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TECHNICAL REPORT 10-13

2009 Annual Report Steelhead Kelt Reconditioning and Reproductive Success

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June 29, 2010

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2009 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). Examination of gamete and progeny viability continued on first-time spawners and reconditioned kelt steelhead. Initial results suggest that egg quantity and/or quality is similar. Reproductive success of three reconditioned kelts that returned to and spawned naturally in Omak Creek has been verified using microsatellite genotyping and parentage analysis of juvenile *O. mykiss*. Yakima River kelts have been successfully identified to stream origin using microsatellite genotyping and GSI (Genetic Stock Identification) analysis. Identification of areas with higher rates of iteroparity such as Satus Creek will increase the statistical power for detection of kelt progeny. The Columbia River Inter-Tribal Fish Commission along with the Nez Perce Tribe and the University of Idaho have expanded research into the Snake River 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 Nez Perce Tribe is developing a steelhead kelt master management plan for the Snake 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. Improvements were made to the juvenile bypass at Lower Granite Dam to improve passage and collection of adult steelhead outmigrating as kelts. Additionally, we are designing an approach to identify specific hormones associated with reproductive status for screening kelts and assigning them to specific management actions.

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, Jake Aleck, Michael Fiander, Carrie Skahan, OJ Davis, and Bob Gleason), 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, and Richard McConville. We would also like to thank Mr. Porter and his students at Warrenton High School for their assistance with food production and necropsies.

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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, 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 2006). 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 2006 and 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 2006).

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 in 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 that have had the biggest impact 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. mykiss*, at rates estimated to be as high as 79% for populations in the Utkholok River of Kamchatka, Russia 1994-96 (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), exceeded 17% (NMFS 1996). 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 to encourage reinitiation of feeding, 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 (reproductive success and gamete/progeny viability) to determine the extent to which reconditioned kelts are contributing to subsequent generations. The ultimate 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 4 sections:

- **Section 1:** Describes the evaluation of various management strategies that could be used as tactics for steelhead restoration programs.
    - In-River at Yakima River (Control)
    - Transporting Unfed kelts
    - Transporting Fed kelts
    - Long-term reconditioning
        - Yakima River (Prosser, WA)
        - Omak Creek (Okanogan River)
        - Warm Springs River
    - Management Scenario Evaluation

- **Section 2:** Includes an evaluation of progeny and gamete viability of Skamania stock steelhead in the Hood River.
    - 2007 (kelts)
    - 2008 (kelts)
    - 2009 (First time and initial kelt spawning)
    - 2010 (first-time spawners)

- **Section 3**: Field study of reproductive success of reconditioned kelt steelhead in the following basins:
    - Omak Creek
    - Yakima River
    - Warm Springs River

- **Section 4** Snake River Basin kelt steelhead evaluations. The Nez Perce Tribe and two University of Idaho groups are conducting studies on kelt steelhead.
    - The Nez Perce Tribe
        - Coordination of basin efforts,
        - Development of a master plan to address the BiOp requirement to increase B-run steelhead abundance using kelt steelhead.

D Idaho Cooperative Fish and Wildlife Research Unit

    - Idaho Cooperative Fish and Wildlife Research unit
        - Describing the general physiology and endocrinology of kelts from the point of spawning through the early migration period using hatchery and non-hatchery origin stocks.
        - Physiological and endocrinological profiles from downstream migrating wild stocks captured at Lower Granite Dam versus fish from upriver sites.
        - Physiology of fish collected at Lower Granite Dam and transported via barge or truck to locations below Bonneville Dam.
    - Dr. James Nagler's lab
        - Establish and validate assays for plasma and tissue level bioindicators of reproductive status, growth, and stress in steelhead kelts and post-spawning rainbow trout.
        - Construct a profile of post-spawning recovery and reproductive redevelopment in a hatchery model of Snake River B-run kelt steelhead held in captivity using non-lethal sampling.
        - Compare reconditioning profiles of kelt steelhead at different locations in the Columbia basin using non-lethal sampling.
        - Determine the sequence of events in reproductive rematuration in post-spawning rainbow trout.
        - Compare reproductive rematuration in post-spawning rainbow trout and kelt steelhead.
        - Determine whether ghrelin administration stimulates appetite and growth in rainbow trout.

References:

Behnke, R.J. 1992. Native trout of western North America. American Fisheries Society Monograph 6.

Bell, G. 1980. The costs of reproduction and their consequences. The American Naturalist 116(1):45-76.

Evans, A.F., and R.E. Beaty. 2001. Identification and enumeration of steelhead (*Oncorhynchus mykiss*) kelts in the juvenile collection systems of Lower Granite and Little Goose dams, 2000. Annual Report to U.S. Army Corps of Engineers, Walla Walla District, for Contract No. DACW-00-R-0016. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.

Evans, A.F., R.E. Beaty, D.R. Hatch, J. Blodgett, and D. Fast. 2001. Kelt reconditioning: A research project to enhance iteroparity in Columbia Basin steelhead (*Oncorhynchus mykiss*). 2000 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2000-017. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.

Fleming, I.A. 1998. Pattern and variability in the breeding systems of Atlantic salmon (Salmo salar), with comparisons to other salmonids. Canadian Journal of Fisheries and Aquatic Sciences 55:59-76.

Gunsolus, R.T. and G.J. Eicher. 1970. Evaluation of fish-passage facilities at the North Fork project on the Clackamas River in Oregon. Research report to the Fish Commission of Oregon, Oregon Game Commission, United States Bureau of Commercial Fisheries, United States Bureau of Sport Fisheries and Wildlife, and Portland General Electric.

Hatch, D.R., P.J. Anders, A.F. Evans, J. Blodgett, B. Bosch, D. Fast., and T. Newsome. 2002. Kelt reconditioning: A research project to enhance iteroparity in Columbia Basin steelhead (Oncorhynchus mykiss). 2001 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2000-017. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.

Hatch, D.R., R. Branstetter, J. Blodgett, B. Bosch, D. Fast, and T. Newsome. 2003b. Kelt reconditioning: A research project to enhance iteroparity in Columbia Basin steelhead (Oncorhynchus mykiss). Annual Report to the Bonneville Power Administration for Contract No. 00004185. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland OR.

ISRP (Independent Scientific Review Team) 1999. Scientific issues in the restoration of salmonid fishes in the Columbia River. Fisheries 24(3):10-19.

Keefer, M.L., R.H. Wertheimer, A.F. Evans, C.T. Boggs, and C.A. Peery. 2008. Iteroparity in Columbia River summer-run steelhead (*Oncorhynchus mykiss*): implications for conservation. Canadian Journal of Fisheries and Aquatic Sciences 65:2592-2605.

Narum S.R., D. Hatch, A.J. Talbot, P. Moran, and M.S. Powell. 2008. Iteroparity in complex mating systems of steelhead *Oncorhynchus mykiss* (Walbaum). Journal of Fish Biology 72:1-16.

Nehlsen, W., J.E. Williams, and J.A. Lichatowich. 1991. Pacific salmon at the crossroads: Stocks at risk from California, Oregon, Washington, and Idaho. Fisheries 16: 4-21.

NMFS (National Marine Fisheries Service). 1996. Status review of west coast steelhead from Washington, Idaho, Oregon, and California. Seattle, WA.

NMFS (National Marine Fisheries Service). 1997. Endangered and threatened species: Listing of several evolutionary significant units (ESUs) of west coast steelhead. Final rule. Federal Register 62:43937.

NMFS (National Marine Fisheries Service). 1999. Endangered and Threatened Species: Threatened Status for Two ESUs of Chum Salmon in Washington and Oregon, for Two ESUs of Steelhead in Washington and Oregon, and for Ozette Lake Sockeye Salmon in Washington; Rules. Final rule. Federal Register 57:14517

NPPC (Northwest Power Planning Council). 1986. Compilation of information on salmon and steelhead losses in the Columbia River Basin. Portland, OR. 252 p.

NPPC (Northwest Power Planning Council). 1995. 1994 Columbia River Fish and Wildlife Program (revised 1995). Portland, Oregon.

NRC (National Research Council). 1996. Upstream: Salmon and society in the Pacific Northwest. National Academy Press, Washington D.C.

Olsen, E.A., 2008. Hood River and Pelton Ladder Evaluation Studies. Annual Report 2007 to U.S. Dept. of Energy, Bonneville Power Administration, Project No.1988-053-04. Prepared by the Oregon Department of Fish and Wildlife, Portland, OR.

Savvaitova K.A., K.V. Kuzishchin, S.V. Maksimov, and S.D. Pavlov. 1996. Population Structure of Mikizha, Salmo mykiss in the Utkholok River (Western Kamchatka). Journal of Ichthyology 37(3):216-225.

Seamons T.R., Guest Lecture. Individual lifetime reproductive success of repeat spawning vs. one-time spawning steelhead. Pacific Coast Steelhead Management Meeting. Pacific States Marine Forecast Comission. Port Townsend, WA. 7 March 2006

Taborsky, M. 2001. The evolution of bourgeois, parasitic, and cooperative reproductive behaviors in fishes. Journal of Heredity 92:100-110.

Section 1: Management Scenario Evaluation

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Goals

The premise 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 may 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. Furthermore, 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 for release back into study streams.

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 and the long-term treatment was replicated geographically.

<u>Yakima In-River Release</u>
A systematically selected portion (1 in 10) of the kelts that would have been suitable for reconditioning were PIT-tagged and released immediately back to the Yakima River to act as a control group. These PIT-tagged kelts provide baseline data and an opportunity to compare Hockersmith et al. (1995) reported repeat spawner rates inferred from steelhead scale pattern analysis from the Yakima River.

Transport Unfed (No-term) Treatment

In this treatment we directly transport steelhead kelts around the hydro-system and evaluate success by measuring survival to the ocean and survival to return. 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 last occurred in 2008 but we continued to monitor acoustic and PIT tag detections from the No-term releases throughout the Columbia River Basin.

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, they may have a predatory effect on migrating salmonid smolts. It is also important to assess whether transportation impacts the homing ability of these fish. To address these concerns, all steelhead kelts were PIT-tagged with a portion also receiving 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.

Transport Fed kelts (Short-Term) Treatment

Short-term reconditioning is defined as the period of time needed (approx. 3-12 weeks) for kelts to initiate post-spawn feeding, followed by the transportation of kelts around mainstem hydroelectric facilities for release, and rematuration in the Pacific Ocean. Successful expression of iteroparity in steelhead may be limited by post-spawning starvation and downstream passage through the mainstem corridor. Thus, short-term reconditioning may augment iteroparity rates by initiating the feeding response while still allowing kelts to naturally undergo gonadal recrudescence in the estuary and marine environments. Since short-term reconditioned fish were also transported and released below Bonneville Dam, PIT-tag and hydro-acoustic tags were used to assess fish survival (based on detection or lack of), movement, distribution, travel time, velocity, as well as residence time in the estuary. The transport fed (Short-term) release last occurred in 2008 but we continued to monitor return acoustic and PIT tag detections from the fed (Short-term) releases throughout the Columbia River Basin.

Long-term Reconditioning Treatment

We define long-term reconditioning as holding and feeding post-spawn steelhead until the upstream migrating runs appear, typically in mid to late October for the Yakima River. The fish are released to over-winter and return to the spawning sites on their own volition. 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 Omak Creek, Warm Springs River, and Young's Bay.

Study Area

Kelt Reconditioning

Prosser Hatchery is located on the Yakima River at river kilometer (Rkm) 75.6, downstream from Prosser Dam, and adjacent to the Chandler Juvenile Evaluation Facility (CJEF) where steelhead kelts are collected for the in-river, no-term, short-term, and long-term treatments (Figure 1). The Yakima River is approximately 344 km in length and enters the Columbia River at Rkm 539. 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). The Prosser Hatchery is operated by the Yakama Nation (YN), with a primary function of rearing, acclimation, and release of fall chinook salmon *O. tshawytscha*, and is also used for coho salmon *O. kisutch* rearing prior to acclimation and release in the upper Yakima River Basin. Long-term reconditioned fish 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.

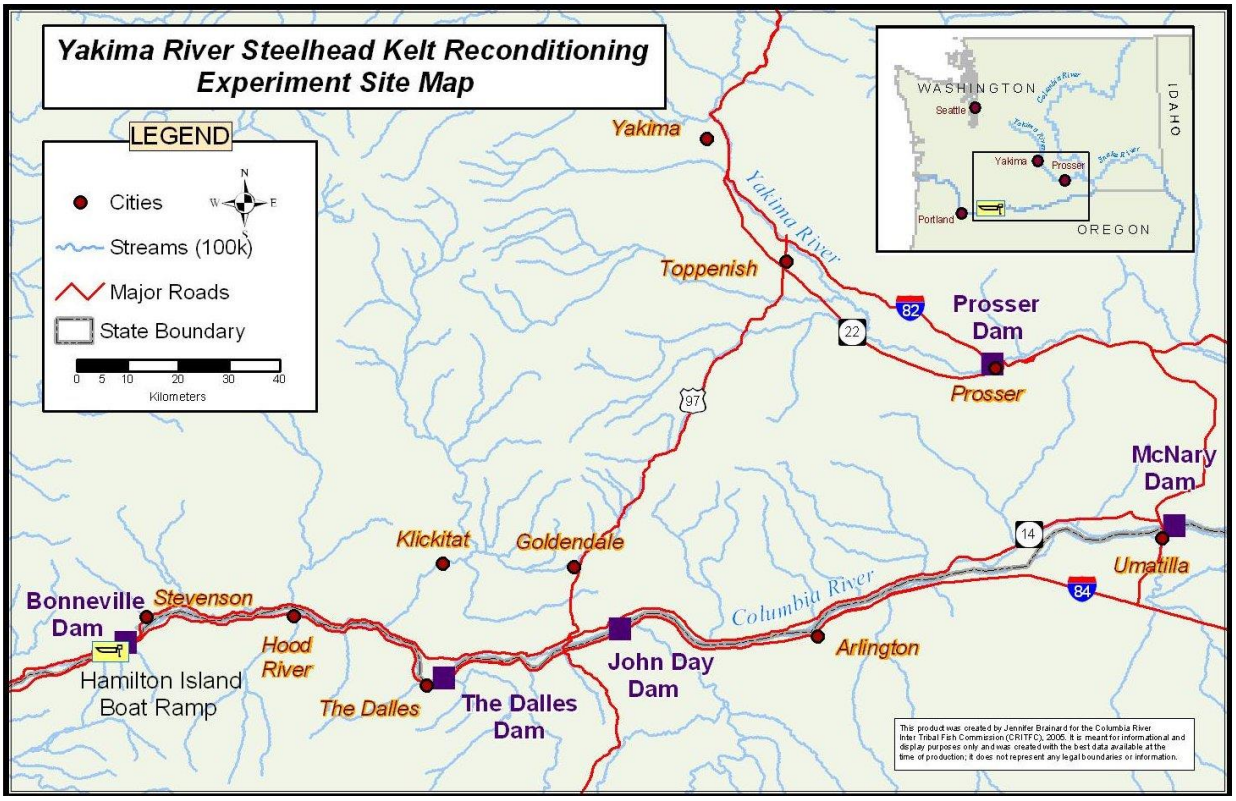


Figure 1: Reconditioning site (Prosser, WA) and release site (Hamilton Is. Boat Ramp) for No-term and Short-term reconditioning experiments.

Steelhead for the transport treatments were trucked and released at the Hamilton Island Boat Ramp (Rkm 231) located downriver from Bonneville Dam on the Washington shore of the Columbia River in 2008. 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. In 2009 we continued to monitor for the return migration of experimental groups in the lower Columbia River using acoustic telemetry technology (Figure 2) (Rkm 138 to 0.) (Appendix B). Migration behavior, return rates, and timing were evaluated for these treatments.

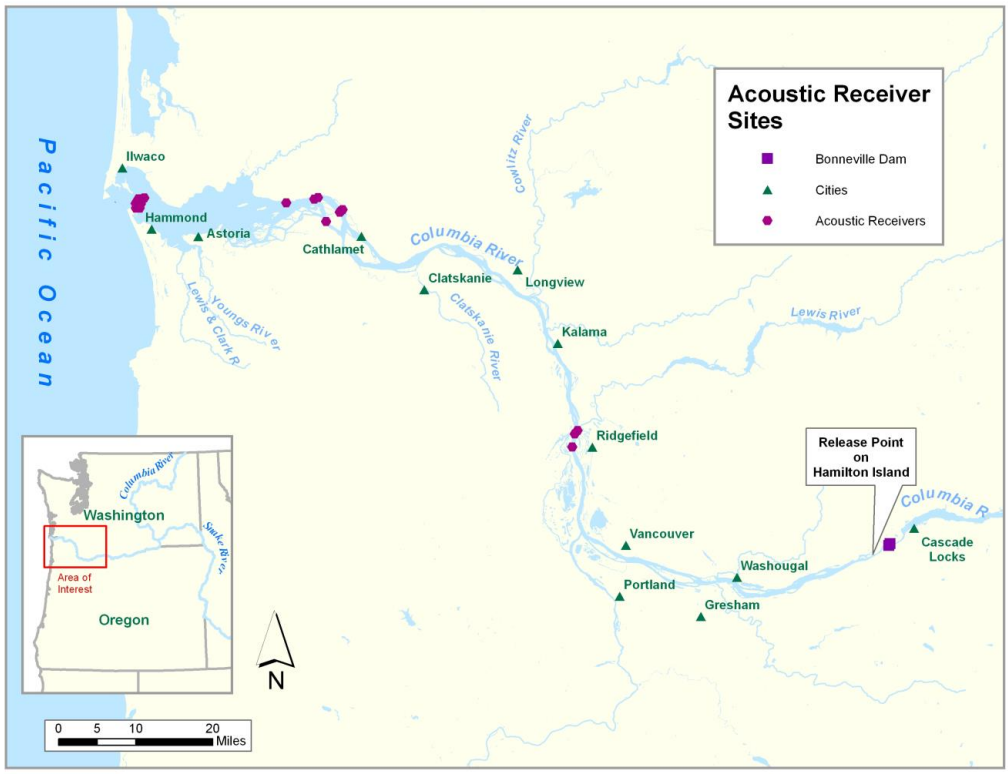


Figure 2. Lower Columbia River acoustic receiver deployment 2009.

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 3). 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.

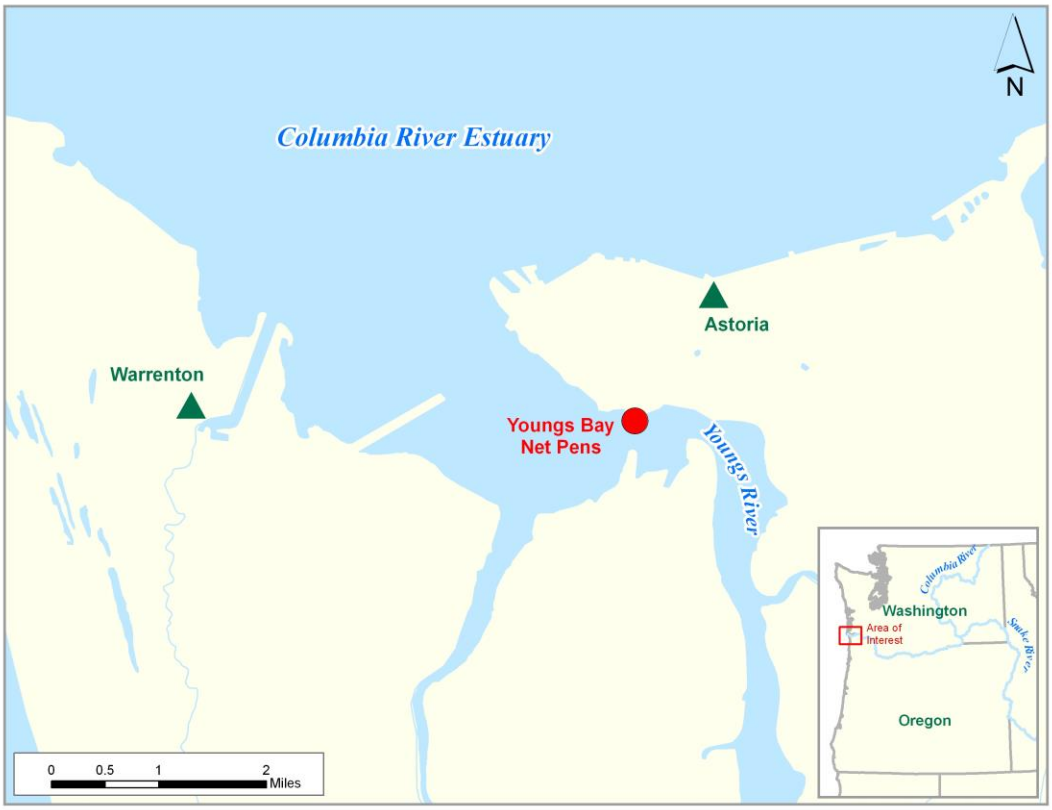


Figure 3: Young's Bay Net Pen locations for lower Columbia River steelhead kelt reconditioning 2009.

The three additional groups of kelt steelhead collected for long-term reconditioning were from Omak and Bonaparte creeks (tributaries to the Okanagon River) with reconditioning at Cassimer Bar Hatchery, Warm Springs River (tributary to the Deschutes River) with reconditioning at Warm Springs National Fish Hatchery (WSNFH), and the Hood River (tributary of the Columbia River) with reconditioning at the Parkdale Fish Facility.

Omak Creek, a tributary to the Okanogan River, is located in Okanogan County in North Central Washington. It is approximately 35.4 km ending at its confluence with the Okanogan River (Figure 4). It runs entirely within the reservation of the Colville Confederated Tribes (CCT). Bonaparte Creek is a tributary to the Okanogan River, which closely parallels State Route 20 east of Tonasket. Omak Creek and Bonaparte kelt steelhead were reconditioned at the Cassimer Bar Hatchery located at the confluence of the Okanogan River. Currently the CCT operate the Cassimer Bar Hatchery. The facility was originally constructed in 1994, as a sockeye salmon *O. 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.

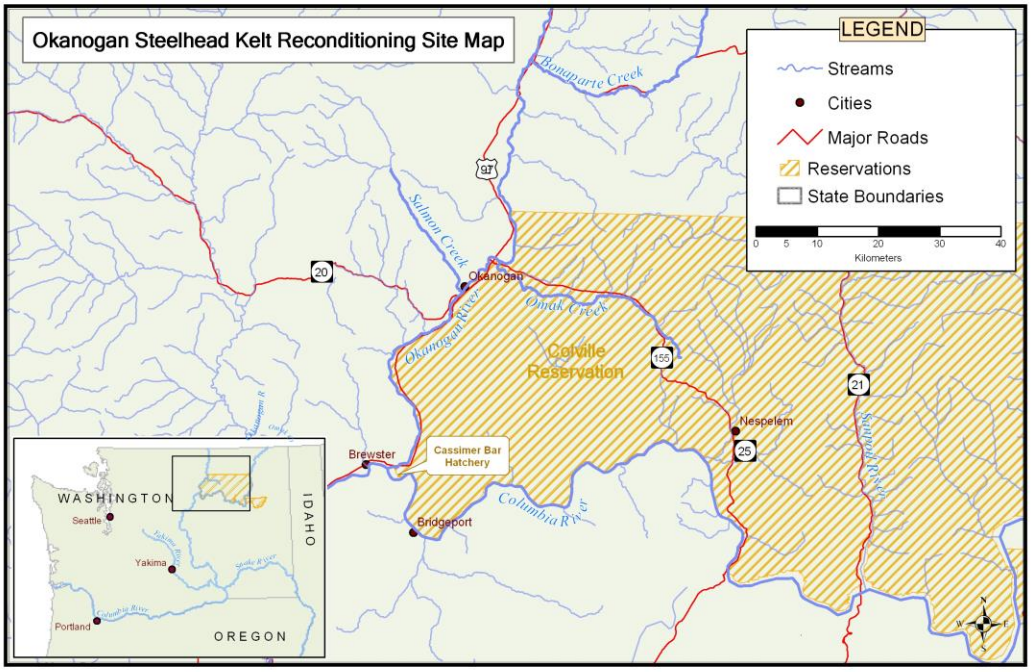


Figure 4. Map showing the locations of Omak Creek as well as the Confederated Tribes of the Colville Reservation.

The Warm Springs National Fish Hatchery is located within the boundaries of the Confederated Tribes of Warm Springs Reservation. The reservation covers 240,000 ha. It is located on the eastern slopes of the Cascade Mountains. The reservation boundaries run from the crest of the Cascades to the north and west, the Deschutes River to the east and the Metolius River to the south.

Work was concentrated on Warm Springs River and Mill Creek (Figure 5). The Warm Springs River (44 51 29.79 lat. -121 04 0.62 lon.) is the largest river system within the reservation. The river flows for 85 kilometers and drains 54,394 hectares. It enters the Deschutes River at Rkm 135. The WSNFH is located at Rkm16. Fish were captured, airspawned, and held for reconditioning at WSNFH. Mill Creek is a major tributary to the Warm Springs River. Its confluence is at Rkm 32. Two weirs were placed in Mill Creek (Rkm 25.2 and 26.5) to inhibit immigration and emigration from the reintroduction site.

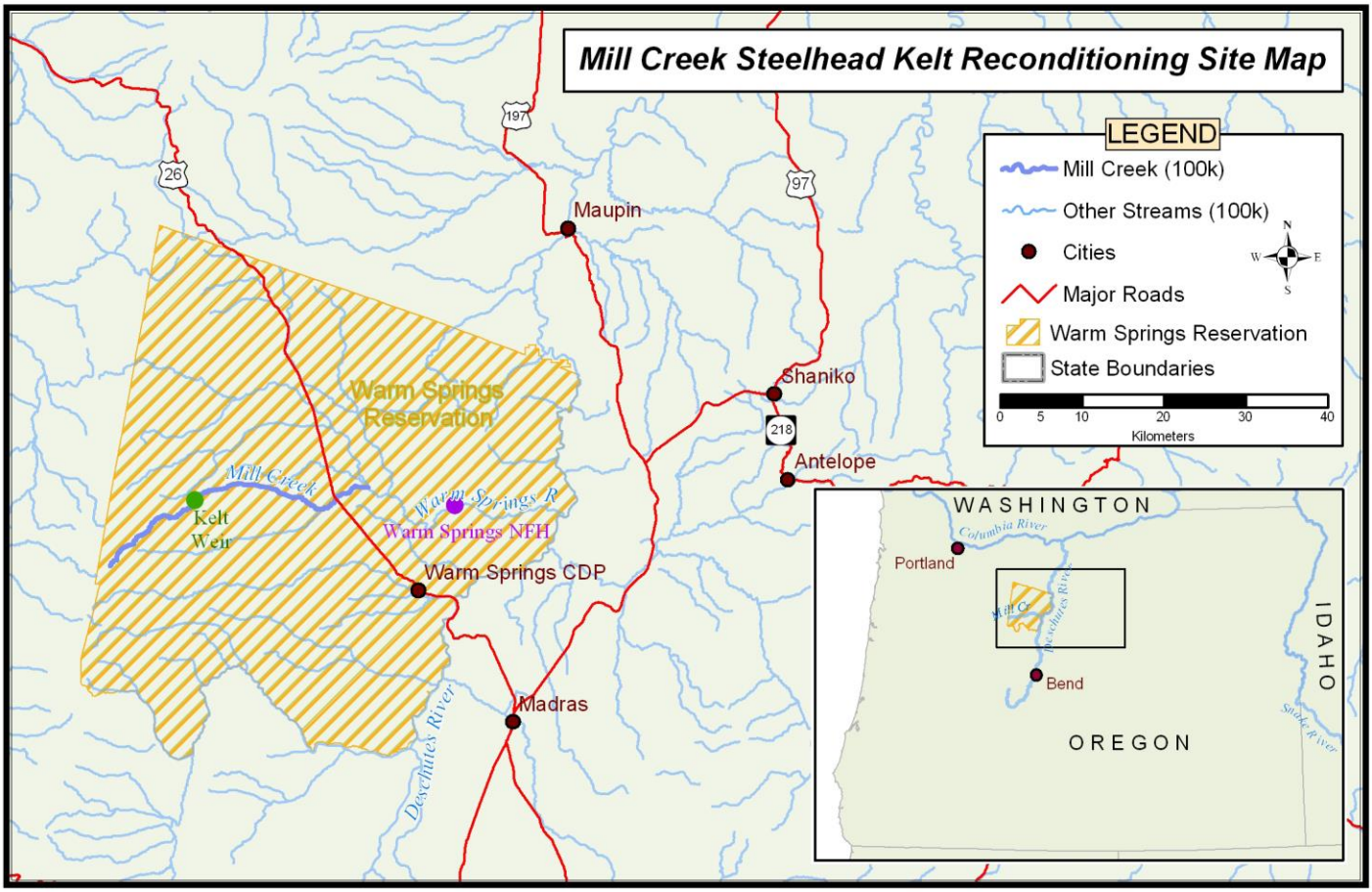


Figure 5: Warm Springs project area 2009.

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 6). The Hood River is a tributary of the Columbia River 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. Adult steelhead collection for the Parkdale Fish Facility is 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).

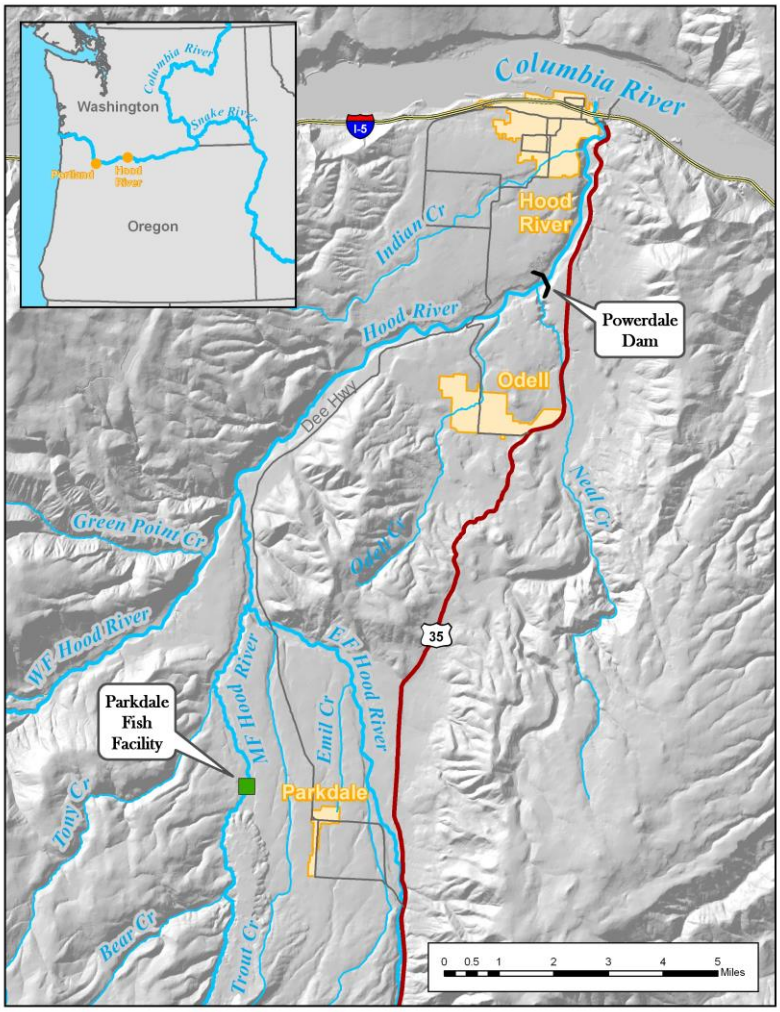


Figure 6. Location of Parkdale Fish Facility and Powerdale Dam/ Fish Trap.

Methods

Kelt Collection and In-Processing

Yakima River

After spawning naturally in tributaries of the Yakima River, a portion of the steelhead kelts that encounter Prosser Dam during emigration are diverted into an irrigation channel that directly connects to the Chandler Juvenile Evaluation Facility (CJEF) (Figure 7). Fish screens divert migratory fishes away from the irrigation canal to reduce mortality associated with agriculture. Once diverted into the CJEF, emigrating kelts were 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 for processing or release back to the river). Yakama Nation (YN) staff monitored the Chandler bypass separator 24 hours a day from April 9, 2009- July 7, 2009.

Figure 7. Chandler Juvenile Evaluation Facility adult separator.

All adult steelhead arriving at the CJEF separator were dipnetted off the separator (Figure 7) and placed into a water-lubricated PVC pipe slide that was directly connected to a temporary holding tank 20' (l) x 6' (w) x 4'(h) containing oxygenated well water (57^0F or 13.8^0C). Post-spawned steelhead kelts were identified (Evans and Beaty 2001) 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. All specimens visually determined to be prespawn individuals were immediately returned to the Yakima River.

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 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. Steelhead kelts this year were not administered antibiotics initially this year based on the documented lack of prophylactic benefit in other species (Grondel et al. 1987, Al-Ankari, 2005, Studnicka et al., 2000, Serezli et al., 2005, Tafalla et al. 1999). However antiobiotic treatment was resumed in June by the Yakama Nation after high infestation and mortality was observed.

In-River Release

A systematic sample (1 of 10) of kelts suitable for reconditioning, were instead 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 from the Yakima River (Hockersmith et al. 1995). In-river release specimens were selected systematically throughout the duration of the steelhead kelt run.

Adult Collection

Omak and Bonaparte Creeks

The Omak Creek weir (Rkm 0.8) is utilized to collect broodstock and steelhead kelts for reconditioning (Figure 8). This stock is being used by the Cassimer Bar Hatchery to develop a naturalized steelhead broodstock for the OkanoganRiver and Omak Creek. To increase the total number of kelts available for reconditioning, kelts were also collected from the Bonaparte Creek weir which is a tributary of the Okanogan River.

Kelts are collected for reconditioning in either of two ways at Cassimer Bar: 1) males and females collected for broodstock that survive spawning are put into the kelt tank for reconditioning. 2) kelts exiting Omak Creek or Bonaparte Creek are collected at the trap site of the respective creek and transported to the Cassimer Bar hatchery. All anadromous *O. mykiss,* regardless of up or downstream movement and not 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 received salt treatments on a regular basis to help prevent against fungus and copepod infestation.

Figure 8: resistance board weir located on Omak Creek.

Warm Springs River

The initial sampling plan approved by the Warm Springs Tribal Fish and Wildlife Committee stipulated that 150 fish had to pass the weir before collection could proceed. Because of the low run this year, collection occurred only at the tail end of the run. We began collecting steelhead at WSNFH on May 11, 2009 (Figure 9). The Warm Springs National Fish Hatchery (WSNHF) weir was checked daily by WSNFH staff until May 29, 2009.

We targeted pre-spawn migrating wild adults. Prior to moving fish to the reconditioning tank, we collected information on fish regarding origin (hatchery or wild), sex, physical condition, coloration and fork length. Fish were also inspected marks (e.g., tags and secondary marks). We based condition on the presence and amount of fungus. Good, fair, and poor condition was based on < 25%, 25 - 50%, and 50 - 100% fungus, respectively. Due to the late start, we included fish in poor condition.

Figure 9: Warm Springs National Fish Hatchery Adult Trap

Powerdale Trap

Oregon Department of Fish and Wildlife Employees captured Skamania-run steelhead first-time spawners at the Powerdale trap (Figure 10) and trucked them 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 measure the impact of reconditioning. Trapping begins in June and ends in early March for the Skamania project fish. Collection ended when we obtained our target of 20 pairs of first time spawning steelhead.

Figure 10: Powerdale trap on the Hood River.

Young's Bay Net Pens

Twenty kelts captured at the CJEF were trucked to the Young's Bay Net Pens. Temperatures between the reconditioning facilities at Prosser 11.1^{0}C (52^{0}F) and the net pens 11.6^{0}C (53^{0}F) in Astoria were similar. Fish are transferred from the fish hauling truck to a small powered float that has two oxygenated totes (4' x 4' x 4' ft). They are then netted from the totes and released to the net pens.

Reconditioning

Prosser Hatchery

Steelhead kelts retained for the short-term and long-term reconditioning treatments at Prosser Hatchery were held in one of four 20' (d) x 4' (h) circular tanks (Figure 11). Loading densities were well below the 200 fish carrying capacities of these tanks. Tanks were fed oxygenated 13.8^{0}C (57.^{0}F) well water at 200 gallons/minute. Short-term reconditioned kelts that were released in 2008 were fed a diet of krill for the duration (3-5 weeks) of their captivity. Their were no short-term fish in 2009.

Figure 11: Steelhead kelt reconditioning tanks Prosser, WA.

Long-term reconditioned fish at Prosser Hatchery were initially fed frozen krill for 2.5 weeks then slowly switched over to Moore-Clarke Trout Broodstock pellets until release. Krill is utilized as a starter feed due to the readiness of kelts to consume this specific feed. Steelhead kelts are then slowly moved over to the Moore-Clark pellets to improve nutrition in the diet.

Cassimer Bar

One 22' circular tank was used to recondition Omak Creek steelhead kelts (Figure 12). Water was circulated at 120 gallons/minute at an average temperature of 13.3^{0}C (56.0^{0}F). Kelts were then separated by sex into circulars. 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 12: Steelhead kelt at Cassimer Bar Hatchery. Reconditioning tanks to left and right w/ sampling area in center.

Warm Springs National Fish Hatchery

The Warm Springs River kelts were held in a circular fiberglass tank, 16' (d) X 4' (h) at WSNFH (Figure 13). We attempted to mimic a natural setting by incorporating a small log, rocks, hiding locations and a small cedar tree in the tank (Figure 14). Artificial cover was also made by using sheets of plywood and 2' X 6' lumber. The tank was supplied with filtered water from the Warm Springs River. Kelt tank temperatures were regulated using a mix of chilled (May thru 18 August 2009) and unchilled river water (post 19 August). An input approximately 60 gallons/minute was maintained during majority of the study. We also treated the water with Formalin once per day at a rate of 45 ml per minute to prevent infestations.

Figure 13: Steelhead kelt reconditioning tank at WSNFH

Figure 14: Inside view of the tank.

Parkdale

Skamania run steelhead kelts were held in a 40'l x 8'w x 4'd raceway at 400 gal/min until ripened and ready for spawning (Figure 15). 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) with water flow at 60 gal/min for reconditioning and were held there until late September were they were placed back into the raceway for the duration of the winter season until the following year's spawning (Figure 16).

Figure 15: Raceways where kelts are held from late fall to early spring.

Figure 16: Kelt seasonal (late spring to early fall) reconditioning circulars

Young's Bay

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 17).

Figure 17: 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 Cassimer Bar Hatchery, Warm Springs National Fish Hatchery, Young's Bay and the Parkdale Fish Facility (Hatch et al. 2004). Steelhead kelts are initially fed krill and then slowly transitioned to pellet feed, Feeding occurs 2-3 times a day to satiation, and is monitored to prevent overfeeding which causes pollution in the holding. Hatchery managers and project staff are allowed to modify protocols as needed to improve survival.

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. During the period of time between the first kelt arriving on station until the last kelt being processed, fish are fed natural foods. Krill coated with cod liver oil, and squid, provide a rich source of nutrients that the fish would 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 hand made food. As hand made food is introduced, natural food is also 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.

WSNFH

Modifications of the original Prosser diet (e.g., squid, pellets) were made throughout the season to improve survival. Generally fish were fed to satiation 2-3 times a day. We observed each feeding event to determine if fish were eating. Initially, kelts were fed krill or krill coated with "Mikes" Extra Strength Glo Scent Shrimp Oil, with squid and pellets added later to the diet.

Parkdale

Fish were fed at Parkdale initially upon entering the Parkdale facility 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.

Young's Bay

Due to concerns about raw feed being deposited into the Young's Bay River kelts held were fed a mashed mix of krill and Bio-Oregon brood pellets (formed into a noodle like consistency using a meat grinder) three times daily for the duration of the holding time.

Kelt Mortalities

The following data were collected on all kelts that died during the reconditioning process at all facilities. 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. A majority of the no-term and short-term reconditioning releases were fitted with an acoustic receiver to compare release strategies. All surviving specimens retained for reconditioning at the time of release were classified as either feeding or non-feeding based on weight change during captivity.

Unfed and Fed transport treatment groups were released at the Hamilton Island Boat Ramp (Rkm 231), below Bonneville Dam in 2008. Acoustic arrays were deployed in 2009 and will be again in 2010 to detect skip-year spawning fish.

Fish in the long-term experiments were released in late October 2009 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 of Prosser Dam (Yakima River, 75.6 Rkm) and 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.

Prior to release, growth measurement data and rematuration status were recorded on all released individuals. Reconditioning success was based on the proportion of fish that survived the reconditioning process, the number of fish that successfully rematured (based on ultrasound examinations), and the number of fish that were detected actively migrating above Prosser Dam.

Comparison of Treatments Using Biotelemetry

Acoustic Telemetry

There were 49 kelts from the No-term and 50 kelts from the Short-term reconditioning experiments at Prosser Hatchery that had a coded Vemco© V16-4H acoustic transmitter surgically implanted intraperitoneally (body cavity) using standard surgical procedures in 2008. The weight impact of the tag on adult fish was nominal with its length at 65 mm and weight in water at 10g, which constitutes on average 0.25% of the fishes total body weight. In an internal implantation, an incision just smaller than the transmitter 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. Closure of the incision is accomplished 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.

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 B). Each acoustic tag has a unique bandwidth pulse that provides individual identification codes. The complete array was deployed in early March 2009 and was retrieved late October the same year. This year's array placement remained nearly identical to previous year's (Branstetter et al. 2008) (Appendix B) with the exception of a newly placed array that was used for detecting returning kelts due to it's relative isolation and tight navigation channel to protect against receiver attrition. This arrangement would provide us data on survival and timing in-river, to the estuary, and to the ocean while attempting to reduce loss of receivers. Using acoustic telemetry data we can compare Unfed and Fed reconditioning experiments to assess fish return rates, movement, distribution, travel time, velocity, as well as residence time in the estuary (Appendix B).

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.

Management Scenario Evaluation

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 7 years of data from Prosser Hatchery, 3 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).

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

General Population Characteristics

Yakima River

A total of 621 live kelts were captured between April 9 and July 7, 2009. Of these captures, 13 were mortalities in the bypass, 20 were immediately culled due to poor condition, 58 were used in the Yakima River in-river release, 510 were retained for long-term reconditioning/release, and 20 fish were used for the Young's Bay net-pen reconditioning. Collection was continuous throughout the outward migration, with peak collection occurring on May 5, 2009 (Figure 18). The total number of kelts captured represented 18% (621 of 3,450) of the previous Yakima River spawning migration based on fish ladder counts obtained from Prosser Dam for the period July 1, 2008 through June 30, 2009.

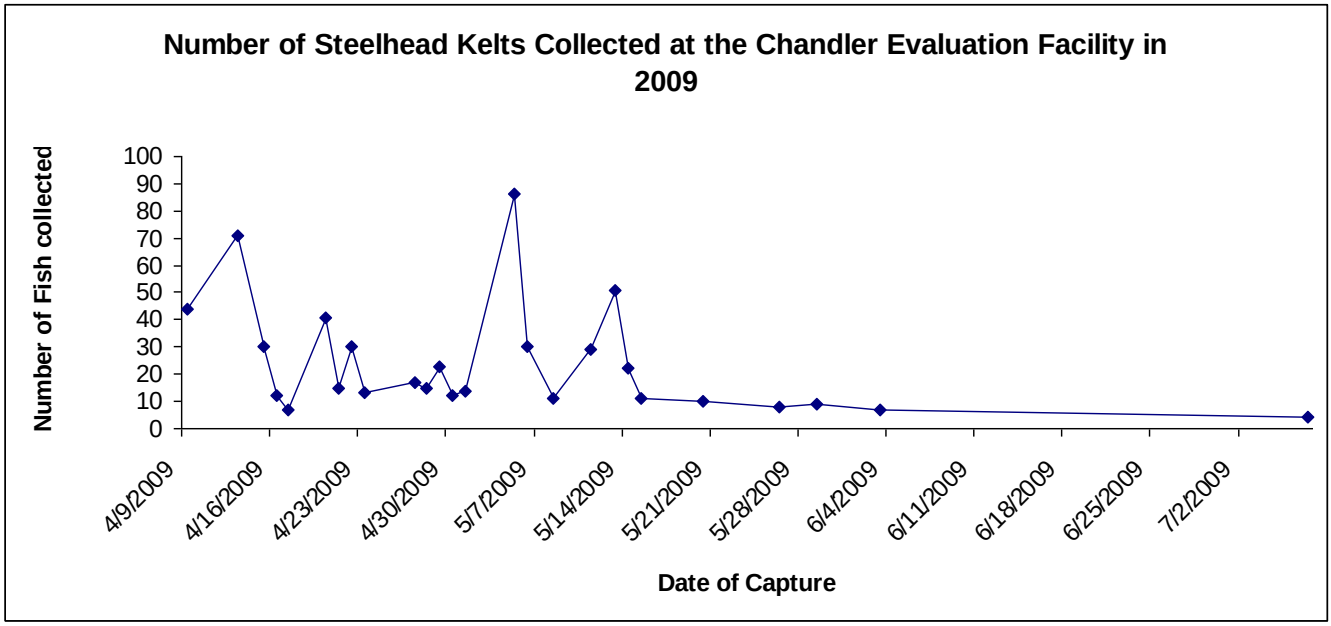


Figure 18: Steelhead Collection at Prosser, WA 2009.

The overwhelming majority of kelts captured were female which is consistent with previous findings. Based on visual observations, in 2009 569 (91%) of the kelts were female, 51 (8%) were male, with 2 fish not Identifiable. Most Yakima River kelts collected during 2009 were classified as being in good (n=227, 37%) or fair (n=365, 59%) condition, with the remaining fish classified as poor (n=30, 4%) including 20 fish immediately culled, 13 mortalities which occurred in the bypass, and 3 which were retained for the long-term reconditioning). Coloration was predominately intermediate (n=333, 54%) or bright (n=274, 44%) with a small percentage that were dark (n=15, 2%).

Omak Creek

The first fish was enumerated on March 2009 at Bonaparte Creek and the last fish released upstream was seen June 2009. The first kelt was collected on April with peak collection occurring in May. Total there were 28 fish enumerated at Bonaparte Creek in 2009 of which we had 21 males and 7 females. There were 3 first-time spawners (2 males and 1 female) that were retained for broodstock and 4 kelts (1 male and 3 females) that were captured. These fish were all retained for reconditioning.

The first fish was enumerated at Omak Creek was in March 2009 and the last fish put upstream was seen June 2009. The first kelt was collected in April with peak collection occurring in May. Forty summer steelhead were collected at the Omak Creek trap with a ratio of 2.6 males for each female was observed (29 males; 11 females). Four steelhead were identified as originating from the Cassimer Hatchery (3 males; 1 female). In addition to the 40 fish that passed upstream of the trap, 5 male and 5 female summer steelhead were transported to the Cassimer Hatchery as kelts (3 male; 1 female) or broodstock (2 male; 4 female).

Warm Springs River

The total steelhead count to the Warm Springs River was 188. The run into WSNFH was 70% wild (132 fish) and 30% hatchery (Figure 19). The first hatchery fish entered WSNFH in November 2008. The first wild fish was captured 2 February 2009. The peak of the run was on April 27, when 39 fish entered the facility (USFWS unpublished).

Eight wild steelhead kelt out-migrants were captured at the WSNFH. The majority (75%) of fish captured were female. Fish were categorized as fair (37.5%) and poor (62.5%) condition for both sexes. While females were 50% fair and 50% poor

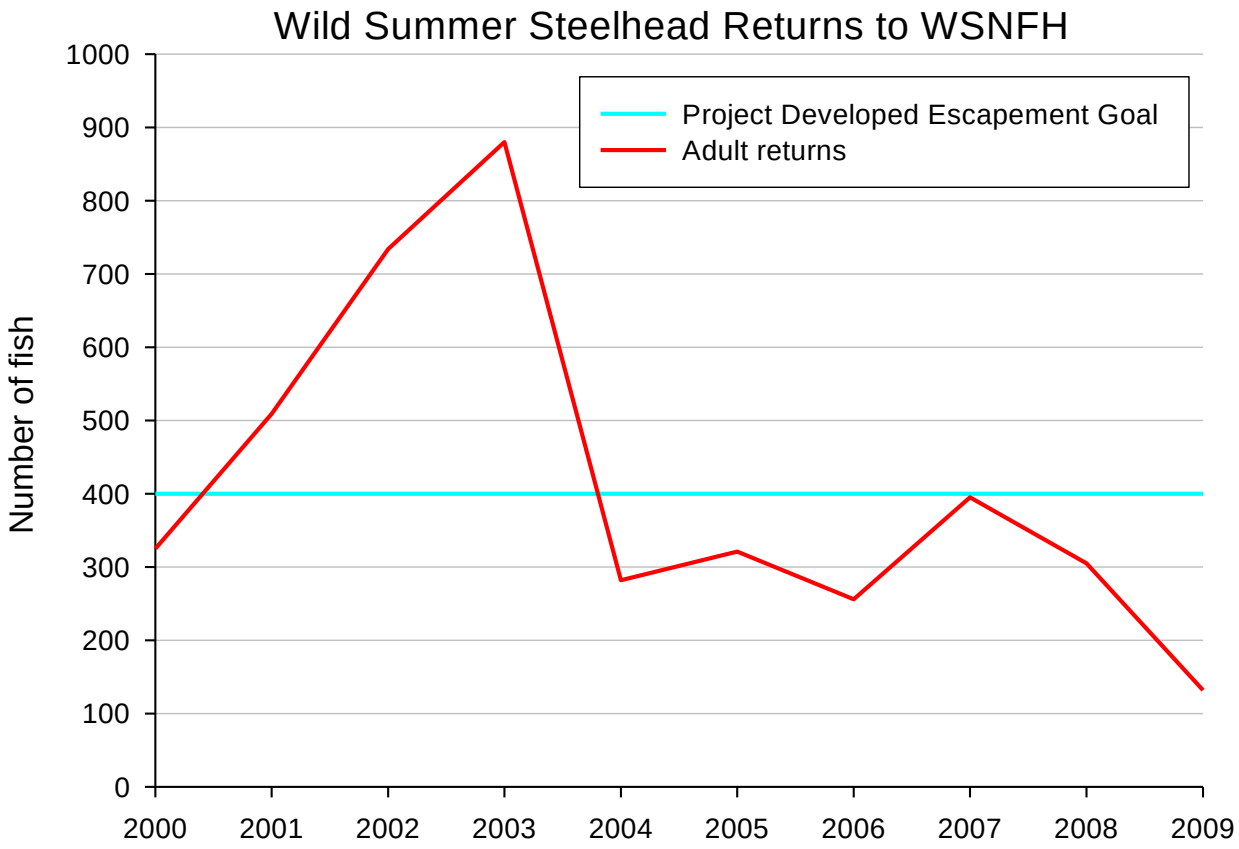


Figure 19. Wild summer Steelhead returns to WSNFH, 2000 - 2009.

The Hood River

The first fish was captured on June 24, 2009. Trapping ended March 2, 2010. A total of 43 steelhead first-time spawners were captured (13 males and 30 females). All of these fish upon initial inspection appeared to be in good to excellent shape with little to no tissue scrapping or damage.

Long-Term Reconditioning and Survival to Release or Spawning

Prosser Fish Hatchery

After induction into the Prosser Hatchery reconditioning facility, 1/3 of the mortalities occurred around the Memorial Day (May 18 to May 26) week at which point antibiotics (oxytetracyclin) were administered to control a furunculosis outbreak. The rest of the mortalities occurred steadily over time with the exception of September when fish had to be re-sampled for disease screening before release. A total of 27.6% of the fish collected for long-term reconditioning survived to October 29, 2009 at which time 140 individuals were released into the Yakima River (Table 1) (Figure 20). Fish were released below Prosser Dam (75.6 Rkm).

Table 1: Long-term reconditioning results by tank 2009.

	Long-term				
					Total
	C1	C2	C3	C4	
Held for Reconditioning	127	128	127	128	510
Released	46	35	32	27	140
Known mort after Rls					
Survival Rate	36.2%	27.3%	25.2%	21.1%	27.5%
Pct with wt gain					89.7%
Avg wt gain/loss (lbs)					1.87

Figure 20: Steelhead Kelt at Prosser prior to release after long term reconditioning.

To date, 45 (32.1%) fish from the long-term release were detected by PIT tag presence at Prosser Dam. Most migratory movements occurred throughout November but there were two additional fish detected migrating upriver in January and in March of 2010.

Cassimer Bar

Only 2 of 17 fish collected in 2009 and reared at Cassimer Bar survived to release (Figure 21). Both were female fish. Copepods were extremely problematic which may have resulted from not applying antibiotics or Ivermectin at Cassimer in 2009.

Figure 21: Reconditioned Kelt at Cassimer Bar prior to release.

Warm Springs National Fish Hatchery (WSNFH)

In 2009 one out of six (all female) fish survived from capture to release at Warm Springs National Fish Hatchery.. One mortality occurred during the air spawning process. The cause of the other four mortalities is unknown but may be related to severe fungus growth on the exterior of the body and stress. . The majority of fish did not initiate feeding. Two fish started eating prior to spawning. After airspawning two fish began to feed after 5 days.

Average water temperature (unchilled) for the kelt tank from 18 May – 28 May was 12.0 ^{o}C. Average water temperature (mixed) for from 29 May – 18 August was 10.1 ^{o}C. Water was not chilled after 18 Aug as WSNFH needing the chilled water for the adult spring Chinook holding ponds and egg incubation, and average water temperature through 23 September was raised to 13.6 ^{o}C (Figure 22.),

The surviving reconditioned female was released into Mill Creek on 28 September 2009 at Rkm 25.2. Upon capture her fork length was 57 cm. Upon release her fork length was 62 cm. No weight was collected at capture to minimize handling stress. Upon release weight was 2,090 grams (Figure 23). Prior to release a treatment of 0.2 ml of OTC and 0.5 ml of Ivermectin was given. A full duplex PIT tag (code 3D9.1BF2536826) was inserted into the pelvic girdle.

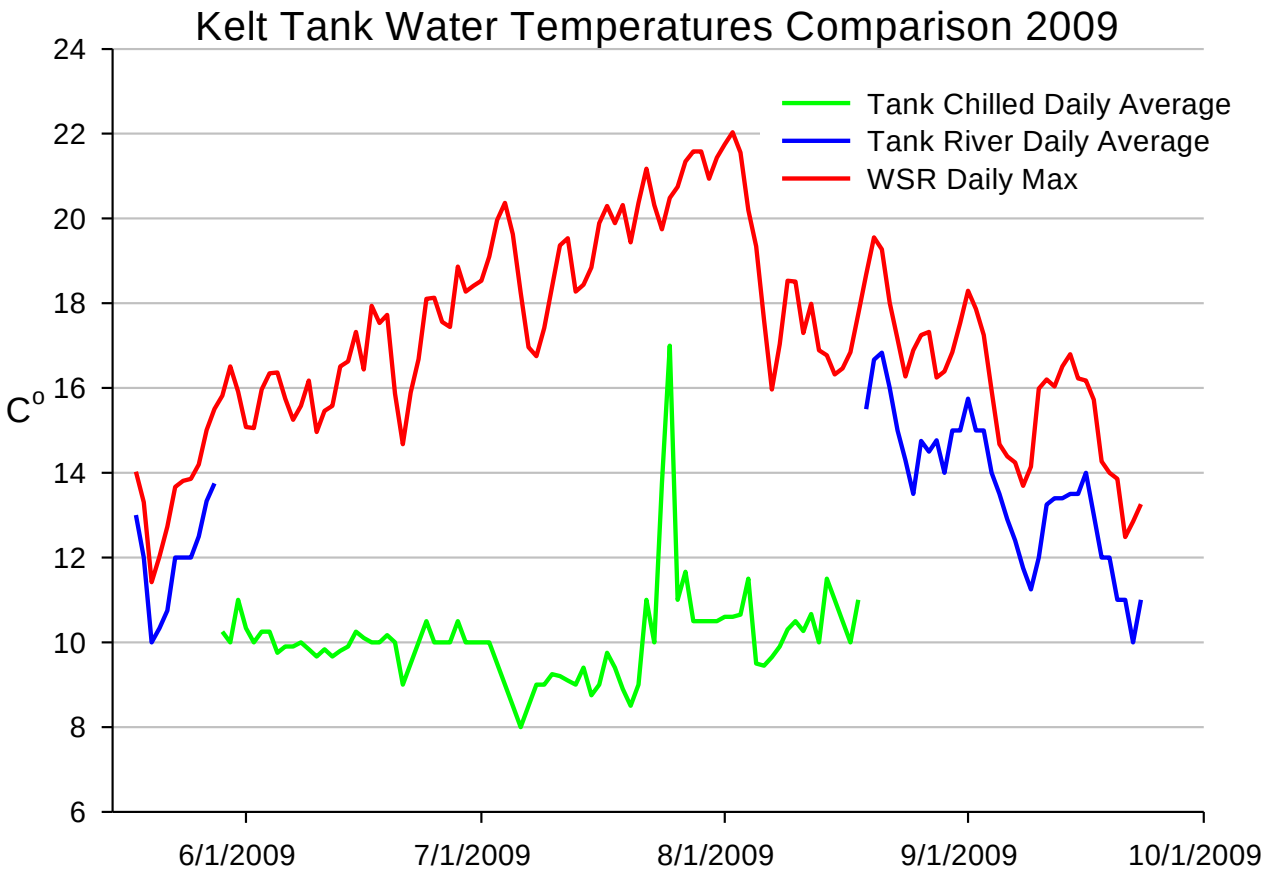


Figure 22. Comparison of kelt tank and Warm Springs River temperatures.

Figure 23: Warm Springs kelt sampling at time of release.

Parkdale (One 2007 female, Seven 2008 females) steelhead kelts already on site were feeding vigorously and appeared visually healthy. There were nine prespawn mortalities from the 2009 Skamania broodstock of which four of these were males and five females. All 2009 male broodstock were sacrificed for milt collection. This left us with twelve females available for spawning of which four survived.

Young's Bay Net Pens

There were 8 Skamania steelhead kelts already on hand from the previous years studies

Water temperatures in Young's Bay increased the weekend of Memorial Day Weekend 2009 likely from warmer temperatures occurring upriver, causing the mortality of approximately half the fish present, with additional mortalities the following week. . We opted to release the remaining 2 kelts into the mainstem of the Columbia River to avoid further fish stress.. Necropsies performed on the fish by ODFW personnel did not prove definitive cause of death due to the rapid decay of the carcasses in the brackish water, but they did find furunculosis bacteria which would coincide with the outbreak at the Prosser, WA fish facility.

Acoustic Tag Detection of Fed and Unfed Transport Fish

We did not detect any steelhead kelts that were released in previous years attempting to return in 2009.

Evaluating Management Scenarios

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 (A) 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) (Figure 24). The proportion of repeat spawners in the run at large at Bonneville Dam is based on scale pattern interpretation of 6 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 0.54%. This indicates that iteroparity is very low in steelhead populations above Bonneville Dam. The return rate to Bonneville Dam of kelts tagged and released in-river at Prosser Hatchery is 3.77% that 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 up river stocks. The comparison group tagged and released at Lower Granite Dam returned to Bonneville Dam at a rate of 0.65%. This is quite low and not statistically different ($p=0.433$) from the run at large at Bonneville Dam.

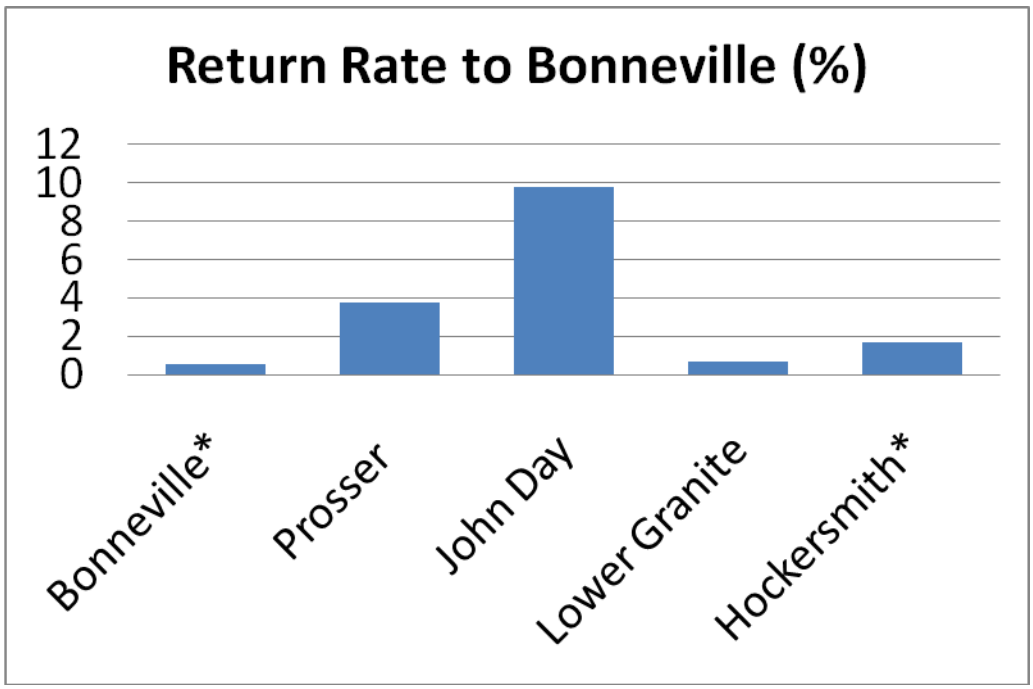


Figure 24. The return rate to Bonneville Dam of repeat spawners from various locations used as "controls" or comparison groups. Note that Hockersmith is a return rate to Prosser Hatchery not Bonneville Dam. Starred groups are based on scale pattern analysis; the remaining groups are based on returns of PIT tagged fish.

Treatment Groups

Kelts collected and transported and released below Bonneville Dam include groups from John Day Dam, Prosser Dam (both fed and unfed groups), and Lower Granite Dam. Return rates to Bonneville Dam for these treatments were 12.55%, 5.81%, 2.96%, and 2.10% for Lower Granite, Prosser Fed, Prosser Unfed, and Lower Granite Dam groups respectively, based on weighted means over all years available (Figure 25).

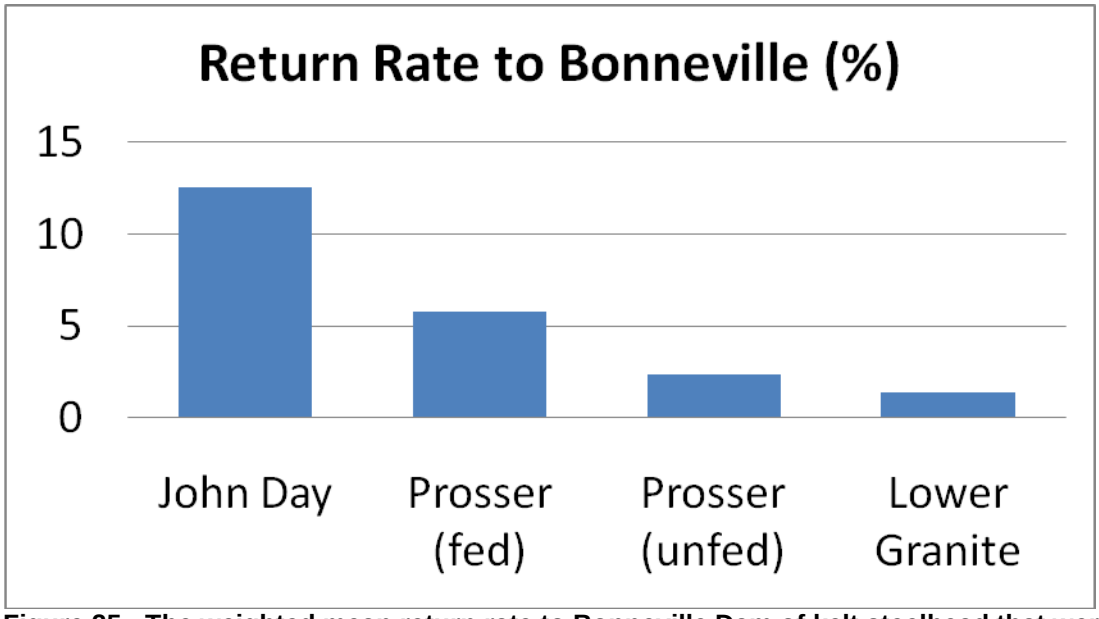


Figure 25: The weighted mean return rate to Bonneville Dam of kelt steelhead that were transported downstream.

To calculate relative transportation benefits we divided the return rates to Bonneville Dam for transport groups by rates from the control groups. Any number greater than 1 is a positive benefit and any number less than 1 is a negative benefit. Kelts transported from Lower Granite Dam showed the highest transport benefit (2.11) relative to in-river control groups (Figure 26) followed by Prosser Fed groups (1.54), and the John Day group (1.29). The Prosser Unfed group had a transport benefit of 0.79 indicating that the in-river control group actually returned at a relatively higher rate. Interestingly, all groups showed transportation benefits relative to the repeat spawners in the run at large at Bonneville Dam ranging from 18.22 at John Day Dam to 2.57 at Lower Granite Dam.

Survival from release through migration to the ocean was estimated for several of the Prosser transport groups using sequential detections of acoustic tags. Release to ocean survival estimates were 46.89% for unfed groups and 48.68% for fed groups. The highest survival was 70.37% for the fed group released in 2007 and the lowest survival was 10.71% for the 2005 fed group. Based on these data significant mortality occurs on transported kelts between Bonneville Dam and the Pacific Ocean reach. In future years we are going to experiment with releasing some transported groups closer to the ocean in hopes of boasting survival to the ocean therefore improving benefits of transporting kelts.

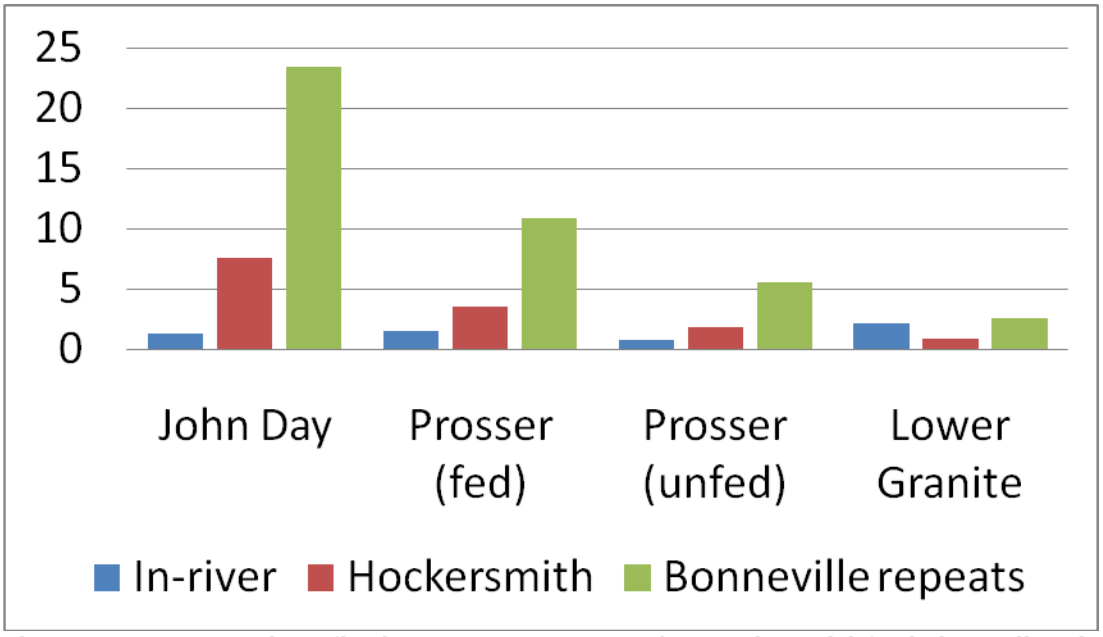


Figure 26: Transport benefits (treatment return rate / control metric) for kelt steelhead collected at John Day, Prosser, and Lower Granite dams.

Survival of long-term reconditioned groups was lower in 2009 relative to other years, likely the result of not inoculating kelts at arrival. Inoculations upon kelt arrival were omitted after fish health experts questioned the benefit of preventative inoculations. However, as the high mortality seen in 2009 could not be attributed to other factors, we think it likely that the omission of inoculations may be the cause. We plan to return to the original protocol of inoculating all kelts on arrival in 2010 and beyond.

Survival through long-term reconditioning was highest for fish reconditioned at Prosser Hatchery. The weighted mean survival estimate of 37.90% as calculated over 10 years

indicates that steelhead kelts can be successfully reconditioned. Other locations also have exhibited good survival of long-term reconditioned kelts, with the caveat high mortalities seen in 2009.

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 a 10.04 times increase in survival over fish left untreated in the river (Figure 27). Additionally, this is a bit of an underestimate since the in-river control group is measured as a return to Bonneville Dam and the long-term reconditioned kelts are released back into the Yakima River. The in-river control group must transverse and additional 225 miles of river to reach the long-term treatment's release site. Compared to the proportion of repeat spawners in the run at large at Bonneville Dam, long-term reconditioned kelts at Prosser Hatchery had an increased survival 70.78 times greater than untreated fish. In Omak Creek, reconditioning led to a 32.95 times increase in survival (Figure 28). Long-term reconditioning shows great promise as a tool for restoration based on these data.

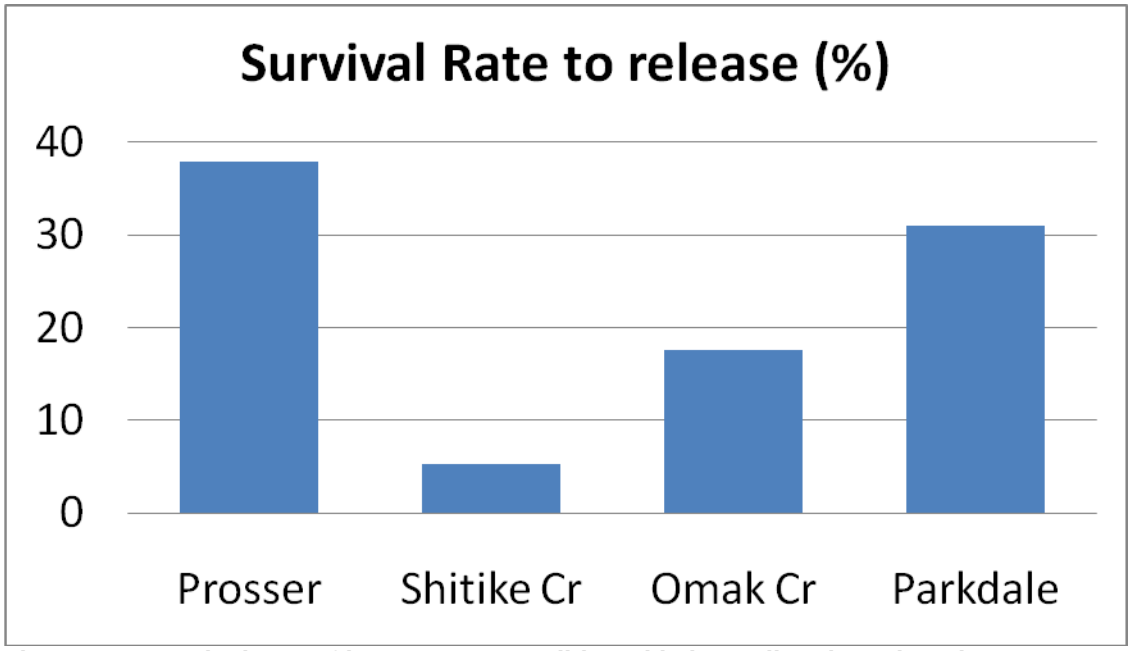


Figure 27: Survival rate of long-term reconditioned kelt steelhead at 4 locations.

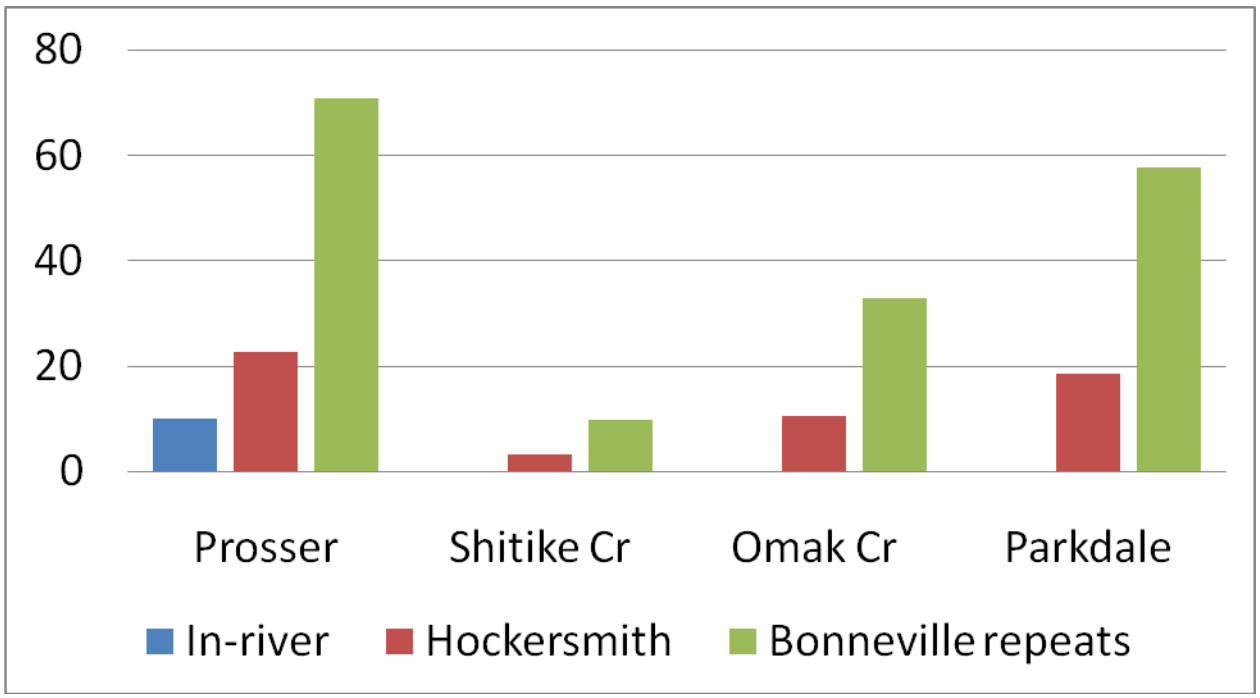
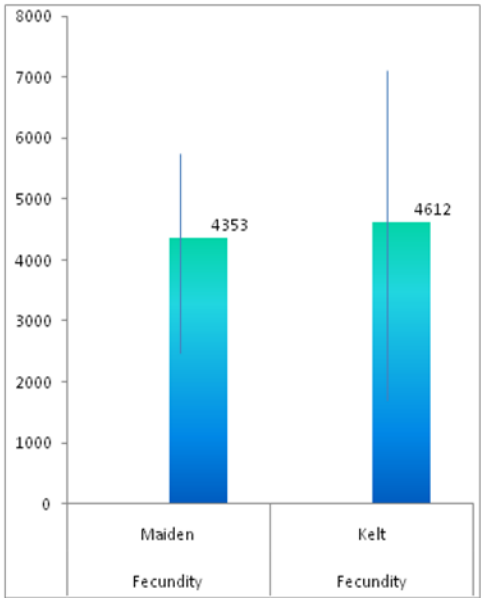


Figure 28: Long-term reconditioning benefits calculated by dividing long-term survival rates by control group metrics.

A concern regarding long-term reconditioning is the potential impacts that the artificial rearing environment could have on reproductive success. We are conducting reproductive success studies in Omak Creek and in the Yakima River which eventually should address this question but at this point we will summarize some of the gamete and progeny work that we have conducted at Parkdale Hatchery using Skamania stock summer steelhead. These fish were collected in the Hood River and placed in Parkdale Hatchery prior to what was assumed to be their first spawning event. After the fish ripened they were air spawned and then reconditioned for one to two years depending on their maturation schedule. After maturation following reconditioning they were again air spawned for compassion to their first spawning event. Measures of fecundity, fertilization, and fry survival rates between the first and kelt spawning events were not statistically different (Figure 29).



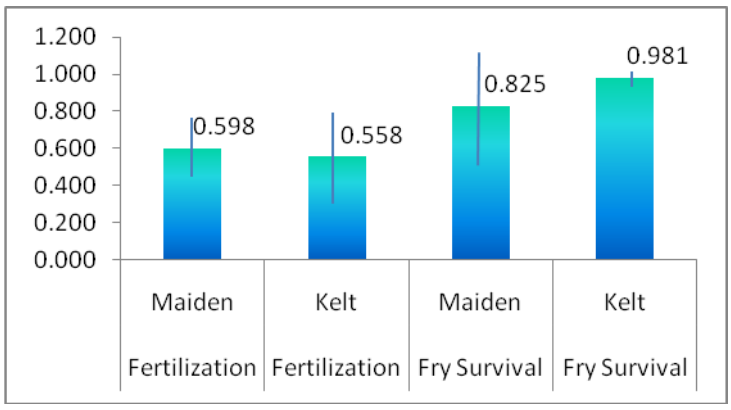


Figure 29: Mean fecundity, fertilization rates, and fry survival for maiden and kelt spawning at Parkdale Hatchery. N=15.

References

Al-Ankari A.S. 2005. Immunomodulating Effects of Black Seed and Oxytetracycline in Pigeons. Immunopharmacology and Immunotoxicology, 27;515-520, 2005.

Branstetter R., J. Stephenson, D. Hatch, J. Whiteaker S.Y. Hyun, B. Bosch, D. Fast, J. Blodgett, and T. Newsome, L. Hewlett-Dubisar, J. Graham, J. Lovetang, R. Dasher,C. Fisher, 2008. Steelhead Kelt Reconditioning and Reproductive Success. 2007 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-00. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.

Evans, A.F. 2002. Steelhead (Oncorhynchus mykiss) kelt outmigration from Lower Granite Dam to Bonneville Dam: Abundance, downstream conversion rates, routes of passage, and travel times. Annual Report to U.S. Army Corps of Engineers, Walla Walla District, for Contract No. DACW68-01-0016. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.

Evans, A.F., and R.E. Beaty. 2000. Identification and Enumeration of Steelhead (*Oncorhynchus mykiss*) Kelts at Little Goose Dam Juvenile Bypass Separator, 1999. Annual Report to U.S. Army Corps of Engineers, Walla Walla District, for Contract No. DACW68-99-M-3102. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.

Evans, A.F., R.E. Beaty, D.R. Hatch, J. Blodgett, and D. Fast. 2001. Kelt reconditioning: A research project to enhance iteroparity in Columbia Basin steelhead (*Oncorhynchus mykiss*). 2000 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2000-017. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.

Evans, A.F., R.H. Wertheirmer, M.L. Keefer, C.T. Boggs, and C.A. Peery. 2008. Transportation of steelhead kelts to increase iteroparity in the Columbia and Snake rivers. North American Journal of Fisheries Management 28:1818-1827.

Grondel J.L., J.F.M. Nouws, W.B. van Muiswinkel. 1987. The influence of antibiotics on the immune system: immuno-pharmokinetic investigations on the primary anti-SRBC response in carp, Cyprinus carpio L., after oxytetracycline injection.

Hatch, D.R., P.J. Anders, A.F. Evans, J. Blodgett, B. Bosch, D. Fast., and T. Newsome. 2002. Kelt reconditioning: A research project to enhance iteroparity in Columbia Basin steelhead (Oncorhynchus mykiss). 2001 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2000-017. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.

Hatch, D.R., R. Branstetter, and S. Narum. 2003a. Evaluate steelhead (Oncorhynchus mykiss) kelt outmigration from Lower Granite Dam to Bonneville Dam and test the use of transportation to increase returns of repeat spawners. Annual Report to US Army Corps of Engineers, Walla Walla District, for Contract No. DACW68-00-C-0027. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland OR.

Hatch, D.R., R. Branstetter, J. Blodgett, B. Bosch, D. Fast, and T. Newsome. 2003b. Kelt reconditioning: A research project to enhance iteroparity in Columbia Basin steelhead (Oncorhynchus mykiss). Annual Report to the Bonneville Power Administration for Contract No. 00004185. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland OR.

Hockersmith, E., J. Vella, L. Stuehrenberg, R.N. Iwamoto, and G. Swan. 1995. Yakima River radio-telemetery study: steelhead, 1989-93. Project Number 89-089, Contract Number DE-AI79-89BP00276, Technical Report to U.S. Department of Energy, Bonneville Power Administration, Division of Fish and Wildlife, Portland, Oregon.

Howell, P., K. Jones, D. Scarnecchia, L. LaVoy, W. Kendra, and D. Ortmann. 1985. Stock assessment of Columbia River anadromous salmonids Volume II: steelhead stock summaries stock transfer guidelines - information needs. Final Report to U.S. Department of Energy, Bonneville Power Administration, Project No. 83-335.

Langford, T.E., J.M. Fleming and N.P.James, 1977. The tracking of salmonids in the Afon Seiont River System, using ultrasonic tagging techniques. Central Electricity Research Laboratories, Lab. Note RD/L/N51/77, 16 p.

Miranda, D., J. Whiteaker, and J. Fryer. 2005. Age and Length Composition of Columbia Basin Chinook, Sockeye, and Coho Salmon at Bonneville Dam in 2004. Technical Report for the Columbia River Inter-Tribal Fish Commission, Portland, Oregon.

Serezli R., H. Cagirgan, I. Okumus, S. Akhan, F. Balta. 2005. The Effect of Oxytetracycline on Non-Specific Immune Respones in Sea Bream (Sparus aurata L. 1758). Turkish Journal of Veterinary Animal Science. 29 (2005) 31-35.

Studnicka M., A.K. Siwicki, M. Morand, A. Rymuszka, A. Bownik and E. Terech-Majewska. 2000. Modulation of nonspecific defence mechanisms and specific immune responses after suppression induced by xenobiotics. Journal of Applied Ichthyology. 16 (2000), 1-7.

Tafalla, C., B. Novoa, J.M. Alvarez, and A Figueras.1999. In vivo and in vitro effect of oxytetracycline treatmen on the immune response of turbot, *Scophtalmus maximus* (L.). Journal of Fish Disease 22, 271-276.

Torbek, C., J. Mainord, and J. Whiteaker. 2009. Age and Length Composition of Columbia Basin Chinook and Sockeye Salmon and Steelhead at Bonneville Dam in 2008. Technical Report for the Columbia River Inter-Tribal Fish Commission, Portland, Oregon.

Whiteaker, J., J. Fryer, and J. Doyle. 2006. Age And Length Composition Of Columbia Basin Chinook And Sockeye Salmon And Steelhead At Bonneville Dam In 2005. Technical Report for the Columbia River Inter-Tribal Fish Commission, Portland, Oregon.

Whiteaker, J., and J. Fryer. 2007. Age and Length Composition of Columbia Basin Chinook and Sockeye Salmon and Steelhead at Bonneville Dam in 2006. Technical Report for the Columbia River Inter-Tribal Fish Commission, Portland, Oregon.

Whiteaker, J., and J. Fryer. 2008. Age and Length Composition of Columbia Basin Chinook and Sockeye Salmon and Steelhead at Bonneville Dam in 2007. Technical Report for the Columbia River Inter-Tribal Fish Commission, Portland, Oregon.

Section 2: Gamete and Progeny Viability

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Introduction

Reproductive success is difficult to observe in the field, particularly for steelhead. Migration occurs during high water periods impacting our ability to operate temporary weirs and detect spawners and redds. Fish tend to stage in larger systems prior to high water and spawning contributions can occur from resident populations. The operation of weirs and traps are often compromised by Spring flow regimes, and rarely catch a large proportion of the spawning population. Logistical issues related to the complexity of steelhead life history, and incomplete understanding of all variables has limited the success of obtaining easily quantifiable results. Consequentially, we are also investigating gamete and progeny viability of reconditioned kelt steelhead in the lab. The design is to collect hatchery-origin prespawn adults and place them in a hatchery. 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 and Omak). This experiment utilizes a replicated, repeated measures experimental design, to assess and compare egg and progeny viability of reconditioned vs. first time spawners. Long-term reconditioning and subsequent captive spawning provides us with means to obtain 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 first 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). The Hood River is a tributary of the Columbia River 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. This facility is co-managed by The Confederated Tribes of Warm Springs and the Oregon Department of Fish and Wildlife.

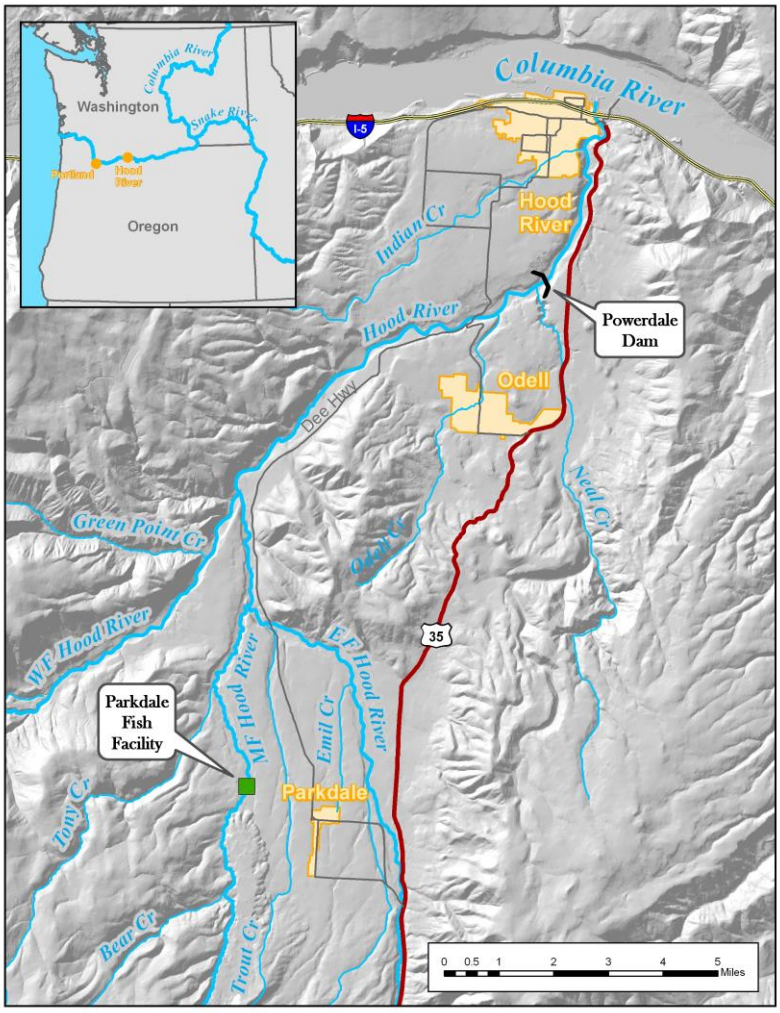

Figure 1. Location of Parkdale Fish Facility and Powerdale Dam/ Fish Trap.

Methods

From February to June, staff sorted fish biweekly for ripeness based on previous experience with this stock. Male gametes were collected manually and cryogenically stored (Cloud & Osborne 1997) prior to fertilization. Female gametes were collected by air-spawning (Leitritz and Lewis 1980). 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). If disease screens were determined to be positive for any of the parents, the eggs were immediately disposed of and the female brood fish if still alive euthanized. The exception to this was that we kept kelt progeny in an isolation trough so that we could still obtain measures on these fish. 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) from two different males to avoid against any disease positive males destroying an entire batch of eggs. The use of cryopreserved milt in all experimental matings allows us to spawn the same female with the same males in multiple years to evaluate any reconditioning effect on a female kelt's eggs. Each egg group was held in isolation baskets. Post 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. A subsample of eggs (N=20) were collected on day 15 (average of 120 Temperature Units) which 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 picking troughs (14'l x 16.5" w x 4.5"d at 15gal/min) as single female groups (34" x 16.5" or 55.5" x 16.5 depending on stocking density) for space saving purposes. They were fed Biovita starter feed #0 every hour during daylight hours for the first 4 weeks to satiation then gradually moved to 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" quick net 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 administering a fatal dosage of MS-222.

Surviving females were reconditioned at the Parkdale Fish Facility and spawned a second time with cryopreserved milt from the same male combinations to assess the effect of reconditioning on viability and early juvenile survival. Females were administered another dosage of ivermectin after completion of air spawning and placed in 10' d x 4.5 'l tanks at 50gal/ min for summer holding. Fish were checked again in late spring (May) for the presence of copepods and administered additional ivermectin treatment if copepods were present. Fish were started on krill but readily accepted pelleted feed and were fed 3-4 times daily until satiation. By mid-fall (October) the female kelts were moved back to the raceway and placed with incoming brood. Feeding was discontinued in mid December to prepare for spawning.

Results and Discussion

Spawning

2010 Broodstock First-time Spawning

We spawned 15 females and cryopreserved the milt of 8 males. Six females were terminated due to the presence of IHN in the ovarian fluid. Two females were terminated due to lack of maturation during the spawning season. Spawning and juvenile data will be available in the 2011 Annual Report.

2009 Broodstock First-time Spawning and Reconditioning Results/ Kelt Spawning

We successfully spawned 12 first time spawners. Egg take for the kelts averaged 3200 eggs with a maximum take of 4864 eggs and a minimum of 0 eggs (3 fish that did not produce eggs). Based on keel presence/absence there was an average fertilization rate of 60.0% (minimum=10.5%, maximum=85.7%). Post cold shock eggs were compared against fertilization rates and these numbers were very close with the average survival rate of 64% (Minimum=29.0%, Maximum=90.0%).

Typically, half to just over half of the juvenile mortalities occurred immediately post-hatch with an average of 1 or 2 mortalities per group, a week thereafter. Juvenile fish steadily increased in length and weight over the 10-week period growing an average 0.07 grams and 1.5 millimeters per week. The juvenile growth is much slower at the Parkdale Hatchery than at other larger production hatcheries due to the cool water conditions present at the facility (5.5 ^{0}C).

Currently, there are 4 female kelts along with 2 (potentially dud) fish that did not spawn in 2009 that have survived reconditioning. The other 6 females appeared to have died from *C. shasta* infections. We anticipate that the 4 surviving 2009 brood are skip spawners and will spawn in 2011 while the 2 fish that did not originally spawn in 2009 are being held to determine if they are incapable of producing eggs.

2007 Broodstock Reconditioning

The 2007 brood steelhead kelt that spawned in 2009 increased her egg clutch by approximately 2000 eggs (Branstetter et al., 2009) for a total of 6604 estimated eggs. The cold shock data was close to the keel data (Branstetter et al. 2009) with egg mortality increasing slightly by an additional 5%.

Juvenile growth factors for this particular kelt are difficult to measure due to the difference in water temperature between Parkdale and the University of Idaho Aquaculture Research Institute (ARI). Differences in the survival of the 2007 juveniles (86%) and 2009 (99%) juveniles put this female at roughly the same reproductive capacity at 3100 progeny produced (+/- 500 eggs). The survival difference is likely due to complications regarding transport and the water quality at the University of Idaho Aquaculture Research Institute.

When comparing kelts against their maiden spawning event event it appears with the exception of the 2006 steelhead kelt (Branstetter et al. 2009), the 2007 fish showed a small decline in egg survival (Figure 2) while improving in egg production by an additional 2000 eggs (Figure 3)and juvenile survival increased from 86% to 99%. When comparing the two different spawning years the reduced egg survival and the increase in egg production results in an approximate decline of 848 fish produced from the first time spawning, but when considering juvenile survival the difference is much smaller at a decline of 350 fish produced in the kelt spawning year. Although, as mentioned before the survival difference may be a result of water quality differences between Parkdale and the ARI facilities.

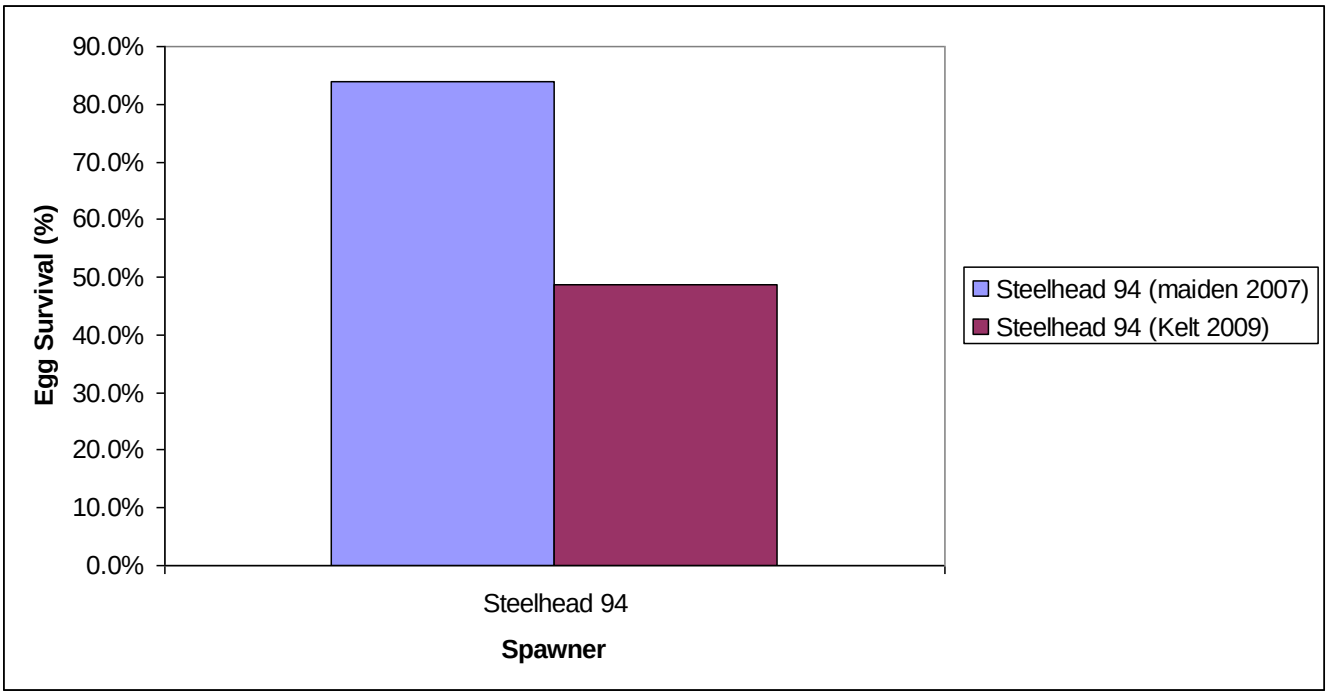

Figure 2: Steelhead 94 Egg Survival (maiden spawning in 2007 to kelt spawning in 2009).

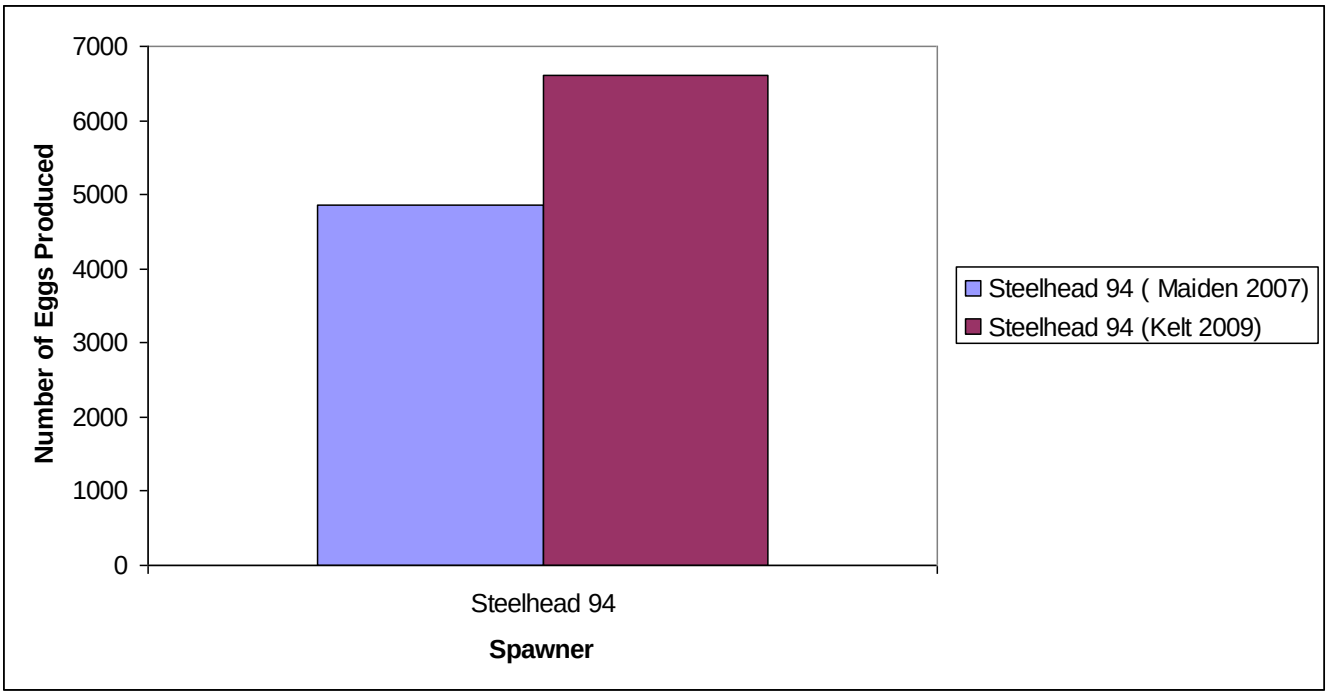

Figure3: Steelhead 94 Egg Production from maiden spawning 2007 to kelt spawning in 2009.

2008- Reconditioning and Kelt Spawning

There were 7 kelts that survived reconditioning with 3 kelts that spawned in 2009. Each of these fish had gained around 1kg each. One of the kelts (2828) died shortly after spawning due to IHN according to the ODFW pathology lab. The other female perished from what appears to be an inability to feed as she was extremely emaciated and did not eat, she also did not spawn. The progeny of 2828 were held in an isolation trough to insure that there would be no possible contamination to the other progeny.

The three spawning kelts produced an average of 5000 eggs; this is an increase of 500 eggs from the 2008 maiden spawning event. In addition to the increase in egg production, egg survival also increased an average of 25% when compared against the maiden spawning event. Post shock mortality showed a nominal increase in mortality (+12 fish) for kelt ID: 2828 but decreased in the other females (-3). Kelt ID : 2828 progeny increased in average juvenile weight (+362 mg) while the other two females juveniles decreased in weight by approximately 10g. All three juvenile groups increased in length on average of 25 mm.

The 2008 kelt brood improved in all aspects from their maiden spawning from egg production (Figure 4) and survival to juvenile weight gain. Egg production increased but not by a large amount, the largest increase was 2843 with an increase of 624 eggs (Figure 5).

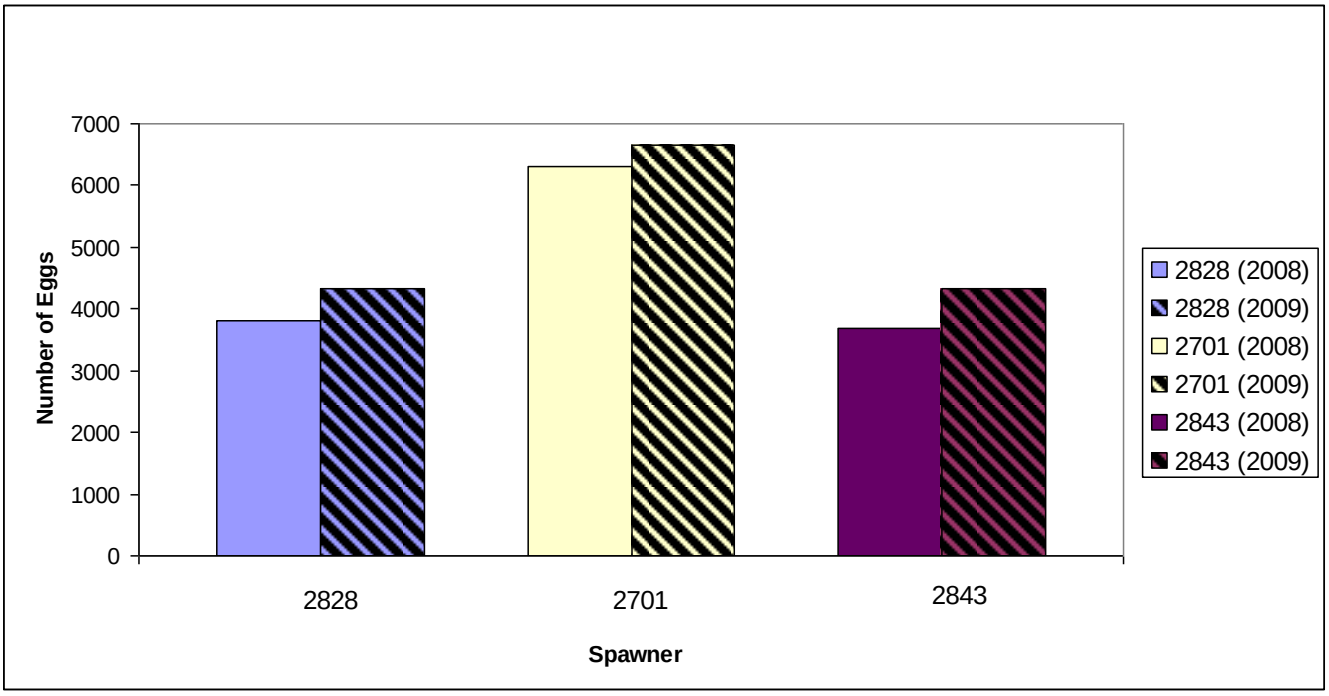

Figure 4: 2008 maiden spawning versus 2009 kelt spawning egg production.

There was a large increase in egg survival from the maiden spawning year in two of the kelts spawning year, with 2701 almost doubling in egg survival, while 2828 showed a small increase (Figure 5).

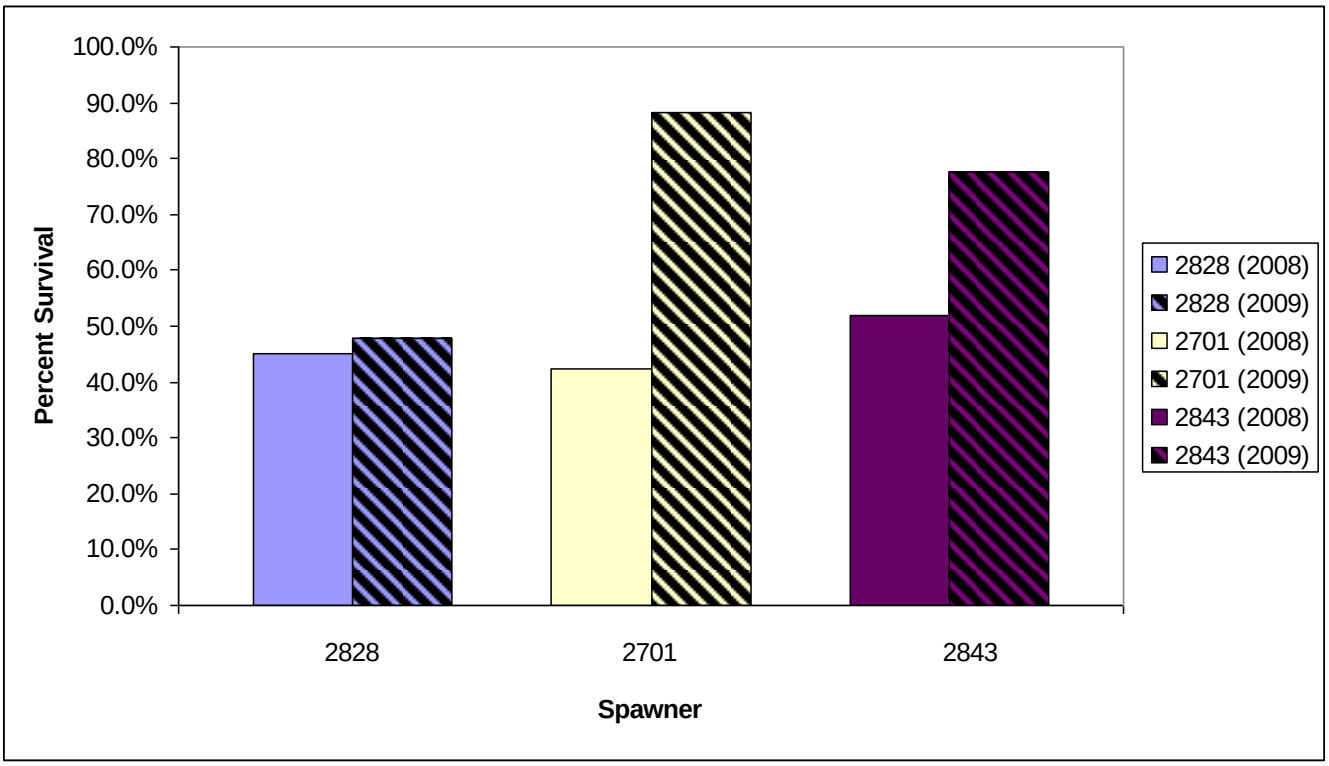

Figure 5: 2008 maiden spawning versus 2009 kelt egg survival.

There were 5 fish from the 2008 brood still alive in 2010, all of these fish spawned in 2010. Results from the 2010 spawning and rearing will be available in the 2011 annual report.

Maiden Spawners vs. Kelt Spawners and Spawning Year

Comparing the long-term reconditioning kelts against the incoming maiden brood, the kelts perform as well as the best spawners. Kelt spawners produced on average 1500 more eggs than maiden spawners and variation was also less (Figure 6).

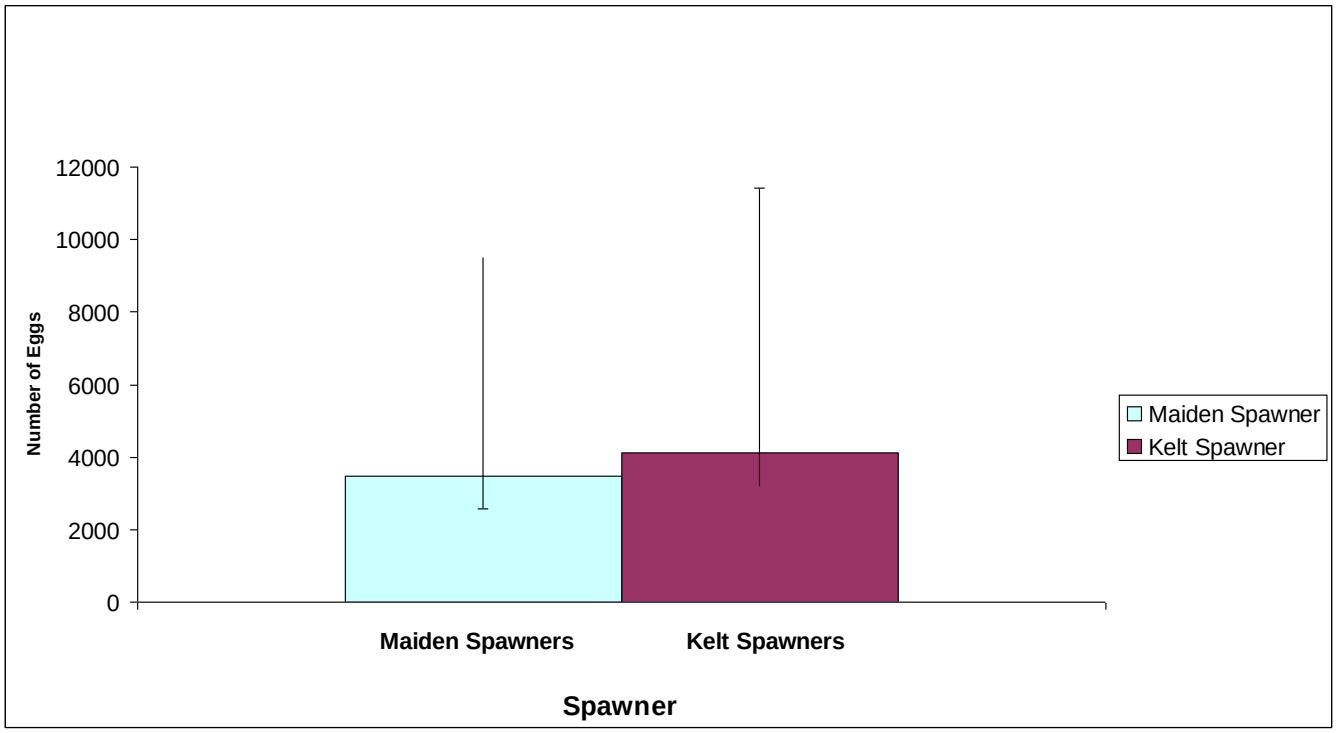

Figure 6: Egg Production Maiden vs. Kelt Spawner 2006-2009.

Fertilization success rates for maiden and kelt spawners were not significantly different (Figure 7).

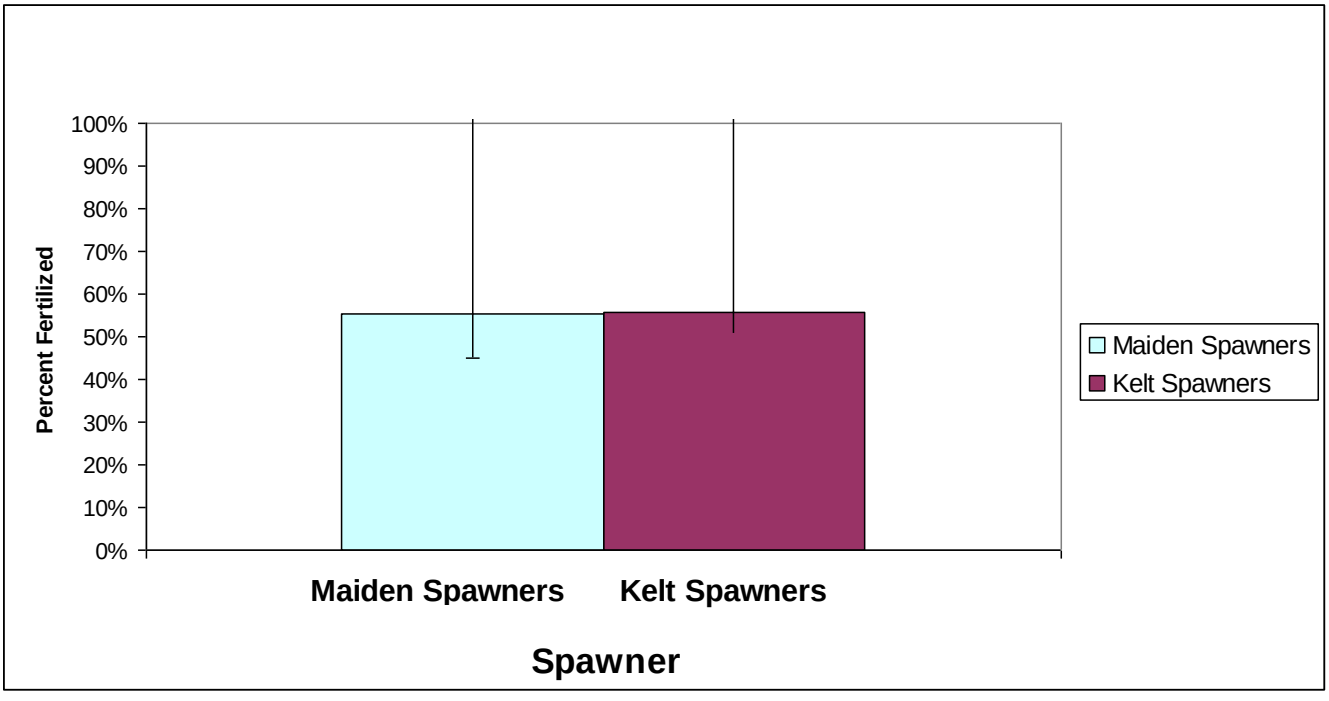

Figure 7: Fertilization Success 2006-2009.

Juvenile survival was higher for progeny from kelts though not significantly; this may be an effect of rearing at the ARI facility which had higher mortality rates. When ARI survival rates

are removed there is minor difference in survival first time survival versus kelt survival just slightly higher -3% (Figure 8).

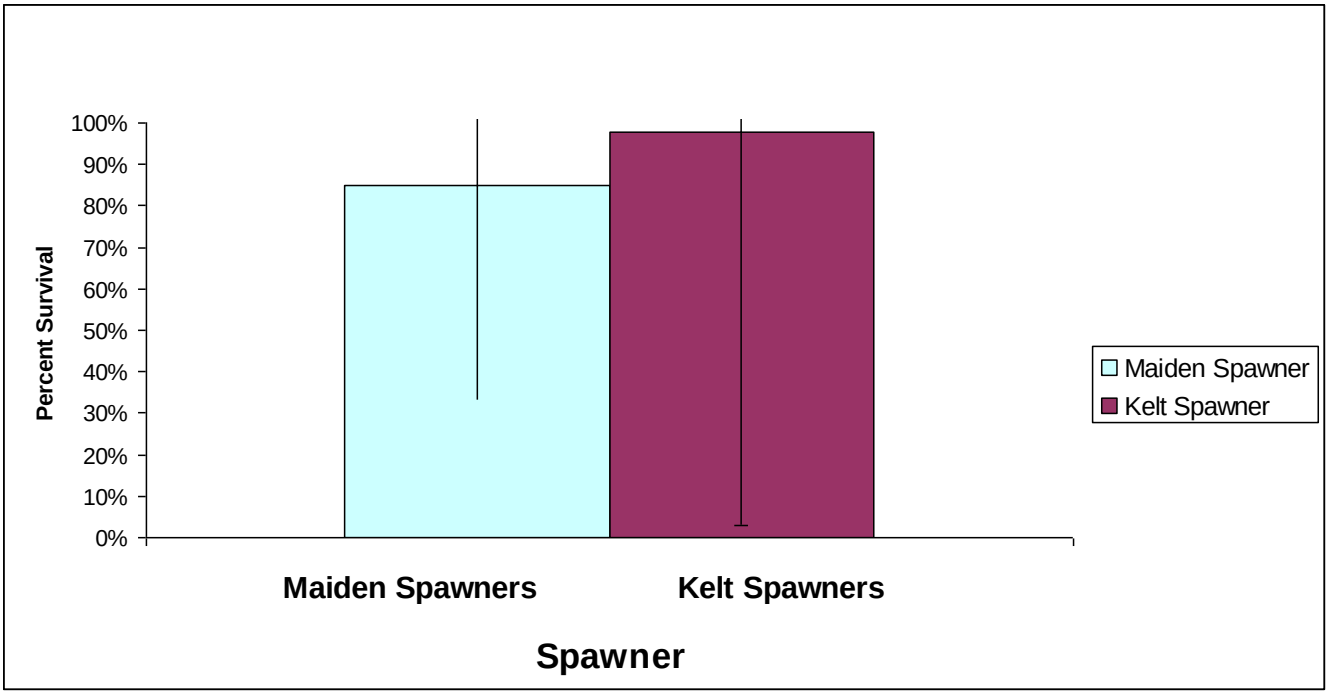

Figure 8: Juvenile Survival 2006-2009.

When considering that there are maiden spawning fish that do not produce any viable offspring this may mean that steelhead kelts are an even more valuable resource for insuring population stability. This phenomenon has also been observed in Snow Creek, WA (Seamons and Quinn, 2010) where steelhead kelts actually performed better than first time spawning fish.

References

Branstetter, R.D., J. Stephenson, D. R. Hatch(PI), J. Whiteaker, S.–Y., Hyun, B. Bosch, D. Fast, J. Blodgett, T. Newsome, L. M. Hewlett-Dubisar, J. Graham, R. Dasher, C. Fisher. 2009. Steelhead Kelt Reconditioning and Reproductive Success. 2007 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-00. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.

Cloud, J.G. and C. Osborne. 1997. Cryopreservation of Salmonid Sperm. Department of Biological Sciences, University of Idaho.

Leitritz, E. and R.C. Lewis. 1980. Trout and Salmon Culture. Division of Agricultural Sciences. University of California.

Oregon Department of Fish and Wildlife. 2008. Grande Ronde Basin Spring Chinook Salmon Captive Broodstock Program. 2008 Annual Operating Plan. Northeast Region Fish Research and Development, Oregon Department of Fish and Wildlife, La Grande.

Pennel W. and Barton B.A., (Eds.) 1996. Principles of Salmonid Culture. Elsevier. Amsterdam, Netherlands.

Seamons, T. R. and T.P. Quinn, 2010. Sex-specific patterns of lifetime reproductive success in single and repeat breeding steelhead trout (Oncorhynchus mykiss). Behavorial Ecology Sociobiology 64:505–513

Wedemeyer G.A., (Ed). 2002. Fish Hatchery Management Second Edition. North American Journal of Fisheries. Bethesda, Maryland.

Section 3: Reproductive Success

Reproductive Success of Omak Creek Reconditioned Steelhead Kelts

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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.

Ho: Reproductive success among natural-origin, hatchery-origin, and reconditioned kelt steelhead is equal within and among streams.

METHODS

Anadromous adults were collected via an adult trap at a semi-permanent weir on Omak Creek. A PIT tag antennae array was also operated upstream of the confluence with the Okanogan River. Downstream juvenile migrants were collected with a screwtrap during the spring. Electrofishing techniques during the fall were used to target resident populations, 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.

Three reconditioned kelts were released in October 2005, one male and two females. 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). 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. The first was captured in Omak Creek on April 10th, passed upstream and was captured again on May 3rd after spawning. After the second capture, it was taken to the hatchery for reconditioning. The second was captured April 26th, 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, but was not observed at the adult trap.

None of the four reconditioned kelts from Bonaparte Creek were detected again. Both Bonaparte and Omak creek had low water flows in March of 2008. 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.

Tissue 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), 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.

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 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.

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

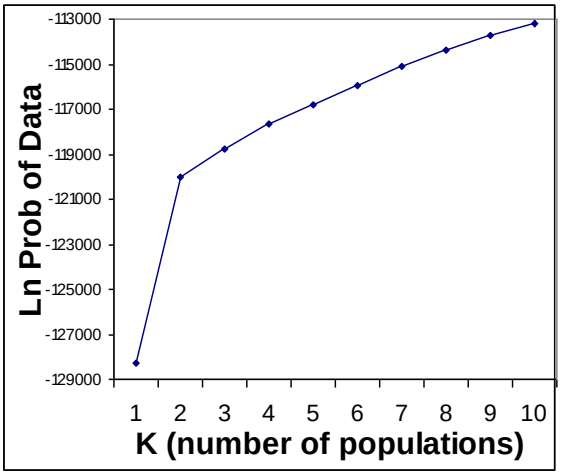
Statistical analysis was performed on 1,693 samples collected in Omak and Bonaparte creeks between 2004 and 2008. Sample numbers and population statistics are reported for each sample collection in Table 1. Departures from Hardy-Weinberg equilibrium (critical level =0.05 /16 loci = 0.00313) and or linkage disequilibrium (critical level =0.05 /120 pairwise comparisons = 0.00042) were seen in most population collections, commonly as a heterozygote deficit. Additional statistical analysis proceeded as normal as population admixture is not unexpected, and Hardy-Weinberg equilibrium is not needed for the additional analyses completed.

Table 1. 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	7.57	11	82
Omak Anadromous	2005	104	0.8149	0.7932	13.5	7.81	1	19
Omak Anadromous	2006	89	0.8166	0.8118	13.6	8.00	0	3
Omak Anadromous	2007	70	0.8273	0.8153	13.6	8.18	0	7
Omak Anadromous	2008	50	0.8147	0.8075	13.4	8.11	0	1
Omak Anadromous	2009	51	0.8188	0.8105	12.4	7.87	0	4
Bonaparte Anadromous	2006	11	0.8135	0.8443	7.9	7.80	0	0
Bonaparte Anadromous	2007	59	0.8233	0.8167	12.8	8.05	1	3
Bonaparte Anadromous	2008	28	0.8181	0.8000	10.9	7.90	0	3
Omak above falls	2005	21	0.8328	0.7796	10.0	7.88	0	0
Omak above falls	2006	45	0.7676	0.7904	9.7	6.55	2	13
Omak above falls-Haley	2008	25	0.8079	0.7660	9.7	7.39	0	0
Omak above falls-Lobe	2008	67	0.7260	0.7148	10.1	6.18	2	11
Omak above falls	2009	23	0.7761	0.7446	8.6	6.77	2	13
Omak below falls	2005	78	0.8270	0.7999	12.3	7.69	8	63
Omak below falls	2006	93	0.8443	0.8098	13.8	8.44	3	18
Omak below falls	2007	93	0.8437	0.8322	14.4	8.52	1	8
Omak below falls	2008	43	0.8300	0.8196	12.1	8.04	1	17
Omak Screwtrap	2006	96	0.8386	0.7968	13.9	8.13	7	26
Omak Screwtrap	2007	278	0.8263	0.8115	15.7	8.08	10	45
Omak Screwtrap	2008	326	0.8285	0.8202	15.8	8.12	9	35
Omak Screwtrap	2009	69	0.8337	0.8445	13.9	8.26	1	6
Omak Fry	2008	28	0.7476	0.7716	8.1	6.28	1	22

Structure results supported multiple populations with the largest change in Ln probability of data between K values of 1 and 2 (Figure 1). 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 further population substructure.

Figure 1. Ln Probability of Data. For each value of K =1-10, three ln Probability values are graphed. The large increase between K=1 and K=2 is considered to be the only one of significant interests.



Structure results for **Figure 2 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. Each individual is shown as blue (Anadromous), yellow (Resident), or as a portion of both. Results for each site are condensed across all years.**

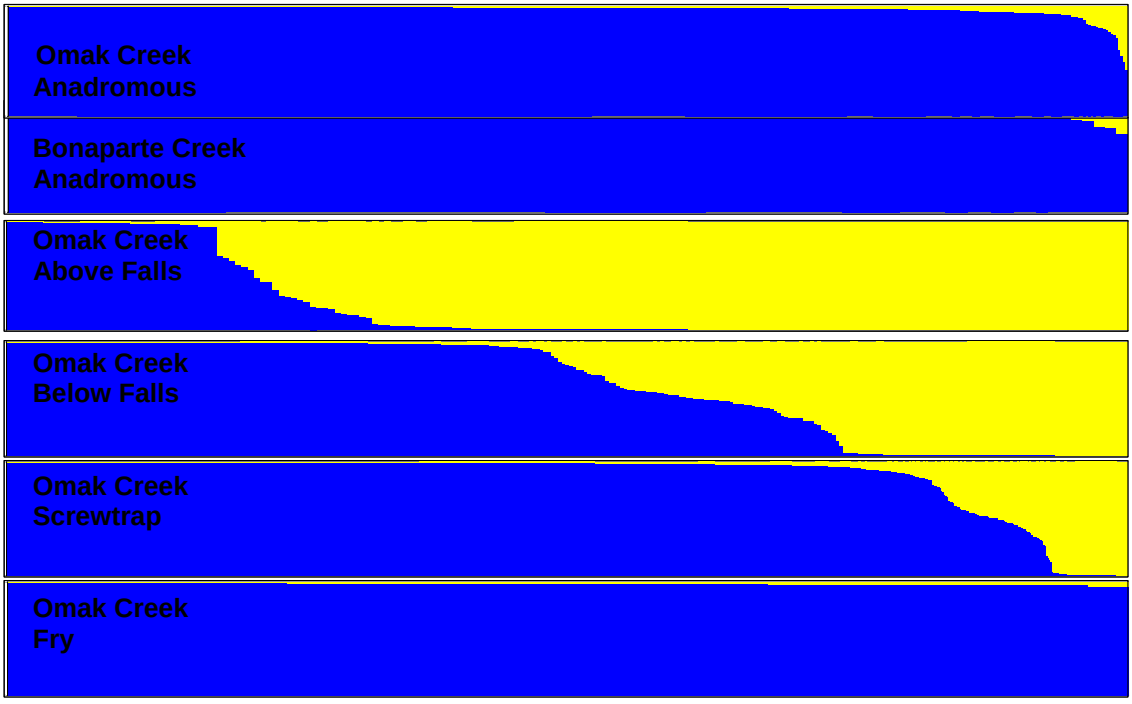
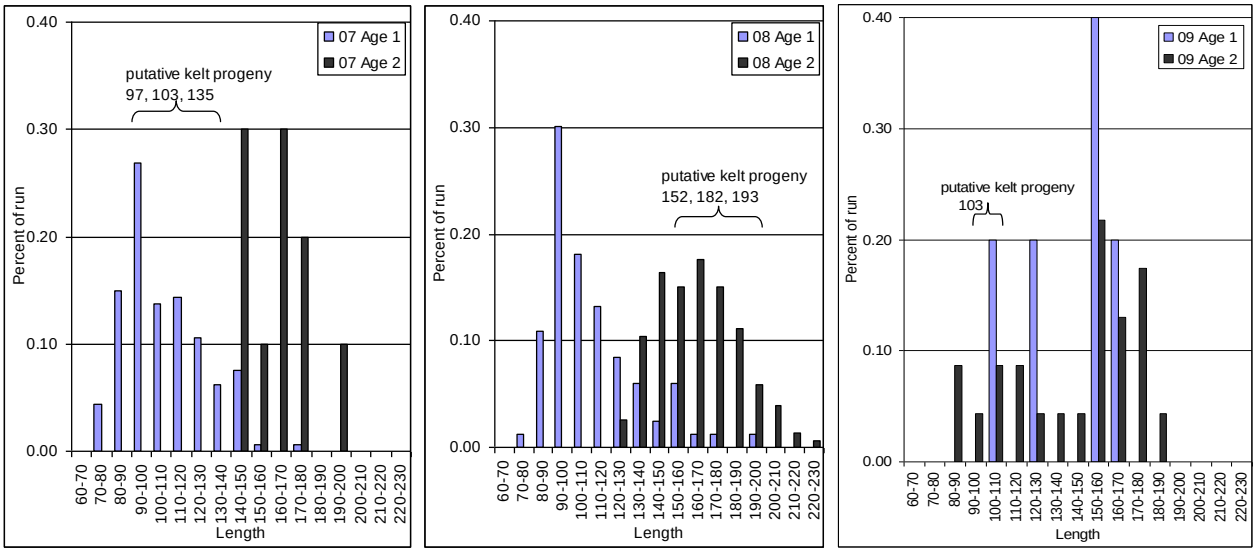


Table 2. Population assignments by structure. Percentage of each collection assigning to the group labeled as anadromous (Anad), Resident (Res), or Mixed. Mixed is defined as having intermediate values of less than 0.70 or 0.90 for both the anadromous and resident groups.

		0.70 cutoff			0.90 cutoff		
Collection	Year	Anad	Resi	Mixed	Anad	Resi	Mixed
Omak Anadromous	2004	0.99	0.00	0.01	0.98	0.00	0.02
Omak Anadromous	2005	1.00	0.00	0.00	0.98	0.00	0.02
Omak Anadromous	2006	1.00	0.00	0.00	0.99	0.00	0.01
Omak Anadromous	2007	0.99	0.00	0.01	0.93	0.00	0.07
Omak Anadromous	2008	0.96	0.00	0.04	0.88	0.00	0.12
Omak Anadromous	2009	1.00	0.00	0.00	0.90	0.00	0.10
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	0.96	0.00	0.04
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-Haley	2008	0.24	0.72	0.04	0.24	0.64	0.12
Omak above falls-Lobe	2008	0.06	0.91	0.03	0.06	0.82	0.12
Omak above falls	2009	0.57	0.39	0.04	0.57	0.22	0.22
Omak below falls	2005	0.56	0.31	0.13	0.53	0.28	0.19
Omak below falls	2006	0.55	0.32	0.13	0.47	0.31	0.22
Omak below falls	2007	0.62	0.17	0.20	0.60	0.14	0.26
Omak below falls	2008	0.23	0.37	0.40	0.19	0.35	0.47
Omak Screwtrap	2006	0.67	0.21	0.13	0.60	0.20	0.20
Omak Screwtrap	2007	0.86	0.09	0.05	0.83	0.09	0.08
Omak Screwtrap	2008	0.90	0.03	0.07	0.85	0.02	0.13
Omak Screwtrap	2009	0.71	0.06	0.23	0.71	0.01	0.28
Omak Fry	2008	1.00	0.00	0.00	1.00	0.00	0.00

Figure 3 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 the 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.

Figure3. Length frequency histograms for fish sampled in 2007, 2008, and 2009. Percent of run for each length classes is reported for known age (via parentage) juveniles captured in the screwtrap. Range of putative kelt progeny is displayed to demonstrate relationship with known age fish.



Of the 28 samples collected as emergent fry, at least one parent was assigned to 26. 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 or 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 3. 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

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 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 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 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 as 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 2006 and 2008.

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 may continue in future years, so other options should be considered. 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.

References

Arterburn, J., K. Kistler and R. Dasher. 2005. Okanogan Basin Steelhead Spawning Ground Surveys. Colville Confederated Tribes Fish and Wildlife, Anadromous Fish Division, Omak WA.

Falush, D., M. Stephens and J.K. Pritchard. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164:1567-1587.

Fisher, C., and J. Arterburn. 2004. Improvements of anadromous fish habitat and passage in Omak Creek; Includes 2003 steelhead surveys in Omak Creek. 2002-2003 Annual Report, Project No. 200000100, 23 electronic pages, (BPA Report DOE/BP-00005103-1).

Guo S.W., and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. Biometrics 48:361-372.

Haldane J.B.S. 1954. An exact test for randomness of mating. Journal of Genetics 52:631-635.

Kalinowski S.T. 2005. HP-Rare: A computer program for performing rarefactions on measures of allelic diversity. Molecular Ecology Notes 5:187-189.

Kalinowski S.T., M.L. Taper, and T.C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases confidence in paternity. Molecular Ecology 16:1099-1106.

Marshall, T.C.J. Slate, L. Kruuk, and J.M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology 7:639-655.

McConnell, S., L. Hamilton, D. Morris, D. Cook, D. Paquet, P. Bentzen, and J. Wright. 1995. Isolation of salmonid microsatellite loci and their application to the population genetics of Canadian east coast stocks of Atlantic salmon. Aquaculture 137:19-30.

Olsen, J.B., P. Bentzen, M.A. Banks, J.B. Shaklee, and S.Young. 2000. Microsatellites reveal population identity of individual pink salmon to allow supportive breeding of a population at risk of extinction. Transactions of the American Fisheries Society 129:232-242.

Park, S.D.E. 2001. Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection [Ph.D. thesis] University of Dublin.

Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.

Rousset, F., and M. Raymond. 1995. Testing heterozygote excess and deficiency. Genetics 140:1413–1419

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution. 43: 223-225

Stephenson J.J., M.R. Campbell, J.E. Hess, C. Kozfkay, A.P. Matala, M.V. McPhee, P. Moran, S.R. Narum, M.M. Paquin, O. Schlei, M.P. Small, D.M. Van Doornik, J.K. Wenburg. 2008. A centralized model for creating shared, standardized, microsatellite data that simplifies inter-laboratory collaboration. Conservation Genetics 10:1145-1149.

Weir B.S. 1990. Genetic data analysis. Sinauer Publ., Sunderland, MA.

Genetic Analysis of Yakima Subbasin Oncorhynchus mykiss

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INTRODUCTION

Genetic structure of populations of *Oncorhynchus mykiss* from the Yakima River basin was determined to act as a baseline in order to assign unknown steelhead kelts to population of origin. This effort expands on previous genetic studies of *O. mykiss* (Campton and Johnston 1985, Busack et al. 2006) in an attempt to identify the most favorable sites to perform kelt reproductive success. While kelt reconditioning is currently being done in the Yakima River, the reproductive success of post reconditioned kelts has not yet been shown. Tributaries to the Yakima River that produce a proportionally larger number of kelts will be targeted as sites for reproductive success studies.

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 and by collection type (putative first time spawners at Prosser 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), 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 both the 2008 and 2009 effort. Table 1 shows the 85 SNPs that were genotyped during both years. SNPs not genotyped for both years are not included in this report. Genotypes were collected using a Fluidigm EP1 instrument in conjunction with Fluidigm SNP Genotyping Analysis software.

Table 1. SNP loci used.

Marker Name	Reference	Marker Name	Reference
Omy_myclarp404-111	Unpublished N. Campbell	Omy_gh-334	Campbell et al. 2009
Omy_Omyclmk436-96	Unpublished N. Campbell	Omy_gh-475	Campbell et al. 2009
Omy_113490-159	Unpublished C. Garza	Omy_hsc715-80	Campbell & Narum 2009b
Omy_114315-438	Unpublished C. Garza	Omy_hsf1b-241	Campbell & Narum 2009b
Omy_121006-131	Unpublished C. Garza	Omy_hsf2-146	Campbell & Narum 2009b
Omy_121713-115	Unpublished C. Garza	Omy_hsp47-86	Campbell & Narum 2009b
Omy_123044-128	Unpublished C. Garza	Omy_hsp70aPro-329	Campbell & Narum 2009b
Omy_123048-119	Unpublished C. Garza	Omy_hsp90BA-193	Campbell & Narum 2009b
Omy_127236-583	Unpublished C. Garza	Omy_hsp90BA-229	Campbell & Narum 2009b
Omy_128693-455	Unpublished C. Garza	Omy_IL17-185	Unpublished J. DeKoning
Omy_130295-98	Unpublished C. Garza	Omy_IL1b-163	Unpublished J. DeKoning
Omy_130524-160	Unpublished C. Garza	Omy_IL6-320	Unpublished J. DeKoning
Omy_187760-385	Unpublished C. Garza	Omy_inos-97	Unpublished J. DeKoning
Omy_95489-239	Unpublished C. Garza	Omy_LDHB-1_i2	Aguilar & Garza 2008
Omy_96222-125	Unpublished C. Garza	Omy_LDHB-2_e5	Aguilar & Garza 2008
Omy_97077-73	Unpublished C. Garza	Omy_LDHB-2_i6	Aguilar & Garza 2008
Omy_97660-230	Unpublished C. Garza	Omy_mapK3-103	Unpublished N. Campbell
Omy_97865-196	Unpublished C. Garza	Omy_mcsf-268	Unpublished J. DeKoning
Omy_97954-618	Unpublished C. Garza	Omy_mcsf-371	Unpublished J. DeKoning
Omy_aldB-165	Campbell et al. 2009	Omy_myoD-178	Campbell et al. 2009
Omy_aldB-414	Campbell et al. 2009	Omy_nach-200	Unpublished J. DeKoning
Omy_ALDOA_1	Aguilar and Garza 2008	Omy_NaKATPa3-50	Campbell et al. 2009
Omy_aromat-280	Unpublished J. DeKoning	Omy_nkef-241	Campbell et al. 2009
Omy_arp-630	Campbell et al. 2009	Omy_nkef-308	Campbell et al. 2009
Omy_aspAT-123	Campbell et al. 2009	Omy_nramp-146	Campbell et al. 2009
Omy_aspAT-413	Campbell et al. 2009	Omy_Ogo4-212	Campbell et al. 2009
Omy_b1-266	Sprowles et al. 2006	Omy_OmyP9-180	Sprowles et al. 2006
Omy_b9-164	Sprowles et al. 2006	Omy_Ots208-138	Campbell et al. 2009
Omy_BAC-B4-126	Unpublished S. Young	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_R0917-230	Sprowles et al. 2006
Omy_cd59b-112	Unpublished J. DeKoning	Omy_R1175-137	Sprowles et al. 2006
Omy_colla1-525	Unpublished J. DeKoning	Omy_rapd-132	Sprowles et al. 2006
Omy_cox1-221	Campbell et al. 2009	Omy_rapd-167	Sprowles et al. 2006
Omy_cox2-335	Unpublished J. DeKoning	Omy_sSOD-1	Brunelli et al. 2008
Omy_crb-106	Sprowles et al. 2006	Omy_star-206	Unpublished J. DeKoning
Omy_CRBF1-1	Aguilar and Garza 2008	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 were dropped (Omy_myclarp404-111, Omy_Omyclmk436-96). The remaining 99 loci (16 microsatellites, 83 SNPs) 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). When pairwise comparisons showed linkage disequilibrium, the locus with lower heterozygosity was dropped (Table 2). An exception was made for STRUCTURE analysis which used a dataset that included linked loci.

Table 2. SNP loci dropped due to linkage, along with the alternative locus kept.

Dropped	Kept
Omy_127236-583	(Omy_121006-131)
Omy_BAC-B4-126	(Omy_BAC-B4-324)
Omy_nkef-308	(Omy_nkef-241)
Omy_Ogo4-212	(Ogo4.1)
Omy_aldB-414	(Omy_aldB-165)
Omy_crb-106	(Omy_tgfb)
Omy_nach-200	(Omy_113490-159)
Omy_rapd-132	(Omy_rapd-167)
Omy_CRBF1-1	(Omy_tgfb-207)
Omy_R1175-137	(Omy_R0917-230)

Of the 2,332 initial samples in the study, 238 were removed due to duplicate genotypes, missing data, or hybridization with cutthroat trout. Samples removed by category include the following: duplicate samples (n=3), samples with greater than four incomplete genotypes for 16 of the microsatellites or 10 incomplete genotypes for 96 of the SNPs (n=196), and samples with evidence of cutthroat hybridization (n=39). Data for these fish are not included in the statistical analysis, although cutthroat hybridization is reported along with population statistics in Table 3. Fish sampled at both Prosser and again at Chandler were included in the analysis for each collection. This left 2,094 remaining samples for further statistical analyses.

Locations with multiple collections were tested for population differentiation (Weir and Cockerham 1984) using GENEPOP. Collections were pooled when there was no evidence of 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 to significant values were not made as the large number of comparisons makes 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 used. 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.

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 samples 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 Facility were then assigned to baseline stocks. Stock proportions of fish collected as kelts at the Chandler Facility were compared to stock proportions of adults collected the previous year at Prosser Dam during the fall upstream migration.

RESULTS

Statistical analysis was performed on 2,094 samples. Basic population statistics are reported in Table 3. 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, Ahtahnum Creek, Naches River). Mixture collections (Prosser 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. Cutthroat trout alleles were seen in eleven collections including adults at Prosser in 2007. Teanaway River had the highest incidence of cutthroat trout influence with 15 samples indicating sampling of hybrid, introgressed, or pure cutthroat trout.

Table 3. Population Statistics. Each collection is reported in terms of sample size (N), number of samples removed due to evidence of cutthroat sampling or introgression (cut), number of microsatellite alleles (A_{msat}), microsatellite allelic richness (AR_{msat}), microsatellite private allelic richness (PAR_{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	cut	A_{msat}	AR_{msat}	PAR_{msat}	H_E	H_O
Satus 06-09	206	2	13.88	7.16	0.10	0.3152	0.3163
Teanaway 05	81	15	12.38	7.59	0.15	0.3394	0.3352
Toppenish 06-09	232	2	13.50	6.53	0.08	0.2981	0.2914
Toppenish 09 Fall	88	0	9.50	5.88	0.04	0.2803	0.2821
Ahtanum 01	78	6	12.94	7.84	0.10	0.3645	0.3627
Ahtanum 06	84	0	13.94	8.08	0.13	0.3719	0.3591
Ahtanum 07-10	28	1	11.06	7.95	0.22	0.3752	0.3809
LR Snake 05, 08	46	1	11.25	7.52	0.08	0.3395	0.3321
Naches 04, 06	136	3	14.50	7.78	0.14	0.3418	0.3362
NFL Naches 08	21	1	9.25	7.34	0.05	0.3113	0.3099
Nile 05,08	59	2	11.56	7.44	0.11	0.3471	0.3459
Pileup	21	3	8.63	6.90	0.04	0.3208	0.3272
Quartz 05,08	26	0	9.50	7.27	0.04	0.3400	0.3384
Chandler 2006	89	0	14.13	7.68	0.11	0.3287	0.3254
Chandler 2008	305	0	18.06	8.01	0.15	0.3481	0.3365
Chandler 2009	262	0	16.31	7.70	0.12	0.3364	0.3215
Prosser 2007	160	3	16.31	8.16	0.16	0.3586	0.3349
Prosser 2008	87	0	14.00	7.72	0.09	0.3378	0.3261

Results for Hardy-Weinberg equilibrium are reported in Table 4. 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 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 4. 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 (Proportion), and the number and proportion showing evidence of heterozygote deficit (Deficit) or excess (Excess).

Collection	Comps	Proportion		Deficit		Excess	
Satus 06-09	87	9	0.1034	8	0.0920	5	0.0575
Teanaway 05	86	6	0.0698	6	0.0698	2	0.0233
Toppenish 06-09	86	13	0.1512	9	0.1047	2	0.0233
Toppenish 09 Fall	75	11	0.1467	5	0.0667	3	0.0400
Ahtanum 01	87	10	0.1149	7	0.0805	2	0.0230
Ahtanum 06	88	12	0.1364	11	0.1250	2	0.0227
Ahtanum 07-10	82	4	0.0488	4	0.0488	2	0.0244
LR Snake 05, 08	82	4	0.0488	7	0.0854	1	0.0122
Naches 04, 06	86	5	0.0581	8	0.0930	0	0.0000
NFL Naches 08	62	3	0.0484	2	0.0323	2	0.0323
Nile 05,08	82	10	0.1220	4	0.0488	3	0.0366
Pileup	70	6	0.0857	2	0.0286	3	0.0429
Quartz 05,08	73	5	0.0685	3	0.0411	0	0.0000
Chandler 2006	86	4	0.0465	5	0.0581	0	0.0000
Chandler 2008	89	15	0.1685	19	0.2135	1	0.0112
Chandler 2009	89	18	0.2022	16	0.1798	1	0.0112
Prosser 2007	89	17	0.1910	21	0.2360	1	0.0112
Prosser 2008	88	10	0.1136	13	0.1477	2	0.0227

Pairwise Fst values are shown in Table 5. Number of loci with p≤ 0.05 for each pairwise comparison are shown in Table 6. 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 averate 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 5. Pairwise Fst values between populations. Fst values greater than 0.020 are highlighted. Comparisons within Naches tributaries, Ahtanum collection years and adult collections are shown as bordered blocks.

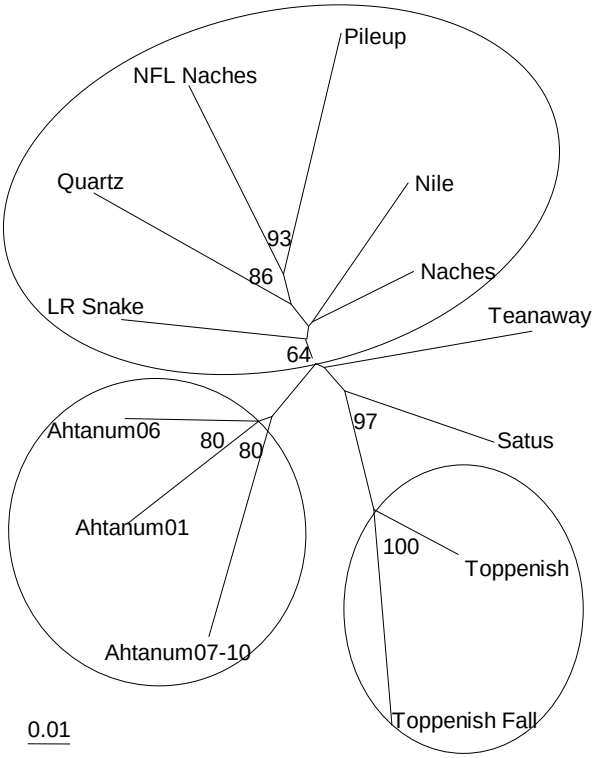
	Satus 06-09	Teanaway 05	Toppenish 06-09	Toppenish 09Fall	Ahtanum 01	Ahtanum 06	Ahtanum 07-10	LR Snake 05,08	Naches 04, 06	NFL Naches 08	Nile 05,08	Pileup	Quartz 05,08	Chandler 2006	Chandler 2008	Chandler 2009	Prosser 2007
Teanaway 05	0.021																
Toppenish 06-09	0.027	0.038															
Toppenish 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					
Chandler 2006	0.002	0.015	0.018	0.041	0.019	0.015	0.023	0.013	0.011	0.013	0.014	0.030	0.019				
Chandler 2008	0.008	0.014	0.020	0.041	0.011	0.008	0.013	0.009	0.007	0.012	0.009	0.024	0.017	0.003			
Chandler 2009	0.007	0.016	0.014	0.030	0.017	0.013	0.018	0.012	0.009	0.015	0.011	0.024	0.017	0.002	0.002		
Prosser 2007	0.012	0.012	0.020	0.040	0.011	0.008	0.013	0.011	0.007	0.013	0.009	0.023	0.018	0.006	0.001	0.004	
Prosser 2008	0.010	0.012	0.016	0.039	0.015	0.009	0.017	0.008	0.004	0.011	0.007	0.023	0.014	0.002	0.001	0.001	0.002

Table 6. 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 tributaries, Ahtanum collection years, and adult collections 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	Quartz 05,08	Chandler 2006	Chandler 2008	Chandler 2009	Prosser 2007
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					
Chandler 2006	17	39	40	46	41	44	31	26	31	17	34	24	24				
Chandler 2008	44	35	54	62	34	29	24	26	36	20	30	26	21	16			
Chandler 2009	39	39	47	52	46	41	29	24	38	18	32	22	24	10	8		
Prosser 2007	55	37	59	55	38	30	23	28	36	19	29	23	28	23	9	18	
Prosser 2008	46	28	47	57	32	26	25	16	22	16	21	18	21	15	10	5	11

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



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. Ahtanum, Naches, Teanaway 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 or Chandler peak which 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.

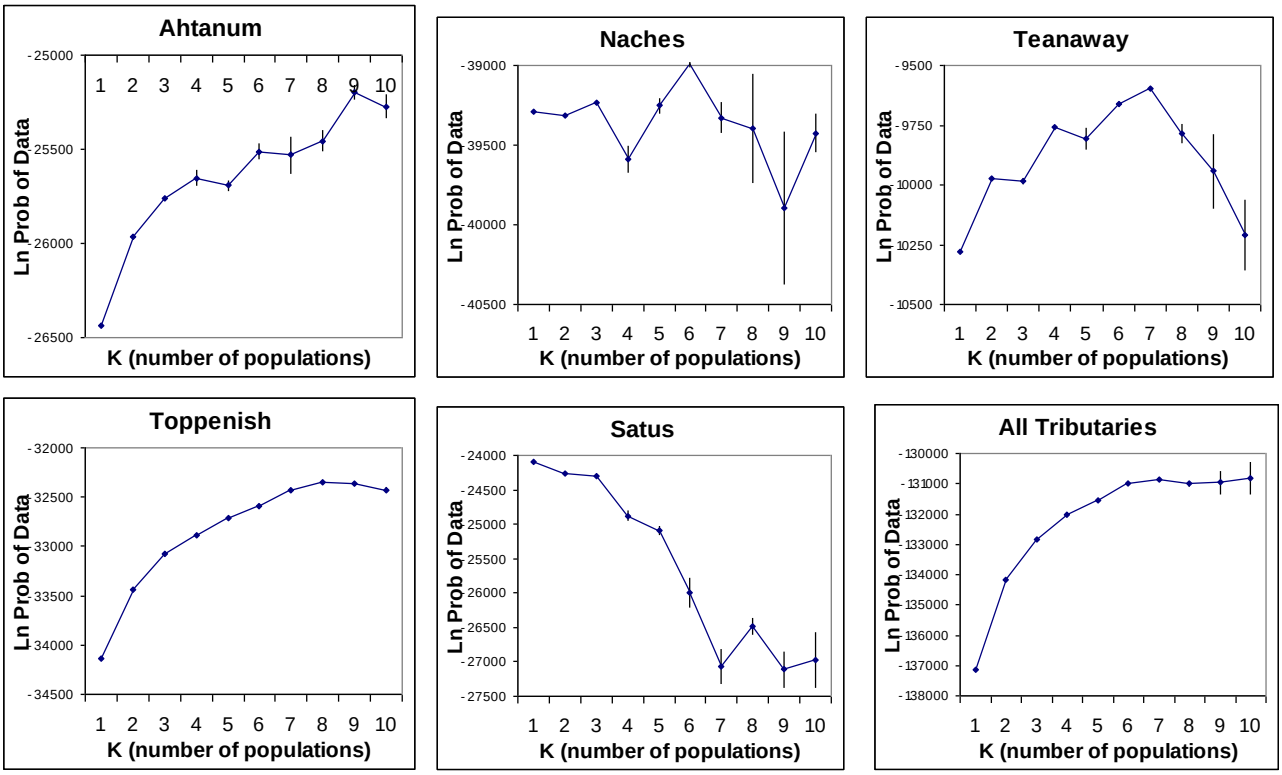
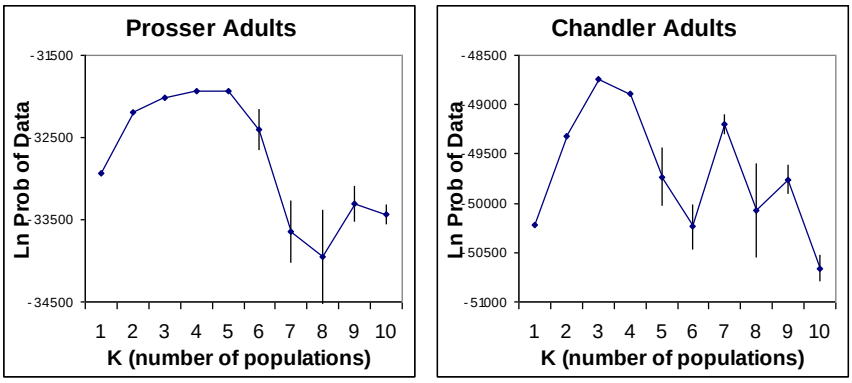


Figure 2b. Ln probability of data for mixed adult collections. For each value of K from 1-10, the average of four iterations of ln probability values is graphed. Standard deviation is graphed for each K value.



Results for 100% simulations are reported in Table 7 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 2006 to 0.9999 in Toppenish 2009 Fall.

Table 8. 100% simulations to both the population of origin and reporting groups. Inter-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 8. Corresponding assignments to reporting groups are in Table 9. Assignment rates back to population of origin averaged only 60.1%, but increased to 90.3% for assignment to reporting groups.

Table 8. 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 are assigned to their population of collection. Inter-reporting group assignments 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	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 9. 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 are assigned to their population of collection. Inter-reporting group assignments are shown as bordered blocks

	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 assignment of unknown fish to each of the five primary tributaries are shown as graphs in Figure 3, and numerically in Table 10 for the two run years with both the upstream (Prosser) and kelt (Chandler) components. Of note, assignments differed when comparing adults collected at Prosser Dam during the upstream migration, and subsequent downstream migrating kelts collected at the Chandler Facility. During the 2007/2008 upstream migration, assignment to Satus Creek increased from 11.85% at Prosser to 30.02% at Chandler. During the 2008/2009 run, assignments increased from 14.51% to 30.08%. Assignment to Satus Creek was highest in the Chandler 2006 collection; however there is no upstream component to compare. Also, stock proportions were highly similar among years within each group of either upstream fish or kelts, except in 2006 when a much smaller sample size of kelts was collected at Chandler (n=89).

Figure 3. Proportional mixed stock assignments. The proportion of each collection of unknown origin fish assigned to each reporting group. Unknown fish consist of adults collected at either Prosser Dam during the upstream spawning migration, or as outmigrating kelts at the Chandler Facility. Reporting groups consist of the five tributary collections.

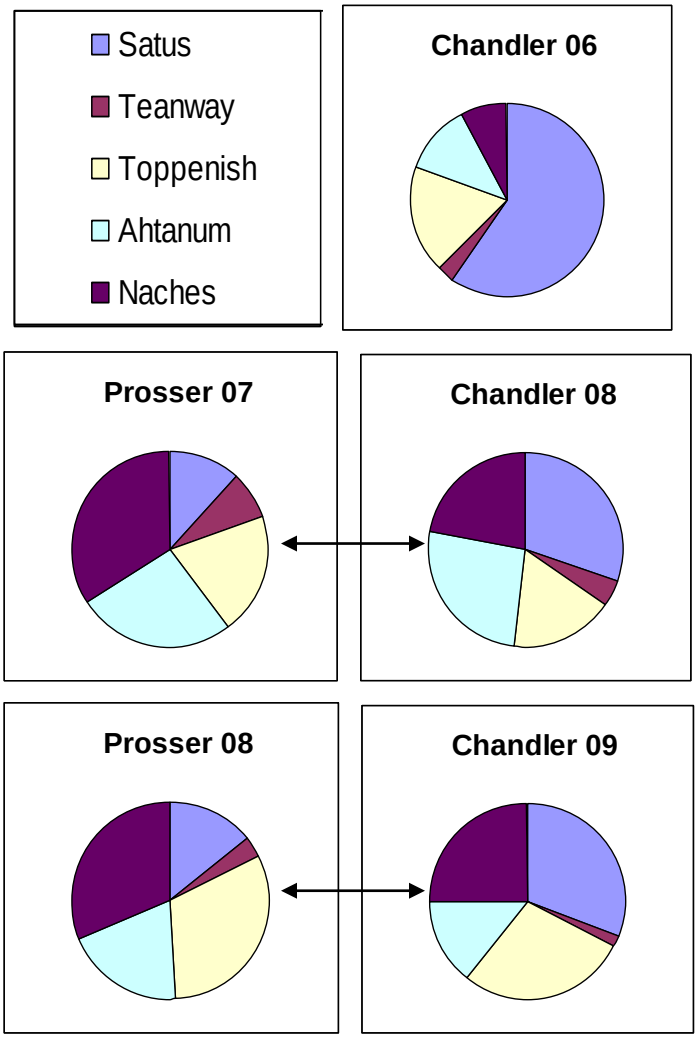


Table 10. Proportional mixed stock assignments. The proportion of each collection of unknown fish assigned to the five reporting units. Unknown fish are listed across the top and consist of adults collected at either Prosser Dam during the upstream spawning migration in the fall, or outmigrating kelts at the Chandler fish trap. 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 07		Chandler 08	
Satus	0.1185	(0.0602, 0.1678)	0.3032	(0.2436, 0.3615)
Teanway	0.0776	(0.0339, 0.1340)	0.0435	(0.0145, 0.0745)
Toppenish	0.2006	(0.1352, 0.2690)	0.1698	(0.1296, 0.2117)
Ahtanum	0.2630	(0.1796, 0.3364)	0.2641	(0.1719, 0.2943)
Naches	0.3403	(0.2566, 0.4313)	0.2194	(0.1856, 0.2970)

	Prosser 08		Chandler 09	
Satus	0.1451	(0.0522, 0.2071)	0.3083	(0.2284, 0.3742)
Teanway	0.0314	(0.0000, 0.0896)	0.0203	(0.0034, 0.0508)
Toppenish	0.3152	(0.2227, 0.3983)	0.2772	(0.2229, 0.3338)
Ahtanum	0.1932	(0.1017, 0.2958)	0.1422	(0.0955, 0.2059)
Naches	0.3150	(0.2283, 0.4434)	0.2521	(0.1836, 0.3226)

DISCUSSION

Analyses clearly show that there are multiple distinct populations of *O. mykiss* in the Yakima 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. While there was evidence for only 2-3 populations in the adult samples, the results showed 4-5 likely populations amongst 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, Naches and Toppenish 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 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.

Proportional assignments were highly successful for all five populations, indicating a powerful genetic baseline for Genetic Stock Identification. Self assignment in Ahtanum Creek had previously been low. Improvement was likely influenced by not pooling all collection years, and the inclusion of the 2001 collection.

The proportion of fish assigned to the Satus group was highest when looking at kelt samples. Satus Creek is the lowest tributary in the drainage. Its proximity to the Chandler Juvenile Evaluation Facility where kelts are collected may explain its high representation in kelt samples. After collection, kelts are reconditioned and are expected to return to their population of origin in subsequent spawn years. Therefore, adults returning to Satus Creek should have the highest proportion of reconditioned kelts in the spawning population.

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 Satus Creek, with high proportions of kelts, will make parentage analysis more powerful and lead to better estimates of relative reproductive success.

Genetic analysis in the Yakima Basin will be continued in 2010 to confirm and improve upon results from 2008 and 2009. GSI work will focus on examining differences between upstream migrants collected in fall versus spring at Prosser Dam. Previously these adults were only sampled during the fall migration due to logistical issues. Spring collections started in 2010 and will be compared to their fall 2009 counterparts to test for differential run timing between five tributaries.

Analysis in 2010 will start transitioning towards detection of reproductive success in reconditioned kelts. We intend to focus on Satus Creek as results heretofore have indicated that it should have the highest proportion of reconditioned kelts. To assist in differentiation of pre and post kelt reconditioning spawning events, we are targeting age-0 samples collected in the fall. After this age length overlaps between ages classes may preclude accurate aging.

Although data is not included in this report, samples from Roza dam were also genotyped as part of a cooperative agreement between CRITFC and WDFW (Washington Department of Fish and Wildlife). Samples and genotypes used in this report were collected and shared by both agencies. This collaboration allowed additional samples to be ran and will make best use of baseline data. Genetic sampling in 2010 will continue this collaboration to maximize the efficiency of both labs.

References

Aguilar A., and C. J. Garza. 2008. Isolation of 15 single nucleotide polymorphisms from coastal steelhead, Oncorhynchus mykiss (Salmonidae). Molecular Ecology Resources 8:659-662

Anderson, E.C., R.S. Waples, S.T. Kalinowski. 2008. An improved method for estimating the accuracy of genetic stock identification. Canadian Journal of Fisheries and Aquatic Sciences 65:1475-1486.

Brunelli, J.P., G.H. Thordaard, R.F. Leary, and J.L. Dunnigan. 2008. Single Nucleotide Polymorphisms Associated with Allozyme Difference between inland and coastal rainbow trout. Transactions of the American Fisheries Society 137:1292-1298.

Busack, C., A. Fritts, T. Kassler, J. Loxterman, T. Pearsons, S. Schroder, M. Small, S. Young, C. Knudsen, G. Hart, P. Huffman, "Yakima/Klickitat Fisheries Project Genetic Studies; Yakima/Klickitat Fisheries Project Monitoring and Evaluation", 2005-2006 Annual Report, Project No. 199506325, 205 electronic pages, (BPA Report DOE/BP-00022370-5)

Campbell N.R., S.R. Narum. 2009b. Identification and characterization of heat shock response related single nucleotide polymorphisms in *O. mykiss* and *O. tschawytscha*. Molecular Ecology Resources 9:1460-1559.

Campbell N.R., K. Overturf., S.R. Narum. 2009. Characterization of 22 novel single nucleotide polymorphism markers in steelhead and rainbow trout*. Molecular Ecology, Resources* 9:318-322.

Campton, D. E., and J. M. Johnston. 1985. Electrophoretic evidence for a genetic admixture of native and non-native rainbow trout in the Yakima River, Washington. Trans. Amer. Fish. Soc. 114:782-793.

Cavalli-Sforza, L.L., and Edwards, W.W.F. 1967. Phylogenetic analysis; models and estimation procedures. Evolution 32:550-570.

Falush, D., M. Stephens and J.K. Pritchard. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164:1567-1587.

Guo S.W., and E.A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. Biometrics 48:361-372.

Haldane J.B.S. 1954. An exact test for randomness of mating. Journal of Genetics 52:631-635.

Kalinowski S.T. 2005. HP-Rare: A computer program for performing rarefactions on measures of allelic diversity. Molecular Ecology Notes 5:187-189.

Kalinowski S.T., M.L. Taper and T.C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases confidence in paternity. Molecular Ecology 16:1099-1106.

Langella, O. 2001. Populations 1.2.24: population genetic structure (individuals or populations distances, phylogenetic trees). Available: http://www.pge.cnrs.gif.fr/bioinfo/populations/.

Olsen, J.B., P. Bentzen, M.A. Banks, J.B. Shaklee, and S.Young. 2000. Microsatellites reveal population identity of individual pink salmon to allow supportive breeding of a population at risk of extinction. Transactions of the American Fisheries Society 129:232-242.

Page, R.D.M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. 12:357-358

Park, S.D.E., 2001. Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection [Ph.D. thesis] University of Dublin.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.

Rousset, F., and M. Raymond. 1995. Testing heterozygote excess and deficiency. Genetics 140:1413–1419

Stephenson J.J., M.R. Campbell, J.E. Hess, C. Kozfkay, A.P. Matala, M.V. McPhee, P. Moran, S.R. Narum, M.M. Paquin, O. Schlei, M.P. Small, D.M. Van Doornik, J.K. Wenburg. 2008. A centralized model for creating shared, standardized, microsatellite data that simplifies inter-laboratory collaboration. Conservation Genetics 10:1145-1149.

Sprowles A.E., M.R. Stephens, N.W. Clipperton, and B.P May. 2006. Fishing for SNPs: A Targeted Locus Approach for Single Nucleotide Polymorphism Discovery in Rainbow Trout. Transactions of the American Fisheries Society 135:6 1698-1721.

Weir B.S., 1990. Genetic data analysis. Sinauer Publ., Sunderland, MA.

Weir B.S., and C.C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:L1358-1370.

Genetic Structure of Oncorhynchus mykiss populations in the Deschutes Basin

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INTRODUCTION

Genetic structure of *Oncorhynchus mykiss* populations in the Deschutes Drainage was examined in regards to potential kelt reconditioning and egg outplanting projects. This effort expands on previous genetic studies of *O. mykiss* (Matala et al. 2008). We attempted to examine genetic structure with four primary goals: 1-Describe population structure of *O. mykiss* within the Warm Springs River. 2-Determine the proportion of resident and anadromous juveniles outmigrating from the Warm Springs River. 3-Expand the baseline of standardized genetic data for *O. mykiss* in the Deschutes River drainage. 4-Determine the effect of straying on native populations of *O. mykiss*. While not all goals are possible at this point, it is hoped that the data here will contribute to future efforts including those by other genetic laboratories.

METHODS

Samples were collected using multiple methods (adult trap, screw trap, electrofishing), and include multiple stages (parr, smolt, adult). Anadromous adults in Warm Springs River were sampled at the Warm Springs National Fish Hatchery during the upstream migration. Separate collections were made for both unmarked, and marked (strays) fish. Emigrating juveniles in Warm Springs River were collected using a rotary screwtrap. These samples are thought to include both resident and anadromous fish. Additional samples were collected using electrofishing methods in Beaver Creek and Mill Creek, tributaries to the Warm Springs River. Samples from Shitike Creek were taken from upstream migrants of adult fish, and downstream migrants of juveniles. Anadromy of adults was determined by length at time of capture. Additional tributaries within the Deschutes were represented by juvenile collections at Buck Hollow and Trout Creek. Juveniles from Round Butte Hatchery were sampled to represent potential in-basin strays.

The genetic methods described here account for efforts at the Hagerman Fish Culture Experiment Station. However, extraction and microsatellite genotyping was also performed at the U.S. Fish and Wildlife Abernathy Fish Technology Center. For additional information on that effort, see Matala et al. (2008). Standardized microsatellite genotypes (Stephenson et al. 2008) were used to facilitate data sharing.

Samples were collected and stored in ethanol for preservation of DNA. 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 13 standardized microsatellite loci (Stephenson et al. 2008). 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 using a Fluidigm EP1 instrument in conjunction with Fluidigm SNP Genotyping Analysis software. Prior to any statistical analysis, two loci used for detection of cutthroat trout hybrids (Omy_myclarp404-111, Omy_Omyclmk436-96), and one sex determining marker (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). Critical values were corrected to 0.0055 (BYFDR at 4850 comparisons; Narum 2006). When pairwise comparisons showed linkage disequilibrium for greater than half the collections, the locus with lower heterozygosity was dropped (Table 2). Loci with low diversity (heterozygosity of less than 0.05) at all populations were also dropped.

Table 1. SNP loci used.

Marker Name	Reference
Omy_SEXY1	Unpublished M. Campbell
Omy_myclarp404-111	Unpublished N. Campbell
Omy_Omyclmk436-96	Unpublished N. Campbell
Omy_113490-159	Unpublished C. Garza
Omy_114315-438	Unpublished C. Garza
Omy_121006-131	Unpublished C. Garza
Omy_121713-115	Unpublished C. Garza
Omy_123044-128	Unpublished C. Garza
Omy_123048-119	Unpublished C. Garza
Omy_127236-583	Unpublished C. Garza
Omy_128693-455	Unpublished C. Garza
Omy_130295-98	Unpublished C. Garza
Omy_130524-160	Unpublished C. Garza
Omy_187760-385	Unpublished C. Garza
Omy_95489-239	Unpublished C. Garza
Omy_96222-125	Unpublished C. Garza
Omy_97077-73	Unpublished C. Garza
Omy_97660-230	Unpublished C. Garza
Omy_97865-196	Unpublished C. Garza
Omy_97954-618	Unpublished C. Garza
Omy_aldB-165	Campbell et al. 2009
Omy_aldB-414	Campbell et al. 2009
Omy_ALDOA_1	Aguilar and Garza 2008
Omy_aromat-280	Unpublished J. DeKoning
Omy_arp-630	Campbell et al. 2009
Omy_aspAT-123	Campbell et al. 2009
Omy_aspAT-413	Campbell et al. 2009
Omy_b1-266	Sprowles et al. 2006
Omy_b9-164	Sprowles et al. 2006
Omy_BAC-B4-126	Unpublished S. Young
Omy_BAC-B4-324	Unpublished S. Young
Omy_cd28-130	Unpublished J. DeKoning
Omy_cd59-206	Unpublished J. DeKoning
Omy_cd59b-112	Unpublished J. DeKoning
Omy_colla1-525	Unpublished J. DeKoning
Omy_cox1-221	Campbell et al. 2009
Omy_cox2-335	Unpublished J. DeKoning
Omy_crb-106	Sprowles et al. 2006
Omy_CRBF1-1	Aguilar and Garza 2008
Omy_cxcr-169	Unpublished J. DeKoning
Omy_dacd1-131	Unpublished N. Campbell
Omy_e1-147	Sprowles et al. 2006
Omy_g1-103	Sprowles et al. 2006
Omy_g12-82	Unpublished J. DeKoning
Omy_gadd45-332	Unpublished N. Campbell
Omy_gdh-271	Campbell et al. 2009
Omy_gh-334	Campbell et al. 2009
Omy_gh-475	Campbell et al. 2009
Omy_GHSR-121	Unpublished N. Campbell
Omy_gluR-79	Unpublished N. Campbell
Omy_hsc715-80	Campbell & Narum 2009b
Omy_hsf1b-241	Campbell & Narum 2009b
Omy_hsf2-146	Campbell & Narum 2009b
Omy_hsp47-86	Campbell & Narum 2009b
Omy_hsp70aPro-329	Campbell & Narum 2009b
Omy_hsp90BA-193	Campbell & Narum 2009b
Omy_hsp90BA-229	Campbell & Narum 2009b
Omy_IL17-185	Unpublished J. DeKoning
Omy_IL1b-163	Unpublished J. DeKoning
Omy_IL6-320	Unpublished J. DeKoning
Omy_inos-97	Unpublished J. DeKoning
Omy_LDHB-1_i2	Aguilar & Garza 2008
Omy_LDHB-2_e5	Aguilar & Garza 2008
Omy_LDHB-2_i6	Aguilar & Garza 2008
Omy_mapK3-103	Unpublished N. Campbell
Omy_mcsf-268	Unpublished J. DeKoning
Omy_mcsf-371	Unpublished J. DeKoning
Omy_metA-161	Unpublished N. Campbell
Omy_metB-138	Unpublished N. Campbell
Omy_myoD-178	Campbell et al. 2009
Omy_nach-200	Unpublished J. DeKoning
Omy_NaKATPa3-50	Campbell et al. 2009
Omy_ndk-152	Unpublished N. Campbell
Omy_nkef-241	Campbell et al. 2009
Omy_nkef-308	Campbell et al. 2009
Omy_nramp-146	Campbell et al. 2009
Omy_Ogo4-212	Campbell et al. 2009
Omy_OmyP9-180	Sprowles et al. 2006
Omy_Ots208-138	Campbell et al. 2009
Omy_Ots249-227	Campbell et al. 2009
Omy_oxct-85	Unpublished J. DeKoning
Omy_p53-262	Unpublished N. Campbell
Omy_pad-196	Unpublished N. Campbell
Omy_PEPA-i6	Aguilar & Garza 2008
Omy_R0917-230	Sprowles et al. 2006
Omy_R1175-137	Sprowles et al. 2006
Omy_rapd-132	Sprowles et al. 2006
Omy_rapd-167	Sprowles et al. 2006
Omy_SECC22b-88	Unpublished N. Campbell
Omy_sSOD-1	Brunelli et al. 2008
Omy_star-206	Unpublished J. DeKoning
Omy_stat3-273	Unpublished J. DeKoning
Omy_tgfb-207	Unpublished J. DeKoning
Omy_tlr3-377	Unpublished J. DeKoning
Omy_tlr5-205	Unpublished J. DeKoning
Omy_u07-79-166	Unpublished S. Young

Table 2. SNP loci dropped due to linkage or low heterozygosity.

Dropped	Reason
Omy_nkef-241	Linked to Omy_nkef-308
Omy_BAC-B4-126	Linked to Omy_BAC-B4-324
Omy_tgfb-207	Linked to Omy_CRBF1-1
Omy_aldB-414	Linked to Omy_aldB-165
Omy_hsp90BA-229	Linked to Omy_hsp90BA-193
Omy_Ogo4-212	Linked to Ogo4
Omy_121006-131	Linked to Omy_127236-583
Omy_121006-131	Linked to Omy_dacd1-131
Omy_127236-583	Linked to Omy_dacd1-131
Omy_121006-131	Linked to Omy_SECC22b-88
Omy_187760-385	Low Heterozygosity
Omy_ALDOA_1	Low Heterozygosity
Omy_nramp-146	Low Heterozygosity

Of the 1,098 initial samples in the study, 5 were removed due to duplicate genotypes. An additional 47 were removed due to incomplete genotypes as defined by either four or more missing microsatellites or 10 missing SNPs. Data for these fish were not included in the statistical analysis. This left 1,046 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 of separation. Otherwise separate collections were treated and reported independently. Adults collected at Warm Springs weir and juveniles at the Warm Springs Screw trap were left separate regardless of results. Alternatively, for structure analysis, collections within the same tributary or locations were pooled.

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 13 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). Bonferroni corrections to critical values were made following Rice 1989.

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, the program STRUCTURE v.2.0 (Pritchard et al. 2000, Falush et al. 2003) was used. 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 replicates each at a burn-in of 100,000 with 300,000 iterations.

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 samples and collections were re-sampled from the baseline, treated as unknowns, and assigned to stocks. Results were reported for both 100% proportion simulations and individual assignment success. Reporting units were created to include all collections within each location. The Warm Springs reporting unit included all fish collected within the Warm Springs River except strays.

Proportional mixed stock assignments were performed using microsatellite data from 161 Columbia River Basin populations. However, previous work has already shown this baseline to have limited power to discriminate populations in reporting groups for the id Columbia and Lower Snake Rivers that includes 30 different collections (J Hess, personal communication).

RESULTS

Statistical analysis was performed on 1,046 samples. Basic population statistics are reported in Table 3. The number of samples per collection ranged from 28 to 150, with a minimum of 49 per sampling location. The highest number alleles (13.69) were seen in Shitike Creek anadromous adults, while the highest allelic richness was seen in the Warm Springs Screw Trap (10.55).

Table 3. Population Statistics. Each collection is reported in terms of sample size (N), number of microsatellite alleles (A_{msat}), microsatellite allelic richness (AR_{msat}), microsatellite private allelic richness (PAR_{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.

Collection	N	A_{msat}	AR_{msat}	PAR_{msat}	H_E	H_O
Beaver-Lower	39	10.54	9.82	0.08	0.3045	0.2962
Beaver-Upper	49	10.38	9.27	0.03	0.3012	0.3002
Buck Hollow	62	11.92	10.22	0.17	0.3116	0.3045
Mill	158	13.23	10.02	0.09	0.3139	0.3098
Shitike Anad	140	13.69	10.29	0.13	0.3131	0.3046
Shitike Res	98	11.00	8.60	0.06	0.2633	0.2592
Trout	51	11.62	10.52	0.12	0.3077	0.3028
Round Buttte	45	9.69	9.18	0	0.3036	0.2954
Upper Main 07	32	9.62	9.44	0.05	0.3304	0.3116
Upper Main 06	28	9.85	9.85	0.02	0.3471	0.3339
Warm Sp Anad	44	11.54	10.44	0.26	0.3164	0.2981
Warm Sp Screwtrap08	80	13.15	10.55	0.08	0.3153	0.3128
Warm Sp Screwtrap09	73	12.77	10.50	0.09	0.3157	0.3078
Warm Sp Strays 05	57	11.00	9.54	0.07	0.3119	0.3164
Warm Sp Strays 06	90	12.46	10.31	0.04	0.3120	0.3008

Results for Hardy-Weinberg equilibrium and Linkage disequilibrium are reported in Table 4. Hardy-Weinberg results are reported for each collection as the number of loci out of equilibrium, as well as the number showing evidence of either a deficit or excess of heterozygotes. Both uncorrected (p=0.05) and Bonferroni corrected (p=0.0005, Rice 1989) critical values are shown.

After Bonferroni correction for multiple tests (Rice 1989) departures from Hardy-Weinberg disequilibrium were infrequent. As many of the collections were potentially sampled from mixed populations, this is somewhat unexpected.

Table 4. Hardy-Weinberg equilibrium. Each collection is reported as the number of loci showing departures from Hardy-Weinberg (H-W), and the number and proportion showing evidence of heterozygote deficit (Deficit) or excess (Excess). Significant results are reported for both uncorrected (p=0.05), and corrected (p=0.0005) critical values.

	H-W		Deficit		Excess		Linkage	
Collection	**0.05**	**0.0005**	**0.05**	**0.0005**	**0.05**	**0.0005**	**0.05**	**0.0055**
Beaver-Lower	4	1	4	1	2	0	135	11
Beaver-Upper	7	0	6	0	1	0	296	55
Buck Hollow	6	0	4	0	1	0	252	30
Mill	14	1	7	0	1	0	422	87
Shitike And	6	1	9	2	0	0	198	27
Shitike Res	7	0	4	0	4	0	179	23
Trout	4	0	4	0	3	0	159	10
Round Butte	2	0	4	0	3	0	207	26
Upper Main 07	5	0	4	0	1	0	226	28
Upper Main 06	2	0	3	0	0	0	147	19
Warm Sp. Anad	10	0	11	0	1	0	153	14
Warm Sp Screwtrap08	7	0	7	0	1	0	203	14
Warm Sp Screwtrap09	3	0	4	0	0	0	183	24
Warm Sp Stray 05	3	0	2	0	2	0	131	16
Warm Sp Stray 06	7	0	8	0	1	0	183	20

Pairwise Fst values are shown in Table 5. Number of loci with p≤ 0.0005 (.05/95) for each pairwise comparison are shown in Table 6. The majority of pairwise comparisons have greater than 5 loci out of equilibrium demonstrating statistically significant population differentiation. Particularly high values are seen for comparisons involving the Upper Mainstem, Shitike resident, and Round Butte Hatchery Collections.

Table 5. Pairwise Fst values between populations. Fst values greater than 0.020 are highlighted.

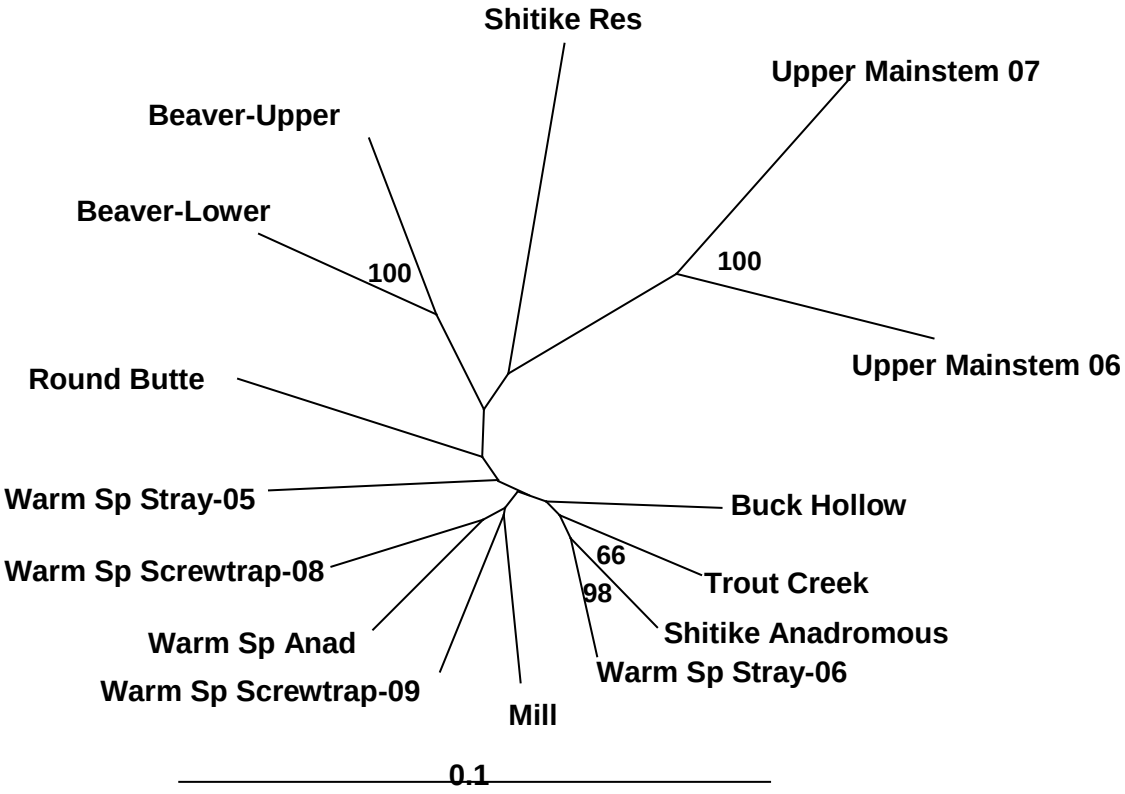
	Beaver Lowel	Beaver Upperl	Buck Hollow	MillI	Shitike Anadl	Shitike Resl	Trout	Rouind Butte	Upper Main07	Upper Main 06	Warm Sp Anadl	Warm Sp Screwtrap08	Warm Sp Screwtrap09	Warm Sp Stray05
Beaver Lower	0.0065													
Buck Hollow	0.0156	0.0202												
Mill	0.0112	0.0108	0.0105											
Shitike Anad	0.0138	0.0215	0.0032	0.0116										
Shitike Res	0.0646	0.0694	0.0669	0.0532	0.0678									
Trout	0.0132	0.0210	0.0044	0.0114	0.0008	0.0796								
Round butte	0.0322	0.0384	0.0173	0.0286	0.0138	0.0838	0.0168							
Upper Main07	0.0325	0.0353	0.0292	0.0280	0.0300	0.0411	0.0312	0.0344						
Upper Main06	0.0644	0.0765	0.0663	0.0647	0.0677	0.0625	0.0705	0.0689	0.0127					
Warm Sp Anad	0.0086	0.0148	0.0097	0.0082	0.0066	0.0624	0.0077	0.0253	0.0261	0.0587				
Warm Sp Screwtrap08	0.0071	0.0105	0.0088	0.0061	0.0087	0.0593	0.0082	0.0277	0.0256	0.0570	0.0006			
Warm Sp Screwtrap09	0.0097	0.0164	0.0097	0.0064	0.0121	0.0345	0.0135	0.0268	0.0148	0.0464	0.0044	0.0044		
Warm Sp Stray05	0.0223	0.0351	0.0126	0.0247	0.0089	0.1089	0.0061	0.0310	0.0527	0.0903	0.0196	0.0193	0.0297	
Warm Sp Stray06	0.0085	0.0167	0.0037	0.0089	0.0014	0.0758	0.0016	0.0217	0.0327	0.0712	0.0042	0.0034	0.0108	0.0040

Table 6. Significant comparisons. For pairwise comparison, the number of loci at P<0.000526 (0.05/95) is shown. Numbers greater than 5 are highlighted.

	Beaver Lowel	Beaver Upperl	Buck Hollow	Millll	Sritike Anadl	Shitike Resl	Trout	Rouind Butte	Upper Main07	Upper Main 06	Warm Sp Anadl	Warm Sp Screwtrap08	Warm Sp Screwtrap09	Warm Sp Stray05
Beaver Lower	1													
Buck Hollow	4	8												
Mill	6	6	10											
Shitike Anad	6	11	3	17										
Shitike Res	20	26	30	32	37									
Trout	1	6	1	5	0	28								
Round butte	10	15	6	16	5	30	4							
Upper Main07	10	13	5	14	9	16	6	10						
Upper Main06	18	26	21	32	28	31	22	22	1					
Warm Sp Anad	1	5	1	0	2	24	0	4	4	15				
Warm Sp Screwtrap08	0	2	2	1	9	34	0	11	7	18	0			
Warm Sp Screwtrap09	0	4	5	2	7	17	3	7	3	18	0	1		
Warm Sp Stray05	5	10	2	15	4	36	0	12	18	22	6	10	11	
Warm Sp Stray06	2	8	2	7	0	33	0	9	11	22	0	2	4	0

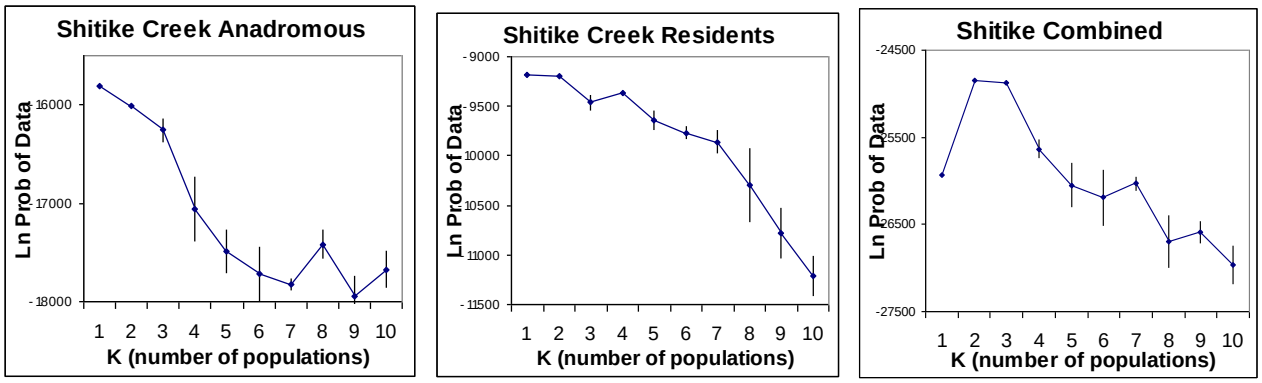
The relationship of collections is shown in the neighbor joining dendrogram in Figure 1. Structure is not well demonstrated here as indicated by the lack of high (greater than 50% bootstrap values) for the majority of nodes. The only tributary grouping with a supporting bootstrap value is seen between Trout Creek and Shitike Creek Anadromous. Additionally Shitike Creek was grouped with the Warm Springs strays sampled in 2006. Strays are known to occur in Shitike Creek, and its proximity to Trout Creek would facilitate gene flow through straying between the two locations or from a common external source of strays.

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).



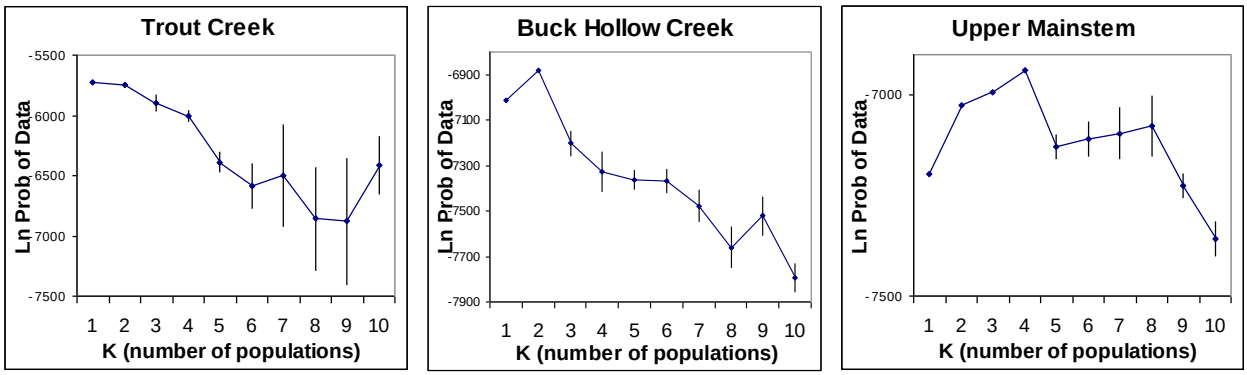
The ln probablitiy for number of populations (K) within each collection is shown in Figures 2-6. Results for both the anadromous and resident components of Shitike Creek fail to support evidence of more than 1 population when analyzed separately. In contrast, when both collections were pooled and analyzed together, an increased probability was seen for k=2. This is consistent with prior genetic (Branstetter et al. 2008) and otolith (Zimmerman et al. 2000) analysis.

Figure 2. Ln probability of data for Shitike Creek collections. Shitike Creek is reported for both anadromous, residents and a combined collection. 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.



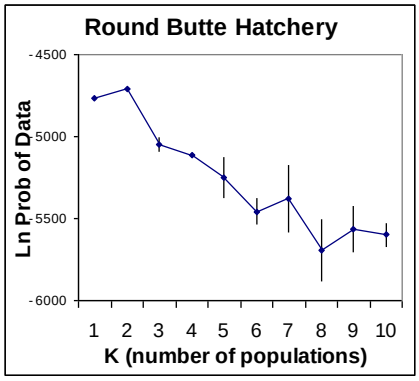
Within the three other natural populations outside of Warm Springs, only Trout Creek had results consistent with a single population (Figure 3). Buck Hollow Creek has week evidence of a second population present within the collection. The upper Mainstem has evidence of between two and four populations present.

Figure 3. Ln probability of data for Buck Hollow, Trout Creek and upper Mainstem Collections.



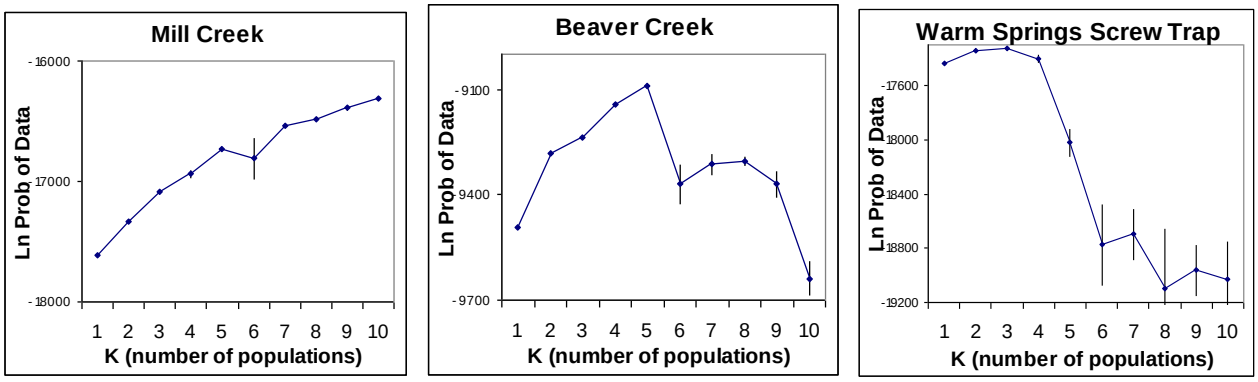
Although the Round Butte Hatchery collection has a slightly higher Ln probability of data when k=2 (Figure 4), the effect is minimal and it may also be better described as containing a single population.

Figure 4. Ln probability of data for Buck Hollow, Trout Creek and upper Mainstem Collections.



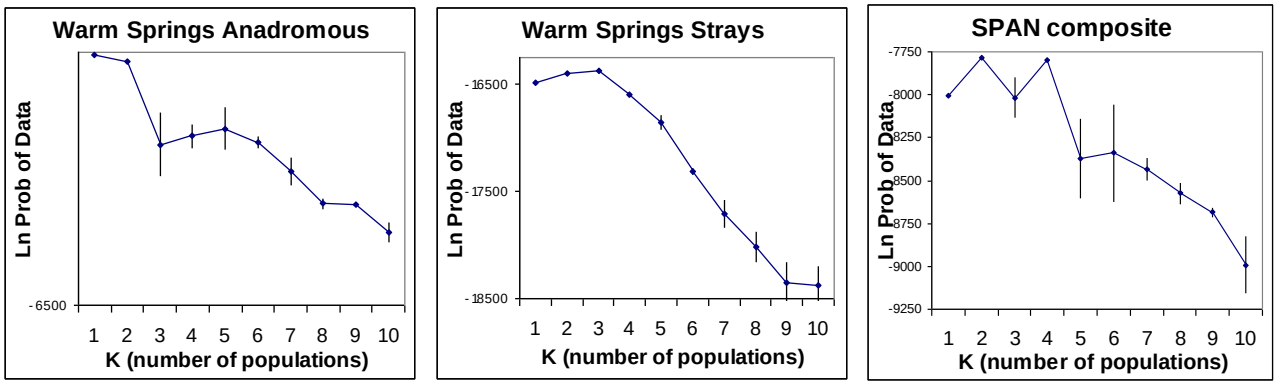
All three Warm Springs juvenile collections showed at least minimal evidence for the presence of greater than one population present (Figure 5). In particular, Beaver Creek has a large increase in Ln Probability of data between k=1 and k=2, indicating the presence of at least two populations in the collection. This is consistent with population differentiation results from earlier. Mill Creek shows a gradually increasing Ln Probability of data, which does not support greater than K=1. Ln probability of data increases with k=2 in the Warm Springs Screw Trap. The relatively smaller increase at k=3 is not enough to conclude that greater than one or two populations were present.

Figure 5. Ln probability of data for Juvenile Collections within warm springs.



Anadromous unmarked adults within the Warm Springs River did not show evidence of a population greater than one (Figure 6), but this may be a result of a small sample size (n=44). Samples collected from marked adults (strays) showed weak evidence of 2-3 populations present.

Figure 6. Ln probability of data for Warm Springs collections.



Assignments at k=2 for juveniles captured at the Warm Springs trap were quantified to compare STRUCTURE group assignment with anadromy designations by field personnel. Of 74 fish labeled as rainbow trout in the field, 21% were labeled as population 1, and 47% as population two. The remaining had samples intermediate (<0.70) assignment values. Comparatively, of 79 fish labeled as steelhead smolts in the field, only 3% were assigned to population 1 while 65% assigned to population 2 with the remained having intermediate values. The relatively high assignment rates of smolts to population 2 may indicate it as the anadromous grouping. It is possible that the 47% of fish that were labeled as rainbow trout in the field, but assigned to population 2, were also of the anadromous grouping, but lacked the smolting/anadromous phenotype at the time of capture.

Results for 100% simulations are reported in Table 7 for both the population of origin, and the assigned reporting group. Average assignments to either the population (0.6168) or reporting unit (0.8139), were poor. Assignments were particularly low for Buck Hollow (0.5726), Shitike Creek anadromous (0.7120), Trout Creek (0.2843), and Warm Springs Anadromous (0.7471), even when the reporting group was used. Assignment rates for Warm Springs strays were also low, but this is not unexpected if baseline groups contain multiple populations.

Table 7 100% simulations to both the population of origin and reporting groups

	Population Group	Reporting Unit
Beaver-Lower	0.4072	0.9610
Beaver-Upper	0.6535	0.9973
Buck Hollow	0.5726	0.5726
Mill	0.9095	0.9781
Shitike Anad	0.7120	0.7120
Shitike Res	0.9870	0.9889
Trout	0.2843	0.2843
Round Butte	0.8960	0.8960
Upper Main 07	0.8656	0.9209
Upper Main 06	0.8650	0.9982
Warm Sp Anad	0.1723	0.7471
Warm Sp Screwtrap 08	0.4569	0.8690
Warm Sp Screwtrap 09	0.5022	0.9242
Warm Sp Stray 05	0.4905	0.8111
Warm Sp Stray 06	0.4770	0.5474
Average	0.6168	0.8139

Individual self assignments to reference populations are shown in Table 8. Corresponding assignments to reporting groups are in Table 9. Assignment rates back to population of origin averaged only 40.2%, increasing to 61.5% for assignment to reporting groups.

Table 8. Individual assignments to reference populations. The number of fish assigning to each baseline collection is listed. Each row lists where samples were collected. Columns list where individuals assigned. Bold numbers are assigned to their population of collection.

	Beaver-Lower	BeaveriUpper	Buck Hollow	Mill	Shitike Anad	Shitike Res	Trout	Round Butte	Upper Main 07	Upper Main 06l	Warm Sp Anad	Warm Sp Screwtrap08	Warm Sp Screwtrap09	Warm Sp Stray 05	Warm Sp Stray 06	IA to pop
Beaver-Lower	**10**	6	0	2	2	0	0	0	0	0	0	2	1	3	3	34.5%
Beaver-Upper	2	**19**	0	2	0	0	0	0	0	0	0	4	2	0	0	65.5%
Buck Hollow	0	0	**16**	3	11	2	3	2	2	0	1	1	3	1	8	30.2%
Mill	1	2	4	**62**	5	0	4	1	0	0	3	8	12	3	9	54.4%
Shitike Creek Anad	0	1	6	2	**31**	4	8	5	1	0	2	5	7	9	13	32.3%
Shitike Creek Res	0	0	1	0	1	**61**	0	1	0	0	0	0	1	1	0	87.1%
Trout	0	0	4	3	10	0	**8**	0	0	0	0	1	1	5	4	22.2%
Round Butte	0	0	0	1	3	0	2	**14**	1	0	1	0	0	0	0	63.6%
Upper Main 07	0	0	0	0	3	1	4	1	**8**	5	0	0	0	0	0	33.3%
Upper Main 06	0	0	1	0	0	0	2	0	1	**15**	0	0	0	0	0	78.9%
Warm Sp Anad	0	0	0	4	3	2	0	0	1	0	**1**	5	3	2	6	3.4%
Warm Sp Screwtrap08	4	3	0	12	2	0	0	1	1	0	5	**11**	7	2	9	19.0%
Warm Sp Screwtrap09	2	1	2	5	7	9	1	0	1	0	4	8	**9**	2	2	16.7%
Warm Sp Stray 05	1	0	1	0	2	0	5	1	0	0	0	0	0	**16**	13	41.0%
Warm Sp Stray 06	2	0	7	1	8	0	5	0	0	0	2	10	3	17	**14**	20.3%
Average																40.2%

Table 9. 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 are assigned to their population of collection.

	Warm Springs	Buck hollow	Round Butte	Shitike Anad	Shitike Res	Trout	Upper main	Warm Strays	IA to Group
Beaver-Lower	**21**	0	0	2	0	0	0	6	72.4%
Beaver-Upper	**29**	0	0	0	0	0	0	0	100.0%
Buck Hollow	8	16	2	11	2	3	2	9	30.2%
Mill	**88**	4	1	5	0	4	0	12	77.2%
Shitike Anad	18	6	5	**31**	5	8	1	22	32.3%
Shitike Res	4	1	1	1	**62**	0	0	1	88.6%
Trout	5	4	0	10	0	**8**	0	9	22.2%
Round Butte	2	0	**14**	3	0	2	1	0	63.6%
Upper Main 07	2	0	1	3	1	4	**13**	0	54.2%
Upper Main 06	0	1	0	0	0	2	**16**	0	84.2%
Warm Sp Anad	**14**	0	0	3	3	0	1	8	48.3%
Warm Sp Screwtrap08	**43**	0	1	2	0	0	1	11	74.1%
Warm Sp Screwtrap09	**30**	2	0	7	9	1	1	4	55.6%
Warm Sp Stray 05	1	1	1	2	0	5	0	**29**	74.4%
Warm Sp Stray 06	18	7	0	8	0	5	0	**31**	44.9%
Average									61.5%

Table 10 shows assignment to reporting group over the Columbia River Basin. The major proportion of all collection assigned to the Mid Columbia and Lower Snake reporting unit (Mid Col LowerSnake). Because the baseline condensed all Deschutes River sub-basin populations into a single reporting group, determination of strays from nearby areas surrounding the Deschutes River was not possible.

Table 10. Proportional mixed stock assignments. The proportion of fish assigned to reporting groups across the Columbia Basin. Each row lists where samples are from. Columns list where individuals were assigned to. Bold numbers are assigned to their population of collection. HoodCanal/Puget Sound, Willamette and Big White Salmon not shown, as no proportion assigned to them.

	Lower Columbia	Lower Columbia su	MidCol LowerSnake	Klickitat	Yakima	Upper Clearwater	Grand Ronde	Imnaha	MF SF Salmon	Upper Salmon	Lower Salmon	Upper Columbia
Beaver	0.02		0.84		0.02			0.05		0.05	0.02	
Buck Hollow			1.00									
Mill Creek			0.96					0.00	0.00	0.03		0.01
Shitike Anad			0.94			0.02		0.01	0.00	0.01	0.02	
Shitike Res			0.98			0.01						0.01
Trout			0.93			0.02		0.01		0.04		
Round Butte			1.00		0.00							
Upper Main		0.02	0.90		0.01			0.01		0.02		0.05
Warm Sp Anad	0.02		0.90							0.07		0.01
Warm Sp Screwtrap	0.01		0.93	0.01	0.01	0.02			0.01		0.02	
Warm Sp Stray			0.93	0.00		0.02	0.01		0.01	0.02	0.01	

DISCUSSION

Analysis shows that there are multiple distinct populations of *O. mykiss* in the Deschutes River Basin. The genetic structure of those populations is less clear. Three populations show consistently high Fst values as compared to all other populations. Shitike Creek Residents had the highest with an average Fst=0.0664. The lowest Fst value for Shitike Creek was 0.0345 when compared to juveniles collected at the Warm Springs Screwtrap in 2009. Upper Mainstem collections had an average Fst=0.0492, with a minimum of 0.0148 when compared to juveniles collected at the Warm Springs trap in 2009. Round Butte Hatchery had an average Fst=0.0333. Round Butte pairwise comparisons with Fst<0.02 were seen in Buck Hollow (Fst=0.0173), Shitike Creek Anadramous (Fst=0.0138), and Trout Creek (Fst=0.0168). This may be due straying of round butte fish, but it is also possible that Round Butte broodstock were collected from these populations.

In addition to the structure detected by pairwise comparisons between sample collections, there may be internal structure within the collections. The upper Mainstem, Buck Hollow, and Beaver Creek in particular show evidence of further possible distinct populations as seen by STRUCTURE results. This may be expected within the Upper Mainstem as it is downstream of discrete locations, and may include multiple populations. However this is not supported by a clear deficiency of heterozygotes as would be expected in an admixed population. The same goes for Buck Hollow, although with a sample size of only 62, statistical power may be lacking. In Beaver Creek Fst between Upper and Lower collections was small (0.0065) and differentiation was supported by only a single locus at a corrected p= 0.0005. Although Beaver

Creek may contain two populations, there is little evidence to confirm that differences are biologically significant, or that it is stratified by upper and lower collection sites.

Tributary collections of populations thought to be anadromous steelhead provided minimal evidence of distinct populations. When Shitike Creek Residents, Upper Mainstem juveniles, and Round Butte Hatchery were removed from analysis the average Fst dropped from 0.0285 to 0.0109. Of particular note, there is little to distinguish strays from unmarked fish collected within Warm Springs River, the average Fst between the collections being only 0.0119. GSI analysis shows that strays appear to originate from within the reporting group, but this reporting group encompasses many populations outside of the Deschutes River sub-basin. Furthermore, the proportion assigned (0.93) is similar to the rate of unmarked fish (0.90) assigned to the same reporting group. This corroborates previous concerns regarding the effect of straying native populations (Hand and Olson 2004, Matala 2008).

Genetic analysis of *O. mykiss* in the Deschutes River sub-basin will continue in 2010. In particular, the Abernathy Fish Technology Center has ongoing projects that we will continue to collaborate with through sharing of samples and genotypes.

References

Aguilar A., and C. J. Garza. 2008. Isolation of 15 single nucleotide polymorphisms from coastal steelhead, Oncorhynchus mykiss (Salmonidae). Molecular Ecology Resources 8:659-662

Anderson, E.C., R.S. Waples, S.T. Kalinowski. 2008. An improved method for estimating the accuracy of genetic stock identification. Canadian Journal of Fisheries and Aquatic Sciences 65:1475-1486.

Branstetter R., J. Stephenson, D. Hatch, J. Whiteaker S.Y. Hyun, B. Bosch, D. Fast, J. Blodgett, and T. Newsome, L. Hewlett-Dubisar, J. Graham, J. Lovetang, R. Dasher,C. Fisher, 2008. Steelhead Kelt Reconditioning and Reproductive Success. 2007 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-00. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.

Brunelli, J.P., G.H. Thordaard, R.F. Leary, and J.L. Dunnigan. 2008. Single Nucleotide Polymorphisms Associated with Allozyme Difference between inland and coastal rainbow trout. Transactions of the American Fisheries Society 137:1292-1298.

Busack, C., A. Fritts, T. Kassler, J. Loxterman, T. Pearsons, S. Schroder, M. Small, S. Young, C. Knudsen, G. Hart, P. Huffman, "Yakima/Klickitat Fisheries Project Genetic Studies; Yakima/Klickitat Fisheries Project Monitoring and Evaluation", 2005-2006 Annual Report, Project No. 199506325, 205 electronic pages, (BPA Report DOE/BP-00022370-5)

Campbell N.R., S.R. Narum. 2009b. Identification and characterization of heat shock response related single nucleotide polymorphisms in O. mykiss and O. tschawytscha. Molecular Ecology Resources 9:1460-1559.

Campbell N.R., K. Overturf., S.R. Narum. 2009. Characterization of 22 novel single nucleotide polymorphism markers in steelhead and rainbow trout. Molecular Ecology, Resources 9:318-322.

Campton, D.E., and J. M. Johnston. 1985. Electrophoretic evidence for a genetic admixture of native and non-native rainbow trout in the Yakima River, Washington. Trans. Amer. Fish. Soc. 114:782-793.

Cavalli-Sforza, L.L., and Edwards, W.W.F. 1967. Phylogenetic analysis; models and estimation procedures. Evolution 32:550-570.

Falush, D., M. Stephens and J.K. Pritchard. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164:1567-1587.

Guo S.W., and E.A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. Biometrics 48:361-372.

Haldane J.B.S. 1954. An exact test for randomness of mating. Journal of Genetics 52:631-635.

Hand, D.M. and D.E. Olson. 2004. Creating a Sanctuary for Wild Steelhead Trout Through Hatchery Operations. Published as part of the proceedings of the Wild Trout VIII Symposium, Yellowstone National Park.

Kalinowski S.T. 2005. HP-Rare: A computer program for performing rarefactions on measures of allelic diversity. Molecular Ecology Notes 5:187-189.

Kalinowski S.T., M.L. Taper and T.C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases confidence in paternity. Molecular Ecology 16:1099-1106.

Langella, O. 2001. Populations 1.2.24: population genetic structure (individuals or populations distances, phylogenetic trees). Available: http://www.pge.cnrs.gif.fr/bioinfo/populations/.

Matala, A.P., W. R. Ardren, D. Hand, D. Olson, and H. Schaller. 2008. Population structure and genetic characteristics of summer steelhead (Oncorhynchus mykiss) in the Deschutes River Sub-basin, Oregon. U. S. Fish and Wildlife Service, Abernathy Fish Technology Center Report.

Narum, S.R. 2006. Beyond Bonferroni: Less conservative analyses for conservation genetics. Conservation Genetics 7:783-787.

Olsen, J.B., P. Bentzen, M.A. Banks, J.B. Shaklee, and S.Young. 2000. Microsatellites reveal population identity of individual pink salmon to allow supportive breeding of a population at risk of extinction. Transactions of the American Fisheries Society 129:232-242.

Page, R.D.M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. 12:357-358

Park, S.D.E., 2001. Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection [Ph.D. thesis] University of Dublin.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.

Rousset, F., and M. Raymond. 1995. Testing heterozygote excess and deficiency. Genetics 140:1413–1419

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution. 43: 223-225

Stephenson J.J., M.R. Campbell, J.E. Hess, C. Kozfkay, A.P. Matala, M.V. McPhee, P. Moran, S.R. Narum, M.M. Paquin, O. Schlei, M.P. Small, D.M. Van Doornik, J.K. Wenburg. 2008. A

centralized model for creating shared, standardized, microsatellite data that simplifies inter-laboratory collaboration. Conservation Genetics 10:1145-1149.

Sprowles A.E., M.R. Stephens, N.W. Clipperton, and B.P May. 2006. Fishing for SNPs: A Targeted Locus Approach for Single Nucleotide Polymorphism Discovery in Rainbow Trout. Transactions of the American Fisheries Society 135:6 1698-1721.

Weir B.S., 1990. Genetic data analysis. Sinauer Publ., Sunderland, MA.

Weir B.S., and C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:L1358-1370.

Zimmerman, C. E., and G.H. Reeves. 2000. Population structure of sympatric anadromous and non-anadromous Oncorhynchus mykiss: evidence from spawning surveys and otolith microchemistry. Canadian Journal of Fisheries and Aquatic Sciences 57:2152-2162.

Section 4: Snake River Research and Master Plan Development

Developing Strategies to Improve Survival and Return Recruitment of Steelhead Kelts from Snake River Stocks

Semi-Annual Report for the Columbia River Inter-Tribal Fish Commission

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Background

Within their native range, steelhead trout *Oncorhynchus mykiss* are iteroparous, and the proportion of fish that survive to spawn again varies from ~ 40% to < 1%. In the Snake and Columbia River systems, fish passage facilities at hydro dams were not well designed to accommodate downstream migrating, post spawning steelhead adults (kelts). Aside from inadequate bypass facilities, reservoir flow conditions are reported to affect efficient passage of kelts downriver (Keefer et al. 2008). Historical rates of iteroparity in Columbia and Snake River steelhead stocks before hydro development are not well documented, but recent estimates range from 1.6 to 17% (Hatch et al. 2003).

Tagging studies conducted at Lower Granite Dam (Evans 2002; Hatch et al. 2003; Boggs and Peery 2004; Keefer et al. 2008) documented high kelt mortality during downstream migration. Of the PIT-tagged fish, 70% and 95% were unaccounted for between Lower Granite and Bonneville Dams. Recent estimates of iteroparity in the Snake River steelhead stocks are estimated at below 2% (Boggs and Peery 2004; Evans et al. 2008; Keefer et al. 2008).

Kelts tagged and released at John Day Dam in the lower-Columbia River have performed appreciably better than Snake River kelts, and 10% or more of tagged fish have been detected returning to spawn again (Wertheimer et al. 2003; Evans et al. 2008). Repeat spawning rates as high as 17% have been observed in the Kalama River located below Bonneville Dam (Leider et al. 1986). Groups of downstream migrating kelts from the Yakima River have been collected and held in captive reconditioning facilities and portions of these fish now survive to spawn again (Branstetter et al. 2007). However, few studies have quantified the physiological or endocrinological changes of kelts during the post-spawning recovery process. Increased survival of kelts was obtained through collection and transporting kelts below the hydro dams within in the Columbia River corridor (Evans et al. 2008).

The Lower Granite Dam bypass collection facility currently provides access to the largest number of kelts anywhere in the Columbia River Basin (Evans et al. 2004), making it an excellent location for increased research and monitoring of threatened summer-run steelhead. Collection, transportation, and reconditioning of kelts from Lower Granite Dam (or other bypass facilities in the Lower Snake River) has the potential to benefit all spawning aggregates upstream of the dam.

Research focused on the physiology, health and reproductive potential of spawned out steelhead kelts is needed to establish viable management protocols that can be used to maximize the contribution of these animals to the population through increased iteroparity.

Goal of Project

This is the first year of a multi-year project to study the physiology, health and condition of both A and B-run steelhead kelts with a goal of evaluating the feasibility and success of strategies for rehabilitating and handling kelts in the Snake River system. The project

will develop the background science and facilities needed to provide metrics and testable hypotheses to improve the survival of kelts. Through our research, we will develop and test protocols for collection and transportation of kelts, and pose strategies that can be employed to rehabilitate selected fish to maximize their contribution to the next spawning generation.

Objectives - Year 1

• Objective 1. Obtain basic information describing the general physiology and endocrinology of kelts from the point of spawning through the early migration period using hatchery and non-hatchery origin stocks.

• Objective 2. Describe and obtain physiological and endocrinological profiles from downstream migrating wild stocks captured at Lower Granite Dam, and compare and contrast these profiles with fish from upriver and downriver sites.

• Objective 3. Evaluate the physiology of fish collected at Lower Granite Dam and transported via barge or truck to locations below Bonneville Dam.

• Objective 4. Evaluate the physiology and endocrinology of small groups of kelts held for 2 months or more, and compare results with groups of fish from other sites, and with models from fish migrating through river corridors.

Summary of Progress by Objective and Task

Objective 1. Obtain basic information describing the general physiology and endocrinology of kelts from the point of spawning through the early migration period using hatchery and non-hatchery origin stocks.

Task 1.1. Construct a model of the physiology and endocrinology of B-run steelhead from the point of spawning to up to three weeks post spawning, using kelts from Dworshak National Fish Hatchery (DNFH).

Sampling of fish from DNFH at spawning in 2009 began 20 January and terminated on 21 April. Sampling days were coordinated to overlap with steelhead spawning at the hatchery. In January, we sampled fish from two groups of early fish (entering the hatchery in October 2008). These fish were anesthetized in 100 mg/L buffered MS222, live spawned, sampled for blood at the hatchery, and PIT tagged. Groups sampled in February to April were removed from production fish that were anesthetized with CO_2 so that fish not spawned could be released to the fishery in the river with no risk for human consumption. The use of CO_2 appeared to compromise the fish health when combined with transport and sampling activities.

On sampling dates, small groups of sampled fish (7 to 16) were transported in a 400 gallon transport tank to the University of Idaho (U of I) fisheries wet lab for further holding (Table 1). During holding in the wet lab, the fish were offered krill and shrimp, and their feeding behavior was observed and monitored. Tanks were inspected for feces and uneaten food. In the laboratory we sampled additional blood from kelts at selected intervals to follow changes in the blood plasma parameters over time (Table 2). At the last sampling interval, we euthanized the remaining kelts with 250 mg/L buffered MS222, sampled blood, performed a necropsy of all internal organs, removed a gill sample for analysis of Na^+K^+-ATPase, and removed samples of liver, spleen, gonad, stomach and intestine for later histological and endocrinological profiles. Samples for histology were fixed and stored in 10% neutral buffered formalin. Tissues for endocrinological profiles were stored frozen (-80°C). Samples of the liver, skin and muscle were removed and frozen (-20°C) for later proximate analysis of lipid, protein, and ash content. We photographed each fish, and recorded general condition and content of the gastrointestinal tract. Fish that died were examined by necropsy, photographed, and samples of skin, muscle and liver were retained for proximate analysis. Having these fish in the U of I laboratory provided us with the opportunity to sample spawned fish and train graduate students and staff unable to travel to DNFH in procedures for fish care and handling, blood sampling, blood preparation and storage, necropsy, and tissue preparation and storage.

The results from sampling DNFH fish at the hatchery and in the U of I wet lab will be included in the annual report. We will compare results from DNFH spawned fish with samples obtained from migrating kelts sampled at Clearwater River weirs or at Lower Granite Dam bypass.

Table 1. Summary of blood sampling dates at DNFH. Fish were spawned live at the hatchery and transported to the University of Idaho laboratory for further sampling, or transported to the U of I for sampling and spawning. * Spawned carcasses were sampled at the hatchery on 21 April for skin, muscle and liver tissues only.

Dworshak spawning take	Date of collection	Number of fish sampled	Number with full necropsy samples
1	20-Jan	9	6
2	27-Jan	16	13
3	17-Feb	8	3
4	24-Feb	8	5
6	11-Mar	8	0
7	18-Mar	8	1
8	25-Mar	8	0
9	1-Apr	7	7
10*	21-Apr	30	

Table 2. Analyses by metric category performed on plasma sampled from steelhead trout kelts. Assays are conducted using an autoanalyzer at Gritman Hospital Laboratory, Moscow, ID.

Electrolytes	Enzymes and nutritional factors	Hormones
Sodium	ALP	Cortisol
Potassium	ALT	Total T4
Chloride	AST	
Calcium	LDH	
Magnesium	Cholesterol	
Phosphorous	Creatine kinase	
	Lipase	
	Triglycerides	
	Total Protein	
	Glucose	

Task 1.2. Construct a model of the physiology and endocrinology and migration success of kelts from stocks from at least two tributaries of the Clearwater River watershed, from the point of capture at local weirs within the tributary reaches.

We collaborated with Idaho Department of Fish and Game (IDFG) scientists and managers to sample downstream migrating fish at two weirs in the Clearwater River basin: Crooked River Weir on the South Fork Clearwater River, and Fish Creek on the Lochsa River. The two weirs provide the opportunity to obtain kelts from known locations and documented management histories in the Snake River drainage. Steelhead trout were extirpated from the South Fork of the Clearwater River in 1927 with the construction of the Harpster Dam. The dam was removed in 1962, and a B-run steelhead population was reestablished before the lower Snake River dams were in place, although the reestablishment was limited due to habitat degradation. In 1984, a long term habitat restoration program began and a permanent weir was built on Crooked River to collect spawning steelhead trout and Chinook salmon. The weir and associated adult trap collect upstream migrating fish except when the pickets are pulled due to early season or high flows. Natural/wild fish are passed upstream of the weir. The trap has been used for evaluating and counting Chinook and steelhead trout intermittently over the past several years. Idaho Fish and Game has used the weir to monitor steelhead migrations more seriously, and to accomplish steelhead monitoring, pickets are placed in the system earlier in the season. Spawning steelhead typically appear at the weir in Mid April to the end of May. Spawned out kelts migrate downstream from the end of April to the end of June.

The Fish Creek weir is located on Fish Creek, a tributary to the Lochsa River. The Fish Creek weir was installed in 1993, and has been used to collect data on migrating steelhead trout and Chinook salmon and limit the passage above the weir to only naturally/wild spawning fish. The Fish Creek steelhead trout population is considered a pure B-run. The weir is constructed with pickets placed in the river in early March and the weir is removed in mid-November. The Fish Creek weir remains in place unless high flows and debris destroy the weir. Upstream migrating steelhead trout begin to arrive at the weir in early April, and kelts are encountered typically at the end of April through June.

In April, U of I staff met with IDFG weir operators, provided them with all necessary clinical equipment and supplies, and training for sampling blood from kelts at the weirs. The U of I maintained communications with weir operators, and coordinated retrieval of blood plasma collections.

In 2009, the Crooked River weir had nearly continuous high flows and a large debris load. As a result, the weir was not in operation for much of the migration season and IDFG staff documented few steelhead trout moving upstream. No kelts were sampled at this site in 2009. The number of kelts each year has varied, and we are planning to provide training and equipment for IDFG at this site for studies next year.

At the Fish Creek weir, IDFG staff collected samples from 5 kelts in April before high water flows and debris disabled the weir. In June, the IDFG staff reconstructed the weir and it was in operation again by 15 June. We provided dry ice to staff for sample storage. Starting on 16 June, an additional 47 fish were collected at the weir for a season total of 52 kelts sampled. The average length of the female fish was 78 cm and median length was 79 (range 62 - 86 cm; Table 3). The average length of male kelts was 74, and median length was 69 (range 62 - 94 cm). Some of these kelts appeared to be below the range considered average B-run fish of 78 – 86 cm by Idaho Department of Fish and Game. We were able to document a total of 23 fish with PIT tags at the Fish Creek weir, 14 of which were anesthetized and tagged with 20 mm PIT tags in the pelvic girdle at the time of downstream migration. The remaining 9 kelts had been PIT-tagged as juveniles and had returned as adults. Of these 9 fish tagged as juveniles, 6 were dead at the weir. A total of 17 PIT-tagged kelts migrated downstream from the Fish Creek weir, 10 fish were noted in good condition and 7 were in poor condition. One female fish (83 cm) in poor condition tagged on 15 May 20009 at Fish Creek was detected during downstream migration at McNary Dam on 27 May 2009. From the PIT tag data, the travel time for this fish to migrate from Fish Creek weir to McNary (474 Rkm) was 12 days.

Table 3. Number and fork length of steelhead kelts collected at Fish Creek Weir by week and sex. From 17 May to 12 June, the weir was not in place due to high water and debris.

Week	Female		Male	
	N	Average fork length (range)	N	Average fork length (range)
26 Apr- 2 May	1	74	0	
3 – 9 May	0		1	94
10- 16 May	3	84 (83-86)	0	
14-20 June	14	79 (74-84)	5	71 (62-84)
21 – 27 June	20	77 (63-83)	4	72 (67-85)
28 June – 4 July	2	(71-78)	0	
5-11 July	2	(62-85)	0	
Totals	42	78 (62-86)	10	74 (62-94)

We collected blood samples from 25 downstream migrating kelts at the Fish Creek weir. Ten fish sampled for blood were weak and died after sampling. Of the live fish sampled for blood, 7 were in poor condition, and 8 were in good condition, and 19 were females (Table 4). All of the plasma will be processed over the next 10 weeks at Gritman Medical Center for the same profile as fish from Dworshak (Table 2)

Table 4. Condition of fish collected at the Fish Creek weir by week and condition during 2009. Codes: F = female; M = male. The weir was not in operation from 17 May through 12 June.

Week	Good		Fair		Poor		Dead	
	M	F	M	F	M	F	M	F
26 April – 2 May						1		
3 – 9 May	1							
10 – 16 May		1				2		
14 – 20 June	1	2				2	4	10
21 - 27 June	2	8			1	1	1	11
28 June - 4 July								2
5 – 11 July								2
Total	4	11			1	6	5	25

Objective 2. Describe and obtain physiological and endocrinological profiles from downstream migrating wild stocks captured at Lower Granite Dam, and compare and contrast these profiles with fish from upriver and downriver sites.

Task 2.1. Obtain baseline physiology of naturally spawning (wild) fish and marked hatchery origin steelhead kelts emigrating from Snake River tributaries and collected at Lower Granite Dam .

The sampling of emigrating kelts at Lower Granite Dam (LGD) began on 2 April and the last samples were taken 26 June. Because of permitting delays, no samples were taken from 6 April to 12 May. During each week of sampling, the research team from U of I provided a kelt collection schedule to the staff at LGD, and hatchery or wild fish were sampled. Because of the limited size of the concrete holding tank at the dam (maximum of 25 fish) for many of the sampling dates, we asked the operator of the Corps of Engineers fish separator to assess and collect target fish groups to be diverted (e.g. females; good condition fish; hatchery fish). As a result, we do not have a representative sample of the entire population emigrating past LGD via the juvenile bypass or spill.

We collected data and samples from a total of 317 fish, and 85.5% were wild or natural origin (Table 5). The hatchery fish were euthanized in 250 mg/L buffered MS222 for a full necropsy and tissue analysis using procedures used for samples of DNFH origin fish (Objective 1). Wild fish were sampled for blood, and PIT-tagged and a fin clip removed for genetic stock identification.

Table 5. Summary of number of wild/natural (adipose fish present) and hatchery origin (adipose fin removed) steelhead kelts sampled from LGD bypass by sex. Most of sampling was targeted on female fish, and therefore the number of males sampled is not representative of their proportion in the run.

	Wild or natural fish (percent of total)	Hatchery origin fish (percent of total)	Total number (percent by sex)
Female	233	41	274 (86.4)
Male	38	5	43 (13.6)
Total	271 (85.5)	46 (14.5)	317

To sample wild fish, a processing station was assembled with equipment for tagging and sampling. Fish were netted individually from the concrete holding tank, placed into a fish bag and carried to a 150 L anesthetic bath with 100 mg/L MS222, buffered with $NaHCO_3$ to assure pH of 7.2-7.8. Supplemental oxygen was supplied to the tank with diffuser stone and compressed oxygen, and water temperature was maintained within 2 °C of the river temperatures. Fish were interrogated with a PIT tag reader and tabletop antennae for presence of previously applied tags. The external condition (very poor,

poor, fair, good, very good) of each fish was recorded. Once anesthetized, a fish was placed onto a V-board for processing. Fork length (cm) was measured and a fin clip was removed from the right pelvic fin for future genetic analysis (Narum et al. 2008), and preserved in absolute ethanol. Each kelt was placed ventral side up for sampling of blood from the caudal vessel, and a submersible pump provided water to profuse the gills of the fish during blood sampling and tagging. Approximately 1.5 to 2 mL blood was removed from the caudal vessel with a 3 mL, heparanized syringe fitted with a 21 gauge, 1.5 inch needle. Syringes were inverted to assure mixing of heparin and then placed onto wet ice until they could be processed.

A PIT tag (20mm X 3.05mm, 134.2kHz ISO, BioMark, Inc. Boise ID) was then applied to each fish to be released using needles and tags disinfected with 95% ethanol. To protect workers, the needles were capped with tubing between uses. Each tag was inserted into the pelvic girdle of the fish, using an implanter syringe, with an 8-gauge, 2.0-inch needle.

Fish were then placed into a tared bag and weighed (nearest 0.1 kg) with a Chatillion scale. After weighing, each fish was transferred to a recovery tank (circular, 4 foot diameter, with flow through water). We manually replaced the water in these tanks every 2-3 fish. Recovered fish were placed into the treatment release scheduled for that day (in-river release into a watered release tube directed to the river near the barge slip; truck tranpsort below Bonneville Dam; or barged and released below Bonneville Dam.

In the LGD laboratory, blood samples were processed by dividing them equally into two, 2.0mL centrifuge tubes, and centrifuged for 10 minutes to separate blood cells from plasma fraction. We stored 500µl of plasma in a screw top 1.2mL cryogenic vial for later analysis of enzymes, electrolytes, and selected hormones (Table 2). An additional 75µl sample of plasma was saved for analysis of cortisol (Carl Schreck laboratory, Oregon State University, Corvallis). The remaining plasma was pipetted into a 1.7mL vial for other potential hormone analyses. One of the two tubes with blood cells was retained for later analysis of red blood cells. Blood plasma and blood cells were placed in a cooler with dry ice until they could be transported and stored frozen –80°C for later analysis.

Wild fish were sampled for blood, fin clipped for genetic profiles, PIT tagged and released to migrate downstream in the river, or selected for groups to be transported to below Bonneville. Transportation routes were either within a net pen/transport barge, or inside a 400-gallon tank truck (Table 6). The net pen (nylon mesh supported by an aluminum frame, 2.1 X 1.2 X 2.4 m) was located within a hold of a juvenile transport barge (Evans et al. 2008). Barged fish were collected early to accommodate the 9 AM departure time. The trip by barge was less than 40 hours and was approximately 471 Rkm. Trucked fish were sampled and then loaded into the truck by hand. To reduce stress during truck transit, 0.5% NaCl was added to the tank. Water temperature and oxygen levels in the transit truck were monitored during the 6-8 h trip at least twice; water temperature was maintained with small buckets of ice. Fish were released to the

Columbia River at the Hamilton Island Boat ramp just below Bonneville Dam on the Washington shore.

Table 6. Summary of the number of release dates, total fish, and mortality recorded in three treatment groups of steelhead kelts PIT tagged and sampled for blood at LGD from 2 April to 26 June. Barged kelts were transported on 3, 11, and 17 June. Trucked kelts were transported 21 & 28 May, and 12 June. All groups of transported fish were inspected for mortality at the time of release below Bonneville Dam, and survival is reported.

Release or transport treatment	Number of releases	Total fish by treatment		Percent survival
		Live	Dead	
In-river	18	176	2	99
Barge	3	29	3	90
Truck	3	39	0	100
Total	244	5		98

The average length of all fish sampled at LGD was significantly smaller than for fish sampled at DNFH (Figure 1). The majority of fish collected at the dam appeared to be A run steelhead kelts, with a mode fork length of 58 cm. These data were collected from small groups of fish that were diverted from the bypass by system operators, and held in a tank for sampling. Extrapolations from our data to the downstream migrating populations should be made cautiously, since nearly all sampling was from mid May through June, and collections were directed for specific target groups, such as hatchery or wild fish, females, and in some samples we targeted only fish in good condition. At the time of sampling we assessed and rated fish condition as: very poor, poor, fair, good, and very good. We rated 65.6% of the sampled kelts in good to very good condition, with the remainder rated fair (21.8%) and poor to very poor (12.7%; Table 7).

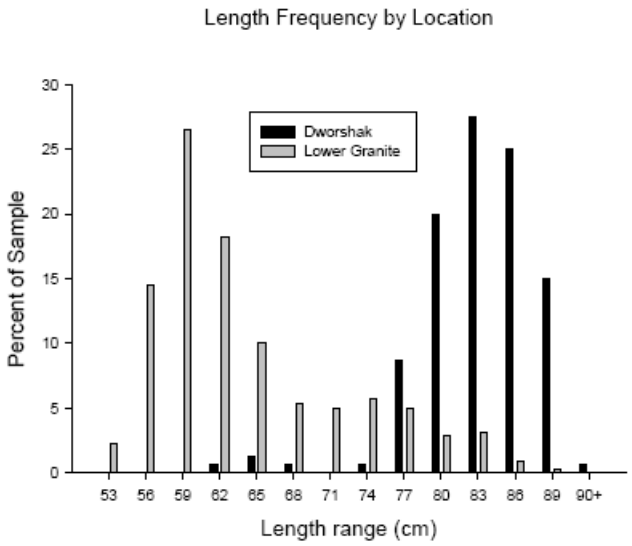

Figure 1. Length frequency of steelhead kelts sampled at DNFH and LGD. Bins were 3 cm, and ending points are indicated. Note that the majority of kelts sampled at LGD bypass were not B-run fish.

Table 7. Condition of kelts sampled at LGD by origin. The number of fish in each category is included.

Origin	Number of fish by condition					Total fish
	Very Poor	Poor	Fair	Good	Very good	(percent)
Wild/natural	4	27	53	110	77	271 (85.5)
Hatchery	0	9	16	9	12	46 (14.5)
Percent	1.3	11.4	21.8	37.5	28.1	

Over the sampling season at LGD, we noted a number of wounds and trauma primarily on the head region of the fish (43 of all fish sampled or 13% of the fish; Figure 2). We observed no differences between wounds on hatchery or wild/natural fish samples. Our observations on fish condition are biased by the procedures used for collecting fish for sampling at the bypass separator. On most dates, we requested fish of good condition for tagging, and hatchery or wild/natural fish were selected on certain dates for study. Over the sampling season, we improved our methods for estimating and recording the extent of injury to achieve a more consistent ranking of condition among all staff. We observed several fish with very severe wounds (Figure 2), and many wounds appeared recent. We plan to prepare for a consistent coding for grading and evaluating external condition of kelts at weir and dam sites in the coming year, and will train all staff in the ranking procedures. The flows at the forebay of LGD and conditions within the bypass system could be factors affecting injury of kelts (Figure 3). We will pursue these

relationships with more comprehensive sampling of the downstream migrating kelts in future years. Our sampling will be limited to fish entering the bypass system, and therefore may not reflect conditions of fish migrating over the dam at the removable spillway weir.

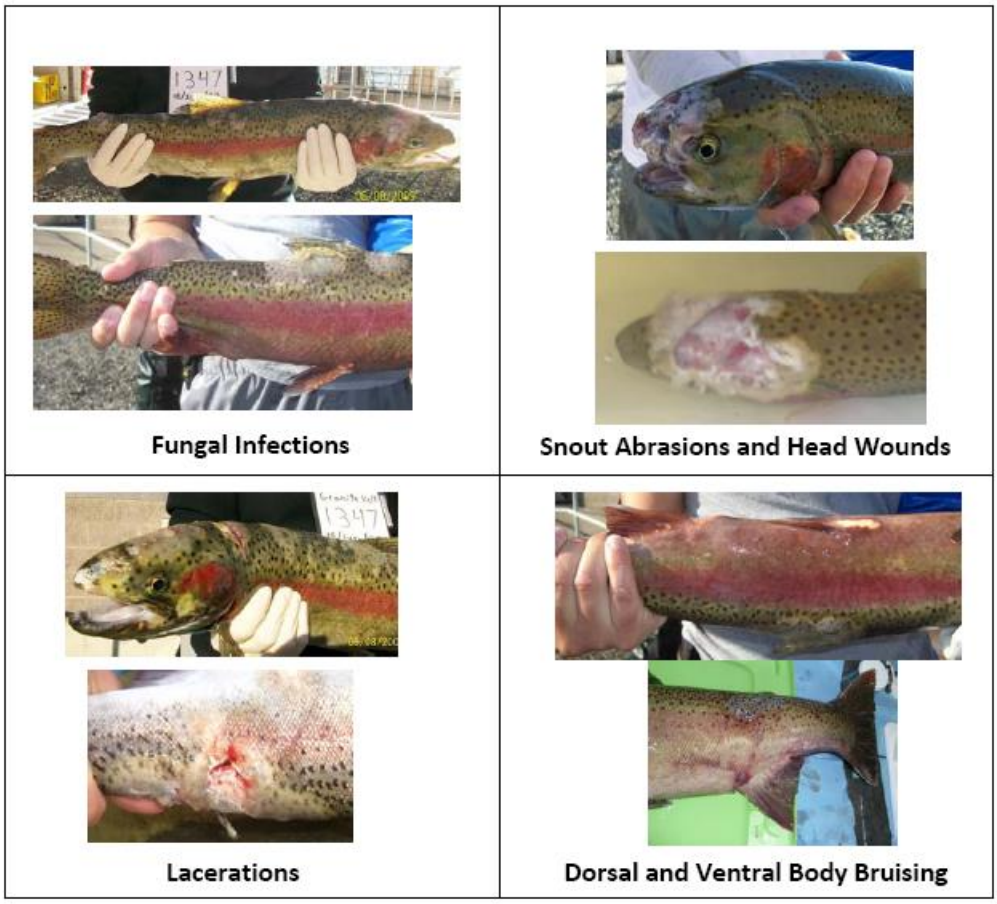

Figure 2. Examples of trauma and fungus infections in kelts sampled at LGD in May and June, 2009.

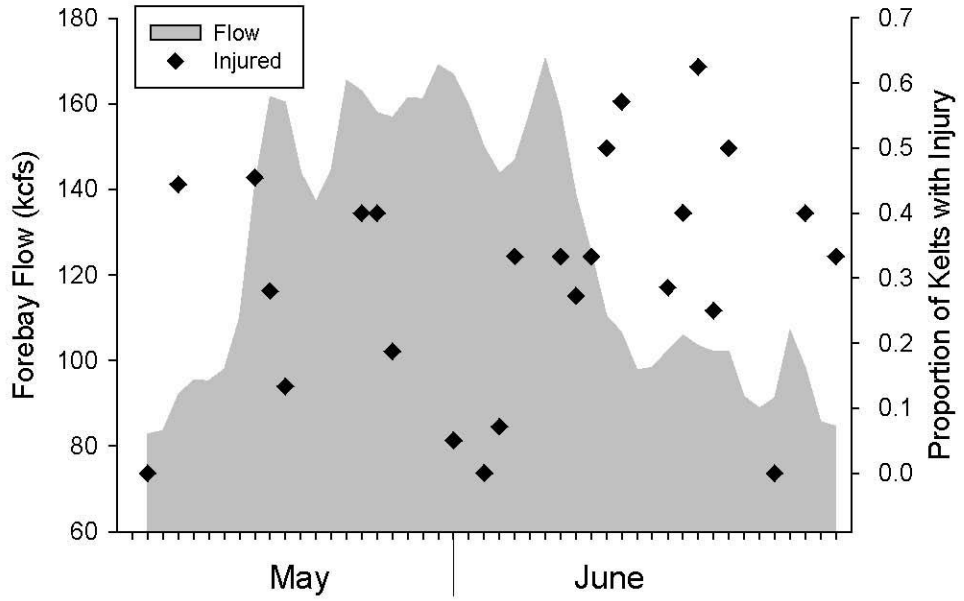

Figure 3. Relationship between proportion of kelts sampled each day observed with injuries and daily flows at the forebay of Lower Granite Dam from 12 May to 26 June, 2009.

Objective 3. Evaluate the physiology of fish collected at Lower Granite Dam and transported via barge or truck to locations below Bonneville Dam.

Task 3.1. Explore the effect of transportation to enhance the survival and probability of repeat spawning by steelhead kelts emigrating down the Snake and Columbia Rivers. Sampling methods include tagging fish and sampling blood to assess condition of kelts transported via truck and barge and compared with non-transported kelts.

During 2009, we applied PIT-tags to 244 wild or natural origin kelts collected at the juvenile bypass system at LGD. Kelts were PIT tagged and released in one of three treatment groups that included in-river release, barge transport from LGD to below Bonneville Dam, and truck transport to release at Hamilton Island boat ramp below Bonneville Dam. The majority of fish were placed into the in-river release treatment. We entered all PIT tag information into the PTAGIS retrieval system. We located 16 of the 62 kelts multiple times (13 fish 2 times; 2 fish 3 times) in the hydro system for a total of 81 detections recorded in the retrieval system. One barged kelt was detected by the portable TWX array in the Columbia River estuary (Table 7).

Table 7. Number of PIT tag detections for kelts tagged and released below LGD, and percent of detections at each site. Detection sites are listed for 6 dams in the Snake and

Columbia River hydrosystem, and one portable array in the lower Columbia River. All detections are counted, of which there were 16 multiple detections. * Portable array detection was on a barged fish.

Dam or array	Count	Percent of detections
Little Goose Dam	23	28.4
Lower Monumental Dam	17	21.0
Ice Harbor Dam	8	9.9
McNary Dam	8	9.9
John Day Dam	4	4.9
Bonneville Dam	20	24.7
Portable array*	1	1.2

Of the 176 kelts, 62 PIT-tagged kelts were detected one or more times during their river outmigration for a detection rate of 35% (Table 8). None of the kelts in poor condition were detected during outmigration.

Table 8. Total number of PIT-tagged kelts released to migrate downstream at LGD, by fish condition at time of tagging. The percent of PIT-tagged kelts detected was calculated based on the number of fish in each condition.

Condition	Total fish released	Fish detected	Percent of fish by condition
Very poor	5	0	0
Poor	16	0	0
Fair	38	12	31.6
Good	71	34	47.9
Very good	46	16	34.8
Total	62	176	35.2

Travel times for the kelts were determined from all collections within the data set. The travel times for kelts from LGD to Bonneville dam ranged from 7.46 - 19.43 days with an average time of 11.1 days (Table 9). The average travel rates of the kelts released from Lower Granite was also determined by dividing the distance from LGD to detection date in days. The range for the average rate of migration was 13.64 km/day - 61.82 km/day (Table 9).

Table 9. Summary of average time to migrate between hydro facilities with PIT tag detection.

Dam	Days	km	Travel rate (km/d)
Little Goose	2.70	60	24.58
Lower Monumental	3.50	106	32.46
Ice Harbor	4.89	157	34.29
McNary	6.44	225	36.27
John Day	7.62	348	46.89
Bonneville	11.13	461	43.49

We observed a difference in the travel rates estimated for kelts migrating through the Snake River versus those observed moving through the Columbia River system (Figure 3). Kelts recorded traveling through the lower Snake River moved at slower rates than kelts moving through the lower Columbia River. Sample sizes were small, and we have not posed causative factors for these observations.

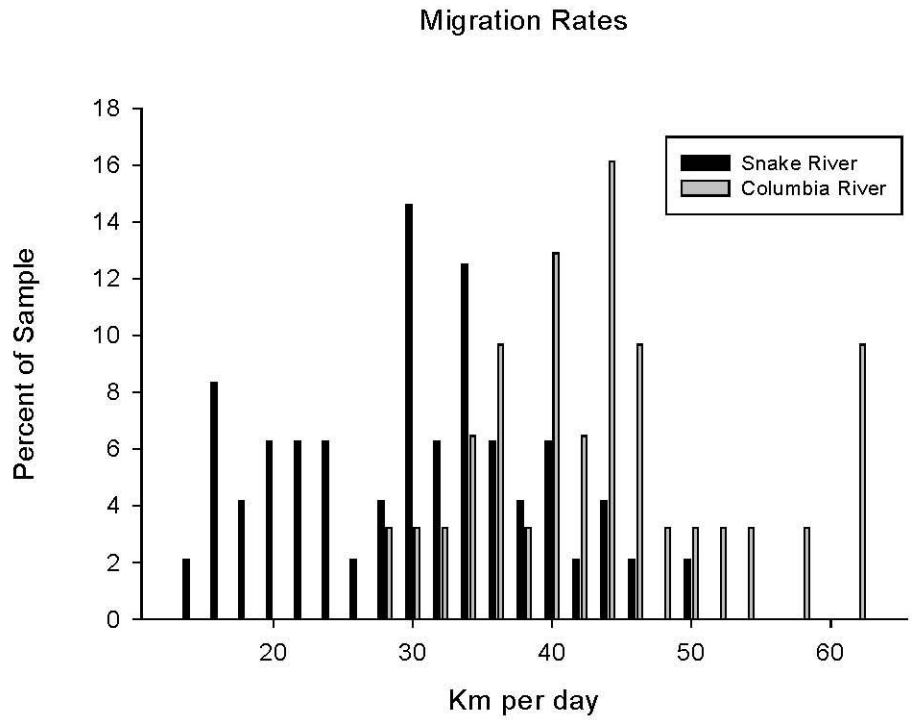

Figure 3. Distribution of individual migration rates for PIT tagged kelts detected at hydro systems, as they migrated downstream through the Snake and Columbia River systems,

We were unable to evaluate the physiology of fish after their release at Lower Granite, or after downstream transportation by barge or truck. Due to the limited size of the holding and recovery facilities at LGD we elected to cancel tagging fish with acoustic tags for downriver release below Bonneville. In addition, we did not locate a suitable site to hold fish for sampling of blood parameters after barge or truck transport. We have made plans to modify and improve substantially the facilities at LGD for 2010. We have worked with the Corp of Engineers and the U of I mechanical staff on feasible designs for improved holding and sorting. We must find a suitable location to hold and process transported fish below Bonneville if we are to evaluate kelt physiology and stress in transported fish. Furthermore, our sampling during this season was limited to treatments on wild or natural fish (with adipose fins) because of our use of MS222 anesthetic, and complications of drug clearance and legal withdrawal periods.

Objective 4. Evaluate the physiology and endocrinology of small groups of kelts held for 2 months or more, and compare results with groups of fish from other sites, and with models from fish migrating through river corridors.

Task 4.1. Determine the series of physiological changes that occur during long term reconditioning in DNFH captive steelhead kelts.

During March through April, we prepared a site at Dworshak National Fish hatchery for four, 15 foot diameter fiberglass tanks. The tanks were shipped in sections and assembled on site. Assembly, plumbing and site preparation took approximately 6 weeks. Plumbing for the tanks was designed and provided by contractors. The water system was connected to a fire hydrant water source.

On April 21, 2009, 50 pre-spawn steelhead trout were collected from the DNFH trap and transported to two of the 15 ft. diameter circular holding tanks. These fish were allowed to recover for approximately 2 weeks, and hand spawning was scheduled on 5 and 6 May. Due to a variety of factors steelhead mortality was high throughout this experiment. These factors include but are not limited to: handling stress, late treatment for fungal infection, flow rate problems in the tanks, poor water quality and gas bubble trauma as well as limitations in fish and tank care. Prior to spawning, 12 steelhead died and were not sampled. The carcasses from these fish were stored frozen for later necropsy. The gas super saturation was created with unplanned releases of water from Dworshak Dam, and pressurization from the pumps used in the fire suppression system that was used for water supply to tanks. Because of the exposure the fish showed signs of fungal infection, and head burns. Personnel from the Idaho Fish Health Center detected gas bubble disease in coho fry on 16 April (Cori Sampson, USFWS personal communication).

On 5 and 6 May 38 steelhead were crowded, moved to an anesthesia tote (100mg/L of MS-222 buffered). The anesthetized fish were measured, sampled for blood, spawned, PIT tagged and returned to recovery tank. Immediately after sampling, eight steelhead died and these mortalities were sampled for liver, spleen, GI tract, and muscle tissues on site. The remaining steelhead recovered in the two holding tanks and were monitored and fed daily. Steelhead kelts were fed 60-120g of krill daily and treated with formalin drips every other day to control fungal growth. Steelhead mortality continued following spawning, until the final steelhead died on 5 June. No blood samples were collected beyond the final spawning date.

All mortalities after spawning were bagged and frozen for later necropsy at the U of I fisheries wetlab. We collected samples of liver and muscle tissues for proximate analysis. We inspected and characterized the condition of internal organs, weighed the spleen, liver, GI tract, and ovaries.

In June we invited Dr. Barnaby Watten, USGS Leetown Science Center, to evaluate the water system of the circular kelt tanks, and recommend technology for improving the rearing system. One recommendation by Dr. Watten to lessen the impact of dissolved gas was the installation of a portable vacuum degasser (Watten et al. 1994). This system had been used successfully in other hatchery systems to remove super saturated gasses in hatchery inflow systems. Dr. Watten shipped a prototype of the degasser to DNFH and we conducted several trials of the portable system on 23- 24 June. In addition, we used a limestone sand packed column as a way to increase the alkalinity of the inflow water to improve buffering capacity and elevate pH. A design for an improved system will be prepared and submitted separately to CRITFC.

Acknowledgements

We thank Dr. Andrew Pierce, CRITFC, for his contributions in sampling design, sampling efforts, and tank assembly for all phases of these studies. We are grateful to the staff from the Nez Perce Tribe and US Fish and Wildlife Service at DNFH for cooperation with sampling of fish and logistics of site preparation for holding tanks; the Corps of Engineers staff at LGD for their support and cooperation with sampling and transporting kelts; and Idaho Department of Fish and Game staff for operating weirs and sample collections in the Clearwater River. Rob Bohn provided invaluable assistance with sampling and care of kelts in the holding tanks at DNFH. Tom Ruehle, NMFS Pasco office, provided the transport tank and truck used in studies. The National Science Foundation provided funding and support for the three Research Experience for Undergraduates (REU) student interns for the summer.

References

Boggs, C.T. and C.A. Peery. 2004. Steelhead (*Oncorhynchus mykiss*) kelt abundance, condition, passage, and survival in the lower Snake and Columbia rivers, 2003.Report to US Army Corps of Engineers, Walla Walla District. Contract No. DACW-02-D-0005.

Branstetter, R., J. S Stephenson, D. R. Hatch, J. Whiteaker, S–Y. Hyun, B. Bosch, D. Fast, J. Blodgett, T. Newsome, L. Hewlett, J. Lovetang, M. Gauvin, R. Dasher, and C. Fisher. 2007. Steelhead Kelt Reconditioning and Reproductive Success. Annual Report for 2007. Project Number: 2007-401-00, Contract Number: 0030769. Bonneville Power Administration. Portland.

Busby, P. J., Wainwright, T. C., Bryant, G. J., Lierheimer, L. J., Waples, R. S., Waknitz, F. W. & Lagomarsino, F. W. 1996. Status review of west coast steelhead from Washington, Idaho, Oregon, and California. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-NWFSC-27. Available at http://www.nwfsc.noaa.gov/publications/techmemos/tm27/tm27.htm

Evans, A.F. 2002. Steelhead (*Oncorhynchus mykiss*) kelt outmigration from lower Granite Dam to Bonneville dam: Abundance, downstream conversion rates, routes of passage, and travel times, 2001. Submitted by CRITFC to U.S. Army Corps of Engineers Walla Walla District for Contract No. DACW68-01-0016.

Evans, A.F., M.S. Fitzpatrick, and L.K. Siddens. 2004. Use of ultrasound imaging and steroid concentrations to identify maturational status in adult steelhead. North American Journal of Fisheries Management 24: 967–978.

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. North American Journal of Fisheries Management 28:1818-1827.

Hatch, D.R., R. Branstetter, and S. Narum. 2003. Evaluation of steelhead (*Oncorhynchus mykis*) kelt outmigration from Lower Granite Dam to Bonneville Dam and test the use of transportation to increase returns of repeat spawners. Submitted by CRITFC to U.S. Army Corps of Engineers Walla Walla District for Contract No. DACW68-00-C-0027.

Keefer, M. L., R. H. Wertheimer, A. F. Evans, C. T. Boggs, and C. A. Peery. 2008. Iteroparity in Columbia River summer-run steelhead (*Oncorhynchus mykiss*): implications for conservation. Canadian Journal of Fisheries and Aquatic Sciences 65: 2592-2605.

Leider, S.A., M.W. Chilcote, and J.J. Loch. 1986. Comparative life history characteristics of hatchery and wild steelhead trout (*Salmo gairdneri*) of summer and winter races in the Kalama River, Washington. Canadian Journal of Fisheries and Aquatic Sciences 43:1398-1409

Narum, S. R., D. Hatch, A. J. Talbot, P. Moran, and M. Powell. 2008. Iteroparity in complex mating systems of steelhead *Oncorhyncus mykiss* (Walbaum). Journal of Fish Biology 72:45-60.

Watten, B. J., W. J. Ridge, and V. Mudrak. 1994. Effect of subatmospheric pressure on the performance of an automated packed-column nitrogen desorption system. Aquaculture Engineering 13:41-58.

Wertheimer, R.H., P.L. Madson, and M.R. Jonas. 2003. Evaluation of Steelhead Kelt Project Abundance, Condition, Passage, and Conversion Rates through Lower Columbia River dams, in 2002. U.S. Army Corps of Engineers, Portland District Fish Field Unit, Bonneville Lock and Dam, Cascade Locks, OR.

Developing Strategies to Improve Survival and Return Recruitment of Steelhead Kelts from Snake River Stocks

Quarterly Report

Contract No C08-22

Purchase Order No C0802240

Prepared by

Christine Moffitt, Principal Investigator

Research Staff:

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Submitted to

Columbia River Inter-Tribal Fish Commission

Doug Hatch, Contract Officer

Idaho Cooperative Fish and Wildlife Research Unit

University of Idaho, Moscow, ID 83844-1141

15 April 2010

Executive Summary

This quarter we completed plans for field studies to be conducted in 2010, and continued our analysis of data collected in 2009. We hired staff to assist with sampling at Lower Granite Dam, and Idaho Weirs. We completed the construction and preparations of new and improved trapping and holding facilities at Lower Granite Dam. We received our permit from Washington Department of Fish and Wildlife for collections at Lower Granite Dam, and received transport permits for Oregon and Washington. CRITF staff provided for us the NOAA letter of determination to cover sampling of stocks at risk at Lower Granite Dam.

Progress by Objective

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.

In 2009, we lethally sampled 124, B-run steelhead from Dworshak National Fish Hatchery at the time of spawning or shortly after spawning and sampled tissues for histology, and proximate analysis. Blood plasma samples were analyzed for various biochemical and hormonal constituents. In 2009, we lethally sampled downstream-migrating hatchery origin kelts for histological assessment, proximate analysis and otoliths at the Lower Granite Juvenile Bypass Facility from May through June. As with the B-run DNFH samples only fish with a known cause and time of death were used for analysis as post-mortem effects can alter body composition. In addition, fish at LGD suspected of a non-anadromous resident life history (stocked Rainbow Trout) were censored from our analyses. We completed our analysis of lipid content of all fish sampled. All somatic muscle lipids were low, but kelts from Lower Granite Dam had significantly lower content proportion of lipids than did those sampled at Dworshak, median 0.19% (Table 1).

Table 1. Summary of results of proximate analysis of somatic muscle samples from below the dorsum from hatchery origin fish sampled at Dworshak National Fish Hatchery and Lower Granite Dam in 2009.

Location	Lipids %	Ash %	Protein %	Water %
		Lower Granite Dam		
Mean	0.31	1.40	15.83	82.46
Median	0.19	1.41	15.82	82.70
Range	0.06-1.47	1.06-1.71	12.55-19.28	78.16-86.18
		Dworshak National Fish Hatchery		
Mean	1.76	1.54	19.57	77.13
Median	1.73	1.44	19.68	77.13
Range	0.43-4.77	1.08-2.60	15.49-24.53	71.56-81.64

In contrast we were able to sample a small number of fall, pre-spawning migrants from the Salmon River in November. The lipid content of the muscle tissues in these fish was significantly higher than either of the samples collected from hatchery origin fish at DNFH or at LGD (Table 2).

Table 2. Summary of results of proximate analysis of somatic muscle samples from below the dorsum from wild caught fish from the Salmon River in November 2009.

Location	Lipids %	Ash %	Protein %	Water %
Mean	3.36	1.98	21.20	73.46
Median	3.34	2.03	21.26	73.42
Range	2.85-4.20	1.63-2.25	20.55-21.79	72.72-73.99

The histological samples from 2009 have been embedded, sectioning, mounted and stained. We have developed a draft approach for scoring for the liver, and the anterior intestine. We will be grading the cellular structure of the liver hepatocytes, and ranking of the extent of vacuolation in field. In the anterior intestine, we will be evaluating the infiltration of eosinophilic monocytes, vacuoles, and size and arrangement of goblet cells. The analysis of the sagittal otoliths revealed a hard central area (aberrancy) in many of these hatchery origin fish, especially the A run fish. We are pursuing this with further analyses of the eoliths.

As part of a collaboration with Idaho Department of Fish and Game, the Nez Perce Tribe, and Washington Department of Fish and Wildlife, we collected blood samples from 28 upstream migrating natural origin fall migrants at Lower Granite Dam in November of 2009. We were also able to collect blood from 30 upstream migrating pre-

spawning wild steelhead in March of 2010. All these fish were PIT tagged and sampled for genetic identify by collaborating scientists. We have evaluated the plasma samples from the fall collection at Lower Granite Dam and as expected several nutritional factors were significantly different from fish sampled as kelts in the spring (Figure 1).

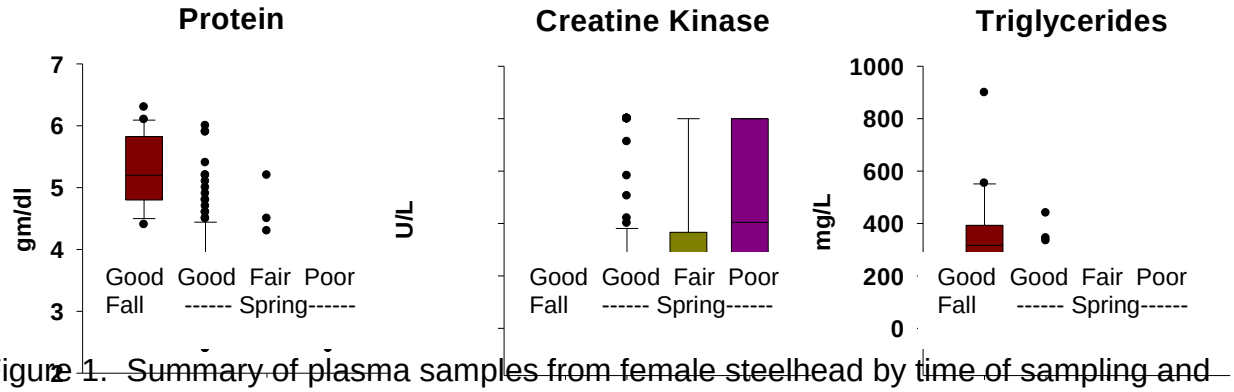

Figure 1. Summary of plasma samples from female steelhead by time of sampling and fish condition. Fall samples (N=20) were from 20 good condition fish, November 2010; Spring samples were from 165 good condition, 41 fair condition, and 23 poor condition female fish sampled May – June 2009.

We have begun to evaluate the effects of time of collection on samples from Lower Granite Dam in 2009. Our models of natural origin fish are developed from non-lethal blood samples, and observations of fish condition. Samples of natural origin fish migrating downstream at the Fish Creek weir showed a wide range of responses of the parameters evaluated, and they were related to fish condition.

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.

This year we planned for increased sampling from weirs in the Clearwater River drainage. Our work with Idaho Fish and Game on the Potlatch River system at four weirs has already been successful in obtaining samples. As of 9 April, we collected blood plasma samples from 31 kelts from the Potlatch River system. These fish are PIT tagged, and exiting the system. All the sampled fish have come from Little Bear Creek, but there are fish above the weir at Big Bear Creek as well. Graduate student Byan Jones started in January, and is working with IDFG biologists 6 days a week to collect these samples and assess fish condition. More fish are expected in this system, as the low flows this spring have allowed for nearly 100% capture efficiency. We were able to train staff from IDFG that will be working at the Fish Creek Weir, but their operations will not begin until the last part of April.

In an effort to define differences between A an B stocks of steelhead, we plotted several blood metrics against fish fork length to determine if there was any relationship with fork length of fish that may be a factor in separating the A and B stocks of steelhead. We still have failed to determine many metrics that are related to size of fish. We identified two measures that were different in samples of good condition fish in 2009 (Figure 2). We plan to test for differences in much larger sample sizes of B-run fish over the entire downstream migration period in 2010.

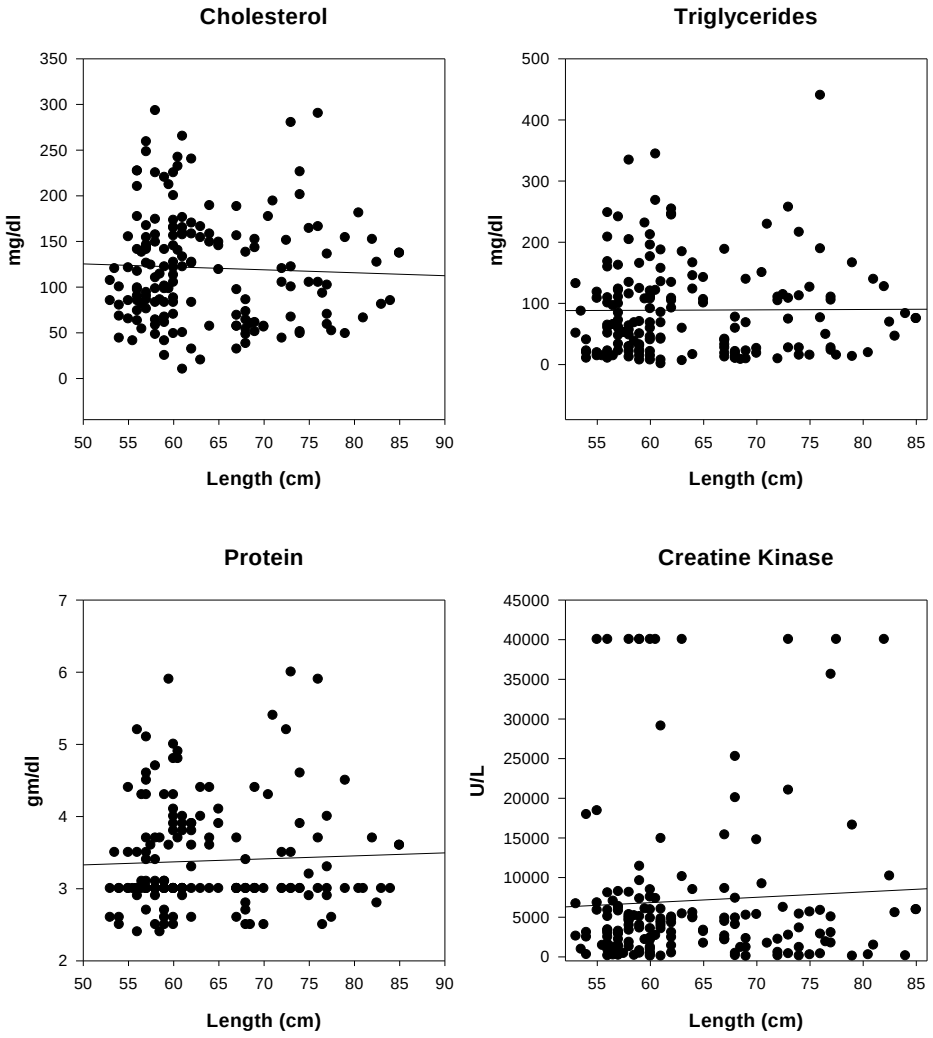

Figure 2. Scatter plot of four plasma metrics versus fork length of kelts at Lower Granite Dam May through June 2009. Linear regression lines are provided for each plot.

We have begun to assess the effects of time of migration on several plasma factors. Four factors were evaluated using good condition female fish sampled in 2009 and few trends are noted (Figure 3).

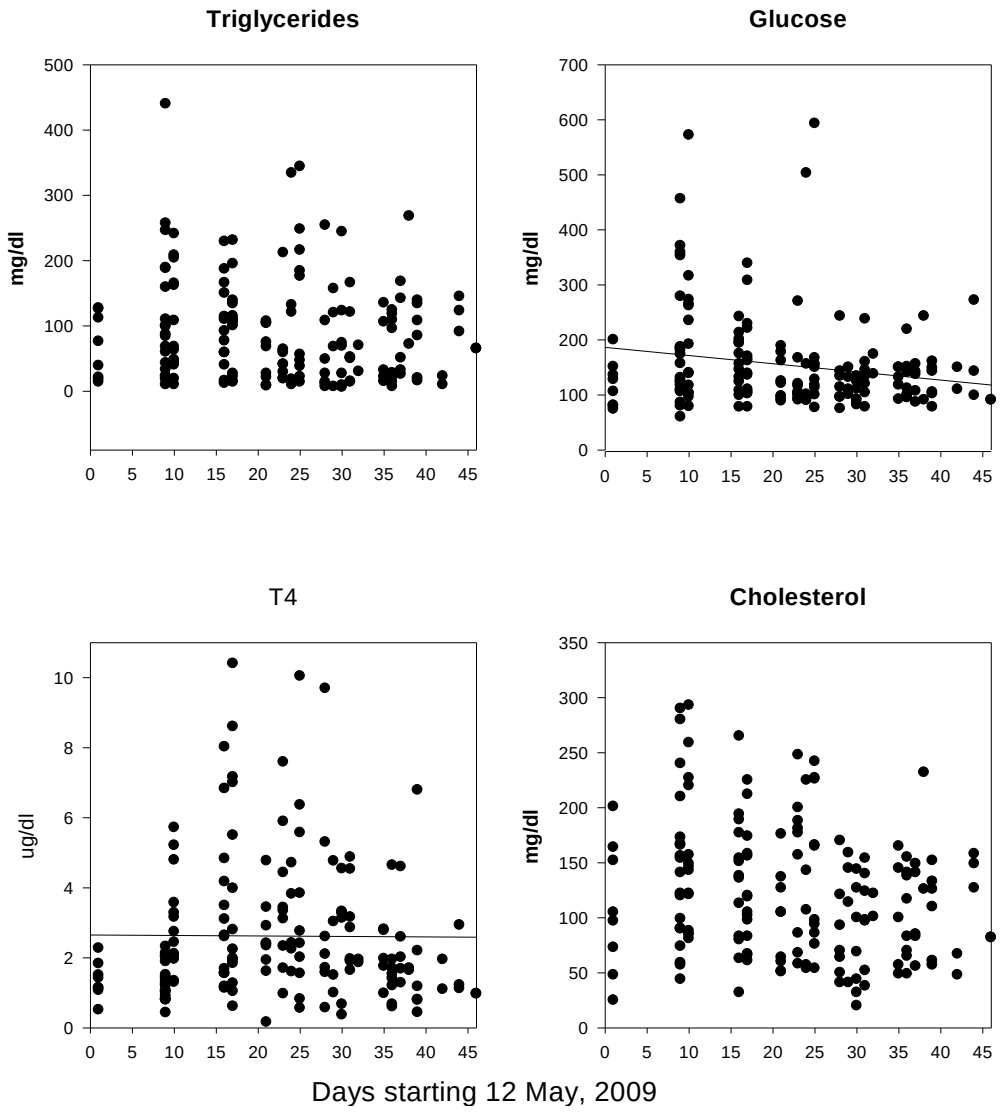

Figure 3. Scatter plots of selected plasma biochemical and hormone parameters from good condition natural origin fish collected from 12 May through 26 June at Lower Granite Dam bypass, 2009.

To provide for adequate facilities for sampling at Lower Granite Dam, the U of Idaho and the U.S. Army Corps of Engineers (COE) staff completed the new sampling and holding facility for kelts at Lower Granite Dam. In February and March, we tested all systems, and made modifications to assure holding and crowding operations will be safe for fish and workers. We attended the COE research coordinating meeting in March, and provided bypass manager Mike Halter necessary completed Hazard Analysis Forms for safety compliance of all our operations from tagging, necropsy, trucking and barge loading. We provided the COE and staff from WDFW copies of all permits and study plans. All U of Idaho workers have completed safety training and have badge identification for access. In March we installed a blower system with air stones in both tanks to provide supplemental air to insure the holding and recovery tank provided life

support systems in case of reduced water flow to the tanks. We installed covers over the tanks, and the COE excavated a loading area at the end of the recovery tank to allow for easier loading of fish from the recovery tank into the hauling tank. The COE will provide a small hauling tank that fits into the bed of the pickup truck to move fish from our tank site to the barge release loading site. This site is fitted with a flexible hose that allows for water delivery of fish into a pipe to load directly into the fish barge hold.

Objective 3. 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.

We have made plans for acoustic tagging of fish to locations below Bonneville Dam. We have set up a tagging and operation system in the work area at the tanks, and have arranged for CRITFC staff to oversee the first applications of acoustic tags. When fish numbers allow, we plan to barge transport 100 kelts per week to release below Bonneville Dam and include acoustic tags with these groups. Our first barges have been delayed due to very low numbers of fish arriving in the juvenile collection and bypass system. We completed a trial run of our small tank trailer (500 gallon) to the Aldrich Point, OR boat ramp, below Bonneville Dam. The trip was very long, and we requested that we switch with the Yakima Tribe to have them haul the large truck there if possible. We have since been in contact with the Yakima Tribe to plan for two loads of PIT-tagged fish (early and mid run fish) to the Aldrich Point boat ramp, with approximately 100 to 150 fish per haul. We will use our small hauling truck and trailer to transport several groups to below Bonneville Dam to the Hamilton Island boat ramp as in 2009.

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

With the new facilities, we are preparing to release into the river larger numbers of PIT tagged natural origin kelts to follow their migration downstream in the Snake and Columbia River system. Tagging began on 1 April. The numbers of fish have been low due to cold weather and increased spill conditions. The U of Idaho staff plans to PIT tag and release up to 250 kelts per day below LGD when numbers increase. Released fish will be tracked as they move down river to the ocean using the PTAGIS database system. These data will be used to relate emigration behavior and physiological condition, flow characteristics, and survival success.

Problems or special needs

We have had to modify our plans for sampling at Lower Granite Dam with the delayed start of tagging and hauling fish. All other aspects of the project are on schedule.

Snake River Long-Term Reconditioning Studies

Andrew Pierce
Columbia River Inter-Tribal Fish Commission

Introduction

The goal of this part of the project is to establish an experimental model for long-term reconditioning of B-run kelt steelhead in the Snake River basin. Most of our effort during 2009 was in project planning, collaboration development, and facilities construction.

Area and Facilities

Kelt reconditioning facilities were constructed at Dworshak National Fish Hatchery (DNFH) in Ahsahka, Idaho. DNFH is located at the confluence of the North Fork and Main stem of the Clearwater River (river kilometer 65). The facility is co-managed by the US Fish and Wildlife Service (USFWS) and the Nez Perce Tribe (NPT). The primary goal of DNFH is to "Conserve and perpetuate the unique North Fork Clearwater River 'B-run' summer steelhead population." DNFH releases 2.11 – 2.21 million B-run steelhead smolts per year (USFWS 2009). The facility also rears Coho salmon (O. kisutch), Chinook salmon (O. tshawytscha) and rainbow trout.

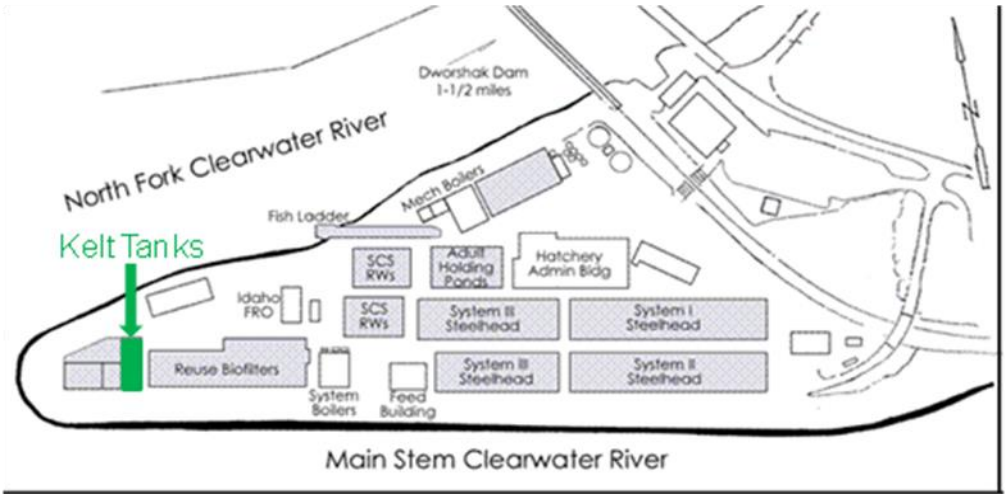

Figure 1: Location of experimental kelt reconditioning tanks at Dworshak National Fish Hatchery. Figure modified from USFWS 2009.

DNFH Kelt Reconditioning Tank Construction

Four 15 foot diameter tanks were installed at DNFH. Water was provided from a fire suppression line at a flow rate of 50 gpm per tank. During Spring 2009 reconditioning trials, kelt tank flow rates varied due to hatchery operations. To allow adjustment to varying flows, a valve and flow meter were installed on the inflow plumbing. Tank outflows were plumbed to the DNFH settling pond. Tanks were provided with both an internal standpipe and an external vented vertical loop to control tanks level.

Figure 2: Experimental kelt reconditioning tanks at Dworshak National Fish Hatchery. (A) Kelt tank overview. (B) Fire hydrant water supply. (C) Inflow control valve. (D) Inflow flow meter.

Tank level was set at 3 ft 6 inches. Covers were installed to prevent fish from jumping out and provide shade. Platforms were installed to facilitate fish feeding and sampling, and a shed was constructed for storage of equipment and supplies. An air blower and aeration system were installed.

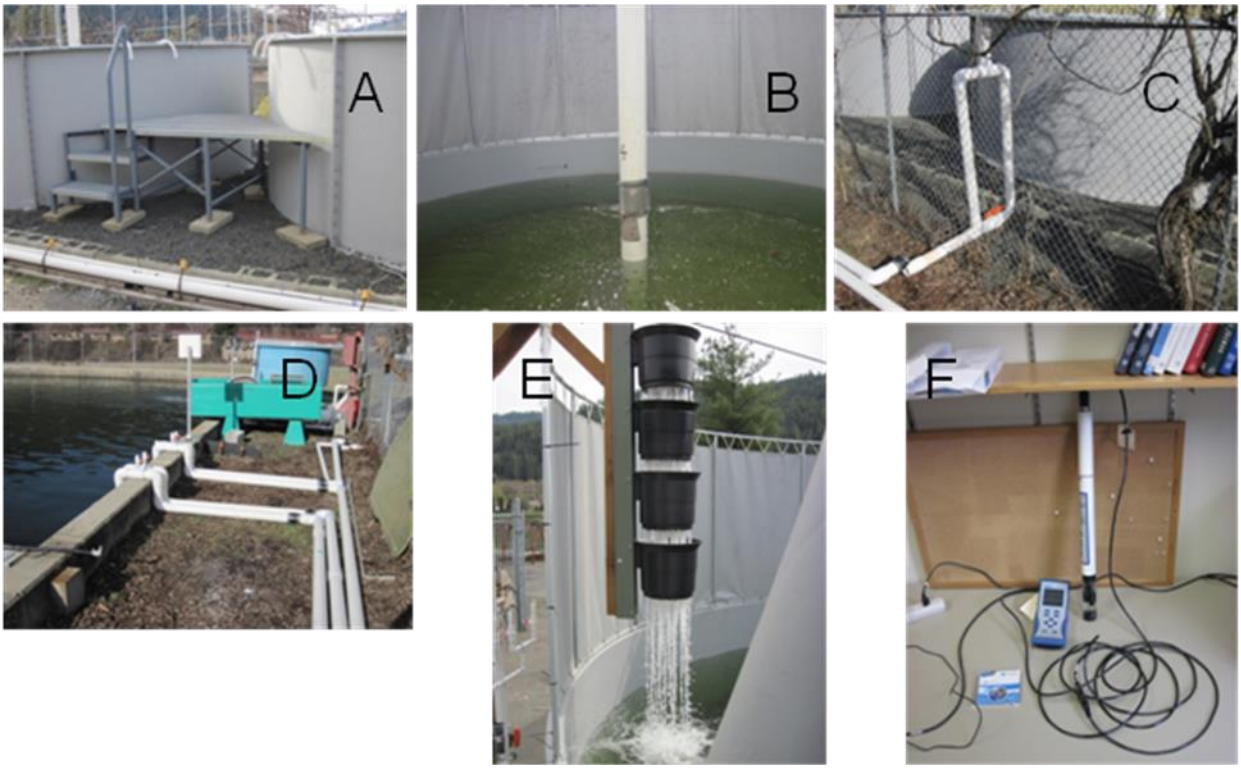

Figure 3: Experimental kelt reconditioning tanks at Dworshak National Fish Hatchery. (A) Platform. (B) Tank in operation showing standpipe. (C) Outflow loop. (D) Discharge to settling pond. (E) Tank in operation showing degassing column and cover. (F) Hach Hydrolab water quality monitoring system.

During Spring 2009 reconditioning trials, kelt mortalities displayed signs of gas bubble disease, including overinflated swim bladders and hemorrhaging along the lateral line and operculae. Levels of gas supersaturation in kelt tanks were not monitored during Spring 2009. To prepare for 2010 reconditioning trials, a packed column degassing system was installed on the inflow to kelt tanks. A Hach Hydrolab water quality monitoring system was purchased and installed. With this setup, gas supersaturation levels in kelt tanks are controlled to less than 20 mmHg under the inflow gas conditions encountered so far (Fig 4). This meets recognized standards for sensitive animals (Colt 2000).

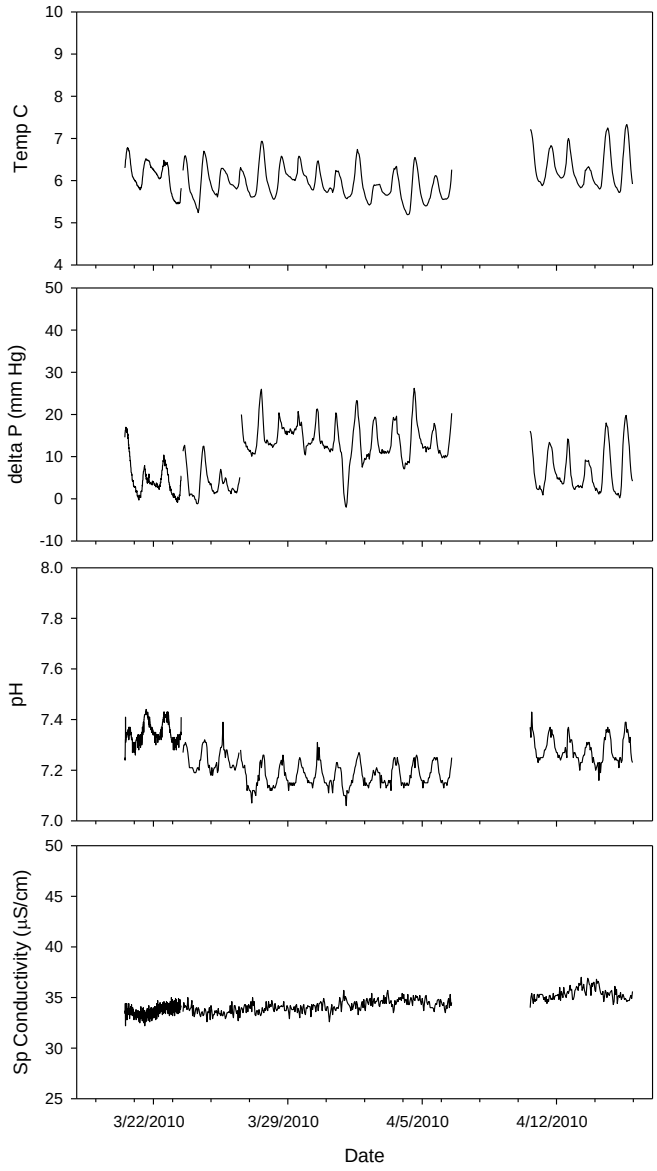

Figure 4: Water quality parameters measured in DNFH kelt tanks. The probe was moved to a tank without a degassing column from 3/26/10-4/06/10.

Kelt Source for Experimental Reconditioning at DNFH.

Strategies for obtaining B-run steelhead kelts for the pilot scale reconditioning program were discussed extensively with collaborators. We intended to stock our four tanks with 25 female kelts each for a total of 100 fish. We first planned to non-lethally spawn female hatchery origin fish returning to the ladder at DNFH, transfer them to our tanks, and attempt reconditioning. However, the disposition of the eggs from the fish needed to meet the policy requirements and operational restrictions of both of the co-managers of DNFH. NPT policy requires that a conservation or enhancement use be a use is made of all viable gametes harvested. Based on fecundity estimates for DNFH fish, this

meant that rearing space for approximately 600,000 eggs to at least the fry stage would need to be found. DNFH production was not willing to include eggs harvested by non-lethally spawning female steelhead into hatchery production, due to concerns regarding egg quality. Our pilot scale project did not have the personnel or other resources to rear a large number of juvenile fish. Due to these issues, we were not able to obtain fish at DNFH for our studies. As an alternative, we proposed to collect female hatchery origin B-run kelts at Lower Granite Dam (LGR). This proposal was accepted by the DNFH co-managers. We initiated a collaboration with the NPT and the University of Idaho for the collection of fish and LGR and transport to DNFH.

Kelt Reconditioning Physiology Studies

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.

Post-Spawning 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.

Hagerman

We began an experiment in collaboration with Professor Ron Hardy, Director of the Aquaculture Research Institute (ARI) in Hagerman, ID, to examine the physiology and endocrinology of post-spawning rainbow trout. On 1/14/10, 30 post-spawning female fish were PIT tagged and sampled for blood. Beginning 1/28/10 and at four week intervals thereafter, these fish are scheduled to be sampled for blood. Five additional fish are scheduled to be killed at each sampling point and tissues (pituitary, liver, ovary, and digestive tract) taken for gene expression assays.

Moscow

We began an experiment at the ARI fish holding facility at the University of Idaho in Moscow, ID, using 150 3-year-old post spawning female rainbow trout obtained from Troutlodge in Sumner, WA. These fish had been fasted for approximately 1 month prior to spawning, and were all hand stripped on 2/23/10 (S. Nepper, Troutlodge, personal communication). Fish were transported to Moscow on 2/25/10 and divided into 6 tanks in a recirculating system. Fish were initially sampled and PIT tagged on 3/4/10 and

3/5/10. After the initial sampling, tanks were assigned one of two treatment groups (3 replicate tanks per treatment): one group fed a control level of feed (0.5% of fish body mass per day), and a second group fed restricted ration (0.1% fbm/d). The goal of these treatments is to produce one group of fish that carries out a normal reproductive cycle, in which energy (body lipid content) is not a limiting factor, and a second group of fish that is energy restricted. Fish are scheduled to be sampled every four weeks, with all fish non-lethally sampled for blood and muscle lipid levels, and 5 fish per treatment killed for tissues at each sampling.

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 River 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. In addition to samples taken from kelts in reconditioning programs, blood samples were obtained from hatchery steelhead at spawning at DNFH, and from downstream migrating kelts at LGR.

Table 1. Blood samples taken from kelts in 2009 and early 2010.

Location	Dates	Event	Blood Samples
Prosser	March-June 2009	Intake	496
Prosser	06/22/09	Sampling	31
Prosser	08/02/09	Sampling	32
Prosser	10/29/09	Release	134
Prosser	March-April 2010	Intake	~800
Parkdale	6/22/09	Sampling	16
Parkdale	9/23/09	Sampling	23
Parkdale	12/16/09	Sampling	16
Parkdale	Feb 2010	Intake	3
Parkdale	Mar 2010	Intake	14
Cassimar	6/23/09	Sampling	1
Dworshak	Feb-April 2009	Spawning	99
LGR	April-June 2009	Migration	308

During the 2010 season, we hope to obtain blood samples from kelts being reconditioned at Dworshak at six week intervals, and samples from a subset of kelts being reconditioned at Prosser at similar intervals. We will continue blood sampling

kelts at Parkdale and Cassimar Bar. In addition, we will obtain non-lethal muscle lipid measurements with the Distell Fish Fatmeter from as many kelts as possible at intake at Prosser, and during our reconditioning study at Dworshak.

METHODS

Plasma Hormone Level Assays

Fish plasma levels of vitellogenin and estradiol-17β (E2) are indicators of reproductive development, Plasma vitellogenin concentrations will be assayed using a rainbow trout vitellogenin ELISA kit (Biosense, Cayman Chemical, Ann Arbor, MI). Plasma samples will be appropriately diluted and triplicate technical replicates assayed in the ELISA according to the manufacturer's instruction manual provided with the kit. Plasma E2 concentrations will be assayed by radioimmunoassay using a commercially available kit (Coat-A-Count Estradiol, Diagnostic Products, Los Angeles, CA) at the Center for Reproductive Biology Assay Core Laboratory (Department of Animal Sciences, Washington State University, Pullman, WA). Plasma samples will be solvent extracted twice with diethyl ether before use in the RIA protocol. Fish plasma levels of insulin-like growth factor-I (IGF-I) and growth hormone (GH) are indicators of metabolic status and growth rate. Radioimmunoassays for salmonid plasma IGF-I and GH will be established using commercially available components from Novozymes GroPep, Inc., according to published methods (Shimizu, et al. 2000; Peterson, et al. 2003). We have established a collaboration with Prof. Larry Riley at the University of California, Fresno to measure plasma levels of ghrelin, an appetite regulating hormone. We will establish assays for free and total cortisol using size exclusion spin columns according to published methods (Barry et al. 2001).

Tissue Gene Expression Assays

We will establish assays for measuring RNA transcript abundance in tissue samples using quantitative real-time PCR (Nagler et al. 2000; Pierce et al. 2004). Assays will be established for the following transcripts: growth hormone, somatolactin, IGF-I, IGF-II, GH receptor, somatolactin receptor, vitellogenins, estrogen receptors, ghrelin, leptin, kisspeptins, gonadotropin releasing hormone, and several reference genes: elongation factor 1 alpha and acidic ribosomal phosphoprotein P0.

Distell Fish Fatmeter

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 and 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 and Shearer, 2001; Crossin and Hinch 2005).

RESULTS/DISCUSSION

Experiments on kelt endocrinology and physiology are ongoing. Data on plasma hormone levels and tissue gene expression levels are not yet available. However, some preliminary data on muscle lipid levels measured with the Distell Fish Fatmeter can be presented.

Muscle Lipid Levels

Fatmeter readings were taken on Prosser kelts at release on 10/29/09. Individual specific growth rates (SGR) in weight from intake to release were calculated by the method of Ricker (1979): SGR = 100 * (ln W2 – ln W1)/ days reconditioned, where W2 and W1 are weights in grams at release and intake, respectively. It was not possible to calculate SGRs for all fish due to PIT tag losses. SGRs over the reconditioning period were compared with muscle lipid content measured with the Fatmeter at release using linear regression (Fig. 5). Significant positive correlations were found between SGR and muscle lipid for both female and male fish (females $n=93$, $p<0.0001$, $r^2=0.3602$; males $n=10$, $p=0.0231$, $r^2=0.4955$). This provides initial validation of the Fatmeter as a potentially useful tool for assessing energetic status in kelts. Additional studies using lethal sampling and biochemical measurement of muscle lipid stores are underway. Further, the correlation suggests that fish that grow faster during reconditioning experience greater increases in muscle lipid content.

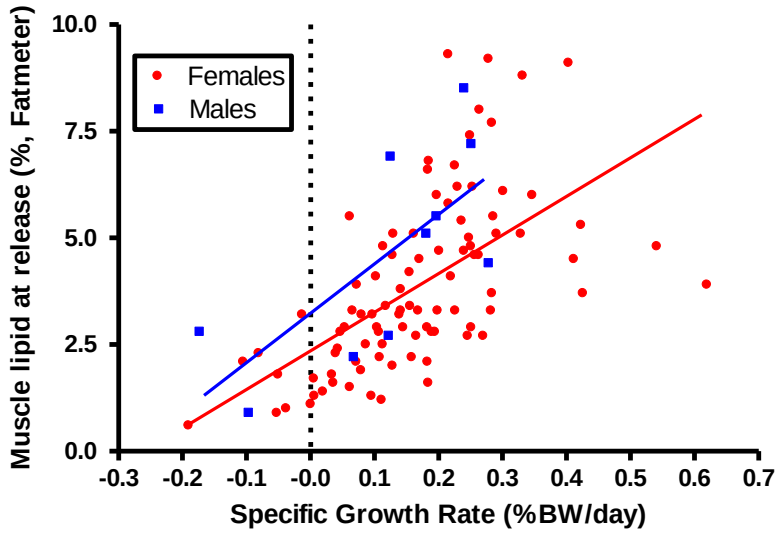

Figure 5: Relationship between individual fish muscle lipid levels measured with the Distell Fish Fatmeter in Prosser kelts at release (10/29/09), and specific growth rate in weight from intake to release.

Muscle lipid content measured with the Fatmeter was compared between post spawning rainbow trout sampled in Moscow on 3/4/10 and 3/5/10, and steelhead kelts taken in to the reconditioning program at Prosser during the early part of the 2010 season (Fig. 6). Muscle lipid levels in kelts were not significantly lower than those in post-spawning rainbow trout. The post-spawning rainbow trout used in this study were fasted for approximately 1 month before sampling, whereas the Prosser kelts had presumably fasted since entering freshwater in July- September. One interpretation of this result is that steelhead reserve a larger proportion of muscle lipid stores for the energetic demands of migration, whereas domesticated rainbow trout mobilize nearly all muscle lipid stores to support ovarian development. This suggests that, at least in terms of lipid stores, post spawning rainbow trout are a valid experimental model for studying kelt reconditioning. In rainbow trout aquaculture broodstock programs, survival to repeat spawning is in the 70-80% range (S. Williams, Moscow ARI, personal communication). Under ideal conditions, similar survival may be possible in kelt reconditioning programs.

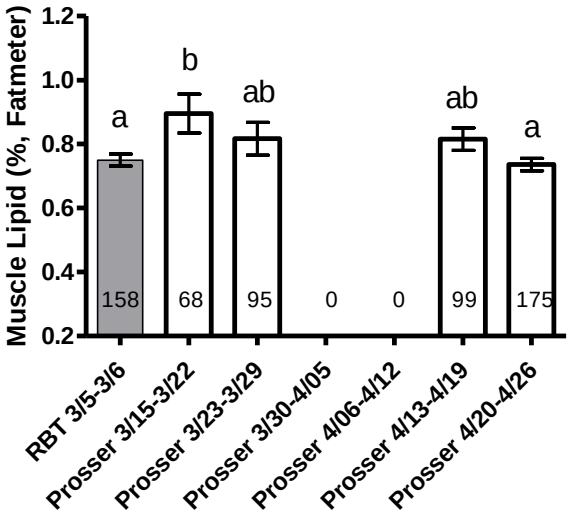

Figure 6: Muscle lipid levels measured with the Distell Fish Fatmeter in post spawning Troutlodge rainbow trout (March 4 and 5 2010) and Prosser kelts at intake in 2010. Bars sharing a superscript letter do not differ significantly (Bonferroni multiple comparison test). The sample number is indicated inside each bar. The Fatmeter was at Lower Granite Dam from 3/30-4/12.

References

Barry T.P., Unwin M.J., Malison J.A., and T.P. Quinn. 2001. Free and total cortisol levels in semelparous and iteroparous chinook salmon. Journal of Fish Biology 59**:** 1673-1676.

Brannon E.L., Powell M.S., Quinn T.P., and A.J. Talbot. 2004. Population Structure of Columbia River Basin Chinook Salmon and Steelhead Trout. Reviews in Fisheries Science 12: 99–232.

Colt J. 2000. Gas Bubble Disease. In: Encyclopedia of Aquaculture, R. Stickney, ed. R. Wiley and Sons, New York, 2000, pp 403-408.

Colt J., and K.D. Shearer. 2001. Evaluation of the Use of the Torry Fish Fatmeter to Non-Lethally estimate Lipid in Adult Salmon. Report of Research to the U.S. Army Corps of Engineers, Portland Dist., Contract Report W66QKZ00805700.

Crossin G.T. and S.G. Hinch. 2005. A nonlethal, rapid method for assessing the somatic energy content of migrating adult Pacific salmon. Transactions of the American Fisheries Society 134: 184-191.

Nagler J.J., Krisfalusi M., and D.G. Cyr. 2000. Quantification of rainbow trout (Oncorhynchus mykiss) estrogen receptor-alpha messenger RNA and its expression in the ovary during the reproductive cycle. Journal of Molecular Endocrinology 25: 243-251.

Peterson B.C., Simpson P.R., Cain K.D., Hardy R.H., Schelling G.T., and T.L. Ott. 2003. Effects of administration of somatostatin-14 and immunoneutralization of somatostatin on endocrine and growth responses in rainbow trout. Journal of Fish Biology 63: 506–522.

Pierce A.L., Dickey J.T., Larsen D.L., Fukada H., Swanson P., and W.W. Dickhoff. 2004. A quantitative real-time RT-PCR assay for salmon IGF-I expression, and its application in the study of GH regulation of IGF-I expression in primary culture of salmon hepatocytes. General and Comparative Endocrinology 135: 401-411.

Ricker, W.E. 1979. Growth rates and models In: Ricker, W.E., Hoar, W.S., Randall, D.J., Brett, J.R. Eds.., Fish Physiology, vol. 8. Bioenergetics and Growth. Academic Press, New York, pp. 738-743.

Shimizu M., Swanson P., Fukada H., Hara A., and W.W. Dickhoff 2000. Comparison of extraction methods and assay validation for salmon insulin-like growth factor-I using commercially available components. General and Comparative Endocrinology 119: 26-36.

U.S. Fish and Wildlife Service (USFWS). 2009. Dworshak, Kooskia, and Hagerman National Fish Hatcheries: Assessments and Recommendations Appendix B: Briefing Document; Summary of Background Information. Final Report, June 2009. Hatchery Review Team, Pacific Region. U.S. Fish and Wildlife Service, Portland, Oregon. Available at: http://www.fws.gov/Pacific/fisheries/Hatcheryreview/reports.html

NPT Steelhead Kelt Reconditioning Project

PROGRESS REPORT

Period Covered: October 2009 to April 2010

Scott Everett
Nez Perce Tribe Department of Fisheries Resources Management

I) MILESTONES/ACCOMPLISHMENTS

- Hired NPT Steelhead Kelt Coordinator to facilitate Master Plan development
- Secured transport permits for Snake River Steelhead Kelt transfers
- Completed Section 12 of DNFH HGMP covering Steelhead Kelt research activities
- Developed expanded scope SOW for FY 2011 contract

II) WHAT WE HAVE DETERMINED SO FAR

Information from numerous on-going projects will contribute to the development of this plan. A review of these projects was compiled in the Kelt Management Plan Drafted by BPA. The preliminary results of those projects are summarized as below.

OPERATIONAL STRATEGIES

1) Enhanced In-River Migration

Dam passage of kelts was predominantly via spillways and surface flow routes

2) Collection and Transportation

Collection, transport and release capabilities have been establish. Transported fish do survive and return rates have been measured.

KELT RECONDITIONING STRATEGIES

1) In-River and Transport Only Strategies

Much variation has been documented the success rates of in-river group versus transported groups between locations and also between years at the same location.

2) Short-Term Treatment

The returns rates of short-term reconditioned kelts have been higher than the transport only group, and the in-river migrants.

3) Long-Term Treatment

Long-term reconditioning has shown to increase the survival of steelhead kelts when compared to the expected survival rates of non-reconditioned kelts.

III) ISSUES OF CONCERN

AVAILABILITY OF DATA AND TIME FAME

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, collection sites, transport, and reconditioning

Stocking
Site selection, release dates, fish 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.

IV) MASTER PLAN UPDATE

MASTER PLAN PROGRESS
Approximately 30% 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.

CURRENT MASTER PLAN OUTLINE
CHAPTER 1: INTRODUCTION

The Purpose of the Master Plan
Management Decision Process
Goals and Objectives
Need for Action
Ecological Significance of Iteroparity
Project History
Relationship to Other Plans, Programs, and Projects in the Region

CHAPTER 2: STATUS OF SNAKE RIVER STEELHEAD

Stock Abundance and Distribution
Life History Diversity
Reproduction and Life Stage Survival

Supplementation and Exploitation

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 4: PROPOSED MANAGEMENT ACTIONS

Development of Alternative Management Actions
Alternative Action Assessment
Description of the Criteria Used to Evaluate Actions
Evaluation of Management Actions Using Established Criteria
Management Action Implementation
Indicators of Success and Failure
Harvest Management

CHAPTER 5: LIMITING FACTORS

Harvest
Hatcheries
Mainstem Snake and Columbia River Hydrosystem
Habitat

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

V) COORDINATION AND MEETINGS

- Clearwater Pre-AOP Meeting, DNFH - Dec 9, 2009
- Dworshak NFH Steelhead Pre-Spawning Coordination, DNFH – Dec. 10, 2009

- University of Idaho Kelt Research Presentation (Zach Penney), Moscow – Mar. 1, 2010
- Research Division Project Leaders Meeting, Lapwai – Feb. 3, 2010
- Corps of Engineers Staff Kelt Operations Meeting, LGR – Feb. 16, 2010
- Clearwater AOP Meeting, DNFH – Feb. 17, 2010
- Kelt Pre-season Activities Coordination Meeting, Yakima - March 9th
- ISAB meeting preparation, Portland - Mar. 15, 2010
- Snake River fall Chinook Coordination Meeting, Lewiston – Mar. 17, 2010
- All Agency Research Meeting, LGR – Mar. 18, 2010
- DNFH Steelhead Kelt Operations Meeting, DNFH – Apr. 12, 2010
- ISAB meeting, Portland – Apr. 2, 2010

In addition to the above meetings, we have conducted monthly coordination conference calls/meetings.

VI)FUTURE PLANNED ACTIVITIES

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

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

Continue the development of necessary M&E components.

Initiate preliminary design documents and appropriate environmental analysis.

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.

Appendix A :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	Transportation (or treatment) Benefit relative to Bonneville natural
											1.66	
In-river	2005	Prosser	67	67				3	4.48	1.19	2.70	25.93
In-river	2006	Prosser	52	52				1	1.92	0.51	1.16	3.12
In-river	2007	Prosser	53	53				3	5.66	1.50	3.41	9.31
In-river	2008	Prosser	88	88				4	4.55	1.20	2.74	6.66
In-river	2009	Prosser	58	58				1	1.72	0.46	1.04	3.88
Total and weighted mean			***318***	***318***				***2.58***	***3.77***	***1.00***	***2.27***	***7.05***
In-river	2002	Lower Granite*	1209	1209				8	0.66	1.01	0.40	
In-river	2003	Lower Granite*	865	865				3	0.35	0.53	0.21	
In-river	2004	Lower Granite*	1138	1138				10	0.88	1.34	0.53	1.52
Total and weighted mean			***3212***	***3212***				***7.36***	***0.65***	***1.00***	***0.39***	***1.22***
In-river	2002	John Day*	***287***	***287***				***28***	***9.76***	***1.00***	***5.88***	***18.22***
Total and weighted mean												
Transported	2002	Lower Granite*	750	750				19	2.53	3.83	1.53	
Transported	2003	Lower Granite*	376	376				3	0.80	2.30	0.48	
Transported	2004	Lower Granite*	982	982				7	0.71	0.81	0.43	2.04
Total and weighted mean			***2108***	***2108***				***10.56***	***1.38***	***2.31***	***0.83***	***2.57***
Transported	2002	John Day*	***271***	***271***				***34***	***12.55***	***1.29***	***7.56***	***23.43***
Total and weighted mean												
Transported (unfed)	2004	Prosser	75	63	15/28	53.57		5	6.67		4.02	19.10
Transported (unfed)	2005	Prosser	98	96	14/57	24.56		1	1.02	0.23	0.61	5.91
Transported (unfed)	2006	Prosser	55	49	31/49	63.27		2	3.64	1.89	2.19	5.89
Transported (unfed)	2007	Prosser	43	38	14/35	40.00		0	0.00	0.00	0.00	0.00
Transported (unfed)	2008	Prosser	100	100	26/49	53.06		3	3.00	0.66	1.81	4.40
Total and weighted mean			***371***	***346***		***46.89***		***2.38***	***2.96***	***0.79***	***1.79***	***5.54***
Transported (fed)	2002	Prosser	479	334				43	8.98		5.41	
Transported (fed)	2003	Prosser	208	187				8	3.85		2.32	
Transported (fed)	2004	Prosser	105	83	11/26	42.31		5	4.76		2.87	13.64
Transported (fed)	2005	Prosser	106	96	6/56	10.71		0	0.00	0.00	0.00	0.00
Transported (fed)	2006	Prosser	56	50	32/50	64.00		0	0.00	0.00	0.00	0.00

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	Transportation (or treatment) Benefit relative to Bonneville natural
Transported (fed)	2007	Prosser	40	38		19/27	70.37	1	2.50	0.44	1.51	4.11
Transported (fed)	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***	***1.54***	***3.50***	***10.84***
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					15.92	16.54	61.74
Total and weighted mean			***4696***	***1780***	***37.90***					***10.04***	***22.83***	***70.78***
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***	***9.83***
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
Total and weighted mean			***136***	***24***	***17.65***						***10.63***	***32.95***
Long-term	2006	Parkdale	1	1.0	100.00						60.24	579.00
Long-term	2007	Parkdale	15	1.0	6.67						4.02	10.81
Long-term	2008	Parkdale	14	7	50.00						30.12	82.21
Long-term	2009	Parkdale	12	4	33.33						20.08	48.87
Total and weighted mean			***42***	***13.0***	***30.95***						***18.65***	***57.80***
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					

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	Transportation (or treatment) Benefit relative to Bonneville natural
Natural repeat	2008	Bonneville Dam	2639					18	0.68			
Natural repeat	2009	Bonneville Dam	2474					11	0.44			
									0.54			

* 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. North American Journal of Fish Management 28:1818-1827.

Appendix B. Lower Columbia River Array Locations

Figure 1: Lower Columbia Acoustic Receiver Arrays 2009

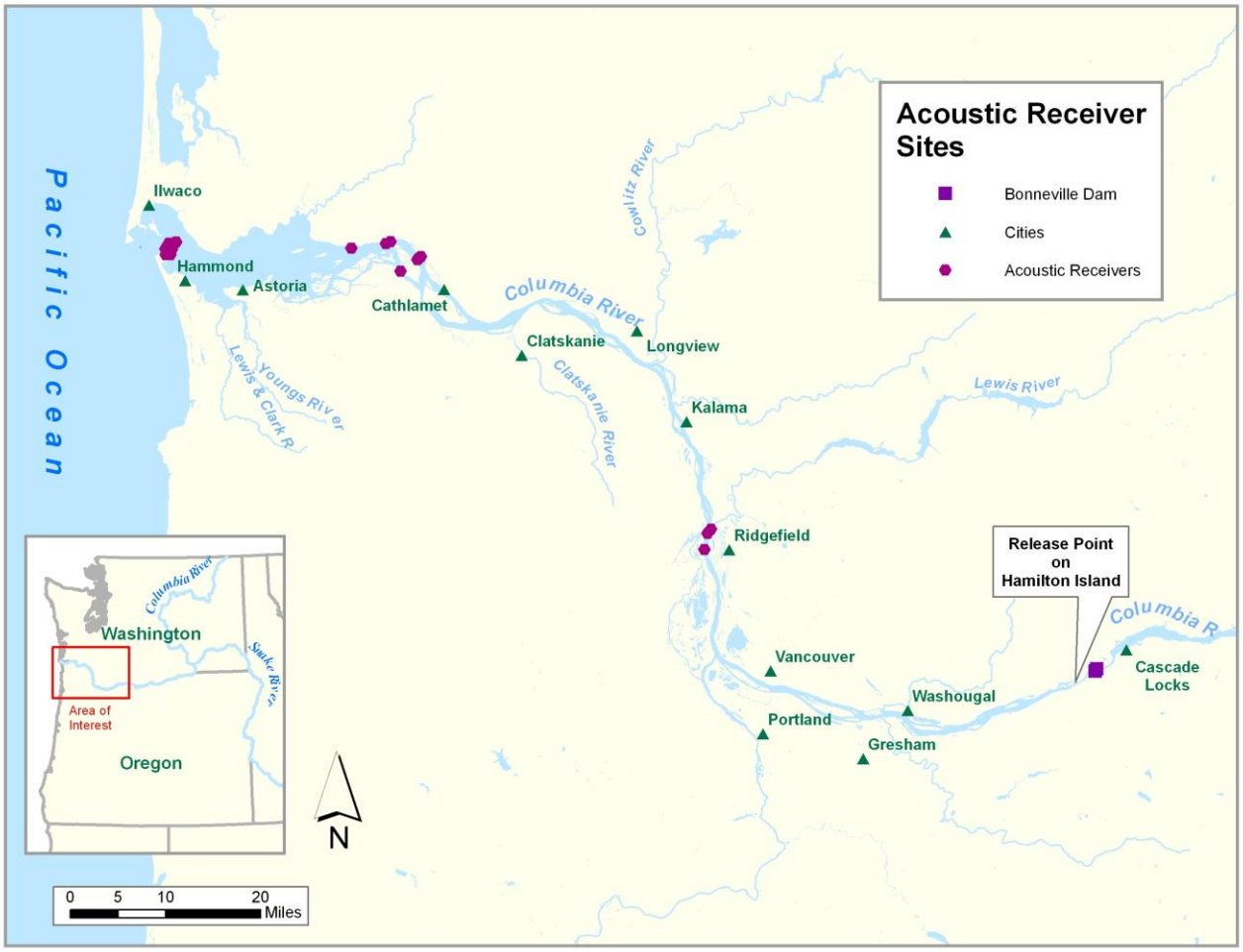

Figure 2. St Helens Array (Rkm 138) 2009.

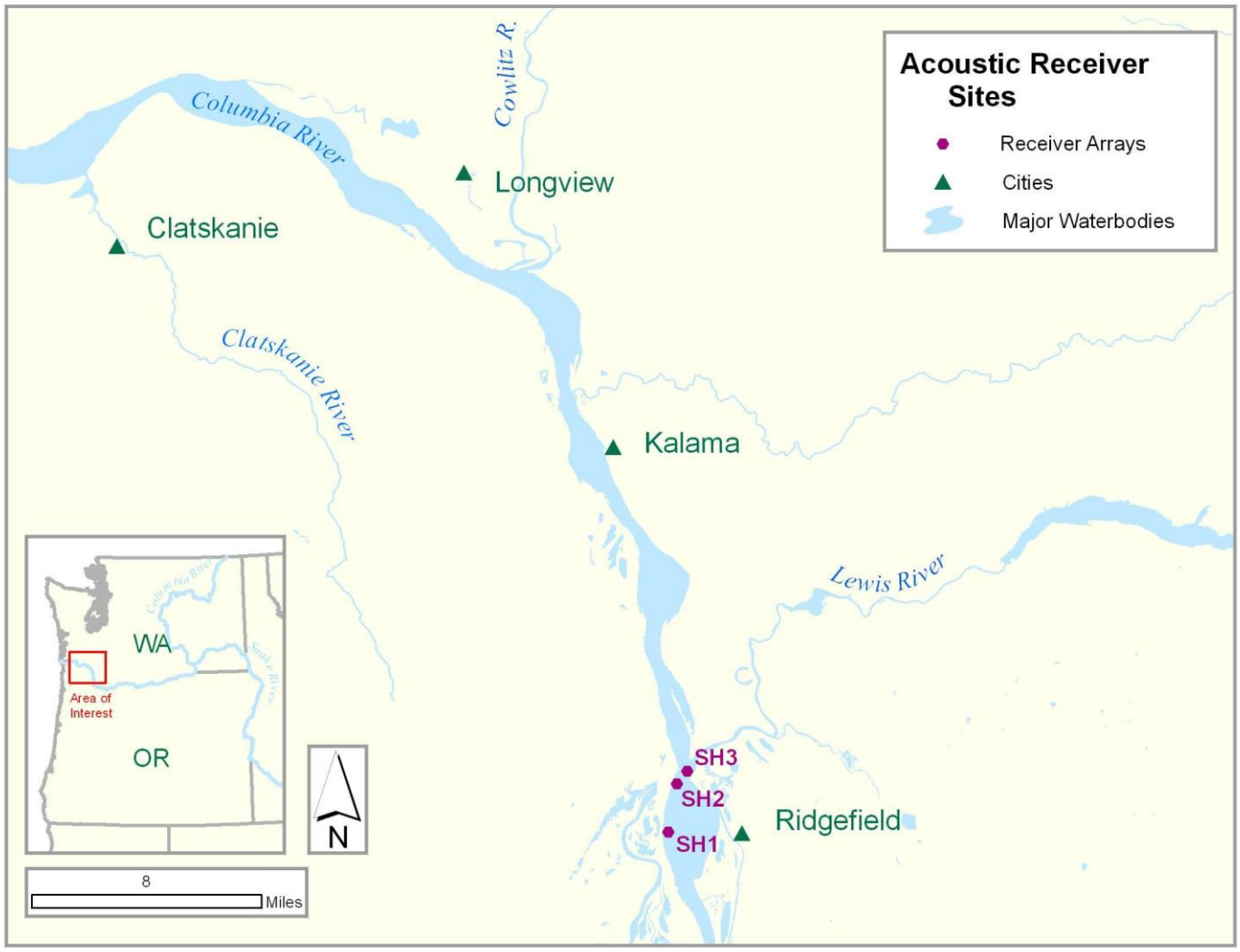

Figure 3: Estuary Acoustic Array Sets (Mouth of the Columbia RM 0 and Estuary Rkm 45).