kelts were air spawned and reconditioned.

In 2005, the surviving reconditioned kelt was measured, weighed, and analyzed for sexual maturation. During the 301 days of reconditioning, the kelt grew 14 cm in length and increased by 1 kilogram in weight. To verify sexual maturation, an ultrasound was performed. Egg production was verified, however number and size of eggs was indistinguishable at the time of analysis. After analysis, the reconditioned kelt was transported to Shitike Creek and released at RKM 5.4. The reconditioned steelhead was apparently not detected again as the PIT tag number (3D9.1BF1F719EB) did not appear in the regional database (http://www.ptagis.org/ptagis).

On October 12, 2007, one reconditioned kelt was released in the Deschutes River near Dry Creek (RKM 148). The ultrasound showed that the reconditioned female had a small undeveloped egg mass. The fish gained 0.8 kg (53% increase) in the reconditioning facility (initial weight, 1.5 kg, Table 4). The reconditioned steelhead was apparently not detected again as the PIT tag number (3D9.1BF25311A3) did not appear in the regional database (http://www.ptagis.org/ptagis).

On September 28, 2009, one reconditioned female was PIT tagged and released into Mill Creek at RKM 27.9, between the weirs that were installed five days previous. The reconditioned female kelt overwintered in Mill Creek between the weirs. Snorkel surveys were conducted to confirm presence of the kelt on March 9 and March 17, 2010. The kelt was observed by a snorkel surveyor on March 17, 2010 near the downstream weir. On March 22, 2010 two male steelhead (65 and 72 cm fork length) were transported from WSNFH and released between weirs on Mill Creek to spawn with the female reconditioned kelt. From April 15 to June 29, 2010, six redd surveys were conducted to determine spawning success, however no redds were observed. Weirs were removed July 9, 2010.

The reconditioned steelhead was apparently not detected after release as the PIT tag number (3D9.1BF2536826) did not appear in the regional database (http://www.ptagis.org/ptagis).

## Tissue Samples Collected for Genetic Analysis

From 2005 to 2009, a total of 1,825 tissue samples from *O. mykiss* collected in Shitike Creek and Warm Springs River were received by CRITFCs Hagerman Genetics Laboratory (Table 10). Of these, DNA was extracted from 84% of the samples. From 2005 to 2008, 667 samples from adult *O. mykiss* were extracted. Of the adult samples, 65% (404/263) were anadromous (Table 11). From 2005 to 2009, 740 samples from juvenile *O. mykiss* were extracted.

**Table 11. Tissue samples from *O. mykiss* collected in Shitike Creek and Warm Springs River, 2005-2009.**

| Year | Stream | Site | Samples[2] | | Fork Length (mm)[3] | | |
|---|---|---|---|---|---|---|---|
| | | | Received | DNA Extracted | Avg. | Min. | Max. |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 2005 | Shitike Creek | weir | 217 | 217 | 459 | 310 | 740 |
| | | screw trap | 58 | 58 | 48 | 38 | 50 |
| | WSR[1] | weir | 61 | 61 | 640 | 160 | 760 |
| 2006 | Shitike Creek | weir | 89 | 89 | 500 | 350 | 800 |
| | | screw trap | 134 | 134 | 80 | 36 | 165 |
| | WSR | weir | 115 | 113 | 631 | 510 | 780 |
| 2007 | Shitike Creek | weir | 162 | 162 | 566 | 290 | 840 |
| | | screw trap | 230 | 130 | 106 | 43 | 205 |
| | WSR | weir | 25 | 25 | 632 | 530 | 710 |
| | | unknown | 68 | 68 | --- | --- | --- |
| 2008 | Shitike Creek | weir | 63 | 55 | 594 | 335 | 780 |
| | | screw trap | 180 | 0 | 132 | 52 | 202 |
| | WSR | screw trap | 86 | 85 | 163 | 56 | 223 |
| 2009 | WSR Beaver | Dahl Pine | 19 | 19 | 62 | 36 | 119 |
| | | Fence Line | 10 | 10 | 70 | 10 | 140 |
| | | Hwy 9 | 1 | 1 | 120 | | |
| | | Solar Jack | 15 | 15 | 67 | 32 | 167 |
| | | Halfway Trail | 49 | 49 | 78 | 35 | 178 |
| | WSR Mill | Old Bridge | 16 | 16 | 56 | 42 | 65 |
| | | Potters Pond | 36 | 36 | 66 | 40 | 165 |
| | | Strawberry Falls | 19 | 19 | 58 | 37 | 122 |
| | | Upper Mill | 94 | 94 | 64 | 37 | 150 |
| | WSR | screw trap | 78 | 74 | 134 | 53 | 230 |

[1]Warm Springs River

[2]Samples received by CRITFC and number of samples for which DNA was extracted (data courtesy of Jeff Stephenson)

[3]Of the samples received

**Table 12. Anadromous (>508mm) and resident (≤508 mm) forms of adult *O. mykiss* samples collected in Shitike Creek and Warm Springs River, 2005 – 2008, for which DNA was extracted.**

| Stream | Year | Anadromous | Resident |
|---|---|---|---|
| Shitike Creek | 2005 | 60 | 157 |
| | 2006 | 38 | 51 |
| | 2007 | 109 | 53 |
| | 2008[1] | 0 | 0 |
| Warm Springs River | 2005 | 60 | 1 |
| | 2006 | 114 | 1 |
| | 2007 | 25 | 0 |

[1]63 samples were received (55 anadromous and 8 resident), but no DNA was extracted.

Genetic analyses performed by CRITFCs Hagerman Lab are summarized in two CRITFC reports, one written in 2007 and the second written in 2009 , each having different objectives (Branstetter and Coauathors 2007; Branstetter and Coauathors 2009). The 2007 report includes population statistics from 791 samples collected from Shitike Creek between 2005 and 2007, an analysis of samples assignment to anadromous or resident forms, and parentage assignments of juveniles (Branstetter and Coauathors 2007). The 2009 report describes genetic population structure of steelhead sampled in Warm Springs (including two tributaries, Beaver and Mill creeks), Shitike Creek and two other Deschutes River tributaries (Buck Hollow and Trout creeks), the upper main stem Deschutes River and steelhead from Round Butte Hatchery using 96 SNP markers (Branstetter and Coauathors 2009). Since interpretation of gene-frequency data requires specialized training and experience, readers may reference tables of population statistics from CRITFCs analyses (Branstetter and Coauathors 2007; Branstetter and Coauathors 2009) if desired and interpretation of results from *O. mykiss* samples collected in Shitike Creek and Warm Springs River will be discussed below.

## Mill Creek Redd Surveys

The CTWSRO fisheries staff conducted redd surveys in Mill Creek late spring 2009 to determine whether steelhead used the area for spawning and then after releasing the kelt, redd surveys were repeated the following spring in 2010. On June 18 and 19, 2009 redd surveys were conducted in Mill Creek from

Passage Way (RKM 34.3) downstream to Old Mill Creek Bridge (RKM 17.9). In June 2009, one steelhead redd was observed in the area that was enclosed by weirs (RKM 30.2 to 27.9) in 2010. To determine the spawning success of the kelt released in Mill Creek fall 2009, redd surveys were conducted April 15 to June 29, 2010 from RKM 30.2 to RKM 27.9, however no redds nor any adult steelhead were observed.

## Discussion

From 2005 through 2009, three steelhead kelts from Shitike Creek and Warm Springs River were successfully reconditioned. The average rate of reconditioning kelts was 6.5% (Table 9). Comparing the number of potential steelhead kelts for reconditioning and survival rate of those experimentally reconditioned at the four long-term reconditioning sites, potential and success for Shitike Creek and Warm Springs River was comparatively low (Table 12). The number of wild kelts available for reconditioning in Shitike Creek (2005-2008) or Warm Springs River (2009) averaged 2.2% and 27% of those available in Yakima River or Omak Creek, respectively. Because few wild kelts were available for reconditioning in Shitike Creek and Warm Springs River, fish in moderate to poor condition were retained for reconditioning. Sites with greater numbers of wild kelts may have selected kelts in better condition and therefore had greater success. While air-spawned, reconditioned Skamania steelhead from Hood River may not be a direct comparison to steelhead spawned in the wild, it was similar to the 2009 effort of air spawning wild steelhead caught at WSNFH. Except for 2007, when all but one of the females being reconditioned at the Parkdale Fish Facility died, likely from copepod infestation, survival rates varied from 33 to 100% (Table 12). The 2009 effort of reconditioning an air-spawned female at WSNHF resulted in one release out of a total of eight kelts (12.5%). However, it could be argued that the two males held for one week, used for air spawning and died after spawning should not be counted in the number attempted for reconditioning, in which case the rate of survival was one release out of six females experimentally reconditioned (16.7%). A second female that was three weeks short of surviving the reconditioning process (Table 8) would have boosted the rate to 33.3%, and entered the range of Parkdale's success rate.

**Table 13. Numbers of kelts experimentally reconditioned and numbers and percentage that survived reconditioning from Yakima River (YR), Omak Creek (OC), Shitike Creek/Warm Springs River (SC) and Hood River (HR), 2005-2009.**

| Year | Kelts Experimentally Reconditioned | | | | Kelts that Survived Reconditioning | | | | % Survival | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | YR | OC | SC* | HR | YR | OC | SC | HR | YR | OC | |
| 2005 | 386 | 51 | 9 | --- | 86 | 3 | 1 | --- | 22.3 | 5.9 | 1 |
| 2006 | 279 | 27 | 4 | 1 | 85 | 2 | 0 | 1 | 30.5 | 7.4 | |
| 2007 | 422 | 43 | 14 | 15 | 221 | 8 | 1 | 1 | 52.4 | 18.6 | 7 |
| 2008 | 472 | 32 | 11 | 14 | 269 | 9 | 0 | 7 | 57.0 | 28.1 | |
| 2009[1] | 510 | 17 | 8 | 12 | 140 | 2 | 1 | 4 | 27.5 | 11.8 | 1 |
| Average | 413.8 | 34.0 | 9.2 | 10.5 | 160.2 | 4.8 | 0.6 | 3.3 | 37.9 | 14.4 | 6 |

[1]In 2009, adult steelhead were collected from Warm Springs River rather than Shitike Creek and air spawned to create kelts for the reconditioning experiment.

[2]Low survival due to copapod infestation.

Many improvements to catching, handling, and reconditioning were made to increase the number of kelts for reconditioning and the rate of survival. In 2007, the upstream trap at the weir on Shitike Creek was repositioned to the left side of stream (looking downstream) where recent high-flow events scoured a channel. The downstream box was situated closer to the right bank with the catch pen connected. These modifications were intended to improve trap efficiency and reduce mortalities. Handling stress was a concern after the first year of the project in Shitike Creek as kelts were handled two times before release into reconditioning tank. To reduce the amount of handling stress in 2006, kelts were processed at the weir, placed in fish bags, and then transported to the WSNFH for in-processing (Lovtang and Hewlett 2006). Another effort to reduce stress was modifying the reconditioning tank to mimic a more natural setting by painting a camouflage pattern and adding structure for cover. Kelts were observed using cover in the reconditioning tank, only coming out to feed and occasionally moving around the tank (Jim et al. 2009). It was observed that individual kelts seemed to have a preference for particular structures in 2009. Once they chose a particular structure, individuals returned to their preferred location. Many attempts were made to entice kelts to feed by manipulating food items offered. Once kelts began to feed on krill it was difficult to transition to feeding on pellets (Lovtang and Hewlett-Dubisar 2008). Pellets were cut into small pieces and inserted into krill and mixed into pellet/krill slurry. These efforts were unsuccessful in transitioning food types. In 2009, kelts were observed eating pellets four times throughout the experiment. Fish fed on squid when introduced into the kelt tank. Efforts were made to combine pellets with squid and krill but were unsuccessful in achieving kelts to accept pellets (Jim et al. 2009).

Unsuccessful efforts to recondition steelhead from Shitike Creek initiated CTWSRO and CRITFC to reorganize scope of the project in 2009 (Jim et al. 2009). The site of collecting steelhead was moved from Shitike Creek to Warm Springs River because of a greater number of steelhead in the latter stream and the immediate proximity to the reconditioning facility. Steelhead counts in Shitike Creek were 20, 61, and 41 in 2006, 2007, and 2008 respectfully (CTWSRO unpublished data). Wild steelhead enumerated at WSNFH were 256 in 2006, 395 in 2007, and 305 in 2008 (WSNFH unpublished data). In 2009, 132 wild steelhead returned, which was only one-third of the goal set by the 2009 study plan; the return of 400 wild adult steelhead was an project-specific goal so as not to impose too great an impact on the run (Hewlett-Dubisar et al. 2009) . Therefore, the anticipated abundance of steelhead for the reconditioning experiment was not realized and only six females and two males were collected.

## Genetic Analysis

Analysis of the 791 samples collected from Shitike Creek between 2005 and 2007 indicated that the resident population of *O. mykiss* was genetically and temporally, in migration of the adult and juvenile stage, distinct from the anadromous form (Branstetter and Coauathors 2007). Sympatric steelhead and resident rainbow trout have been documented shifting from resident to anadromous forms between generations, essentially acting as one population. Based on otolith microchemistry to determine the occurrence of steelhead and resident rainbow trout progeny in adult populations of *O. mykiss*, Zimmerman (2000) observed steelhead of resident rainbow trout origin and resident rainbow trout of

steelhead maternal origin in the Babine River, British Columbia. However, in the Deschutes River, only steelhead of maternal steelhead origin and resident rainbow of resident rainbow trout origin were observed, suggesting that steelhead and rainbow trout constitute reproductively isolated populations (Zimmerman and Reeves 2000). As with Deschutes River, *O. mykiss* in Shitike Creek maintained two distinct populations (Jeff Stephenson, Geneticist Lab Manager, CRITFC, pers. comm.). Through this analysis and using length at time of capture in screw traps, CRITFC determined that larger (ca. 100 to 200 mm fork length) juvenile *O. mykiss* caught early (mid-March-April) were usually anadromous forms, while smaller (ca. 50 to 100mm fork length) juveniles caught during spring were resident forms. This was very practical information for this study because the intent was to target anadromous juveniles and test for parentage, which would indicate reproductive success of returning reconditioned kelts. Self-assignment rates of *O. mykiss* samples from Shitike Creek were analyzed to determine how well genetic techniques correctly identified anadromous and resident forms; self-assignment rates were high for both anadromous steelhead and resident rainbow trout, 97.2% (246/253) and 97.0% (197/203), respectively (Branstetter and Coauathors 2007). Sampling timing was shifted earlier in spring from 2005 to 2007 and more anadromous juveniles were identified in the latter year (57.7% in 2007 compared to 0% in 2005 and 8.1% in 2006, (Branstetter and Coauathors 2007). After anadromous juveniles were identified in samples collected from Shitike Creek in 2007, parentage assignments identified at least one parent for 24 of the 71 anadromous juveniles and one of the 24 samples assigned to two. While the genetic techniques were successful in describing population structure of *O. mykiss* in Shitike Creek, differentiating life-history types, and in determining parentage of juvenile tissue samples collected, analysis did not indicate reproductive success of the reconditioned kelt released in 2005, and no similar analysis is available that would indicate reproductive success of the reconditioned kelt released in 2007.

Results from Genetic Stock Identification (GSI) of lower Deschutes River *O. mykiss* indicates that there are multiple distinct populations, in which significant population differentiation was documented in upper mainstem Deschutes River, Shitike Creek resident, and Round Butte Hatchery collections (Branstetter and Coauathors 2009). Tributary collections of anadromous steelhead provided minimal evidence of distinct populations. GSI analysis indicated that strays outside the Deschutes Basin originated from within reporting groups, including Shitike Creek and Warm Springs River, suggesting that the baseline contained strays. From 1996 to 2001, out-of-basin steelhead strays accounted for over half of the total run ascending Sherars Falls (Olson and Spateholts 2001). Whether out-of-basin strays have been reproductively successful and have introgressed with native steelhead has not been documented.

## Conclusion

In 2005, the Confederated Tribes of Warm Springs Reservation (CTWSRO), in conjunction with the Warm Springs National Fish Hatchery (WSNFH), was selected to participate in evaluating long-term kelt reconditioning (Branstetter and Coauathors 2007) and reproductive success of artificially-reconditioned wild steelhead kelts in Shitike Creek. From 2005 to 2009, three kelts were reconditioned and released. Despite efforts to improve catching, handling, and reconditioning kelts, success rates were low compared to other long-term reconditioning sites. While genetic analyses advanced understanding of population structure of O. mykiss in Shitike Creek and Warm Springs River, tissue samples did not include progeny from reconditioned kelts to document reproductive success. Because of the low numbers of kelts available in Shitike Creek and Warm Springs River and the apparent insufficient quality and poor success rate of kelt reconditioning, these sites were terminated as part of the long-term kelt reconditioning study.

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## Appendix E: Steelhead Kelt Reconditioning Treatments

| Strategy | Year | Location | # Collected | # released | S @ release (%) | # @ ocean | S @ ocean (%) | # @ Bonneville | Return Rate to Bonneville (%) | Transportation (or treatment) Benefit relative to in-river | Treatment benefit relative to Hockersmith 1.66 | Transportation (or treatment) Benefit relative to Bonneville natural |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| In-river | 2005 | Prosser | 67 | 67 | | | | 3 | 4.48 | 1.51 | 2.70 | 25.93 |
| In-river | 2006 | Prosser | 52 | 52 | | | | 1 | 1.92 | 0.65 | 1.16 | 3.12 |
| In-river | 2007 | Prosser | 53 | 53 | | | | 3 | 5.66 | 1.91 | 3.41 | 9.31 |
| In-river | 2008 | Prosser | 88 | 88 | | | | 4 | 4.55 | 1.54 | 2.74 | 6.66 |
| In-river | 2009 | Prosser | 58 | 58 | | | | 3 | 5.17 | 1.75 | 3.12 | 11.63 |
| In-river | 2010 | Prosser | 155 | 155 | | | | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Total and weighted mean* | | | ***473*** | ***473*** | | | | ***1.98*** | ***2.96*** | ***1.00*** | ***1.78*** | ***5.63*** |
| In-river | 2002 | Lower Granite* | 1209 | 1209 | | | | 8 | 0.66 | 0.98 | 0.40 | |
| In-river | 2003 | Lower Granite* | 865 | 865 | | | | 3 | 0.35 | 0.51 | 0.21 | |
| In-river | 2004 | Lower Granite* | 1138 | 1138 | | | | 10 | 0.88 | 1.30 | 0.53 | 1.52 |
| In-river | 2009 | Lower Granite | 178 | 176 | | | | 2 | 1.12 | 1.66 | 0.68 | 1.52 |
| In-river | 2010 | Lower Granite | 1411 | 1399 | | | | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Total and weighted mean* | | | ***4801*** | ***4787*** | | | | ***5.00*** | ***0.68*** | ***1.00*** | ***0.41*** | ***1.29*** |
| In-river | 2002 | John Day* | ***287*** | ***287*** | | | | ***28*** | ***9.76*** | ***1.00*** | ***5.88*** | ***18.57*** |
| *Total and weighted mean* | | | | | | | | | | | | |
| Transported (Hamilton Island) | 2002 | Lower Granite* | 750 | 750 | | | | 19 | 2.53 | 3.83 | 1.53 | |
| Transported (Hamilton Island) | 2003 | Lower Granite* | 376 | 376 | | | | 3 | 0.80 | 2.30 | 0.48 | |
| Transported (Hamilton Island) | 2004 | Lower Granite* | 982 | 982 | | | | 7 | 0.71 | 0.81 | 0.43 | 2.04 |
| Transported (Hamilton Island) | 2009 | lower Granite | 71 | 68 | | | | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| Transported (Hamilton Island) | 2010 | lower Granite | 301 | 301 | 13/108 | | 12.04 | 0 | 0.00 | 0 | 0.00 | 0.00 |
| *Total and weighted mean* | | | ***2480*** | ***2477*** | | | | ***8.97*** | ***1.17*** | ***1.74*** | ***0.70*** | ***2.23*** |
| Transported (estuary release) | 2010 | Lower Granite | 23 | 22 | 4/10 | | 40.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Total and weighted mean* | | | | | | | | | | | | |
| Transported | 2002 | John Day* | ***271*** | ***271*** | | | | ***34*** | ***12.55*** | ***1.29*** | ***7.56*** | ***23.88*** |
| *Total and weighted mean* | | | | | | | | | | | | |
| Transported (unfed Hamilton Island) | 2004 | Prosser | 75 | 63 | 15/28 | | 53.57 | 5 | 6.67 | | 4.02 | 19.10 |
| Transported (unfed Hamilton Island) | 2005 | Prosser | 98 | 96 | 14/57 | | 24.56 | 1 | 1.02 | 0.23 | 0.61 | 5.91 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Transported (unfed Hamilton Island) | 2006 | Prosser | 55 | 49 | 31/49 | 63.27 | 2 | 3.64 | 1.89 | 2.19 | 5.89 |
| Transported (unfed Hamilton Island) | 2007 | Prosser | 43 | 38 | 14/35 | 40.00 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| Transported (unfed Hamilton Island) | 2008 | Prosser | 100 | 100 | 26/49 | 53.06 | 3 | 3.00 | 0.66 | 1.81 | 4.40 |
| Transported (unfed Hamilton Island) | 2010 | Prosser | 124 | 123 | 27/59 | 45.76 | 1 | 0.81 | #DIV/0! | 0.49 | 1.80 |
| *Total and weighted mean* | | | ***495*** | ***469*** | | ***46.70*** | ***2.03*** | ***2.42*** | ***0.82*** | ***1.46*** | ***4.61*** |
| | | | | | | | | | | | |
| Transported (unfed estuary release) | 2010 | Prosser | 113 | 113 | 13/60 | 21.67 | 1.00 | 0.88 | #DIV/0! | 0.53 | 1.98 |
| *Total and weighted mean* | | | | | | | | | | | |
| | | | | | | | | | | | |
| Transported (fed Hamilton Island) | 2002 | Prosser | 479 | 334 | | | 43 | 8.98 | | 5.41 | |
| Transported (fed Hamilton Island) | 2003 | Prosser | 208 | 187 | | | 8 | 3.85 | | 2.32 | |
| Transported (fed Hamilton Island) | 2004 | Prosser | 105 | 83 | 11/26 | 42.31 | 5 | 4.76 | | 2.87 | 13.64 |
| Transported (fed Hamilton Island) | 2005 | Prosser | 106 | 96 | 6/56 | 10.71 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| Transported (fed Hamilton Island) | 2006 | Prosser | 56 | 50 | 32/50 | 64.00 | 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| Transported (fed Hamilton Island) | 2007 | Prosser | 40 | 38 | 19/27 | 70.37 | 1 | 2.50 | 0.44 | 1.51 | 4.11 |
| Transported (fed Hamilton Island) | 2008 | Prosser | 108 | 100 | 28/50 | 56.00 | 7 | 6.48 | 1.43 | 3.90 | 9.50 |
| *Total and weighted mean* | | | ***1102*** | ***888*** | | ***48.68*** | ***21.40*** | ***5.81*** | ***1.96*** | ***3.50*** | ***11.06*** |
| | | | | | | | | | | | |
| Transported (pooled groups) | 2002 | Prosser | 479 | 334 | | | 43 | 8.98 | | 5.41 | |
| Transported (pooled groups) | 2003 | Prosser | *208* | 187 | | | 8 | 3.85 | | 2.32 | |
| Transported (pooled groups) | 2004 | Prosser | 203 | 179 | 26/54 | 48.15 | 10 | 4.93 | | 2.97 | 14.11 |
| Transported (pooled groups) | 2005 | Prosser | 161 | 145 | 20/113 | 17.70 | 1 | 0.62 | 0.14 | 0.37 | 3.60 |
| Transported (pooled groups) | 2006 | Prosser | 99 | 88 | 63/99 | 63.64 | 2 | 2.02 | 1.05 | 1.22 | 3.27 |
| Transported (pooled groups) | 2007 | Prosser | 140 | 138 | 33/62 | 53.23 | 1 | 0.71 | 0.13 | 0.43 | 1.17 |
| Transported (pooled groups) | 2008 | Prosser | 232 | 223 | 54/99 | 54.55 | 10 | 4.31 | 0.95 | 2.60 | 6.32 |
| Transported (pooled groups) | 2010 | Prosser | 237 | 236 | 40/119 | 33.61 | 2 | 0.84 | #DIV/0! | 0.51 | 1.89 |
| *Total and weighted mean* | | | ***1759*** | ***1530*** | | ***45.14*** | ***15.68*** | ***4.38*** | ***1.48*** | ***2.64*** | ***8.33*** |
| | | | | | | | | | | | |
| Long-term | 2000 | Prosser | 512 | 91 | 17.77 | | | | | 10.71 | |
| Long-term | 2001 | Prosser | 551 | 197 | 35.75 | | | | | 21.54 | |
| Long-term | 2002 | Prosser | 420 | 140 | 33.33 | | | | | 20.08 | |
| Long-term | 2003 | Prosser | 482 | 298 | 61.83 | | | | | 37.24 | |
| Long-term | 2004 | Prosser | 662 | 253 | 38.22 | | | | | 23.02 | 109.49 |
| Long-term | 2005 | Prosser | 386 | 86 | 22.28 | | | | 4.98 | 13.42 | 129.00 |
| Long-term | 2006 | Prosser | 279 | 85 | 30.47 | | | | 15.84 | 18.35 | 49.39 |
| Long-term | 2007 | Prosser | 422 | 221 | 52.37 | | | | 9.25 | 31.55 | 86.10 |
| Long-term | 2008 | Prosser | 472 | 269 | 56.99 | | | | 12.54 | 34.33 | 83.56 |
| Long-term | 2009 | Prosser | 510 | 140 | 27.45 | | | | 5.31 | 16.54 | 61.74 |
| Long-term | 2010 | Prosser | 1157 | 404 | 34.92 | | | | #DIV/0! | 21.03 | 78.10 |
| *Total and weighted mean* | | | ***5853*** | ***2184*** | ***37.31*** | | | | ***12.61*** | ***22.48*** | ***71.03*** |
| | | | | | | | | | | | |
| Long-term | 2005 | Shitike Cr | 9 | 1 | 11.11 | | | | | 6.69 | 64.33 |
| Long-term | 2006 | Shitike Cr | 4 | 0 | 0.00 | | | | | 0.00 | 0.00 |
| Long-term | 2007 | Shitike Cr | 14 | 1 | 7.14 | | | | | 4.30 | 11.74 |
| Long-term | 2008 | Shitike Cr | 11 | 0 | 0.00 | | | | | 0.00 | 0.00 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Total and weighted mean* | | | ***38*** | ***2*** | ***5.26*** | | | | ***3.17*** | ***10.02*** |
| Long-term | 2005 | Omak Cr | 17 | 3 | 17.65 | | | | 10.63 | 102.18 |
| Long-term | 2006 | Omak Cr | 27 | 2 | 7.41 | | | | 4.46 | 12.01 |
| Long-term | 2007 | Omak Cr | 43 | 8 | 18.60 | | | | 11.21 | 30.59 |
| Long-term | 2008 | Omak Cr | 32 | 9 | 28.13 | | | | 16.94 | 41.23 |
| Long-term | 2009 | Omak Cr | 17 | 2 | 11.76 | | | | 7.09 | 26.46 |
| Long-term | 2010 | Omak Cr | 13 | 6 | 46.15 | | | | 27.80 | 103.23 |
| *Total and weighted mean* | | | ***149*** | ***30*** | ***20.13*** | | | | ***12.13*** | ***38.33*** |
| Long-term | 2006 | Parkdale | 1 | 1.0 | 100.00 | | | | 60.24 | 162.11 |
| Long-term | 2007 | Parkdale | 13 | 1.0 | 7.69 | | | | 4.63 | 12.65 |
| Long-term | 2008 | Parkdale | 14 | 7 | 50.00 | | | | 30.12 | 73.31 |
| Long-term | 2009 | Parkdale | 9 | 4 | 44.44 | | | | 26.77 | 99.96 |
| Long-term | 2010 | Parkdale | 15 | 4 | 26.67 | | | | 16.06 | 59.64 |
| *Total and weighted mean* | | | ***52*** | ***17.0*** | ***32.69*** | | | | ***19.69*** | ***62.23*** |
| Natural repeat | 2004 | Bonneville Dam | 1146 | | | 4 | 0.35 | | | |
| Natural repeat | 2005 | Bonneville Dam | 579 | | | 1 | 0.17 | | | |
| Natural repeat | 2006 | Bonneville Dam | 1459 | | | 9 | 0.62 | | | |
| Natural repeat | 2007 | Bonneville Dam | 1973 | | | 12 | 0.61 | | | |
| Natural repeat | 2008 | Bonneville Dam | 2639 | | | 18 | 0.68 | | | |
| Natural repeat | 2009 | Bonneville Dam | 2474 | | | 11 | 0.44 | | | |
| Natural repeat | 2010 | Bonneville Dam | 1342 | | | 6 | 0.45 | | | |
| | | | | | | | ***0.53*** | | | |

* Lower Granite and John Day data from Evans, A.F., R.H. Wertheimer, M.L. Keefer, C.T. Boggs, C.A. Peery, and K. Collis. 2008. Transportation of steelhead kelts to increase iteroparity in the Columbia and Snake Rivers.