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Steelhead Kelt Reconditioning and Reproductive Success 2011 Annual Report

Doug Hatch, Ryan Branstetter, Jeff Stephenson, Andrew Pierce, John Whiteaker, and Bill Bosch

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Columbia River Inter-Tribal Fish Commission
503.238.0667
www.critfc.org
729 NE Oregon, Suite 200
Portland, OR 97232

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2011 Annual Report

Edited by:
Douglas R. Hatch
Ryan Branstetter
Jeff Stephenson
Andrew Pierce Ph. D.
John Whiteaker
and
Bill Bosch

Columbia River Inter-Tribal Fish Commission
729 NE Oregon Street, Suite 200
Portland, OR 97232

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Portland, OR 97283-3621
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Executive Summary

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

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

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

scale samples (Seamons and Quinn 2010). In Hood River, repeat spawning summer run steelhead comprise on average 5.7% of the run based on scale pattern analysis (Olsen 2008). Iteroparity rates for Klickitat River steelhead were reported at 3.3% from 1979 to 1981 (Howell et al. 1984). Summer steelhead in the South Fork Walla Walla River have expressed 2% to 9% iteroparity rates (J. Gourmand, ODFW, pers. comm.). Hockersmith et al. (1995) reported that repeat spawners composed 1.6% of the Yakima River wild run and recent tagging data shows average return rates to Bonneville Dam of 3.77

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

This report is divided into 3 chapters:

- **Chapter 1:** Management Scenario Evaluation: Describes the evaluation of various management strategies that could be used as tactics for steelhead restoration programs. Reconditioning post-spawned fish (kelts) in a captive environment to encourage reinitiating feeding, growth, and redevelopment of gonads is evaluated in this study as an approach to restore depressed steelhead populations. To test the efficacy of utilizing steelhead kelt as a management and recovery tool, different scenarios were investigated ranging from little intervention (collect and return fish to river) to high intensity (collect and feed fish in captivity until rematuration).

Transport experiments used Yakima and Snake river kelt steelhead collected near Prosser and Lower Granite dams. The steelhead were transported and released at Hamilton Island near Bonneville Dam (Rkm 233) and near Westport, OR (Rkm 56). Yakima River origin kelt steelhead survival to the ocean was 34% and 37%, for releases at Hamilton Island and Westport, respectively. Snake River origin kelt steelhead survival to the ocean was 7% and 32% for releases at Hamilton Island and Westport, respectively. Mortality in the estuary reach was high for all release groups. Based on return rates to Bonneville Dam of transported kelt steelhead, there are only marginal benefits over leaving the kelts in the river.

Long-term reconditioning survival at Prosser Hatchery, Omak Creek, and Parkdale Hatchery was 33%, 20%, and 22%, respectively. Survival was slightly below the long term average for each location. Long-term reconditioning experiments were also conducted at Dworshak National Fish Hatchery using Snake River origin steelhead. Long-term reconditioning appears to provide a substantial benefit over leaving the kelts in the river. Prosser steelhead had a survival benefit 16.5 times higher than fish left in the Yakima River.

- **Chapter 2:** Reproductive Success Evaluation: Evaluates progeny and gamete viability of maiden and repeat spawner steelhead in the Hood River and field studies of reproductive success of reconditioned steelhead in Omak Creek and the Yakima River. We evaluated fecundity, fertilization rates, and fry growth (length and weight) of maiden and repeat spawners and in general found no statistical difference in these parameters.

  An estimated132 steelhead spawned in Omak Creek in 2010. Successful reproduction has been confirmed for four of the nine reconditioned kelts that were detected returning to Omak Creek. For this year progeny from only 1 of 5 kelts were detected. In the Yakima River, reproduction by reconditioned kelts was confirmed for four adults released in 2010. While this number is lower than expected, statistical power is lacking, and expected contributions were likely confounded by collection methods. The highest percent of expected rate of assignment was seen in kelts collected in 2011 (116.0% of expected). For age-0 samples collected in 2011, these collections represent adults that putatively spawned for the first time. As these collections are comprised of post-spawn adults, they are thought to have successfully completed spawning prior to capture. While reproductive success has been confirmed for reconditioned kelts, we are currently unable to calculate relative reproductive success estimates. The small number of samples that are being successfully assigned limits statistical power to compare reproductive success among other groups such as first time spawners. Increasing the proportion of adult spawners and number of juveniles sampled will help with this issue. A second issue is the lack of unbiased data for first time spawners. Samples collected at the Chandler facility as post-spawn

kelts are putative first time spawners for the year they are collected. However, they are not random samples. Post-spawn kelts have survived the full life cycle and are assumed to have successfully spawned. Alternatively, kelts that are released following reconditioning are still exposed to over-wintering and pre-spawn mortality. Reconditioned kelts detected at Prosser Dam and first time spawner adults sampled at Chandler Dam in the fall are likely a good comparison, but sample sizes for these two groups are low. Additional work is needed to identify a method for comparing success rates of reconditioned kelts to other fish.

A draft Hood River Kelt Management Plan was developed. This plan provides managers with potential uses of kelt reconditioning in the Hood River.

- **Chapter 3:** Snake River Basin kelt steelhead evaluations. The Nez Perce Tribe and two University of Idaho groups are conducting studies on kelt steelhead. Christine Moffitt's laboratory in the Idaho Cooperative Fish and Wildlife Research Unit is developing strategies to improve survival and return recruitment of steelhead kelts from Snake River stocks. In addition, James Nagler's laboratory is investigating the physiology of post spawn rainbow trout (*O. mykiss*), reproductive development of reconditioned steelhead kelts (*O. mykiss*), copepod treatment options, diet supplements, and force feeding and effect on kelt survival. The Nez Perce Tribe and CRITFC are estimating the stock composition of Snake River steelhead kelts captured at Lower Granite juvenile bypass. The Nez Perce Tribe is developing a Kelt Master Plan for the Snake River.

  The University of Idaho (Idaho Cooperative Fish and Wildlife Research Unit) tracked kelts from Snake Basin tributaries to determine the fate of these fish as they move through the hydrosystem utilizing acoustic tags and correlate these movements to hormone profiles. Forty-three kelt steelhead were collected at two Clearwater River tributaries and acoustically tagged. All of these tagged fish were detected at are below the mouth of the Clearwater River at Lewiston, Idaho, and 34 (79%) were detected in the Lower Granite Dam forebay.

  In our efforts to establish time series models of plasma factors from Columbia River summer steelhead stocks, we continued analysis of blood plasma from upstream migrants, sexually mature spawners, and emigrating kelts. Sampling in spawning year 2011 provided blood plasma for nearly every month of the reproductive cycle. Dworshak National Fish Hatchery was particularly important in that blood plasma was sampled from October 2010 to April 2011 and provided a physiological representation of plasma during early to mid-migration, overwintering, and at the time of spawning.

Nutritional factors measured in the plasma change by stage of development and time in steelhead trout sampled during the spawning year 2011. Plasma cholesterol, triglycerides and protein show sequential depletion from September through June. Plasma triglycerides and protein were below detection limits for many of the kelts and for steelhead trout sampled at spawning during the April at Dworshak National Fish Hatchery.

We compared the blood plasma metrics of the good condition kelts tagged in the Potlatch River and Fish Creek in 2010 and 2011. We found consistent trends between tributaries. Most nutritional factors, including cholesterol, triglycerides, and amylase were higher in kelts sampled in 2011. The only nutritional factor that was higher in 2011 was calcium. The tissue damage factors Alanine aminotransferase and aspartate aminotransferase were higher in 2010. The electrolytes sodium and chloride were both higher in both years in the Potlatch River kelts. Magnesium was higher in 2010 in kelts from both tributaries.

In 2009 and 2010, studies were initiated to apply tools from fish physiology and endocrinology to issues in kelt reconditioning. These studies were continued in 2011. By developing and applying indices based on the endocrinology and physiology of reproduction, growth, stress, and osmoregulation in fish, we aim to achieve a detailed understanding of the physiology of reconditioning in kelt steelhead. This knowledge will provide a scientific basis for maximizing the success of kelt reconditioning programs. This research project has goals of establishing post-spawning rainbow trout as a model for studying reconditioning in kelt steelhead, establishing a hatchery model of Snake River B-run kelt steelhead, establishing and validating assays for plasma and tissue level bioindicators of reproductive status, growth, and stress in steelhead kelts and post-spawning rainbow trout, comparing reconditioning profiles of kelt steelhead at different locations in the Columbia basin and rainbow trout using non-lethal sampling, and testing specific interventions such as force feeding in kelts and/or rainbow trout.

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 ESA-listed status of fish in most reconditioning programs. Therefore, we have begun studies on post-spawning physiology in rainbow trout. Our initial goal is to construct a profile of growth and reproductive endocrine physiology in post-spawning female rainbow trout. This can then be compared to profiles from kelts, and treatments to stimulate feeding enhance survival, and increase reproductive maturation can be tested in rainbow trout. In 2011, we continued a study on the physiology of post-spawning rainbow trout, which is presented in Section B.I.

One of the objectives in the CRITFC kelt project under the Columbia Basin Accords is to compare kelt reconditioning at different locations. We are collecting blood samples to compare kelt reconditioning endocrinology and physiology across the Columbia Basin. Our goals are to develop methods for monitoring reproductive development of kelts, selecting fish for reconditioning, and enhancing the survival, growth, and rematuration of kelts in reconditioning programs. In 2011, we conducted studies on reproductive development (Section B.II), a new treatment for the freshwater parasitic copepods (Section B.III), and a pilot study for a supplemented diet (Section B.IV). Invasive or manipulative studies and studies involving lethal sampling are difficult with wild endangered fish. Therefore, we believe that the establishment of a hatchery model for kelt reconditioning is of critical importance in kelt research. We took steps toward establishing this model in 2011 using artificially spawned Dworshak hatchery B-run kelts. Hatchery fish were used to test the effect of force feeding on survival in steelhead kelts (Section B.V), and were our first indication that the use of Ivermectin for copepod control in kelts was problematic (Section B.III). The results of these studies will increase the success of kelt reconditioning throughout the Columbia River Basin.

Acknowledgements

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Chapter 1: Kelt Management Scenarios

Written by
Doug Hatch (PI)
Ryan Branstetter
Jeff Stephenson
Andrew L. Pierce Ph. D.
John Whiteaker
Columbia River Inter-Tribal Fish Commission

Bill Bosch
Joe Blodgett
Dave Fast Ph. D.
Yakama Nation Fisheries

Scott Everett
Nez Perce Tribe

Rhonda Dasher
Colville Confederated Tribes

Albert Santos
Jim Gidley
Chuck Gehling
Confederated Tribes
Of the
Warm Springs Reservation

Introduction

The goal of this group of studies is to develop and evaluate potential strategies that fishery managers could use for steelhead restoration by utilizing the iteroparity life-history to increase steehead populations. Providing assistance to kelts in the form of transportation, feed, captivity, and prophylactic measures will increase the probability that individual steelhead repeat spawn and contribute to population growth. The group of studies includes In-River Release, Transport Unfed, Tansport Fed, and Long term reconditioning. These studies attempt to include measures that span from low intensity and associated costs through relatively high intensity and associated costs. Using data from all these studies we've developed a Management Scenario Evaluation to assist in kelt steelhead management decisions.

Study Descriptions

In-River Release (Yakima and Snake Rivers)

A systematically selected portion of the kelts that would have been suitable for reconditioning were PIT-tagged and released immediately back to the Yakima and Snake Rivers to act as a control group. These PIT-tagged kelts provide baseline survival data and an opportunity to compare current repeat spawner rates to other contemporary and historical estimates elsewhere in the Columbia River basin.

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

In this treatment we directly transport steelhead kelts around the hydro-system and evaluate success by measuring emigration survival to the ocean and survival for a subsequent spawning migration. Given the high mortality rates of unassisted 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 survival to the marine environment. The Transport Unfed kelts (No-term) release was continued in 2011 to compare transport treatments from the Snake River (Lower Granite Dam) and the Yakima River (Chandler Fish Facility).

Transport fed (Short-term) Treatment (Yakima River)

Short term reconditioning is an intermediate cost alternative to boost post spawn steelhead survival by holding kelts in a captive environment, implement feeding and care treatments for 4-6 weeks and then transport them for release below the hydrosystem. The rational for this treatment is to not only provide the survival benefit of transport but to also provide a positive energy balance for the kelt migration and ocean entry. We no longer conduct this study but summary data is included in the management scenario evaluation.

Long-term Reconditioning Treatment

We define long-term reconditioning as holding and feeding post-spawn steelhead in a captive environment to increase kelt survival and future spawning opportunities.

The long-term steelhead reconditioning diet and care treatments were established from the studies conducted in 2001 and 2002 (Hatch et al. 2002 and Hatch et al. 2003b) and continue at reconditioning facilities located in Prosser, WA, St. Maries Acclimation Facility, WA, Parkdale Fish Facility, OR, and Dworshak, ID. These fish are typically released in the fall to over-winter and return to the spawning sites volitionally, with the exception of Parkdale and some fish at Dworshak. This treatment represents the highest cost alternative.

Management Scenario Evaluation

An evaluation of kelt steelhead restoration stratedies 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.

Survival data for each treatment is used to calculate a survival benefit for comparisons between treatments. This evaluation is intended to assist fisheries resource managers in making decisions related to expected survival outcomes that would be anticipated for each intervention. It also demonstrates how interventions that improve kelt steelhead survival can directly increase steelhead spawner abundance.

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Section A: Kelt Collection and In-River Release

Introduction

Steelhead kelts are collected from 4 main areas throughout the Columbia River Basin, Prosser, WA, Lower Granite, WA, Omak, WA, and Parkdale, OR. This section details the capture locations, capture methods, and biological information collected from specimens. A small portion of the kelts collected are released back to the river to determine the baseline steelhead kelt iteroparity rate. This information provides a standard with which to compare the experimental approaches. Leaving steelhead kelts in the river represents the least cost option, which is currently the status quo for the majority of the Columbia River Basin.

Study Area

Yakima River Basin

The Yakima River is approximately 344 km in length and enters the Columbia River at Rkm 539. The basin is 15,928 km^2 and average discharge is 99 m^3/s. Summer steelhead populations primarily spawn upstream from Prosser Dam in Satus Creek, Toppenish Creek, Naches River, and other tributaries of the Yakima River (TRP 1995) (Figure 1).

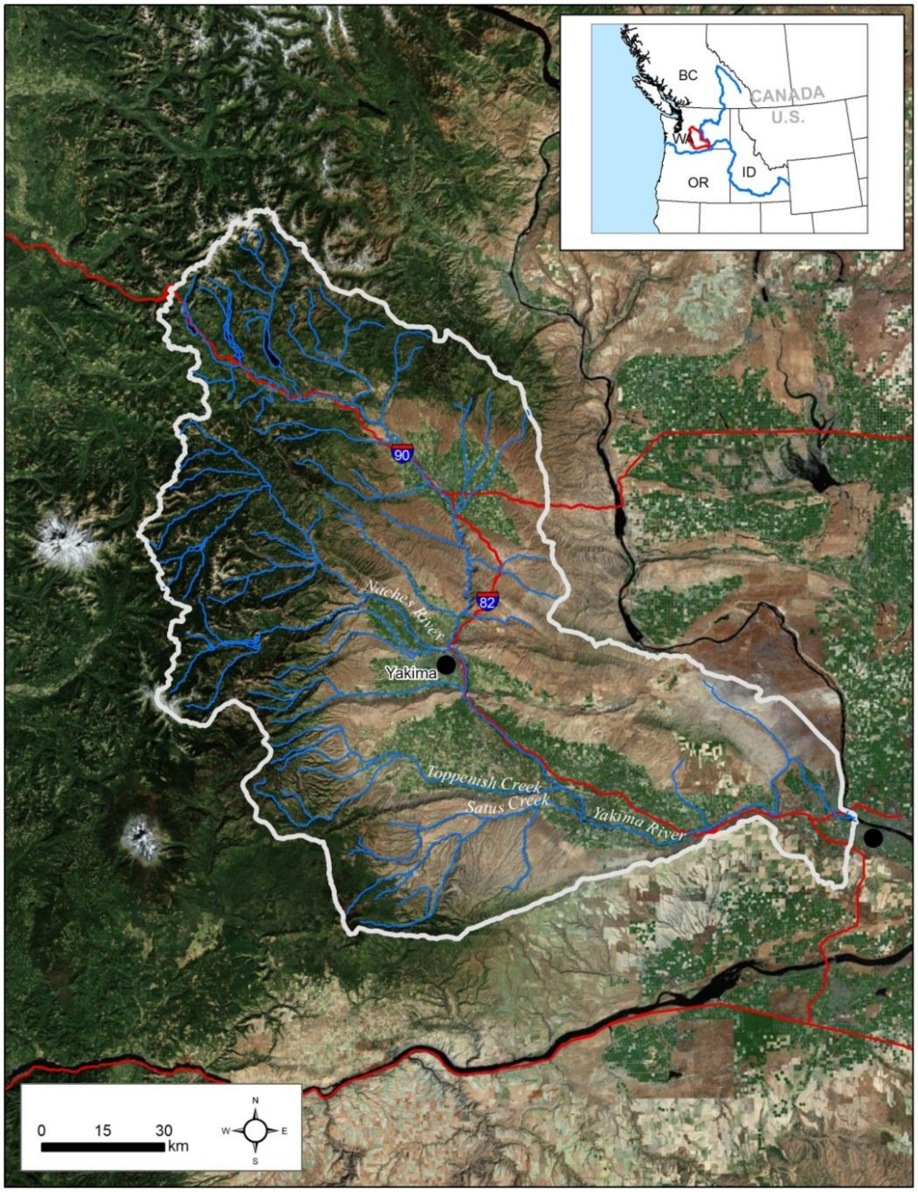

Figure 1: Map of the Yakima River Subbasin.

Snake River Basin

The Snake River watershed is the tenth largest among North American rivers, and covers almost 280,000 km^2 in portions of six U.S. states: Wyoming, Idaho, Nevada, Utah, Oregon, and Washington, with the largest portion in Idaho. Most of the Snake River watershed lies between the Rocky Mountains on the east and the Columbia Plateau on the northwest. The largest tributary of the Columbia River, the Snake River watershed makes up about 41 % of the entire Columbia River Basin. The Snake River enters the Columbia at Rkm 523. Its average discharge at the mouth constitutes 31% of the Columbia's flow at that point. The Snake River's average flow is 1,553 m^3/s. At Anatone, Washington, downstream of the

confluences with the Salmon and Grand Ronde, but upstream of the Clearwater, the mean discharge is 979 m^3/s (Figure 2).

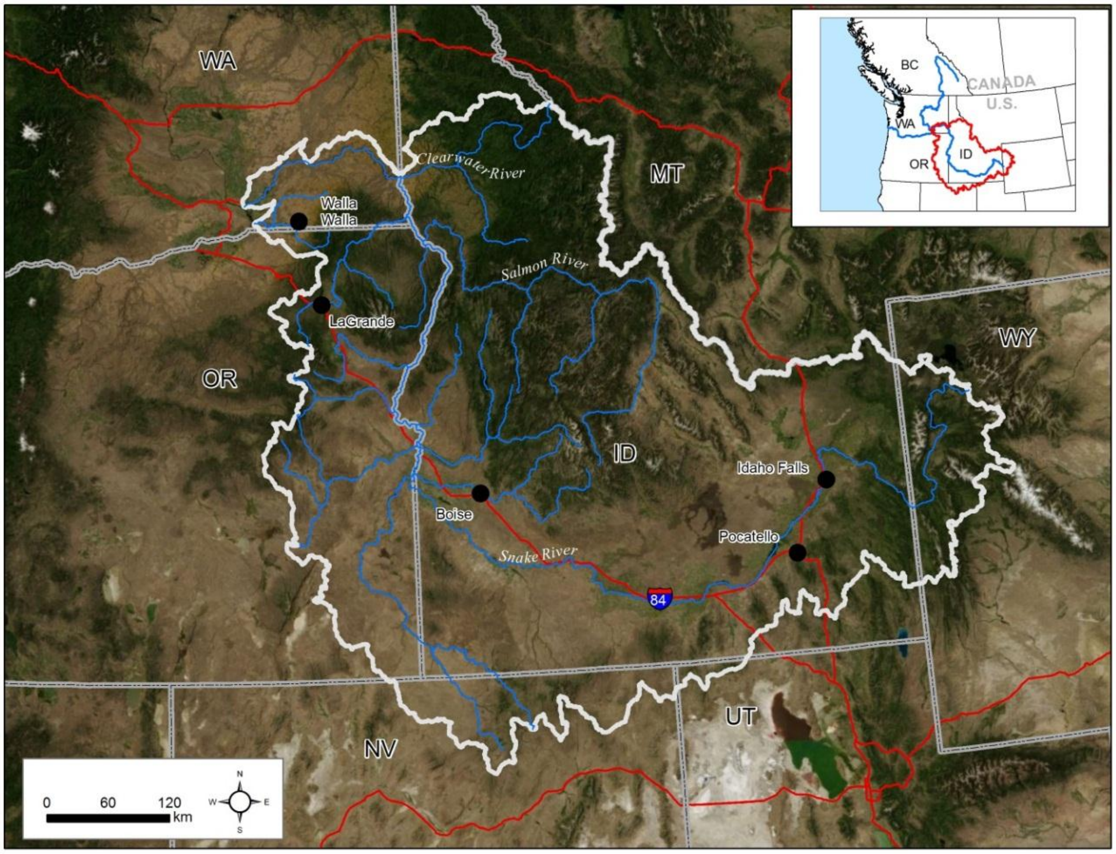

Figure2: Map of the Snake River Basin.

Okanogan River Subbasin

The Okanogan River is a tributary of the Columbia River and the confluence is located at Rkm 858 of the Columbia River. The Okanogan drainage area is 21,238 km^2 with an average discharge rate of 86 m^3/s. Omak Creek, a tributary to the Okanogan River, is located in Okanogan County in North Central Washington, the confluences of Omak Creek is located at Rkm 52 of the Okanogan River. Omak Creek is approximately 35.4 km in length (Figure 3) running entirely within the Colville Confederated Tribes (CCT) reservation boundaries. Bonaparte Creek's anadromous navigable water runs for 1.6 kilometers and is a tributary to the Okanogan River, which flows through the town of Tonasket. Lower Salmon Creek (6.9 kilometers of Salmon Creek) is a tributary of the Okanogan River that has a diversion dam which prevents upstream fish passage. Steelhead naturally spawn in Omak and Bonaparte Creeks with limited natural spawning in Salmon Creek.

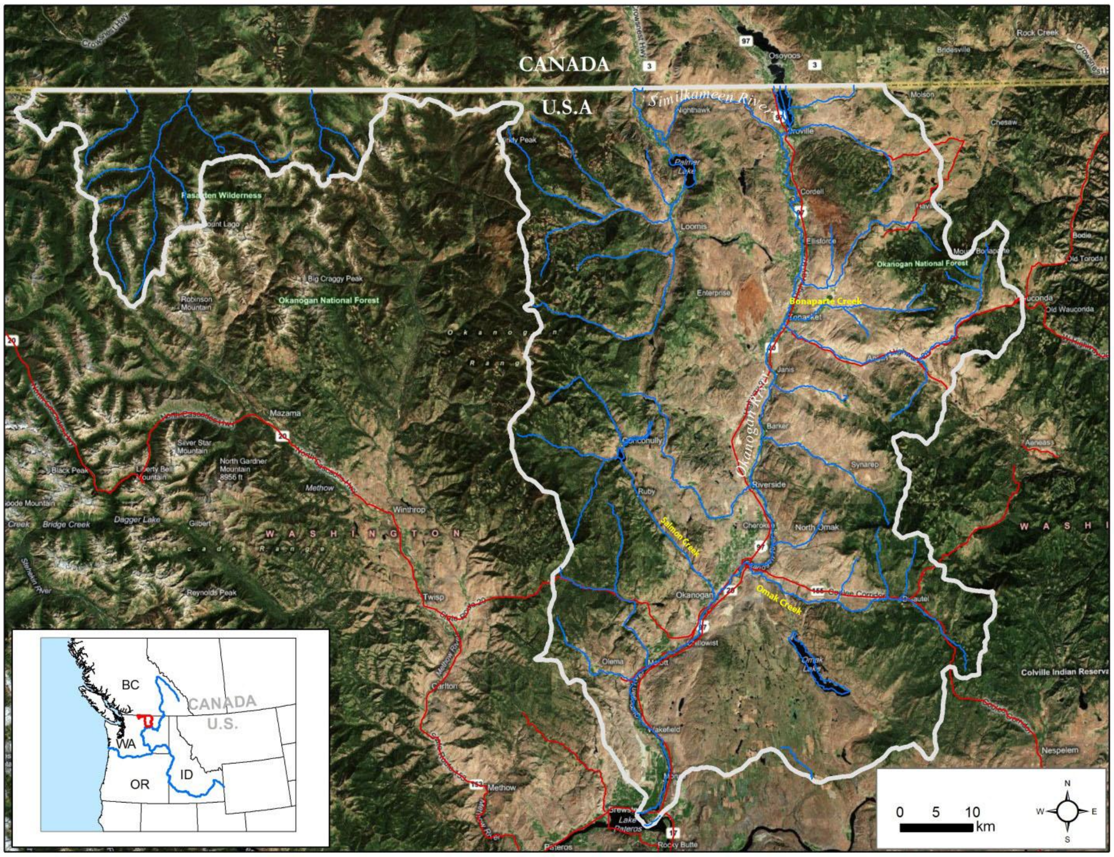

Figure 3: Map of the Okanogan River Subbasin. Omak Creek, Bonaparte Creek, and Salmon Creek in yellow font.

Hood River

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

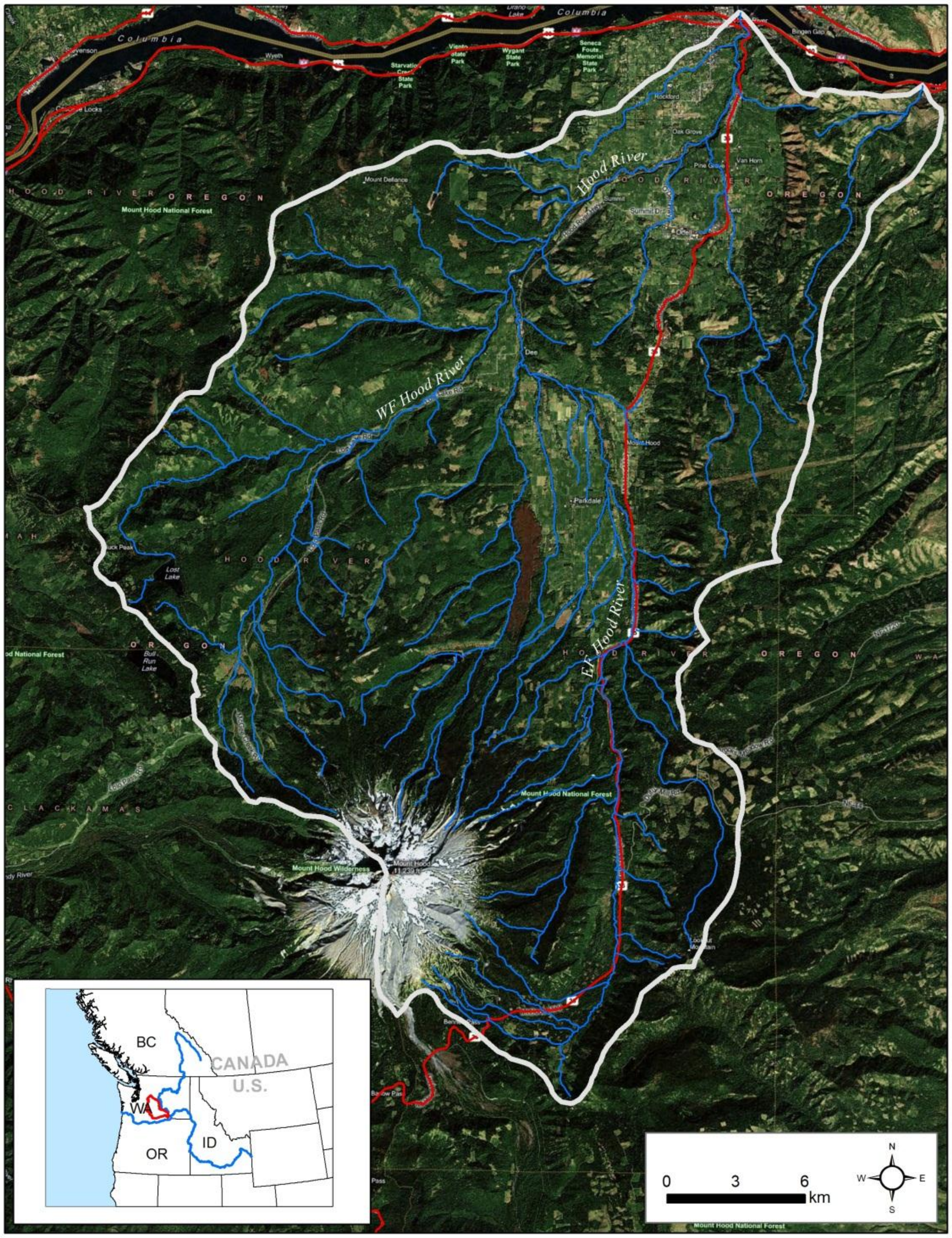

Figure 4: Map of the Hood River Subbasin.

Methods

Kelt Collection and In-Processing

Chandler Juvenile Monitoring Facility (Yakima River)
Post spawn steelhead migrating downriver are inadvertently collected by way of the Chandler Juvenile Migration Facility (CJMF a.k.a Chandler Juvenile Evaluation Facility CJEF)) which diverts migratory fishes away from the irrigation canal into the Chandler Juvenile Migration Facility (CJMF a.k.a Chandler Juvenile Evaluation Facility CJEF)). Once diverted into the CJMF. emigrating kelts are manually collected from a fish separation device (a device that allows smaller juvenile salmonids to "fall through" for processing in the juvenile facility while larger fish can be dipnetted for processing (Figure 5). Yakama Nation staff monitored the Chandler bypass separator during the kelt migration.

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

All collected steelhead are placed into a water-lubricated PVC pipe slide that diverts fish to a temporary holding tank 6.096 m (l) x 1.8288 m (w) x 1.2192 m (h) containing oxygenated well water at 13.8^{0}C (Figure 6). All specimens were 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 prespawn individuals were immediately

released to the Yakima River. All kelt steelehad were processed for the various experimental treatments (in-river release, un-fed transport, or long-term reconditioning).

Figure 6. Chandler Juvenile Monitoring Facility PVC slide and holding tanks.

Following kelt identification, we identified sex, weighed (collected in pounds but converted to kg for this report), measured fork and eye length (cm), assigned 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 afflictions (e.g., head burn, eye damage). Passive Integrated Transponder (PIT) tags, if not already present, were implanted in every fish's pelvic girdle for later identification

The Lower Granite Juvenile Fish Facility (Snake River)

Steelhead kelts migrating from tributaries of the Snake River above Lower Granite Dam that do not emigrate via the Removable Spillway Weir (RSW) are directed by a large bypass system to the Juvenile Fish Facility at Lower Granite Dam where they are collected by Army Corps of Engineer (COE) Staff. Kelts are collected off the adult fishseparator bars and moved to a fish hopper leading to the kelt receiving tank (Figure 7). Both B-run ($\geq$ 70 cm) and A-run (<70 cm) steelhead are selected. In 2011, the separator was manned 24 hours throughout the season. Staff from the Nez Perce Tribe (NPT), University of Idaho (UI), and CRITFC processed fish diverted into the receiving tank by the COE.


A
B
C
D

Figure 7: LGR JFF separator bar screen (A), kelt hopper (B), kelt delivery pipe (C), and kelt receiving tank (D).

The kelt receiving tanks are 6 feet wide by 25 feet long and 6 feet deep. The tanks have built in crowders, which move along a guided track chain. Each crowder has a lower gate panel, which can be raised mechanically. Both tanks have a release chamber with a lifting floor and an exit gate. The exit gates are connected to pipes leading directly to the river. The receiving tank (tank #1) is nearest to the river and has an additional crowder to allow separation of treatment groups. The holding tank (tank #2) has an additional exit gate, which can be connected to a large diameter hose for alternative release locations (figure 8).

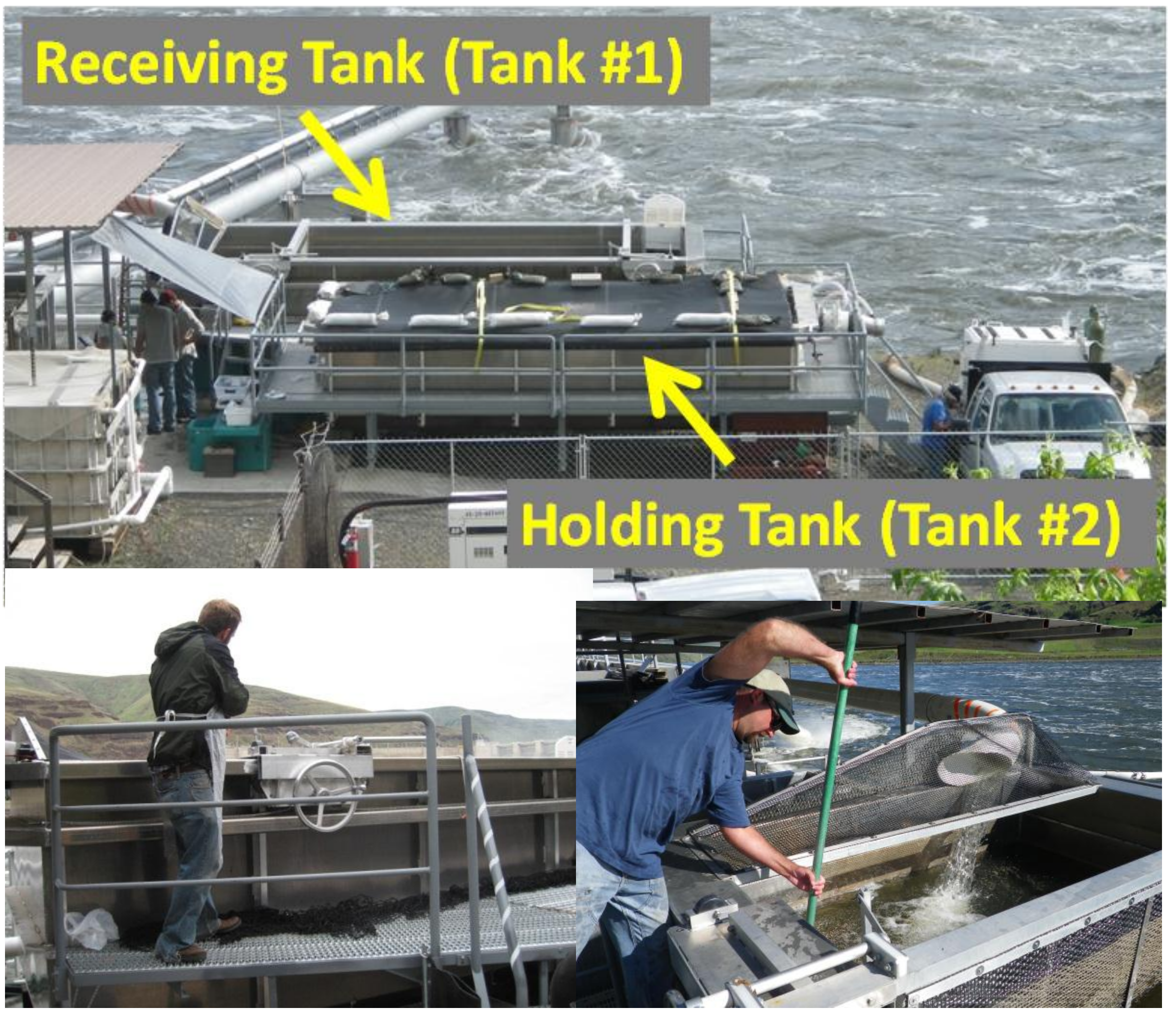

Figure 08: Tanks designed by the UI for holding and sorting kelts at Lower Granite Dam.

Every day, staff from the NPT, UI or CRITFC processed fish. Fish were anesthetized in tricaine methanesulfonate (MS-222) buffered with standard stock solution of sodium bicarbonate to decrease stress and mortality (McCann et al. 1994). Fish were measured, weighed and graded by condition. In assessing the condition, several factors were

considered. The condition rating we used referred to the fish's potential for reconditioning. This rating was based on physical appearance, texture and firmness. This rating used three criteria: color, fungus, and injury. Fish also had blood and tissue samples collected for physiological measures and genetic profiling. All fish that were not moribund received a PIT-tag before being assigned to a treatment or released back to the river.

Omak and Bonaparte Creek weirs

The Omak Creek weir (Rkm 0.8) is utilized to collect broodstock and steelhead kelts for reconditioning (Figure 9). This stock is being used by the Cassimer Bar Hatchery to develop a naturalized steelhead broodstock for the Okanogan River and Omak Creek. To increase the total number of kelts available for reconditioning, a weir with trap was set up at Bonaparte Creek (0.4 Rkm) (Figure 10).

Figure 9: Resistance board weir located on Omak Creek.

Figure 10: Bonaparte Creek capture weir.

All anadromous *O. mykiss,* regardless of up or downstream movement including those selected for broodstock or reconditioning, were sexed, 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.

East Fork Weir (East Fork of the Hood River)

Different from the other kelt reconditioning studies, post spawn steelhead in the Hood River are not collected in the wild, and post spawn hatchery fish are used instead. Oregon Department of Fish and Wildlife Employees capture anadromous winter run steelhead spawners at the resistance board weir located at approximately mile 12.5 of the East Fork of the Hood River (Figure 11). Approximately 40 (20 females and 20 males) winter steelhead were trucked to the Parkdale Fish Facility where they were held until fully ripened. These fish are typically recycled through the fisheries three times before they are terminated and donated to the Oregon State Food Bank Program. ODFW/Parkdale staff attempted to retain fish that visually appeared to be in good condition to maximize the success of spawning and reconditioning. Fish are sexed, weighed, and measured at collection to evaluate the impact of reconditioning. Trapping usually begins in Febuary/March and ends typically in early May. Collection for this study ended when we obtained our goal of 20 pairs of first time spawning steelhead unless there was early mortality and if the trap was still in use we collected additional fish.

Figure 11: Resistance board weir on the East Fork of the Hood River. In River Release

Yakima River

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

Snake River

Steelhead kelts collected at Lower Granite Dam that were not moribund and not selected for reconditioning were PIT-tagged and directly released to the river during the duration of the steelhead kelt seaward migration. This will provide an annual baseline for iteroparity under operation of the current hydrosystem. Results can also be compared against Yakima River rates.

Results and Discussion

General Population Characteristics

Yakima River

A total of 984 live kelts were captured between March 17 and June 8, 2011 at the CJMF. There were 3 kelts discovered dead upon arrival in the bypass. There were 19 kelts in poor condition and 11 prespawn (maiden) steelhead that were released immediately back to the Yakima River on site.. A total of 85 good condition kelts were diverted back to the Yakima River for the direct release. Collection was mostly continuous throughout the outward migration, with peak collection occurring on May 5, 2011 (Figure 12). The bypass was shutdown from April 2-6 and 8-12 for the removal of freshet transported flotsam. The total number of kelts captured represented 15.9% (987 of 6,197) of the Yakima River spawning migration based on fish ladder counts obtained from Prosser Dam for the period July 1, 2010 through June 30, 2011. This collection of Yakima River steelhead kelts is represented by kelts that volunteer into the bypass facility while others migrate over the Chandler dam.

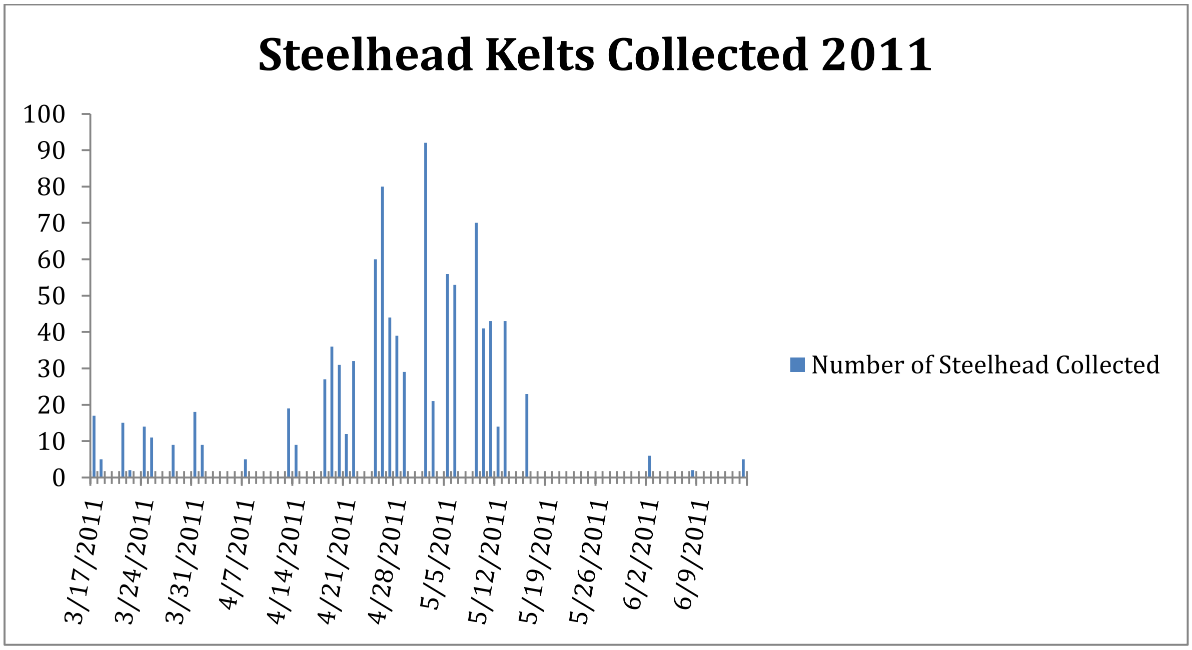

Figure 12: Steelhead Collection at Prosser, WA 2011.

The majority of kelts captured were female which is consistent with previous findings (Hatch et al. 2011). Based on visual observations, in 2011 880 (88%) of the kelts were female, 110 (11%) were male, with 7 fish were not sexed. Yakima River kelts collected during 2011 were classified as being in good (n=312, 31%) or fair (n=664, 67%) condition, with the remaining fish classified as poor (n=20, 2%). Coloration was predominately intermediate (n=487, 49%) or bright (n=480, 48 %) with a small percentage that was dark (n=29, 3 %).

Snake River

A total of 1,974 kelts were intercepted by the LGR JFF between April 12 and June 30, 2011. Collection was mostly continuous throughout the season. The separator was shutdown on a few days for a few hours to clear debris. The peak collection (103 fish) occurred on May 3, 2011 (Figure 13). There were 27 collection/handling mortalities, and 20 fish were lethally sampled. In addition, select fish were outfitted with acoustic tags and placed on barges or transport trucks. Table 1 summarizes the final disposition of kelts collected by the LGR JFF.

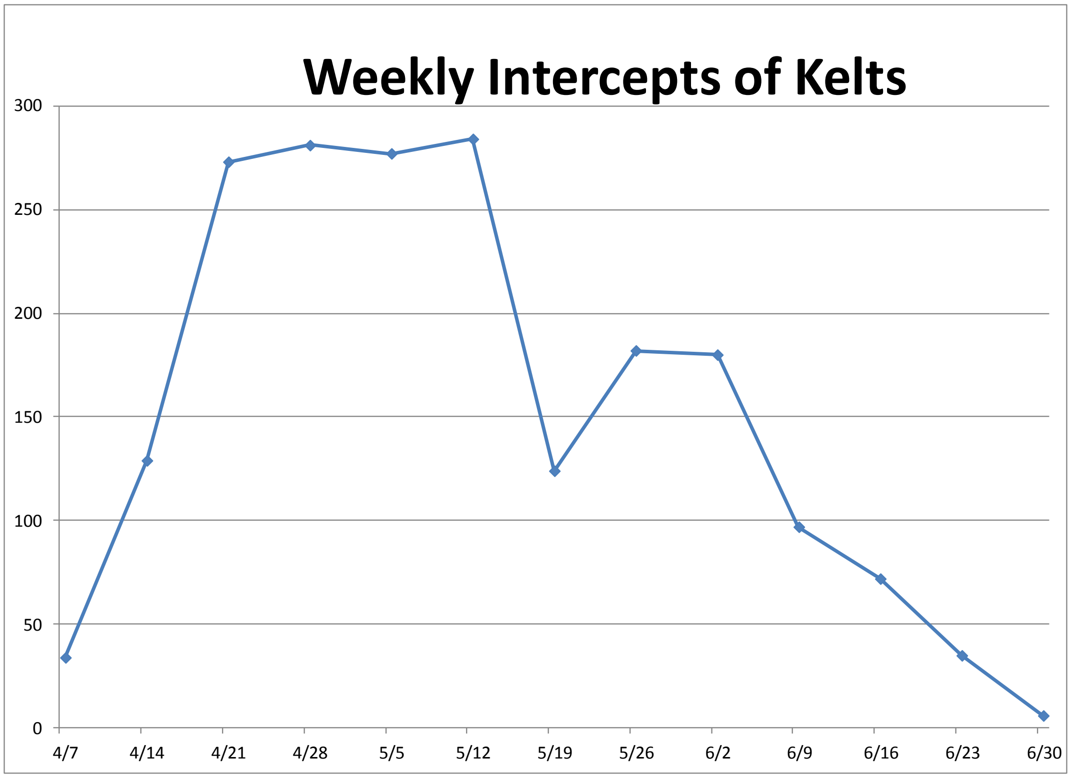

Figure 13: Weekly steelhead kelt interceptions at LGR JFF in 2011.

Table 1: Summary of final disposition of fish collected at LGR JFF in 2011.

Final Disposition	A-run	B-run	Total
Returned to River	1236	379	1615
Barged to Lower Columbia River	76	34	110
Trucked to Lower Columbia river	73	18	91
Transported to DNFH for reconditioning	41	70	111
Lethal sample	9	11	20
Mortality	15	12	27
Total	**1450**	**524**	**1974**

The majority of the fish collected from the Snake River at LGR JFF in 2011 were A-run females in good condition. Most fish were without any major wounds (scraps, cuts, fungal infections) with the majority of them collected in the month of May (Table 2). Unlike 2010, the proportion of fish assessed as fair condition decreased (27% to 17.3%) and the proportion of poor fish increased (12% to 26.6%: Table 3).

Table 2: Condition of Snake River Kelts collected at the LGR JFF in 2011.

	April	May	June
Good	245	630	229
Fair	96	166	78
Poor	177	216	131

Table 3: Condition of steelhead kelts by sex and size at the LGR JFF in 2011.

Female (85.9%)	Good (56.1%)	Fair (17.3%)	Poor (26.6%)	% of collection
< 70 cm	756	147	287	60.5
≥ 70cm	203	166	132	25.5
			Total	**86.0**
Male(21.1%)				
< 70 cm	141	22	93	13.0
≥ 70cm	4	5	12	1.0
			Total	**14**
Total	1104	340	524	1968

Similar to 2010, fish were observed with recent head injuries (Branstetter et al. 2011). These head injuries look very similar in nature (deep tissue wounds), which may indicate something restricting their journey to or through the bypass system (Figure 14). This type of injury is not typically seen in other reconditioning sites and if present, not observed as high a frequency as is found in the Snake River. Overall, the proportion of head injuries was 38.2% (Table 4). The weekly proportion varied between 20% and 60% throughout the majority of the collection season; however, no apparent pattern was observed with discharge (Figure 15)

Figure 14: Typical head injury observed on steelheads kelts at LGR JFF.

Table 4: Percent of head injuries on steelhead kelts at the LGR JFF.

	A-run	B-run	Total
No	47.2 (N=928)	14.6 (N=288)	61.8 (N=1216)
Yes	26.3 (N=518)	11.9 (N=234)	38.2 (N=752)
Total	73.5 (N=1446)	26.5 (N=522)	100 N=1968

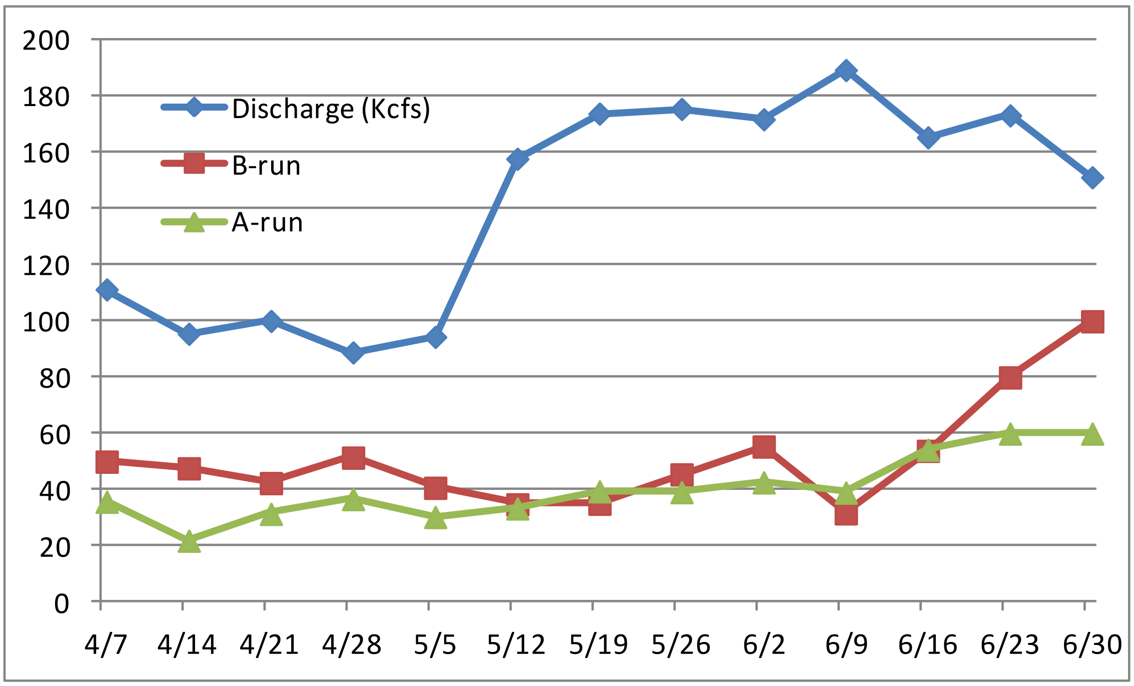

Figure 15: Mean weekly discharge (Kcfs) and percent head injuries observed on steelhead kelts at the LGR JFF in 2011.

Omak Creek

The trap was in operation from March 8 through early August 2011. During the season 58 summer steelhead were captured and 44 were passed above the weir. The other 11 fish were used for the Colville captive broodstock at Winthrop National Fish Hatchery. The 2011 season had the relatively high number of wild fish enumerated since trapping started in 2001. Based on combined redd surveys and weir counts, we estimated that a total of 132 adult steelhead spawned in Omak Creek in 2011 (Miller et al. 2011). The natural-origin ratio was also the highest level seen, with over 85% of the fish returning being of natural origin. There were 26 males and 32 females processed at the trap (Table 5). Weather conditions remained consistent with consistent cold river water temperatures throughout the trapping season with high flows negatively impacting the weir (2-weeks shutdown). In

the downstream trap a total of 27 kelts were collected. There were 16 good condition kelts and 7 (dead-on-arrival/ poor condition). A total of 20 fish (16 females and 4 males) were captured and transported to Cassimer Bar Hatchery for the Colville kelt reconditioning program.

Table 5: Percentage and totals of male, female and wild summer steelhead passed above the Omak Creek weir, 2011.

	Total (N)	Natural (N)	Percent-Natural (%)	Passed above weir (N)
Total	58	50	85.9	44
Males	26	21	36.0	20
Females	32	29	50.8	24

Bonaparte Creek

The trap went in on March 8 water temperatures at that time were very cold at 2.4^{0}C. The weir was fished until May 24. Temperatures remained low throughout the trapping period. Staff did not collect many fish attempting to move into the system and twice there were blowouts of the wier from high spring flows. Colville staff collected 6 kelts that were either in poor condition or dead upon arrival. Colville staff believes that due to the cold water and recurrent high flows most steelhead may have bypassed Bonaparte in 2011 and sought out other steams with more consistent flows and warmer temperatures.

Hood River

A total of 14 males and 23 females winter steelhead were collected for cryopreserving and subsequent air spawning. This was the first year of collection at the East Fork of the Hood River. Fish captured in 2011 had many more dermal scrapes and cuts than fish that were captured at the former Powerdale dam collection site.

In-River Release and Detection Results

Yakima River

2011 In River Release

There was a total of 85 kelts released as in-river fish into the Yakima River in 2011. There were no detections of these fish trying to return in 2011 or early 2012.

2010 In-River Release Detected in 2011

Two in-river fish were detected out of the 155 that were released in the Yakima River in 2010. One of the fish was detected passing Bonneville Dam in early July 2011 and the other passed in early August (Table 6). One of the two fish successfully passed McNary Dam in mid-October 2011 and was detected passing Prosser Dam a week later (Table 6).

Table 6: 2010 Yakima In-River release with return detections by year (2010 and 2011) and the number detected at each dam.

2010 Yakima In- River				
Total Tagged and Released: 155				
Detection Year	Bonneville Dam	McNary Dam	Prosser Dam	% return of total release to Yakima R.
2010	0	0	0	0
2011	2	1	1	0.6%
Total	2	1	1	0.6%

Snake River

2011 In- River Release

There were a total of 1,613 PIT tagged kelts released as in-river fish into the Snake River in 2011.

2011 Downstream Detection After Release in 2011

There were a total of 18% detected (Table 7) migrating downriver. We have had no return migration detections of these fish in 2011. The number of PIT detections decreased after Lower Monumental Dam but remained consistent until Bonneville Dam (Table 8). This was likely due to the large amount of spill that was occurring at these dams, which would decrease the number of kelts that would be volunteering into the bypass facilities where they are detected.

Table 7: Number of kelts PIT tagged and released at Lower Granite Dam in 2011 separated by gender and the presence (Unclipped) or absence (Clipped) of an adipose fin, as well as the number and percent detected at least once in the Snake or Columbia rivers migrating downriver.

Group and condition	Total tagged and released	Number detected migrating downriver	Percent detected
2011 Total	**1613**	**289**	**18**
Males	**260**	**61**	**23**
Unclipped	189	35	19
Clipped	71	26	37
Females	**1353**	**228**	**17**
Unclipped	1006	159	16
Clipped	347	69	20

Figure 8. First (bold), second, and third (in parentheses) detections of kelts tagged and released at Lower Granite Dam in 2011 by Nez Perce Tribe Fisheries personnel. The rows in this table show the number of fish detected for the first time at each dam, then the number and location of subsequent detections of that group of fish. Not included in this table are 9 kelts that were detected ascending the Lower Granite adult fish ladder after release (possibly pre-spawning fish that fell back through the juvenile fish bypass).

2011	Little Goose	Lower Monumental	Ice Harbor	McNary	John Day	Bonneville	Towed array
Little Goose	**120**	19	3	2 (3)	3 (1)	1	0
Lower Mon.		**75**	4	4	4 (1)	0 (1)	0
Ice Harbor			**21**	1	0	0	0
McNary				**23**	1	0	0
John Day					**33**	1	0
Bonneville						**7**	0
Towed Array							**1**

2010 In-River Release

2010 Downstream Detection After Release in 2010

The number kelts PIT-tagged and released in-river in 2010 was 1,398 fish, which was slightly lower than what was release in 2011 (Table 10). General condition of fish along with numbers collected can be found in Appendix (A) and in Branstetter et al 2011 Chapter 3 Section A.

2010 In-River Release Detected in 2011

We detected 5 of the 1,398 Snake River kelts that were PIT-tagged and released in-river in 2010 (Table 10). Initial detections were first recorded at Bonneville Dam with the first one occurring in late-July and the other individuals passing in mostly early August and the last one toward the end of the month. Of the 5 detections at Bonneville, 4 were detected migrating over McNary Dam in mid-August to late-September, and again at Lower Granite Dam in September to late October (Table 10). All of these fish returned as skip spawners.

Table 10: 2010 Snake In-River release with return detections by year (2010 and 2011) and the number detected at each dam.

2010 Snake In-River				
Total Tagged and Released: 1398				
Detection Year	Bonneville Dam	McNary Dam	Lower Granite Dam	% return of total release to Snake R.
2010	0	0	0	0
2011	5	4	4	0.3%
Total	5	4	4	0.3%

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Section B . Transport and Release of Unfed (No-term) Treatment (Yakima and Snake Rivers) Groups.

Introduction

The transportation of kelts represents a possible option to assist steelhead iteroparity rates. Transport may be useful in areas where large numbers (>200) of kelts attempt to leave the basin and budgets and/or facility space may be an issue. Transport is a cheaper option than a long-term reconditioning facility. A portion of the steelhead kelt migration from the Yakima and Snake rivers was used to measure the effectiveness of transport. We used acoustic receivers to determine survival through the lower Columbia River and used PIT-tags to determine how many kelts returned from the ocean to test transport efficacy.

Study Area

Kelt steelhead for the transport treatments were captured and implanted with acoustic tags at the Chandler Juvenile Monitoring Facility (CJMF) in Prosser, WA and at the Lower Granite Dam juvenile bypass near Pomeroy, WA. These fish were either trucked or barged and released at two sites. The first site is the Hamilton Island Boat Ramp (Rkm 233) located downriver from Bonneville Dam on the Washington shore of the Columbia River (Figure 1). The second release site, Westport, OR is located in the upper portion of the Columbia Estuary at Rkm 72 (Figure 1). The lower Columbia River habitat from approximately Rkm 75-0 is typified as an estuarine environment, and is influenced by tidal oscillations from the Pacific Ocean. Acoustic telemetry technology (Figure 2) (Rkm 233 to 0) (Appendix B) was utilized to monitor this release and prior years' releases (2004-2008, 2010-2011). Receivers were grouped into four arrays providing detection gates as fish pass down the river (Figure 2). We opportunistically included detections of an array near Bonneville Dam, which was part of the Sea Lion Predation study. The inclusion of the Bonneville detection array is meant to determine possible immediate release mortality after transport and to detect any returning kelts. The lower Columbia River arrays were partitioned into 3 reaches (River reach from Bonneville Dam (Rkm 231) to near St. Helens, OR (Rkm 150); Estuary Reach extending from St. Helens, Or (Rkm 150) to Westport, OR (Rkm 56); and, River mouth extending from Westport, OR (Rkm 56 to the mouth of the Columbia River (Rkm 0).

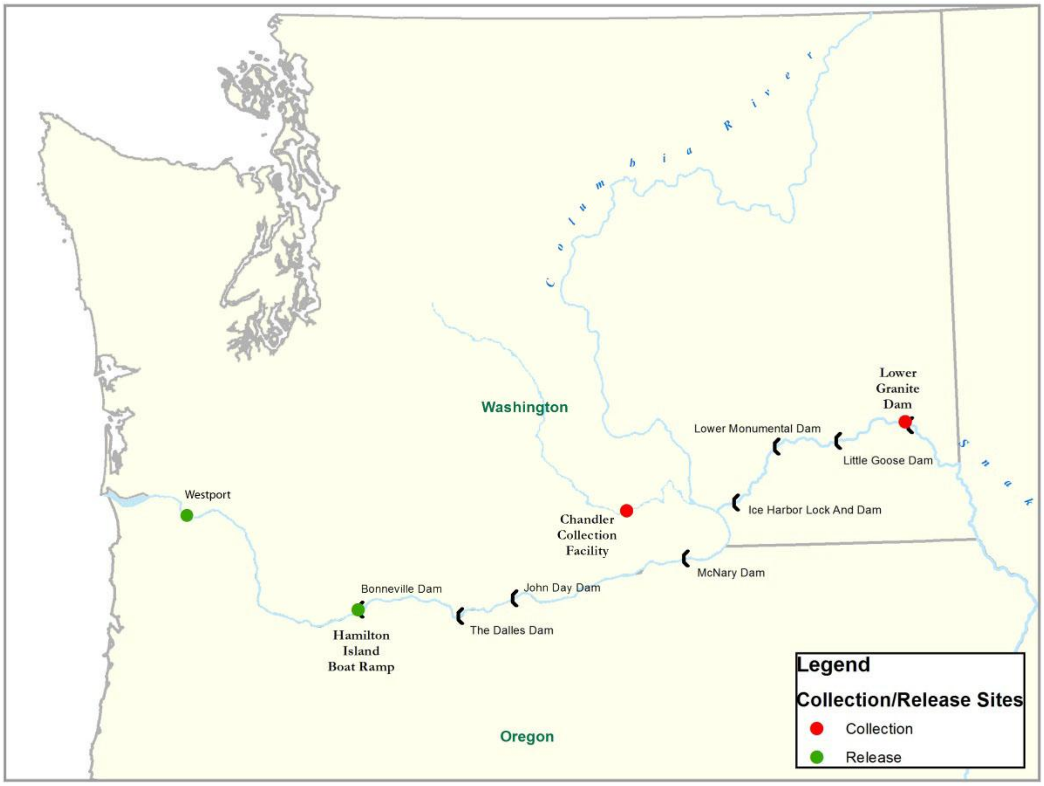

Figure 1. Map of the Columbia River showing kelt collection and release sites for transported steelhead kelts.

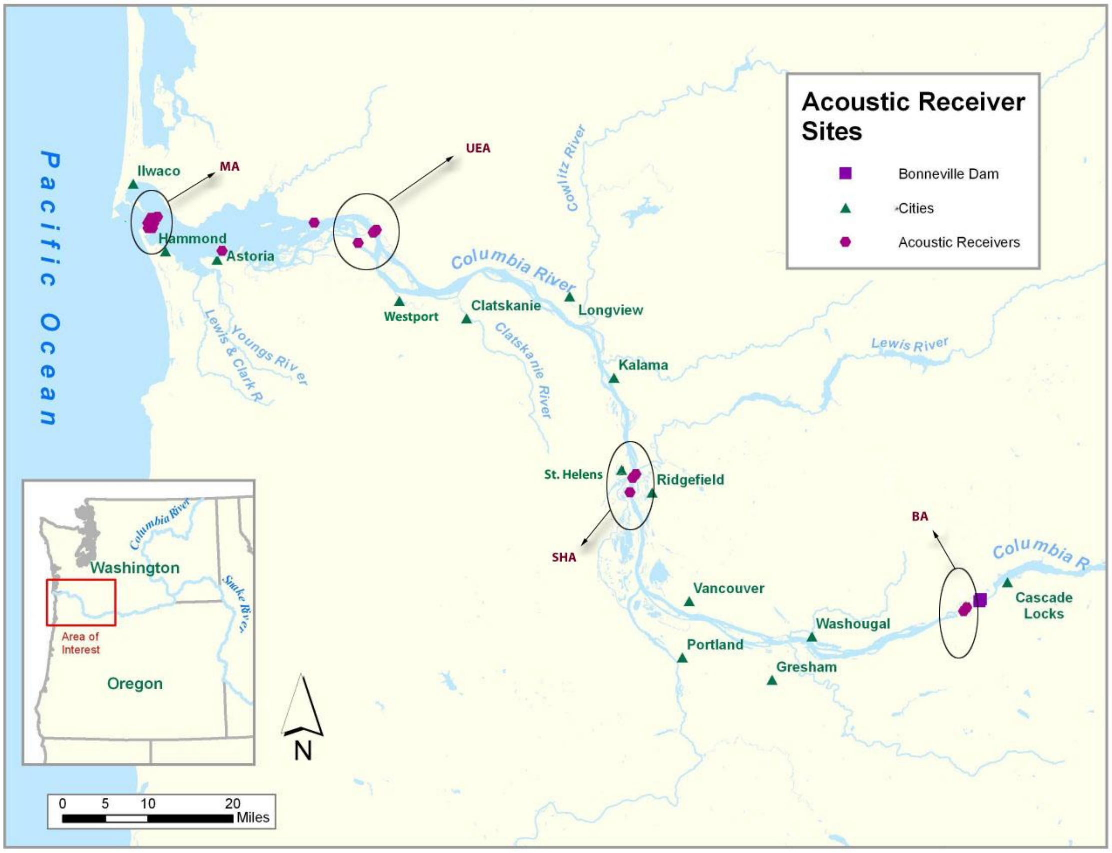

Figure 2. Map of the lower Columbia River showing locations of acoustic receiver arrays (circles) and individual receivers (purple dots) in 2011. BA= Bonneville Array, SHA= St. Helens Array, UEA= Upper Estuary Array, and MA= Mouth Array.

Methods

Capture

Fish captured for this study were collected from juvenile bypass facilities (CJMF and Lower Granite) described in Section A. All fish were scanned for pre-existing (juvenile or early capture) PIT-tags and if none were present they were tagged. Fish condition (based on a modified version of Evans et al. 2003, excellent=bright/good, good=medium->dark/good, fair=medium->dark/fair) and metrics (length, weight) were recorded. All poor condition (head wounds, deep lacerations, and physical deformities) kelts were excluded from acoustic tagging to insure that fish would survive the tagging procedure. At Lower Granite Dam we considered any fish over 70-cm a “B-run” kelt.

Acoustic Tags

A portion of the steelhead kelts captured at the CJMF and Lower Granite juvenile bypass had a coded Vemco© V16-4H acoustic transmitter surgically implanted intraperitoneally (body cavity) using standard surgical procedures that we have used previously (Branstetter et al. 2011). Each acoustic tag has a unique bandwidth pulse that provides individual identification codes. The tags' mass, 10g, constitutes on average 0.25% of the fishes total body weight. This weight value .25% is well below recommended weight percentages and is likely not a physiological issue for the fish (Welch et al. 2007; Jepson et al. 2002; Summerfelt and Mosier 1984). In an internal implantation, an incision (using sterilized scalpel fine blade (Mulcahy 2003)) just smaller than the transmitter (approximately 10 mm) diameter is made into the body cavity, usually on the midline of the ventral surface halfway between the pectoral and pelvic fins (Langford et al. 1977) (Mulcahy 2003). The incision is spread open utilizing a sterilized gloved finger as a dilator. The use of the muscle-splitting approach causes less damage and speeds healing than cutting the muscle tissue all the way through and making a larger incision (Pers. Communication w/ DVM. Flecker). Transmitters and surgical equipment were disinfected using chlorhexidine before placement into the body cavity (Burger 1994; Mulcahy 2003). Once the transmitter is securely inside the fish the original incision is closed with a single interrupted suture (Wagner et Al. 2000). Sterile, non-reabsorbing monofilament suture was used due to concerns of seawater prematurely causing the suture to split (Mulcahy 2003). General anesthetics (MS-222) were used during surgery, and fish were returned to freshwater immediately following surgeries to recover (Mulcahy 2003). 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 (Mulcahy 2003).

Tag loss via encapsulation and post-tag related mortality are not likely a significant issue for tagged steelhead kelts (Summerfelt and Mosier 1984;Welch et al. 2007; Lucas 1989) especially considering the short amount of time they are observed during the migration. Lucas (1989) and Welch et al. (2007) noted that after 3 and 7 months both mortality and tag loss was low for both. We feel confidant that surgical procedures, tag related mortality, and tag shedding are not factors in this experiment.

Release

The unfed transport treatment groups were released at either the Hamilton Island Boat Ramp area (Rkm 233) below Bonneville Dam or at the Westport Boat Ramp (Rkm 72) in 2011. The fish were transported in a large fish transport truck (Yakima River releases and Snake River Westport release) or tug driven barge from Lower Granite Dam (Snake River Hamilton Island release). 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). The complete array was deployed in early March 2011 and was retrieved late October the same year. Array placement remained nearly identical to previous years' with only minor modifications (Branstetter et al. 2011, Branstetter et al. 2009, Branstetter et. al. 2008, and Branstetter et. al. 2007) (Appendix B). This arrangement provides data on reach survival and migration timing in-river, to the estuary, and to the

ocean. Comparisons are made between release groups and between release sites. We assess the releases by survival during the emigration, and fish return rates to Bonneville Dam, movement, distribution, travel time, velocity, as well as residence time in the estuary (Appendix B). A small array will be deployed in 2012 to detect returning fish. Transportation benefits were compared between steelhead stocks (Snake and Yakima rivers).

Detection History

Steelhead kelt detection histories are manually collected from Vemco VR2W receivers. Receivers are moored on anchor/buoy lines by cable ties attached approximately 15-feet below the water's surface. Each array is composed of 3 to 12 receivers deployed in a manner that their combined detection range encompasses a cross section of the river. To retrieve detection data, a boat and crew would download data from each VR2W to a PC, using a Bluetooth connection. Detections were stored using Vemco's proprietary database system and then relevant detection histories filtered and exported to a spreadsheet for analysis. The presence/absence of detection history at each array is used to derive our survival and detection probability values. Emigration timing is also derived from our first and last detections within and between array lines. We assume that fish without detections and fish with detections that remain stationary (over a month) are likely mortalities. Another assumption is that detections made in July-August after having a final detection at the mouth of the river (several weeks after release) are likely fish returning from the ocean. Automatic adult PIT-tag detectors are present in all ladders at Bonneville, McNary, and Prosser dams, and weirs on smaller systems to provide additional survival and movement information.

Estimating Survival and Detection Probability

Survival and detection probability was calculated for each release reach independently using a maximum likelihood function, except for the river mouth reach where survival and detection probability cannot be separated. At the river mouth we can approximate survival estimates by assuming that detection probability is 1 and therefore the product is a minimum survival estimate.

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

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

Where parameter vector $\boldsymbol{\theta} = (s_{g1}p_1, s_{g1}(1-p_1)s_{g2}p_2)$. First element of vector $\boldsymbol{\theta}$ (i.e., $\boldsymbol{\theta}_{(1)} = s_{g1}p_1$) means the probability that a fish from stage 1 survives to next stage, and also is detected at the next stage. The second element $\boldsymbol{\theta}_{(2)}$ (= $s_{g1}(1-p_1)s_{g2}p_2$) indicates the

probability that a fish from stage 1 survives to stage 2, is not detected at stage 2, survives from stage 2 to stage 3 and finally is detected at stage 3.

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

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

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

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

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

By definition, the likelihood function of parameters, $L(\theta, \lambda_g \mid n_{g12}, n_{g13}, n_{g23})$ is eq. 3.

Ignoring constants with respect to parameters, the likelihood function of parameters is

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

Note that this likelihood function has three parameters as variables: s_{g1}, p_1, and λ_g. For convenience of the calculation of maximum likelihood estimates (MLEs) of those three parameters and the variances, we take the natural logarithm for the likelihood function of eq. 4. The conversion to the log likelihood form is straightforward so we don't show it here. Finally, implementing the log likelihood function to software, Automatic Differentiation Model Builder (ADMB) (Fournier 2000), we differentiate the log likelihood function with respect to parameters to obtain the MLEs, and further calculate the Hessian matrix for calculation of the variances.

Results and Discussion

Capture and Condition of Kelts

There were a total of 190 kelts collected at CJMF and another 201 kelts at Lower Granite Dam (Table 1) for transport studies. All steelhead kelts captured for this portion of the study at CJMF on the Yakima River were unclipped and ranged in size from 50 to 75 cm fork length. The steelhead kelts captured for the transport study at Lower Granite Dam were a mix of clipped (53 released at Hamilton Island and 30 at Westport) and unclipped (57 released at Hamilton Island and 61 at Westport) and ranged in size from 51 cm to 84 cm with 13 (Hamilton Island) and 9 (Westport) "B-run" steelhead in the Snake River releases.

One half of the Yakima origin kelt steelhead were rated in excellent and good condition and the other half were judged in fair condition (Table 1). Sixty-two percent of the kelt steelhead collected in the Snake River were in excellent and good condition and 38% were judged as fair or poor (Table 1). Stock composition of this release was similar to the entire run (Chapter 3, Section C) with the majority of these fish likely coming from the upper Salmon River (51%) and the Grande Ronde River (23%).

Table 1. Condition ratings of steelhead kelts from the Yakima and Snake rivers collected and transported. (Includes PIT-tag only and acoustic/PIT-tagged fish).

	Yakima River	Snake River
Excellent	38	50
Good	57	74
Fair	95	56
Poor	0	21
Total	190	201

2011 Release Detection and Survival Estimates

A total of 188 fish were acoustic tagged, transported and then released. There were 94 fish tagged from both the Yakima and Snake Rivers with 47 fish tagged for each release location Hamilton Island and Westport Boat ramp. One acoustic tagged Snake River origin kelt released at Hamilton Island died during transport. Releases occurred between April 27/28th and May 5/6th, 2011 (Table 2). Most transport was via truck with the exception of the Snake River release at Hamilton Island, which was by barge.

The 187 acoustic tags released generated 47,739 detections spread over 29 receivers deployed in the lower Columbia River. In general, detections were similar among river reaches moving downstream until the final reach from the upper estuary to the mouth of the river where detections dropped substantially (Table 2). The Snake River group released at Hamilton Island had the least number of detections to the ocean (3 detected) of all release groups and locations this year (Table 3). The Hamilton Island release from the Yakima River showed a steady decline in detections moving downstream and then a large

drop in the estuary, whereas the Snake River origin kelts declined at a faster rate through the upper reaches, and a substantial drop in the estuary but the rate of loss between the upper estuary and ocean was slightly less for the Snake River origin compared to the Yakima River origin kelts (Figure 3). This suggests that fish from both collection areas were equally vulnerable to the mechanism causing the loss in the estuary. Five B-run kelt steelhead were included in the Hamilton Island release from the Snake River. Detections for these fish were: 5 at Bonneville; 4 at St. Helens; 2 at the Estuary; and, 0 to the Ocean. This depletion pattern is very similar to the Snake River release at large and is consistent with the notion that B-run kelts are functionally similar to their A-run cohorts.

To evaluate how the fish condition rating might relate to migration success, we plotted detection histories by condition ratings (Excellent, Good, Fair) for the releases at Hamilton Island. The Yakima origin fish showed similar detection histories among all condition ratings (Figure 4). Plotting the Snake River origin kelt steelhead (Figure 5) shows that fair condition fish performed relatively poorly. Removing the fair condition component from the Snake River release, results in a detection plot that is more similar to the Yakima origin release particularly for detections at St. Helens and the Estuary arrays (Figure 5). Previous studies have observed very low return rates to Lower Granite Dam of fish in the fair category (Keefer et al 2006. and Evans et al. 2008).

Detection to the ocean of steelhead kelts released at Westport was similar for Snake and Yakima River origin fish (Table 2). The rate of loss between the upper estuary and ocean was the same for both groups. This again suggests that fish from both collection areas were equally vulnerable to the mechanism causing the loss in the estuary. Four B-run kelt steelhead were included in the Westport release from the Snake River. One fish died during transport and detections for the remaining fish were: 3 at the Estuary; and, 1 to the Ocean. This depletion pattern is again very similar to the Snake River release at large as well as the Yakima origin release and is consistent with the notion that B-run kelts are functionally similar to their A-run cohorts.

Our array lines had minimal weather related or human caused disturbances. In the case of receivers outages they were replaced quickly usually within the week. We are confident that detection efficiency was high due to receiver array lines remaining intact during the weeks of releases.

Table 2: Acoustic detections of kelt steelhead from the Yakima River and Snake River transported and released below Bonneville Dam in 2011.

Origin	Release Location	Release Date	Type	Release #	# of Acoustic tags released	Detection at Bonneville Line	Detection at St. Helens line	Detection at Upper Estuary line	Mouth of Ocean
Yakima River (Prosser, WA)	Hamilton Is.	4/28/2011	Truck	1	47	47	45	43	16
Yakima River (Prosser, WA)	Westport Boat Ramp	5/5/2011	Truck	2	47	ND	ND	46	16
Total					94	47	45	89	32
Origin	Release Location	Release Date	Type	Release #	# of Acoustic tags released	Detection at Bonneville Line	Detection at St. Helens line	Detections at Upper Estuary line	Mouth of Ocean
Snake River (Lower Granite Dam)	Hamilton Is.	4/27/2011	Barge	1	47	46	35	25	3
Snake River (Lower Granite Dam)	Westport Boat Ramp	5/4/2011	Truck	2	46	ND	ND	44	14
Total					93	46	35	69	17
Grand Total					187	93	80	158	49

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

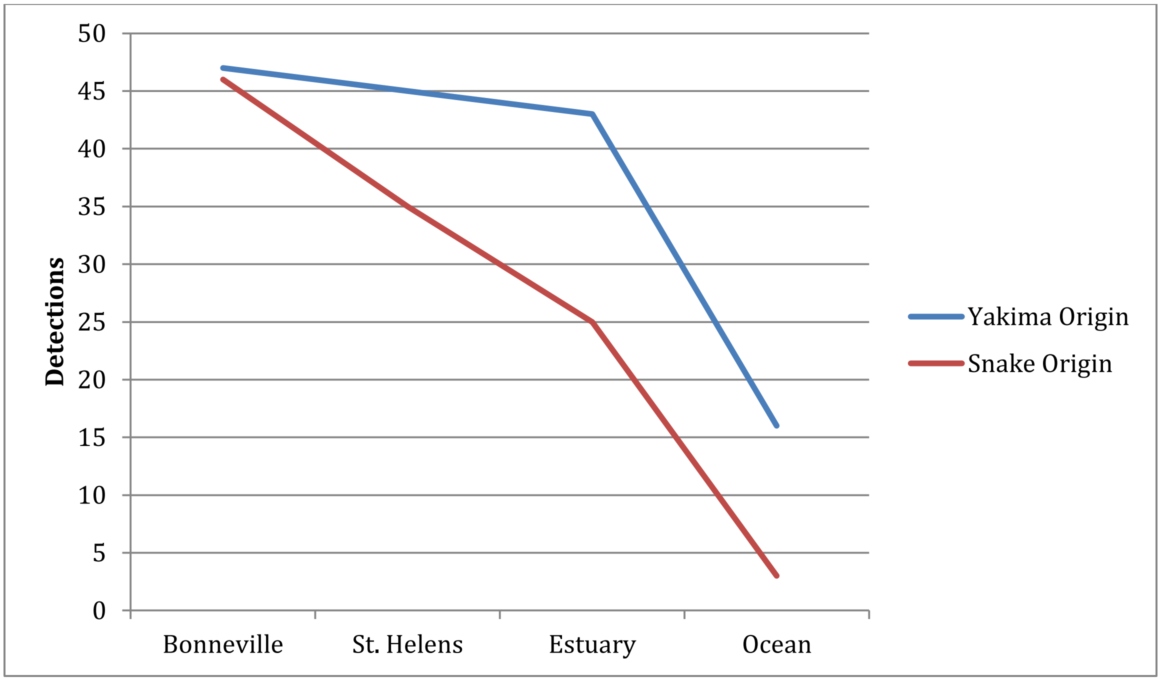

Figure 3. Detections at lower river array locations of acoustic tagged steelhead kelts released at Hamilton Island in 2011.

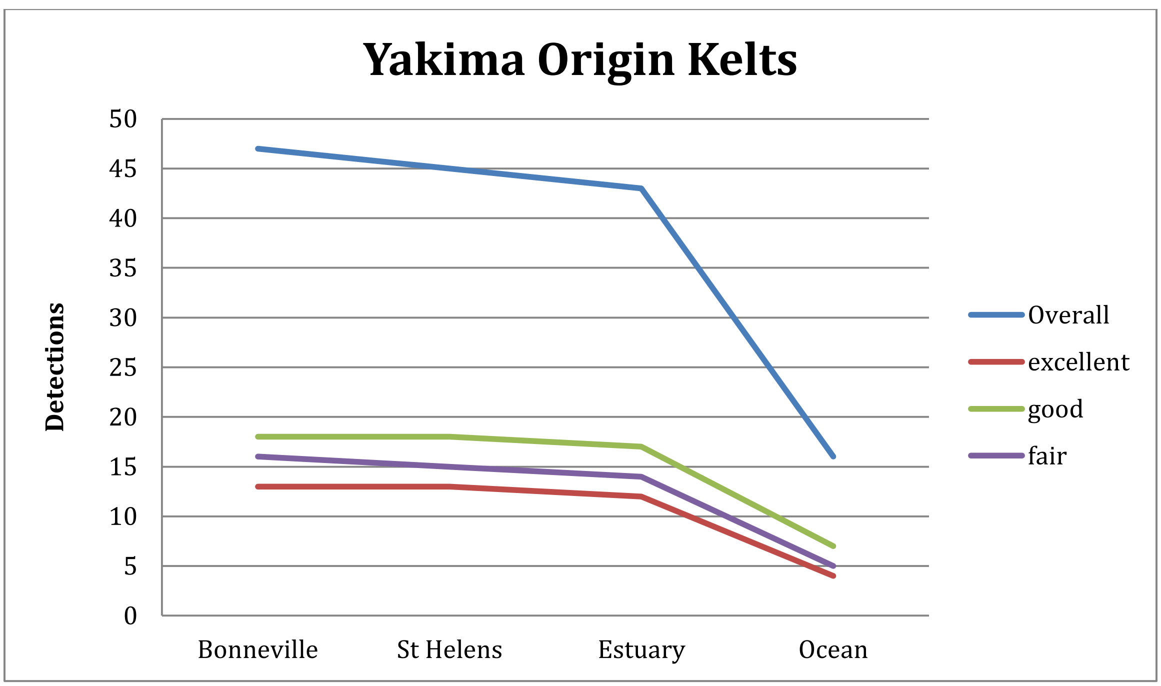

Figure 4. Detections at lower river array locations of acoustic tagged steelhead kelts with assigned condition ratings from the Yakima River released at Hamilton Island in 2011.

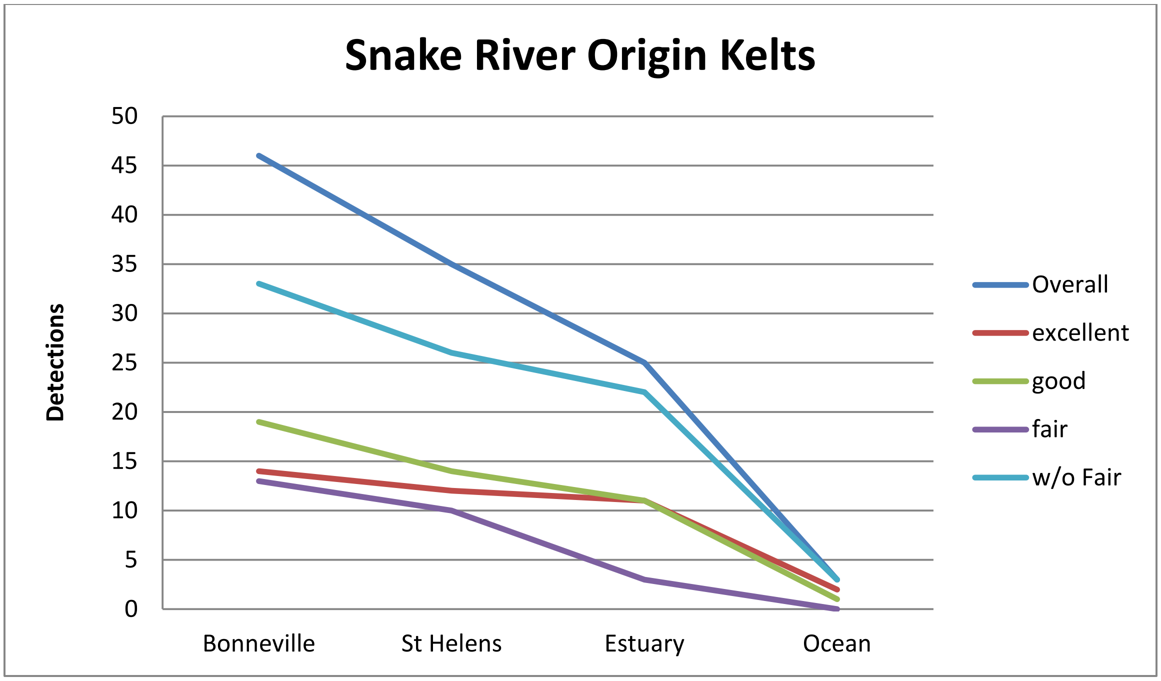

Figure 5. Detections at lower river array locations of acoustic tagged steelhead kelts with assigned condition ratings from the Snake River released at Hamilton Island in 2011.

Survival to the ocean was 34% for both Yakima River origin kelt steelhead releases (at Hamilton Island and Westport) (Table 3 and 4). Survival to the ocean was 6.5% and 30% for the Snake River origin kelts released at Hamilton Island and Westport, respectively (Table 3 and 4). Detection probability at the arrays was 100% for each location and release except at St. Helens for the Snake River release at Hamilton Island where detection probability was 96% (Table 3 and 4).

Survival of Yakima origin kelt steelhead transported and released at Hamilton Island was significantly lower than the 7 year average (Appendix B) for this group (34% verse 45%). This result is somewhat surprising due to presumably favorable out migration conditions. Columbia River flows at Bonneville Dam exceeded 500,000 cfs during a large part of the kelt migration period. In fact reach survival was very high to the estuary (Table 3), but high mortality was observed in the estuary. This heavy loss in the estuary is consistent with data collected by NOAA from tagging spring Chinook salmon (*Oncorhynchus tshawytscha*) (Rub et al. 2011). Rub et al. (2011) reported a spring chinook survival of 36% in the estuary reach in 2010 and 23% survival for the estuary reach in 2011. In recent reports by Bowles et al. (2010a,b) they suggest the possibility that acoustic tags may have been "heard" by some pinnipeds, making these tagged fish more vulnerable to predation. The frequency of coded "pings" emitted by the 69-kHz tags is within the upper audible range of harbor seals, and a relatively low-frequency "click" associated amplitude envelope of the ping may be audible at very close distances to both harbor seals and sea lions (Rub et al. 2011). As least 4 of our acoustic tags were last heard in the East Mooring Basin near Astoria, OR, which is the favored haul out site for pinnipeds in the area. Additionally, one tag was moving downriver and then made an abrupt and rapid return to Bonneville Dam.

This pattern is consistent with a pinniped predation event. Survival of the Yakima origin kelts released at Westport (Table 4) was significantly higher than last year (34% verse 22%). However, the heavy loss in the estuary is similar to losses experienced by the Hamilton Island release.

Survival to the ocean of Snake River origin kelt steelhead transported and released at Hamilton Island (6.5%) (Table 4) and was lower than the 2010 survival of 12% for the same release location (Appendix A). Survival to the ocean for the Westport release was also lower than the previous year (30% verse 40%). However, given the very low return rates of Snake River origin kelt steelhead left in the river (mean 0.58%), transporting fish to the estuary could be yield survival benefits.

Table 3. Survival and standard deviation (SD) by reach for Yakima River and Snake River kelts transported and released near Hamilton Island in 2011.

	Yakima River		Snake River	
Reach	**Survival (SD)**	**Detection Probability (SD)**	**Survival (SD)**	**Detection Probability (SD)**
Release to St. Helens	0.95 (0.02)	1 (0)	0.77 (0.06)	0.96 (0.03)
St. Helens to Estuary	0.97 (0.02)	1 (0)	0.68 (0.07)	1 (0)
Estuary to River Mouth (Survival x Probability)	0.36 (0.07)		0.12 (0.06)	

Table 4. Survival and standard deviation (SD) by reach for Yakima River and Snake River kelts transported and released near Westport, OR in 2011.

	Yakima River		Snake River	
Reach	**Survival (SD)**	**Detection Probability (SD)**	**Survival (SD)**	**Detection Probability (SD)**
Release to Estuary	1 (0)	1 (0)	0.91 (0.04)	1 (0)
Estuary to River Mouth (Survival x Probability)	0.36 (0.07)		0.33 (0.07)	

Kelt Migration Rates

Migration rates were calculated for all kelt steelhead that successfully passed through each river reach. Based on the kelts that successfully migrated to the ocean, the Snake River fish released at Hamilton Island moved the quickest on average 3.8 km/hr (Bonneville to St. Helens) and 4.2 km/hr (St. Helens to Estuary) (Figure 6) compared to Yakima origin fish. This group of fish also had the highest resident time at the Bonneville array with just under an average of 36 hours and then the shortest residence times at each of subsequent arrays downriver (Figure 7). Yakima origin kelts released at Westport moved faster than Snake River fish (Figure 6). At the Westport releases the Yakima River kelts took on average 20 hours longer in the upper estuary array area than Snake River origin kelt steelhead (Figure

7). The Snake River Westport release group had an extended residence time at the river mouth with kelts spending on average almost a day (~ 20hrs) around the mouth before heading entering the ocean (Figure 7).

The total migration rate to ocean (from release to the ocean) for all groups was faster than the 2010 groups by almost double (Branstetter et al 2011). This was likely due the higher Columbia River discharge in 2011.

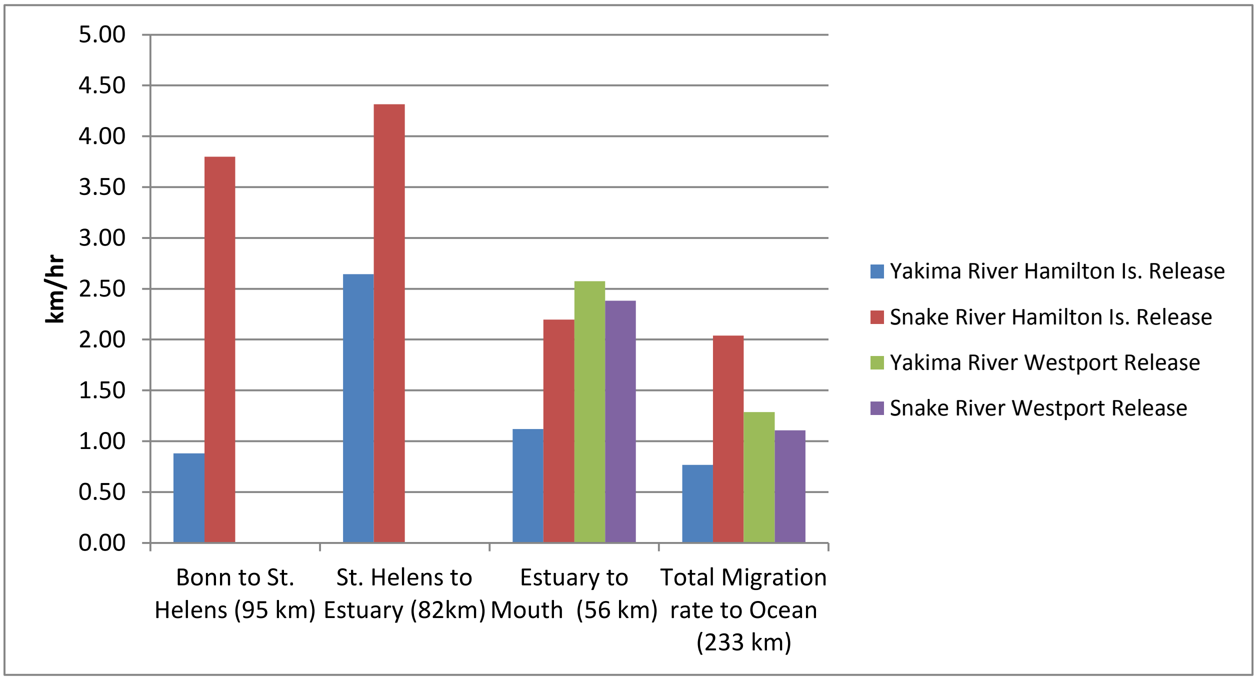

Figure 6: Average travel rate through each river reach for ocean migrators (km/hr). Total migration rate to the ocean includes resident times at arrays.

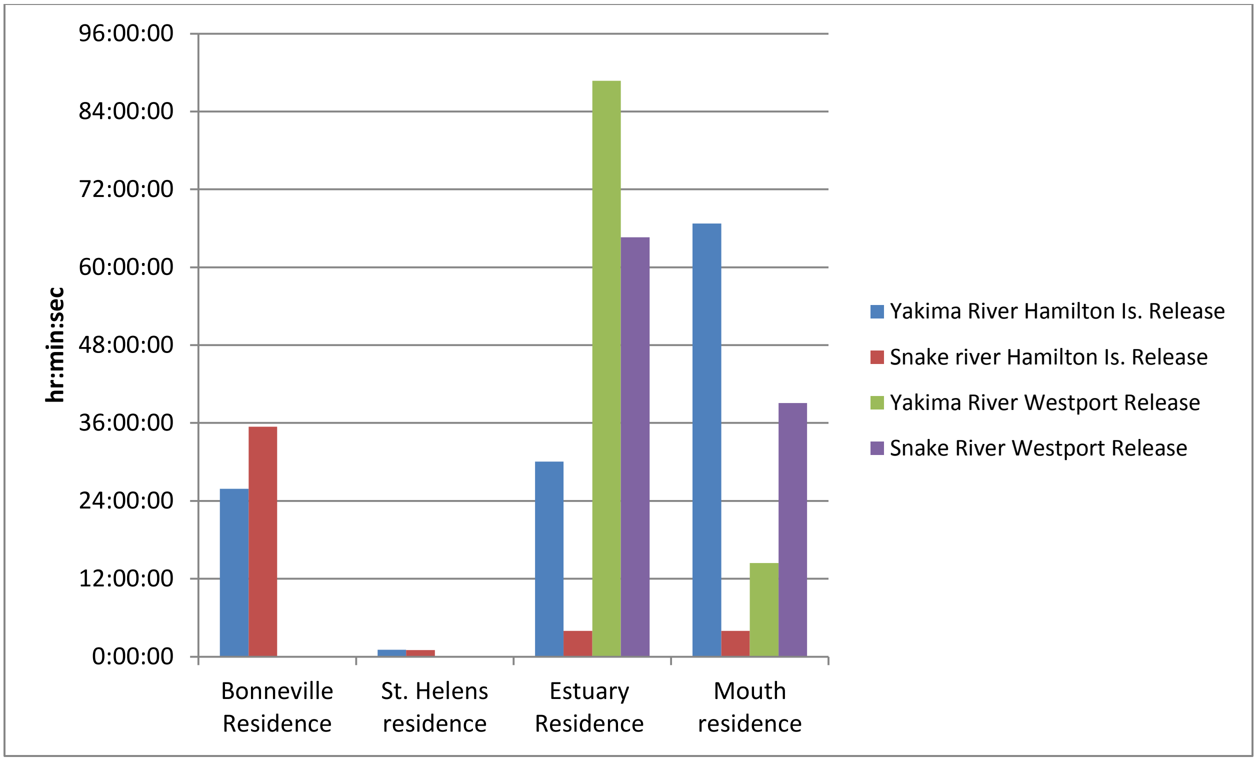

Figure 7: Average residence times at each array for the different releases for ocean migrators (hr:min:sec).

When we compare migration rates for kelt steelhead that were detected at the ocean with fish that weren't, we find generally the same pattern with Yakima origin fish moving slower than Snake River fish (Figure 6 and 8). However regarding the non-ocean migrators in the St. Helens to Estuary reach, the Yakima River fish moved faster than those the successfully reached the ocean and the Snake River fish moved slower than their successfully migrating cohorts.

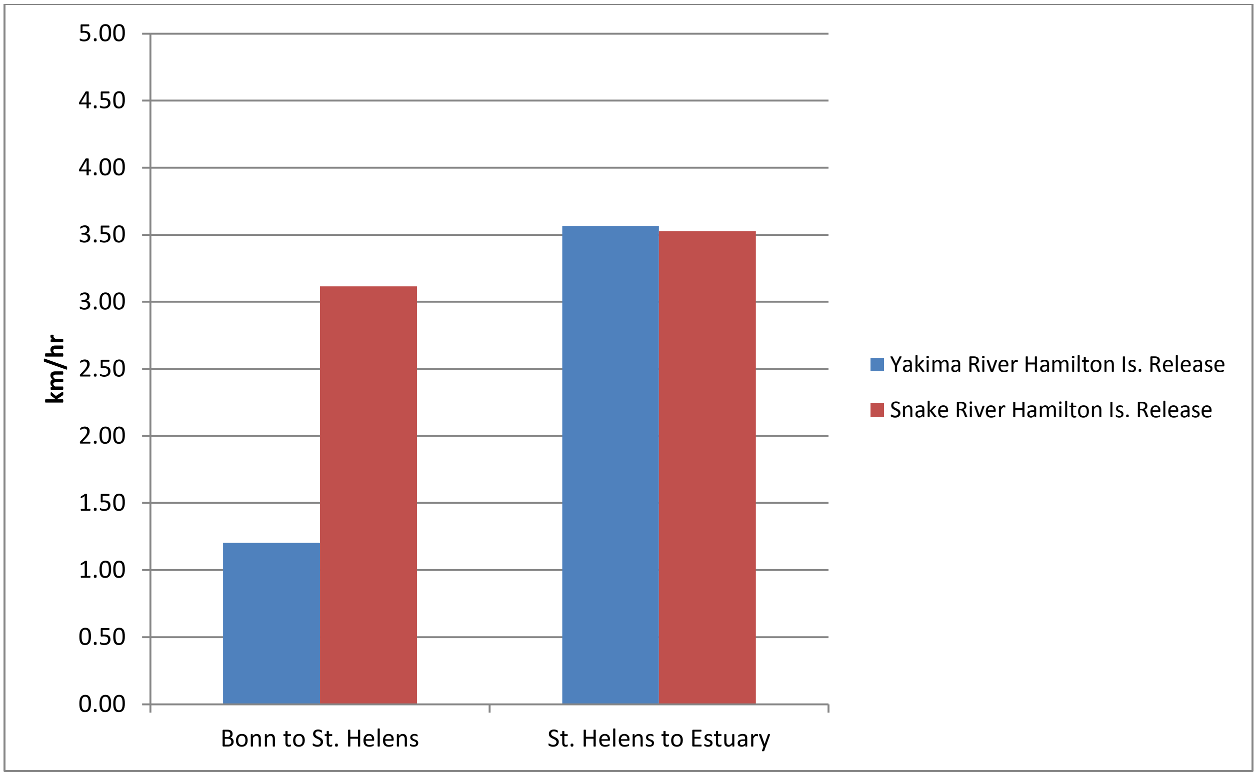

Figure 8: Average travel rate through each river reach for non-ocean migrators (km/hr).

Condition and Migration Rate

In the past we have observed that successful kelt emigrates typically travel more slowly than unsuccessful emigrates. Yakima origin kelt steelhead with different condition ratings had fairly similar migration rates and detection rates to lower river arrays (Table 6). Snake River origin kelt steelhead with different condition ratings differed in migration rate and detection rates to the lower river arrays (Table 6). Reviewing the same data for the Westport releases reveals no obvious differences among kelt steelhead with different condition ratings (Table 7).

Table 6. Condition ratings of kelt steelhead collected at Prosser Dam (Yakima River) and at Lower Granite Dam (Snake River) and released at Hamilton Island.

Release Location: Hamilton Island (233 rkm)						
Fish Origin:	**Yakima River**			**Snake River**		
Condition	**Number of Kelts at Release**	**Number of Kelts Detected to Ocean**	**Avg travel rate to ocean (km/hr)**	**Number of Kelts at Release**	**Number of Kelts Detected to Ocean**	**Avg travel rate to ocean (km/hr)**
Excellent	13	4	0.65	14	2	1.00
Good	18	7	0.63	20	1	2.13
Fair	16	5	1.35	13	0	ND
Total	47	16		47	3	

Table 7. Condition ratings of kelt steelhead collected at Prosser Dam (Yakima River) and at Lower Granite Dam (Snake River) and released at Westport.

Release Location: Westport (56 rkm)						
Fish Origin:	**Yakima River**			**Snake River**		
Condition	**Number of Kelts at Release**	**Number of Kelts Detected to Ocean**	**Avg travel rate to ocean (km/hr)**	**Number of Kelts at Release**	**Number of Kelts Detected to Ocean**	**Avg travel rate to ocean (km/hr)**
Excellent	18	5	0.41	17	5	0.28
Good	12	5	0.37	23	7	0.25
Fair	17	6	0.24	6	2	0.28
Total	47	16		46	14	

2010 Acoustic Release Return Detection

Two Snake River origin tags were detected at the river mouth in June and July that may have been previously tagged kelts attempting to return but they were only single detections with no subsequent upstream detections so these cannot be considered reliable detections.

PIT-tag Detections From Returning Fish

All kelt steelhead in the transport studies receive a PIT tag prior to release. Detections of these tags at Bonneville are used to calculate return rates for treatments. During 2011, two PIT tag detections were recorded at Bonneville Dam. One detection was from a fish released in 2010 that was a Yakima origin kelt Hamilton Island release. The other detection was a 2011 Yakima origin estuary (Westport) release. These detections result in return

rates to Bonneville Dam for transported fish of 0.81 for the 2010 Hamilton Island release and 1.11 for the 2011 Westport release. These return rates are very poor compared to previous years (Appendix B).

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Section C. Long-term Reconditioning Treatment

Introduction

Long-term reconditioning is the process where kelt steelhead are collected during their seaward migration in the spring, held and cultured in a large tank, and released in the fall of the same year as the steelhead run is returning from the ocean. Long-term reconditioning is the most intensive and successful kelt steelhead treatment that we have evaluated. We are conducting studies of long-term reconditioning at 4 locations with fish collected from the Yakima, Snake, Hood, and Okanagon (Omak Creek) rivers. This details results of long-term reconditioning studies at each of these locations in 2011.

Study Area

Prosser Hatchery

Prosser Hatchery is located on the Yakima River just downstream of Prosser Dam at (Rkm) 75.6. This facility is part of the The Yakima/Klickitat Fisheries Project, a supplementation project designated by the NPPC as the principle means of protecting, mitigating, and enhancing the anadromous fish populations in the Yakima and Klickitat Subbasins. Prosser Hatchery was constructed in 1994 with a primary function of rearing, acclimating, 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 (Figure 1). Sturgeon and lamprey are also experimentally reared at this location.

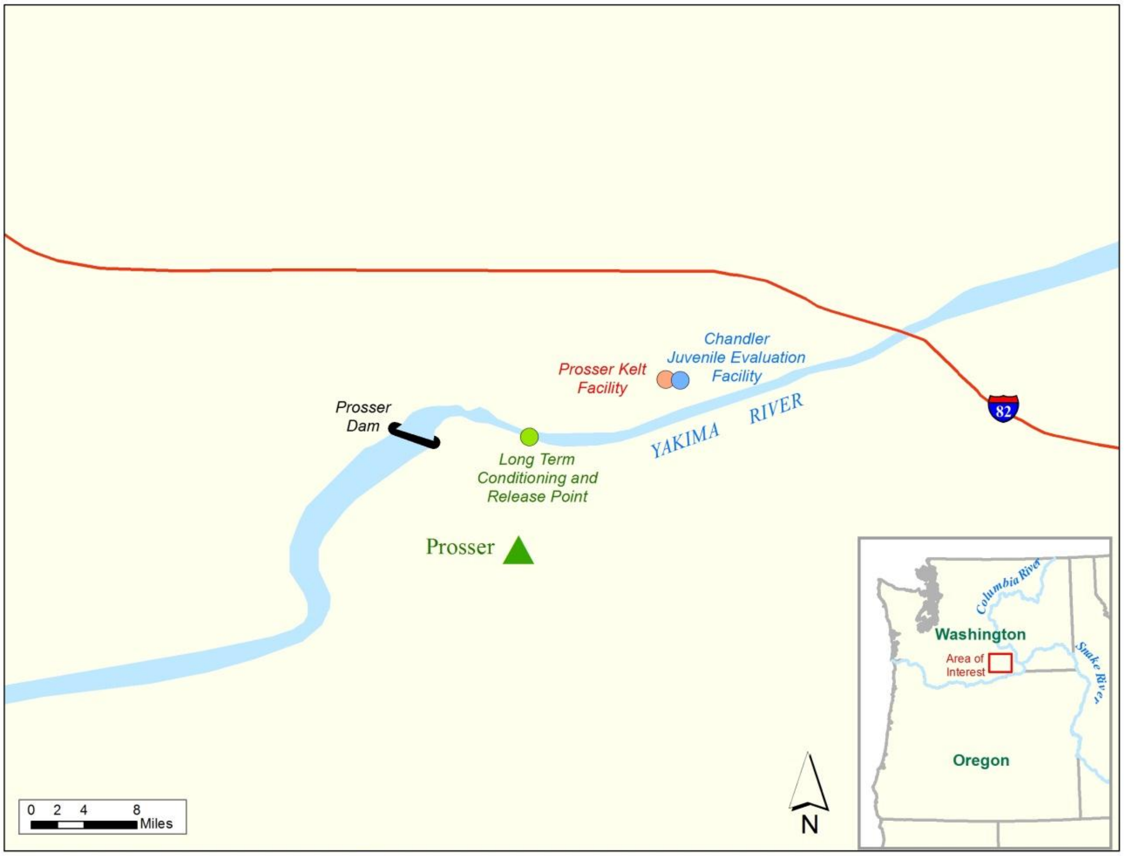

Figure 1: Map showing the location of Prosser Dam and the kelt reconditioning facility at Prosser, WA.

Dworshak Fish Hatchery

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

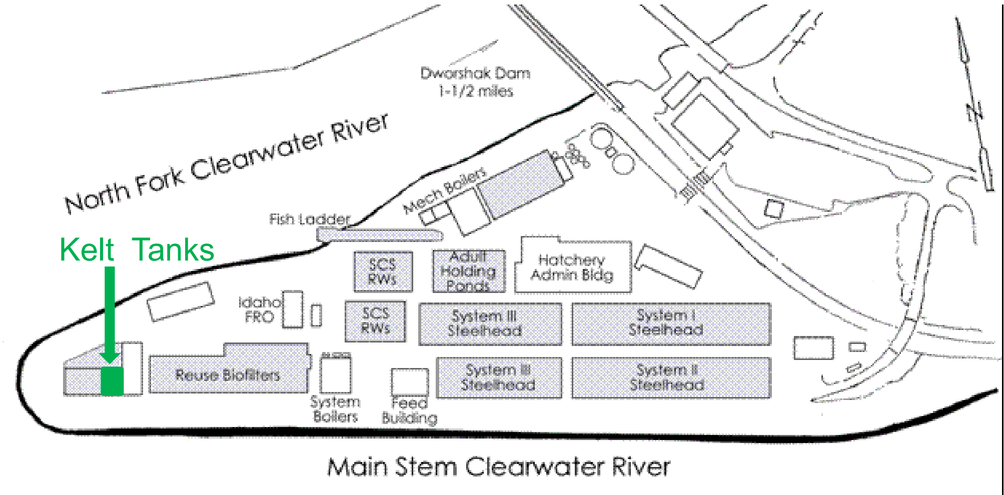

Figure 2: Map showing the location of experimental kelt reconditioning tanks at Dworshak National Fish Hatchery. Figure modified from USFWS 2009.

St. Maries's Mission Acclimation Pond

Omak Creek kelt steelhead were reconditioned at the St. Maries acclimation pond located at RM 5.0 of Omak Creek below Mission Falls near the town of Omak (Figure 3). The Colville Confederated Tribes operate the facility and this was the first year reconditioning kelt steelhead at the site as the prior reconditioning site was decommissioned. The facility was originally constructed in 2002, as a spring chinook acclimation facility. Spring chinook smolts are acclimated on site from March until release in April.

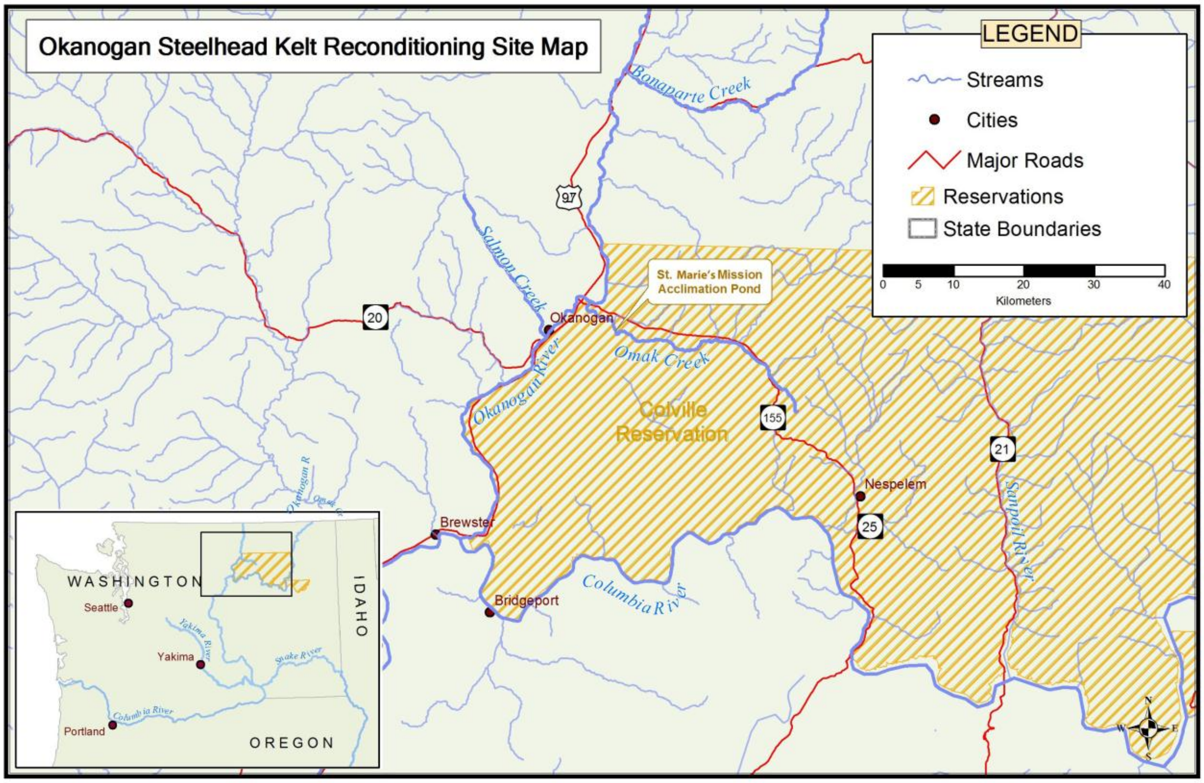

Figure 3: Map showing the locations of Omak Creek, the St. Marie's Mission Acclimation Pond, and the Confederated Tribes of the Colville Reservation.

Parkdale Fish Facility

Steelhead kelt reconditioning for the Hood River was performed at the Parkdale Fish Facility located at Rkm 5.6 on the Middle Fork of the Hood River (Figure 4). The Bonneville Power Administration originally built the facility in 1998 as an adult holding facility for ESA listed summer/winter steelhead and spring Chinook (reared at Oak Springs). Currently, the facility produces winter steelhead (reared at Oak Springs) and spring Chinook (reared at Parkdale and Oak Springs) for supplementation and terminal fisheries (non-indian sport, Tribal commercial and Tribal subsistence fisheries). The facility is co-managed by Confederated Tribes of the Warm Springs Reservation and Oregon Department of Fish and Wildlife.

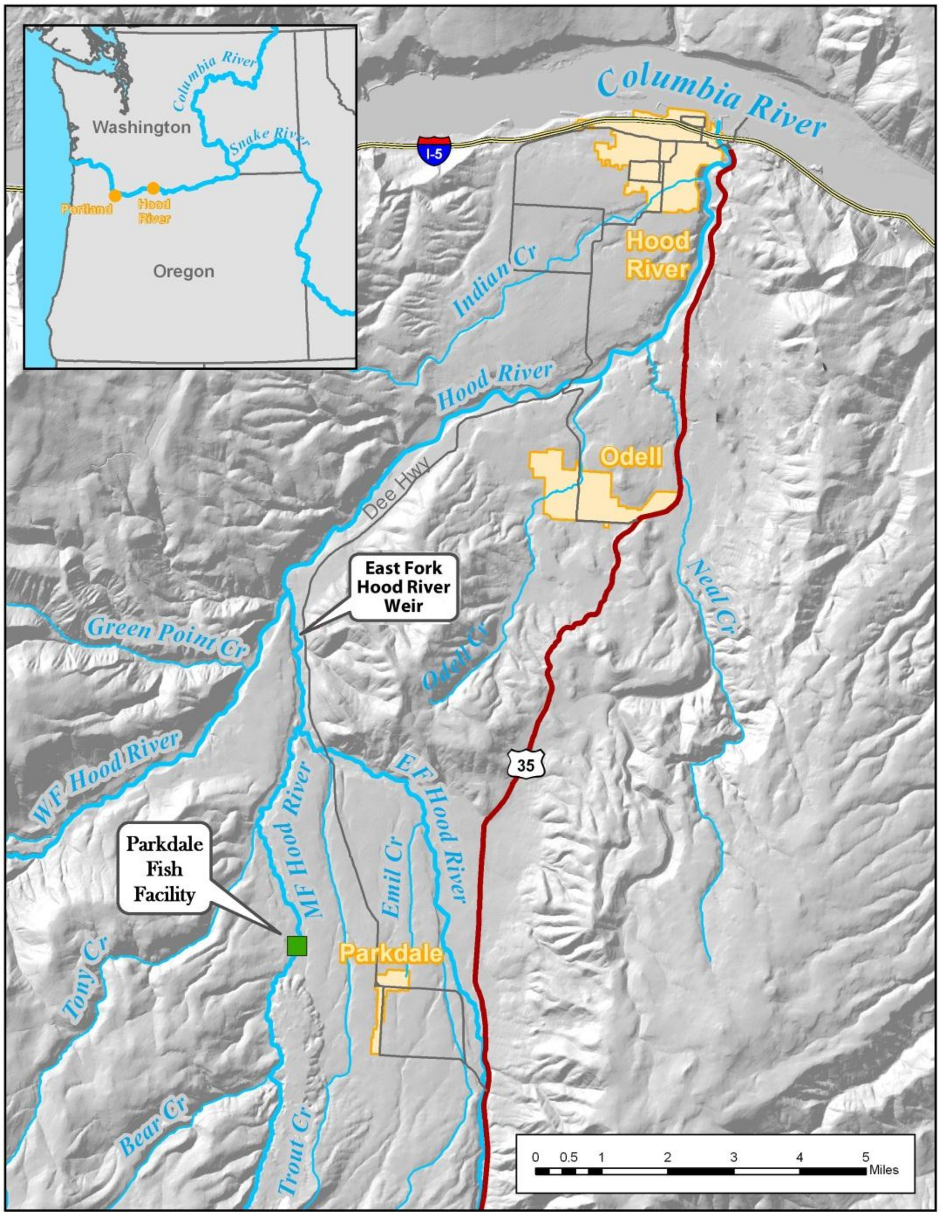

Figure 4: Map showing the location of Parkdale Fish Facility and East Fork Hood River weir.

Methods

Long-term Reconditioning Facilities

Prosser Hatchery

Transport to Prosser Fish Hatchery

Kelts are captured at the CJEF and then placed in a holding tank (Section A). 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 in good condition was 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) (Section A). Kelts in the holding tank are dip netted and placed into a trailer-mounted tote and moved by a Kawasaki mule to the hatchery (Figure 5). Steelhead kelts retained for the long-term reconditioning treatments were held in one of four 6.096 m (d) x 1.219 m (h) circular tanks (Figure 5). Loading densities were approximately 2/3rd of the 300 fish carrying capacities of these tanks. Tanks were fed oxygenated 13.8^{0}C well water at 757 liters/minute.

Figure 5: Steelhead kelt reconditioning tanks at Prosser Hatchery, Prosser, WA.

All kelts held for an extended period of time in reconditioning tanks are susceptible to severe infestations of parasitic copepods, which can be lethal to cultured fishes in confined environments. The parasitic copepod *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. For parasite control fish received a treatment of Ivermectin™ which is used throughout the Columbia River Basin for copepod control (Johnson and Heindal 2000). The Ivermectin™ was diluted with saline (1:30) and injected into the fish's esophagus using a small (1cc) plastic syringe. In 2011, we suspected that a portion of the fish mortalities were likely a result of Ivermectin toxicity. The CRITFC and Yakama Nation initialized a small trial of emamectin benzoate that was administered via injection to the peritoneal cavity for the treatment of copepods (Glover et al 2010). Study results are presented in Chapter 3, Section B.III. All fish held for long-term reconditioning received an intramuscular injection, based on weight, of the antibiotic oxytetracyclin.

Another health concern for fish that may have dermal abrasions, lesions, or lacerations is the increased chance for fungal infections. Untreated, fungal infections can be lethal to kelts that have weakened immune systems that normally would be able to fight off such infections. The drug Formalin is administered approximately five times a week (depending on fungal growth) at 1:6,000 for 1 hour in all reconditioning tanks to treat and prevent fungal outbreaks in cultured kelts.

Dworshak National Fish Hatchery

Transport to Dworshak from Lower Granite Dam

Fish destined for DNFH were dipped netted from the adult holding tank at Lower Granite Dam and placed in a transport truck. Nets were large enough to handle active adult steelhead and consisted of a soft cotton or natural fiber mesh. The transport truck had a 1.514-kiloliter tank fitted with supplemental regulated, compressed oxygen that was fed via air stones; also a 12-volt powered tank aeration pump was used to circulate oxygenated water. Stress Coat® or PolyAqua® was used to replace the natural protective slime coating that may have been compromised by handling. In addition, salt was added to reduce osmoregulatory stress. Temperature and dissolved oxygen levels were monitored during transport. Loading densities were kept to a minimum; no more than 20 kelts were transported at one time.

On-Station Air Spawning

Due to the difficulties collecting an adequate number of kelts from the LGR JFF in 2010, additional steelhead were collected from the adult ladder at DNFH. Steelhead were crowded into collection baskets and anesthetized in tricaine methanesulfonate (MS-222). CO_2 was not used because it presents sub-lethal stresses that were likely to be adverse to survival of the kelts. Sorted steelhead were emptied on to a large stainless steel table and assessed by observing several physical factors prior to being selected for air spawning and reconditioning. Fish health was evaluated by: 1) maturation level - only very ripe females 2) morphological fitness – no physical injuries on the body surface, no obvious fungus present, no fin rot or head burn.

Selected fish were transferred to an area set aside for the air-spawning procedure. Air-spawning was conducted following a technique demonstrated by CTWSRO staff. Low-pressure compressed air was injected into the fish using a 20-gauge needle. Eggs were

allowed to flow freely or gently massaged out. Each female's eggs were collected in a bucket with a distinct identification tag and given to DNFH for fertilization and incubation. Standard fish health sampling occurred on these fish to meet the DNFH spawning criteria routinely employed at the hatchery, this included ovarian fluid and genetic sampling.

While sedated, fish were sampled for blood, body lipid levels, PIT tagged and photographed. Blood (1.5 – 2 ml) was drawn from the caudal vessels using sterile 18 gauge, 1.5 inch needles fitted to heparinized syringes. Body lipid levels were measured by applying a Torrey Fish Fatmeter to the outside of the fish. Tagging needles and PIT tags were disinfected before each use by soaking them in 70% ethyl alcohol, and subsequently dried. A 20 mm PIT tag was inserted with a sterile 8-gauge trocar midway between the pelvic fins. Length and weight were recorded. Fish received an injection of oxy-tetracycline. In addition, 50% of the fish were force-fed a slurry of fresh frozen cyclop-eeze using a gastric gavage tube and syringe (Chapter 3, Section B.V). After sampling, each fish was placed in a recovery tank for observation prior to transfer to the kelt reconditioning tanks.

Reconditioning Facility and Treatment

Four 15-foot diameter tanks are located at DNFH (Figure 6). River water was provided from a fire maintenance line at a flow rate of 50 gpm per tank. Tank outflows are plumbed to the DNFH settling pond. Tanks are outfitted with both an internal standpipe and an external vented vertical loop to control water level. A four-bucket Koch ring packed column-degassing system supported by external posts is installed on the inflow to each kelt tank. An aeration system is also installed. Flow, temperature, and dissolved gas levels were constantly monitored using a hydrolab.

A prophylactic treatment oxy-tetracycline is administered to all kelts when transferred to the tanks. Formalin treatments (baths) are applied routinely to control fungus. Feeding begins after initial sampling. Fish are first presented with krill until the feeding response is well established. Then fish are given a higher lipid content kelt/broodstock feed.

Figure 6: Experimental kelt reconditioning tanks at DNFH with anti-jump containment curtains and four bucket Koch ring packed columns.

St. Marie's Acclimation Pond

Transport to St. Maries

Kelts collected for reconditioning are captured exiting Omak Creek at, or above the trap site and transported to the St. Maries Acclimation Facility. Fish were transported by 400-gallon tank truck to St. Maries's from the Omak Creek weir. Polypond water conditioner was used to protect slime coat and the addition of 3% salt was used to help calm fish.

Reconditioning Facility and Treatment

St. Maries Mission Pond is 21.945m (l) x 3.657m (w) x 1.219 (h) this pond is served by a screened gravity feed from Omak Creek and a well that delivers 11^{0}C water up to 2.082 kpm (Figure 7). The top is covered in a shade cloth (60% reduction) to reduce stress and assists in algal reduction. Salt was placed at the head of the pond to prevent against fungus and to reduce algea growth in the pond. An initial injection of liquamyacin was

administered based on recommended WDFW rates. Every 2-months, fish were checked for copepods, and given ivermectin solution if copepods were present. Feed consisted of Squid, prawns, and sand shrimp with a top coating of Vitamin C to provide for immune system enhancement.

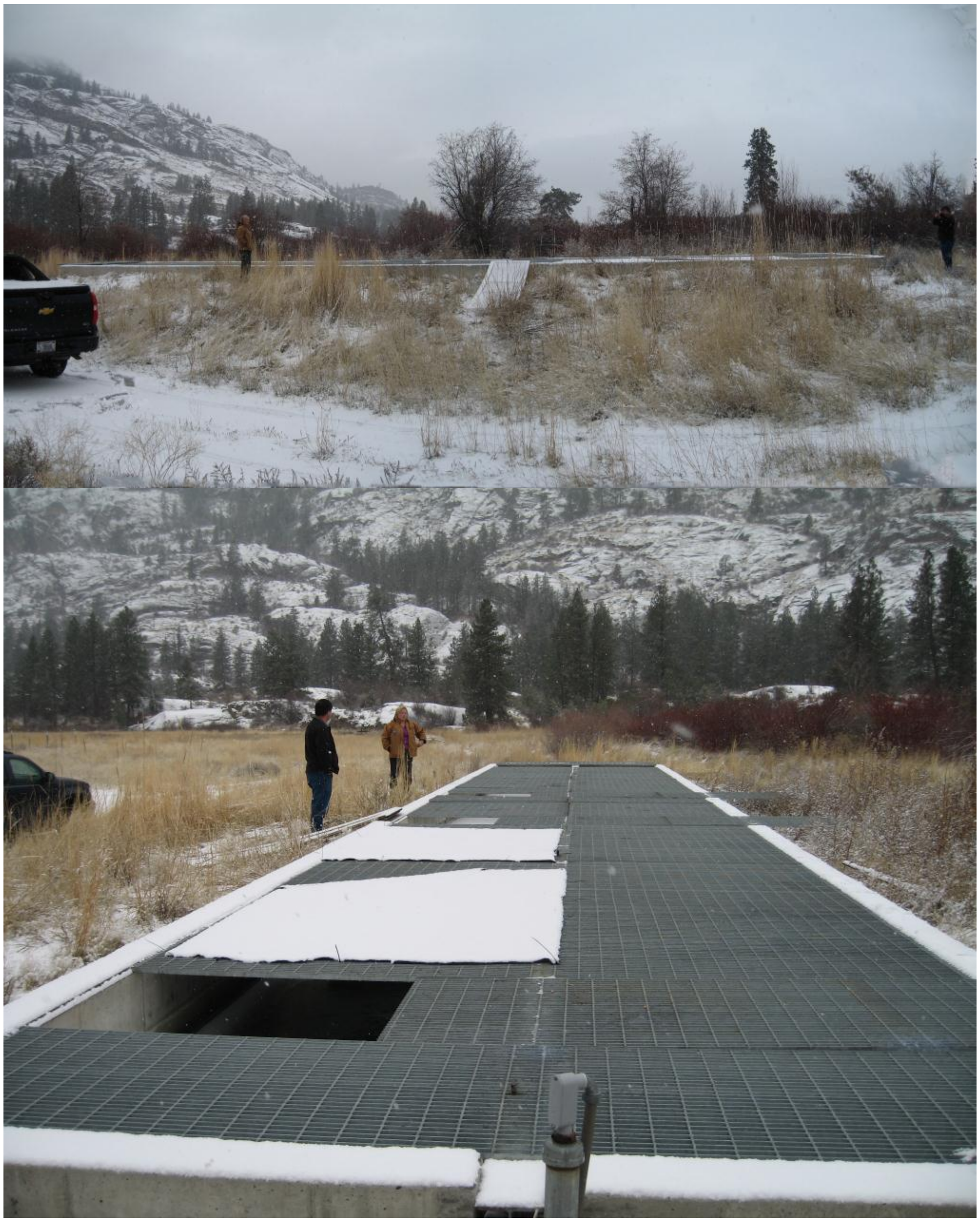

Figure 7: St. Maries Acclimation Pond Omak, WA. Top (side profile), bottom (above)

Parkdale Fish Facility

Transport to Parkdale Fish Facility

Adult steelhead were collected at the East Fork of the Hood River located at Rkm 20 south of the city of Hood River, Oregon by the Oregon Department of Fish and Wildlife (ODFW). Fish were transported by truck to the Parkdale Fish Facility.

Reconditioning Facility and Treatment

Different than the other reconditioning locations, maiden steelhead are collected at Hood River, held and air spawned at Parkdale Hatchery and then reconditioned. Winter and remaining Skamania (collection ended in 2010) summer- run steelhead kelts are placed into a 12.192m(l) x 2.438m (w) x 1.219m (h) raceways at 1.514 kpm until ripened and ready for spawning (Figure 8). 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 (1.219m (h) x 3.048 (d)), segregated by run, with water flow at .227 kpm for reconditioning and held until late September when they were placed into the raceway for the duration of the winter season until the following year's spawning (Figure 9). Post spawn females were administered another dosage of Ivermectin after completion of air spawning. Fish were checked again in late spring (May) for the presence of copepods and administered additional Ivermectin treatment if copepods were present. Steelhead kelts from earlier brood years were given Ivermectin and antibiotics in September after chinook were moved from nurseries and raceways disinfected.

Figure 8: Parkdale Fish Facility raceways where kelts are held from late fall to early spring.

Figure 9: Circular tanks at Parkdale Fish Facility used seasonally (late spring to early fall) for reconditioning kelts.

Feeding

Modified versions of the feeding and holding protocols developed at Prosser Hatchery are utilized for long term reconditioning at Dworshak Hatchery, St. Maries Acclimation Pond, and the Parkdale Fish Facility (Hatch et al. 2004). Generally, at most all sites, kelts are offered parboiled flash frozen Antarctic krill (*E. superba*), 3 times daily and fed to satiation. The krill is fed to fish for approximately 4 to 6 weeks and depending typically on the timing of incoming kelts. Kelts then are transitioned to a pellet based diet, which is typically a modified Moore-Clark trout broodstock pellet (slower sinking rate). Pellets were administered 3-5 times daily at a rate of 1-2% body weight or until fish seemed satiated. Hatchery managers and project staff are allowed to modify protocols as needed to improve survival. The typical difference at the multiple locations is variation in feeding rate along with the duration of natural feed. At St. Maries natural food is primary food type fed to kelts, while at Parkdale, natural feed is offered throughout the reconditioning process. These facilities also offer additional natural feed types such as squid, additionally at St. Maries they top coat feed with cod liver oil. Fish at Parkdale are fed Bio-Oregon Biobrood pellets instead of the Moore-Clark pellets.

Kelt Mortalities

On discovery of a mortality, fish were collected, scanned for PIT tags, recorded in the database, 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 sanitized and reused viable acoustic tags whenever possible. The Lower Columbia Fish Health Center (Prosser), Washington Department of Fish and Wildlife Pathology (Omak), Oregon Department of Fish and Wildlife Pathology (Parkdale) provided disease-monitoring services to insure the health of reconditioned steelhead kelts.

Steelhead Kelt Status and Release

All surviving kelts, prior to release in late fall were scanned for PIT tags, weighed, and measured for fork-length. Reconditioning success was based on the proportion of fish that survived the reconditioning process. Reconditioned kelts were classified as either feeding or non-feeding at the status check based on weight change. Long-term reconditioned fish located at the Prosser Fish Hatchery 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 that are actively migrating to spawning grounds. At Dworshak any unclipped (wild) kelts that survive reconditioning are released in the fall. Fish in the long-term experiments at St. Marie's are released approximately at Rkm 1 of the Okanagon River. Based on previous releases the 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. Fish were then released to the river to coincide with fall spawning migration and others were retained or terminated. PIT tag antenna arrays were monitored for subsequent migration data. The only non-release groups are the Parkdale Fish Facility Steelhead and any hatchery fish reconditioned at Dworshak, which were retained or terminated for important physiological data for future indices or fish health reasons. Please See Chapters 2 (Section A) and 3 (Section B) for further details.

Results

Long-Term Reconditioning and Survival to Release or Spawning

Prosser Fish Hatchery

There were 223 (32.8%) of the total collected (n=680) for the long-term reconditioning that survived to the pre-release processing on October 13, 2011 (Table 1)(Figure 10). A total of 136 long-term reconditioned fish were released to the Yakima River on October 26, 2011. The remaining fish were retained for additional experiments conducted at Prosser facility. Steelhead kelts were released at a later date than usual, due to the presence of a WDFW fishery near the release site. This release site is useful for determining parentage analysis (See Ch 2. Section C). Most migratory movements occurred in late October through November but there were additional kelts detected migrating upriver in February/March of 2012. . As of May 2012, 58 (42.6%) fish from the long-term release were detected by PIT tag presence migrating past Prosser Dam.

Table 1: Long-term reconditioning results by tank 2011 at Prosser Hatchery.

Long-term Reconditioning									
	Tank								Long-term
	C1	C2	C3	C4	S1	S2	S3	S4	Total
Held for Reconditioning	150	150	149	149	21	21	20	20	680
Suviving fish on 10/13/2011	49	68	30	49	9	4	4	11	223
Survival Rate	32.4%	45.3%	19.9%	32.7%	42.9%	19.0%	20.0%	55.0%	32.8%

Figure 10. Long term reconditioned kelt steelhead from the Yakima River just prior to release.

Skip Spawning Long-Term Reconditioned Kelts

A skip spawning migration pattern was observed in 13 previously long-term reconditioned kelts from 2010 that had PIT-tag detections at Bonneville Dam in the summer/fall (July – September) of 2011. Later 11 of these fish were detected at Prosser Dam. The Prosser detections migrated in two pulses, a fall and a spring group. This same distribution is demonstrated annually by the steelhead run at large. The majority of these fish detected at Prosser (7 kelts) had no upstream detections after release in 2010. It is assumed that these fish, instead of spawning that year, migrated to the ocean to continue reconditioning (natural) and presumably spawned in the winter/spring of 2012. We have previously noticed this type of behavior in PIT-tag records. The remaining 4 kelts were previously detected at Prosser Dam following their release in 2010. Presumably these 4 fish spawned in 2011 and then successfully emigrated to the ocean where they naturally reconditioned. If so, this would be the 3rd spawning event (minimum) for these fish. This sequential spawner strategy has been observed previously in long-term reconditioned fish at Prosser, as well as at Parkdale.

Dworshak National Fish Hatchery.

Reconditioning

Lower Granite Juvenile Fish Facility Steelhead

A total of 111 fish were transferred from the LGR JFF to DNFH for reconditioning (Table 2). Fish survival averaged less than four days after transfer to the reconditioning tanks. On June 22, 2011 a valve was discovered partially open that allowed domestic (chlorine treated) water to enter the kelt tanks. After the valve was closed, the average fish survival increased (Figure 11). Unfortunately, only 6 new fish were transferred after June 22, 2011. Of these, 2 (unclipped) were released to the Snake River in the fall of 2011 to coincide with the returning steelhead run.

Table 2: Snake River steelhead kelts collected at LGR JFF and transferred to DNFH for reconditioning in 2011.

	A-run	B-run	Total
Adipose Clipped	4	5	8
Un-Clipped	37	65	103
Total	**41**	**70**	**111**

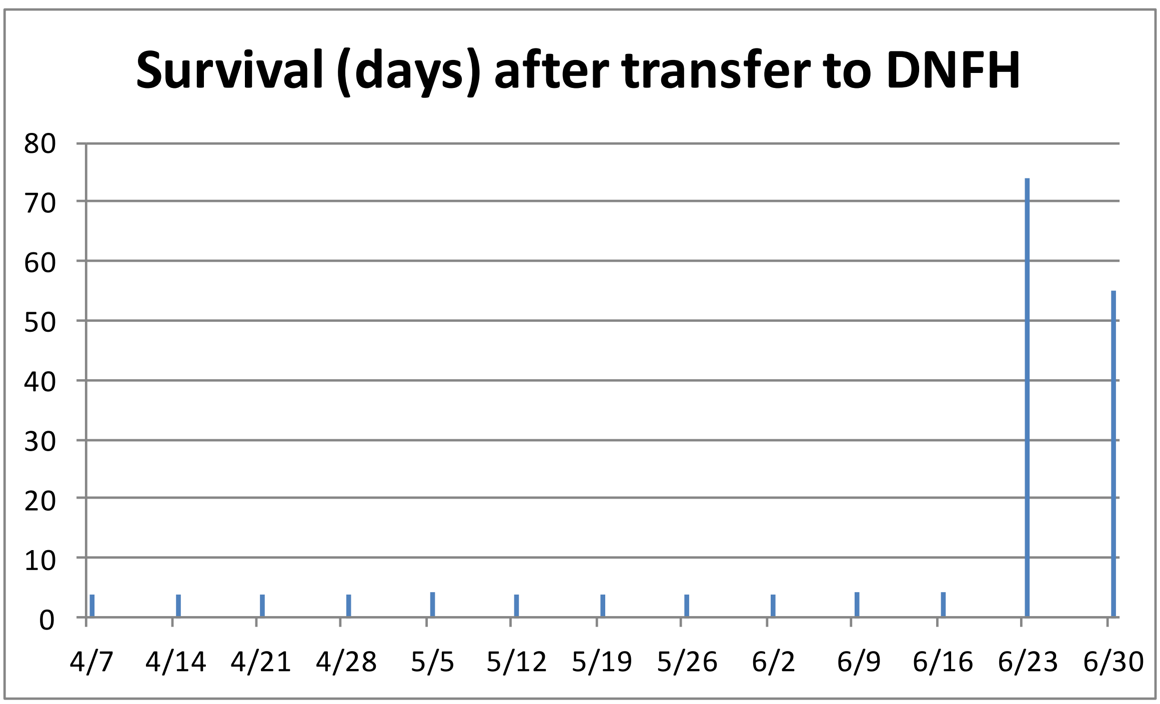

Figure 11: Mean weekly survival (days) of steelhead kelts transferred from LGR JFF to DNFH for reconditioning in 2011.

Air-Spawned Steelhead

A total of 92-steelhead were air-spawned. Fifteen fish were collected on January 11, and 77 were collected on February 1, 2011. Eggs from the first fifteen fish were incorporated into DNFH production program. Eggs from 73 of the remaining fish were outplanted as eyed-

eggs on March 5, 2011 into several sites within the Lolo Creek drainage in the Clearwater subbasin of Idaho.

On March 28, 2011, DNFH conducted a shutdown of the North Fork Clearwater River main pump that supplies the hatchery with river water. This river pump is responsible for charging the fire maintenance line that supplies the kelt tanks with water. In order to maintain flow to the kelt tanks, a portable diesel pump was employed with over 100 feet of 3 inch hose (Figure 12). This portable pump was successful in supplying water to the kelt tanks for several days; however, it required careful monitoring. As a result of pumping directly from the river, the tanks had high turbidity and sediment build-up.

Figure 12. (A) Portable diesel pump along the north bank of the mainstem Clearwater River. (B) Over 100 feet of hose was necessary to supply the kelt tanks with water. (C) Pressure supply valve and outflow lines to the kelt tanks.

St. Marie's Fish Acclimation Site

Reconditioning

There were 20 post spawn kelts that were trucked to St. Marie's Acclimation Pond of which 0 were hatchery fish and 20 were natural origin. In September we released 4 steelhead kelts into the mouth of the Okanagon River (4 females). Most of these fish were in good to excellent condition (Figure 13). In July, there was a lighting strike that disabled the pump and backup systems. This resulted in the kelts going without fresh water for an estimated period of 24-hours. There was some instant and delayed mortality that was attributed towards this event (5 females, 1 male). Tribal fisheries staff began implementing redundant systems with battery and generator backups at the end of 2011 so that fish would not be lost to future natural events.

Figure 13: Long-term reconditioned Omak Creek Kelt just prior to release.

Parkdale Hatchery

Summer Run (Skamania) Steelhead Reconditioning

Skamania kelt collections were discontinued in 2010 with the dismantling of Powerdale Dam, ODFW stopped releasing smolts prior to the dam demolition. The remaining fish in the reconditioning program are remnants of these last captures at Powerdale and are continuing to be reared to get final spawning data.

2010 Brood

This is the final group of Skamania kelts that were collected at the Powerdale Dam. There were 6 remaining Skamania female steelhead by the beginning of 2011. There were 3 mortalities that winter of 2011. We are still in the process of attempting to spawn the remaining kelts; there are 3 surviving kelts that will be candidates for "skip-spawning" in 2012.

2009 Brood

Two steelhead kelts successfully reconditioned. The remaining 2 did ripen that year and were successfully spawned. This was our first group that had all skip spawners.

2008 Brood

There were 2 fish remaining at the beginning of 2011, all of which were successfully spawned in 2011. (Table 3). At the end of 2011 both of these fish had perished likely from old age (6-7 year old fish). This group of fish have been extraordinary survivors and done well being reconditioned. This group of fish though small in numbers, may give us an idea of the reproductive viability of "sequential" kelt spawners which we have detected moving through the hydrosystem (Table 3).

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

Brood Year	2006	2007	2008	2009	2010
Aive as of 1/2012	0	0	0	1	3
Maiden spawn	1	15 (2 culled IHN)	14	12 (3 culled IHN)	22 (7 culled IHN)
1st sequential spawning	1	1	4	0	0
Succ. Recon Rate %	100%	16%	50%	22%	TBD (20% max)
Skip Spawner kelt	0	1	3	2	TBD
% skip spawner of reconditioned fish	0%	8%	21%	22%	TBD
2nd sequential spawning	0	0	2	TBD	TBD
3rd sequential spawning	0	0	2	TBD	TBD
4th sequential spawning	0	0	2	TBD	TBD

Winter run (Locally Adapted Broodstock) Steelhead Reconditioning

The winter run steelhead are a locally adapted broodstock that are used for supplementation. These fish in 2011 were captured at the East Fork Hood River resistance board weir trap. In 2012 there should be enough spawners (counting sequential and skip spawners) for a full accounting of the2010 winter brood.

2011 Brood

This was the first year of collection of kelts at the East Fork of the Hood River weir. Many of the kelts captured had dermal abrasions and light lacerations likely due to collection location and time (late winter/early spring) instead of summer/early fall. We lost about 1/3rd of the fish to fungal infections in 2011. The rest were lost either to cold-water disease or *C. shasta* infections.

2010 Brood

This was the first year of reconditioning winter steelhead at Parkdale; these fish were captured at the Powerdale trap. At the end of 2011 a total of 5 female kelts were spawned. By the end of the 2011 we had 2 remaining fish (Table 4).

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

Brood Year	2010	2011
Aive as of 1/2012	2	5
Maiden spawn	22 (3 culled IHN)	23
1st sequential spawning	5	TBD
Succ. Recon Rate %	26%	TBD 22%
Skip Spawner	2 max	TBD
% kelt skip spawner of reconditioned fish	11% max	TBD

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Section D. Management Scenario Evaluation

Introduction

Management scenarios have consisted of collecting and transporting unfed or fed kelt steelhead downstream and releasing them below Bonneville Dam and rejuvenating kelts by holding them in large tanks and feeding them until the next season's upstream run occurs when the kelts are liberated. We present 9 years of data from Prosser Hatchery, 5 years from Lower Granite, and 1 year from John Day Dam (Evans et al. 2008) to evaluate the benefits of transporting kelt steelhead around the hydrosystem. To evaluate success of various management strategies we compared kelt return rates (for transported treatments) and survival rates (for long-term reconditioned kelts) with several "control" groups. Control groups included returns of in-river treatments (fish that were tagged and released back in the river) when available, composition of repeat spawners in the run at large sampled at Bonneville Dam, and values from the literature (Hockersmith et al. 1995). In 2010, (Branstetter et al. 2011) we compared all treatments and locations across all years, so this year we primarily compare results for just 2011 but also compare means across years.

Methods

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

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

Results and Discussion

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

Comparison groups

Our comparison or control groups consisted of 1. The proportion of repeat spawners in the run at large, at Bonneville Dam; 2. The return rate to Bonneville Dam of fish PIT tagged and released at Prosser Hatchery; 3. The return rate to Bonneville Dam of fish PIT tagged and released at John Day Dam; The return rate to Bonneville Dam of fish PIT tagged and released at Lower Granite; and, the reported proportion of repeat spawners in the run at Prosser Dam based on scale pattern interpretation (Hockersmith et al. 1995) (Table 1). The proportion of repeat spawners in the run at large at Bonneville Dam is based on scale pattern interpretation of 8 years of data collected from over 12,000 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 0.51%. This indicates that iteroparity is very low in steelhead populations above Bonneville Dam and in 2011 the return rate was 0.38%. The return rate to Bonneville Dam of kelts tagged and released in-river at Prosser Hatchery in 2011 was 0.00 but the 7 year average of 2.87% is much higher than the run at large at Bonneville Dam suggesting the Yakima River fish may exhibit higher than average iteroparity rates relative to other tributaries. Repeat spawner composition in the Yakima River run based on scale pattern analysis (Hockersmith et al. 1995) was reported at 1.66%. This estimate differs from the other control groups in that it is measured at Prosser Hatchery not at Bonneville Dam but further supports the notion that Yakima River steelhead exhibit higher iteroparity rates relative to the run at large measured at Bonneville Dam. The Bonneville Dam return rate of kelt steelhead tagged and released at John Day Dam was 9.76%. This is very high relative to other sites and includes only a single year (2002). Kelt returns in 2002 were the highest ever recorded for transported fish collected at Prosser Hatchery and Lower Granite Dam as well suggesting that the return rate measured at John Day Dam is likely at the high end of the range. It also indicates that when environmental conditions are conducive, high iteroparity rates can be achieved in upriver stocks. The comparison group tagged and released at Lower Granite Dam returned to Bonneville Dam in 2011 at a rate of 0.0%. This is consistent with low return rates of in-river kelts from other locations in 2011. The 6-year mean return rate to Bonneville Dam for kelts tagged and released at Lower Granite Dam is 0.58%. This is quite low and not statistically different (pooled variance t test; t=1.511; df=11; p=0.159) from the run at large at Bonneville Dam.

Table 1. The return rate in 2011 and the mean from available years to Bonneville Dam of repeat spawners from various locations used as "controls" or comparison groups. Note that Hockersmith is a return rate to Prosser Hatchery not Bonneville Dam. Starred groups are based on scale pattern analysis; the remaining groups are based on returns of PIT tagged fish.

Return Rate timeframe	Bonneville*	Prosser	John Day	Lower Granite	Hockersmith*
2011	0.38	0.00	-	0.00	-
mean	0.51	2.87	9.76	0.68	1.66

Treatment Groups

Transported treatment groups in 2011 included kelts collected at Lower Granite Dam and Prosser Dam. For each of these treatment collection locations we used two different release locations: Hamilton Island (below Bonneville Dam where previous transport groups were released) and Westport, OR boat ramp, located at approximately river mile 43.

No kelts were detected returning to Bonneville Dam from fish collected at Lower Granite Dam and transported to Hamilton Island or to Aldrich Point. The 6-year mean return rate to Bonneville Dam for fish collected at Lower Granite Dam and transported is 1.12. Two kelts (1 fish from each release location) were detected returning to Bonneville Dam from fish collected at Prosser Dam and transported to Hamilton Island and Westport. Return rates of Prosser collected fish to Bonneville Dam were 1.00 for the Hamilton Island release and 1.11 for the Westport release. Both of these return rates are lower than the 9-year mean return rate of 4.05.

Only limited transport benefits can be calculated for the 2011 returns because of the low or zero return rates for transport and in-river groups. The kelts collected at Prosser Hatchery and transported to Hamilton Island had treatment benefits of 0.78, 2.63, and 0.60 relative to the control metrics of in-river, the steelhead run at large at Bonneville Dam, and the Hockersmith value of 1.66, respectively. The Prosser kelts released at Westport, OR showed similar treatment benefits of 0.86, 2.92, and 0.67 relative to in-river, the steelhead run at large at Bonneville Dam, and the Hockersmith value of 1.66, respectively. Remember that any number greater than 1 is a positive benefit and any number less than 1 is a negative benefit. Neither release location resulted in returns to Bonneville Dam substantial enough to yield benefits over control/comparison groups. Transport benefits for Snake River origin kelts collected at Lower Granite Dam are very low and difficult to measure due to very low returns to Bonneville Dam from these treatment groups. In 2011, no fish returned to Bonneville Dam from either the Hamilton Island or Westport, OR release of Snake River origin kelt steelhead. No Snake River origin kelt steelhead have returned to Bonneville Dam from 2010 or 2011 releases in the estuary. No Snake River origin kelt steelhead have returned to Bonneville Dam from 2009 through 2011 releases. These very marginal treatment benefits suggest that in most years collecting kelt steelhead then transporting and releasing them below the hydrosystem has very limited benefits. Trucking fish these long distances likely impacts long term survival of transported kelt steelhead.

Survival from release to the ocean was estimated from both collection areas Lower Granite and Prosser dams and both release sites, in 2011 using sequential detections of acoustic tags. For the kelts collected at Lower Granite Dam, survival to the ocean was 6.5% and 29.8% for the Hamilton Island and Westport, OR release sites, respectively. For the Prosser Dam collected kelts survival to the ocean was 34.0% for both for both the Hamilton Island and Westport, OR release sites. The 7-year mean survival from release at Hamilton Island to the ocean is 44.9%. These low survival rates could be a result of transportation stress on

the fish or river environment impacts. For the Snake River origin kelts we found that releasing the fish closer to the ocean resulted in higher survival to the ocean, however the lack of returns to Bonneville Dam lead has to discontinue evaluating transportation as a management option.

In 2011, survival of long-term reconditioned groups was 33.1% for Prosser, 20.0% for Omak, and 21.7% for Parkdale. Survival for the Prosser and Parkdale fish was slightly below average, and survival for Omak fish equaled the mean survival for that site (20.1%). Overall this convincingly indicates that steelhead kelts can be successfully reconditioned at a variety of locations.

We calculated the benefits of long-term reconditioning in the same manner as we did the transport benefits but instead of return rate to Bonneville we used survival to release for the long-term treated fish. Fish reconditioned at Prosser Hatchery had an 12.9 times survival advantage over the 7 year average return rate to Bonneville Dam for fish left in the river (Figure 1). We used the 7 year mean for comparison since the 2011 group had 0 returns to Bonneville Dam, therefore, the within year comparison is a minimum estimate. Compared to the proportion of repeat spawners in the run at large at Bonneville Dam, long-term reconditioned kelts at Prosser Hatchery had a 72.2 times survival advantage, those from Omak Creek had a 39.4 times advantage, and steelhead from Parkdale had a 57.5 times advantage (Figure 2). Long-term reconditioning shows great promise as a tool for restoration based on these data.

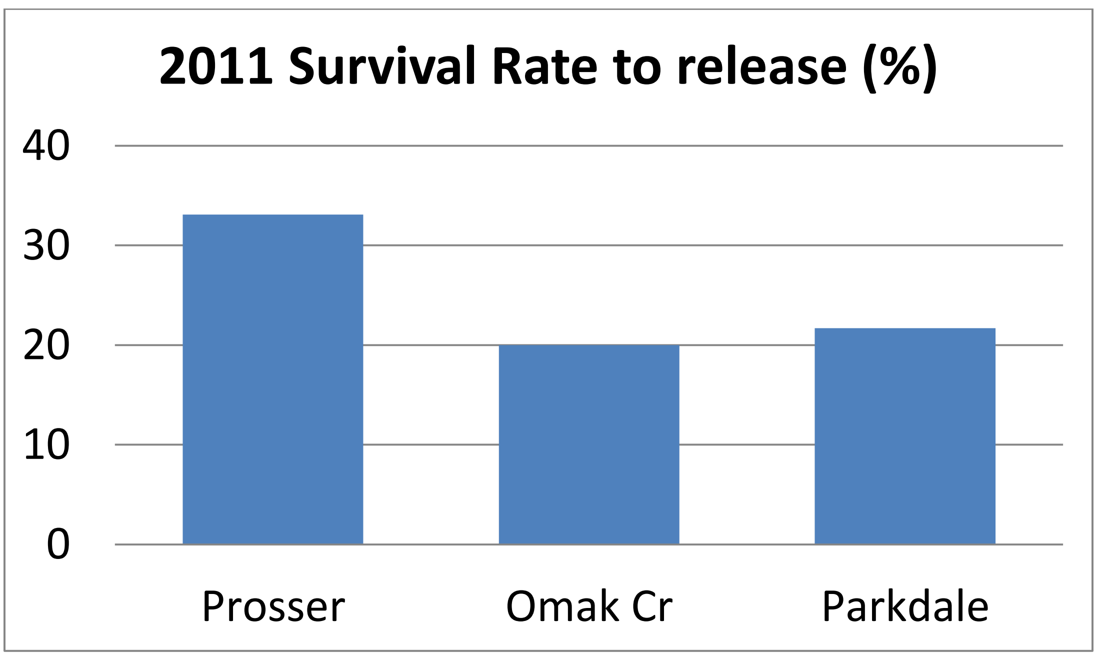

Figure 1: Survival rate of long-term reconditioned kelt steelhead at 3 locations in 2011.

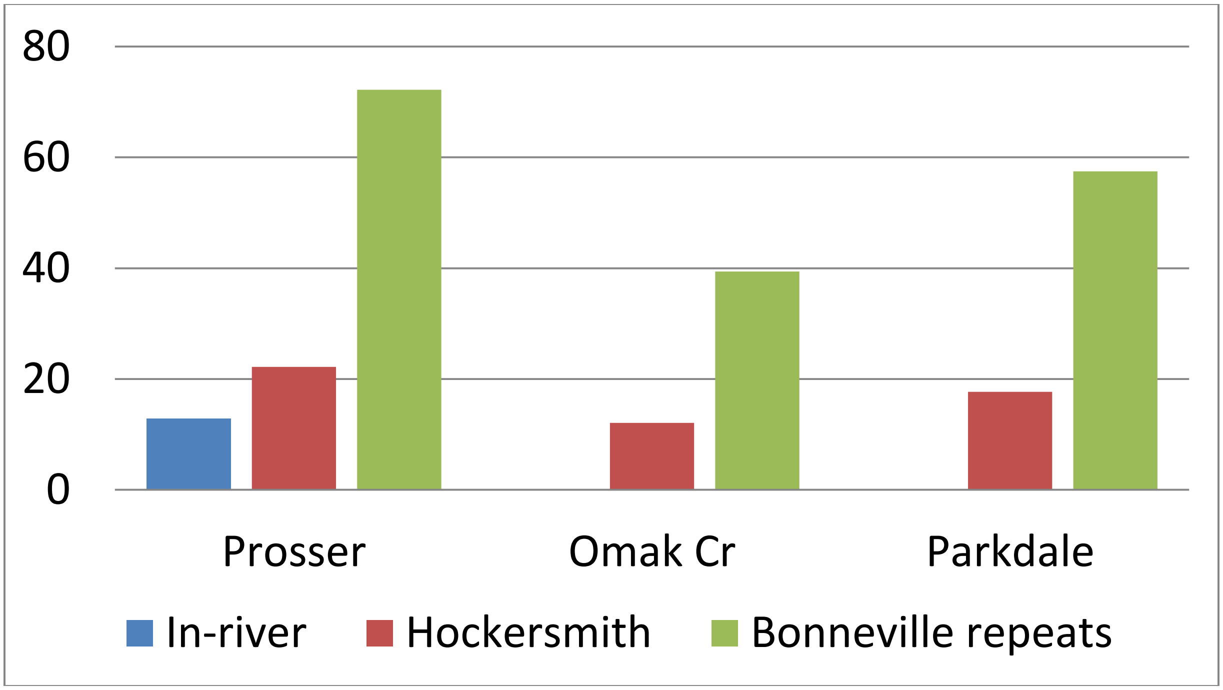

Figure 2: Long-term reconditioning benefits for 2011, calculated by dividing long-term survival rates by control group metrics.

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Chapter 2: Reproductive Success Evaluation

Section A: Steelhead Kelt Gamete and Progeny Viability In a Hatchery Setting

Written by

Doug Hatch
Ryan Branstetter
Jeff Stephenson
Andrew Pierce
John Whiteaker
Neil Graham
Columbia River Inter-Tribal Fish Commission

Albert Santos
Jim Gidley
Confederated Tribes of the Warm Springs Reservation

Introduction

Reproductive success is difficult to observe in the field. Steelhead, in particular, are problematic as migration and spawn timing is associated with high flow events in the Spring. This limits the operation of weirs and traps and makes direct observation of spawning difficult. In addition to the difficulties of sampling migratory anadromous adults, resident *O.mykiss* can represent a substantial portion of the parents spawning with anadromous forms (Araki et al. 2007), and are often unsampled. Consequently, we are investigating gamete and progeny viability of reconditioned kelt steelhead in a hatchery setting where variables can be controlled or minimized. The design is to collect hatchery-origin prespawn adults and transport them to the hatchery to study them in a more controlled environment. We initially began this experiment utilizing Skamania stock steelhead, which is a highly aggregated commercial stock. In 2010 we also collected locally adapted winter run steelhead for comparative purposes while phasing out the Skamania portion of the experiment. In 2011, only winter run steelhead were collected.
Fish are held until they are ripe. Females are air spawned and the eggs are fertilized with cryopreserved milt. Offspring are raised for several weeks while recording various measures of quality. After air spawning, females are placed in tanks and reconditioned in a manner similar to the other long-term reconditioning treatments (Prosser, Omak, and Dworshak).

This experiment utilizes a replicated, repeated measures experimental design to assess and compare egg and progeny viability of maiden versus reconditioned spawners. Long-term reconditioning and subsequent captive spawning provides valuable quantitative data on gonad processes, maturation rates and juvenile survival. Data resulting from this research will greatly contribute to the evaluation of reconditioning as a conservation tool. The hypothesis we are testing is:

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

Study Area

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

Figure 1: Parkdale Fish Facility. Kelt Round tank left of center. Raceways where kelts are held. Hatch house were kelts are spawned and eggs incubated.

Methods

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

Figure 2: Airspawning female steelhead at Parkdale Fish Facility. Pictured left to right are Ryan Branstetter, Jim Gidley, and Albert Santos.

Organ tissue and gamete samples were collected from post spawn males. Ovarian fluid samples were collected during air spawning and submitted to the ODFW pathology lab to screen for infectious diseases including Infectious Hematopoietic Necrosis virus (IHNV) and Bacterial Kidney Disease (BKD). The result of a positive screen from either parent prompted the disposal of eggs and euthanasia of all possibly infected juveniles. 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 held in an isolation basket and mixed with thawed cryopreserved milt (ODFW 2008) from two different males. This minimized the loss of sample units as a result of positive disease tests (Figure 3). Disease screens can take upwards of 4-6 weeks to be fully processed. Surviving females were reconditioned at the Parkdale Fish Facility and spawned a second time with cryopreserved milt from the same male combinations.

Figure 3: Utilizing cryopreserved milt to fertilize steelhead eggs.

Water hardened eggs were disinfected with a diluted solution of iodaphor povadine (1:100 ppm) (Argentyne) prior to placement into vertical stack incubators. Eggs were incubated at 5.5^{0}C water and treated with formalin 3 times weekly at 1:600 for 15 minutes. Eggs were subsampled (N=20) on day 15 (120 temperature units), which developmentally, is at the epiboly stage. The collected eggs are 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 one of the 5 fiberglass picking (aka California trough) troughs (4.2672 m (l) x 41.91 cm (w) x 11.43 cm (d) troughs. These troughs were originally used to incubate eggs prior to the advent of vertical stack incubators but in this case work as good rearing troughs for the fry life stage. The single female groups are placed into a subdivided section within the troughs (88.36cm x 41.91cm or 140.97 cm x 41.91 cm depending on stocking density) for isolation purposes (Figure 4). Single pass water is fed via downspout flowing at 56.78 liters/min with a constant temperature 5.5^{0}C. Fry were fed Biovita starter feed #0 to satiation every hour during daylight hours for the first 4 weeks

then gradually moved to Biovita #1 and #2 at 4 times daily to satiation for the remaining 6-10 weeks. Fry were sampled by collecting two 20.32 cm random quick-netted subsamples of juveniles every week for 10 weeks. These fish were anesthetized with MS-222 to reduce stress and simplify sampling. Wet weights and lengths were measured on 20 individuals from the collection. At the end of the 8-10- week period, all juvenile fry were euthanized with the administration of a fatal dosage of MS-222. All mortalities and intentional terminations were landfilled.

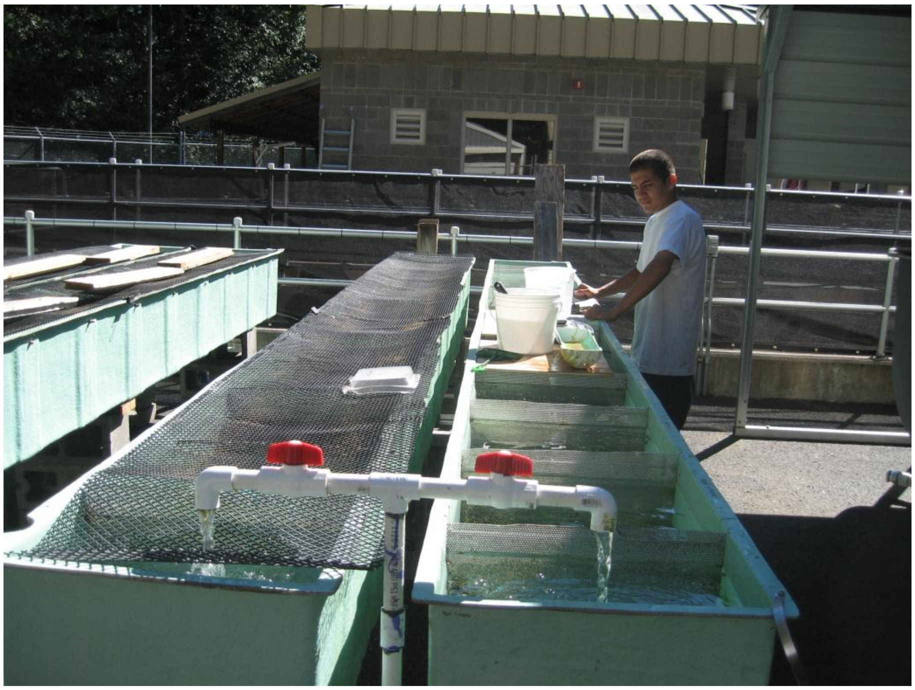

Figure 4: CRITFC-intern (Hardo Lopez) sampling juvenile fish from picking trough to collect weight and length measures.

Results and Discussion

2011 Winter Steelhead Broodstock Maiden Spawning

Eggs

The estimated average fecundity of the 23 female 2011 brood maidens was 5,249 eggs per spawner with a minimum of 2,783 and maximum of 8,401. Average fertilization success based on eyed egg survival was 91%. Keel samples taken at day 15 demonstrate that there was little to no egg loss with samples indicating there was a minimum of 71% fertilization success rate.

Juveniles

The average starting weight was at 0.23 g per fish with an average increase of 0.31 grams for an average ending weight of 0.54 grams at week 6. The average starting length of fish was 3.1 cm with an average increase of .7 cm with a final length measure of 3.8 cm over a 6-week period. At week 7 a backup technician on the project cleaned the trough screens too vigorously, which bumped the screens out of their track, allowing fish to move back and forth between isolation screens leaving data during this period unreliable.

The fry mortality rate was 4.2% over 6-weeks of rearing. This is slightly higher than in previous years at Parkdale, where average mortality was 1-2%. Mortalities occurred throughout the 6-week experiment and were highest after transfer from vertical stack incubators to the picking troughs. Occasionally, juveniles would get caught under the cleaning brush and/or aquarium vacuum and would perish shortly afterwards

2011 Sequential Spawners

There were two groups that had sequential spawners, the 2010 winter brood fish and the 2008 kelt Skamania brood. This was the first sequential spawning for the 2010 brood, while the remaining 2008 sequential spawners were on their 4th straight year of spawning.

2010 Winter Brood

In this section we compare the performance of artificially reconditioned repeat spawner winter steelhead with their previous maiden performance using measures of fecundity, fertilization rates, fry length change, and fry weight change over ten weeks. In addition, we compare the performance of the artificially reconditioned repeat spawner winter steelhead with maiden spawners that were collected and air spawned in 2011. The same performance metrics were used in this comparison.

Eggs

The estimated average fecundity of the 5 female 2010 winter brood sequential spawners was 4,096 eggs per spawner with a minimum of 2,320 eggs and maximum of 5,256 eggs produced (Table). During the maiden spawning these fish produced an average of 3,767 eggs (Table).

Table 1: Average egg production and fertilization from maiden and sequential spawning 2010 winter brood.

Brood Year 2010		
Fish: 2768, 2785,2808, 2820,2821		
Spawning Year	Egg Production	Fertilization
2010	3767	38%
2011	4096	41%

This fecundity increase for the repeat spawners is consistent with (Seamons and Quinn 2010), but is not statistically significant (t = -0.730, df = 4, p = 0.506)(Table X). Average fertilization success based on eyed egg survival was 41% for the repeat spawning compared to 38% for the maiden spawning (Table 2). This difference was not statistically significant (t= -0.110, df = 4, p= 0.917) (Table 2).

Table 2: Statistical comparison of maiden 2010 versus 2011 sequential steelhead spawnings from 5 individuals using 2 egg measures.

Variable	Mean Difference	95% C.I. Lower limit	95% C.I. Upper limit	Standard deviation of difference	t	df	P value
Egg production	-329.6	-1.592	923	1,009	-0.73	4	0.506
Fertilization	-0.03	-0.796	0.735	0.617	-0.11	4	0.917

We compared the egg production of the five kelt spawners versus their first spawning along with 17 additional maiden spawners from 2010. The 22, 2010 maiden spawners average fecundity was 5,249, with a SD=1,332 and the kelt average fecundity was 4,096, with a SD=1,194. This high variance resulted in no significant difference compared to the artificially reconditioned repeat spawners using a two-sample t test (t = -1.775, df =25, p = 0.088). The 23 maiden spawners average fertilization rate were 69%, with a SD=12.2% compared to kelt rates of 41%, with a SD=26.8%. Fertilization rates in the 2011 maiden group were significantly higher than the repeat spawner group (t = -3.706, df =26, p = 0.001) (Table 3).

Table 3. Statistical comparison egg production and fertilization rates of maiden and sequential steelhead spawnings from 23 individuals (23 maidens, 5 sequential spawners).

Variable	Mean Difference	95% C.I. Lower limit	95% C.I. Upper limit	t	df	P value
Egg production	-1,152	-2,490	185.0	-1.775	25	0.088
Fertilization	-0.281	-0.437	-0.125	-3.706	26	0.001

Juveniles

The change in weight for fry was compared at week 4 instead of the standard week 10 due to a logistical issue in 2010, which left a sampling gap at weeks 5 and 6 and a technician error in 2011, which left week 7-10 unreliable. The average change in fry weight for sequential spawners was 0.114 g. This compares to the average change in fry weight from the maiden spawning of 0.150 g. This difference was not significantly different (t = -0.036, df = 4, p = 0.449) (Table 4). The change in fry weight from the maiden spawners averaged .044 g, with a SD= 0.044. This weight change was not statistically significant (t = -1.000, df = 41, p = 0.323) (Table 5).

The average change in fry length for sequential spawners was 0.4 cm, compared to 0.462 cm for their maiden spawning. This difference in fry length was not statistically significant (t = -0.060, df = 4, p = 0.607) (Table 4). All of the metrics measured on maiden spawning and repeat spawning fish, so far, indicate that the artificial reconditioning is not adversely impacting the winter run steelhead reproductive performance or juvenile quality. The 2011 maiden spawners average change in fry length was 0.397 cm, with a SD=0.126 (Table 5). This change in length was not a statistically significant (t = -0.045, df =41, p = 0.964) from kelt and 2010 and maiden spawnings (Table 5).

Table 4: Statistical comparison of maiden 2010 versus 2011 sequential steelhead spawnings from 5 individuals using 2 juvenile growth metrics.

Variable	Mean Difference	95% C.I. Lower limit	95% C.I. Upper limit	Standard deviation of difference	t	df	P value
Fry wt	-0.036	-0.155	0.083	0.096	-0.838	4	0.449
Fry length	0.060	-0.239	0.359	0.241	0.557	4	0.607

Table 5. Statistical comparison of maiden and sequential steelhead spawnings from 41 individuals (38 maidens (2010 and 2011), 5 sequential spawners) using 2 juvenile success metrics.

Variable	Mean Difference	95% C.I. Lower limit	95% C.I. Upper limit	Standard deviation	t	df	P value
Fry wt	-0.020	-0.060	0.020	0.044	-1.000	41	0.323
Fry length	-0.003	-0.120	0.115	0.126	-0.045	41	0.964

2008 Skamania Broodstock Sequential Spawning Comparisons

Two of the Skamania fish ID 2701 and 2843 repeat spawned in 2011. These fish were spawned as maidens in 2008 and sequentially spawned in 2009, 2010, and 2011. These two fish have survived the longest of any steelhead kelt in the reconditioning programs to date. These sequential spawners have been retained because they are our best equivalent to understand multiple (more than 2 spawnings) sequential spawners that are sometimes detected by PIT-tag antennas at the Columbia River Dams making multiple spawning run returns to the Yakima. Typically, the sequential spawners in the Yakima have usually been through a round of reconditioning and are detected a year or two later after release, with an additional year after that. These kelts at Parkdale also represent the maximum capability to continuously recondition kelts in a long-term reconditioning program. These fish finally died in the fall of 2011. Tests for pathogens were negative, leaving old age (6-7 years old, estimated) as the likely cause of death.

Egg

The egg production from in 2011 for fish 2701 was 7,392 eggs and 5,712 for fish 2843. This represented an increase per spawner of 1074 and 2016 eggs respectively for the 2011 sequential spawn over the maiden spawning in 2008 (Figure 5). Sequential spawner fish 2701 had mostly improved in egg production over time but by the third spawning event there was a modest reduction (approximately 600 eggs) in egg production with a final albeit much larger increase (approximately 1300 eggs) in 2011 (Figure 5). Fish 2843's sequential spawning egg production improved +700-1000 eggs annually, with the exception of 2011, which was nearly identical to 2010 (Figure 5). The egg production from 2008-2011 accounted for a total of 26,424 eggs produced from fish 2701 and 19,440 eggs from fish 2843.

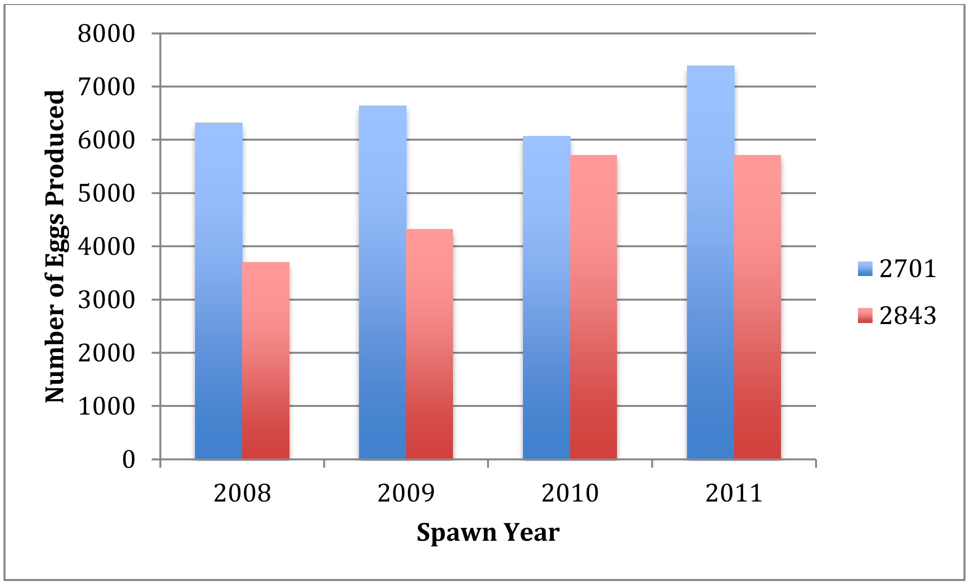

Figure 5: Maiden and sequential egg production of 2008 Skamania Fish ID 2701 and 2843 by year (2008-2011).

Post shock survival was 26.7% (fish 2701) and 86.0% (fish 2843) with keel samples recorded at 28.3% (fish 2701) and 90.0% (fish 2843). When comparing the 2008 maiden versus the 2011 sequential spawners eyed egg survival was 15% lower for fish 2701 than in its 2008 maiden spawning (Figure 6). Spawner 2843 demonstrated an increase over its 2008 spawning by 34% (Figure 6). The eyed egg percentages for the sequential spawners both dramatically increased in the 2nd spawning event in 2009, with fish 2701 having more than doubled and fish 2843 increasing by over 30% (Figure 6). The two fish diverged in 2010 with fish 2701 having a large decline (just under 70%) in eyed eggs while 2843 had a modest increase of about 10% (Figure 6). Interestingly, the percentage of eyed eggs from fish 2701 increased by almost 20% in 2011, while fish 2843 declined in nearly half (Figure 6).

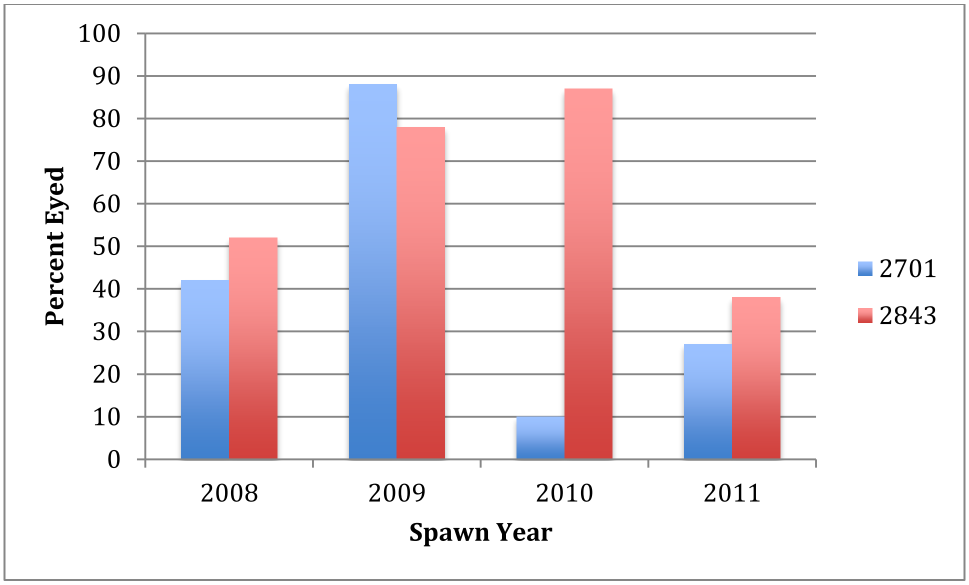

Figure 6: Maiden and sequential percent of eyed egg after cold shock by year (2008-2011) of 2008 Skamania Fish ID 2701 and 2843.

Comparing the 2008 maiden spawning to the 2011 sequential spawning egg production combined with the fertilization rates results in a difference of -39.9% for fish 2701 and a +216.4 % for fish 2843. Although, it should be noted, that any production from fish is positive even though differences may exist from year to year.

Juvenile

The growth of progeny from Fish ID 2701 and 2843 improved with each spawning event through their third spawning (Figure 7, 8, 9, and 10). Juvenile growth from the fourth spawning was less than that of the third indicating that possibly there was an environmental, age related, or unknown effect that occurred. All repeat spawning events yield progeny that displayed greater growth than from the maiden spawning. The average increase in weight for the 2011 brood juveniles was 0.78 grams while the average length increase was 1.57cm (Figures 7, 8, 9, and 10). When comparing the 2011 juveniles versus the 2008 progeny the difference is positive in weight with an increase of +0.14 g (fish 2701) and +0.24 g (fish 2843) (Figures 7 and 8).

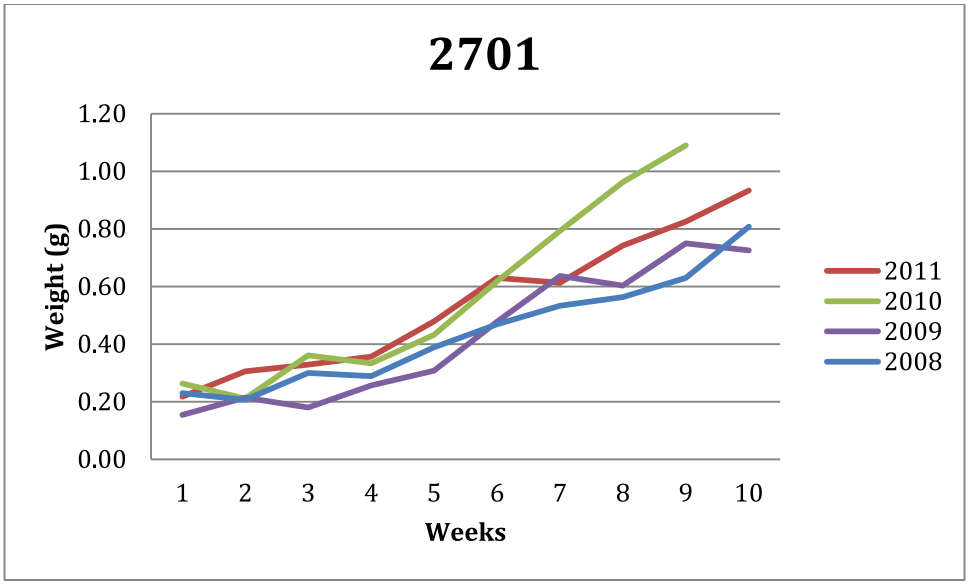

Figure 7: Sequential spawner 2701, 4-years cumulative data on weight (g) (2008-2012) over a 10-week period.

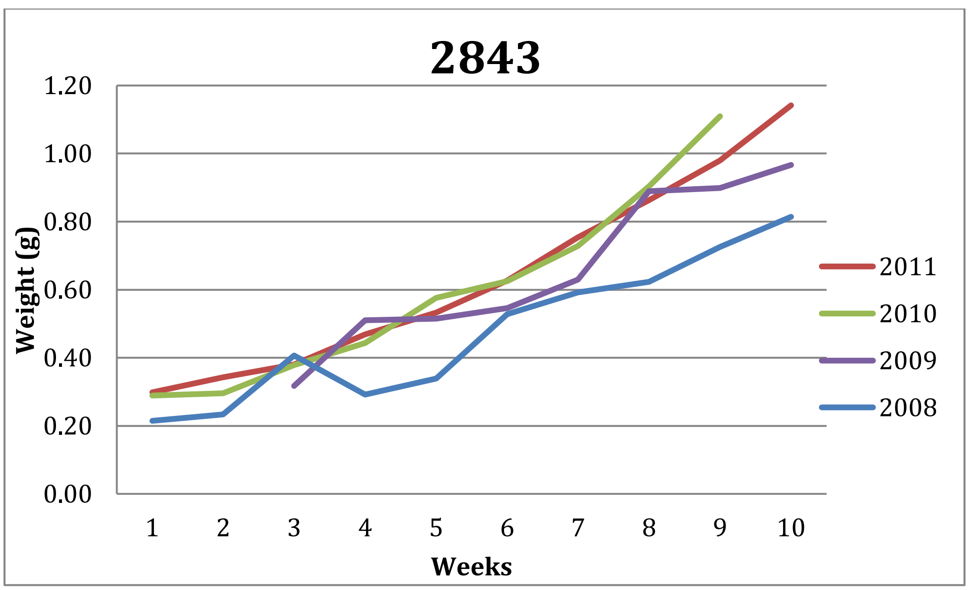

Figure 8: Sequential spawner 2843, 4-years cumulative data on weight (g) (2008-2011) over an 8-10 week period.

Much like weight, the best year for length gains, for both groups, was in 2010 resulting in progeny from fish 2701 being almost 1cm larger, and progeny from fish 2843 being approximately 0.5cm larger than their comparable maiden spawnings in 2008 (Figures 9 and 10). The major difference between the groups was in 2011; fish 2701 had its second best length gains while fish 2843 had length gains that were slightly lower than what they were in 2009 (Figures 9 and 10). Length difference between 2008 and 2011 was also positive at +0.3 cm (2701) and +0.4cm (2843) (Figures 9 and 10).

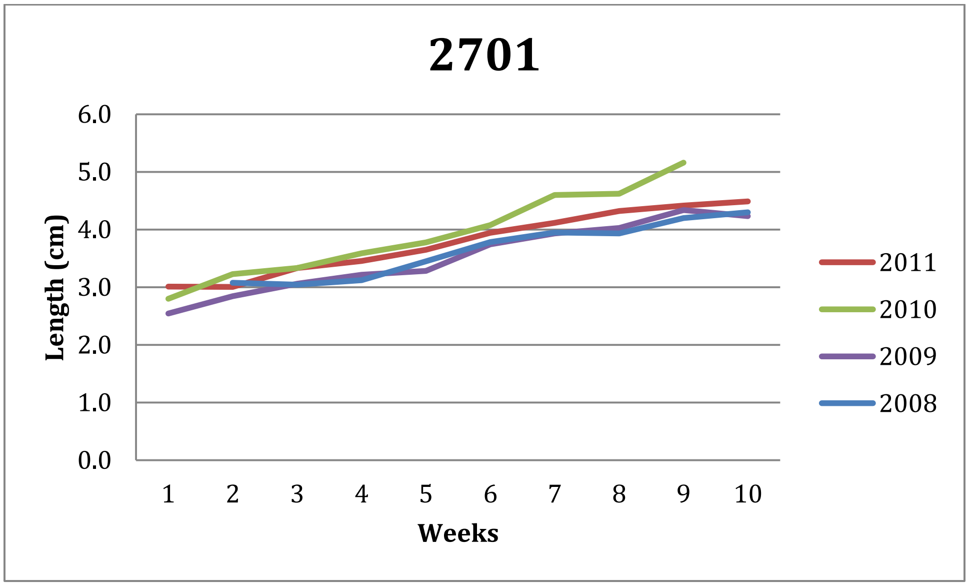

Figure 9: Sequential spawner 2701, 4-years cumulative data on length (cm) (2008-2011) over a 9-10 week period.

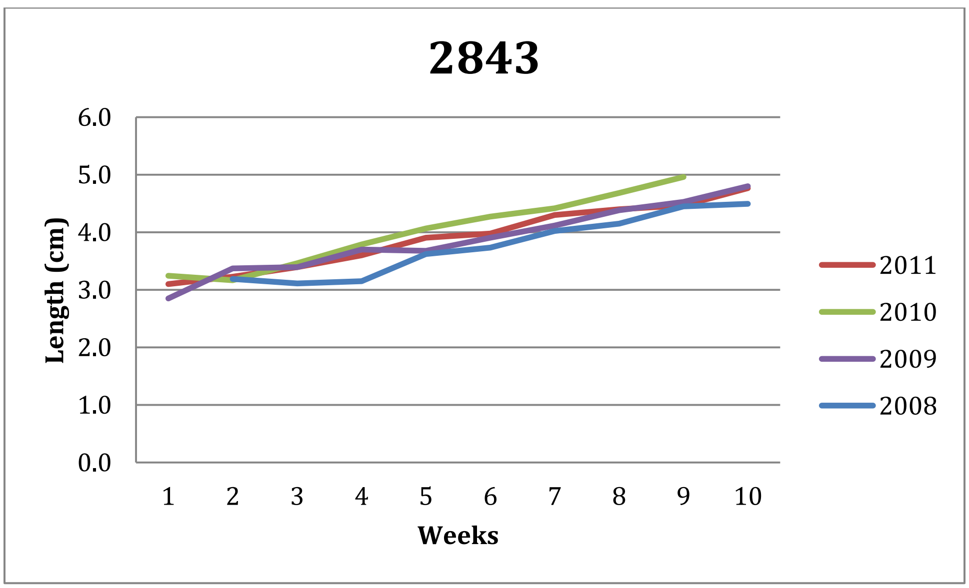

Figure 10: Sequential spawner 2843, 4-years cumulative data on length (cm) (2008-2011) over a 9-10 week period.

Cumulative Skamania Maiden Spawners versus Skamania Kelt Spawners all years.

In this section we compare maiden and kelt spawners using a general comparison of maiden fish versus kelt spawners in terms of fecundity, fertilization rates, fry weight and length gain. In most of these comparisons kelts performed better, similar to maiden steelhead, or slightly underperformed. Holding space at Parkdale is low which limits the number of fish that we can recondition on site. This limits statistical power (sequential and skip spawner sample size at 13 post maiden spawning events).

Repeated Measures General Comparison of Skamania Spawners 2006-2011

Comparing the long-term reconditioned kelts against the incoming maiden brood, the sequential and skip spawners perform as well as the best maiden spawners. Sequential spawners produced 900+ more eggs than maiden spawners (Table 6). The number of eggs produced continued to increase in the multiple sequential spawners by year from +2000 in the 3rd spawning and nearly +3000 in the final spawning (table 6). In Quinn *et al.* (2010) sequential spawners were also observed to produce more eggs than 3-year-old maiden spawners, which they suggest, is a result of the increased size of the female fish from the time of its maiden spawning.

Average fertilization rate for the maiden spawning event (60%) was slightly higher than average fertilization rates following reconditioning (49%) (Table 7). In Seamons and Quinn (2010) sequential and skip spawners were observed to produce slightly more adult offspring than maiden spawning fish. This could mean that even though initial fertilization is lower, positive kelt juvenile growth factors may confer a slight survival advantage for kelt progeny.

Table 6: Mean egg production from 2006-2011 with mean for each spawning number and year.

	Year						
Spawning	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**Mean**
1	4618	3646	4061	2745	3767		3767
2		3208		6160	5569	3952	4722
3					5892		5892
4						6552	6552
Mean	4618	3427	4061	4453	5076	5252	5233

Table 7: Mean egg fertilization based on eyed eggs from 2006-2011 with mean for each spawning number and year

	Year						
Spawning	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**Mean**
1	75%	64%	38%	67%	57%		60%
2		17%	49%	68%	50%	64%	49%
3					49%		49%
4						33%	33%
Mean	75%	40%	43%	67%	52%	48%	48%

In 2006 and 2007 fish were reared at the University of Idaho Aquculture Reasearch Institute. The ARI had higher water temperatures and different rearing containers that

could not be replicated at Parkdale (Branstetter et al 2007 and Branstetter et al. 2008) so these juvenile growth values should not be compared against kelts raised at Parkdale. kelt progeny on average put on weight better than maiden progeny in a 10-week period (Table 8). The biggest year difference in weight was the skip spawing kelt progeny from the 2008 brood in 2010. These fish were 0.26 g heavier than the maiden progeny from that year (Table 8). Kelt progeny on average also grew longer than the maiden spawning progeny (Table 9). The largest difference comes from the 2008 broodstock. In 2009, the 2008 brood sequential spawners were slightly longer than the maiden spawning brood that year (Table 9). In 2010, the skip spawning and 3rd time sequential 2008 brood spawners had the largest difference in growth for all juvenile fish at Parkdale at 0.2-0.3 cm difference from the 2010 maiden brood (table 9). This difference was 0.4 cm and 0.5 cm different from their 2008 maiden spawning which represents a sizeable difference (table 9) compared against other years and other spawners. Though in 2011, the 4th time spawners began to show a marked decline in progeny size compared against the average maiden juveniles (-0.2) in 2008 and the average of all maiden juveniles (-0.3) (Table 9). It appears that the sequential and skip spawners peaked at the 5-6 year age mark and the 2nd or 3rd spawning event depending if that fish was a skip or sequential spawner (Tables 8 and 9).

Table 8: Mean juvenile weight gain (grams) from 2006-2011 with mean for each spawning number and year. Spawning 1 is the maiden spawning year.

	Year						
Spawning	**2006***	**2007****	**2008**	**2009**	**2010**	**2011**	**Mean**
1	0.60	3.33	0.73	0.77	0.81	NB	1.25 (.77)***
2		3.16	0.72	0.74	1.07		1.42 (.84)***
3					0.82		0.82
4						0.78	0.78
Mean	0.60	3.24	0.73	0.76	0.90	0.78	1.07

NB=No Brood Reared

*** Raised at University of Idaho ARI. Chilled Water Temperature at 11.1^{0}C. Reared in small circular tanks.**

**** Raised at University of Idaho ARI. Non-chilled water temperature at 14.4^{0}C. Reared in small boxes.**

***** Numbers in parentheses represent values only from Parkdale.**

Table 9: Mean juvenile length from 2006-2011 with mean for each spawning number and year

	Year						
Spawning	**2006***	**2007***	**2008**	**2009**	**2010**	**2011**	**Mean**
1	NA	NA	1.6	1.6	1.8	NB	1.7
2				1.7	2.1		1.9
3					2.0		2.0
4						1.4	1.4
Mean	NA	NA	1.6	1.7	1.9	1.4	1.7

*** Raised at University of Idaho ARI. No lengths collected. NA=Not Available**

NB=No Brood Reared

Mortality

Skamania kelt progeny post hatch mortality was the lowest out all the progeny in 2011 at Parkdale with an average of 4.4% mortality for both groups. Though in previous year 2008 and 2009 mortality has usually been around 2%.

Discussion

This portion of the steelhead kelt program was initially started to determine if reconditioned steelhead kelts were capable of reproduction and to subsequently quantify that production. We have demonstrated that kelts can successfully reproduce in a hatchery setting and based on the small sample size that there appears to be little (positive difference) to no difference in the reconditioned summer steelhead. We have just begun looking at these same conditions for supplemental winter steelhead and results look positive. What we have detected at Parkdale fits what was observed in Quinn et al. 2010, when they suggested that steelhead kelts may be foregoing active growth and instead directing the majority of energy into egg production. This makes sense, based on the fact that steelhead are not a long lived species. After post-spawn recovery, energy that is obtained through feeding is likely primarily allocated towards reproduction with any secondary or excess going towards growth. We have observed the females at Parkdale getting larger primarily in girth and in slightly so in length. This larger size contributes to the increased production of eggs and improved growth factors of the progeny (weight and length). These factors should confer a numeric and size advantage over first time spawners. Our data suggests that these progeny are longer and heavier than their maiden cohorts, which would likely influence the timing of smolting (Beckman et al. 1998) and possibly confer a survival benefit in the riverine and later, estuarine environments (Zabel and Achord 2004) and subsequent survival to adulthood (Quinn 2005). Even with some of the sequential or skip spawners having slightly lower gamete or progeny yields from their maiden spawning, they would still be contributing potentially thousands more juvenile fish towards populations. We have observed reproduction in both the Omak Creek and Yakima River systems. Ideally following progeny throughout the rearing and release would be ideal. Currently a proposed addition to the management plan has been drafted in cooperation with the Confederated Tribes of Warm Springs Indian Reservation Parkdale Fish Facility and the CRITFC to raise kelt progeny to release and track return migration via PIT-tags (Appendix D). This would provide egg to adult data on kelt progeny throughout its life cycle, which could then be compared against the maiden spawn and the whole population progeny.

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Section B: Parantege Analysis at Omak Creek

Written By:

Jeff Stephenson
Shawn Narum
Ryan Branstetter
Doug Hatch
Columbia River Inter-Tribal Fish Commission

Rhonda Dasher
Confederated Tribes of the Colville Indian Reservation

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: 1) Do reconditioned kelts produce viable offspring that contribute to recruitment, 2) How does kelt reproductive success compare with natural first time spawners; and, 3) How does kelt reproductive success compare with hatchery origin spawners? In this study we utilize DNA markers and pedigree analysis to address these questions for kelt steelhead in Omak Creek, a tributary to the Okanogan River. The answers to these questions will be important in determining if kelt reconditioning is a viable restoration tool that will aid in the recovery of ESA listed steelhead populations in the Columbia River Basin.

Methods-Sample Collection

Adult steelhead were collected as upstream or downstream (kelts) migrants via an adult trap at a semi-permanent weir on Omak Creek. A PIT tag antennae array was also operated upstream of the Omak Creek confluence with the Okanogan River. Juvenile outmigrants were collected with a rotary screwtrap located downstream of the weir during the spring. Age-0 juveniles (juveniles collected in the same calendar year as the spawning event) were collected in the fall using electrofishing techniques. Age-0 status was determined by length (fish <100mm) during fall collections. In 2011, electrofishing started at the PIT tag array and continued approximately 100 yards upstream of the adult trap (NMFS 2000 Electrofishing Guidelines).

Reconditioning efforts and subsequent detections of returning adults are quantified in Table 1. Juvenile sampling and genotyping was designed to preferentially sample fish of appropriate age to the post-reconditioning spawning event. Details for detections at each year can be found in the following text. In 2011 at least 5 of the 6 fish reconditioned in 2010 were detected crossing the PIT tag antennae array in Omak Creek.

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

Capture	Release		Detection		Potential contribution to age classes									
Year	Year	n	Year	n	2013	2007	2008	2009	2010	2011	2012	2013	2014	2015
2005	2005	3	2006	1		Age 1	Age 2	Age 3						
2006	2006	1	2007	0										
2007	2007	8	2008	3			Age 0	Age 1	Age 2	Age 3				
2008	2008	9	2009	0										
2009	2009	<5	2010	0										
2010	2010	6	2011	5						Age 0	Age 1	Age 2	Age 3	
2011	2011	4	2012								Age 0	Age 1	Age 2	Age 3

Methods-Genetic Analysis

Fin 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. Genotyping efforts prior to 2011 used microsatellite markers described in Stephenson et al. (2008) and Blankenship et al. (2011). Current genotyping efforts utilize the PBT (parentage based tagging) panel of SNP (Single Nucleotide Polymorphism) markers described in Hess et al. (2012).

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). 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). 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 minimize incorrect assignments, simulations were performed to determine a 99.0% confidence LOD value. Assignments were excluded unless they met the critical LOD value of 2.75, had a minimum of 92 loci comparisons, and zero mismatches.

Sample completion rates (S_{cr}) were based off the number of genotyped adults divided by steelhead escapement estimates as reported in Miller et al. (2011, 2012). Expected rates of

assignment were graphed by calculating a range of S_{cr} for parental collections, as well as the expected values for BY2010 and By2011.

Probability of Both parents = $S_{cr} * S_{cr}$
Probability of only one parent = $2*((S_{cr}) - (S_{cr} * S_{cr}))$
Probability of No parents = $(1-S_{cr}) * (1-S_{cr})$

Results-Genetic Analysis

A total of 528 samples were successfully genotyped. Numbers for each collection, by location and year, can be seen in Table 2. Departures from Hardy-Weinberg equilibrium (critical level =0.05 /95 loci = 0.00053) or linkage disequilibrium (critical level =0.05 /4465 pairwise comparisons = 0.000012) were seen at higher rates in the juvenile collections. This may be the result of kinship or the Wahlund effect resulting from population admixture of distinct resident and anadromous populations (Branstetter et al. 2011). Parentage analysis proceeded as normal as it does not require Hardy-Weinberg equilibrium to be informative.

Table 2. Population Statistics. Each collection is reported in terms of sample size (n), expected heterozygosity (H_E), observed heterozygosity (H_O), number of loci out of Hardy-Weinberg equilibrium (HW), and number of pairwise loci comparisons showing linkage disequilibrium (LD).

	n	H_E	H_O	HW	LD
Omak Anadromous Adults 2010	188	0.4261	0.4233	0	9
Omak Anadromous Adults 2011	65	0.4241	0.4239	0	3
Omak Screwtrap 2011	101	0.4340	0.4156	1	15
Omak Electrofish 2011	174	0.4288	0.4334	4	88
Total	528				

Table 2 shows the expected and observed accuracies of successful parentage assignments for each of the juvenile classes. Samples at the screwtrap were successfully assigned to both parents 27.7% of the time, and to a single parent 12.9% of the time. Juvenile samples collected by electrofishing were successfully assigned to both parents 5.7% of the time, and to a single parent 29.3% of the time.

Table 2. Successful parentage assignments to either 1 or both parents.

	n	1 parent		2 parent		Total	
Omak Screwtrap 2011	101	13	12.9%	27	26.7%	41	40.6%
BY 2010 Expectations			9.7%		47.4%		57.1%
Percent of expected			132.8%		56.4%		
Omak Electrofish 2011	174	51	29.3%	9	5.2%	61	35.1%
BY 2011 Expectations			21.4%		28.9%		50.3%
Percent of expected			137.2%		17.9%		

Of the 61 samples collected by electrofishing, 2 were assigned back to a single reconditioned kelt detected returning in 2011. Progeny from two other reconditioned kelts

were seen in the screwtrap samples at lengths consistent with reproduction as a first time spawner.

Table 3 shows a summary of reproductive success attributed to fish that went through the reconditioning process and returned to spawn again. Successful reproduction has been confirmed for four of the nine reconditioned kelts that were detected returning to Omak Creek. For this year progeny from only 1 of 5 kelts were detected.

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
OmyOCS2011j-0175	Age-0	77	2011	2011	Omy-AE27	Female
OmyOCS2011j-1012	Age-0	78	2011	2011	Omy-AE27	Female

Discussion

Reproduction by reconditioned kelts was confirmed for four individuals. Two progeny were seen from a female kelt reconditioned in 2010 and detected at the PIT tag array in 2011. A female observed digging below the weir in 2008 had progeny detected as an age-0 emergent fry in 2008 and age-1 in 2009. One of the females returning in 2006 was also shown to reproduce with the detection of an age-2 progeny in 2008. 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.

Expected rates of assignment compared to actual assignment rates in 2011 differed in both juvenile collections, as expected. Screwtrap samples collected in 2011 may represent parental classes from 2008, 2009, and 2010, of which only the 2010 parents are included in this analysis. Additionally, previous analysis has shown that juvenile collections in Omak Creek may include juveniles of both resident and anadromous origin parents.

All samples collected by electrofishing were under 100mm and assumed to be age-0 samples, and as expected, assigned back only to 2011 adults. Observed assignment versus expected assignment rates varied, but likely as a result of sampling location. Electrofishing efforts were targeted at areas primarily downstream of the adult trap, and were more likely to contain unsampled parents. The fact that 35% of juveniles assigned to at least one adult may be higher than expected under random circumstances, given that most of the genotyped adults were collected at and expected to spawn above the adult trap. Of 132 fish

that likely spawned in Omak, it is estimated that 52 spawned below the adult trap (Miller et al 2012). The six reconditioned kelts detected crossing the PIT tag array, but not sampled at the adult trap represent the only genotyped parents expected to spawn only below the trap.

Fish detected at the trap may have already spawned, or moved downstream following release upstream of the trap (fall back). Alternatively, juveniles may be moving downstream in Omak Creek or leaving the creek and entering the Okanogan River prior to collection in the fall. While we targeted the lower section of Omak Creek due to PIT tag detection of reconditioned kelts, we may not be representatively sampling for their progeny.

Determination of kelt reproductive success is dependent upon separation of first and second time spawning events. While some years (2007 and 2008) have shown discrete age-1 and age-2 histograms in spring collections at the screwtrap, variable growth rates preclude reliable age assignment by length. Potential alternatives include 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. The successful sampling of age-0 fish in fall 2011 demonstrated the ability to sample juveniles at a length that is identifiable, and will be repeated in future years.

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Section C: Yakima River Parentage Analysis

Written By

Jeff Stephenson
Shawn Narum
Ryan Branstetter
Doug Hatch
Columbia River Inter-Tribal Fish Commission

David Fast
Bill Bosch
Todd Newsome
Tim Resseguie
Joe Blodgett
Yakama Nation Fisheries Program

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: 1) Do reconditioned kelts produce viable offspring that contribute to recruitment, and 2) How does kelt reproductive success compare with natural first time spawners? In this study we utilize DNA markers and pedigree analysis to address these questions for kelt steelhead in the Yakima River and its tributaries. Addressing these questions will be important in determining if kelt reconditioning is a viable restoration tool that will aid in the recovery of ESA listed steelhead populations in the Columbia River Basin.

Methods-Sample Collection

Anadromous steelhead were collected as upstream migrating adults at Prosser Dam, or downstream kelt migrants at the Chandler Juvenile Monitoring Facility. Age-0 juveniles (juveniles collected in the same calendar year as the spawning event) were collected in the fall using electrofishing techniques (NMFS 2000 Electrofishing Guidelines). Age-0 status was determined by length (Toppenish <100mm, Satus <90mm) during fall collections. Sampling was targeted near areas where steelhead spawning was observed. Sample numbers for each collection can be seen in Table1.

Methods-Genetic Analysis

Samples were collected and stored in ethanol for preservation of DNA. Genetic analysis was conducted at the Hagerman Fish Culture Experiment Station in Hagerman, ID. DNA was extracted from tissue samples using standard manufacturer's protocols from Qiagen® DNeasy™ extraction kit. Genotyping efforts prior to 2011 used microsatellite markers described in Stephenson et al. (2008) and Blankenship et al. (2011). Current genotyping efforts utilize the PBT (parentage based tagging) panel of SNP (Single Nucleotide Polymorphism) markers described in Hess et al. (2012).

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.

To evaluate genetic diversity, expected and observed heterozygosity were calculated using Excel Microsatellite Toolkit (Park 2001). 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). 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 minimize incorrect assignments, simulations were performed to determine a 99.9% confidence LOD

value (natural log of the overall likelihood ratio). A Sampling rate of 0.185 was used for simulations as determined by dividing the total number of genotyped fish expected to spawn in 2011 (1140) by the Prosser Dam escapement estimate of (6195). Parental assignments were excluded unless they met the critical LOD value of 9.9 (From 99.9% confidence value), had a minimum of 92 loci comparisons, and zero mismatches.

Expected assignment rates for single parent matches, two parent matches and total number of matches were calculated using sample completion rates (S_{cr}) which were based off the number of genotyped adults expected to spawn in 2011 divided by steelhead escapement estimates. Adults expected to spawn in 2011 include all 2011 kelt collections, upstream migrants collected at Prosser in fall of 2010, and kelts collected in 2010 that were successfully reconditioned, released and subsequently detected by PIT tag moving upstream at Prosser Dam. Because the majority of adult samples were collected as kelts, samples are biased towards females. This creates a bias in the expected probability of one versus two parent assignments, but will still allow comparisons between locations.

Probability of Both parents $= S_{cr} * S_{cr}$

Probability of only one parent $= 2*((S_{cr}) - (S_{cr} * S_{cr}))$

Probability of No parents $= (1-S_{cr}) * (1-S_{cr})$

Expected parental assignments for adult collection types were calculated similar to juvenile collection estimates but were standardized to the number of successful adult assignments to any progeny. Because adults collected at Prosser in the spring of 2010 were expected to spawn in 2010 and not 2011, expected rate of assignment was set to 0.0%.

Results-Genetic Analysis

A total of 2218 samples were successfully genotyped. Numbers for each collection, by location and year, can be seen in Table 1. Departures from Hardy-Weinberg equilibrium (critical level =0.05 /95 loci = 0.00053) were seen only in adult collections that are comprised of multiple populations. Linkage disequilibrium (critical level =0.05 /4465 pairwise comparisons = 0.000012) was seen in both the adult collections, and both Toppenish collections). Parentage analysis proceeded as normal as it does not require Hardy-Weinberg or Linkage equilibrium.

Table 1. Population Statistics. Each collection is reported in terms of sample size (n), expected heterozygosity (H_E), observed heterozygosity (H_O), number of loci out of Hardy-Weinberg equilibrium (HW), and number of pairwise loci comparisons showing significant linkage disequilibrium (LD).

	Lat	long	n	HE	HO	HW	LD
Satus, above Logy Cr.	46.179	-120.497	149	0.4058	0.4136	0	6
Satus, Dry Cr.	46.255	-120.416	49	0.4047	0.4244	0	8
Satus, at Logy Cr.	46.207	-120.482	20	0.4088	0.4156	0	0
Satus, at Wooden bridge	46.017	-120.651	29	0.4059	0.4016	0	0
Toppenish, Above Wesley Rd	46.331	-120.761	144	0.3625	0.3687	0	12
Toppenish, Simcoe Creek	46.403	-120.833	50	0.3921	0.4037	0	6
Chandler kelt 2011			945	0.4220	0.4107	4	12
Chandler kelt 2010			556	0.4194	0.4078	2	5
Prosser upstream 2010 Spring			170	0.4203	0.4082	1	0
Prosser upstream 2010 Fall			106	0.4271	0.4228	0	0
Total			2218				

Across all juvenile collections, 122 offspring were successfully assigned to at least 1 adult. Nine offspring were successfully assigned to both a female and male adult. Table 2 shows the expected, observed and percent of expected parentage assignments for each of the juvenile classes. Percent of expected ranged from 30.4% in Satus, Dry Creek, to a high of 111.7% in Toppenish Creek above Wesley Rd. Percent of expected over all populations was 82.4%.

Table 2. Expected and observed parentage assignments for each juvenile collection.

	n	1 parent		2 parent		Total	
Satus, above Logy Cr.	149	34	22.8%	2	1.3%	36	24.2%
BY 2011 Expectations			30.2%		3.4%		33.6%
Percent of expected			75.7%		39.2%		72.0%
Satus, Dry Cr.	49	5	10.2%	0	0.0%	5	10.2%
BY 2011 Expectations			30.2%		3.4%		33.6%
Percent of expected			33.8%		0.0%		30.4%
Satus, at Logy Cr.	20	3	15.0%	0	0.0%	3	15.0%
BY 2011 Expectations			30.2%		3.4%		33.6%
Percent of expected			49.7%		0.0%		44.7%
Satus, at Wooden bridge	29	7	24.1%	0	0.0%	7	24.1%
BY 2011 Expectations			30.2%		3.4%		33.6%
Percent of expected			80.0%		0.0%		71.9%
Toppenish, Above Wesley Rd	144	47	32.6%	7	4.9%	54	37.5%
BY 2011 Expectations			30.2%		3.4%		33.6%
Percent of expected			108.2%		142.0%		111.7%
Toppenish, Simcoe Creek	50	17	34.0%	0	0.0%	17	34.0%
BY 2011 Expectations			30.2%		3.4%		33.6%
Percent of expected			112.8%		0.0%		101.3%
All Drainages	441	113	25.6%	9	2.0%	122	27.7%
BY 2011 Expectations			30.2%		3.4%		33.6%
Percent of expected			85.0%		59.6%		82.4%

For all parentage assignments, at least 1 juvenile sample was assigned to 61 adults. The majority of adults had either 1 or 2 juveniles, while 6 adults had 7 or greater offspring (Figure 1). Observed versus expected parentage assignment rates for each adult collection are seen in Table 3. Relative percent of expected was highest in the Chandler kelt 2011 collections (116.0%), and lowest in the Prosser upstream 2010 fall collection (8.2%). As anticipated no progeny from fish in the Prosser upstream 2010 spring group were observed.

Figure 1. Distribution of offspring across all successful adults

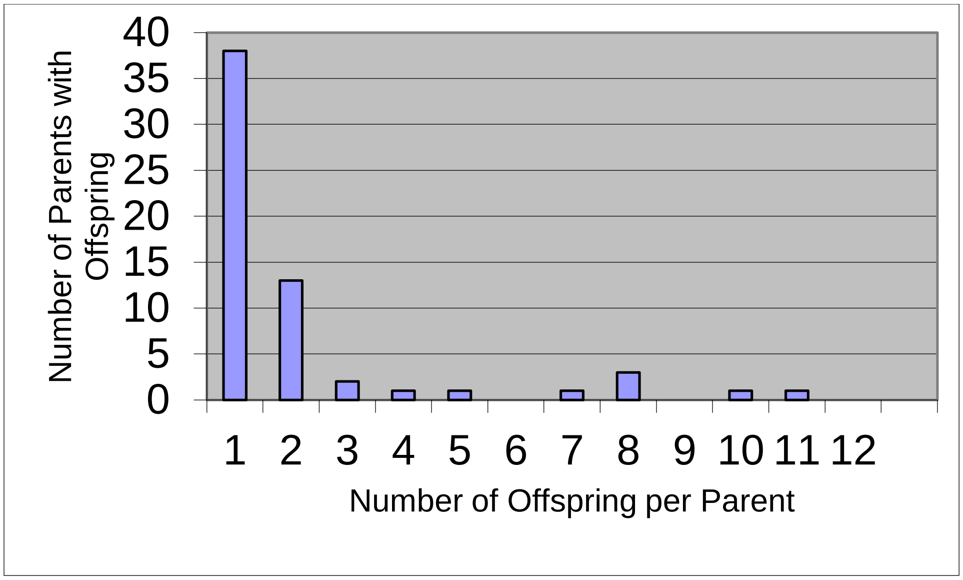

Table 3. Expected and observed parentage assignments for each adult collection. Note that the 2011 kelt collections represent the maiden spawning event for the 2011 Fall electrofish efforts.

	n	Assignments	
Chandler kelt 2011 (maidens)	945	126	96.2%
BY 2011 Expectations		108.6	82.9%
Percent of expected			116.0%
Chandler kelt 2010	89	4	3.1%
BY 2011 Expectations		10.23	7.8%
Percent of expected			39.1%
Prosser upstream 2010 Fall	106	1	0.8%
BY 2011 Expectations		12.18	9.3%
Percent of expected			8.2%
Prosser upstream 2010 Spring	0	0	0.0%
BY 2011 Expectations		0	0.0%
Percent of expected			NA

Four juveniles were assigned back to a reconditioned kelt released in fall of 2010. Table 4 shows a summary of reproductive success attributed to fish that went through the reconditioning process and returned to spawn again.

Table 4. Summary of reproductive success. Each detection of reproductive success reported below

Progeny	Stage	Length	Sample year	Brood Year	Kelt ID	Kelt Gender
OmyTopp11-0046	Age-0	73	2011	2011	OmyYRC10k-0550	Female
OmyTopp11-0055	Age-0	65	2011	2011	OmyYRC10k-0339	Female
OmyTopp11-0125	Age-0	84	2011	2011	OmyYRC10k-0475	Female
OmySat11-0005	Age-0	49	2011	2011	OmyYRC10k-0169	Female

Discussion

Reproduction by reconditioned kelts was confirmed for four adults released in 2010. While this number is lower than expected, statistical power is lacking, and expected contributions were likely confounded by collection methods. The highest percent of expected rate of assignment was seen in kelts collected in 2011 (116.0% of expected). For age-0 samples collected in 2011, these collections represent adults that putatively spawned for the first time. As these collections are comprised of post-spawn adults, they are thought to have successfully completed spawning prior to capture. Fish collected at Prosser in fall of 2010 had a much lower percent of expected rate of assignment at only 8.2%. This indicates that reproductive success of upstream migrants is not directly comparable to that of downstream migrants. Possible explanations include a high rate of fallback at Prosser Dam, high capture rates during fisheries, or high predation prior to spawning.

Percent of expected assignment rates by juvenile sampling location were highest in the two Toppenish collections at 111.7% and 101.3%. Rates within the Satus Creek drainage varied between 30.4% and 72.0%. The differential rates indicate that sampling location influences the proportion of progeny that would be expected to assign back to sampled adults. This may indicate a biased collection method for adults. Kelts are collected only at the Chandler juvenile collection facility. Adults not entering the Chandler facility are not sampled. While previous research has shown differential run timing of kelts (Branstetter et al. 2011), it is unknown if sampling rate is also biased due to emigration routes. Future work using radio-tagged fish may help determine differences, if any, between migration patterns in each stock.

Of the four reconditioned kelts later assigned to a juvenile progeny, only one was detected moving upstream over Prosser Dam following post-conditioning release. PIT tag detections may not be 100% complete at the Dam, and tag shedding has remained a concern in the kelt program. Misassignment of the correct parent also cannot be ruled out. While these fish likely also spawned in 2010, the offspring lengths are consistent with Age-0 collections from the 2011 brood year.

Collection of Age-0 juveniles in the fall was successful in showing reproductive success of reconditioned kelts. The collection of Age-0 juveniles should continue in future years as the primary method of offspring collections. The data in this report indicates that sampling location affects the success rate, but a temporal effect cannot be ruled out, so sampling should continue over multiple areas for the 2012 juvenile collection efforts.

While reproductive success has been confirmed for reconditioned kelts, we are currently unable to calculate relative reproductive success estimates. The small number of samples that are being successfully assigned limits statistical power to compare reproductive success among other groups such as first time spawners. Increasing the proportion of adult spawners and number of juveniles sampled will help with this issue. A second issue is the lack of unbiased data for first time spawners. Samples collected at the Chandler facility as post-spawn kelts are putative first time spawners for the year they are collected. However, they are not random samples. Post-spawn kelts have survived the full life cycle and are assumed to have successfully spawned. Alternatively, kelts that are released following reconditioning are still exposed to over-wintering and pre-spawn mortality. Reconditioned kelts detected at Prosser Dam and first time spawner adults sampled at Chandler Dam in the fall are likely a good comparison, but sample sizes for these two groups are low. Additional work is needed to identify a method for comparing success rates of reconditioned kelts to other fish.

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

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

Contract No C11-32
Purchase Order No C11003240

Prepared by

Christine Moffitt, Principal Investigator

Graduate Students: Bryan Jones, Zachary Penney

Staff: Boling Sun

EPSCoR student intern: Heath Hewett

Idaho Cooperative Fish and Wildlife Research Unit

University of Idaho, Moscow, ID 83844-1141

AI. Quarterly Report 1 January – 30 March 2012

Executive Summary

During the past quarter, we continued data analysis and synthesis of information on aspects of the project. Graduate students Zach Penney and Bryan Jones presented oral papers at the annual meeting of the Idaho Chapter of the American Fisheries Society (AFS). Our EPSCoR intern Heath Hewett presented a poster on steelhead otoliths at the AFS poster session. Zach Penney attended and presented a poster on his research at the Pacific States Marine Fisheries Commission steelhead meeting in March. We interacted with Chris Pinney of the U.S. Corps of Engineers about their contract for acoustical tagging of kelts at Snake River weirs and Lower Granite Dam. Contract recipient Pacific Northwest National Laboratory has hired Bryan Jones to assist with tagging starting in April because of his experience and familiar with the sampling locations and staff of collaborating agencies and tribes. Zach Penney presented his revised dissertation proposal to his graduate committee and has scheduled his written comprehensive examination for the week of 23 April, and oral examination was scheduled for 21 May. His dissertation will be completed in the spring of 2013. Bryan Jones will complete his master's thesis after kelt tagging has ceased and will defend his thesis early in the fall semester. Jones anticipates continuing his work for PNNL and provide assistance for tagging and migration studies of steelhead kelts in the Snake River system in 2013.

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.

Our goal is to quantify and model energy expenditure and transformations in steelhead using samples from lethal and non-lethal methods. This quarter, student Zach Penney continued his comparisons of the physiological capacity for iteroparity between inland Snake River and coastal Situk River steelhead kelts using nutritional and energetic metrics from blood plasma. All analysis has focused on good condition female kelts because of sampling constraints (Table 1).

We sampled coastal steelhead kelts from the Situk River weir in southeast Alaska (Figure 1) in collaboration with the Alaska Department of Fish and Game in May 2011. Blood plasma metrics of Situk kelts were compared with good condition natural origin (adipose present) from locations within the Snake River. We used samples of kelts collected from three locations in 2010: 1) Lower Granite Dam juvenile bypass kelt facility; 2) Several Potlatch River weirs; and 3) two weirs on the upper Clearwater River (Figure 2). Kelts from the upper Clearwater River weirs were large B-run sized fish, and kelts from the Situk River were also very large sized fish.

The kelts collected from the Lower Granite Dam juvenile bypass system from 1 April 1 to 2 July were from mixed stocks with unknown spawning locations or migration history. A random sample of 50 good condition kelts was selected from the data collected at this site to r Granite reduce type II error due to limited sample sizes at other locations. Known stocks of steelhead were sampled from locations within the Clearwater River system in collaboration with Idaho Department of Fish and Game. Kelts from the Potlatch River system were sampled from 18 March 18 to 21 May 21 at four weirs on the following tributaries: Little Bear, Big Bear, East Fork, and West Fork. Kelt sampling in the upper Clearwater River tributaries occurred from 29 April to 2 July on Fish Creek, tributary to the Lochsa River, and on Crooked River, tributary to the South Fork of the Clearwater River.

Table 1: Median, ranges of detectable values and the number below limits of detection (BDL) and percent BDL by plasma metrics indicative of nutritional status. Size range of fish sampled is provided. All samples were from natural origin good condition female steelhead kelts.

				Nutritional plasma factors				
System	N		Length (cm)	Protein (g/dL)	Calcium (mg/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Glucose (mg/dL)
Lower Granite Dam juvenile bypass facility	50	Median	60.5	2.9	9.1	66.0	43.0	85.0
		Range	52.0 - 83.0	2.4 - 3.5	6.0 - 18.2	5.0 - 186.0	12.0 - 243.0	49.0 - 163.0
		No. BDL (%)	0 (0)	37 (74)	0 (0)	0 (0)	9 (18)	0 (0)
Potlatch River weirs	47	Median	68.9	2.7	9.2	87.0	82.5[a]	86.0[a]
		Range	60.0 - 76.0	2.4 - 3.8	7.0 - 16.2	33.0 - 280.0	9.0 - 281.0	39.0 - 264.0
		No. BDL (%)	0 (0)	32 (70)	0 (0)	0 (0)	0 (0)	0 (0)
Upper Clearwater River weirs	25	Median	75.0	2.9	8.8	100.0	75.0	107.0
		Range	62.0 - 81.0	2.5 - 3.6	3.8 - 10.5	28.0 - 178.0	10.0 - 176.0	31.0 - 176.0
		No. BDL (%)	0 (0)	7 (28)	0 (0)	0 (0)	0 (0)	0 (0)
Situk River weir	24	Median	79.5	3.7	12.8	130.5	233.5	136.5
		Range	61.0 - 87.5	2.7 - 6.0	8.1 - 15.6	55.0 - 242.0	55.0 - 576.0	64.0 - 191.0
		No. BDL (%)	0 (0)	2 (8)	0 (0)	0 (0)	0 (0)	0 (0)

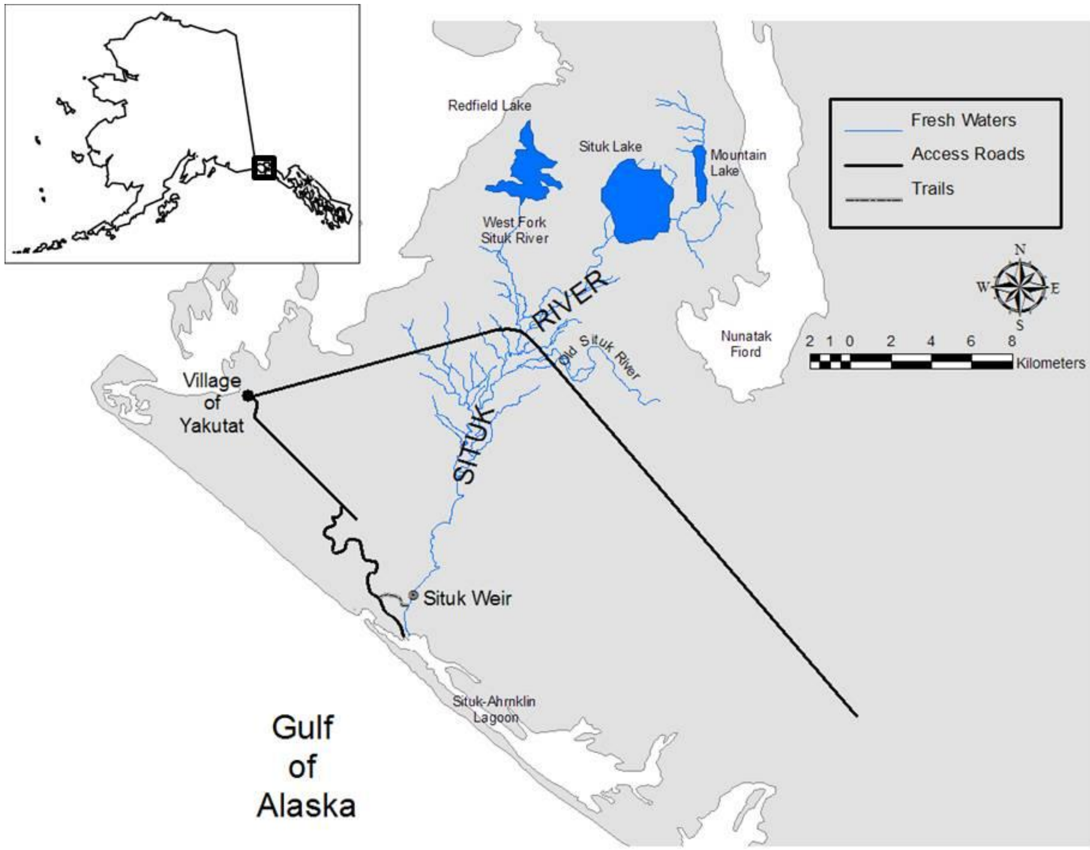

Figure 1. Location of the weir on the Situk River in southeast Alaska where coastal stocks of steelhead kelts were sampled in May 2011.

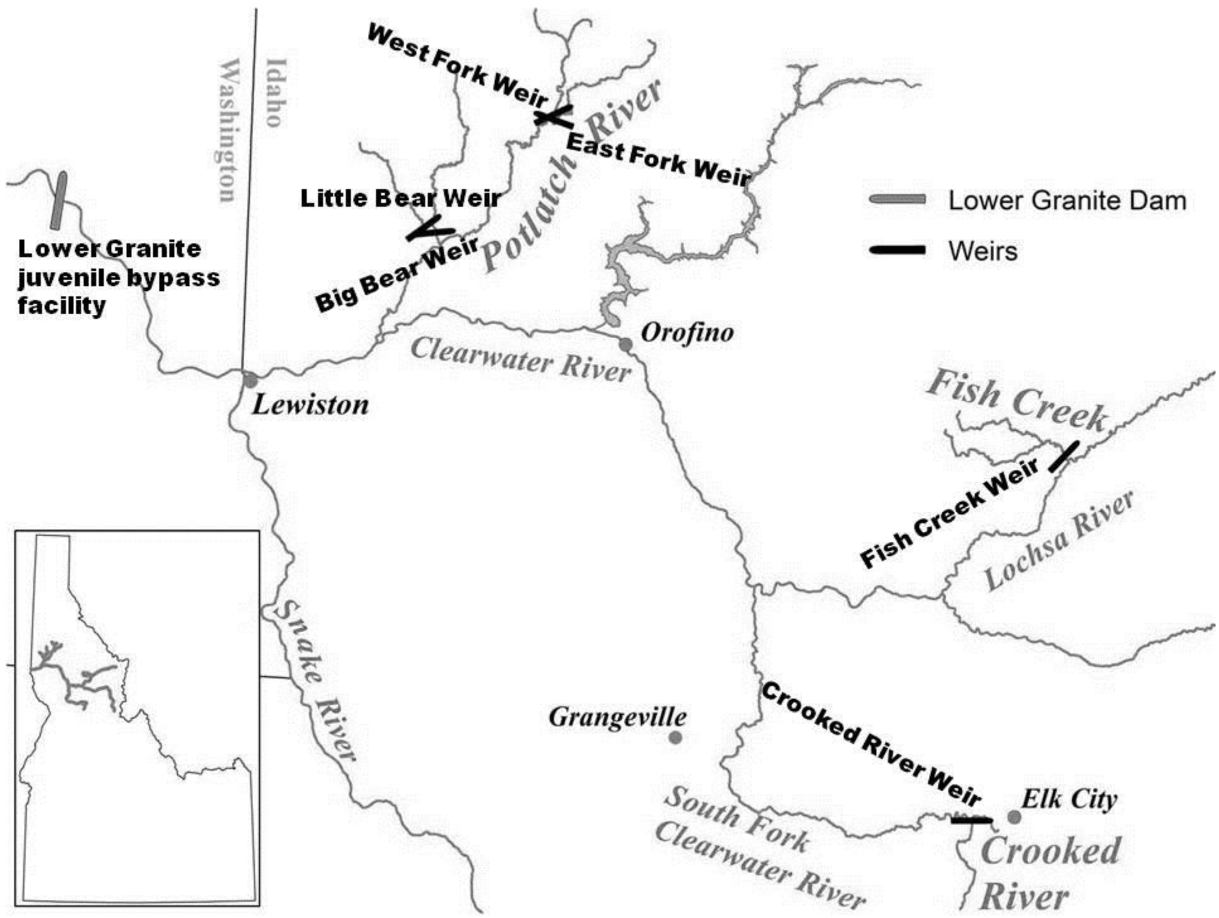

Figure 2. Location of sampling sites for inland summer-run Snake River steelhead kelts. Locations include: Lower Granite Dam juvenile bypass facility; Big Bear weir, Little Bear weir, West Fork weir, and East Fork weir on the Potlatch River; Fish Creek weir, and Crooked River weir.

We explored the variation in our data with principle components analysis, and evaluated relationships among plasma biochemical attributes of nutritional, tissue damage, stress, and electrolytes. Nutritional factors included total plasma calcium, protein, cholesterol, triglycerides, alkaline phosphatase, and amylase. Tissue damage factors included aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH). Stress factors included glucose, sodium, and chloride. Electrolyte factors included magnesium, phosphorus, and potassium. Total plasma protein was not included in principle components analysis due to high numbers of kelts that were below detection limits (<2.5 g/dL) for plasma protein. As a result, we performed chi-square tests on the proportion of kelts below detection limits between the four sample sites.

We found that 56% of the variance was accounted for in three principle components (Table 2). We found the highest loadings were with triglycerides (0.40), cholesterol (0.42), calcium (0.40), and glucose (0.34) of the 14 plasma metrics tested. These loadings suggest that nutritional factors with the exception of glucose accounted for the majority of variance among the Situk and Snake River kelts. Median values for protein (when detectable), cholesterol, triglycerides, calcium, and glucose were consistently higher in Situk kelts than Snake River kelts. (Table 1 & Figure 3). Multiple comparisons with a Kruskal-Wallis test showed that triglycerides, cholesterol, calcium, and glucose were significantly higher in Situk kelts than in Snake River basin kelts, except for plasma cholesterol comparisons between the Situk and upper Clearwater River kelts (Table 2).

We evaluated the differences in proportion of plasma protein samples below the limits of detection with chi-square tests of independence. We found no significant differences between kelts from the Situk and upper Clearwater River, and no significant differences between kelts from the Potlatch River and Lower Granite Dam. However, the proportion of female kelts from the Situk and upper Clearwater River with plasma protein below detection limits was significantly lower than samples from kelts at Lower Granite Dam juvenile bypass facility or the Potlatch River weirs (Table 1).

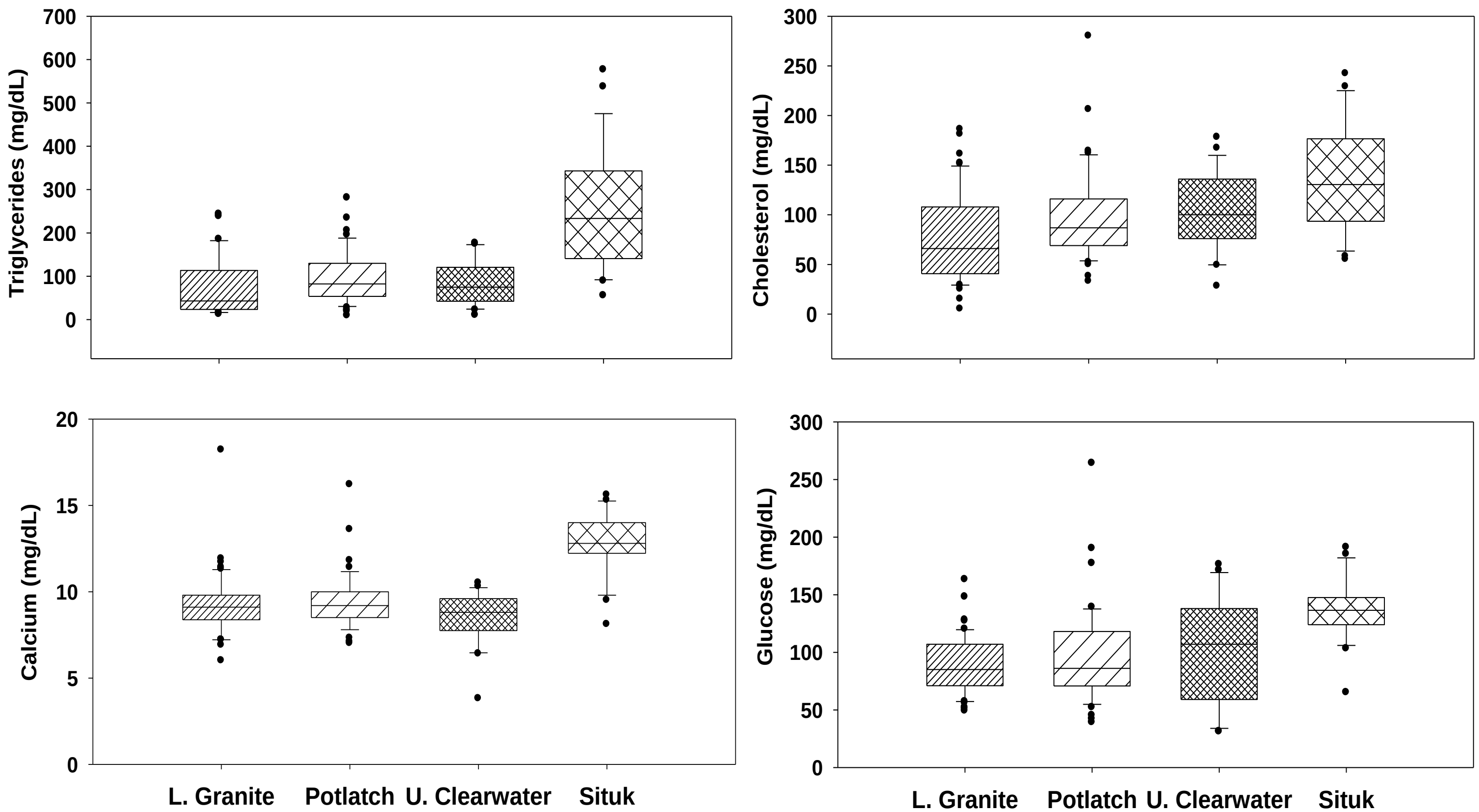

Figure 3: Boxplots of plasma triglycerides, cholesterol, calcium, and glucose, separated by location of sample. All samples were natural origin good condition female steelhead kelts. Sample numbers were as follows: Lower Granite Dam juvenile fish bypass (N = 50), Potlatch River weirs (N = 47), upper Clearwater River weirs (N = 25), and Situk River weir(N = 24).

Table 2: Principle component loadings for nutritional, tissue damage, stress factor, and electrolyte factors in the blood plasma of natural origin good condition coastal female Situk River and inland Snake River steelhead kelts.

Plasma metric	PC 1	PC 2	PC 3
Calcium	0.3967	-0.1849	0.0162
Cholesterol	0.4228	0.2146	-0.059
Alkaline Phosphatase.	0.1555	0.5243	0.0373
Amylase	-0.248	0.1637	0.2143
Triglycerides	0.3961	0.1937	-0.1004
ALT – alanine aminotransferase	0.0703	-0.2408	-0.4165
AST – aspartate aminotransferase	0.2705	0.1475	-0.3751
LDH – lactate dehydrogenase	-0.0896	0.4611	0.0744
Glucose	0.3375	-0.0190	-0.1015
Sodium	0.2861	-0.2654	0.4231
Chloride	0.2768	-0.3682	0.3034
Magnesium	0.2168	0.2713	0.3846
Potassium	-0.1240	-0.0784	0.0115
Phosphorous	-0.0230	0.0453	0.4355

Table 3: Summary of P-values for multiple comparisons of plasma metrics with Kruskal-Wallis tests. All samples were from natural origin good condition female kelts from coastal Situk River and inland Snake River stocks.

	Permutation P-values			
Comparison	Cholesterol	Triglycerides	Calcium	Glucose
Situk vs Lower Granite Dam	<0.01	<0.01	<0.01	<0.01
Situk vs Potlatch	<0.01	<0.01	<0.01	<0.01
Situk vs upper Clearwater	0.07	<0.01	<0.01	0.01
Potlatch vs Lower Granite Dam	0.11	0.56	0.98	0.80
Potlatch vs upper Clearwater	0.98	0.94	0.13	0.89
Upper Clearwater vs Lower Granite Dam	0.08	0.99	0.30	0.40

Nutritional plasma metrics were highest in coastal Situk River kelts. Although, glucose has been identified as a stress factor in feeding juvenile salmonids, we speculate that glucose is likely related to nutrition in fasting kelts. Further examination of the categorization of specific plasma metrics into nutritional, tissue damage, stress, and electrolyte factors using multivariate methods such as factor analysis will be conducted.

The coastal Situk River steelhead populations have two run components: a stream-maturing fall-run and an ocean-maturing spring-run. It was not possible to determine the entry timing of Situk kelts, and thus we could not relate nutritional factors to variations in gonadal maturation. The Situk River is a short system (35.6 km) compared to the Snake River, where inland steelhead swim over 750 km to reach natal spawning systems. Our results provide physiological evidence that nutritional and energetic factors in the blood plasma of coastal kelts are significantly higher than inland steelhead.

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.

At tributary sites in 2010 we collected blood samples from 235 kelts. This sample size was greater than samples in 2009 (n= 15) or 2011 (n= 43) and allows for more robust data analyses. We used principle components analysis (PCA) of these samples to explore covariation within data. We found 59% of the variation was explained by three principle

components (Table 4). Plasma calcium, sodium, chloride, and magnesium formed one component we have called electrolytes; ALT, AST, and LDH formed another likely characterizing tissue damage, and cholesterol, triglycerides, calcium, glucose, and alkaline phosphatase were groups as nutritional components. Calcium grouped with the electrolytes and the nutritional factors. Phosphorus, amylase, and potassium did not group with any of the other parameters within the first three factors. Protein was also excluded because of the high number of values below the detection limits of the laboratory equipment.

Table 4. Summary of principle component analysis for blood plasma biochemical parameters of kelts captured at Clearwater River tributary weirs in 2010. The parameters accounting for the majority of the covariation in the first three components are highlighted in bold.

	PC 1	**PC 2**	**PC 3**
Cholesterol	0.398	-0.144	**0.850**
Triglycerides	0.285	-0.046	**0.684**
Calcium	**0.677**	-0.226	**0.421**
Glucose	-0.047	-0.128	**0.448**
Alkaline phosphatase	0.063	0.345	**0.417**
Phosphorus	0.139	0.344	-0.274
Amylase	-0.178	0.287	-0.068
Sodium	**0.900**	-0.323	0.031
Potassium	-0.184	0.325	-0.234
Chloride	**0.905**	-0.354	-0.028
Magnesium	**0.433**	0.020	0.145
ALT	-0.220	**0.524**	-0.123
AST	-0.165	**0.736**	0.082
LDH	-0.173	**0.983**	-0.058
	Electrolytes	Tissue Damage	Nutrition

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.

In our previous analyses of kelt migration, we tried to account for river flow as a factor affecting migration rate using river discharge as a surrogate for water particle velocity. This approach uses the assumption that water particle velocity is positively correlated with river discharge. Recently biologists from the Fish Passage Center provided us a series of regression models that allow a prediction of water transit time through Lower Granite Reservoir, and other mainstem Snake and Columbia river reservoirs. Water transit time through Lower Granite Reservoir can be predicted using the following regression model:

$$y = -2.015 \ln(x) + 11.88 \qquad (1)$$

where y is days of water transit through Lower Granite Reservoir, and x is the average discharge (KCFS) at Lower Granite dam during the migration of each kelt. We divided the length of the reservoir (51 km) by the estimated days of water transit time, to yield an estimated water transit rate (km/day) that could then be compared to migration rate (km/day) of each kelt through Lower Granite Reservoir.

To investigate the relationship between kelt nutritional status and migration rate we divided kelts sampled and tagged in 2011 into groups of high and low cholesterol. We then plotted kelt migration rate with the predicted water transit rate for each kelt (Figures 4 – 5). We found most kelts from both the Potlatch River and Fish Creek in 2011 migrated faster than speed of the water within Lower Granite Reservoir, but sample sizes from the Potlatch were small (N = 9, vs N = 27 for Fish Creek).

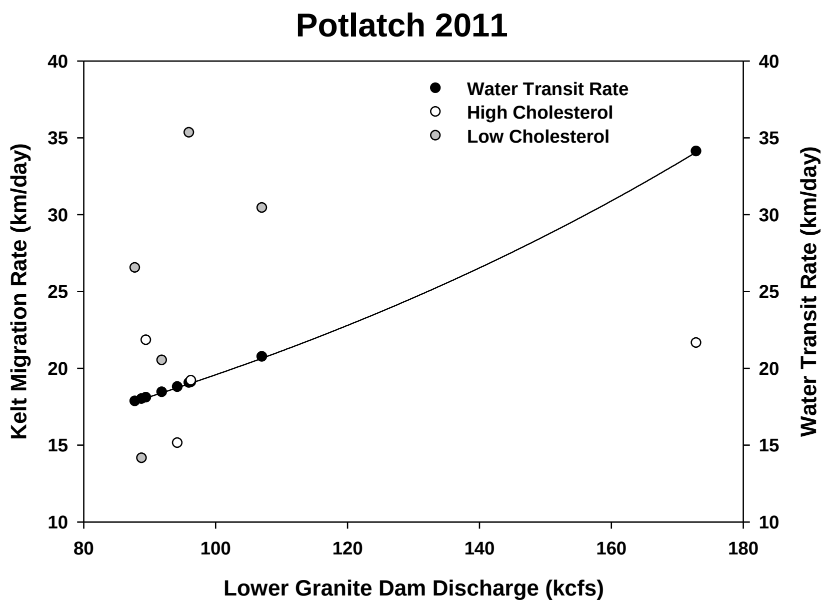

Figure 4. Migration rates of Potlatch River kelts through Lower Granite Reservoir compared with water transit rates during these same times (line connecting black dots). Kelts were separated by cholesterol level into high and low plasma cholesterol.

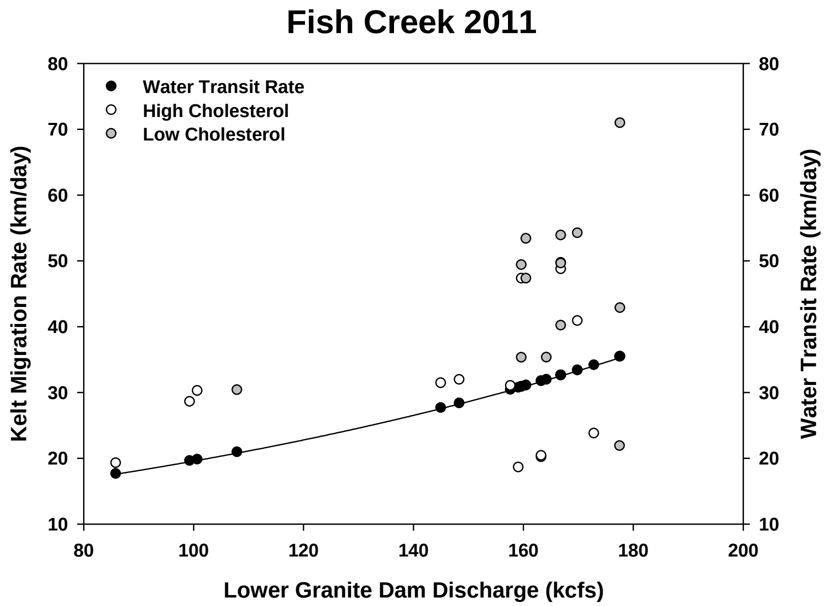

Figure 5. Migration rates of individual Fish Creek kelts through Lower Granite Reservoir compared with water transit rates during the same time (line connecting black dots). Kelts were separated by cholesterol level into high and low plasma cholesterol.

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

2010 migration- The nature of the runoff in 2010 allowed for access to kelts during the entire kelt migration. At the Little Bear weir, we observed a pulse of migration after water temperatures reached 14°C (Figure 6). We plan to analyze these and other data for any relationships with water temperatures. Limited data from the other Potlatch River weirs precluded analysis at other sites.

The timing of the kelt migrations in 2010 varied between sites. We observed that kelts from the tributaries that were lower in elevation migrated earlier than did kelts from higher elevation tributaries. Kelts from Little Bear Creek were first to emigrate with downstream migrations beginning in March. The kelts at Fish Creek were the last to migrate downstream, and median migration was in mid June (Figure 7).

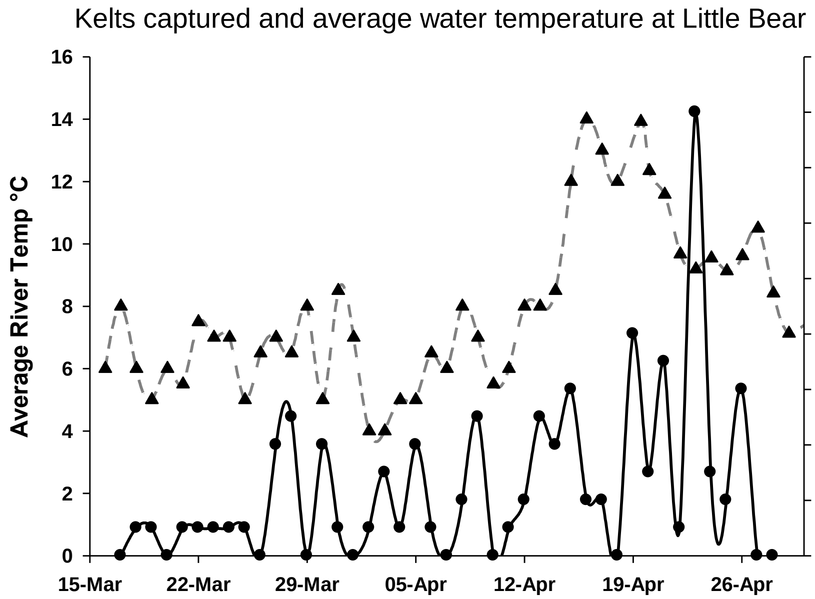

Figure 6. Number of kelts captured at the Little Bear weir, Potlatch River, 2010, by day (solid black line, dots). Average daily water temperature (dotted grey line, triangles) at the site is provided.

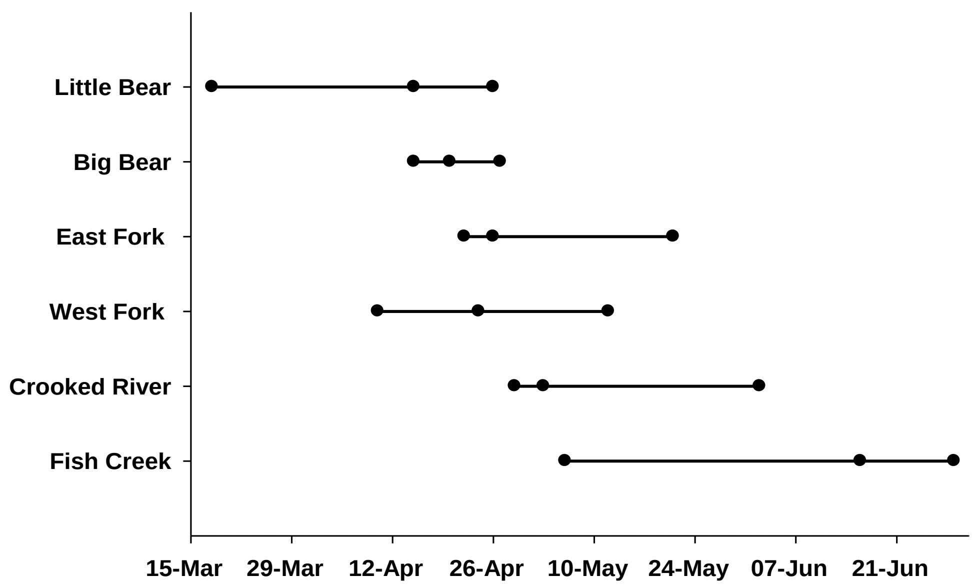

Figure 7. Timing of kelt emigration in 2010 from tributaries of the Potlatch River (Little Bear, Big Bear, East Fork and West Fork), Crooked River, a tributary of the South Fork of the Clearwater River, and Fish Creek, a tributary of the Lochsa. The black dot at the far left of each line indicates the date of the first kelt capture, the middle dot provides the date when 50% of the fish had migrated downstream, and the right dot indicates the date when the last kelt was captured.

2011 migration - We worked with collaborators from Idaho Department of Fish and Game to capture kelts at these same weirs in 2011. Record level snow melt and flows precluded our sampling the complete run and for several weeks we were unable to access any fish. From April to the end of June 2011 we obtained 43 steelhead kelts for our studies. These fish were sampled for blood, surgically implanted with acoustic transmitters, held for recovery, and released alive to migrate downstream (Table 5).

We selected only good condition kelts for tagging. The majority of fish tagged were from Fish Creek and from fish at the end of June. Of the 43 fish tagged, one kelt from Little Bear Creek, and 5 from Fish Creek died after being tagged but before release. Three of these mortalities were due to the oxygen running out in the holding tank. The causes of the other three mortalities are unknown, but the blood samples should provide some insight into their physiological status.

Table 5. Number of fish tagged by location and week sampled at Clearwater tributary weirs in 2011

Sample week	Location					
	Little Bear	Big Bear	West Fork	East Fork	Fish Creek	Crooked River
10-16 April	**1**	0	0	0	0	0
17-23 April	**3**	0	0	0	0	0
24-30 April	**5**	0	0	0	**1**	0
1-7 May	**1**	0	0	**2**	**3**	0
8-14 May	**1**	0	0	0	**3**	0
19-25 June	0	0	0	0	**4**	0
26 June -2 July	0	0	0	0	**19**	0
Total	11	0	0	2	30	0

At Big Bear Creek, and Crooked River, no good condition kelts were captured, as the flows were too high during the most likely time of kelt migration. Personnel from Idaho Department of Fish and Game unable to install the weir on the West Fork of the Potlatch River weir at all due to high flows. All three of these weirs were severely damaged (or completely destroyed and rebuilt as in the case of Little Bear) for a large portion of the kelt run. For this reason we were unable to reach our target of 40 kelts tagged in the Potlatch River. We reached the target of 30 kelts from Fish Creek. The median dates for kelt migration at the three weirs that we tagged fish were compared between the years, and the median dates are much later for 2011 (Figure 8).

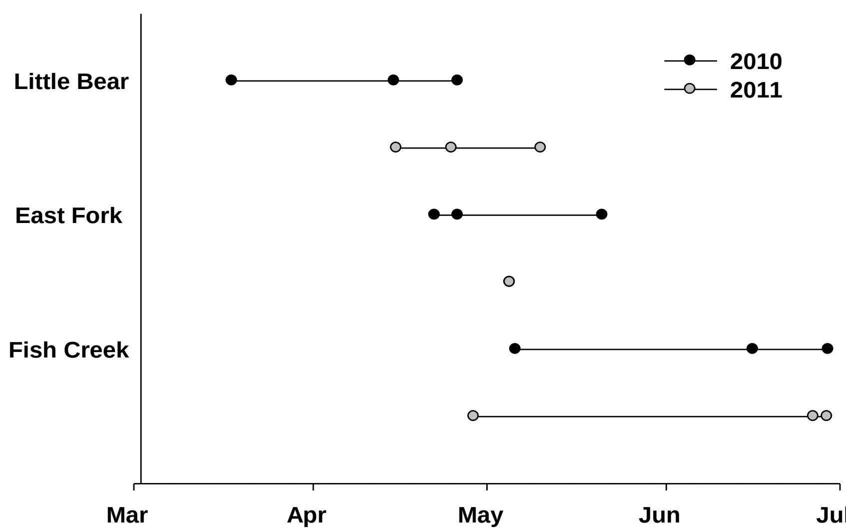

Figure 8. Date of first kelt captured (first dot), date when 50% of the kelts had been captured, and date of the last kelt captured in 2010 compared to 2011 for three tributary weirs of the Clearwater River.

In preparation for the acoustic tagging of kelts in 2011 we obtained permission and permits from various agencies and organizations to deploy acoustic receivers at locations

within the Clearwater River and Lower Granite Reservoir (Figure 9). The receivers within the reservoir are suspended under buoys. To anchor the receivers in the Clearwater River, we used pulley systems strung with steel cable and anchored to the bottom at one end and to various shore and bridge based structures at the other end. Data was downloaded from each receiver on a weekly basis once tagging of kelts began.

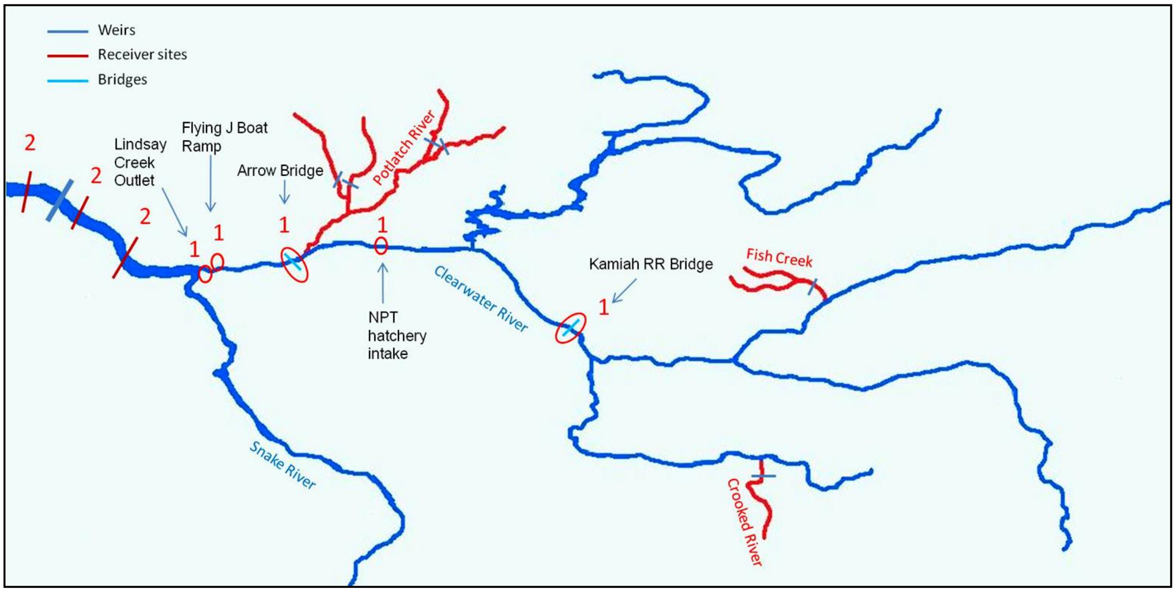

Figure 9. Map of weir and receiver sites (single or double receiver gates are indicated by 1 or 2) in the Clearwater River and lower Snake River reservoirs (Lower Granite Reservoir and Lake Bryan).

All 43 kelts that were released from the tributary weirs were successful in migrating through the Clearwater River and to the mouth of Lower Granite Reservoir (Table 6). At least 4 of the kelts from the Potlatch River appear to have died within the reservoir.

Table 6. Number of kelts tagged from each tributary, and the location at which each fish was last detected. Note: Data with (*) should be considered preliminary and will likely change as new data are downloaded.

		Fish last detected by location				
Site	Total sampled	Detected	Clearwater mouth	Mid reservoir	Lower reservoir	Below LGR
Potlatch	13	13	1	3	5	4*
Fish Creek	30	30	1*	6*	21*	2*
Total	43	43	2	9	26	6

We are currently waiting for data from the receivers below Lower Granite Dam that are monitored by NOAA fisheries. Additionally the last kelts tagged at Fish Creek could still be migrating through Lower Granite Reservoir and past the dam, so those data are not complete. The pattern speed of migration was slow initially after release, and then

migration speed increased. Rates appeared to decrease upon entry into the reservoir (Figure 10).

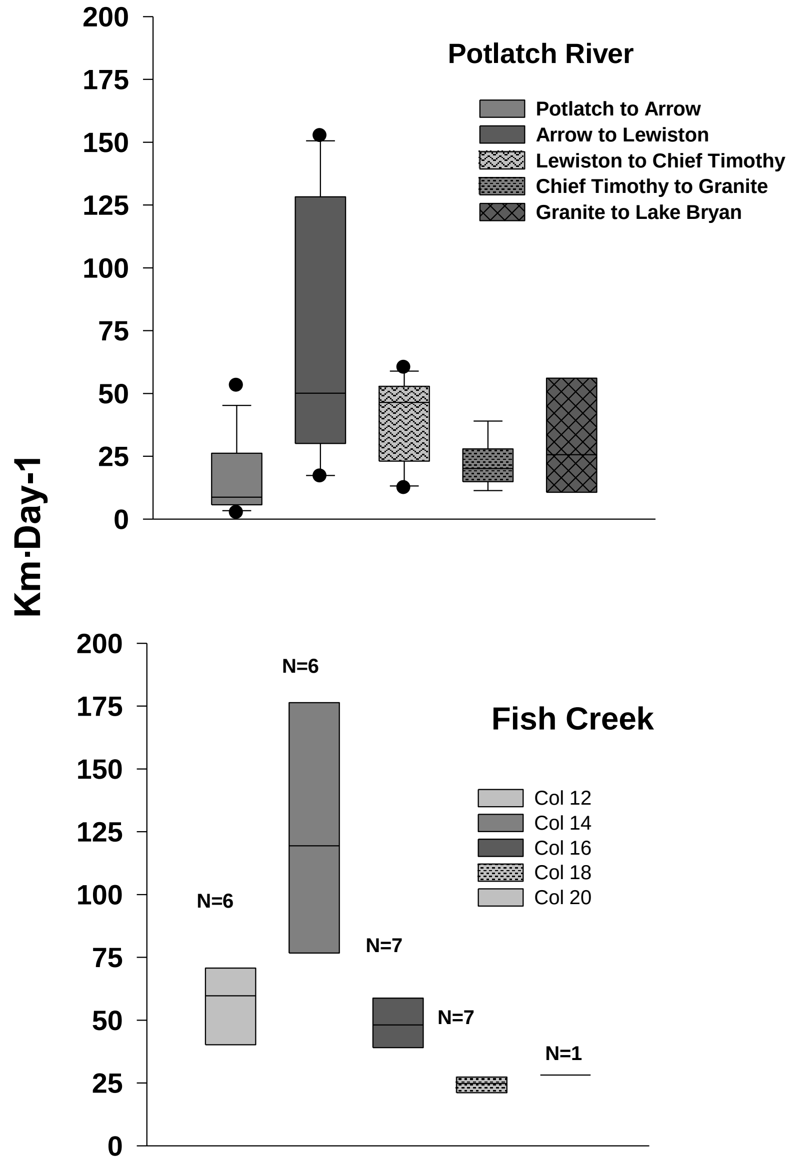

Figure 10. Downstream migration rates for acoustic tagged steelhead from the Potlatch River and Fish Creek tributaries to the Clearwater River. Location of river segments are provided on Figure 9. The 23 kelts tagged at the end of June are not included in this figure.

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.

In 2010 we PIT-tagged and released 1,398 kelts at the juvenile fish bypass at Lower Granite Dam (LGR). Of those tagged fish, 129 of which were detected migrating at the Little Goose Dam (LGS) juvenile fish bypass facility. This was the site generating the most PIT-tag detections that year, (Bonneville Dam, generated the second most detections with 54 kelts detected at that site). We calculated travel rates (km/day) between release at LGR and detection at LGS for the 129 kelts detected there. We calculated water transit time within the LGS reservoir (Lake Bryan) using the following regression model:

$$y = -2.3232 \, \mathrm{Ln}(x) + 13.721 \qquad (2)$$

where y is days of water transit from LGR to LGS and x is the average discharge (KCFS) at LGS during the migration of each kelt. We then divided the length of Lake Bryan (60 km) by the estimated days of water transit time for each kelt to yield an estimated average water transit rate (km/day) which could then be compared to the migration rate of each kelt through Lake Bryan.

We plotted migration rates and estimated water transit rates for the 129 kelts detected at LGS. We separated these kelts by condition (Figure 11), cholesterol level (Figure 12), and sex (Figure 13). As was observed in the migration of acoustic tagged kelts in 2011, the majority of the kelts PIT-tagged at LGR and detected at LGS in 2010 traveled faster than the estimated speed of the water in Lake Bryan. There are no obvious trends in migration rate of kelts related to condition, cholesterol level, or sex.

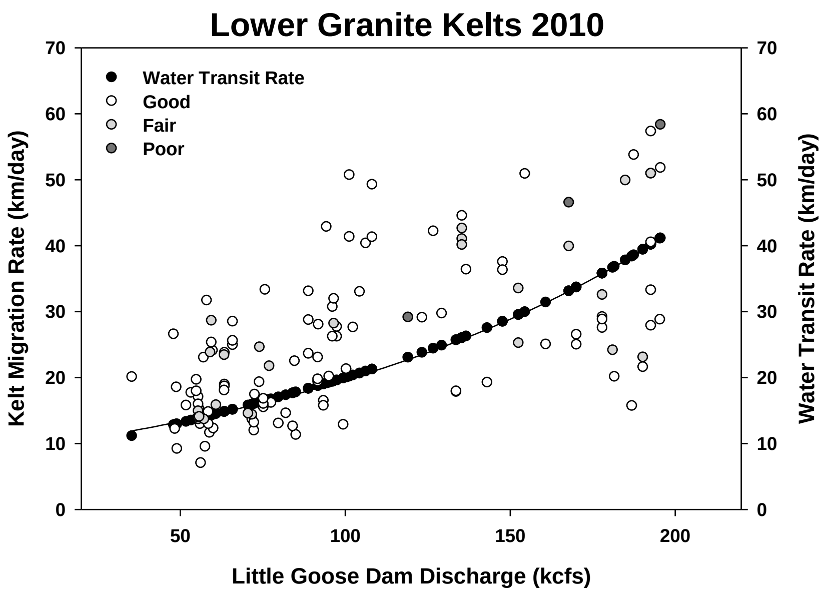

Figure 11. Migration rates of kelts tagged at Lower Granite Dam to detection at Little Goose Dam compared to estimated water transit rate within the reservoir. Kelts were separated by condition.

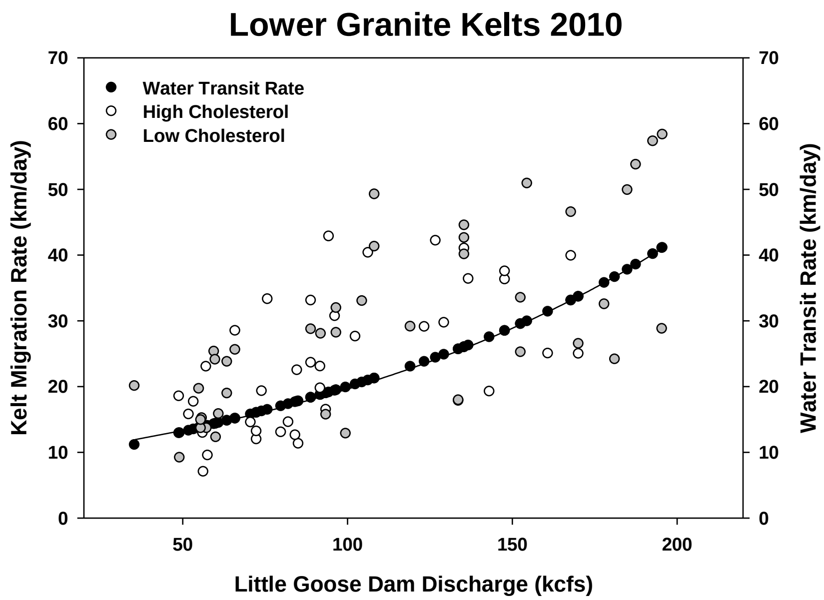

Figure 12. Migration rates of kelts tagged at Lower Granite Dam to detection at Little Goose Dam compared to estimated water transit rate within the reservoir. Kelts were separated by plasma high or low cholesterol.

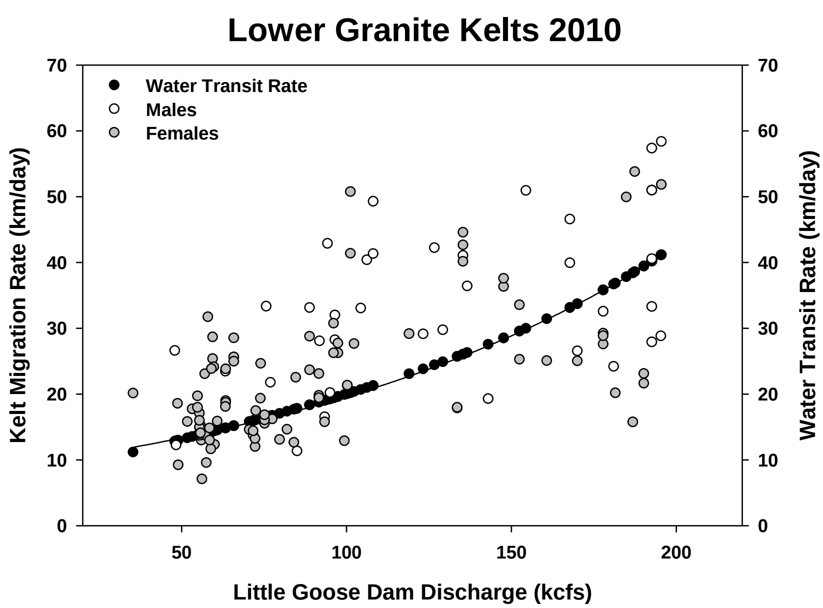

Figure 13. Migration rates of kelts tagged at Lower Granite Dam to detection at Little Goose Dam compared to estimated water transit rate within the reservoir. Kelts were separated by sex.

Problems or Needs for Future Work

We plan to prepare a statement of work and budget for our final segment of support on the kelt project. This contract goal will be to support student Zach Penney to complete his dissertation and to provide support for manuscript preparation and publication costs. Bryan Jones will complete his master's thesis this summer, and defend it in the fall. We plan for active interactions with Jones and Jessica Buelow as we prepare and submit manuscripts for publication during the coming contract year.

AII. Quarterly Report 1 April – 30 June 2011

Executive Summary

Activities this quarter focused on completion of data analysis, and drafting manuscripts summarizing research findings. Graduate student Bryan Jones took leave to work with Pacific Northwest National Laboratory to provide important leadership in acoustic tagging (JSATS) of kelts from multiple tributaries in the Snake/Clearwater River watersheds. Zachary Penney completed his written and oral comprehensive exams and has returned to preparation of manuscripts summarizing the data from our studies. He has prepared a summary of trends in proximate constituents, and energy depletion in tissues of B run steelhead trout from Dworshak National Fish Hatchery. We began preparation of a draft manuscript from a portion of the thesis work of student Jessica Buelow.

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.

We are synthesizing and comparing data collected in 2009, 2010, and 2011 from steelhead trout sampled at various times before, during and post spawning to model the physiological transitions of inland steelhead trout during the reproductive cycle. This quarter, we continued analysis of proximate and total energy measures from liver, white muscle, and whole bodies sampled from locations in the Columbia and Snake River drainage (Table 1).

In our determination of proximate constituents (water, ash, lipid, and protein), we ignored carbohydrates because carbohydrates constitute less than 0.5% of the somatic tissues of salmonids. The measurements of water and ash content of liver and white muscle tissues were obtained in our laboratory in Moscow. Lipid content, total energy (KJ/g) and the proximate constituents of whole bodies were determined at the Hagerman Fish Culture Experiment Station. Nearly all estimates of protein content were determined by subtraction (protein = 100 - water - ash - lipid). This approach is most often used in energy balance, as direct measures of protein using the Kjedahl method can be more variable.

Variations in energy content during sexual maturation, spawning, and kelt migration was evaluated using proximate analysis and bomb calorimetry. Lipid and protein are the primary energy sources in salmonid tissues. The separation of lipid and protein using proximate analysis provides detailed information about how these two specific energy sources are used throughout the reproductive cycle, but does not give direct estimates of total energy. In contrast, bomb calorimetry provides a direct measure of total energy content via reporting the combined sum of lipid and protein energy for a given tissue. However, when only proximate data are available, mass-specific caloric equivalents

for lipid and protein have been used to estimate total energy content in fish tissues (Brett 1995). Caloric equivalents of lipid and protein in fish tissues have been reported to be nearly identical to direct measures determined bomb calorimetry (Craig et al. 1978; Hendry and Berg 1999). Because we determined total energy content (direct method) and separated lipid and protein in tissues using proximate analysis we compared total energy content determined from bomb calorimetry to those calculated by using caloric equivalents (indirect method).

We used the reported caloric equivalents by Brett (1995) to calculate total energy for each gram of tissue, which was 26.4 KJ/g and 20.1 KJ/g for lipid and protein, respectively. We obtained strong correlation between these values when considering the white muscle and whole bodies (all $r^2 \geq 0.95$). The comparisons among liver tissues were not as strong ($r^2 = 0.70$; Figure 1). It is possible that liver may contain higher proportions of carbohydrates than white muscle and whole body considering its physiological importance in glycogen storage. We are conducting additional literature query to better understand why these comparisons were not as robust.

In general, we determined total energy estimated by bomb calorimetry was higher than total energy determined from the use of caloric equivalents for protein and lipid, thus suggesting that the use of calorific equivalents may underestimate the total energy content of varying fish tissues.

To obtain the most robust model of energy use over time in sexually maturing, spawning, and kelt steelhead trout, we are working with samples collected from Dworshak National Fish Hatchery (DNFH) in 2010, and 2011. All steelhead trout sampled from DNFH originated from the hatchery as smolts, thus provide data from a known stock with a known spawning location. All other samples collected from our research were from mixed stocks of unknown origin, and samples of pre spawning fish were limited in number.

The analysis of proximate constituents has provided insight into the metabolic process and the stasis of energy in overwintering steelhead trout. Since we had the most robust and balanced samples from male and female steelhead over spawning year 2010 and 2011, we plotted and modeled the estimates of protein and lipid in samples of white muscle over several monthly intervals, and also evaluated this with respect to average monthly water temperatures.

Table 1 Summary of paired lethal samples of liver, white muscle, and whole body tissues separated by spawning year, site of collection, months of collection, maturity phase, proximity to spawning location, and length (cm).

Proximate and energetic analysis					Liver		White muscle		Whole body	
Spawning Year	Site of Collection	Month of collection	Maturity phase	Proximity to spawning location	<70cm	>70cm	<70cm	>70cm	<70cm	>70cm
2009	Dworshak National Fish Hatchery	Jan - May	Spawning	known	2	67	2	67	0	0
	Lower Granite Dam juvenile fish bypass	May - Jun	Kelt	unknown	33	4	33	4	0	0
2010	Mackay Bar	Oct	Upstream migration	unknown	5	1	5	0	0	0
	Dworshak National Fish Hatchery	Jan - Apr	Spawning	known	2	111	2	111	0	0
	Nez Perce Tribal Hatchery	May	Spawning	known	2	3	2	3	0	0
	Lower Granite Dam juvenile fish bypass	Apr - Jul	Kelt	unknown	79	17	80	17	12	6
2011	Zone 6	Aug - Sep	Upstream migration	unknown	5	8	5	8	5	8
	Mackay Bar	Oct	Upstream migration	unknown	5	4	5	4	5	1
	Dworshak National Fish Hatchery	Oct	Upstream migration	known	10	5	9	5	0	0
	Dworshak National Fish Hatchery	Nov-Dec	Overwintering	known	0	0	2	29	0	0
	Dworshak National Fish Hatchery	Jan-Apr	Spawning	known	0	0	1	59	0	0
	Lower Granite Dam juvenile fish bypass	Apr - Jun	Kelt	unknown	0	0	10	10	0	0
				Total	143	220	156	317	22	15

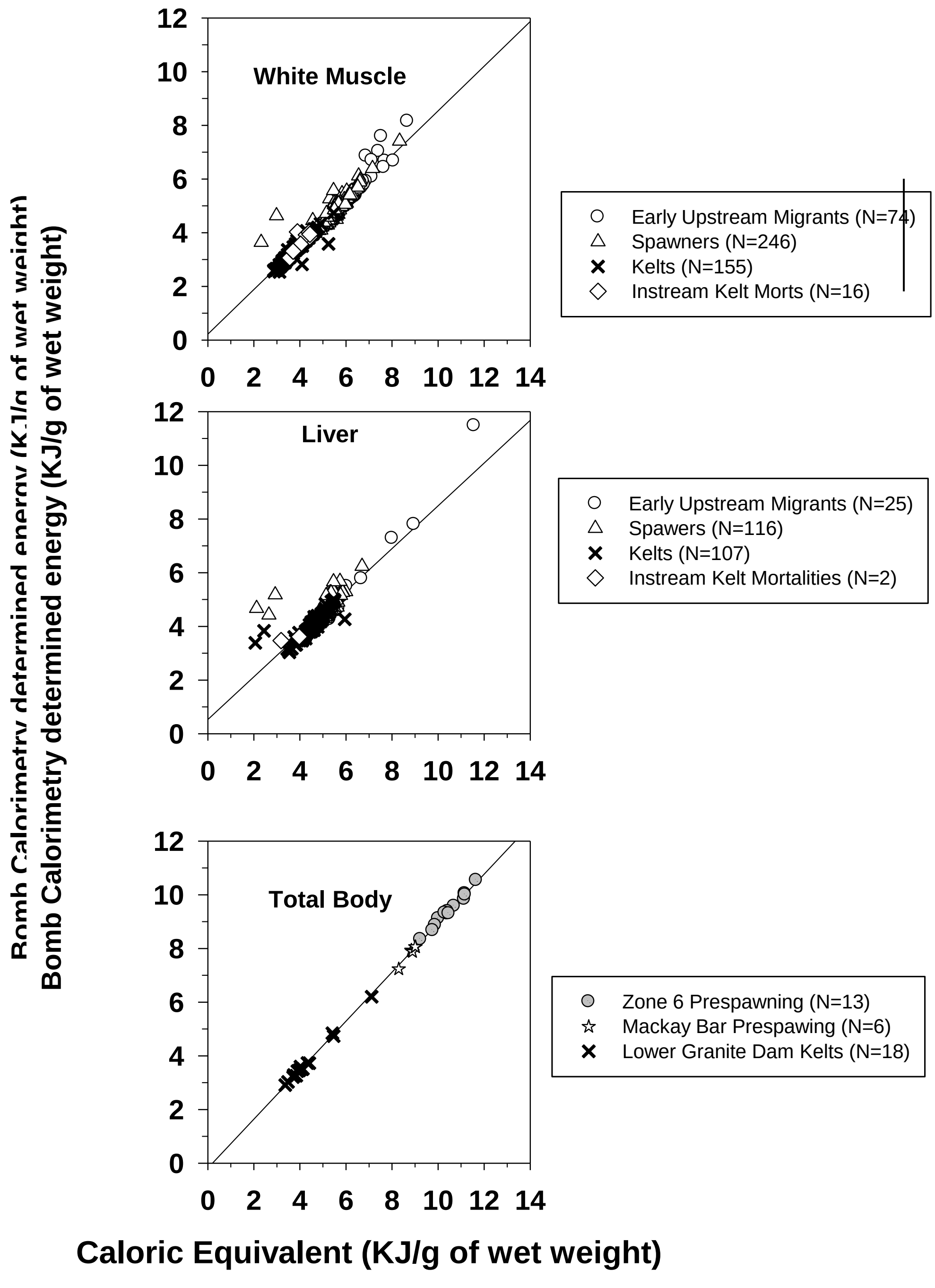

Figure 1. Scatterplot of total energy values for white muscle, liver, and whole body tissues determined from bomb calorimetry versus estimates using caloric equivalents for lipid and protein.

From January through April water temperatures remained below 6°C, and little change in constituents occur until April. However, the samples obtained in spawning year 2011 show depletion occurs in lipid and protein stores from October into the winter (Figures 2 and 3). The lipid fraction of samples in spawning year 2011 showed higher variation than the values estimated for protein. The total energy from bomb calorimetry provides the cleanest profile of these changes over time, and documents the little change observed from January through March (Figure 4).

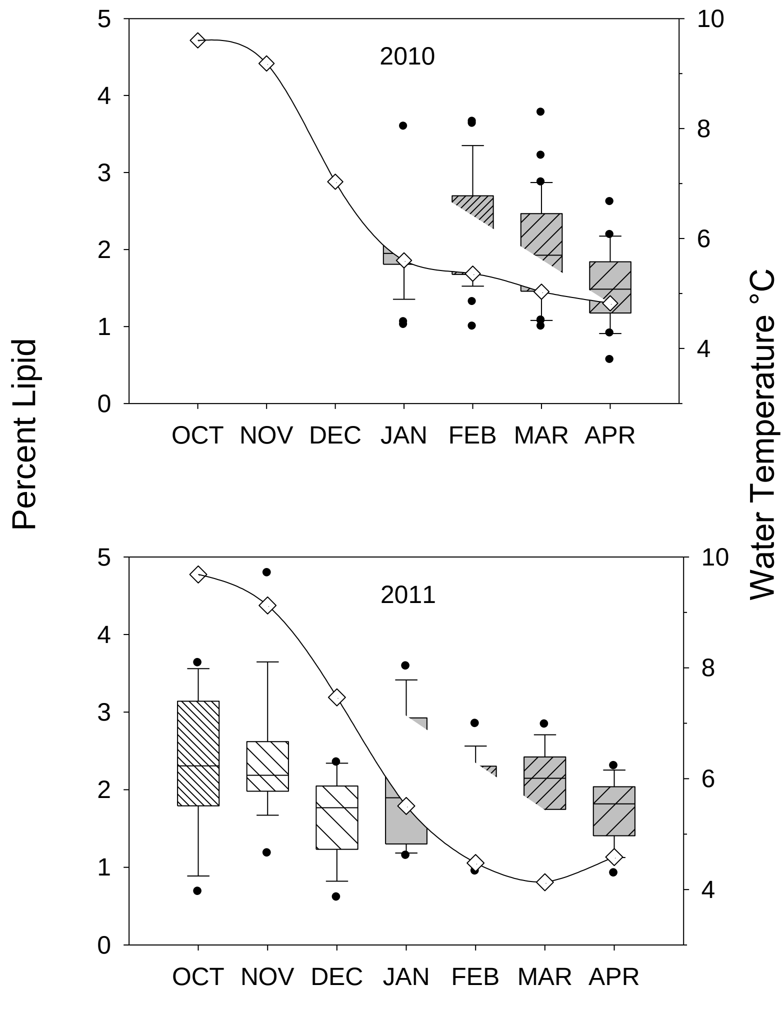

Figure 2. Percent lipid in samples of white muscle from tissues sampled at DNFH over two spawning years, 2010, and 2011. Water temperatures are plotted on the right axis (white diamonds). Samples in the fall of 2011 were collected from fish entering the hatchery, as a result of IHNV sampling that required lethal samples.

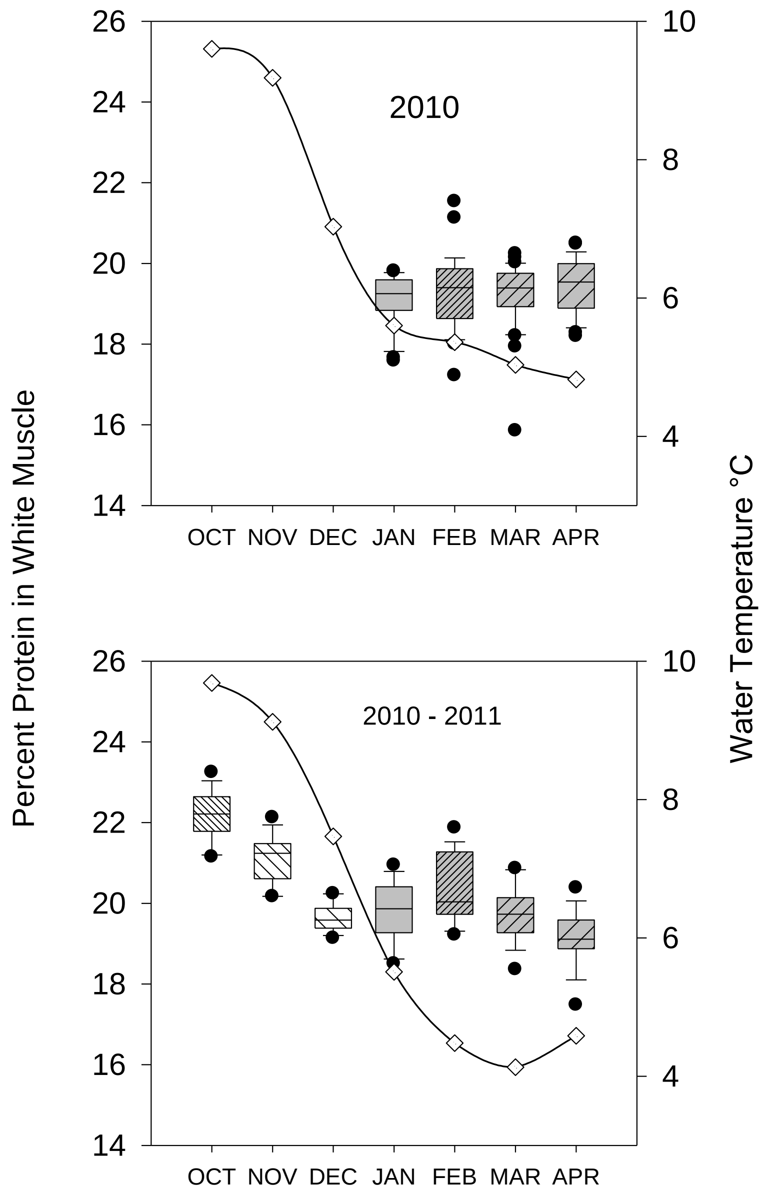

Figure 3. Profile of protein content in samples for white muscle removed from lethally sampled steelhead trout from Dworshak National Fish Hatchery, spawning years 2010 and 2011.Water temperatures are plotted on the right axis (white diamonds). Samples in the fall of 2011 were collected from fish entering the hatchery, as a result of IHNV sampling that required lethal samples.

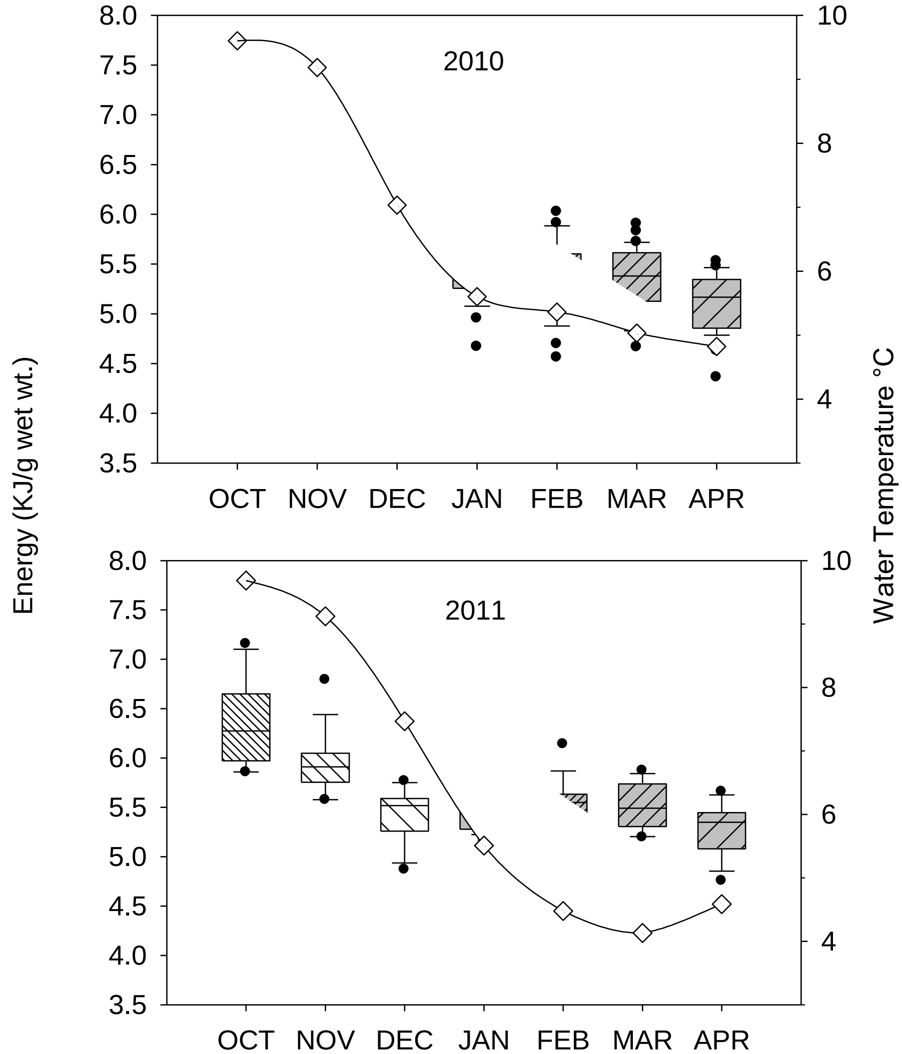

Figure 4. Profile of total energy content (determined via bomb calorimetry) in samples of white muscle removed from lethally sampled steelhead trout from Dworshak National Fish Hatchery, spawning years 2010 and 2011.Water temperatures are plotted on the right axis (white diamonds). Samples in the fall of 2011 were collected from fish entering the hatchery, as a result of IHNV sampling that required lethal samples.

We modeled tissue energy with a GLM model to consider the tissue energy content in samples from steelhead form January through April over two years with a model: Y = month + spawning year +month*spawning year, to test the significant differences between years and month. We found highly significant main effects and no significant interaction effect (Table 2). The significance of month was driven by the differences in April, versus all other time periods (Table 3).

Table 2. Summary of GLM analysis of measured energy content (KJ/g wet weight) in the white muscle of steelhead trout sampled over two spawning years at DNFH, January – April.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Spawning year	1	0.84814	0.84814	9.98	<.0019
Month	3	1.76883	0.58961	6.94	<.0002
Spawning year*month	3	0.04130	0.04130	0.16	0.9217

Table 3. Tukey groupings of least squared means of energy (Kj/g wet weight) across four months of sampling, with means ranked from highest to lowest.

Month	Grouping	LS Mean	DF
January	A	5.45512	41
March	A	5.40956	45
February	A	5.38091	44
April	B	5.17023	44

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.

We are preparing a manuscript to compare the physiological metrics measured in kelt migrants at Lower Granite Dam with those of a typical juvenile seaward migrating smolt. This will be the first manuscript we anticipate submitting for publication from our research. Much of this work was included in Jessica Buelow's thesis.

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 will continue our analysis of these parameters when Bryan Jones returns to complete his master's thesis later this summer.

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.

We have not completed any further analysis of these data, and will complete this during the next quarter.

Problems or Needs for Future Work

We have continued data analysis and preparation of manuscripts. We expect to provide completion reporting in the next contract cycle. We hope to receive our new contract soon, as the month of June is halfway completed.

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Craig, J.F., M.J. Kenley, and J.F. Talling. 1978. Comparative estimations of the energy content of fish tissue from bomb calorimetry, wet oxidation, and proximate analysis. Freshwater Biology 8:585-590.

Hendry, A. P., and O. K. Berg. 1999. Secondary sexual characters energy use, senescence, and the cost of reproduction in sockeye salmon. Canadian Journal of Zoology 77:1663–1675.

Section B: Kelt Reconditioning Physiology Studies

Andrew L. Pierce, Ph.D.
Douglas Hatch
Ryan Branstetter
Columbia River Inter-Tribal Fish Commission

James J. Nagler, Ph.D., Professor
Lucius K. Caldwell, Ph.D. Candidate
Tim Cavileer, Ph.D., Research Scientist
Josh Boyce, Ph.D., Postdoctoral Fellow
Kali Turner, Undergraduate Researcher
Josh Egan, Undergraduate Researcher
Department of Biological Sciences and Center for Reproductive Biology
University of Idaho, Moscow, ID

Bill Bosch
David Fast, Ph.D.
Joe Blodgett
Yakama Nation Fisheries

Scott R. Everett
James Paddlety
Nez Perce Tribe Fisheries

Introduction

In 2009 and 2010, studies were initiated to apply tools from fish physiology and endocrinology to issues in kelt reconditioning. These studies were continued in 2011. By developing and applying indices based on the endocrinology and physiology of reproduction, growth, stress, and osmoregulation in fish, we aim to achieve a detailed understanding of the physiology of reconditioning in kelt steelhead. This knowledge will provide a scientific basis for maximizing the success of kelt reconditioning programs. This research project has goals of establishing post-spawning rainbow trout as a model for studying reconditioning in kelt steelhead, establishing a hatchery model of Snake River B-run kelt steelhead, establishing and validating assays for plasma and tissue level bioindicators of reproductive status, growth, and stress in steelhead kelts and post-spawning rainbow trout, comparing reconditioning profiles of kelt steelhead at different locations in the Columbia basin and rainbow trout using non-lethal sampling, and testing specific interventions such as force feeding in kelts and/or rainbow trout.

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 ESA-listed status of fish in most reconditioning programs. Therefore, we have begun studies on post-spawning physiology in rainbow trout. Our initial goal is to construct a profile of growth and reproductive endocrine physiology in post-spawning female rainbow trout. This can then be compared to profiles from kelts, and treatments to stimulate feeding enhance survival, and increase reproductive maturation can be tested in rainbow trout. In 2011, we continued a study on the physiology of post-spawning rainbow trout, which is presented in Section B.I.

Steelhead Kelt Physiology Studies

Columbia basin steelhead vary greatly in life history, migration distance, and genetic stock (Brannon, et al. 2004). CRITFC and our collaborators are implementing kelt reconditioning projects at Omak Creek on the upper Columbia, on the Hood River at Parkdale, on the Yakima River at Prosser, and in the Snake River Basin at Dworshak. One of the objectives in the CRITFC kelt project under the Columbia Basin Accords is to compare kelt reconditioning at different locations. We are collecting blood samples to compare kelt reconditioning endocrinology and physiology across the Columbia Basin. Our goals are to develop methods for monitoring reproductive development of kelts, selecting fish for reconditioning, and enhancing the survival, growth, and rematuration of kelts in reconditioning programs. In 2011, we conducted studies on reproductive development (Section B.II), a new treatment for the freshwater parasitic copepods (Section B.III), and a pilot study for a supplemented diet (Section B.IV). Invasive or manipulative studies and

studies involving lethal sampling are difficult with wild endangered fish. Therefore, we believe that the establishment of a hatchery model for kelt reconditioning is of critical importance in kelt research. We took steps toward establishing this model in 2011 using artificially spawned Dworshak hatchery B-run kelts. Hatchery fish were used to test the effect of force feeding on survival in steelhead kelts (Section B.V), and were our first indication that the use of Ivermectin for copepod control in kelts was problematic (Section B.III). The results of these studies will increase the success of kelt reconditioning throughout the Columbia River Basin.

References:

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.

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

Lucius K. Caldwell
James J. Nagler
Department of Biological Sciences and Center for Reproductive Biology
University of Idaho, Moscow, ID

Andrew L. Pierce
Columbia River Inter-Tribal Fish Commission

Introduction

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

Methods

On 3 March 2011, 150 two-year-old female rainbow trout were obtained from a commercial broodstock operation immediately after their second spawning. Fish were held at University of Idaho (Moscow, ID) in twelve re-circulating tanks (volume approximately 1000L per tank) that share a common head-tank. The group of fish was split into two treatment groups, a control group and a feed-restricted group. The control group received the equivalent of 0.5% of body mass in feed per day, administered over the course of five days per week (*i.e.,* 0.7% of body mass on Thursday–Monday). The feed-restricted group received the equivalent of 0.1% of body mass in feed per day, administered on one day per week (*i.e.*, 0.7% of body mass on Monday). This feeding schedule for the feed-restricted fish was chosen to minimize dominance effects within the tank that may otherwise have prevented all fish in a tank having access to the limited amount of feed. Also, this scheme avoided potentially confounding physiological samples with acute feeding responses by preventing fish from being fed the day before sampling.

At the time of each sampling, all fish from a tank were netted and isolated into a 254 L double walled insulated fish tote (Daco, Kent, WA); water in the tote was aerated with compressed atmospheric air delivered via an airstone. Fish were individually netted from the isolation tote, anesthetized by immersion into a 100 L cooler (Igloo Products Corporation, Katy, TX) filled with tank-water containing 60 mg L^{-1} tricaine methanesulfonate (Finquel®/MS-222®, Argent Chemical Laboratories, Redmond, WA) buffered with 120 mg L^{-1} $NaHCO_3$ (Fisher Scientific, Hampton, NH), and then sampled. At the initial sampling date (9 March 2011), all fish were implanted with PIT tags to provide each individual with a unique identification code. For all fish, mass was measured to the nearest 0.01 kg with a digital balance, fork length was measured to the nearest 0.1 cm with tape measure, muscle lipid content was measured using a handheld microwave-based fat-

meter (Distell, Fauldhouse, UK), and blood was collected from all fish, using a 1.5” 20-guage needle connected to a 3.0 mL syringe (BD, Franklin Lakes, NJ). Needles and syringes were pre-coated with heparin by aspirating and then dispensing 3.0 mL of 100 mg mL^{-1} ammonium heparin (Sigma-Aldrich, St. Louis, MO) suspended in ultrafiltered H_2O. Whole-blood samples were held on ice until, at regular intervals (< 30 m), they were centrifuged (Microfuge E, Beckman Coulter, Palo Alto, CA) at 15,850 x g for 5 m. Plasma was pipetted off, collected into 2 mL polypropylene cryovials (VWR International, LLC, Radnor, PA), and held on ice until after sampling, then frozen in a -80ºC freezer.

At each sampling, 12 fish (six per treatment group) were deeply anesthetized in 100 mg L^{-1} MS-222® buffered with 200 mg L^{-1} NaHCO3, and then euthanized by decapitation. The torso was opened with a scalpel or scissors via a sagittal incision along the midline of the abdomen. Ovaries were then dissected and weighed to the nearest 0.1 g using an electronic balance. Every five weeks thereafter, all remaining fish were sampled.

To measure plasma concentration of the reproductive steroid 17ß-estradiol (E2), plasma samples were extracted consecutively with methyl tert-butyl ether (MTBE) (Fisher Scientific, Hampton, NH). Briefly, 100 µL plasma from each sample was pipetted into a 10 mL glass tube (Fisher Scientific, Hampton, NH), 4.0 mL MTBE was added to each tube, samples were vortexed for 1 m, and then samples rested for 7 m for phase separation to occur. After phase separation was visually observed, a small amount of liquid N_2 was used to fill the bottom of a foam cooler, and the rack of samples was set into the cooler so the aqueous phase was barely touching a layer of liquid N_2. After 1 m, the aqueous phase was visually inspected to ensure that it was frozen, and the solvent fraction was then poured off into a 5 mL glass tube. The MTBE extracted phase was allowed 10 m to equilibrate to room temperature, then placed into a 55°C water bath for approximately 2 h, until all solvent had volatilized. A second plasma extraction was then performed, using 3.0 mL MTBE, as described above; this second extract was pooled with the first extract. Once the solvent from the second extract had completely volatilized (approximately 2.5 h more), the residue from the pooled extracts of 100 µL plasma was resuspended in 250 µL E2 zero calibrator, yielding a 1:2.5 dilution factor for the extract. Average extraction efficiency was 83%, as determined by RIA values for extracted versus unextracted standard samples included with the RIA kit.

Resuspended plasma extracts were analyzed for 17ß-estradiol (E2) concentration using an antibody-coated tube E2 radioimmunoassay (RIA) kit (Coat-A-Count®, Siemens, Munich, Germany), per the manufacturer’s instructions. Sensitivity for the assay was 8 pg mL-1.

All data were analyzed using one- and two-way ANOVA to detect effects of treatment, time, and treatment by time. If effects were detected, Tukey’s multiple comparisons test or one-tailed Student’s t-test was used to detect specific differences and assign significance. All analyses were performed using the JMP statistical package (JMP, Version 9; SAS Institute Inc., Cary, NC, 1989-2012). Specific growth rate for mass (SGR-M) was calculated as $\frac{\ln({}^{mass_2}/_{mass_1})}{days\ between\ measurements} \times 100$. Fulton’s condition factor (k) was calculated as $(\frac{mass}{length^3}) \times 1000$.

Results

Within 10 weeks of initiating treatment, female fish that were fed the restricted ration exhibited lower total fish mass, SGR-W, fork-length, *k*, muscle lipid content, liver mass, and plasma concentration of E2 compared to female fish fed the maintenance ration (Figures 1-6). Within 15 weeks, female fish fed a restricted ration had reduced ovarian mass compared with female fish fed a maintenance ration (Figure 7). Within 10 weeks, female fish fed a restricted ration had reduced plasma concentrations of E2 compared with female fish fed a maintenance ration (Figure 8).

Discussion

As would be expected, total fish mass and specific growth rate for mass were different between fish fed a full ration and fish fed a restricted ration, suggesting that restricting feed after spawning reduced growth in post-spawned female rainbow trout. Body condition (Figure 4) and muscle lipid content (Figure 5) were similarly affected by restricting feed availability, and the two measurements likely assess similar aspects of fish physiology. This suggests that the mass:length ratio (representing an approximation of fish girth) reliably indicates energy stores. The difference in muscle lipid content and liver mass for fish in the two groups suggests that, in addition to generating reductions in total fish mass, feed restriction also affected body composition. In light of these observations, it appears that during the 30 weeks of this experiment, rainbow trout mobilized energy that was stored as lipid (in both muscle and liver tissue) to fuel critical metabolic processes as they prepared for the next reproductive cycle.

As we hypothesized, markers of reproduction were affected by restricting feed availability. By 15 weeks, ovary mass was greater in fish fed a full maintenance ration compared with fish fed a restricted ration; this difference persisted and increased through the end of the experiment. Additionally, within 10 weeks, plasma E2 concentrations were greater in fish fed a full maintenance ration than they were in fish fed a restricted ration. Again, these differences persisted and increased through the end of the experiment. Taken together, these results suggest that restricting feed availability in post-spawned female rainbow trout impacts the reproductive cycle negatively. Fish that were fed a full ration appeared to prepare for reproduction normally, while those that were fed a restricted ration exhibited unchanging ovary size and steroid output.

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

Figures:

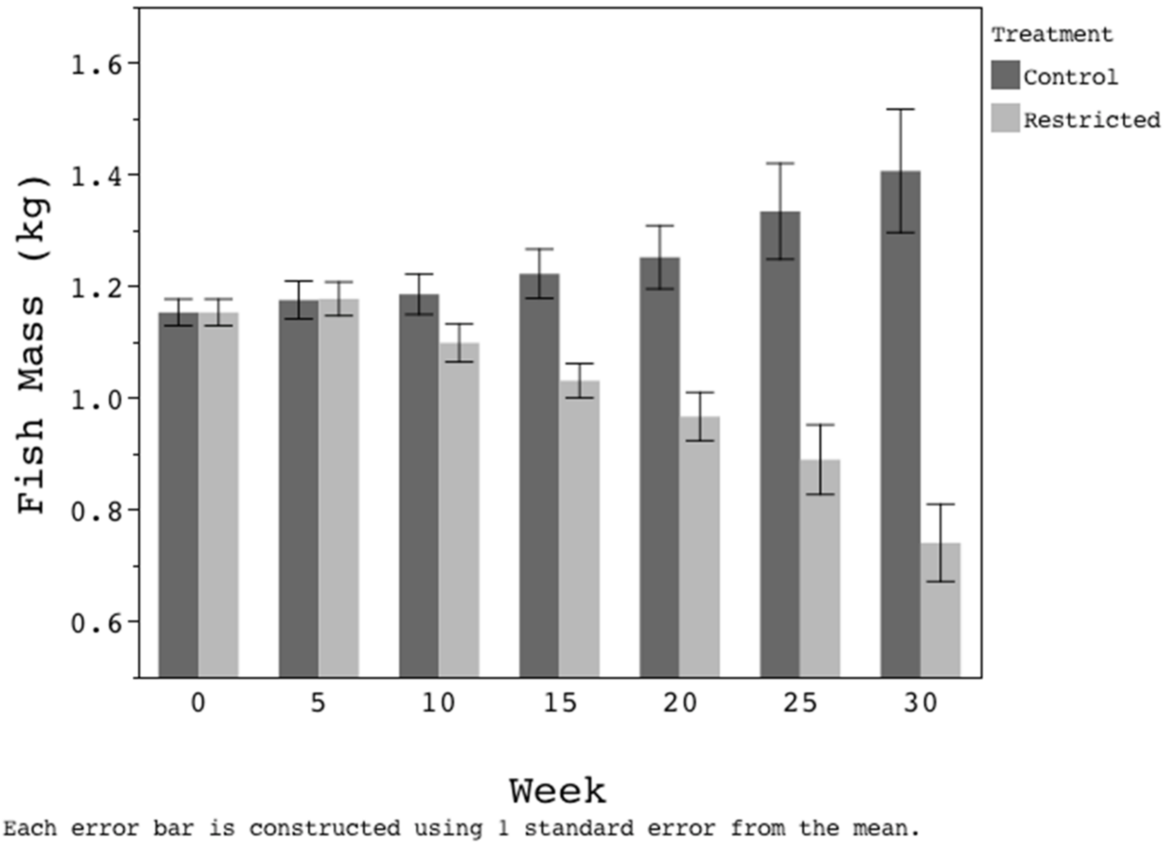


Figure 1: Total fish mass was affected by feed availability; fish that were fed a restricted ration exhibited lower total body mass within 10 weeks.

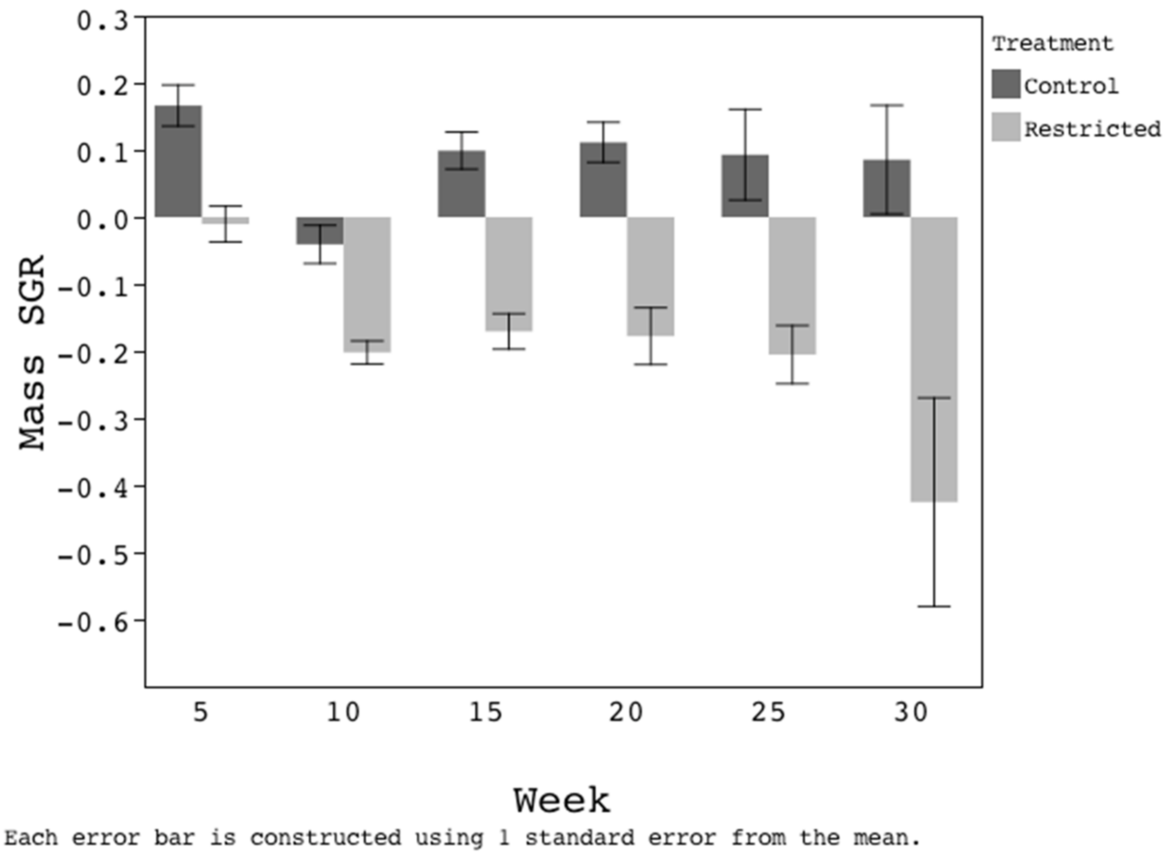


Figure 2: Specific growth rate for fish mass was affected by feed availability; fish that were fed a restricted ration exhibited lower growth rates within 5 weeks.

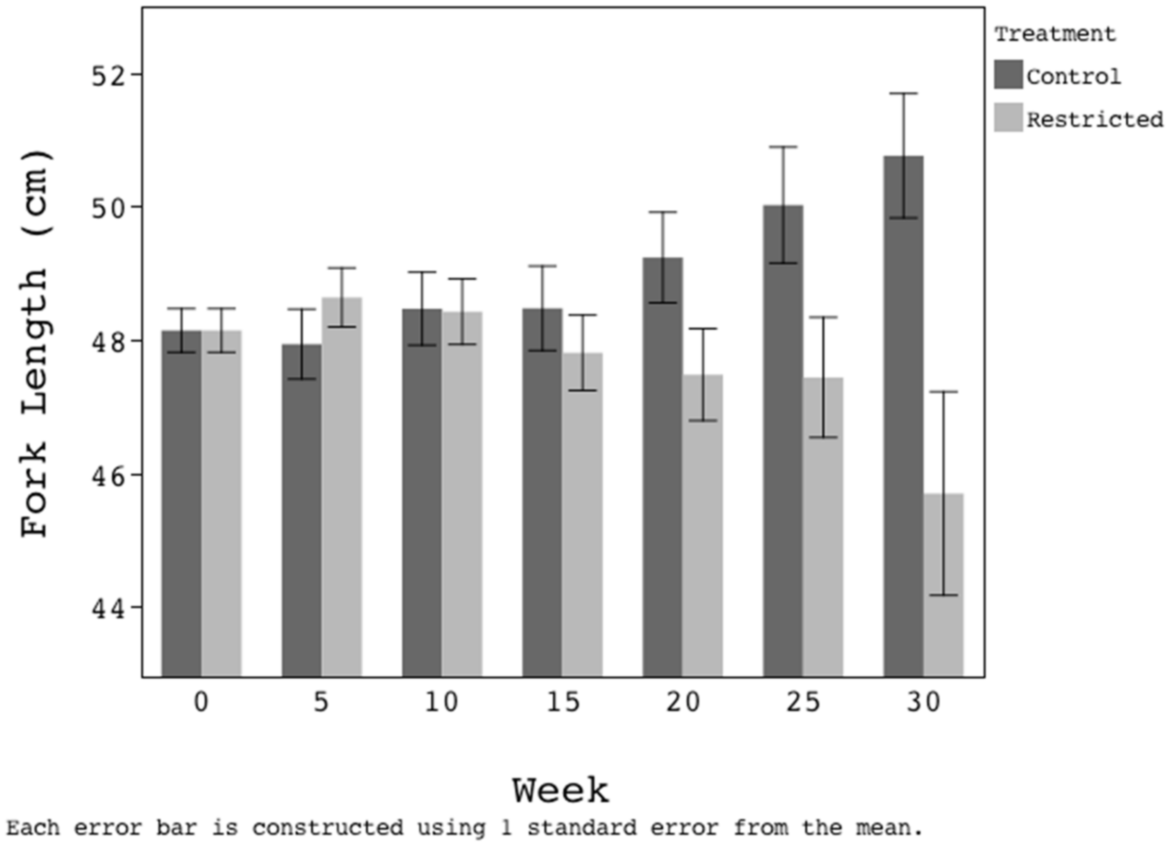


Figure 3: Fork length was affected by feed availability; fish that were fed a restricted ration exhibited lower total body mass within 15 weeks, indicating a combination of size-selective mortality and reduction in length of surviving fish.

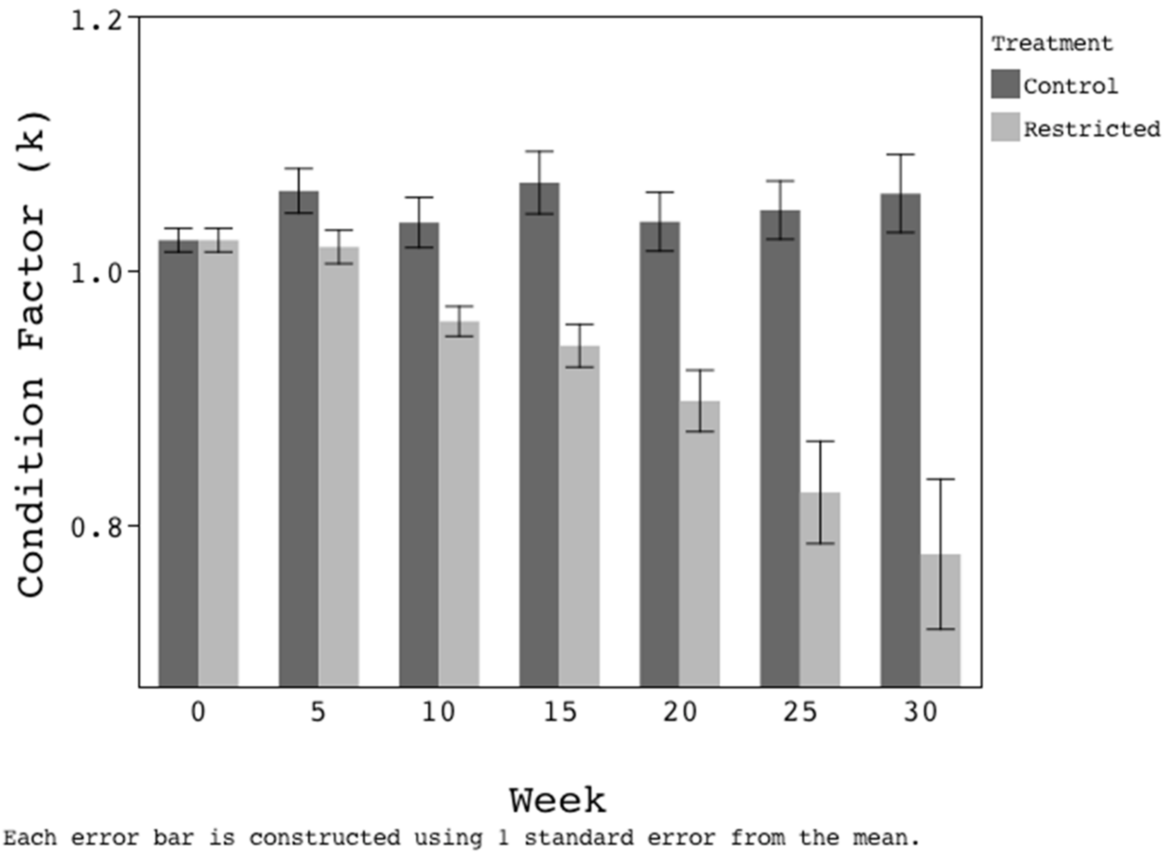


Figure 4: Fulton's condition factor (*k*) was affected by feed availability; fish that were fed a restricted ration exhibited lower *k* within 10 weeks.

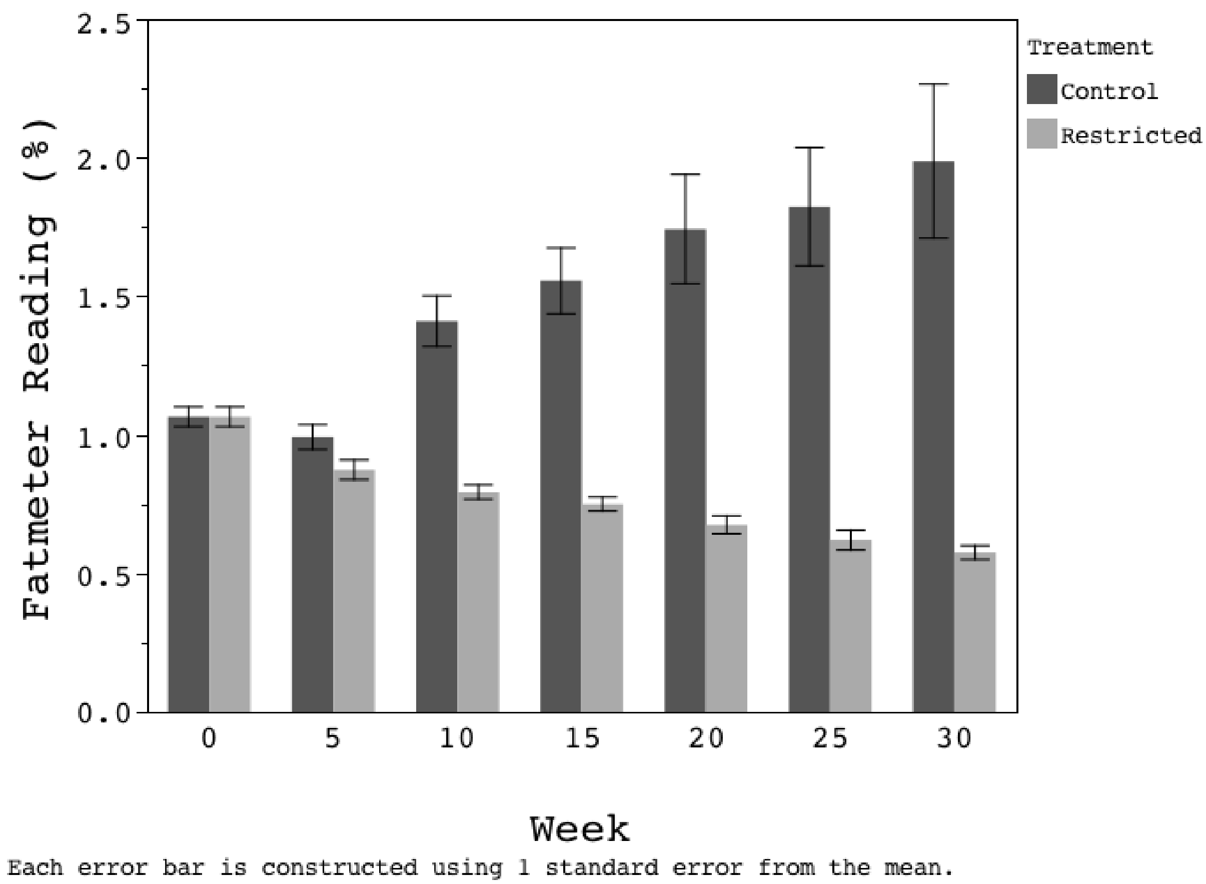


Figure 5: Muscle lipid content was affected by feed availability; fish that were fed a restricted ration exhibited lower lipid levels within five weeks.

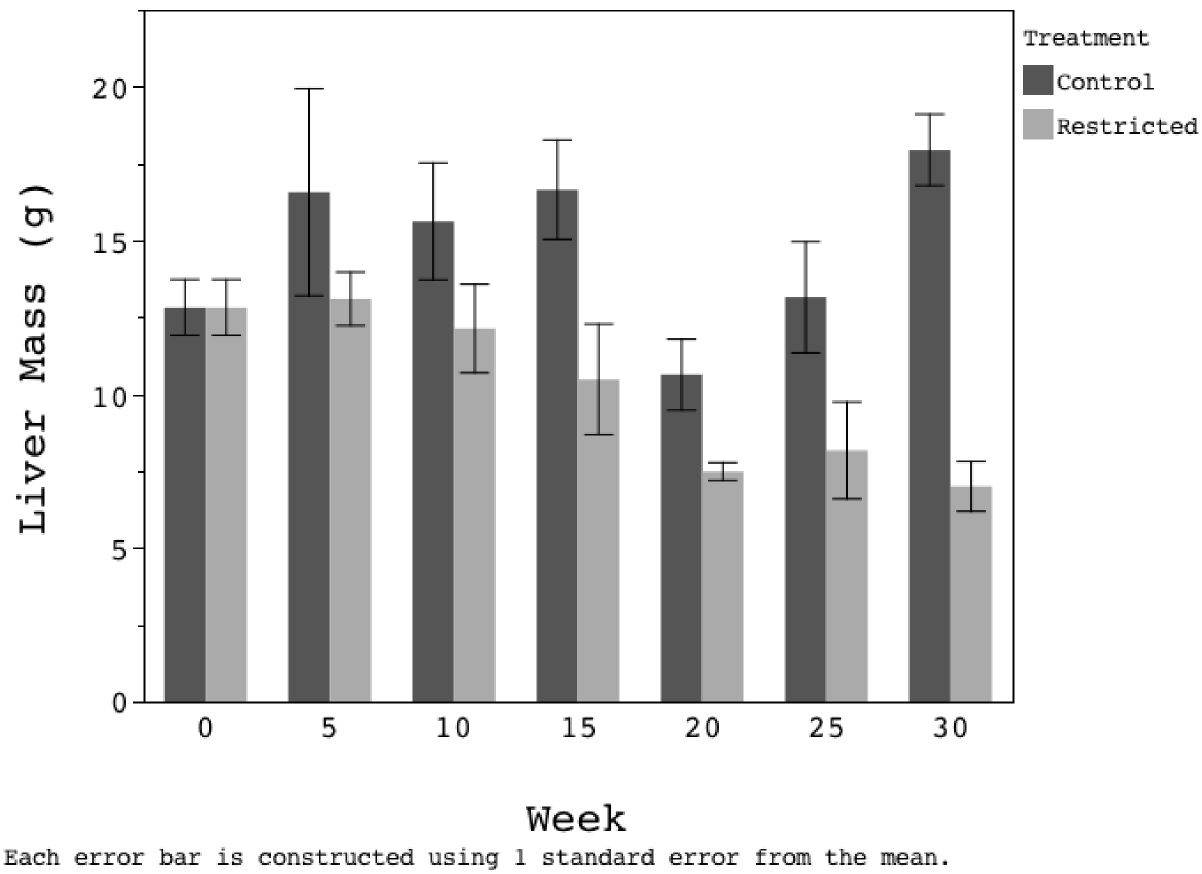
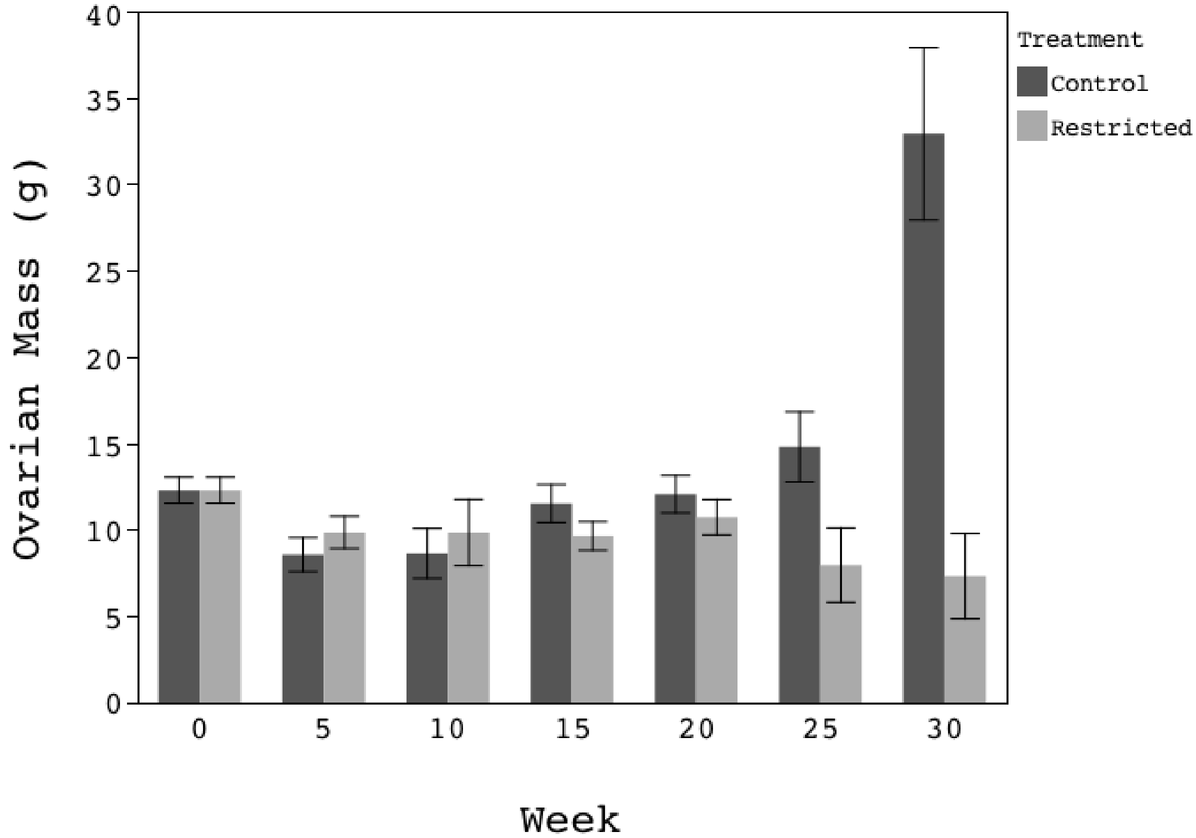


Figure 6: Liver mass was affected by feed availability; fish that were fed a restricted ration exhibited lower liver mass within five weeks.



Each error bar is constructed using 1 standard error from the mean.

Figure 7: Fish ovarian mass was affected by feed availability; fish that were fed a restricted ration exhibited lower ovarian mass within 15 weeks.

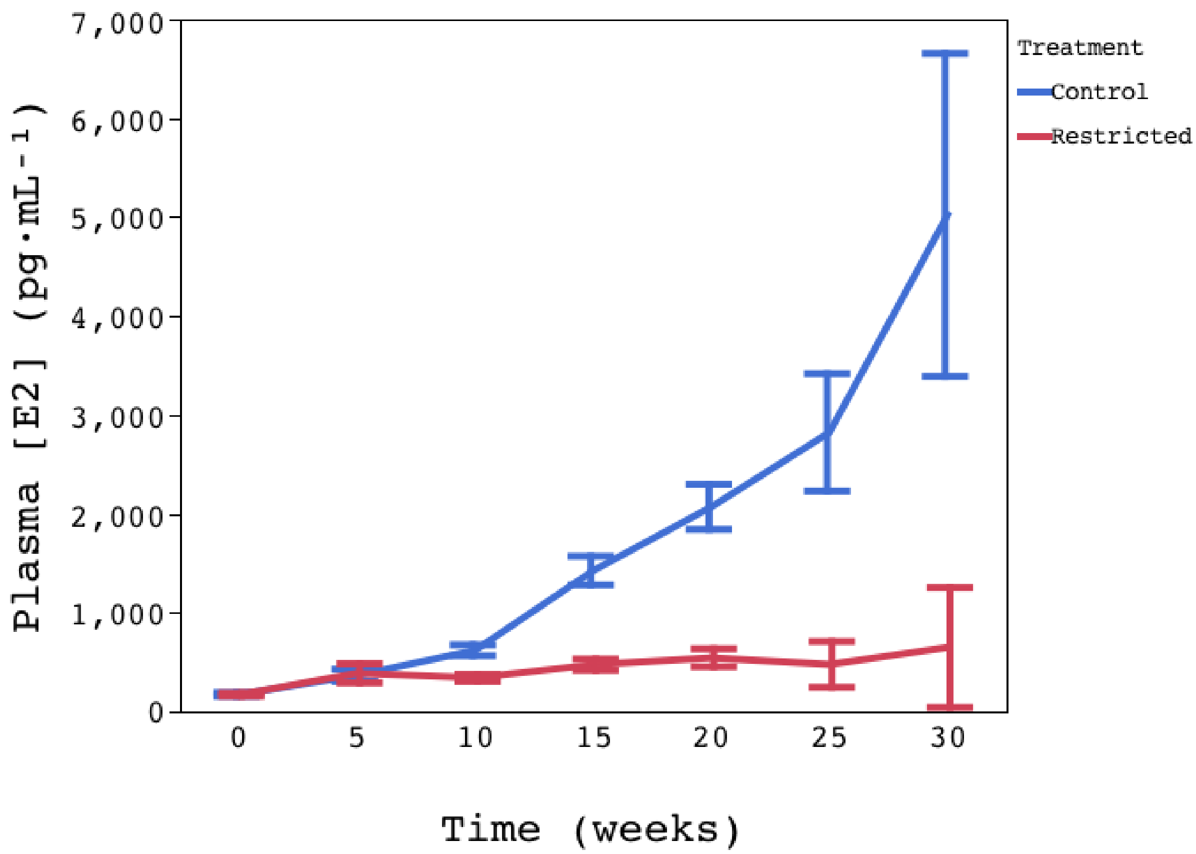


Figure 8: Plasma 17ß-estradiol (E2) was affected by feed availability; fish that were fed a restricted ration exhibited lower plasma E2 within 10 weeks.

Section B.II: Reproductive development in kelt steelhead

Andrew L. Pierce, Ph.D.
Ryan Branstetter
Douglas Hatch
Columbia River Inter-Tribal Fish Commission

Tim Cavileer, Ph.D.
Lucius K. Caldwell
Josh Boyce, Ph.D.
James J. Nagler, Ph.D.
Department of Biological Sciences and Center for Reproductive Biology
University of Idaho, Moscow, ID

Bill Bosch
David Fast, Ph.D.
Joe Blodgett
Micheal Fiander
Yakama Nation Fisheries

Introduction

During 2011, laboratory analysis of blood samples collected during 2009, 2010, and 2011 continued. Analysis of previous results indicated that both maturing and non-maturing female reconditioned steelhead were produced by the reconditioning project at Prosser, and that plasma vitellogenin level indicated maturation status from August onward (Branstetter *et al.* 2010). Based on our studies at Parkdale Hatchery on the Hood River, and on studies on in-river repeat spawning steelhead, we believe that believe that non-maturing fish are skip spawners, fish which will mature the following season (Keefer *et al.* 2008; Branstetter *et al.* 2010). In 2011, one tank of fish at Prosser was blood sampled prior to release and maturation status determined by measurement of plasma vitellogenin level. Maturing fish were used for an experiment, and non-maturing fish were held over the winter to assess maturation the following season. Results from our studies on reproductive development in kelt steelhead were presented at American Fisheries Society annual meeting in Seattle in September of 2011.

Methods

Fish plasma levels of vitellogenin and estradiol-17β (E2) are indicators of reproductive development. Plasma vitellogenin concentrations were assayed using a rainbow trout vitellogenin ELISA kit (Biosense, Cayman Chemical, Ann Arbor, MI). Plasma samples were appropriately diluted and duplicate technical replicates assayed in the ELISA according to the manufacturer's instruction manual provided with the kit. Plasma E2 concentrations were 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 were solvent extracted twice with diethyl ether before use in the RIA protocol.

On Sept 8, 2011 73 fish from tank C2 at Prosser were blood sampled, 66 females and 7 males. Plasma vitellogenin levels were assayed in samples from female fish. Most samples from Prosser from 2009 and 2010 have now been run. Laboratory assays for samples from 2011 are ongoing. Samples run and yet to be run are listed in Table 1.

Table 1. Blood samples from Prosser kelts run and yet to be run.

		2009	2010	2011
Vitellogenin	Blood Samples	226	236	508
	Run	226	236	67
	Remaining	0	0	441
Estradiol	Blood Samples	226	236	508
	Run	226	234	0
	Remaining	0	2	508

Results and Discussion

Plasma vitellogenin levels in female fish sampled Sept 8, 2011 showed that maturing and non-maturing fish were present (Fig. 1). Vitellogenin levels were lower than levels previously measured at release time in mid-October. Vitellogenin increases during maturation, and lower levels would be expected with the earlier sampling point. Fish classified as immature were held through the winter at Prosser. At least one fish with at vitellogenin level in the 0.01 to 0.1 mg ml^{-1} range was found to have eggs in spring of 2012 (J. Blodgett, personal communication). Therefore, fish with vitellogenin levels in this range were classified as borderline. We may need to adjust our cutoff for maturation downward to include these fish for blood samples taken before release. The estimated maturation percentage for tank C2 in 2011 was 54%, omitting borderline fish. This maturation percentage is intermediate between the values obtained for 2009 and 2010. Many more samples remain to be run from 2011 before analysis can be completed (Table 1). In particular, whether fish with a borderline vitellogenin level on 9/8/11 were maturing at release time remains to be determined. Further studies are required to investigate whether reproductive life history is set before intake of kelts into the reconditioning program, or can be influenced by diet and fish husbandry practices (see Section B.IV). The survival percentage for non-maturing C2 fish held over the winter was 64%. This relatively high survival percentage suggests that these fish have restored lost energy and reconditioned. A trial is currently underway to determine what proportion of these fish will mature for the 2012-2013 migration and spawning season.

Analysis of plasma E2 levels in fish from 2010 showed that E2 levels increased before vitellogenin levels in maturing fish (Fig 2). Since E2 drives liver vitellogenin production, E2 would be expected to increase before vitellogenin during oogenesis. This suggests that E2 may indicate maturation status before vitellogenin. If these results are confirmed, we may be able to use E2 as a screen for maturation status. The reagent cost for the E2 assay is approximately $5.50 per sample for E2, versus $22.00 per sample for vitellogenin. Laboratory analysis of E2 levels in samples from 2011 is underway, and statistical analysis of plasma E2 levels from 2009 samples remains to be completed.

Figures

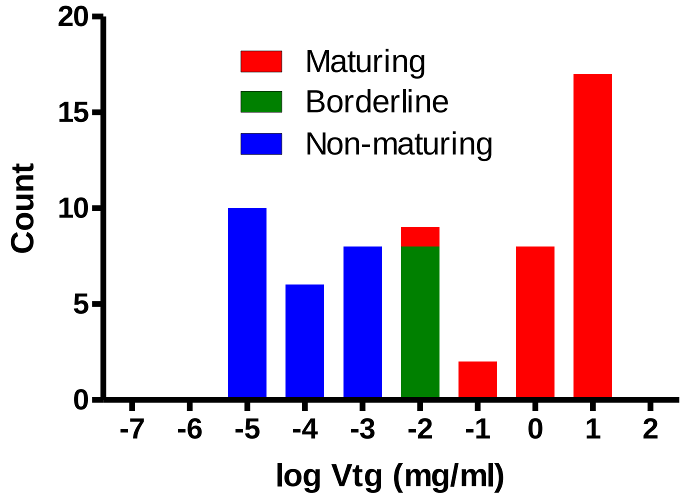


Figure 1. Maturation status of female Prosser tank C2 fish in 2011. Fish were blood sampled on 9/8/2011, and plasma vitellogenin level measured.

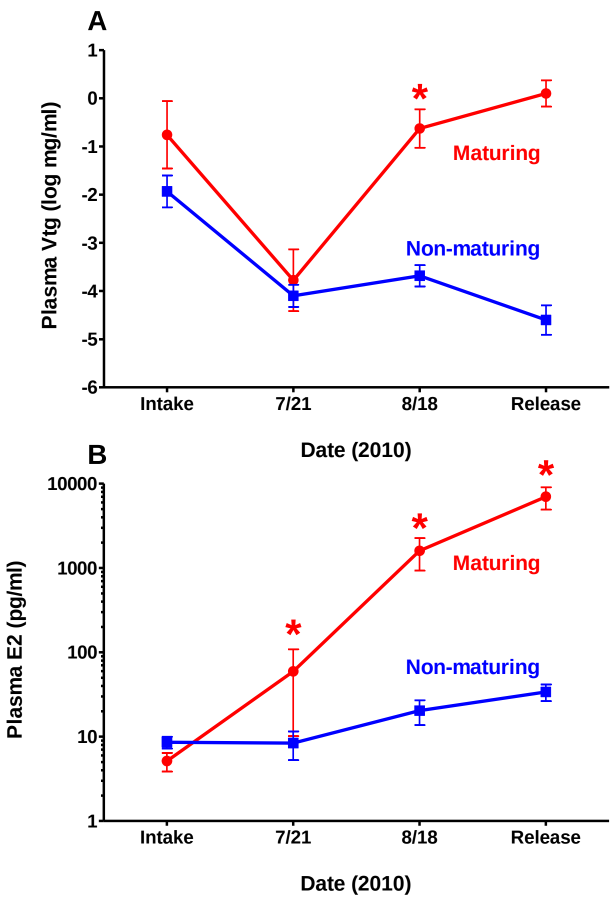


Figure 2. Time course of changes in blood vitellogenin (A) and estradiol (B) level in female Prosser S tank fish in 2010. Fish with vitellogenin levels greater than 0.1 mg ml at release were classified as maturing. Points with an asterisk are significantly different between maturing and non-maturing fish (t-test, $p < 0.05$).

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Section B.III: Safety and efficacy of ivermectin gavage versus emamectin benzoate injection for control of parasitic copepods Salmincola californiensis in kelt steelhead

Andrew L. Pierce, Ph.D.
Ryan Branstetter
Douglas Hatch
Columbia River Inter-Tribal Fish Commission

Josh Egan
Kali Turner
Lucius K. Caldwell
Josh Boyce, Ph.D.
James J. Nagler, Ph.D.
Department of Biological Sciences and Center for Reproductive Biology
University of Idaho, Moscow, ID

Bill Bosch
David Fast, Ph.D.
Joe Blodgett
Michael Fiander
Yakama Nation Fisheries

Scott Everett
James Paddlety
Nez Perce Tribe Fisheries

Introduction

Infestation of fish with freshwater copepod ectoparasites (*Salmincola californiensis*) is a chronic problem in kelt steelhead reconditioning programs. In large adult fish, parasites attach primarily to the gills, and may reach levels of 100-300 adult females' kg^{-1} fish. Heavy parasite loads cause mortality, reduce growth, and may impair reproductive development (McGladdery & Johnston 1988; Duston & Cusack 2002; Branstetter *et al.* 2007). Negative effects of infestation are primarily due to reduced gas exchange capacity at the gill epithelium. If not adequately controlled, copepod infestation often causes high mortality in steelhead kelts in reconditioning programs during July and August (Branstetter *et al.* 2007).

Treatment with ivermectin for copepods in Columbia Basin kelt reconditioning projects was initiated in 1999 (Evans *et al.* 2001). Ivermectin is a member of the avermectin family of chemicals, which are macrocyclic lactones produced by the soil bacterium *Streptomyces avermitilis*. Avermectins interact with GABA receptors and chloride channels in the nervous systems of invertebrates, causing paralysis and death of invertebrate parasites. Treatment protocols used administration of ivermectin into the stomach using a tube inserted down the esophagus (gavage), based on treatments developed for salmon captive broodstock programs (Johnson & Heindl 2001; Roberts *et al.* 2004). According to these authors, ivermectin gavage at 2 doses of 200 µg kg^{-1} 14 days apart in rainbow trout, and 3 doses of 200 µg kg^{-1} 21 days apart in Chinook salmon were effective at reducing copepod infestation and did not cause mortality or sublethal toxic effects. However, lethal and sublethal toxic effects of ivermectin in salmonids at similar dosages have been described by other investigators (Palmer *et al.* 1987; O'Halloran *et al.* 1992; Johnson *et al.* 1993). We experienced heavy mortality in hatchery origin kelts held at Dworshak National Fish Hatchery (DNFH) after ivermectin gavage (Fig. 1).

Emamectin benzoate (emamectin) is an alternative to ivermectin that has been developed for control of sea lice (marine copepod parasites) in Atlantic salmon aquaculture. Emamectin is a synthetically modified avermectin. For sea lice control, emamectin is formulated as Slice ™, a feed additive. The standard protocol for administration of Slice in feed is 50 µg emamectin kg^{-1} fish body weight, fed for 7 days, resulting in a total dose of 350 µg kg^{-1}, which is effective for control of sea lice (Canadian Govornment 2007).

Administration of emamectin by feeding following the standard protocol has been shown to be effective at reducing freshwater gill copepods in salmonids (Duston & Cusack 2002; Carty *et al.* 2010). In juvenile Atlantic salmon and rainbow trout, signs of toxicity were not found with administration by feeding at levels 3-4 times the standard, but sublethal toxicity was found at administration levels 7-8 times the standard (Roy *et al.* 2000). The 96 hour LC_{50} value (exposure to substance in water) was 174-194 µg l^{-1} for emamectin for rainbow trout, versus 3-4.8 µg l^{-1} for ivermectin (Halley *et al.* 1989; Roy *et al.* 2000).

A recent study in Atlantic salmon shows that administration of emamection by intraperitoneal injection is well tolerated, and reduces variability in dosage rate due to

variation in feed consumption by fish (Glover *et al.* 2010). Intraperitoneal injection results in tissue levels of emamectin higher than administration of the same dose by feeding. Injection of emamectin at levels of 100 – 800 µg kg^{-1} did not result in mortality or signs of sublethal toxicity in Atlantic salmon. Emamectin had a half life of approximately 11 days in Atlantic salmon juveniles at 9-14 °C.

These considerations suggest that emamectin may be better than ivermectin for control of copepods in kelt steelhead. Administration of emamectin in treated feed is not likely to be effective in kelts, since the fish do not feed well. Intraperitoneal injection seems like a better method for treating kelts than gavage, since it is easier to precisely control the dosage, less stressful for the fish, and less likely to transfer pathogens between fish. Therefore, we conducted an experiment to compare the efficacy and safety of injection of emamectin versus the standard treatment of ivermectin gavage for control of copepods in kelt steelhead in the reconditioning program at Prosser.

Methods

Ivermectin and Emamectin preparation

Emamectin benzoate PESTANAL analytical standard and propylene glycol carrier were purchased from Sigma. Ivermectin 1% solution Vetrimec was purchased from a veterinary supplier. Ivermectin was diluted in sterile 0.9% saline solution to 0.33 mg ml^{-1} (Evans *et al.* 2001). Emamectin benzoate was prepared as a sterile 907 µg/ml solution in propylene glycol. This concentration of EB was selected so that injection of 0.1 ml per pound of fish resulted in a dose of 200 µg per kg of fish.

DNFH Fish Husbandry, Copepod Treatment, and Mortality

Hatchery origin female steelhead returning to DNFH were artificially spawned and reconditioned for 46 days as described in Section C, following protocols established in kelt projects at Prosser and Parkdale (Evans *et al.* 2001; Branstetter *et al.* 2007). Fish were not treated for copepods at spawning. On March 21, 2011, 35 surviving kelts were sampled and treated with ivermectin. Ivermectin was given by gavage at a dose of 400 µg kg^{-1}, based on the total recommended dose for rainbow trout (Roberts *et al.* 2004). Mortality was recorded daily.

Prosser Fish Husbandry, Copepod Treatment, and Mortality

Wild Yakima River steelhead kelts were captured and reconditioned at Prosser, WA, during the 2011 season following established protocols (Evans *et al.* 2001; Branstetter *et al.* 2007). Fish in the general population of kelts were housed in four 20' diameter tanks. Fish were stocked alternately into each tank, so that tanks did not differ significantly in terms of fish density or arrival date. Three tanks of fish (C1, C3, and C4) were given ivermectin at intake following standard protocols. Fish stocked into tank C2 were alternately given ivermectin by gavage or emamectin by injection at intake. Ivermectin was given by gavage at a dose of 100 µg kg^{-1}, based on the experience of project personnel (J. Blodgett, personal

communication). Emamectin was injected at a dose of 200 µg kg^{-1}. Fish in tank C2 were sampled on July 19th and Sept 8th. C2 fish were blood sampled on both of these dates. During the July 19th sampling, fish were given the same copepod treatment as they received at intake. During both the July 19th and Sept 8th sampling, a photograph was taken of the left gill with the opercular cover raised. The number of copepods visible on the gill was counted. Mortality was recorded daily. Only fish positively identified by PIT tag code from intake to exit (mortality or release) were included in the analysis. Maturation status at release for tank C2 fish was determined by measurement of blood vitellogenin level (Section B.II).

Results

Rapid mortality occurred after treatment of DNFH hatchery steelhead kelts with ivermectin at 400 µg kg^{-1} (Fig. 1). 67% of fish died within 12 days, after which the mortality rate decreased. Beginning the day after ivermectin treatment, fish were observed to be lethargic and dark in color (Fig. 2). Some fish were not able to maintain an upright position in the water, and repeatedly lost equilibrium and turned on their sides. Some fish were observed lying on their sides on the bottom of the tank. However, these fish did not die immediately: opercular movements continued and fish would get up and swim away if disturbed.

Prosser kelts treated at intake with emamectin had significantly lower mortality than fish treated with ivermectin at 100 µg kg^{-1}(Fig. 3a). Most mortality occurred during the first 20 days after intake. At 20 days after intake and copepod treatment, ivermectin treated fish had 25 to 42% mortality, whereas emamectin treated fish had 9% mortality. After the 7/19 sampling and treatment, elevated mortality again occurred in ivermectin treated fish for approximately 20 days (Fig. 3b). However, steady mortality in emamectin treated fish occurred for approximately 50 days, eventually resulting in a similar cumulative mortality rate in the two groups of fish after the 7/19 sampling. Over the entire reconditioning period, tank C2 emamectin treated fish had 46% mortality, versus 59% for tank C2 ivermectin treated fish (Fig 4). The other three ivermectin treated tanks had 65%, 66%, and 81% mortality.

Emamectin treated fish had significantly fewer copepods than ivermectin treated fish. After one copepod treatment, the number of copepods visible on the left gill of emamectin treated fish ranged from 0 to 3, whereas the number of copepods on the left gill of ivermectin treated fish ranged from 0 to 11. After two copepod treatments, the number of copepods visible on the left gill of emamectin treated fish was 0 to 3, and for ivermectin treated fish 0 to 12. After two emamectin treatments, 38 of 39 fish had no copepods visible on the left gill, and one fish had 3 copepods visible.

The maturation rate of female tank C2 fish treated with emamectin was not significantly different than that of fish treated with ivermectin (Table 1; Chi-Squared test $p = 0.79$). In tank C2 fish, muscle lipid levels at release, and the specific growth rate in weight over the reconditioning period were higher in emamectin treated fish than ivermectin treated fish

(data not shown). However, these differences were not significant (muscle lipid $p = 0.3$; growth rate $p = 0.1$).

Table 1: Maturation of tank C2 ivermectin and emamectin treated female kelts. Chi-Squared test $p = 0.79$

	Mature	Non-mature
Ivermectin	**10**	**13**
Emamectin	**15**	**23**

Discussion

Treatment of kelt steelhead and other adult and subadult salmonids held in freshwater for parasitic copepods is necessary to prevent high mortality due to heavy copepod infestations during the summer (Branstetter *et al.* 2007). Administration of ivermectin by gavage has been the standard treatment for copepods in the Columbia River Basin for over 10 years, based on protocols established in captive broodstock programs (Johnson & Heindl 2001; Roberts *et al.* 2004). Based on these studies, we administered ivermectin to hatchery origin steelhead kelts at DNFH at a dose of 400 μg kg^{-1}. Very high mortality occurred after ivermectin administration at this dose (Fig. 1), much of which can be attributed to the toxic effect of ivermectin on the nervous system of the fish. Fish showed signs of central nervous system depression including lethargy, inability to maintain equilibrium, and lying on the bottom of the tank. These signs, as well as the characteristic dark coloration that appeared after treatment, are associated with ivermectin toxicity (Johnson *et al.* 1993). Similar signs are seen after ivermectin administration in kelt steelhead held for reconditioning at Prosser (J. Blodgett, personal communication). Administration of a lower dose of ivermectin, 100 μg kg^{-1}, to kelt steelhead at Prosser was also associated with elevated mortality over an approximately 20 day period (Fig 3). An untreated control could not be employed in these studies, because it is known that failure to treat kelts for copepods will lead to high mortality. However, administration of the ivermectin replacement emamectin caused significantly lower mortality. The reduced mortality in emamectin treated fish was sustained after intake, whereas after the July 19th copepod treatment, mortality in ivermectin treated fish occurred more rapidly than that in emamectin treated fish, but the eventual mortality rate was not different between the two treatments. One explanation for these results could be that ivermectin accelerated mortality in fish in a weakened state that would have eventually died after the July 19th treatment. At intake, all kelts are in a weakened state, and may have experienced elevated mortality due to ivermectin treatment. Our results suggest that switching from ivermectin to emamectin for copepod control may lead to an increase in survival of approximately 15%.

Both ivermectin and emamectin controlled copepods adequately in this study (Fig 4). The most heavily infested fish had 20-30 copepods on the gills, compared to a maximal infestation rate of 200-600 parasites for fish of this size. However, emamectin was significantly more effective than ivermectin, and after two treatments, emamectin had

virtually eliminated copepods (Fig 4b). This difference may be due to a difference in the toxicity of the two compounds to the parasites, the dose of each compound employed, or the administration method. The ivermectin dose employed at Prosser had been reduced below the recommended dose for copepod control, due to issues with toxicity (J. Blodgett, personal communication)(Roberts *et al.* 2004). Administration of ivermectin by gavage may lead to variability in dosage between fish, due to regurgitation of the solution after treatment. Consistent with this idea, the number of copepods on the gills of ivermectin treated fish showed higher variability than emamectin treated fish. Practically speaking, our study shows that emamectin injection is more effective that ivermectin gavage, at the indicated doses, for control of copepods in kelt steelhead.

Kelt steelhead may be more sensitive to the toxic effects of ivermectin than other salmonids species, and kelts may be particularly sensitive at intake. The toxicity of ivermectin to both invertebrate parasites and their vertebrate hosts is due to central nervous system depression. Ivermectin depresses central nervous system activity by increasing signaling at GABA synapses and other mechanisms. GABA is a major inhibitory neurotransmitter, and excessive GABA signaling leads to paralysis and death. Ivermectin is kept out of the central nervous system by the blood brain barrier in most higher vertebrates; however, this does not appear to be the case in fish (Katharios *et al.* 2004). In addition, ivermectin is lipophilic, and tends to concentrate in tissues with high lipid content. Kelt steelhead at intake are extremely depleted in lipid stores. Much of the lipid remaining in the body is in the brain, in the form of myelin, which is not used for energy because it is essential for brain function. Therefore, ivermectin may concentrate in the brain of kelt steelhead, which would increase its neurotoxic effects.

While it is possible that issues with ivermectin toxicity are specific to kelt steelhead, it seems unlikely that lethal and sublethal toxic effects are completely absent in other salmonid species. Indeed, due to issues with toxicity, ivermectin use has been discontinued in Atlantic salmon aquaculture, and is not recommended for other salmonid species (Duston & Cusack 2002). Given the availability of emamectin, a less toxic alternative, it is somewhat surprising that ivermectin use has continued in the Columbia River Basin.

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Figure Legends

Figure 1. Mortality of DNFH hatchery origin kelts after treatment of with ivermectin by gavage at 400 µg kg^{-1}.

Figure 2. Dark appearance of DNFH kelt mortalities after treatment with ivermectin. The dark appearance tended to fade post-mortem, but is still visible in patches on the ventral surface of the fish.

Figure 3. Mortality of Prosser kelts after ivermectin or emamectin treatment. (A) Mortality of all large tanks from intake through 7/18. Fish in tanks C1, C3, and C4 were given ivermectin (100 µg kg^{-1}) at intake, and fish in tank C2 were alternately given ivermectin (100 µg kg^{-1}) or emamectin (200 µg kg^{-1}) at intake. (B) Mortality of ivermectin and emamectin treated fish in tank from the 7/19 sampling 10/13 (release).

Figure 4. Copepod numbers in tank C2 ivermectin and emamectin treated fish. (A) 7/19 sampling, Mann-Whitney test p = 0.0016. (B) 9/8 sampling, Mann-Whitney test p = 0.0028.

Figure 1

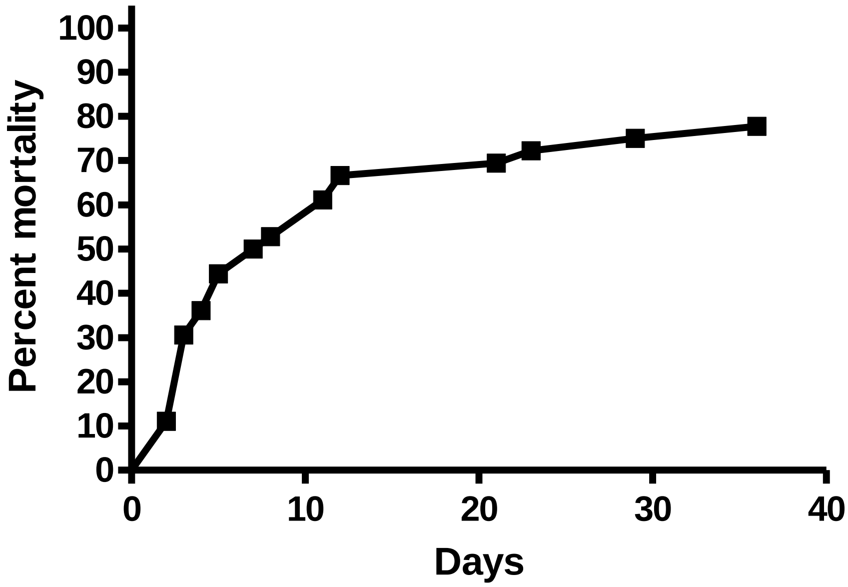
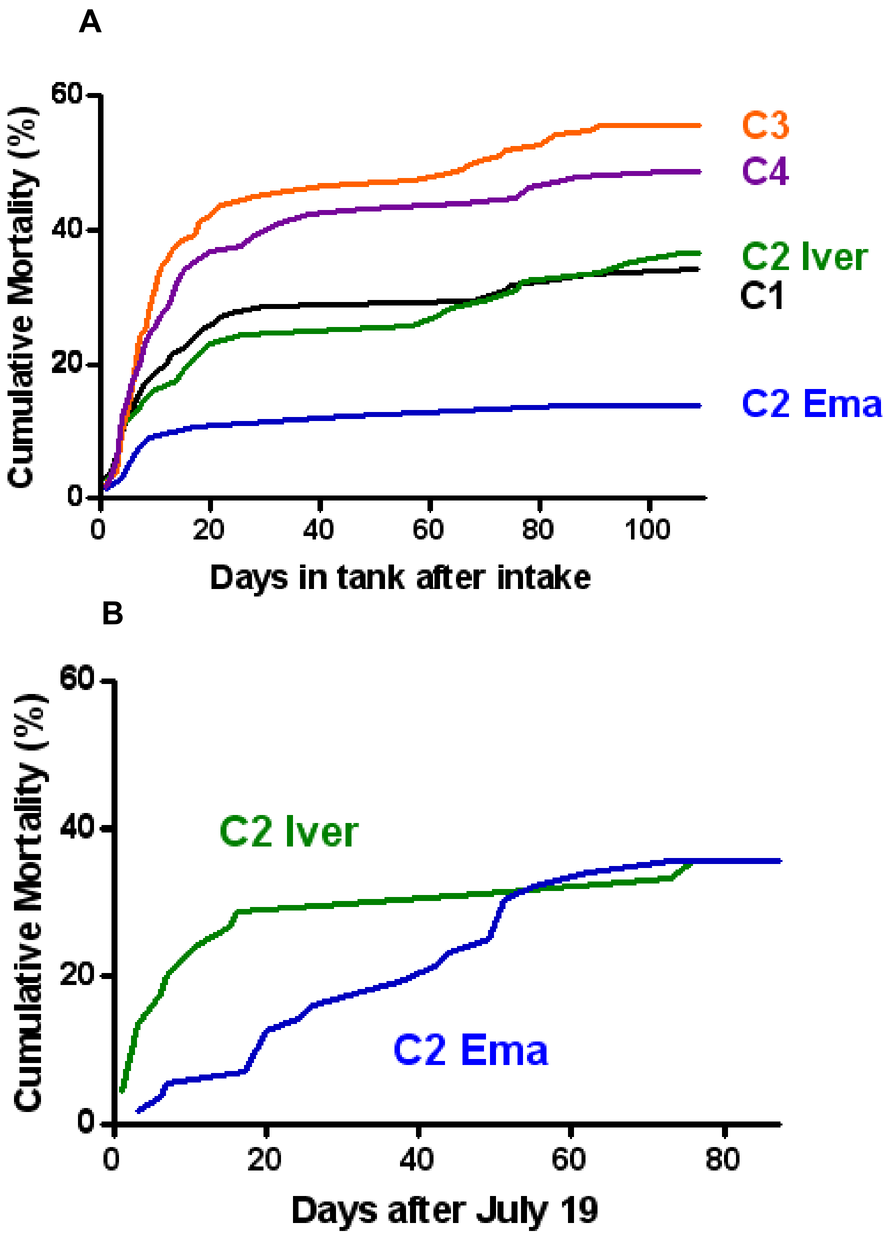


Figure 2

Figure 3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Cumulative Mortality (%)
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Days in tank after intake
C3
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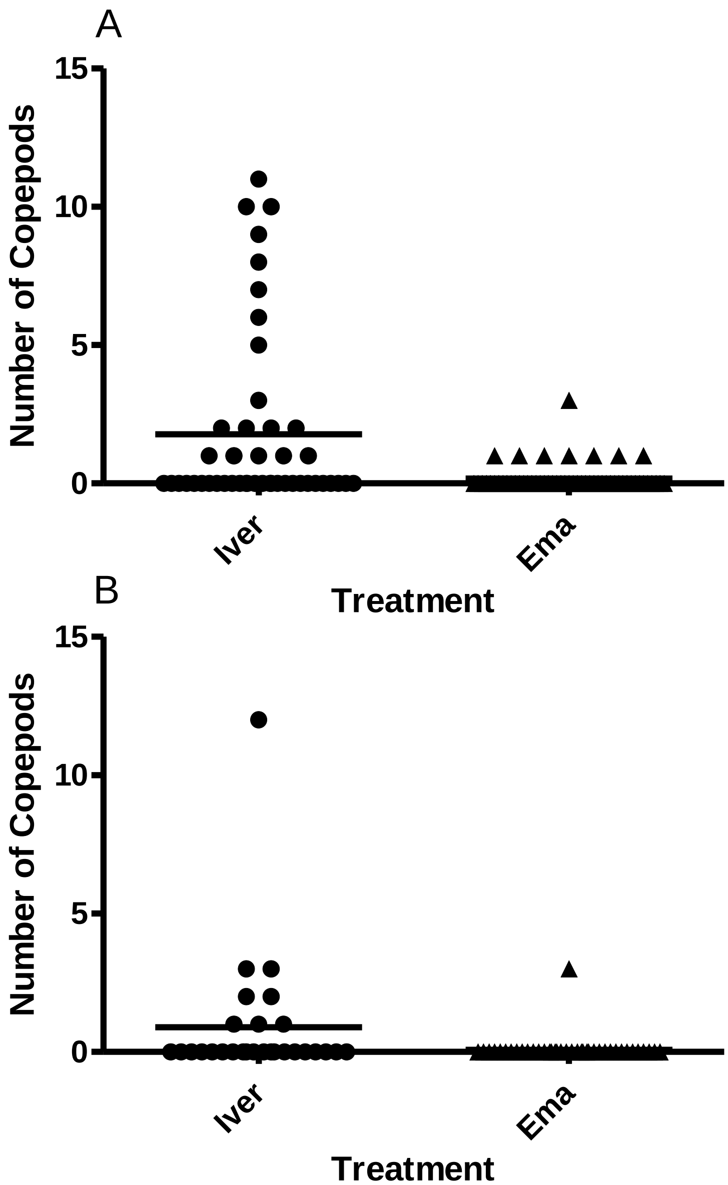
C2 Iver
C1
C2 Ema
B
Cumulative Mortality (%)
60
40
20
0
0
20
40
60
80
Days after July 19
C2 Iver
C2 Ema

Figure 4


A
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10
5
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Number of Copepods
Iver
Ema
Treatment
B
15
10
5
0
Number of Copepods
Iver
Ema
Treatment

Section B.IV: Effects of supplemented diets on kelt steelhead

Andrew L. Pierce, Ph.D.
Ryan Branstetter
Douglas Hatch
Columbia River Inter-Tribal Fish Commission

Bill Bosch
David Fast, Ph.D.
Joe Blodgett
Micheal Fiander
Yakama Nation Fisheries

Introduction

Studies conducted in 2009 and 2010 at the reconditioning project at Prosser showed that muscle lipid levels in the fish at release are strongly related to whether fish show characteristics associated with successful spawning after release (Branstetter *et al.* 2010). Female fish with high muscle lipid levels at release were more likely to be consecutive spawners undergoing active ovarian development at the time of release, whereas females with lower muscle lipid levels at release were more likely to be skip spawners, fish with undeveloped ovaries that would spend an additional year in the ocean prior to maturation in the natural environment (Keefer *et al.* 2008). Both female and male fish with high muscle lipid levels at release were more likely to be detected migrating upriver after release, and reconditioned kelts that were recaptured during downriver migration the spring after release were fish that had very high muscle lipid levels at release. These findings suggest that treatments which increase muscle lipid levels in the fish at release time will increase the proportion of kelts that migrate and spawn successfully in the river after release.

There is a strong relationship between dietary lipid levels and carcass lipid levels in salmonids (Halver & Hardy 2002). Thus, supplementing our diet with additional fish oil might be effective at increasing muscle lipid levels. The feeding motivation of kelts is low at intake into reconditioning. Cyclopeeze is a microscopic copepod harvested from an Arctic freshwater lake which contains appetite stimulants (Argent Laboratories 2011). Tests during 2010 suggested that coating feed items with cyclopeeze increased feeding activity in kelt steelhead. In addition, cyclopeeze contains immune system stimulants, and has been found to improve egg quality in salmonid broodstock. Fish oils also increase palatability of pelleted diets. In order to determine whether a fish oil and cyclopeeze supplemented diet might benefit kelt steelhead, we conducted a preliminary diet trial at Prosser in 2011.

Methods

Kelt diet pellets were purchased from BioOregon. Pellets were topcoated with menhaden oil and freeze dried cyclopeeze to produce the “orange” diet. Cyclopeeze and menhaden oil were purchased from Argent Laboratories. Argent also recommended addition of spirulina, a nutritive algae. To test the effect of spirulina, an additional “green” diet with spirulina topcoated onto pellets was produced. The percentage of ingredients and calculated nutritional composition of the diets is listed in Table 1.

Table 1. Composition of experimental diets.

	Ingredients (%)				Composition (% dry wt)			
	Pellets	Cyclopeeze	Oil	Spirulina	Protein	Lipid	Carbohydrate	Ash
Standard	100	0	0	0	47.8	21	22.2	9.1
Orange	82.5	14.5	3.1	0	46.5	26	19.9	7.9
Green	80.4	14.2	3.1	2.3	46.9	25	20	7.9

Kelt steelhead arriving at Prosser during the spring of 2011 were processed and stocked into tanks following standard procedures. All fish were scanned for PIT tags at intake, and tagged if no existing tag was found. Fish fed the experimental orange and green diets were stocked into four small tanks (tanks S1-S4, 12' diameter, 20 fish per tank), whereas fish fed the standard pellets were stocked into four large tanks (tanks C1-C4, 20' diameter, 149-150 fish per tank). Two S tanks were randomly assigned to the orange diet, and the other two to the green diet. Half of the fish in tank C2 were given an experimental copepod treatment at intake (Section B.III), and these fish were blood sampled twice during the summer. Therefore, tank C2 was not included in analysis of the effect of feeding treatment. The other three C tanks were fed standard pellets. Fish were fed *ad libitum*. All fish were fed krill for an initial period of approximately one month before pellets were introduced. Fish were transitioned from krill to pellets by feeding a mixture of the two following standard procedures established at Prosser. Mortality was recorded daily. Only fish positively identified by PIT tag from intake to exit (mortality or release) were included in the analysis. Muscle lipid levels were measured with the Fatmeter, and specific growth rate in weight was calculated as $\frac{\ell n\,(mass_2 / mass_1)}{days between measurements} \times 100$. Detections of fish after release were obtained by merging queries of the PTAGIS database and detections at the Denil ladder at Prosser.

Results

Mortality did not differ substantially between feeding treatments (Fig. 1). Fish in all treatments experienced high mortality during the first 20 days of reconditioning, after which the mortality rate decreased. Fish fed the orange diet had higher muscle lipid levels at release than fish fed either the standard diet or the green diet (Fig. 2). However, this difference was not statistically significant (ANOVA, $p = 0.0946$). Fish fed the orange diet had a significantly higher specific growth rate in weight than fish fed standard pellets or the green diet (Fig 3; ANOVA $p = 0.0288$, Bonferroni multiple comparison test, standard diet versus orange diet $p < 0.05$). There was a significant positive correlation between growth rate over the reconditioning period and muscle lipid level at release (Fig. 4; linear regression $p < 0.0001$, $r^2 = 0.4819$). There was a significant relationship between diet treatment and detection of fish post release (Table 2; Chi-Squared test $p = 0.0022$). Fish

detected migrating upriver after release comprised 30% of standard diet fish, 42% of green diet fish, and 77% of orange diet fish.

Table 2. Released kelts detected versus not detected by diet treatment. Tank C2 standard diet fish were excluded from the analysis. Chi-Squared test p = 0.0022.

	Standard Diet	Orange Diet	Green Diet
Detected	34	10	5
Not Detected	79	3	7
Total	113	13	12

Discussion

This study shows that the Cyclopeeze and fish oil supplemented "orange" diet is promising. This was a pilot study, and fish fed the orange diet had to be housed in smaller tanks than fish fed the standard diet. Thus, the diet effect is confounded with a potential tank effect. However, fish in the small tanks generally have higher mortality and reduced growth compared to fish in the larger tanks, and show more eye and fin injuries, likely due to rubbing and collisions with tank walls (J. Blodgett, personal communication). Fish fed the orange diet had lower mortality and higher release muscle lipid levels and growth rates over the reconditioning period than fish fed the standard diet, although these differences were not always statistically significant. In addition, fish fed the orange diet were observed to be in exceptionally good shape at release time in terms of eye injuries, fin injuries, and skin coloration and health. All of these changes were in the opposite direction from the expected tank effect, suggesting that they were due to the diet. The increase in growth rate is most likely due to increased feed consumption by orange diet fed fish. Both cyclopeeze and fish oil increase the palatability of salmonid diets (Halver & Hardy 2002; Argent 2011). The increase in muscle lipid levels may be due to either increased feed consumption or the higher lipid level in the orange diet. Fish oil contains a lipid profile similar to that found in fish muscle tissue. After metabolic energy requirements are met, dietary lipids of appropriate types are deposited directly in storage depots, whereas other types of lipids and dietary energy from non-lipid components of the diet must be processed. The improvement in eye, fin, and skin health may be due to increased healing ability in orange diet fed fish. Cyclopeeze contains immune system stimulants and micronutrients that may aid in healing of injuries.

Orange diet fish were detected migrating upriver after release at an exceptionally high rate (Table 2). Nearly 80% of orange diet fish were detected, versus 30% of standard diet fish, similar to previous years. This is an exciting result, but it is preliminary and based on a small number of fish. The high migration rate suggests that a very high proportion of orange diet fish were reproductively maturing at release time. The reproductive status of these fish can be determined by measurement of blood vitellogenin and estradiol levels. Measurement of these factors in 2011 release samples is ongoing (Section B.II).

The addition of spirulina to the orange diet ("green" diet) decreased the benefit of the orange diet. Muscle lipid levels and growth rates in green diet fed fish were very similar to those of the standard diet fish. Since the nutritional composition of the green diet is almost identical to that of the orange diet (Table 1), this is most likely due to reduced feed consumption of the green diet fish as compared to the orange diet. Adult steelhead in the ocean are carnivorous, and do not normally consume algae. Addition of spirulina to the diet may have reduced the motivation of fish to consume pellets. However, green diet fish did show reduced mortality, compared to standard diet fish, and were detected migrating upriver after release at a higher rate than standard diet fish. This suggests that there may be benefits of the supplemented diet that are independent of increased feed consumption. These are likely due to the cyclopeeze and fish oil components of the diet, since orange diet fish experienced similar mortality and migrated at a higher rate than green diet fish.

The significant regression between growth rate over the reconditioning period and muscle lipid level measured at release shows that Fatmeter readings are informative about the growth status of fish at release time (Fig. 4). Changes in body weight and muscle lipid levels are both presumably driven by feed intake. Measurement of muscle lipid level at release provides a rapid, simple, and objective measure of the status of the fish at release time. Fish with higher muscle lipid levels at release are more likely to spawn successfully in the river. It would be very interesting to compare Fatmeter readings from Prosser kelts at release to those of maiden fish on their upriver migration through Prosser dam.

In conclusion, this pilot study showed that a diet supplemented with cyclopeeze and fish oil may be very beneficial for kelt steelhead. Cyclopeeze is quite expensive; however, these benefits may be large enough to justify the cost of the supplemented diet. A larger scale diet study is currently underway.

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Figure Legends

Figure 1. Mortality of fish fed standard, orange, and green diets at Prosser during 2011.
Figure 2. Release muscle lipid levels in fish fed standard, orange, and green diets at Prosser in 2011. ANOVA $p = 0.0946$.
Figure 3. Specific growth rate in weight (SGRW) in fish fed standard, orange, and green diets at Prosser in 2011. Treatments not sharing a letter differ significantly (Bonferroni multiple comparison post-hoc test, $p < 0.05$).
Figure 4. Relationship between SGRW and muscle lipid levels at release in fish fed the standard, orange, and green diets at Prosser in 2011. Linear regression $p < 0.0001$, $r^2 = 0.4819$.

Figure 1

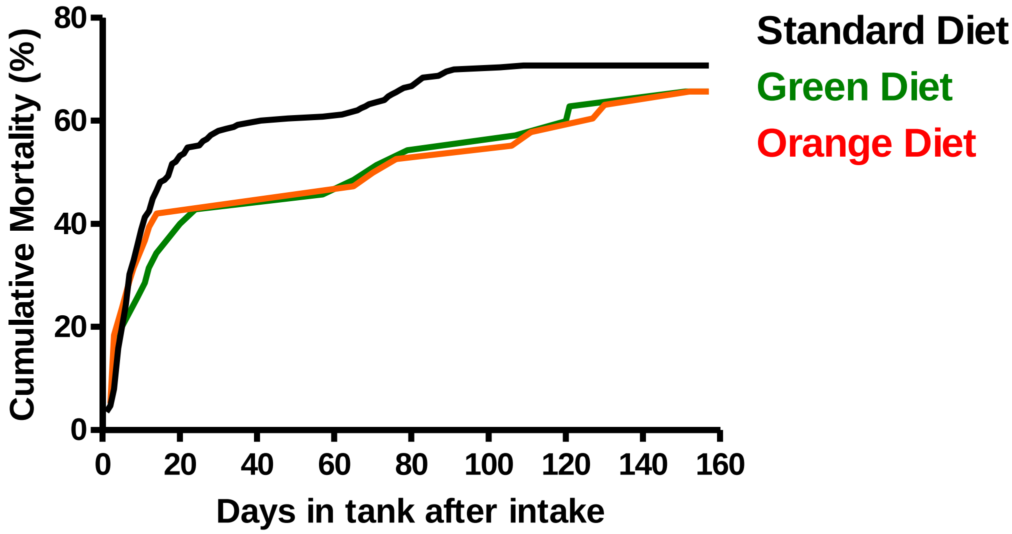


Figure 2

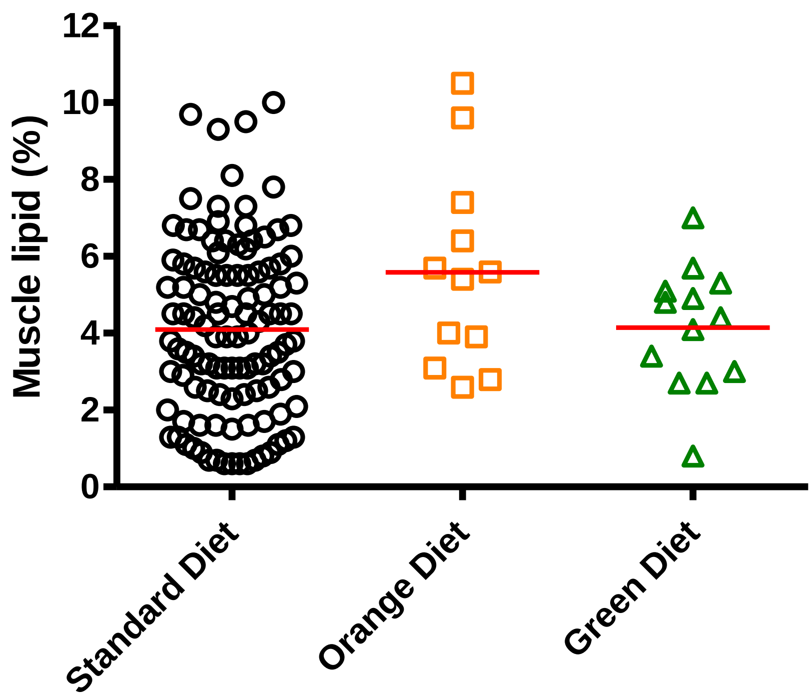


Figure 3

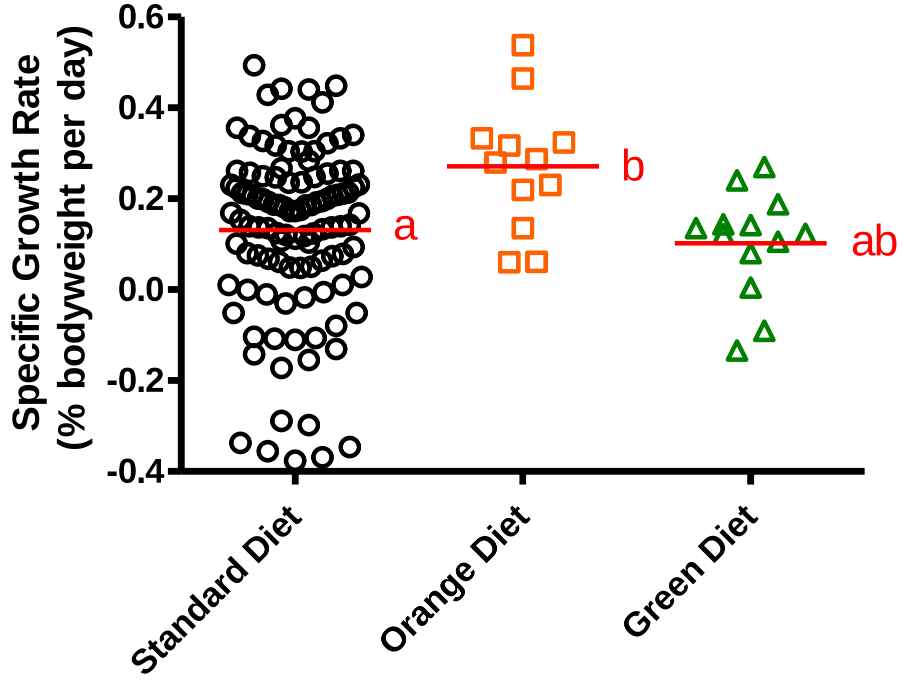
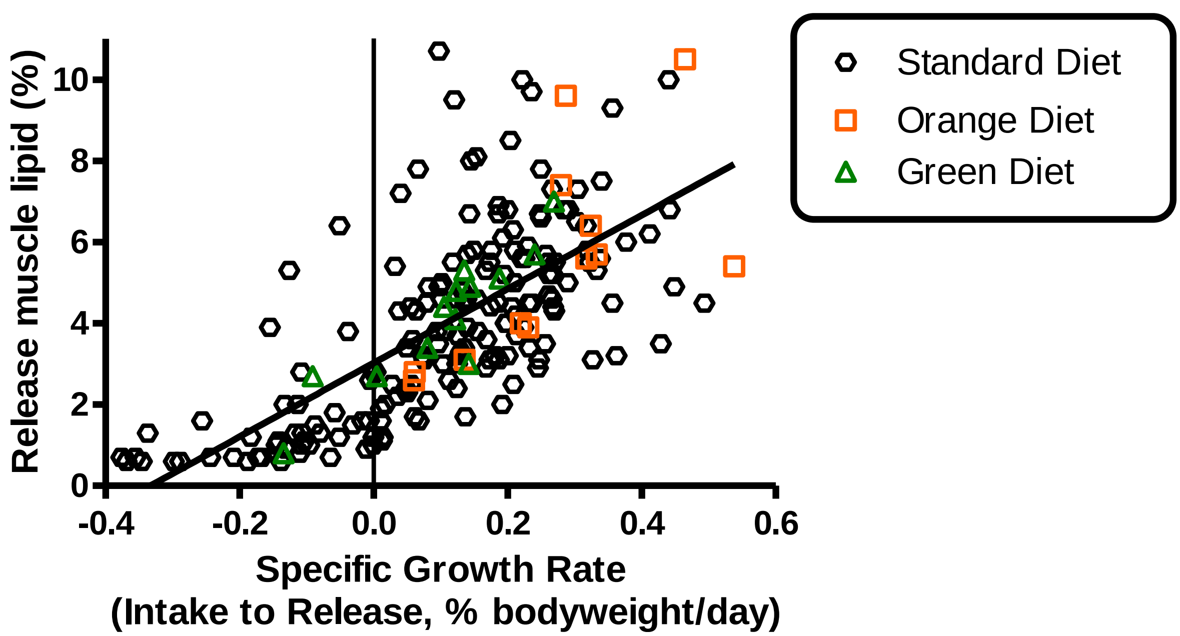


Figure 4



Section B.V: Effects of force feeding on artificially spawned hatchery steelhead kelts

Andrew L. Pierce, Ph.D.
Ryan Branstetter
Douglas Hatch
Columbia River Inter-Tribal Fish Commission

Josh Egan
Lucius K. Caldwell
Josh Boyce, Ph.D.
James J. Nagler, Ph.D.
Department of Biological Sciences and Center for Reproductive Biology
University of Idaho, Moscow, ID

Scott Everett
James Paddlety
Nez Perce Tribe Fisheries

Introduction

Force feeding using a tube inserted down the esophagus into the stomach is standard practice in projects with the goal of rescuing and rehabilitating injured and starving mammals and birds. While the handling associated with frequent force feeding may be problematic with fish, this method has been employed in Atlantic salmon kelt reconditioning projects (Eales *et al.* 1991). Force feeding of precocious Chinook salmon parr results in post-spawning survival (Bernier *et al.* 1993). To determine whether force feeding at intake might benefit steelhead kelts, we conducted a trial with artificially spawned hatchery kelts at Dworshak National Fish Hatchery (DNFH).

Methods

Mature adult female hatchery origin steelhead were collected from the adult ladder at DNFH during January and February of 2011 and air spawned following techniques developed by CTWSRO staff as described (Section C). Ripe females without significant injuries or fungal lesions were selected. Fish were sedated with MS-222. While sedated, fish were sampled for blood, body lipid levels, PIT tagged and photographed. All fish received an injection of oxytetracycline. Alternate fish were force fed 3-5 ml of slurry of cyclopeeze using a gavage tube and syringe. Fish were then stocked into reconditioning tanks. Fish were fed krill and treated 3 days per week with formalin to control fungus. Mortality was recorded daily. Only fish positively identified by PIT tag number were included in the analysis. Mortality rates were analyzed for the 35 days following spawning. Analysis was restricted to this time to avoid any confounding factors from problems with the Fire Maintenance Water System which supplies the kelt tanks, which occurred later (Section C). Most mortality in the kelt reconditioning project at Prosser occurs during the first month of reconditioning (Evans *et al.* 2001). Randomly selected mortalities were necropsied and stomachs examined. The number of fish air spawned and force fed is listed in Table 1.

Table 1. Fish spawned and force-fed during 2011 at DNFH.

	Jan 11, 2011	Feb 1, 2011
Spawned	15	78
Force Fed	8	37
Unfed	7	41

Results and Discussion

Mortality over the first 35 days after air-spawning was not significantly different between force fed and unfed fish for either the Jan 11 or the Feb 1 fish (Fig. 1). However, for both groups, mortality was higher for force fed fish, and this difference was close to being statistically significant (p = 0.1698 and p = 0.2330). These results provide no support for a benefit from force feeding under our experimental conditions. Fresh-frozen cyclopeeze

was selected for force feeding because it is highly nutritious, easy to digest, and forms a slurry. Supplementation of pelleted feed with cyclopeeze was beneficial at later points in reconditioning in Prosser kelts (Section B.IV). Therefore, it seems unlikely that the negative results in the present study were due to feeding with cyclopeeze. It is possible that the negative effect of force feeding was due to processes associated with restoration of gut function after prolonged fasting. The stomachs of kelt steelhead are atrophied at spawning, and restoration of a functioning digestive system takes energy. Necropsy of mortalities did not show stomach injuries or inflammation in force fed fish. Stomach contents were partially digested; however, cyclopeeze was still present in the stomach 35 days after force feeding. This suggests that there was a delay in digestion associated with the restoration of stomach function. The metabolic demands of digesting the large bolus of food delivered into the stomach may have outweighed benefits obtained from the food. Feeding is essential for kelts to survive and recondition. However, the timing and amount of food consumed may be important. Further studies are required to examine whether force feeding at a later time and with smaller amounts of food might be beneficial.

Figure Legends

Figure 1. Mortality of force fed and unfed artificially spawned hatchery fish at DNFH in 2011 over the first 35 days post-spawning. (A) Jan 11 spawning, log-rank test p = 0.1698; (B) Feb 1 spawning, log-rank test p = 0.2330.

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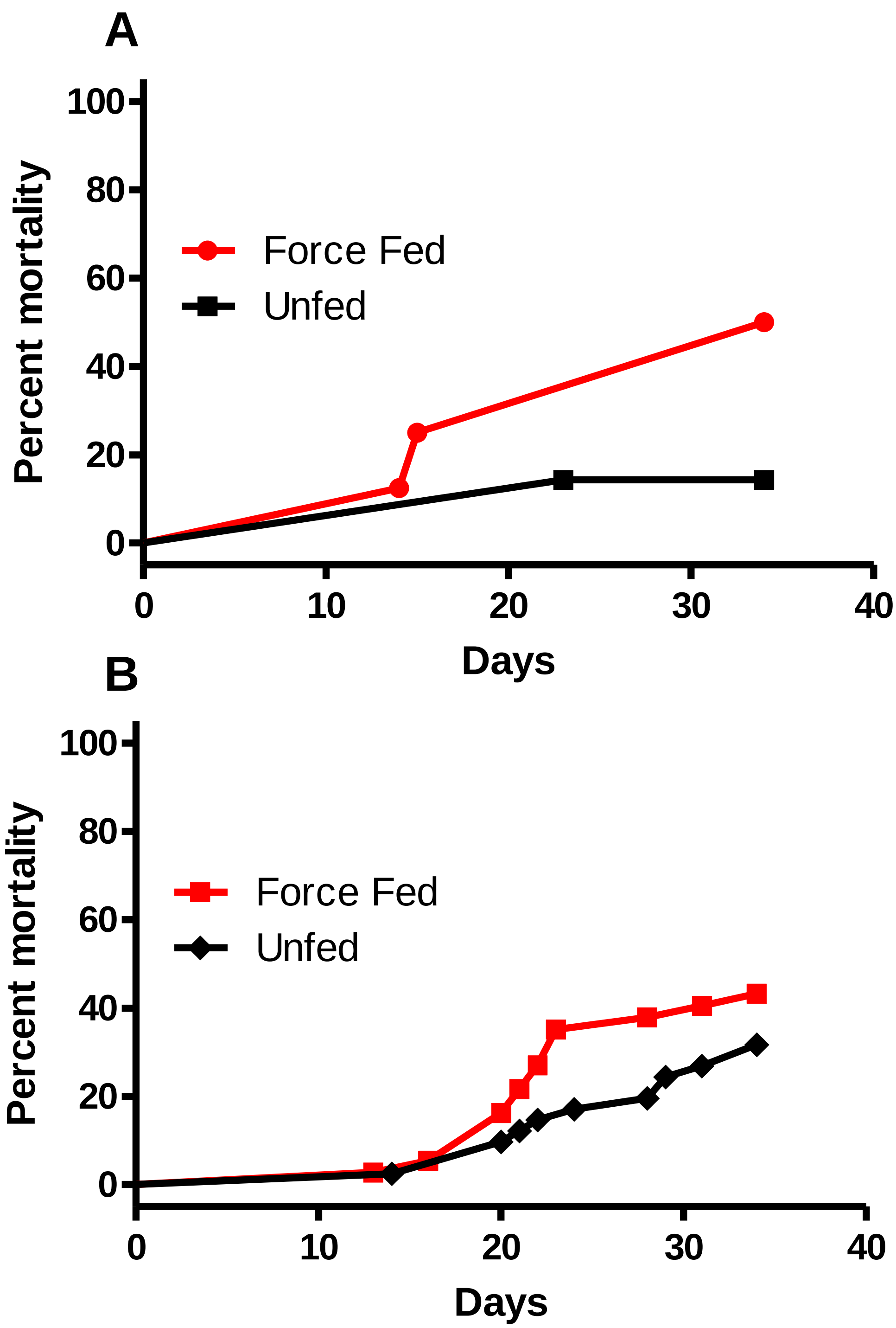
Figure 1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Section C. Genetic stock identification (GSI) to evaluate stock-of-origin from a mixed fishery sample of kelt steelhead sampled at Lower Granite Dam, Snake River Basin

Prepared by:
Andrew P. Matala

Columbia River Inter-Tribal Fish Commission
3059-F National Fish Hatchery Road,
Hagerman, Idaho 83301

Introduction

Post-spawn steelhead trout (*Oncorhynchus mykiss*), known as kelt steelhead are found in many watersheds and subbasins throughout the Snake River Basin, but in variable numbers. The spatially distributed differences in incidence of kelts, and potential for iteroparity (repeat spawning migrations), have been shown to be coincident with particular life history attributes including size and age related to A-run and B-run spawning populations (Narum et al. 2008). In this study we used single nucleotide polymorphism (SNP) genotypic data to evaluate genetic structure among Snake River *O. mykiss* stocks, and to conduct an analysis of genetic stock composition among kelt steelhead sampled at Lower Granite Dam (LGD) between 2009-2011. The objective of this study was primarily to estimate stock proportions in a mixed stock sample, providing a better understand of the origins of post-spawn steelhead among the major subbasins (e.g., Clearwater River, Salmon River, Grande Ronde), but also to relate information about the behavior and population characteristics for genetically assigned kelt stocks.

Methods

The SNP marker panels, laboratory and genotyping methodologies, and descriptive statistics used in these GSI analyses are described in detail in Hess et al. (2011) and Hess et al. (2012). A total of 187 SNP loci were used in GSI analyses, and were pared from the original 192 to exclude a sex determining marker, three *O. clarkii* hybrid determining markers, and one of a pair of linked loci. Natural origin kelt steelhead were sampled and genotyped (n=2,900) from across the downstream migration season in coordination with other ongoing monitoring and evaluation efforts at LGD (pers. comm. Scott Everett and Zach Penny of the Nez Perce Tribe). Field data including sample date, fork length, sex, disposition and overall condition were recorded by Nez Perce and University of Idaho biologists and staff. Overall condition ratings included "poor", "fair" and "good". Briefly, the rating is based on fish color, fungal load, and presence of injuries (e.g., head wounds; pers. comm. Scott Everett, Nez Perce Tribe).

The CRITFC and Idaho Department of Fish and Game (IDFG) SNP baseline for steelhead trout is the frame of reference for determining kelt origins (Hess et al. 2011, Hess et al. 2012, Ackerman et al. 2011). The baseline provides a genetic characterization of all major subbasins in the Snake River Basin, and is typically based on multiple collections (watersheds) per subbasin such that all potential contributing stocks or discrete populations are represented. However, for a mixed-stock sample, the assignment of individual fish to specific populations of origin (within a watershed for example) is routinely less accurate than assignment to "reporting groups" that represent larger aggregates of populations (Hess et al. 2011). Therefore, the use of GSI is a regional application for identifying origins, and draws on the scope of demographic influences such as migration, as well as local adaptations to delineate groups of genetically similar populations.

Allele frequency variation observed in the baseline was used to define or infer distribution of populations and productivity boundaries. The resulting reporting groups (RG's) used for GSI were comprised of groups of collections or populations. The RG's were ultimately defined on the basis of multiple, interrelated sources of information: 1) the genetic similarity of populations based on structure analyses, 2) major population groups (MPG's) determined by managers, 3) the geographic structure of the Snake River (i.e. adjacency of watersheds), and 4) the assignment accuracy of baseline populations using varying reporting group iterations. In the latter, modifications of the reporting groups were constructed to evaluate changes or increases in assignment accuracy for different combinations of populations within each RG until an optimum level of accuracy was achieved.

A power analysis was first conducted for GSI to assess the resolving power (or assignment accuracy) of the steelhead SNP baseline for differentiating all representative stocks in that baseline. The program ONCOR version 1.0 (available at: http://www.montana.edu/kalinowski) was used to conduct tests of 100% simulations. This was achieved by simulating a "fishery mixture sample" for each baseline population, where 100% of the individuals in the sample are from the same population. ONCOR uses the method of Anderson et al. (2007) to simulate mixture genotypes based on observed population allele frequencies, and estimates the probability of occurrence in the baseline population being evaluated. For the 100% simulations, the mixture sample size parameter was set at 200 and the number of iterations was set at 1000.

In addition to 100% simulations, a 'leave-one-out' (LOO) individual assignment procedure was performed on the baseline using the method of Rannala and Mountain (1997) as implemented in ONCOR. In this jackknife method each individual is sequentially removed from the baseline, the allele frequencies in the population are recalculated in the absence of that individual, then the individual is assigned back to the most likely population of origin among all baseline populations. This is done iteratively until each individual has been evaluated; however, ONCOR only considers individuals with a complete genotype (data for all 187 loci). The proportion of individuals that correctly assigned back to their reporting group of origin was calculated for each baseline population; the proportion of individuals among baseline populations that assigned to each incorrect reporting group of origin was also recorded. Both the 100% simulation and the LOO method results return the assignment accuracy to population of origin, and to reporting group of origin.

Following the power analysis, the LOO method was used to assign stock proportions among designated RG's for the LGD mixed stock kelt sample. In determining whether or not a kelt assignment was accurate, both the results of 100% simulation and LOO for the baseline were considered. The LOO method is more conservative, but the 100% simulation results are calculated using all sampled individuals in the baseline, therefore potentially retaining a greater percentage of the original population information. Based on these combined results, an assignment threshold was adopted for kelt mixed-stock samples where only those individual assignments identified with 80% or greater probability were considered accurate.

Results

Baseline Power Analysis

Based on previously described criteria, the baseline populations were partitioned into 10 reporting groups (RG) for analysis (Table 1). Reporting groups are consistently color coded in figures throughout this document. Baseline assignment accuracy for LOO tests by population ranged from 25% in the Lower Snake RG (Asotin Creek#1) to 100% for seven collections in the M. F. Clearwater RG, one in the S. F. Salmon RG (Stolle Meadows), and five collections in the M. F. Salmon RG. Mean assignment accuracy by RG ranged from 43.3% in the Lower Snake RG, to 94.6% in the M. F. Clearwater RG (Table 1; Figure 1). With only four exceptions, all populations in the S. F. Salmon, M. F. Salmon, S. F. Clearwater and M. F. Clearwater (28 of 32 populations) exceeded 80% assignment probability.

By comparison, using the 100% simulation method, mean assignment accuracy for the baseline populations (RG of origin) ranged from 58.9% in the Lower Salmon RG (Hazard Creek) to 100% in the M. F. Salmon RG (Marsh Creek). All populations in the S. F. Salmon, M. F. Salmon, S. F. Clearwater and M. F. Clearwater reporting groups exceeded 80% probability of assignment to reporting group of origin. Mean assignment per RG is given in Figure 2.

Kelt Mixture Assignments

Of the 2,900 kelt samples evaluated, only 1,547 (53%) assigned with at least 80% probability (robust assignments; Table 2). Stock proportions estimated using the entire sample (n=2,900) were not complementary with the latter (Figure 3), in that some stocks were overestimated while others were greatly underestimated. Robust assignment of kelts was greatest in the Upper Salmon, M. F. Salmon and Grande Ronde reporting groups (in increasing order), and accounted for 75% of all assignments across the ten RG's. Stock proportions of kelts determined by GSI analysis were compared to the stock proportions determined from estimated total escapement to LGD; both samples included only natural origin returning adult steelhead (no detectable marks or tags). Note that the stock proportions of kelts varied widely from those estimated from total escapement in the same years (Figure 3).

Demographic Correlations

For these exploratory analyses, only robust assignments were considered. Sex ratios were largely consistent across RG's that had sufficient numbers of assigned fish from each gender for comparison. The proportion of kelts identified as female ranged from 74.3% in the S. F. Salmon RG to 93.8% in the Lower Clearwater RG (n=1 male; Table 2). The mean across RG's was 82.4% female, consistent with Keefer et al. (2008). Generally, male kelts

migrated downstream later than females, and the trend was consistent across RG's. Among both sexes, kelts assigned to the S. F. Clearwater RG migrated earliest, while males and females from both the S. F. Salmon and M. F. salmon exhibited significantly delayed migrations compared to the remaining RG's, most notably the Upper Salmon (Table 2, Figure 4). Kelt size (fork length) significantly trended toward larger fish in regions generally considered to support predominantly B-run steelhead populations (e. g., S. F. Salmon River and Clearwater River), and the trend was apparent for both sexes (Figure 5). This is in agreement with previously published studies that describe kelt distribution and characterization based on GSI (Narum et al. 2008).

The condition rating of female kelts was generally poorer in RG's dominated by larger fish (e.g., Clearwater River RG's), where significantly more fish were rated "good" as mean fork length for an RG decreased (Figure 6). Trending condition ratings for male kelts had no clear correlation with factors evaluated in this study (e. g., size), but there were far fewer male kelts sampled overall, and two RG's had to be excluded from comparisons due to insufficient assignment sample sizes. To corroborate the former observation, correlations between fork length and overall condition, and sample date and overall condition were evaluated across the entire kelt sample (n=2,900). All kelts were evaluated as a single group with no partitioning of sex and without association to a reporting group. There was no relationship observed between sample date and condition, however the relationship between kelt size and overall condition rating of individuals (Figure 7) was significant and complementary to the previously described association of mean fork length to overall condition for female kelts across RG's.

Discussion

Baseline assignment accuracy, though variable provided a reasonable degree of power to differentiate the ten designated reporting groups. Some locales or populations exhibited limited power based on lack of population distinction. This was particularly prevalent in the lower reaches and confluences of the main waterways including the Lower Snake, Lower Clearwater, and Lower Salmon rivers. Results may reflect an elevated incidence of straying among these regions relative to others in the baseline, heightened local resident influences, or a combination of factors.

The B-run life history type is typically characterized by age 2-salt fish, and therefore at least 1 year of additional ocean maturation time prior to their first spawning migration compared with the typical A-run fish. Owing to this behavior, B-run fish also exhibit larger average size than A-run. Based on GSI results it appears size is a good predictor of kelt origin by reporting group. It is encouraging that results reflect a high degree of confidence in assignment of individuals (of unknown origin) to the south and middle forks of both the Clearwater River and Salmon River, where mean size of fish is significantly larger. This is important because within these regions most if not all populations are generally considered to support B-run productivity, while outside these regions populations are believed to be predominately of an A-run life history (Busby et al. 1996; Narum et al. 2008; Ackerman et al. 2011).

Among the kelts representing in this data set, a significant correlation was observed between fish size and the proportion of fish rated "good" in overall condition, where larger fish were generally in poorer condition. A likely contributing factor is size selectivity at the Lower Granite Dam bypass. The bypass entrance orifices are undersized (12") and these small orifices are thought to result in a high incidence of head wounds on large fish (affecting the condition rating). Moreover, entrances may be selecting for smaller fish and excluding larger ones. In a related study, 90% of B-run steelhead that were acoustically tagged at a weir in Fish Creek (Middle Clearwater River) were detected in the Lower Granite Dam forebay, but none of the fish were collected in the bypass (pers. comm. Christine Moffitt, University of Idaho and Doug Hatch, CRITFC).

In contrast to size, neither a later date of downstream migration (i.e. fish presumably in the system for a longer duration), nor migratory distance appear to have a strong association with overall condition of the kelts. Note there was a high proportion of "good" condition kelts in the Upper Salmon RG (Figure 5) at the extreme of the migration corridor. The migration time of Upper Salmon River kelts identified by GSI is highly protracted comparatively, but may be reflective of the dominant numbers of kelts assigning to that region overall (50% of the kelt mixture up from 16.8% of the total return; Figure 3). The delayed migration of south and middle fork Salmon River kelts may be a function of, or coincident with a later mean date of adult return migration to LGD. Lastly, note that the assignment proportions were markedly different for the estimated total escapement across RG's compared to the reporting group of origin for the kelt sample (Figure 3). With refined methods and more data it may be possible to confidently estimate relative rates of iteroparity among RG's based on such comparisons. Monitoring of the relative abundances of both A-run and B-run forms will inform specific management of each, and has important implications for conservation.

These results describing the distribution of kelts to their regions of origin, and related group distinctions (characteristics) are biologically intuitive based on prior information (Busby et al. 1996, Keefer et al. 2008, Narum et al. 2008). We will continue to update the baseline to strive for the highest level of population and reporting group resolution possible. Additional kelt samples in following years will be valuable in evaluating the consistency (or temporal variability) of the observations presented here.

Acknowledgements

I am grateful for the dedicated sampling efforts of LGD crews (U of I, Nez Perce, IDFG) and the quality data provided. This study benefited greatly from resources provided by Christine Moffitt, Jessica Beulow and Zach Penny. Thank you to Stephanie Harmon for diligent and reliable laboratory processing and collection of genotypic data. Data and information provided by Mike Ackerman and IDFG made a substantial contribution to these analyses.

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Table 1. Populations included in the Snake River *O. mykiss* SNP baseline. Populations or collections in the baseline were partitioned into discrete reporting groups (RG's) for GSI based on assignment probability in leave-one-out bootstrap tests. The baseline serves as the reference for identifying reporting group of origin for the mixed stock samples of kelt steelhead sampled at Lower Granite Dam. Age is juvenile (J) or adult (A), all collections are natural-origin fish and all are assumed anadromous. Baseline sample size (n) is shown with actual numbers retained in ONCOR leave-one-out analysis (LOO). Accuracy is the assignment accuracy to reporting group of origin (see figure 1 for mis-assignment proportions. Bolded values are those exceeding 80% accuracy.

Collection	ID	RG	Sample Year/s	lat	long	stage (age)	n (LOO)	accuracy
Asotin	1	Lower Snake	2000	46.3442	-117.0551	J	49 (28)	25.0%
George	2	Lower Snake	2010	46.3029	-117.1168	A	95 (44)	50.0%
Asotin	3	Lower Snake	2008; 2010	46.3228	-117.1368	A	98 (45)	44.4%
Alpowa	4	Lower Snake	2010	46.4076	-117.2198	A	98 (53)	45.3%
Tucannon	5	Lower Snake	2005; 2010	46.3097	-117.6572	A	105 (58)	51.7%
mean								*43.3%*
Lapwai/Mission	6	Lower Clearwater	2000	46.3672	-116.736	J	49 (24)	66.7%
W. F. Potlatch	7	Lower Clearwater	2009-10	46.8054	-116.4182	A	85 (50)	64.0%
E. F. Potlatch	8	Lower Clearwater	2008; 2010-11	46.7984	-116.4194	A	156 (86)	75.6%
Big Bear	9	Lower Clearwater	2007-08; 2010-11	46.6306	-116.6562	A	98 (51)	54.9%
Little Bear	10	Lower Clearwater	2007-08; 2010-11	46.6372	-116.678	A	151 (61)	**80.3%**
mean								*68.3%*
Clear	11	S. F. Clearwater	2000	46.0486	-115.7814	J	45 (24)	70.8%
Crooked	12	S. F. Clearwater	2007-08	45.8211	-115.5272	A	104 (43)	**86.0%**
Tenmile	13	S. F. Clearwater	2000	45.8057	-115.6833	J	46 (27)	**85.2%**
John's	14	S. F. Clearwater	2000	45.8224	-115.8887	J	36 (15)	**86.7%**
mean								*82.2%*
Colt	15	M. F. Clearwater	2000	46.4311	-114.5395	J	38 (19)	**100.0%**
Storm	16	M. F. Clearwater	2000	46.4607	-114.5467	J	38 (20)	**100.0%**
Crooked Fork	17	M. F. Clearwater	2000	46.5251	-114.6786	J	44 (22)	**90.9%**
Lake	18	M. F. Clearwater	2000	46.4632	-114.9965	J	47 (19)	**100.0%**

Fish	19	M. F. Clearwater	2010	46.3336	-115.3471	A	99	(50)	**92.0%**
Canyon	20	M. F. Clearwater	2011	46.2161	-115.5559	J	46	(25)	80.0%
Selway	21	M. F. Clearwater	2008	45.6921	-114.7175	J	76	(35)	**97.1%**
Little Clearwater	22	M. F. Clearwater	2008	45.7441	-114.7895	J	59	(31)	**100.0%**
Whitecap	23	M. F. Clearwater	2008	45.8689	-114.7205	J	76	(32)	**100.0%**
Bear	24	M. F. Clearwater	2000	46.0191	-114.8378	J	35	(13)	**100.0%**
N. F. Moose	25	M. F. Clearwater	2000, 2004	46.1634	-114.9006	J	92	(45)	**95.6%**
Three Links	26	M. F. Clearwater	2000	46.0981	-115.0728	J	47	(24)	**100.0%**
Gedney	27	M. F. Clearwater	2000	46.0583	-115.3141	J	45	(19)	**94.7%**
O'Hara	28	M. F. Clearwater	2000	46.0809	-115.5179	J	47	(23)	73.9%
mean									*94.6%*
Joseph	29	Grande Ronde	2011	46.0278	-117.0177	A	45	(16)	50.0%
Crooked	30	Grande Ronde	2001	45.977	-117.555	J	95	(45)	68.9%
Elk	31	Grande Ronde	2000	45.7053	-117.1529	J	45	(31)	**80.6%**
Little Minam	32	Grande Ronde	2000	45.4004	-117.6722	J	48	(23)	69.6%
Lostine	33	Grande Ronde	2000	45.5521	-117.4898	J	45	(19)	**89.5%**
Menatchee	34	Grande Ronde	1999	46.0075	-117.3651	J	68	(14)	57.1%
Wenaha	35	Grande Ronde	2001	45.9453	-117.4513	J	93	(36)	66.7%
Captain John	36	Grande Ronde	2000	46.1515	-116.934	J	56	(32)	71.9%
mean									*69.3%*
Big Sheep	37	Imnaha	2001	45.5574	-116.8345	J	61	(18)	72.2%
Camp	38	Imnaha	2001	45.5572	-116.8352	J	23	(12)	50.0%
Cow	39	Imnaha	2000	45.7681	-116.7496	J	44	(26)	61.5%
Lightning	40	Imnaha	2000	45.6554	-116.7265	J	38	(8)	50.0%
mean									*58.4%*
Hazard	41	Lower Salmon	2000	45.1836	-116.2995	J	43	(21)	28.6%
Boulder	42	Lower Salmon	2000	45.2019	-116.3114	J	47	(29)	62.1%
Rapid	43	Lower Salmon	2003; 09	45.3737	-116.3569	A	99	(53)	67.9%
Slate	44	Lower Salmon	2000	45.638	-116.2828	J	46	(27)	59.3%
Whitebird	45	Lower Salmon	2000-01	45.7523	-116.3198	J	59	(28)	46.4%

mean									*52.9%*
E. F. S. F. Salmon	46	S. F. Salmon	2000	45.0127	-115.7129	J	45	(21)	71.4%
Secesh	47	S. F. Salmon	2000	45.0268	-115.7082	J	45	(25)	**84.0%**
Lick	48	S. F. Salmon	2010	45.0692	-115.814	J	39	(19)	**84.2%**
Stolle Meadows	49	S. F. Salmon	2000	44.607	-115.681	J	45	(16)	**100.0%**
mean									*84.9%*
Marsh	50	M. F. Salmon	2000	44.4493	-115.2301	J	59	(24)	**100.0%**
Sulphur	51	M. F. Salmon	2000	44.5526	-115.2974	J	42	(20)	**100.0%**
Rapid (M. F.)	52	M. F. Salmon	2000	44.679	-115.149	J	31	(6)	**100.0%**
Pistol	53	M. F. Salmon	2000	44.7217	-115.1488	J	23	(13)	**100.0%**
Camas	54	M. F. Salmon	2000	44.8918	-114.7222	J	56	(31)	**100.0%**
Loon	55	M. F. Salmon	1999-00	44.5976	-114.8123	J	84	(41)	**95.1%**
Big (upper)	56	M. F. Salmon	2000	45.1523	-115.2975	J	45	(28)	**96.4%**
Big (lower)	57	M. F. Salmon	2000	45.0941	-114.7343	J	46	(18)	**94.4%**
Chamberlain	58	M. F. Salmon	2000	45.4523	-114.931	J	46	(34)	73.5%
Bargamin	59	M. F. Salmon	2000	45.5716	-115.1919	J	46	(18)	72.2%
mean									*93.2%*
Sawtooth Weir	60	Upper Salmon	2005; 10	44.1506	-114.8851	A	105	(56)	76.8%
Valley	61	Upper Salmon	2005	44.2231	-114.9272	J	44	(25)	56.0%
W. F. Yankee Fork	62	Upper Salmon	2004; 08	44.3514	-114.7297	J	117	(84)	66.7%
Morgan	63	Upper Salmon	2000	44.6135	-114.1641	J	37	(21)	**81.0%**
Pahsimeroi Weir	64	Upper Salmon	2006; 10	44.6844	-114.0403	A	96	(51)	70.6%
Hayden	65	Upper Salmon	2009-10	44.8616	-113.6319	J	84	(15)	60.0%
N. F. Salmon	66	Upper Salmon	2010	45.4094	-113.9918	A, kelt	99	(62)	51.6%
mean									*66.1%*

Table 2. Reporting group assignment of individual kelts (origin unknown) sampled at Lower Granite Dam. The total number sampled was 2,900; however, only 1,547 met the individual assignment probability threshold of 80%. Numbers assigned to each reporting group are partitioned by gender. The mean sample date at LGD (ordinal day) among fish assigned to each reporting group serves as a proxy for outmigration timing (see Figure 4).

		Female			Male		
Reporting Group	Total (n)	n	%	date	n	%	date
Lower Snake	92	74	80.4	129	18	19.6	140
Lower Clearwater	16	15	93.8	124	1	6.3	166
S. F. Clearwater	59	47	79.7	118	12	20.3	130
M. F. Clearwater	71	60	84.5	143	11	15.5	151
Grande Ronde	176	141	80.1	137	35	19.9	146
Imnaha	87	77	88.5	138	10	11.5	150
Lower Salmon	21	18	85.7	146	3	14.3	155
S. F. Salmon	35	26	74.3	151	9	25.7	170
M. F. Salmon	217	173	79.7	156	44	20.3	162
Upper Salmon	773	601	77.7	133	172	22.3	145
total	1547	1232	---	---	315	---	---
mean	---	---	82.4	137	---	17.6	148

Figure 1. Results of the leave-one-out (LOO) bootstrap method to test baseline assignment accuracy. Individual fish within each baseline collection were assigned to the most likely collection of origin. Baseline collections (defined by ID # in table 1) are arranged by designated reporting group of origin (X-axis). The Y-axis is the assignment proportion, indicating the proportion of fish within each baseline collection that assigned with highest confidence to each of ten reporting groups identified on the Z-axis (depth).

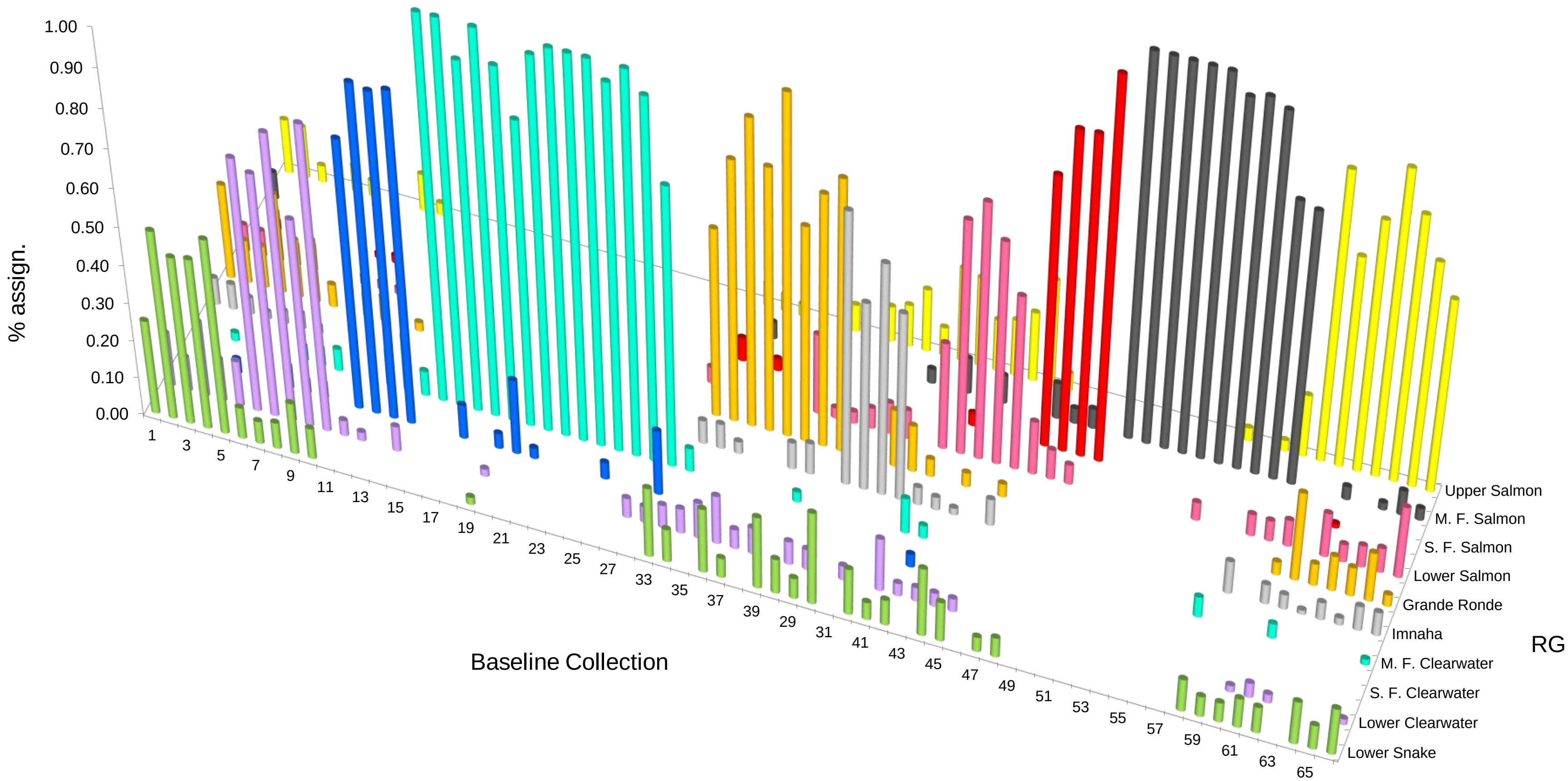


Figure 2. Results of the 100% simulation method to test baseline assignment accuracy. Color coded circles are the mean probability of assignment to reporting group of origin for each baseline collection (defined by ID # in table 1), while star symbols indicate the mean assignment probability to baseline population of origin. The dashed lines mark those collections that fell within the threshold interval of 80-100% mean assignment probability. Mean rate of assignment per reporting group using the 100% simulation method is indicated in the legend.

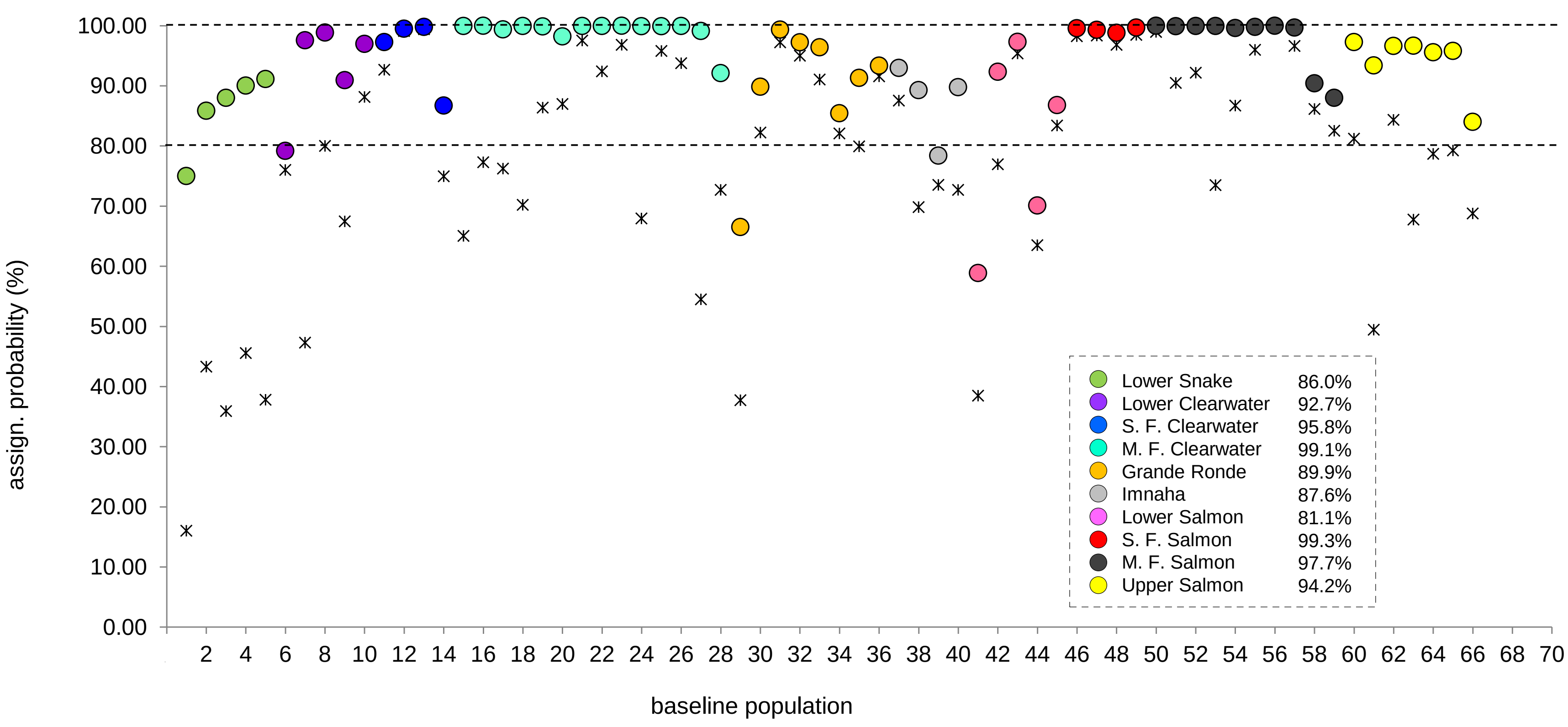


Figure 3. Estimated stock proportions (by reporting group) for total escapement of steelhead sampled at LGD during return years 2009-2010 and 2010-2011 (n=4,182); data provided by Mike Ackerman, IDFG. By comparison, the charts on the right indicate the assigned stock proportions for the kelt mixture comprising all sampled kelts (top; n=2,900), and only those kelts that assigned with ≥80% probability (bottom; n=1,547).

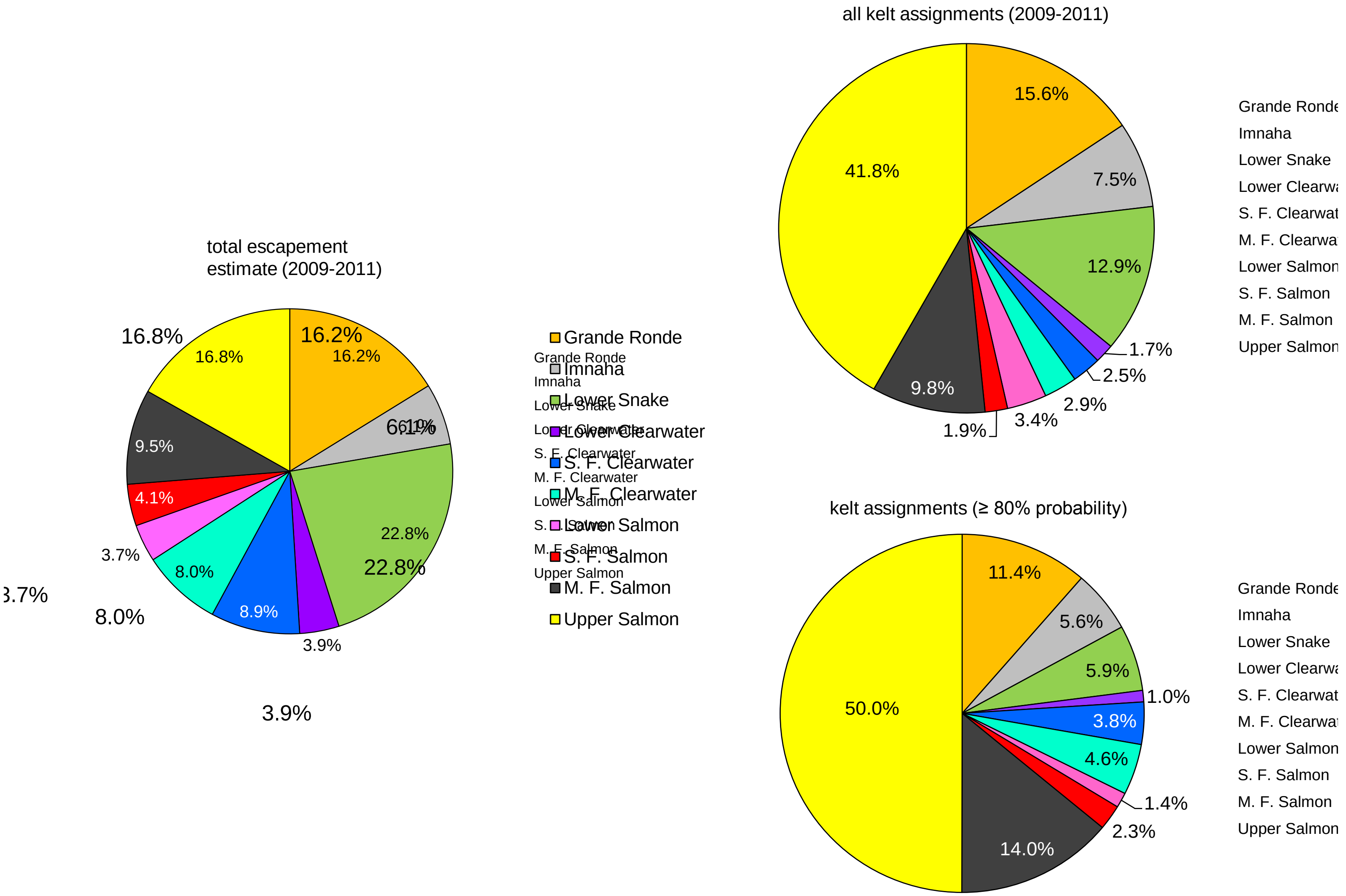


Figure 4. Distribution of sample dates at LGD for downstream migrating kelt steelhead assigned to each stock each stock (reporting group). The dashed line is the mean sample day; note the overall delayed migration time for males across all reporting groups.

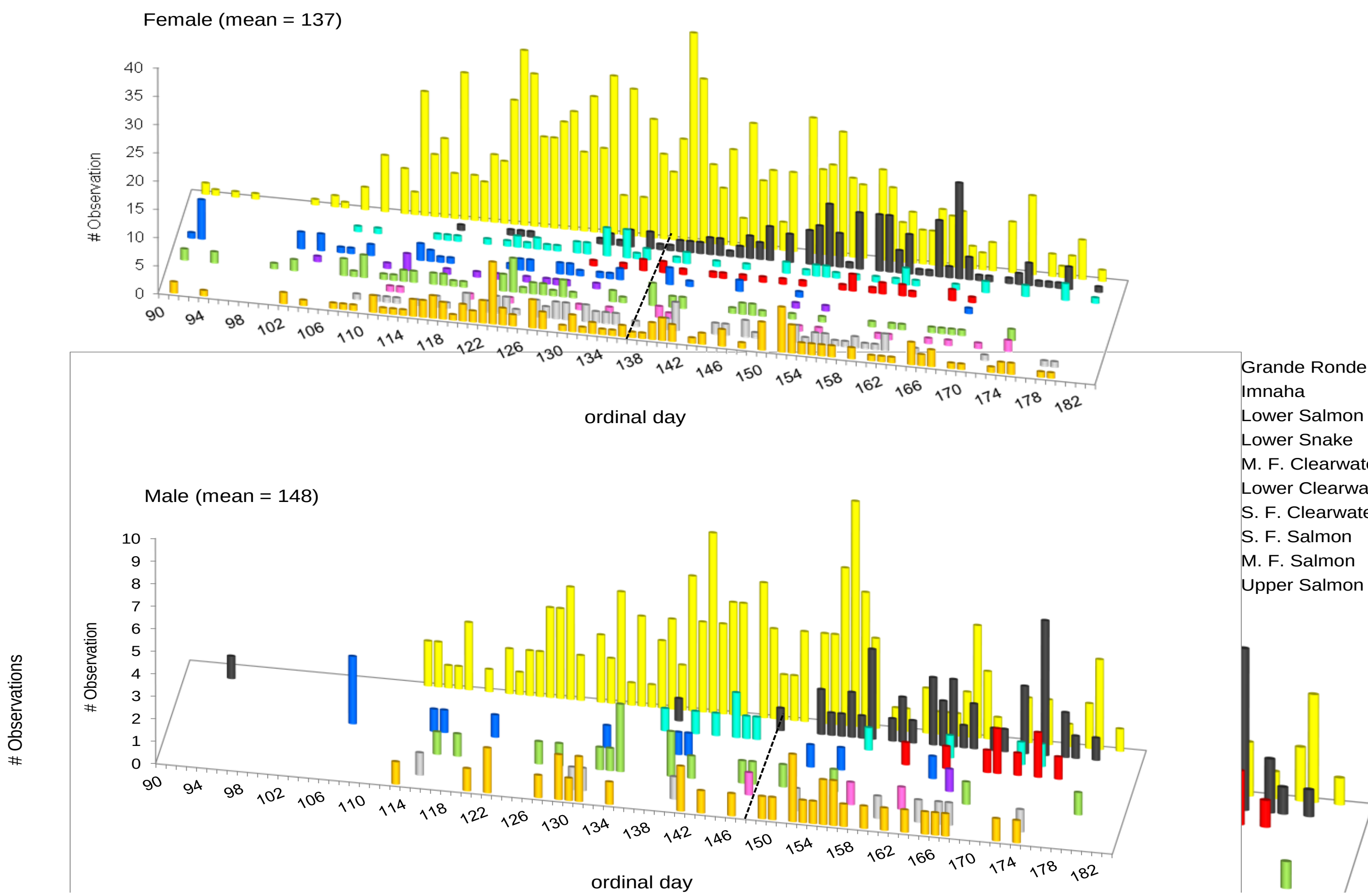
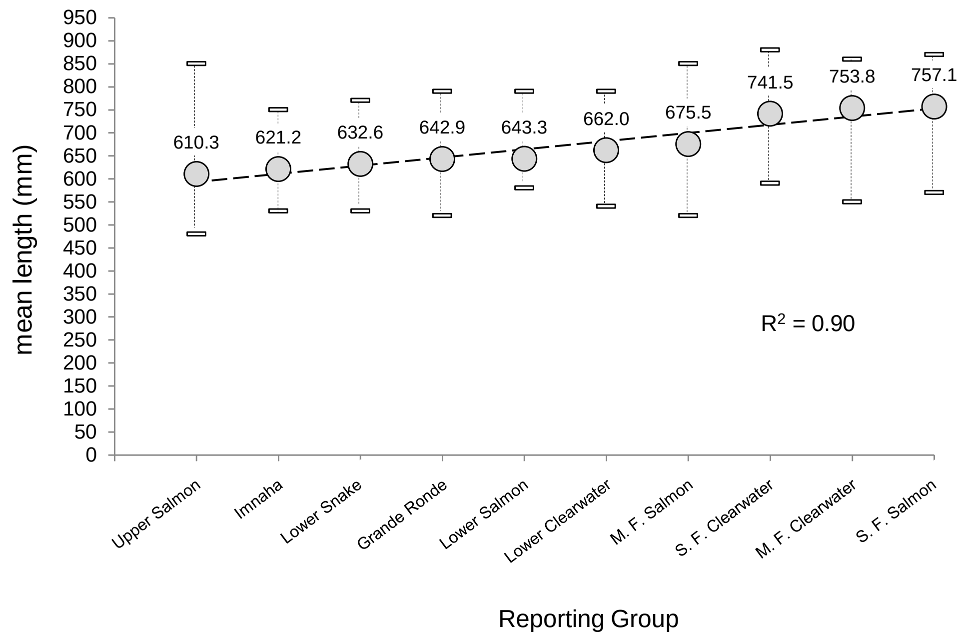


Figure 5. Mean length of fish assigned to each reporting group; a) female b) male. The horizontal bars with dashed lines represent the range of observed lengths. The trend toward larger fish in the middle and south forks of both the Clearwater River and Salmon River was significant for both sexes. The Lower Clearwater and Lower Salmon reporting groups were not included for male kelts due to too few assignments; 1 and 3 respectively.

a) Female



b) male

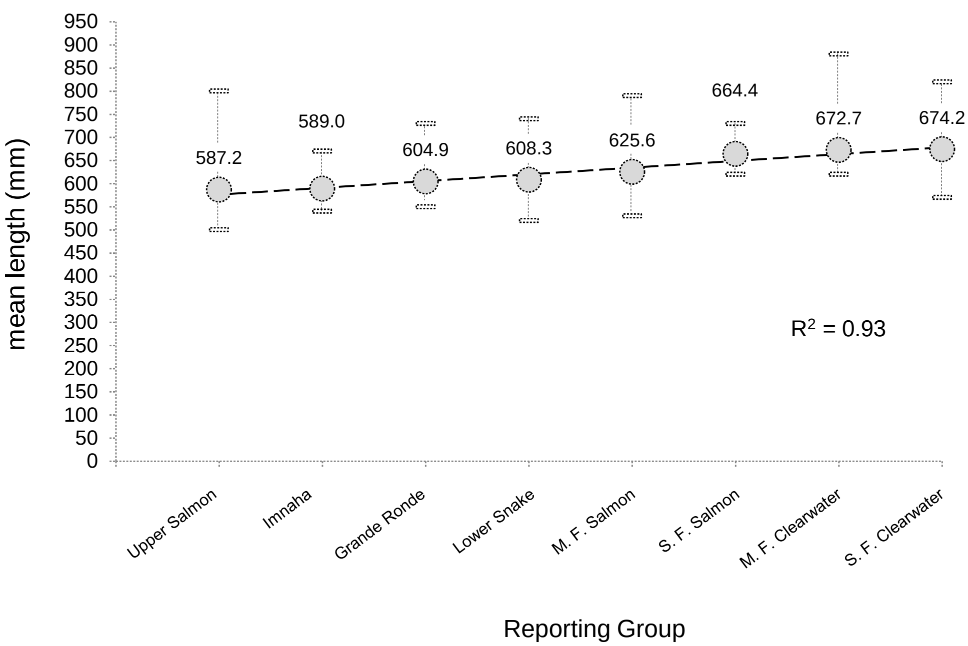
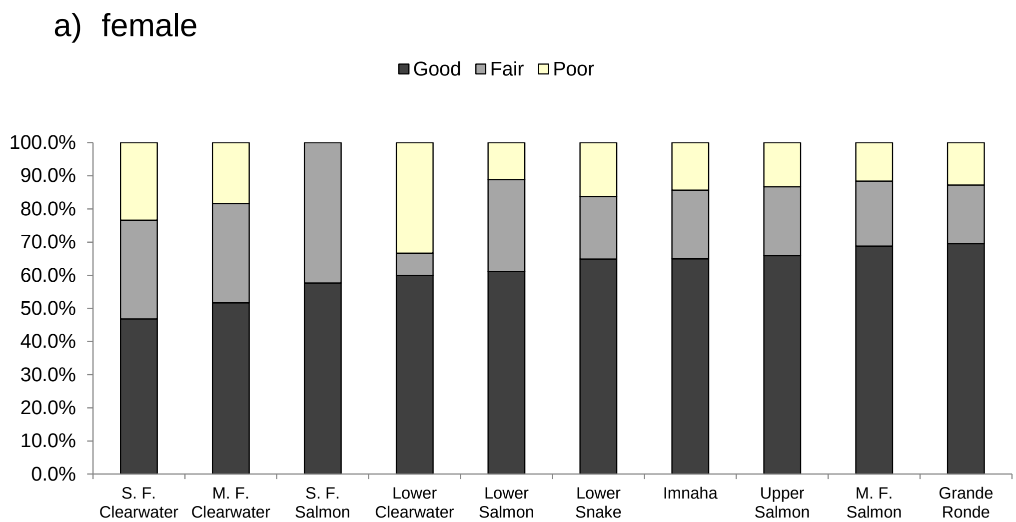


Figure 6. Overall condition of sampled kelts at LGD partitioned by assigned reporting group.

Note that with the exception of M. F. Salmon, the reporting groups associated with larger fish have fewer kelts in "good" condition. Two locations identified by sample size were not included for males owing to limited number of assigned kelts to those RG's. The trend for "good" condition was significant for both sexes; female (R^2 = 0.9147) male (R^2 = 0.8361).



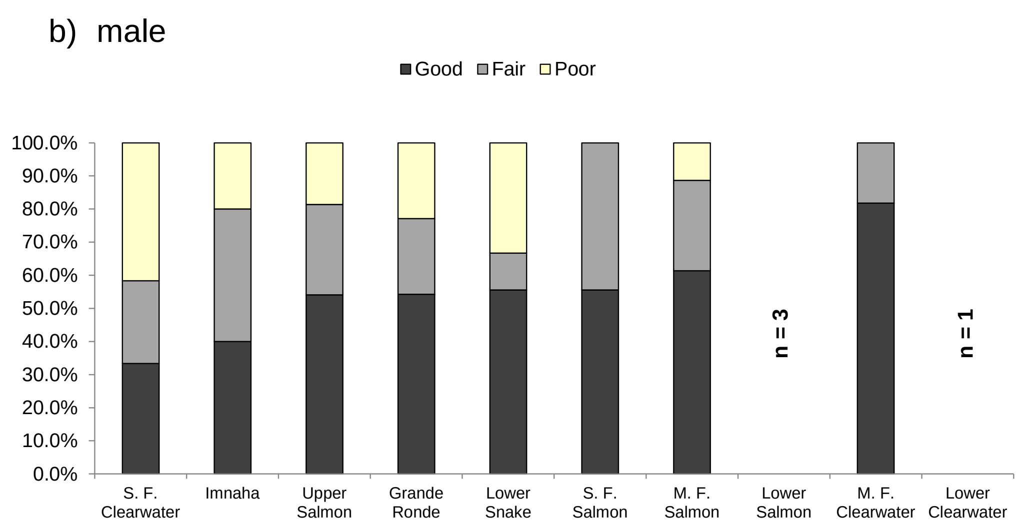
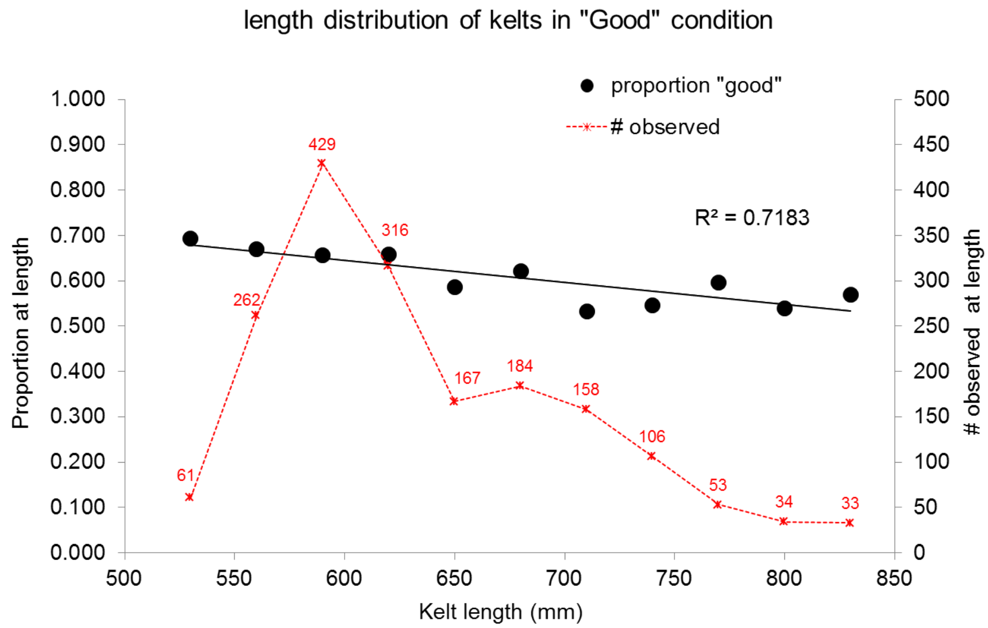
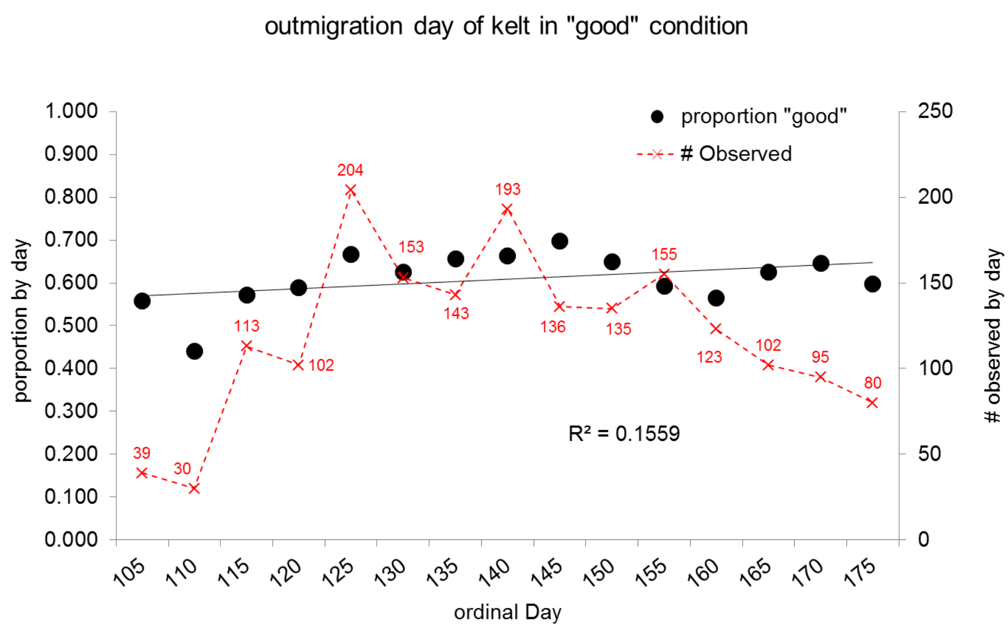


Figure 7. Correlation between life history variables. Condition of kelts ("good") is shown in association with (a) length or size of kelts, and (b) in association with sample date which is treated as a proxy for outmigration time. All sampled kelts (n=2,900) are included in the analysis, but because number of males was limited, results are not partitioned by gender.

a)



b)



Section D: Snake River Kelt Management Plan Update

MASTER PLAN PROGRESS

The Snake River Kelt Management Plan at time of publication of this document is approximately 90% complete. We have approached the drafting of the elements of this document in a very methodical process. With the completion of these elements, the plan will be an effective and fully comprehensive document that represents the Tribal vision for implementation of kelt management in the Snake River Basin. Below, we identify the remaining technical elements that we feel must be finalized before the plan can be published. Publication of the Snake River kelt management plan will be made no later than December of 2012.

CHAPTER 1: INTRODUCTION

- The Purpose of the Master Plan
- Need for Action
- Goals and Objectives
- Project History

Items to complete: Clearly identify critical research needs and their impact on the alternative selection process.

CHAPTER 2: RELATIONSHIP TO REGIONAL PLANS, PROGRAMS AND PROJECTS

- Consistency with NPCC's Master Plan Requirements
- Regional Guidelines
- Comparison to Existing Plans and Projects

Items to complete: Master plan technical element number 15: Provide a completed Hatchery and Genetic Management Plan (HGMP) for the target population(s). This will most likely become components of existing production facilities' HGMP's. Master plan technical element 16: Describe the harvest plan. The target 6% increase of adult steelhead to Lower Granite Dam may allow for additional harvest. Harvest planning will require co-manager input based on effective alternative implementation and will be closely tied to monitoring and evaluation results.

CHAPTER 3: STATUS OF SNAKE RIVER STEELHEAD

- Stock Abundance and Distribution
- Life History Diversity
- Ecological Significance of Iteroparity
- Reproduction and Life Stage Survival
- Supplementation and Exploitation

Items to complete: Update life stage survival with recent data from upper Snake River, Clearwater River and Salmon River steelhead returns. Reported iteroparity rates for most upper Snake River reaches have been below 5%. Recent installation of PIT tag arrays in upper tributaries have resulted in repeat return rates as high as 15%.

CHAPTER 4: INFORMATION USED TO GUIDE MANAGEMENT ACTION SELECTION

- Management Decision Process
- Management Context
- Preliminary Results
  - COE (and others) Operation and Facilities Research
  - Nez Perce Tribe, Yakima (and others) Kelt Reconditioning Research
- Steelhead Kelt Reconditioning Criteria Development
- Integration of Data Sources

Items to complete: Finalize minimum kelt reconditioning criteria. Data are currently being evaluated which will be built into these criteria. Of special note are endocrine and physiological parameters as indicators of potential success of reconditioning kelts.

CHAPTER 5: 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

Items to complete: Much of the past year's master planning effort has focused on this chapter. Alternative site assessment, in conjunction with chapter four's reconditioning criteria development will be updated. Finalize model that uses the above criteria to score various components of each alternative and ranks each alternative. One of the primary indicators of success is the 6% increase of adults to Lower Granite Dam. This target was identified in NOAA Fisheries Supplemental Comprehensive Analysis of Snake River Steelhead, Steelhead Kelt Appendix. The analysis is being reevaluated with updated data. The new spill regime appears to have impacted (lowered) the steelhead kelt interception rate at Lower Granite Dam. Additional effort in collection will be incorporated into the analysis.

CHAPTER 6: LIMITING FACTORS

- Harvest
- Hatcheries
- Mainstem Snake and Columbia River Hydrosystem
- Habitat

Items to complete: This chapter may be reformatted and integrated throughout the Master Plan where appropriate components are discussed. Many of the master plan technical elements are directed at these factors.

CHAPTER 7: 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

Items to complete: The current R, M & E chapter consists of overarching objectives as they relate to steelhead abundance and distribution in the Snake River. On-going research focused on steelhead kelt movement and distribution will be refined in the status monitoring section. Success and failure thresholds will be coordinated with co-managers. These thresholds will be used in a feedback loop that helps direct management approaches as identified in chapter five's model that scores and ranks alternatives.

Appendices.

Appendix A. Cumulative Snake River Downriver PIT-tag Detections 2009-2011

Figure 1. Number of kelts tagged and released into the river at Lower Granite Dam in 2009 and 2010 and the kelts detected during downstream migration at least once in the Snake or Columbia Rivers.

Group and condition	Total tagged	Number detected	Percent detected
2009 Total	**197**	**65**	**33**
Males	**28**	**11**	**39**
Good	15	10	67
Fair	7	1	14
Poor	6	0	0
Females	**169**	**54**	**32**
Good	117	42	36
Fair	35	12	34
Poor	17	0	0
2010 Total	**1398**	**252**	**18**
Males	**374**	**79**	**21**
Good	174	62	36
Fair	114	13	11
Poor	86	4	5
Females	**1024**	**173**	**17**
Good	673	152	23
Fair	250	20	8
Poor	101	1	0.99
2011 Total	**1613**	**289**	**18**
Males	**260**	**61**	**23**
Females	**1353**	**228**	**17**

Appendix B. Acoustic line maps

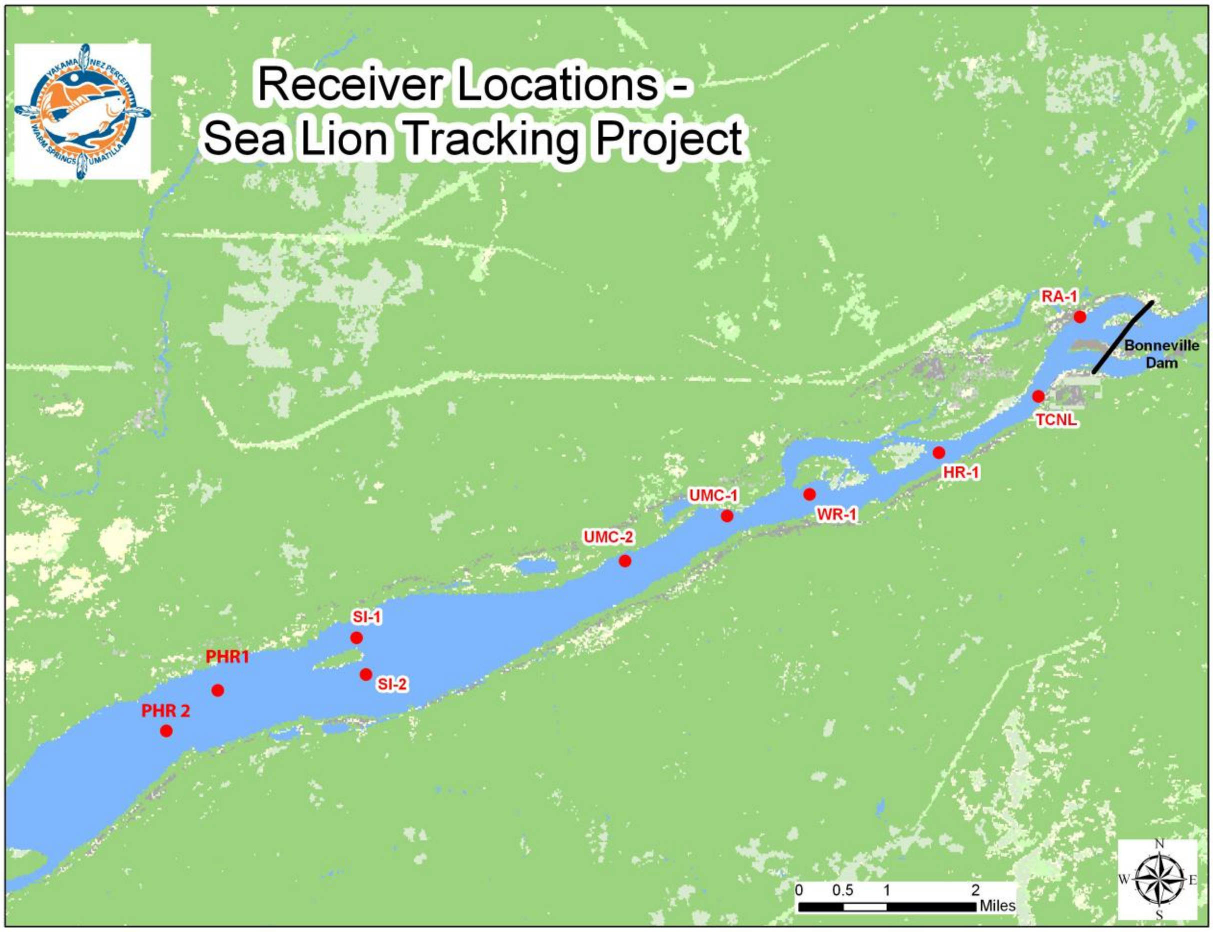


Figure 1: Bonneville Line (Rkm 233) 2011:

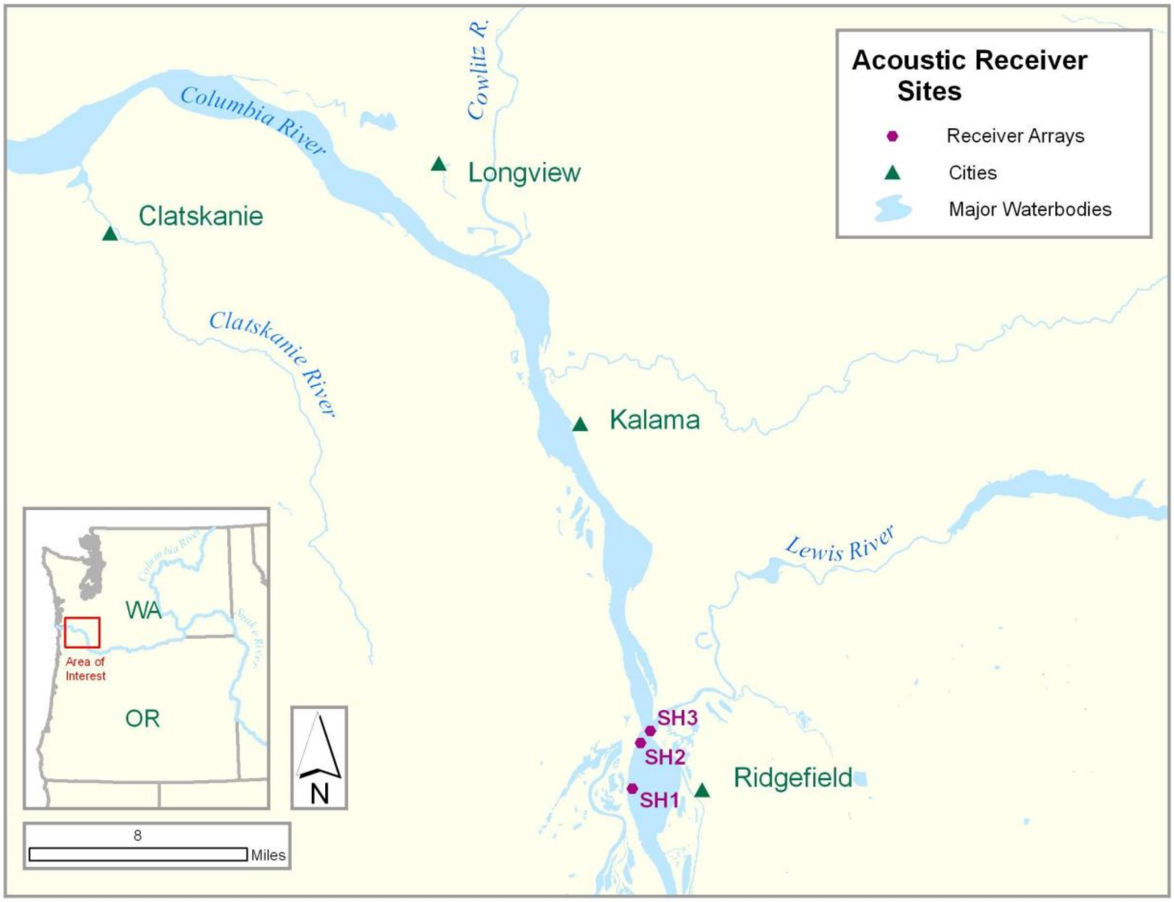


Figure 2: St. Helens Array (RKM 150) 2011.

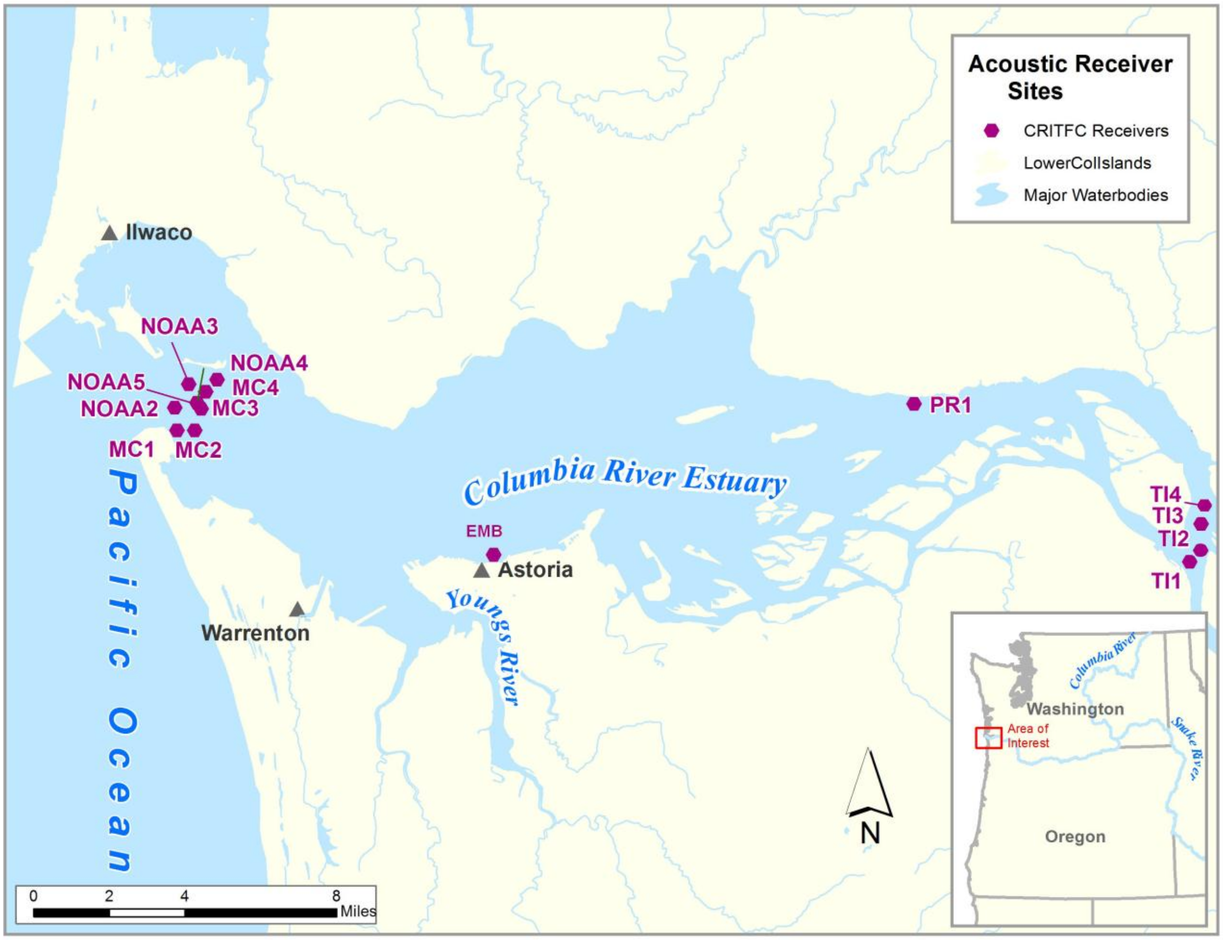


Figure 3: Estuary Acoustic Array Sets (Mouth of the Columbia Rkm 0 and Estuary Rkm 56) 2011.

Appendix C: Steelhead Kelt Reconditioning Treatments

Strategy	Year	Location	# Collected	# released	S @ release (%)	# @ ocean	S @ ocean (%)	# @ Bonneville	Return Rate to Bonneville (%)	Transportation (or treatement)Benefit relative to in-river	Treatment Relative to Hockersmith 1.66	Transportation (or treatment)benefit relative to Bonneville natural
In-river	2005	Prosser	67	67				3	4.48	1.56	2.70	25.93
In-river	2006	Prosser	52	52				1	1.92	0.67	1.16	3.12
In-river	2007	Prosser	53	53				3	5.66	1.97	3.41	9.31
In-river	2008	Prosser	88	88				4	4.55	1.59	2.74	6.66
In-river	2009	Prosser	58	58				3	5.17	1.80	3.12	11.63
In-river	2010	Prosser	155	155				2	1.29	0.45	0.78	2.89
In-river	2011	Prosser	85	85				0	0.00	0.00	0.00	0.00
Total and weighted mean			***558***	***558***				***2.24***	***2.87***	***1.00***	***1.73***	***5.62***
In-river	2002	Lower Granite*	1209	1209				8	0.66	1.13	0.40	
In-river	2003	Lower Granite*	865	865				3	0.35	0.59	0.21	
In-river	2004	Lower Granite*	1138	1138				10	0.88	1.51	0.53	1.52
In-river	2009	Lower Granite	178	176				2	1.12	1.93	0.68	1.52
In-river	2010	Lower Granite	1411	1399				5	0.35	0.61	0.21	0.48
In-river	2011	Lower Granite	1633	1613				0	0.00	0.00	0.00	0.00
Total and weighted mean			***4801***	***4787***				***6.47***	***0.58***	***1.00***	***0.35***	***1.14***
In-river	2002	John Day*	***287***	***287***				***28***	***9.76***	***1.00***	***5.88***	***19.11***
Total and weighted mean												
Transported (Hamilton Island)	2002	Lower Granite*	750	750				19	2.53	3.83	1.53	
Transported (Hamilton Island)	2003	Lower Granite*	376	376				3	0.80	2.30	0.48	
Transported (Hamilton Island)	2004	Lower Granite*	982	982				7	0.71	0.81	0.43	2.04
Transported (Hamilton Island)	2009	Lower Granite	71	68				0	0.00	0.00	0.00	0.00
Transported (Hamilton Island)	2010	Lower Granite	301	301		13/108	12.04	0	0.00	0.00	0.00	0.00
Transported (Hamilton Island)	2011	Lower Granite	109	109		3/47	6.38	0	0.00		0.00	
Total and weighted mean			***2589***	***2586***			***9.21***	***8.59***	***1.12***	***1.74***	***0.67***	***2.19***
Transported (estuary release)	2010	Lower Granite	23	22		4/10	40.00	0	0.00	0.00	0.00	0.00
Transported (estuary release)	2011	Lower Granite	91	90		14/46	30.43	0	0.00	0.00	0.00	0.00
Total and weighted mean			***114***	***112***			***35.22***	***0***	***0.00***	***0.00***	***0.00***	***0.00***
Transported	2002	John Day*	***271***	***271***				***34***	***12.55***	***1.29***	***7.56***	***24.58***
Total and weighted mean												
Transported (unfed Hamilton Island)	2004	Prosser	75	63		15/28	53.57	5	6.67		4.02	19.10
Transported (unfed Hamilton Island)	2005	Prosser	98	96		14/57	24.56	1	1.02	0.23	0.61	5.91
Transported (unfed Hamilton	2006	Prosser	55	49		31/49	63.27	2	3.64	1.89	2.19	5.89

Island)												
Transported (unfed Hamilton Island)	2007	Prosser	43	38		14/35	40.00	0	0.00	0.00	0.00	0.00
Transported (unfed Hamilton Island)	2008	Prosser	100	100		26/49	53.06	3	3.00	0.66	1.81	4.40
Transported (unfed Hamilton Island)	2010	Prosser	124	123		27/59	45.76	1	0.81	0.63	0.49	1.80
Transported (unfed Hamilton Island)	2011	Prosser	100	100		16/47	34.04	0	0.00	0.00	0.00	0.00
Total and weighted mean			***595***	***569***			***44.89***	***1.69***	***2.02***	***0.70***	***1.21***	***3.95***
Transported (unfed estuary release)	2010	Prosser	113	113		13/60	21.67	1	0.88	0.69	0.53	1.98
Transported (unfed estuary release)	2011	Prosser	90	89		16/47	34.04	1	1.11	0.86	0.67	2.92
Total and weighted mean			***203***	***202***			***27.85***	***1.00***	***0.99***	***0.77***	***0.59***	***1.93***
Transported (fed Hamilton Island)	2002	Prosser	479	334				43	8.98		5.41	
Transported (fed Hamilton Island)	2003	Prosser	208	187				8	3.85		2.32	
Transported (fed Hamilton Island)	2004	Prosser	105	83		11/26	42.31	5	4.76		2.87	13.64
Transported (fed Hamilton Island)	2005	Prosser	106	96		6/56	10.71	0	0.00	0.00	0.00	0.00
Transported (fed Hamilton Island)	2006	Prosser	56	50		32/50	64.00	0	0.00	0.00	0.00	0.00
Transported (fed Hamilton Island)	2007	Prosser	40	38		19/27	70.37	1	2.50	0.44	1.51	4.11
Transported (fed Hamilton Island)	2008	Prosser	108	100		28/50	56.00	7	6.48	1.43	3.90	9.50
Total and weighted mean			***1102***	***888***			***48.68***	***21.40***	***5.81***	***2.03***	***3.50***	***11.38***
Transported (pooled groups)	2002	Prosser	479	334				43	8.98		5.41	
Transported (pooled groups)	2003	Prosser	*208*	187				8	3.85		2.32	
Transported (pooled groups)	2004	Prosser	203	179		26/54	48.15	10	4.93		2.97	14.11
Transported (pooled groups)	2005	Prosser	161	145		20/113	17.70	1	0.62	0.14	0.37	3.60
Transported (pooled groups)	2006	Prosser	99	88		63/99	63.64	2	2.02	1.05	1.22	3.27
Transported (pooled groups)	2007	Prosser	140	138		33/62	53.23	1	0.71	0.13	0.43	1.17
Transported (pooled groups)	2008	Prosser	232	223		54/99	54.55	10	4.31	0.95	2.60	6.32
Transported (pooled groups)	2010	Prosser	237	236		40/119	33.61	2	0.84	0.65	0.51	1.89
Transported (pooled groups)	2011	Prosser	190	189		32/94	34.04	1	0.53	0.41	0.32	1.39
Total and weighted mean			***1759***	***1530***			***45.14***	***15.68***	***4.00***	***1.40***	***2.64***	***7.84***
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					5.31	16.54	61.74
Long-term	2010	Prosser	1157	404	34.92					27.06	21.03	78.10
Long-term	2011	Prosser	680	223	32.79					11.54	19.93	87.07
Total and weighted mean			***6533***	***2407***	***36.84***					***12.85***	***22.20***	***72.17***
Long-term	2005	Shitike Cr	9	1	11.11						6.69	64.33

Long-term	2006	Shitike Cr	4	0	0.00			0.00	0.00
Long-term	2007	Shitike Cr	14	1	7.14			4.30	11.74
Long-term	2008	Shitike Cr	11	0	0.00			0.00	0.00
Total and weighted mean			***38***	***2***	***5.26***			***3.17***	***10.31***
Long-term	2005	Omak Cr	17	3	17.65			10.63	102.18
Long-term	2006	Omak Cr	27	2	7.41			4.46	12.01
Long-term	2007	Omak Cr	43	8	18.60			11.21	30.59
Long-term	2008	Omak Cr	32	9	28.13			16.94	41.23
Long-term	2009	Omak Cr	17	2	11.76			7.09	26.46
Long-term	2010	Omak Cr	13	6	46.15			27.80	103.23
Long-term	2011	Omak Cr	20	4	20.00			12.05	
Total and weighted mean			***169***	***34***	***20.13***			***12.12***	***39.44***
Long-term	2006	Parkdale	1	1.0	100.00			60.24	162.11
Long-term	2007	Parkdale	13	1.0	7.69			4.63	12.65
Long-term	2008	Parkdale	14	7	50.00			30.12	73.31
Long-term	2009	Parkdale	9	4	44.44			26.77	99.96
Long-term	2010	Parkdale	15	4	26.67			16.06	59.64
Long-term	2011	Parkdale	23	5	21.74			13.10	57.21
Total and weighted mean			***75***	***22.0***	***29.33***			***17.67***	***57.46***
Natural repeat	2004	Bonneville Dam	1146			4	0.35		
Natural repeat	2005	Bonneville Dam	579			1	0.17		
Natural repeat	2006	Bonneville Dam	1459			9	0.62		
Natural repeat	2007	Bonneville Dam	1973			12	0.61		
Natural repeat	2008	Bonneville Dam	2639			18	0.68		
Natural repeat	2009	Bonneville Dam	2474			11	0.44		
Natural repeat	2010	Bonneville Dam	1342			6	0.45		
Natural repeat	2011	Bonneville Dam	1318			5	0.38		
							0.51		

* 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 D: Hood River Basin Kelt (Steelhead) Management Plan

April, 2012

Confederated Tribes of Warm Springs Reservation

and

Columbia River Inter-Tribal Fish Commission

Table of contents

Introduction

Columbia River *Oncorhynchus mykiss* (hereafter, steelhead), including those inhabiting the Hood River have experienced a dramatic decline in abundance, resulting in the listing of the lower-Columbia Distinct Population Segment (DPS) under the Endangered Species Act (ESA) in 1999 (76 FR 50448). A subset of the species of the family Salmonidae, including steelhead, exhibit iteroparity – the ability to spawn on two or more occasions after a period of recovery. Unfortunately, many of the factors believed to contribute to the decline of steelhead (NRC 1996; *US v. Oregon* 1997; ISRP 1999) have placed significant asymmetric limitations on the opportunity for successful iteroparity. Evidence suggests that passage at mainstem hydropower facilities exerts substantial mortality on kelts that might otherwise survive to spawn (Evans 2002). Kelt survival may also be limited by an increase in energy expenditure accompanying alteration of freshwater habitat (Love 1970). There are no known estimates of the rate of iteroparity of steelhead in the Columbia River Basin (CRB) prior to development, however recent estimates of repeat spawners in the Kalama River (an un-impounded tributary of the lower Columbia River) have been documented as 17% or greater (NMFS1996). In contrast, iteroparity for Klickitat River steelhead, located above just one mainstem Columbia River dam, was reported at 3.3% from 1979 to 1981 (Howell et al. 1984).

In general, efforts to maintain or recover declining steelhead stocks within the CRB have ranged from harvest reduction, habitat restoration, passage improvements at mainstem Columbia River hydro-power facilities, and hatchery propagation. More recently, the use of kelt reconditioning has been investigated as a means to increase total reproductive potential of steelhead populations (Hatch et al. 2010). We are unaware of any existing Master Plan documents that explicitly evaluate the incorporation of kelts as a constituent of a recovery program, thus this document has two goals:

1. Development of a "generic" Master Plan approach to serve as a CRB standard for programs wishing to incorporate kelt reconditioning in fisheries management and recovery programs.
2. Development of a preferred alternative for the use of kelts in Hood River fisheries management.

The Master Plan includes the following sections:

- Section 1: Motivation for the Incorporation of Kelts in CRB Fisheries Management Programs
- Section 2: Alternative Strategies for the Incorporation of Kelts in CRB Fisheries Management Programs.
- Section 3: Integration of kelts into Existing CRB Fisheries Management Programs.

- Section 4: Conclusions.
- Section 5: Hood River Kelt Master Plan

The first four sections of the Master Plan focus in a generic sense on the potential use of kelts in CRB fisheries management programs, while sections three and four apply these generic approaches to the Hood River.

Section 1: Motivation for the incorporation of kelts in crb fisheries management programs

This Master Plan template is intended for application towards steelhead populations that have suffered declines in abundance and are targeted for restoration. In that context, an immediate boost in freshwater productivity may be desirable as a means to offset immediate demographic perils (e.g., adult densities insufficient to ensure access to mates) and loss of genetic diversity. Preferably, kelt reconditioning programs should be accompanied by restoration or remediation targeting the root cause(s) of decline (Seamons and Quinn 2010). In evaluating restoration actions for steelhead, it should be noted that the perils faced by iteroparous species may or may not align perfectly with those faced by semelparous species, thus remediation targeting limiting factors for semelparous species may not be as effective for iteroparous species. For example, habitat restoration actions typically target spawning and juvenile rearing habitat as a means to improve egg and juvenile survival. Fewer habitat actions explicitly focus on attributes that might increase kelt survival. Similarly, fish passage projects at mainstem hydropower facilities generally focus on upstream adult passage improvements, with far less emphasis on guidance and passage for emigrating adults, although this trend appears to be changing (Wertheimer et al. 2008). Thus, it is possible that existing restoration projects may be sufficient to secure demographic stability of a targeted population without simultaneously reestablishing historic rates of kelt contribution. Whether "asymmetric" recovery, as described above, is detrimental has yet to be resolved and is directly related to uncertainty about the genetic basis of iteroparity and its value as a life-history strategy.

It remains unclear whether iteroparity is a genetic trait subject to loss within a population or Distinct Population Segment (DPS) versus a ubiquitous characteristic of the species whose expression is governed solely by environmental influence (i.e., a trait that cannot be "lost"; Seamons and Quinn 2010). Nielsen et al. (2011) analyzed molecular genetic data across various life-history types (single versus repeat-spawning groups) of steelhead inhabiting the Ninilchik River in Alaska. It was concluded that steelhead inhabiting this river formed a single panmictic spawning population, suggesting that iteroparity is a ubiquitous trait, potentially governed solely by environmental conditions that are more or less conducive to survival within and among years. This is consistent with the observation that the expression of iteroparity among interior Columbia River steelhead has persisted despite decades of impoundment-related selection pressures against this life history type. Nonetheless, we are aware of no studies that have quantitatively established the presence or absence of specific genetic factors that might contribute to iteroparity. Given the lack of

conclusive evidence either supporting or negating a genetic component for iteroparity it is reasonable to conclude that enhancing the potential for iteroparity may be a good strategy for risk-aversion within the CRB.

An abundance of literature supports the theoretical value of iteroparity as a strategy to "spread the risk" such that brood year failure is less likely. From that perspective, anthropogenic or environmental factors that limit the successful expression of iteroparity, while detrimental, could be argued to have little impact on long-term diversity. In short, while numerous published literature posit the theoretical benefits of iteroparity (Groot and Margolis 1991, Crespi and Teo 2002; Niemalä et al. 2006) and some document a realized increase in lifetime reproductive success accompanying iteroparity (Seamons and Quinn 2010); it is generally assumed that a loss or reduction in successful iteroparity simply results in a decrease in potential productivity. However, some recent evidence suggests that the loss of successful iteroparity can have profound impacts on not only productivity, but diversity as well. Seamons and Quinn (2010) illustrate both of these potential impacts. The authors studied a small population of coastal steelhead and found that on average, female repeat spawners had 1.9 times the reproductive success of females that spawned only once, while male repeat spawners produced nearly 2.7 times the number of adult offspring as males that spawned only once. Generally, these results support other empirical and theoretical observations regarding the potential benefits of iteroparity. Perhaps more importantly, the authors found that 71% of female repeat spawners typically produced the bulk of their surviving progeny in either the first season or the second spawning event while only 29% of female repeat spawners successfully reproduced during two or more spawning events. Among repeat spawning males none of the repeat spawners produced offspring during their first reproductive attempt; those that did successfully reproduce in their second attempt produced nearly three times as many adult offspring as males that spawned on their first attempt. Although this is a single study of one population of steelhead, if generally applicable the observations profoundly underscore the risks associated with a reduction in iteroparity; namely:

- Successful iteroparity can increase individual lifetime reproductive success by two to three times relative to semelparous individuals.
- A non-negligible fraction of male and female spawners fail to reproduce during their first spawning attempt, suggesting that genetic diversity could be directly reduced when conditions do not support successful iteroparity.

Aside from the documented benefits of iteroparity with regard to productivity and diversity, reestablishment or enhancement of iteroparity has a number of potential management benefits:

1. Increased iteroparity improves fishery opportunities by increasing the total number and mean size of returning adults potentially vulnerable to fisheries.

2. Kelts can be reconditioned and held or captured upon return for use as broodstock, thus decreasing the impact of broodstock collection on escapement for natural spawning.
3. Increased iteroparity may buffer against otherwise reduced escapement when ocean conditions or other factors decrease broodyear strength.
4. Iteroparity can buffer against catastrophic loss associated with rare freshwater events that may vastly reduce freshwater productivity (e.g., unusually high late spring discharge that results in scour).
5. Management actions to increase successful iteroparity may be implemented quickly and could achieve a demographic boost less controversially than artificial propagation and more quickly than habitat or passage improvement.
6. Reconditioning efforts can be focused on natural origin steelhead, thus substantially reducing possible hatchery effects.

Section 2: Alternative strategies for the incorporation of kelts in crb fisheries management programs

This section describes how kelts could be incorporated into CRB fisheries management programs and summarizes results to date from studies evaluating the efficacy of kelt collection, transport, and reconditioning.

2.1 Alternatives for Initiation of a program

A number of strategies could be employed to improve kelt survival and/or incorporate kelts into CRB fisheries management programs. The following alternatives assume that either no propagation program exists for steelhead, or will be initiated simultaneously with initiation of a kelt program:

1. No action – maintain current program with no consideration of kelt transport, reconditioning, or incorporation in production.
2. Capture and transport – capture kelts as they emigrate, and transport them below mainstem hydropower facilities for release and natural rehabilitation.
3. Capture, recondition, release - capture emigrating kelts, hold them for a short period while providing prophylactics and food, and release them to continue their emigration.
4. Capture, recondition, transport – capture emigrating kelts, hold them for a short period while providing prophylactics and food, and transport them below mainstem hydropower facilities for release.
5. Capture, recondition, hold, release for spawning - capture emigrating kelts, hold them for up to a year while providing prophylactics and food, and release them within the watershed of interest where they will presumably spawn.

6. Capture, recondition, hold for broodstock - capture emigrating kelts, hold them for up to a year while providing prophylactics and food, and incorporate them into broodstock, thus reducing or eliminating the need to collect immigrating females to support hatchery production. This alternative might also be used to provide a "backup" option if adult returns do not support broodstock collection goals. Under this scenario, reconditioned kelts could be released or terminated should broodstock goals be met through other means.
7. Capture maiden fish for broodstock, air spawn them and recondition the survivors. These survivors could be used as backup broodstock in subsequent years or released to spawn in wild. This would incorporate kelt reconditioning into a supplementation facility and reduce the "broodstock mining" impact on wild escapement.

2.2 alternatives for existing Propagation programs

Until recently much of the steelhead production in the CRB has focused on a "standard production" regime, consisting of adult broodstock collection, lethal spawning, and juvenile rearing and release. At the very least, these production approaches have neglected the potential for iteroparity and have potentially exerted selective pressure against that trait. Hatch et al. (2010) demonstrated the potential for the use of steelhead kelts as an approach to address population demographic and genetic issues of steelhead recovery in the CRB. Among other considerations, the use of kelts in production programs may serve to decrease concerns regarding artificial propagation (e.g., Waples 1999). In short, whether of hatchery or natural origin, kelts have demonstrated an ability to ascend to spawning grounds, spawn, survive a period of recovery, and return to spawning areas.
If a steelhead propagation program exists, there are a number of additional alternatives for kelt integration:

1. Employ live spawning practices (if they are not currently in place) and release broodstock for emigration as kelts with the potential to return and spawn again.
2. Transport live-spawned broodstock below emigration obstacles.
3. Recondition and release live-spawned broodstock for natural emigration.
4. Recondition, transport, and release live broodstock below emigration obstacles.
5. Recondition, hold, and release broodstock near spawning areas.
6. Recondition, hold, and re-use kelts as broodstock.
7. Recondition kelts for experimental use or utilize their offspring to satisfy research needs.

2.3 Kelt transport and reconditioning studies

The following paragraphs describe the outcome of kelt collection, transport, and reconditioning studies across the CRB with specific emphasis on unresolved questions.

2.3.1 Kelt Transportation

Radio telemetry studies indicate that mortality rates of emigrating kelts range from 20-40% at lower Columbia River dams and from 84-96% for kelts tagged at Lower Granite Dam on the Snake River and tracked to an unspecified location below Bonneville Dam (Wertheimer and Evans 2005). Mortality rates reflect the fact that kelts are often emaciated, having survived a prolonged freshwater migration (up to 1,500 kilometers), but are also a function of energetically costly migration delays, accumulated physiological stress, and delayed effects of injuries sustained during dam passage (Venditti et al. 2000; Budy et al. 2002; Schaller and Petrosky 2007). 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. Evans et al. (2008) compared adult return rates for kelts collected at Lower Granite Dam (LGD) and John Day Dam (JD) and transported below mainstem hydropower facilities to those of kelts left to navigate to the estuary unassisted. The authors found that transported kelts were 2.3 times more likely to return to LGD that fish that were unassisted. However, using a weighted mean, this benefit is reduced to 2.1, and is strongly driven by high kelt returns observed in 2002 (Hatch, D. Columbia River Inter-Tribal Fish Commission, personal communication, 11/9/2011). There was no significant difference in return rate for transported versus unassisted kelts at JD. Branstetter et al (2008) found that 46.7% of adult steelhead captured at Prosser Dam on the Yakima River and immediately transported below Bonneville Dam survived to ocean entry. This value increased to 48.68% for kelts held and fed for 3 to 12 weeks prior to transport. Hatch et al. (2009) report the survival rates of five groups of steelhead kelts:

1. the proportion of steelhead kelts in Bonneville Dam run-at-large (0.54%);
2. the return rate to Bonneville Dam of steelhead PIT tagged and released at the at Prosser Hatchery (2.96% - collected as the emigrated past Prosser Dam);
3. the return rate to Bonneville Dam of steelhead PIT tagged as the emigrated past the John Day Dam (9.76%);
4. the return rate to Bonneville Dam of steelhead PIT tagged as the emigrated past Lower Granite Dam (0.68%); and
5. the reported proportion of repeat spawners in the adult escapement at Prosser Dam (1.66% based on scale pattern analysis).

These groups form baseline against which the authors compare return rates to Bonneville Dam for kelts transported and released below Bonneville Dam from four groups:

1. John Day Dam (12.55%);
2. Prosser Dam (5.81%; short-term reconditioned kelts; kelts held and fed for between 3 and 12 weeks);
3. Prosser Dam (2.42%; unfed kelts); and

4. Lower Granite Dam (1.17%).

Based on this information, the authors calculate a survival benefit for transportation by dividing the return rates to Bonneville Dam for transport groups by rates from the control groups. Any number greater than 1 thus represents a positive benefit and any number less than 1 a negative benefit. Kelts transported from Prosser fed group showed the highest transport benefit (1.96) relative to in-river control groups followed by the Lower Granite Dam group (1.74), and the John Day group (1.29). The Prosser Unfed group had a transport benefit of 0.82 indicating that the in-river control group actually returned at a relatively higher rate.

In general, these calculations indicate that transportation can increase the successful expression of iteroparity, and that short-term reconditioning can offer an even greater survival benefit. While speculative, these observations suggest that kelts collected at Lower Granite Dam are likely emaciated and are generally unlikely to survive emigration, either when assisted (i.e., transported) or left to emigrate unassisted. Alternatively, stress accumulating from capture and handling may offset much of the benefit that might otherwise accrue from transportation. In either case, it is advisable to consider either prophylactic treatment and/or introduction of food and/or short-term reconditioning for collected kelts prior to transportation.

2.3.2 Kelt Reconditioning

As described in Branstetter et al. (2008), kelt reconditioning starts with the introduction of feed to enable short-term survival, restore proteins and lipids depleted by migration and spawning, promote recovery from injuries, and initiate gonadal redevelopment. Researchers have observed that kelts readily initiate feeding on krill, although other food sources are sometimes used, including cod liver oil, anchovies, amphipods, and fish (Branstetter et al. 2008). Generally, kelts are transitioned to pelletized feed following initiation of feeding. Reconditioning can be further partitioned into long versus short-term treatments:

- Short-term reconditioning programs hold kelts for between 3 and 12 weeks during which efforts are made to reinitiate feeding prior to release or transport around hydropower facilities.
- Long-term reconditioning increases the period of holding targeting a release timing that coincides with natural migration to spawning areas.

In addition to the introduction of feed, formalin may be administered to control fungus, ivermectin and more recently ememectin may be used to control Salmincola (a parasitic copepod; Johnson and Heindel 2000, Evans and Beaty 2000), and oxtetracycline may be administered to control bacteria. Reconditioning trials in the CRB support significantly higher kelt survival and re-maturation rates in reconditioned fish relative to in-river controls, with kelt-to-repeat spawner success rates observed in excess of 25% (Hatch et al. 2004).

Hatch et al. (2009) reports a mean survival for kelts subject to long-term reconditioning of 37.9%. Given this survival rate, kelts subject to long-term reconditioning at the Prosser Hatchery had a 10.04 fold increase in survival relative to fish left untreated in the river. 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. These data suggest that long-term reconditioning shows great promise as a tool for restoration.

Gamete and Progeny Viability

Branstetter et al. (2010) report findings from a repeated measures design that compared fecundity, fertilization rate, fry weight, and fry length for eight maiden and kelt steelhead. The authors found no significant differences between maiden and reconditioned kelt steelhead for any of the four metrics, although fry length was marginally greater for progeny of reconditioned kelts ($p = 0.50$). Although sample sizes were low, these results suggest that reconditioned kelts perform as well as maiden broodstock. Additionally, the authors noted a consistent increase in clutch size for reconditioned kelts, including two individuals who were spawned for a third time after successful serial reconditioning.

Relative Reproductive Success

Hatch et al. (2009) used genetic pedigree analysis to document successful juvenile production attributable to one male and two female kelts released in Omak Creek, Washington following long-term reconditioning. Given challenges associated with adult and juvenile sampling, the authors were unable to compare reproductive success for kelts versus maiden spawners.

Research Needs

A number of uncertainties and associated research needs were identified during compilation of this document; including:

- All of the studies summarized in this document utilize available hatchery/holding facilities, it is unclear whether research has specifically addressed the issue of identifying optimal holding and reconditioning facilities.
- The bulk of artificial production programs focus on short-term adult holding and juvenile rearing, far less emphasis has been placed on optimizing factors such as flow rates, water temperature, feed (particularly pelletized feed), and prophylactics for the purpose of long-term adult holding that accompanies kelt reconditioning.
- We found few studies that test the relative reproductive success of kelts in natural and hatchery environments relative to maiden spawners. As such, the potential for programs such as kelt reconditioning and release and/or supplementation of broodstock collection with reconditioned kelts is difficult to gauge.

- Further studies are required to address the potential benefits of short-term prophylactic treatment and reconditioning at Lower Granite Dam prior to kelt transportation.
- We found no projects or literature dedicated to the identification of habitat restoration actions that might directly improve kelt survival.
- Finally, a number of researchers observed non-sequential spawning ("skip spawning") among repeat spawners. For example, skip spawners compose up to 50% of repeat spawners in the Snake River and British Columbia (Keefer et al. 2008). For programs relying on reconditioned kelts for broodstock, or for programs targeting a specified number of reconditioned spawners, it may be necessary to develop methods to identify what fraction of kelts are skip spawners. Branstetter et al. (2010) report on the development of methods utilizing plasma vitellogenin assays to assess maturation status and likelihood of sequential spawning among reconditioned adults. Further development of these methods is a critical research need for effective implementation of many management alternatives that rely on reconditioned kelts.

Section 3: Integration of kelts in crb fisheries management programs

To this point, the Master Plan assumes that increasing the expression of iteroparity is beneficial, practical, and generally without risk. This section examines this assumption in greater detail, describing "natural" kelt abundance and potential risks associated with increasing kelt abundance.

3.1 What is an appropriate Repeat Spawner contribution?

Iteroparity, the ability to spawn on more than one occasion, among CRB steelhead has been documented as far back as 1895 (Evermann 1895), with early accounts of repeat spawners entering natal tributaries (Long and Griffin 1937, Whitt 1954). These accounts indicate that iteroparity extended as far inland as the Snake River Basin (Evermann 1895). Thus, repeat spawners appear to have been a component of presumably all steelhead stocks inhabiting the CRB to some degree. Unfortunately, the fraction of steelhead spawners exhibiting iteroparity is not well documented prior to anthropogenic impacts that might be expected to reduce post-spawn survival and return rates (e.g., harvest, habitat degradation, and dam construction). Whitt (1954) estimated that approximately 2% of adult Clearwater River steelhead were repeat spawners. Unfortunately, Whitt's estimates were conducted after the construction of two mainstem hydropower facilities, and relied on scale analysis, which may have resulted in an underestimate of repeat spawner abundance due to scale reabsorption (Seamons et al. 2009). Emigrating steelhead kelts averaged 58% of annual upstream runs in the Clackamas River from 1956 to 1964 (Gunsolus and Eicher 1970). From 1979 to 1981 3.3% of steelhead escapement in the Klickitat River was composed of repeat spawners (Howell et al. 1984). Repeat spawners composed 1.6% of the Yakima River wild run (from data in Hockersmith et al. 1995). Adult sampling at mainstem

hydropower dams suggests that between 0.5-1.2% and 2.9-9.0% of adult steelhead from the Snake River and tributaries to the Lower Columbia successfully return as repeat spawners (Wertheimer et al. 2008). This is in contrast to the Kalama River, an un-impounded tributary of the lower CRB, where repeat spawners compose in excess of 17% of the escapement (NMFS 1996). Iteroparity estimates for aggregate Columbia River samples (5-6%, across years; Wertheimer et al. 2008) were comparable to rates for British Columbia steelhead (Withler 1966), but were generally lower than those reported across a variety of life history types in Washington (7-11%), Oregon (11- 21%), California (17-23%), and Alaska (21-51%) (Shapovalov and Taft 1954; Busby et al. 1996; Lohr and Bryant 1999). Repeat spawning for Alaska steelhead has reportedly ranged from 11%-38% with an average between 25%-33% (Harding and Brookover 2003). However, on the Situk River in 1994, of 907 steelhead captured and sampled for age and length, 51% were found to be repeat spawners (Johnson 1996).Steelhead iteroparity rates as high as 79% have been documented in in the Utkholok River of Kamchatka, a relatively pristine river system located on Kamchatka peninsula (MSU undated; M. Powell UI and R. Williams, ISRP personal communication; as referenced in Hatch et al. 2010). A more complete table of repeat spawner composition is available in Appendix I.

In general, it is reasonable to assume that current rates of iteroparity in the CRB are likely to have been reduced by mortality of downstream migrating kelts at hydropower dams, which may exert up to 96% mortality on downstream migrants (Evans and Beaty 2001, Wertheimer and Evans 2005). Survival of downstream migrants may also be reduced by tributary, mainstem, and estuary habitat degradation as well as changes in prevailing ocean conditions. Nonetheless, it is unlikely that iteroparity rates in the CRB were historically as high as those documented in the Utkholok River given the trend towards decreasing rates of iteroparity with reduced latitude and increased distance of spawning migration (Withler 1966; Bell 1980; Fleming 1998).

The following sub-sections summarize information that might be useful for generating appropriate iteroparity targets for Snake River and lower Columbia River steelhead populations.

3.1.1 Snake River Kelt Composition

As described above, little direct information is available by which we can identify an "historically appropriate" rate of kelt contribution for CRB steelhead populations. However, for the Snake River, current rates of post-spawn survival and emigration place a boundary on "potential" rates of iteroparity. Between 17-25% of the steelhead run that passes Lower Granite Dam, returns downstream as kelts (Boggs and Peery 2004; Wertheimer and Evans 2005). However, a majority of the upstream adult steelhead migration past Lower Granite Dam is composed of hatchery origin adults (up to 76%: Stouder, Bisson, and Naiman 1997), which are less likely to exhibit iteroparity due to selective harvest and broodstock collection (Wertheimer et al. 2008). Given declines in freshwater habitat quality, which presumably decrease post-spawn survival, mortality associated with passage, and

hatchery-origin escapement, minimum kelt emigration might be expected to have historically been much higher than currently observed values.

3.1.2 Lower Columbia River Kelt Composition

Unlike the Snake River, probable rates of kelt contribution can be more directly inferred from observed kelt contribution in extant lower CRB populations. In the Kalama River, an un-impounded tributary of the lower CRB, kelts compose in excess of 17% of adult escapement (NMFS 1996). Given anthropogenic degradation of freshwater habitat in the Kalama River, coupled with degradation of the Columbia River estuary, this might be reasonably viewed as a lower bound for historic rates of kelt composition in spawning populations for tributaries of the lower Columbia River.

3.1.3 Upper Columbia River Kelt Composition

In the Yakama River, up to 36.9% of the adult steelhead passed above Prosser Dam were intercepted as they attempted to transit the facility during emigration. This is a minimum estimate of the population attempting to emigrate given that kelts can also pass over the spillway. In 2007, 16%, 26%, and 39% of adult steelhead passed above weirs in Shitike Creek, Bonaparte Creek, and Omak Creek were intercepted as emigrating kelts (Branstetter et al. 2008).

3.1.4 Kelt Composition in High Seas Fishery Sampling

Burgner et al. (1992) performed a comprehensive meta-analysis of high seas steelhead interception. Of interest, they found that kelts composed 7.2% of the high seas catch-at-large, finding that an average of 71% of intercepted kelts had spawned once, 21% twice, 8% three times, and one fish four times. Between 1.5 and 1.6 million North American steelhead were estimated to be present in the high seas (Sheppard 1972 and Light 1987). Of these 28%, or roughly 420,000 – 480,000, were estimated to be of CRB origin, and 50% of those were estimated to be of hatchery origin.

3.1.5 Conclusions

As described above, estimating a “historic” rate of iteroparity for CRB steelhead is problematic for the following reasons:

1. Estimates of kelt composition prior to significant anthropogenic alterations within the CRB are uncommon and may be unreliable.
2. Contemporary estimates of iteroparous contribution in the CRB are confounded by anthropogenic influences such as:
    a. hatchery contribution
    b. harvest
    c. hydropower development
    d. habitat degradation

Given these constraints, identifying an appropriate kelt contribution for contemporary steelhead populations is problematic. One could argue that the upper limit for kelt contribution might be provided by natural and largely undisturbed steelhead populations, such as the Utkholok River; suggesting that kelt contribution as high as 79% is acceptable. For lower CRB populations, minimum kelt contribution might be described by populations such as the Kalama River that exits below mainstem hydropower facilities; suggesting that kelt contribution should be no lower than 17%. For upper CRB populations, it may be completely unnecessary to identify lower and upper bounds on iteroparity rates, as they will likely be informed primarily by logistical limitations. Between 17% and 25% of steelhead ascending past Lower Granite Dam are estimated to attempt emigration (Wertheimer et al. 2008). Estimates of annual returns of repeat steelhead spawners vary from 2.9 – 9.0% for kelts tagged at lower Columbia River dams and from 0.5 – 1.2% for kelts for Snake River (Keefer et al. 2008). Thus, even aggressive actions aimed at improving kelt survival and return are unlikely to stimulate iteroparity rates that might be considered "too high."

3.2 potential risks accompanying increased iteroparity

It is unclear whether there are direct "risks" associated with attempts to increase the expression of iteroparity. Clearly, if reconditioned kelts are utilized for broodstock, all of the potential benefits and risks accompanying artificial propagation are equally applicable. Benefits and potential risks accompanying artificial propagation are detailed in numerous documents (e.g., McClure et al. 2008, and references therein), and are not further described here. Two risks that potentially accompany increased iteroparity are examined in detail in this section:

- potential increase in inbreeding
- potential reduction in successful iteroparity should transport or reconditioning efforts fail

3.2.1 Inbreeding

A fundamental assumption underlying conservation genetics theory is that genetic diversity forms the raw material that fuels adaptation necessary to respond to changing environmental stressors (Frankham 1995). The maintenance of genetic diversity is affected by patterns of breeding and survival (Wang, Hard, and Utter 2002). Pacific salmonids in general and CRB steelhead in particular, exhibit a complex life history marked by a prolonged and diverse juvenile freshwater residency, extensive marine rearing, and potentially extended residence in freshwater prior to spawning. The complex life history of steelhead makes them particularly vulnerable to anthropogenic impacts (Allendorf and Waples 1996) given that these impacts can compound across all life history stages, ranging from freshwater habitat loss (Nehlsen et al. 1991), harvest on returning adults (Nehlsen et al. 1991), and hydropower related mortality (Wertheimer et al. 2008).
Given the challenging environment is which steelhead reside, iteroparity is a mechanism that could serve to reduce the loss of genetic diversity. However, it could be argued that an excessive increase in iteroparity might be accompanied by an increase in the risk of

inbreeding, by increasing the variance in reproductive success of individuals. This risk must be placed in the context of CRB steelhead population status. In general, inbreeding arises through two mechanisms: 1) loss of genetic diversity through drift or 2) an increase in the probability of an individual sharing parental genes (i.e., the product of sibling mating). For CRB steelhead populations, the likelihood that these mechanisms are realized is magnified by pervasive declines in abundance. In short, as a population becomes smaller, there is an increased likelihood that offspring from individual pairings will fail to return individuals to the next generation, alternative genetic material will inevitably be lost as the rate of reproductive failure increases, or if population size remains small and reproductive failure among family groups continues (mechanism one, genetic drift: Crow and Kimura 1970). This will tend to increase the mean relatedness of spawning individuals, giving rise to a higher probability that alleles within an individual will be "identical by descent" (IBD, mechanism two: Hard and Hershberger 1995). Thus iteroparity represents a balance; on the one hand iteroparity increases the probability that an individual will pass genetic information to the next generation (a net reduction in the potential for genetic drift), on the other hand the fraction of the population successfully exhibiting iteroparity will (all things being equal) give rise to a greater number of offspring in subsequent generations, increasing the relatedness of individuals in turn increasing the likelihood that alleles at large will be IBD.

As described above, and demonstrated mathematically by Crow and Kimura (1970) genetic drift and relatedness are inextricably linked in a declining population. For steelhead, iteroparity represents a partial solution to genetic drift by increasing the likelihood that an individual will successfully contribute offspring to subsequent generations, but that success is accompanied by the risk of increased relatedness of individuals in subsequent generations. Clearly, successful long-term maintenance of genetic diversity requires a reduction in mortality, which is a major contributor to variance in reproductive success. Long-term maintenance of genetic diversity also requires the reestablishment of populations large enough to reduce the probability of genetic drift. Indeed, this is the goal of current recovery efforts in the CRB. In the interim, managers are faced by a difficult quandary – namely the choice of increasing the rate of successful iteroparity, which may reduce drift but will likely increase relatedness, or waiting until other recovery actions are successful.

Illustrating how declining and/or small population size mechanistically relates to the maintenance/loss of genetic diversity may be useful. Consider the following two alternatives for the same population of interest:

1. as a population declines, variance in reproductive success remains equal, but mean reproductive success declines below 1.0.
2. as a population declines, mean reproductive success declines below 1.0, and variance in reproductive success increases, with certain individuals enjoying much higher or suffering much lower than average reproductive success

In both cases genetic diversity will inevitably be lost from the population, unless factors contributing to mortality are addressed. Very simply, at least half of the individuals fail to contribute genetic material to subsequent generations. However, relatedness increases much more quickly under alternative two, given that even fewer individuals actively contribute genetic material to subsequent generations, and the remaining diversity is contributed by a few highly successful individuals. Unfortunately few studies have been equal to the task of evaluating whether variance in reproductive success increases as population size decreases. However, some recent studies have documented that variance in reproductive success with salmonid populations can be very high (Seamons, Bentzen, and Quinn, 2004, Palstra, O'Connell, and Ruzzante, 2009). As interesting are observations that salmonids exhibit high rates of maternal and paternal polyandry (Garant, Dodson, and Bernatchez, 2001), suggesting that polyandry may be a mechanism to increase genetic diversity of offspring, potentially providing resiliency to fluctuating environmental conditions. Similarly, given the fact that surviving kelts are overwhelmingly female (Wertheimer and Evans 2005) the risk of sibling matings among repeat spawners is dramatically reduced.

In any case, all things being equal, iteroparity might be expected to increase variance in reproductive success, potentially accelerating the loss of genetic diversity. For example, Seamons and Quinn (2010) found that female repeat spawners had 1.9 times the reproductive success of females that spawned only once, while male repeat spawners produced nearly 2.7 times the number of adult offspring as males that spawned only once. More importantly, the authors found that 71% of female repeat spawners produced the bulk of their surviving progeny in either the first or the second spawning event while only 29% of female repeat spawners successfully reproduced during two or more spawning events. Among repeat spawning males none of the repeat spawners produced offspring during their first reproductive attempt. Thus, while iteroparity did result in an increased variance in reproductive success, it also enabled contribution of offspring to subsequent generations for a far greater number of individuals than would have contributed in its absence.

In general, we conclude that genetic drift is an unavoidable consequence accompanying a decline in population size, particularly if that decline is prolonged and/or if the population remains small over a protracted period. Somewhat surprisingly, we found that iteroparity, while it may increase variance in reproductive success, can also dramatically increase the number of individuals and family groups that successfully contribute genetic material to subsequent generations. The high rates of maternal and fraternal polyandry observed for some salmonids, if present in steelhead, may partially mitigate increased variance in reproductive success accompanying successful iteroparity. In practice, this suggests that increased iteroparity would only be detrimental if it accompanies a direct reduction in the productivity of individuals exhibiting a semelparous strategy. Interestingly, given the propensity for repeat spawners to exhibit an earlier spawn-timing (Wertheimer et al. 2008), impacts associated with exceeding carrying capacity (e.g., redd superimposition) are more likely to directly reduce survival of eggs spawned by iteroparous individuals. From

this perspective, the success of iteroparous individuals might be self-governing, decreasing as the contribution of first-time or semelparous spawners increases.

Given our findings, we conclude that there is very limited risk that increasing the successful expression of iteroparity would have a negative impact on genetic diversity. To the contrary, virtually all of the evidence suggests that increasing iteroparity will directly reduce the loss of genetic diversity for imperiled populations that are either declining in abundance and/or exist at a fraction of historical abundance.

3.2.2 Potential Impacts of Failed Transportation and/or Reconditioning

To this point we have assumed that kelt collection and transportation and/or reconditioning successfully improves survival and the potential for expression of iteroparity. As described in Section 2, research to date suggests that kelt collection and transport and/or reconditioning has the potential to dramatically improve the survival of kelts, in turn providing the potential for increased expression of iteroparity. However, failures of these approaches have also been documented. Clearly, a large-scale kelt collection project accompanied by significant mortality could be detrimental. To date, few losses have been reported for kelt capture, transport, and reconditioning projects. Branstetter et al. (2008) reported the loss of 6 males and 50 females resulting from equipment failure. Given the thousands of kelts that have been collected and reconditioned across CRB projects, this rate of loss suggests that while kelt collection, transport, and reconditioning is accompanied by the potential for catastrophic loss, this outcome is largely unrealized. Alternatively, given the documented improvement in survival accompanying these initiatives, a beneficial outcome is far more likely.

Section 4: Conclusions

Existing research on kelt transportation and short and long term reconditioning suggest that these practices have the potential to improve kelt survival and increase the abundance of repeat spawners. Available data suggest that virtually all steelhead populations above mainstem dams in the Columbia River Basin have suffered a reduced contribution of repeat spawners. Given uncertainties about the underlying genetic basis of iteroparity, and the evidence for substantial anthropogenic reduction in realized iteroparity, efforts to improve the survival of kelts may be advisable. In general, we found substantial evidence to support the hypothesis that improving the survival of kelts could be a mechanism to maintain diversity and reduce fluctuations in year-class strength. On the contrary, we found little evidence to support the competing hypothesis that increased iteroparity is likely to reduce genetic diversity as a result of inbreeding.

A number of research needs were identified to further our understanding of kelt life history and improve methods for kelt reconditioning. Many of these research needs are currently being addressed by ongoing studies. However, the next step in the evolution of kelt integration in Columbia River Basin programs requires the judicious incorporation of kelts into existing or newly initiated propagation programs. This effort should be initiated within an experimental framework and accompanied by appropriate research and monitoring to enable quantitative evaluations of the benefits and risks posed by kelt integration.

Section 5: Hood River kelt master plan

5.1 Introduction

The prior sections described why and how kelts might be incorporated into existing or proposed steelhead management programs. This section applies those principles to the Hood River winter and summer steelhead management programs. Context is provided by a summary of existing Master Plans specific to Hood River winter and summer steelhead. An alternative for the incorporation of kelts is then developed for each program, followed by a discussion of the logistics of kelt incorporation. Finally, recommendations for monitoring and research to evaluate the efficacy of the program are developed.

5.2 1991 and 2008 Hood River Production Program Master Plans

The Hood River Production Program (HRPP) is funded by the Bonneville Power Administration (BPA) and has included production programs for winter and summer steelhead and spring Chinook salmon as mitigation for the reduction in abundance of these species accompanying construction of the Columbia River hydropower system (FishPro 2008). The Confederated Tribes of the Warm Springs Reservation (CTWSR) and Oregon Department of Fish and Wildlife (ODFW) completed a Master Plan to guide efforts in 1991 (O'Toole and ODFW 1991a and O'Toole and ODFW 1991b). Following these efforts, Hood River hatchery programs began in 1991 for winter steelhead, 1992 for spring Chinook salmon, and 1997 for summer steelhead. At inception, the spring Chinook salmon program utilized Deschutes River spring Chinook salmon juveniles reared at the Round Butte Hatchery, owing to the fact that the Hood River stock was believed to be largely extirpated by the late 1960's (CTWSR and ODFW 1991). The HRPP summer steelhead program began with adult collections in 1997. The goal of the program was the collection of 160 adults to support production of 150,000 smolts, however the program never progressed beyond an interim strategy that included the collection of only 40 adults supporting the production of 40,000 juveniles. Beginning in 1988, prior to the initiation of the HRPP, up to 75,000 Skamania stock summer steelhead smolts were released into the mainstem and West Fork Hood River; these releases continued through 2009. Conversely, the HRPP winter steelhead program, initiated in 1993, utilized natural origin adults since inception. Up to 64 adults have been collected annually for broodstock, composing no more than 25% of the total natural origin adult escapement. Over the course of the program broodstock composition has ranged from 51% -100% natural origin adults. Juveniles are released in the Middle and East Fork Hood River.

Current production objectives, including the potential for integration of kelts, must be placed in the context of historical production objectives (Table 1). A subsequent Master Agreement (ODFW and CTWSR 1993) reduced the targeted spring Chinook salmon production from 250,000 smolts to 125,000.

Table 1. Hood River production objectives identified in the 1991 HRPP.

Objective	Spring Chinook Salmon		Winter Steehead		Summer Steelhead	
	Natural	Hatchery	Natural	Hatchery	Natural	Hatchery
Adult Escapament To Mouth of Hood River	1,700		5,000		8,000	
Adult Escapement to Natural Production	400		2,400		2,400	
Broodstock Collection	220		90		160	
Harvest (Tribal and Recreational)	1,080		2,510		5,440	
Smolt Production	24,000	250,000		85,000		40,000
Egg to Smolt Survival	NI	NI	NI	NI	NI	NI
Smolt to Adult Return (SAR)	0.68%	0.68%	NI	NI	NI	NI
Pre-Spawn Mortality	10%	10%	NI	NI	NI	NI
Incidental Harvest	63%	63%	NI	NI	NI	NI
Hatchery Origin Escapement to Natural Production	N/A	<100%	NI	25%	NI	50%
NI = Not identified.						

The Hood River Subbasin Plan (Coccoli 2004) revised the original HRPP and Master Agreement goals as follows:

- Spring Chinook Salmon
  - Achieve a mean spawning escapement of 125 natural origin adults annually by 2014, and a mean of 200 adults by 2019.
  - Increase natural smolt production from 15,700 to 20,000 by 2019.
  - Achieve and maintain a naturally spawning stock of spring Chinook salmon adapted to the Hood River.
  - Increase the SAR of hatchery reared smolts.
  - Provide a mean annual tribal and recreational harvest of 2,000 adults by 2019.
- Summer Steelhead
  - Increase SAR to 3% to ensure an annual mean spawning escapement of 600 natural origin adults by 2019.
  - Increase estimated carrying capacity from 13,680 to 20,000 smolts by 2019.
  - Maintain the genetic integrity of natural origin summer steelhead in the Hood River.
- Winter Steelhead
  - Ensure a mean annual escapement of 1,100 natural origin adults by 2019.
  - Maintain the genetic integrity of winter steelhead.

In 2008 a revised HRPP was developed, incorporating the prior HRPP and programmatic review (Underwood et al. 2003). The revised HRPP was developed for the following reasons:

1. The existing HRPP had been active for almost 18 years, suggesting that data were sufficient to validate or modify the original goals based on empirical observations.

2. The goals identified in the Hood River Subbasin Plan in 2004 (Coccoli 2004) necessitated a reevaluation of the HRPP.
3. The eminent removal of the Powerdale Dam, which served both research and management goals, necessitated the identification of alternative strategies to support research needs, adult collection, and removal of hatchery origin adults to maintain project objectives.

The revised HRPP (FishPro 2008) reached a number of conclusions that provide context for deliberations regarding the potential role of kelts in the HRPP:

- Spring Chinook Salmon
    - Production goals identified in the original HRPP were not being achieved, largely because smolt to adult return rates (SAR) assumed in the original document were not being realized. The revised HRPP indicates that failure to achieve the assumed SAR resulted from precocity, straying, and Bacterial Kidney Disease (BKD).
    - The revised HRPP supported comanager recommendations to complete a three year study comparing size at release, precocial maturation, straying, disease prevalence, and SARs for spring Chinook salmon reared at the Carson National Fish Hatchery (Wind River, WA), Round Butte Hatchery/Pelton Ladder (Deschutes River, OR), and the Parkdale Hatchery (Hood River, OR) and subsequently released in the Hood River. Results of this evaluation will identify the most successful rearing option for Hood River spring Chinook salmon production.
    - Within the Hood River, the revised HRPP proposed a new facility at Moving Falls to serve as a broodstock collection facility, spring Chinook salmon acclimation facility, and to enable greater in-basin rearing for spring Chinook salmon pending the outcome of the hatchery trials.
    - The revised HRPP identified the potential for juvenile hatchery origin spring Chinook salmon releases in the Middle Fork Hood River.
    - The revised HRPP recommended increasing spring Chinook salmon hatchery origin juvenile release goals from 125,000 to 150,000 annually beginning in 2010.
    - Finally, the revised HRPP recommended upgrades to the Parkdale Hatchery to support production of 30,000 full-term spring Chinook salmon smolts beginning in 2010.
- Summer Steelhead
    - The revised HRPP recommended cessation of summer steelhead hatchery production beginning in 2008 (final broodstock collection in 2007) for a period of two generations, for the following reasons:
        - Recent relative reproductive success studies (Araki et al. 2007) suggested that hatchery origin steelhead reduce the reproductive success of the population.

        - Escapement of hatchery origin steelhead to natural spawning areas exceeded the 5% recommendation of the Hatchery Science Review Group.
        - Escapement of natural origin summer steelhead was insufficient to support a hatchery program without broodstock collection in excess of 25% of the total escapement.
        - High turbidity during the period of return for summer steelhead limited angler success, reducing the value of that run-type for recreational fisheries. Notably, that assertion is not well supported by harvest records (Table 2).
    - The revised HRPP suggested reevaluating the need for a summer steelhead production program based on the status of the natural summer steelhead population following a two generation period with no hatchery production.
- Winter Steelhead
    - The Revised HRPP recommended continuation of the winter steelhead production program under two conditions: 1) that the program continue to use only natural origin adults for broodstock and 2) that broodstock collection not exceed 25% of the total escapement.
    - The revised HRPP recommended maintaining the current goal of releasing 50,000 winter steelhead smolts, but recommended a program evaluation in 2010.
- General Recommendations:
    - The revised HRPP recommended the construction of two floating weirs, one at Moving Falls on the West Fork Hood River, the other on the lower East Fork Hood River below the confluence with the Middle Fork Hood River, in order to replace trapping opportunities lost due to the removal of the Powerdale Dam.
    - The revised HRPP recommended providing flood protection for the Parkdale Hatchery.

Table 2. Total Hood River harvest from 1996-2006 for spring Chinook salmon and summer and winter steelhead.

1996-2006	Spring Chinook Salmon		Summer Steelhead			Winter Steehead	
	Natural	Hatchery	Natural	Skamania	Local	Natural	Hatchery
Harvest	12	41	1	466	74	2	360
Catch/Release	9	23	353	253		490	143

The 2008 HRPP production and escapement goals (Table 3) are aligned with guidelines established by the Hatchery Science Review Group (HSRG), which specify:

- Hatchery origin adults contribute 5% or less of escapement to spawning areas.
- At a minimum, broodstock must be composed of 10% natural origin adults.
- Effective population size of the combined population must be 500 or greater.

- Proportionate Natural Influence (PNI) must be 0.70 or greater, defined as the percent of natural origin spawners divided by the percentage of hatchery of hatchery origin escapement to spawning areas plus the percentage of broodstock composed of natural origin adults.

The winter steelhead program under the current HRPP is operated within the following regulations:

- Broodstock must be composed of 100% natural origin adults.
- Broodstock collection cannot exceed 25% of the total natural origin escapement.
- Hatchery origin escapement to spawning areas cannot exceed 10% of total escapement.

Table 3. Current production and escapement goals for the HRPP.

Objective	Spring Chinook Salmon		Winter Steehead		Summer Steelhead	
	Natural	Hatchery	Natural	Hatchery	Natural	Hatchery
Adult Escapament To Mouth of Hood River	300	600	656	1,000	510	0
Adult Escapement to Natural Production	205	8	465	24	408	0
Broodstock Collection	20	180	64	0	0	0
Harvest (Tribal and Recreational)	30	318	66	876	51	0
Pre-Spawn Mortality	45	90	66	100	51	0
Smolt Production	15,000	150,000	9,370	50,000	7,500	0
Egg to Smolt Survival	4%	78%	1%	75%	1%	0%
Smolt to Adult Return (SAR)	2.0%	0.4%	7.0%	2.0%	5.0%	0.0%
Pre-Spawn Mortality	15%	15%	10%	10%	10%	0%
Tribal/Recreational/Incidental Harvest	10%	53%	10%	88%	10%	0%

5.2.1 Hood River Production Facilities

Currently, a number of modifications and new construction initiatives are underway to support the production goals identified in the 2008 HRPP. This section summarizes the facilities proposed in the 2008 HRPP, identifies where those plans have been modified, and identifies the final suite of facilities available for production in the Hood River.
The 2008 HRPP includes upgrades to the Parkdale Hatchery consisting of:

1. Adding a pump and plumbing the existing well to the facility.
2. Increasing flow to the incubation building and replacing copper pipe with an inert pipe.
3. Developing new wells and piping to the hatchery building and early rearing troughs.
4. Plumbing Canadian troughs to allow early rearing.
5. Construction of a wastewater treatment system.
6. Installation of a water heater to increase temperature of well water to a more conducive range for incubation.

To date, upgrades 1, 3, 4, and 6 have been completed, upgrades 2 and 5 will not be completed.

In addition, the 2008 HRPP included plans for the construction of a collection/exclusion facility at Moving Falls, which could also serve as a rearing and/or acclimation facility. The Moving Falls facility was to be completed in two stages (intake construction in 2009, subsequent activities in 2014):

- Construct an intake structure with a 5 cfs water right.
- Construct filtration system to limit pathogen (e.g., BKD) introduction.
- Construct six raceways capable of rearing up to 150,000 Chinook salmon smolts at 15 fpp with a maximum density index of 0.16 lbs/ft^3/in.

As built, the Moving Falls facility will include four, rather than six raceways, resulting in a final rearing density of up to 0.2 lbs/ft^3/in.

5.2.1.1 Spring Chinook Salmon Production Program

Adult spring Chinook salmon will be trapped at the Moving Falls fish facility and at the Parkdale Hatchery for broodstock and to meet exclusion criteria. Broodstock captured at Moving Falls will be transported to the Parkdale Hatchery for spawning, where they will be held in two 8'x40'x4' raceways (Table 4). Incubation will take place at the Parkdale Hatchery (Table 5) using pathogen free well water between 44 and 48 degrees Fahrenheit, with temperature control via the proposed heating system.

Table 4. Existing and proposed facilities for the 2008 HRPP spring Chinook salmon production program.

Specification and Facility		Current Condition	Preferred Alternative	
		Parkdale	Parkdale	Moving Falls
Smolt Release	Spring Chinook Salmon	125,000	150,000	150,000
Release Size (fpp)	Spring Chinook Salmon	12-15	15-18	15-18
Water Supply (gpm)	Middle Fork	2,509 (Combined)	0	0
	Rogers Creek		2,509	0
	West Fork	0	0	1,795
	Well	0	275	0
Water Temperature	Eggs	42-52°F	44-48°F	na
	Fry	No	44-48°F	na
	Rearing	No	42°F	36-51°F
Rearing Space	Eggs	8 stacks (64 trays)	10 stacks (70 trays)	0
	Fry	2 (3'x21'x3') Canadian Troughs	8 (3'x21'x3') Canadian Troughs	0
	Offsite Rearing	Round Butte Hatchery	Moving Falls	na
	Number Offsite	125,000	up to 150,000	up to 150,001
	Number Onsite	0	up to 37,000	na
	Rearing	na	1 raceway (8'x40'x4')	5 raceways (15'x50'x4')
	Smolt Acclimation	2 raceways (8'x40'x4')	1 raceway (8'x40'x4')	1 raceways (15'x50'x4')
	Adult Holding	2 raceways (8'x40'x4')	2 raceways (8'x40'x4')	na
Water (gpm)	Eggs	35	45	na
	Fry (300-200 fpp)	Round Butte Hatchery	80	na
	Rearing (April 15)	0	280	1,296
	Offsite Rearing	Round Butte Hatchery	0	0
	Smolt Acclimation	1,500	1,500	0
	Adult Holding	800	800	0
	Max Water	2,335	2,660	1,080
	% of Available Used	93%	96%	83%

Table 5. Incubation facilities proposed for Parkdale Hatchery.

Egg and Incubation	Number
Green Eggs	199,011
Eyed Eggs	169,159
Fry	165,776
Broodtock Collected	200
Females Spawned	60
Males Spawned	60
Fecundity	3,320
Trays	60
Stcaks	9
Flow (gpm)	50

Fish will remain on heated well water until reaching 300 fpp in late March. At that time fish will be moved to eight Canadian troughs for early rearing. In mid-March to early April of the following year, up to 150,000 fish would be transported to the Moving Falls facility and volitionally released at 15-18 fpp. Up to 37,500 of the 150,000 smolts could be held at the Parkdale Hatchery until reaching the target release size (15-18 fpp) and either released from the hatchery, at Moving Falls, or in the Middle Fork Hood River to take advantage of a recently completed project enabling adult passage past a previously impassable falls.

5.2.1.2 Winter Steelhead Production Program

Adult winter steelhead are captured at a resistance board weir located on the East Fork Hood River, which was designed to capture 50% of the upstream migrants. A total of 64 natural origin adults are retained for broodstock and transported to Parkdale Hatchery where they are held from January through June until spawning. Eggs are transported to the Oak Springs Hatchery located on the Deschutes River where they are reared until April of the following year. In April, steelhead smolts are transported from Oak Springs to Parkdale Hatchery and/or an acclimation site on the upper East Fork Hood River where they are released in May.

5.2.1.3 Summer Steelhead Production Program

The HRPP summer steelhead production program was suspended in 2008 primarily owing to chronically low escapement of natural origin adults, which made it difficult to collect sufficient broodstock without exceeding the criterion specifying 25% maximum retention of natural adults for broodstock (Reagan 2010). Additionally, there were concerns regarding the potentially negative influence of artificial propagation on relative

reproductive success and greater than 5% escapement of hatchery origin adults into natural spawning areas (Underwood et al. 2008).

5.3 stock status and limiting factors

This section summarizes the status of cutthroat trout (*Oncorhynchus clarkii*), bull trout (*Salvelinus confluentus*), coho salmon (*O. kisutch*), fall Chinook salmon (*O. tshawytscha*), spring Chinook salmon (*O. tshawytscha*), winter steelhead (*O. mykiss*), and summer steelhead (*O. mykiss*) in the Hood River with greater emphasis on the latter two stocks.

5.3.1 Cutthroat Trout

Based on juvenile capture at Hood River migrant traps, cutthroat trout appear to reside primarily in the East Fork and Middle Fork Hood River. Reagan (2010) reports adult escapement past Powerdale Dam and juvenile cutthroat trout captures in migrant traps in the Hood River Basin (Table 6). The paucity of adults enumerated at Powerdale Dam and generally low encounter rate for juveniles at Hood River migrant traps, suggest that cutthroat trout exist at relatively low abundance in the Hood River. However, given that Hood River cutthroat trout are primarily resident (R. Gerstenberger, CTWSR, personal communication) it is unclear whether the positioning and/or operation of migrant traps is ideally suited to target cutthroat trout.

Table 6. Adult cutthroat trout escapement at Powerdale Dam and number of juveniles collected in Hood River migrant traps.

Adult Escapement at Powerdale		Juvenile Cutthroat Trout Captured in Hood River Migrant Traps					
Calendar Year	Escapement	Mainstem	West Fork	Middle Fork	East Fork	Lake Branch	Neal Creek
1992	5	--	--	--	--	--	--
1993	0	--	--	--	--	--	--
1994	0	2	1	--	14	--	--
1995	0	6	0	1	6	--	--
1996	0	14	1	4	6	--	--
1997	3	14	0	1	3	0	--
1998	0	18	0	5	20	0	--
1999	0	14	0	3	13	0	--
2000	1	13	0	6	11	0	4
2001	1	3	0	2	--	1	--
2002	3	10	0	6	2	0	--
2003	7	5	0	12	14	0	--
2004	4	11	0	6	23	1	--
2005	8	13	0	6	21	1	--
2006	6	5	0	2	7	0	--
2007	2	6	0	3	10	0	--
2008	2	15	0	1	16	0	--
2009	1	16	0	14	25	0	--
2010	7	19	0	17	11	0	--

5.3.2 Bull Trout

Based on juvenile capture at Hood River migrant traps, bull trout appear to reside primarily in the Middle Fork Hood River. Reagan (2010) reports adult escapement past Powerdale Dam and juvenile bull trout captures in migrant traps in the Hood River Basin (Table 7). The paucity of adults enumerated at Powerdale Dam and generally low encounter rate for juveniles at Hood River migrant traps, suggest that bull trout exist at relatively low abundance in the Hood River. However, it is unclear whether the positioning and/or operation of migrant traps is ideally suited to target bull trout.

Table 7. Adult bull trout escapement at Powerdale Dam and number of juveniles collected in Hood River migrant traps.

Adult Escapement at Powerdale		Juvenile Bull Trout Captured in Hood River Migrant Traps			
Calendar Year	Escapement	Mainstem	Middle Fork	East Fork	Lake Branch
1992	6	--	--	--	--
1993	2	--	--	--	--
1994	11	1	--	--	--
1995	11	0	--	--	--
1996	18	0	6	0	--
1997	6	0	11	0	1
1998	18	3	22	0	0
1999	28	0	1	0	0
2000	27	0	1	0	0
2001	12	0	1	0	0
2002	5	2	11	0	0
2003	4	0	0	0	0
2004	10	0	0	0	0
2005	7	0	6	0	0
2006	4	0	1	0	0
2007	6	0	0	0	0
2008	1	0	1	0	0
2009	6	0	0	1	0
2010	7	0	1	0	0

5.3.3 Coho Salmon

Reagan (2010) reports adult escapement past Powerdale Dam for natural, hatchery, and unknown origin coho salmon (Table 8). In general, adult escapement appears to be increasing over time across all categories (natural, hatchery, and unknown), however it is unclear how much of the increase is due to natural production in the Hood River versus potential increased straying from other natural and hatchery origin stocks. Hatchery origin adults account for between 46% and 96% of the total coho salmon escapement to the Hood River, which does not have a hatchery program for coho salmon.

Table 8. Adult coho salmon escapement past Powerdale Dam.

Calendar Year	Natural Origin	Stray Hatchery	Unknown	Hatchery Fraction
1992	22	79	2	0.77
1993	0	28	5	0.85
1994	1	52	3	0.93
1995	11	39	1	0.76
1996	6	20	1	0.74
1997	6	6	1	0.46
1998	5	44	10	0.75
1999	10	19	2	0.61
2000	9	33	0	0.79
2001	20	976	24	0.96
2002	27	66	4	0.68
2003	41	153	13	0.74
2004	126	466	27	0.75
2005	27	263	30	0.82
2006	32	316	18	0.86
2007	50	384	14	0.86
2008	50	189	11	0.76
2009	65	543	25	0.86

5.3.4 Fall Chinook Salmon

Fall Chinook salmon escapement to the Hood River as enumerated at Powerdale Dam has ranged between 10 and 64 adults (Reagan 2010; Table 9). Hatchery origin adults account for between 0% and 40% of escapement to the Hood River, which does not have a hatchery program for fall Chinook salmon. However, much of the fall Chinook salmon spawning is likely to occur below Powerdale Dam, and is not included in these estimates (R. Gerstenberger, CTWSR, personal communication).

Table 9. Adult fall Chinook salmon escapement past Powerdale Dam.

Calendar Year	Natural Origin	Stray Hatchery	Hatchery Fraction
1992	16	5	0.2
1993	6	4	0.4
1994	25	6	0.2
1995	8	4	0.3
1996	13	2	0.1
1997	24	2	0.1
1998	34	4	0.1
1999	16	0	0.0
2000	32	2	0.1
2001	29	10	0.3
2002	33	2	0.1
2003	57	3	0.1
2004	31	3	0.1
2005	42	4	0.1
2006	49	5	0.1
2007	45	0	0.0
2008	21	5	0.2
2009	56	8	0.1

5.3.5 Spring Chinook Salmon

Native spring Chinook salmon in the Hood River were extirpated (O'Toole and ODFW 1991a,b). Current escapement is a result of reintroduction using the Deschutes River stock, and is considered a segregated unlisted population (NOAA 1999: Federal Register Volume 64 Number 56). Adult escapement past Powerdale Dam (Reagan 2010) ranged between 87 and 1,680 adults from 1992 through 2010 consisting of three components; natural origin adults (Table 10), adults returning from the release of Carson stock juveniles (Table 11), and adults returning from Hood River hatchery broodstock (Table 12).

Table 10. Natural origin spring Chinook salmon escapement past Powerdale Dam.

Origin Stock	Calendar Year	Escapement			
		Minijack	Jack	Adult	Total
Natural Hood River	1992	0	0	36	36
	1993	2	0	43	45
	1994	0	1	29	30
	1995	0	0	21	21
	1996	1	1	96	98
	1997	13	1	73	87
	1998	5	1	79	85
	1999	1	3	20	24
	2000	3	0	66	69
	2001	3	4	42	49
	2002	0	2	72	74
	2003	2	11	100	113
	2004	7	13	132	152
	2005	0	7	110	117
	2006	1	4	297	302
	2007	4	4	150	158
	2008	1	1	60	62
	2009	2	23	65	90
	2010	0	4	123	127

Table 21. Carson Hatchery stock spring Chinook salmon adult escapement past Powerdale Dam.

Origin Stock	Calendar Year	Escapement			
		Minijack	Jack	Adult	Total
Hatchery Carson	1992	0	3	413	416
	1993	0	15	445	460
	1994	0	0	265	265
	1995	0	0	38	38

Table 12. Hood River hatchery stock spring Chinook salmon adult escapement past Powerdale Dam.

Origin Stock	Calendar Year	Escapement			
		Minijack	Jack	Adult	Total
Hatchery Deschutes	1993	3	0	0	3
	1994	0	5	0	5
	1995	4	0	24	28
	1996	0	15	0	15
	1997	11	1	279	291
	1998	14	2	17	33
	1999	183	5	89	277
	2000	919	129	21	1,069
	2001	82	499	574	1,155
	2002	11	25	1,028	1,064
	2003	14	15	331	360
	2004	169	183	148	500
	2005	72	77	587	736
	2006	185	36	920	1,141
	2007	544	355	301	1,200
	2008	162	404	840	1,406
	2009	293	446	851	1,590
	2010	0	207	461	668

5.3.6 Winter Steelhead

Natural origin winter steelhead are listed in the Lower Columbia River Distinct Population Segment (DPS), currently considered threatened (Federal Register Volume 70 Number 120). Recent adult escapement past Powerdale Dam and juvenile production (Table 13; Reagan 2010) indicate highly variable freshwater productivity with juvenile production per female spawner ranging from 4.5 to 112.5. Total smolt production ranged from natural spawning (by hatchery and natural origin adults) ranged from 2,176 to 22,509, averaging 7,676 from 1993 to 2007. Average smolt production over this period was roughly 82% of the production target of 9,370 smolts identified in the HRPP and 45% of the estimated 16,970 smolt capacity estimated for the Hood River (Underwood et al. 2003).

Winter steelhead escapement measured at the Powerdale Dam on the Hood River has included individuals of three origins; natural origin adults (Table 14), Big Creek hatchery origin (Table 15), and Hood River hatchery origin (Table 16; Reagan 2010). Natural origin winter steelhead escapement ranged from 206 to 1,059 adults from 1991 through 2010 averaging 508 adults; roughly 77% of the HRPP natural origin escapement goal of 656 adults. Hatchery origin winter steelhead escapement ranged from 99 to 1,818 adults from 1991 through 2010 averaging 548 adults; roughly 55% of the HRPP hatchery origin escapement goal of 1000 adults.

Based on scale analysis, on a calendar-year basis repeat spawners have composed between 1% and 13% (average 5%), 2% and 30% (average 10%), and 0% and 5% (average 2%) for natural origin, Big Creek, and Hood River hatchery stocks, respectively . As expected, female repeat spawners are more common than males, composing 72%, 75%, and 59% of the repeat spawner escapement, respectively by stock.

Table 33. Winter steelhead adult escapement past Powerdale Dam and brood-year juvenile production by age class.

Brood Year	Adult Winter Steelhead			Juvenile Winter Steelhead				
	Male	Female	Total	Age 1	Age 2	Age 3	Age 4	Total
1993	129	225	354	1,052	2,464	874	0	4,390
1994	91	214	305	328	3,169	1,063	0	4,560
1995	82	84	166	166	6,465	1,031	0	7,662
1996	170	200	370	941	19,583	1,971	14	22,509
1997	189	296	485	305	10,471	3,009	55	13,840
1998	122	222	344	29	5,876	1,294	28	7,227
1999	186	255	441	124	2,353	1,283	10	3,770
2000	443	645	1,088	82	3,739	2,072	27	5,920
2001	603	909	1,512	441	5,353	2,714	11	8,519
2002	712	914	1,626	64	4,521	1,290	45	5,920
2003	384	678	1,062	657	8,088	1,074	41	9,860
2004	452	622	1,074	75	2,236	487	0	2,798
2005	222	290	512	14	1,213	938	11	2,176
2006	262	372	634	118	8,058	1,049		
2007	290	494	784	464	6,295			
2008	121	253	374	0				
2009	116	224	340					
2010	401	710	1,111					

Table 44. Natural origin Hood River winter steelhead escapement past Powerdale Dam by calendar year.

Origin Stock	Calendar Year	Escapement			
		Total	Repeat Spawners	% Repeat Spawners	% Female
Natural Hood River	1991-1992	698	50	7.2%	62%
	1992-1993	415	35	8.4%	81%
	1993-1994	404	16	4.0%	86%
	1994-1995	206	18	8.7%	67%
	1995-1996	279	8	2.9%	50%
	1996-1997	290	8	2.8%	100%
	1997-1998	227	11	4.8%	64%
	1998-1999	298	9	3.0%	73%
	1999-2000	921	34	3.7%	51%
	2000-2001	1,015	137	13.5%	85%
	2001-2002	1,059	40	3.8%	75%
	2002-2003	745	40	5.4%	60%
	2003-2004	598	30	5.0%	58%
	2004-2005	345	9	2.6%	82%
	2005-2006	462	11	2.4%	67%
	2006-2007	480	9	1.9%	71%
	2007-2008	339	24	7.1%	79%
	2008-2009	215	28	13.0%	88%
	2009-2010	654	6	0.9%	75%

Table 55. Big Creek stock hatchery origin Hood River winter steelhead escapement past Powerdale Dam by calendar year.

Origin Stock	Calendar Year	Escapement			
		Total	Repeat Spawners	% Repeat Spawners	% Female
Hatchery Big Creek	1991-1992	293	6	2.0%	80%
	1992-1993	205	9	4.4%	71%
	1993-1994	140	5	3.6%	50%

Table 66. Hood River stock hatchery origin Hood River winter steelhead escapement past Powerdale Dam by calendar year.

Origin Stock	Calendar Year	Escapement			
		Total	Repeat Spawners	% Repeat Spawners	% Female
Hatchery Hood River	1994-1995	89	1	1.1%	100%
	1995-1996	274	0	0.0%	0%
	1996-1997	636	4	0.6%	100%
	1997-1998	389	15	3.9%	62%
	1998-1999	313	8	2.6%	75%
	1999-2000	301	16	5.3%	69%
	2000-2001	920	33	3.6%	81%
	2001-2002	1,024	20	2.0%	40%
	2002-2003	553	16	2.9%	62%
	2003-2004	1,027	21	2.0%	48%
	2004-2005	495	22	4.4%	63%
	2005-2006	896	2	0.2%	0%
	2006-2007	445	4	0.9%	100%
	2007-2008	194	1	0.5%	0%
	2008-2009	397	4	1.0%	75%
	2009-2010	1,818	7	0.4%	67%

5.3.7 Summer Steelhead

Natural origin summer and winter steelhead are listed in the Lower Columbia River Distinct Population Segment (DPS), currently considered threatened (Federal Register Volume 70 Number 120). Skamania stock summer steelhead, released from 1988 through 1998, and their descendants are not included in the listed DPS. Recent adult escapement past Powerdale Dam and juvenile production (Table 17; Reagan 2010) indicate highly variable freshwater productivity with juvenile production per female spawner ranging from 0.8 to 37.3. Total smolt production from natural spawning (by hatchery and natural origin adults) ranged from 1,228 to 8,731, averaging 3,857 from 1993 to 2004. Average smolt production over this period was roughly 51% of the production target of 7,500 smolts identified in the HRPP and 28% of the estimated 13,860 smolt capacity estimated for the Hood River (Underwood et al. 2003).

Summer steelhead escapement measured at Powerdale Dam on the Hood River has included individuals of three origins; natural origin (Table 18), Foster stock hatchery origin (Table 19), and Hood River stock hatchery origin (Table 20). Natural origin summer steelhead escapement ranged from 67to 648 adults from 1992 through 2010 averaging 244 adults; roughly 48% of the HRPP natural origin escapement goal of 510 adults. Hatchery origin summer steelhead escapement ranged from 460 to 2,695 adults from 1992 through 2010 averaging 1,245 adults. As of 2009, hatchery production of summer

steelhead was suspended, so there is no current escapement target for hatchery origin summer steelhead. However, the prior HRPP identified a targeted escapement of 8,000 total (natural plus hatchery) summer steelhead adults. From 1992 through 2010, total summer steelhead escapement averaged 1,489 adults, or 19% of the targeted escapement. Based on scale analysis, on a calendar-year basis repeat spawners have composed between 2.5% and 10.4% (average 5.9%), 0% and 4.3% (average 1.9%), and 0% and 3.4% (average 1.3%) for natural origin, Foster stock, and Hood River hatchery stocks, respectively . As expected, female repeat spawners are more common than males, composing 63%, 52%, and 59% of the repeat spawner escapement, respectively by stock.

Table 77. Summer steelhead adult escapement past Powerdale Dam and brood-year juvenile production by age class.

Brood Year	Adult Summer Steelhead			Juvenile Summer Steelhead				
	Male	Female	Total	Age 1	Age 2	Age 3	Age 4	Total
1993	669	1,542	2,211	175	642	411	0	1,228
1994	438	909	1,347	327	1,756	722	0	2,805
1995	752	1,088	1,840	333	3,910	1,293	0	5,536
1996	258	392	650	314	2,800	757	14	3,885
1997	553	931	1,484	427	5,591	2,659	54	8,731
1998	173	339	512	14	2,975	1,030	28	4,047
1999	25	75	100	211	815	955	10	1,991
2000	56	93	149	45	1,501	1,895	27	3,468
2001	37	142	179	44	2,145	1,333	12	3,534
2002	199	326	525	75	2,033	1,102	46	3,256
2003	346	679	1,025	399	3,534	1,584	41	5,558
2004	149	227	376	60	1,558	623	0	2,241
2005	125	194	319	23	1,151	402		
2006	130	164	294	67	1,793			
2007	127	216	343	43				
2008	148	166	314					
2009	225	237	462					
2010	206	146	352					

Table 88. Natural origin Hood River summer steelhead escapement past Powerdale Dam by calendar year.

Origin Stock	Calendar Year	Escapement			
		Total	Repeat Spawners	% Repeat Spawners	% Female
Natural Hood River	1992-1993	477	18	3.8%	67%
	1993-1994	223	10	4.5%	73%
	1994-1995	177	15	8.5%	65%
	1995-1996	122	3	2.5%	10%
	1996-1997	170	6	3.5%	80%
	1997-1998	67	7	10.4%	83%
	1998-1999	119	12	10.1%	82%
	1999-2000	177	10	5.6%	100%
	2000-2001	203	17	8.4%	72%
	2001-2002	480	16	3.3%	61%
	2002-2003	648	42	6.5%	68%
	2003-2004	241	23	9.5%	77%
	2004-2005	203	21	10.3%	68%
	2005-2006	189	7	3.7%	43%
	2006-2007	184	8	4.3%	73%
	2007-2008	213	9	4.2%	33%
	2008-2009	225	11	4.9%	42%
	2009-2010	274	8	2.9%	43%

Table 19. Foster stock hatchery origin Hood River summer steelhead escapement past Powerdale Dam by calendar year.

Origin Stock	Calendar Year	Escapement			
		Total	Repeat Spawners	% Repeat Spawners	% Female
Hatchery Foster	1992-1993	1,671	13	0.8%	77%
	1993-1994	1,068	6	0.6%	50%
	1994-1995	1,562	13	0.8%	60%
	1995-1996	508	10	2.0%	22%
	1996-1997	1,287	14	1.1%	58%
	1997-1998	564	5	0.9%	40%
	1998-1999	524	18	3.4%	56%
	1999-2000	460	13	2.8%	42%
	2000-2001	1,150	13	1.1%	62%
	2001-2002	1,801	24	1.3%	30%
	2002-2003	1,564	28	1.8%	86%
	2003-2004	1,221	28	2.3%	63%
	2004-2005	1,692	18	1.1%	25%
	2005-2006	539	15	2.8%	70%
	2006-2007	426	4	0.9%	100%
	2007-2008	341	2	0.6%	100%
	2008-2009	298	0	0.0%	0%
	2009-2010	492	0	0.0%	0%

Table 20. Hood River stock hatchery origin summer steelhead escapement past Powerdale Dam by calendar year.

Origin Stock	Calendar Year	Escapement			
		Total	Repeat Spawners	% Repeat Spawners	% Female
Hatchery Hood River	2001-2002	404	0	0.0%	0%
	2002-2003	907	28	3.1%	68%
	2003-2004	642	16	2.5%	59%
	2004-2005	1,003	20	2.0%	50%
	2005-2006	645	28	4.3%	42%
	2006-2007	556	4	0.7%	25%
	2007-2008	494	7	1.4%	67%
	2008-2009	606	6	1.0%	50%

5.3.7 Limiting Factors

The primary factors limiting freshwater productivity in the Hood River are believed to be spring freshets and low summer stream flow (Reagan 2010). Mechanistically, spring freshets may scour redds exerting substantial mortality prior to fry emergence, whereas low summer stream flow likely increases mortality of pre-migrant juveniles. In the former case, mortality may be independent of density unless a paucity of spawning habitat increases redd superimposition and/or displaces spawning to marginal habitat. In the latter case, mortality may be density dependent if habitat availability is limited. Given the observed variance in freshwater productivity (smolts per female spawner) observed for

winter and summer steelhead it is difficult to identify whether mortality is best explained by density dependent versus random environmental processes.

A number of habitat improvement actions have been initiated in the Hood River; including:

- Addition of large woody debris and boulders, primarily in the West Fork, Lake Branch, Clear Branch, Robinhood, and McGee Creek (among others).
- Gravel supplementation.
- Riparian fencing.
- bank stabilization.
- Riparian planting.
- Fish screening at water diversions.
- Culvert removal/replacement in Evans Creek, Baldwin Creek, Hutson Pond, and Pinnacle Creek.
- Flow enhancement (3.44 cfs) in the East Fork has been initiated via the Canal pipeline/Neal Creek inverted siphon project.
- The removal of Powerdale Dam, which is anticipated to restore a 500 cfs water right to the state of Oregon, which could remain instream on the mainstem Hood River.

5.3 Potential for Integration of Kelts

This section explores the potential for the integration of kelts into the existing winter steelhead propagation program for the Hood River and the potential to use kelts to re-initiate a summer steelhead production program. As described in sections 5.2.1.2 and 5.2.1.3, current and historical Hood River steelhead production programs have largely relied on Oak Springs Hatchery to rear steelhead. It is assumed that this arrangement will continue for the foreseeable future. Thus, kelt integration focusses primarily on the collection of post-spawned kelts, adult holding, hatchery spawning, and acclimation. It is also assumed that managers wish to maintain a 25% limit on the number of natural origin adults collected for broodstock, and an upper limit of 5% contribution of hatchery origin adults to natural spawning. Lastly, it is assumed that managers wish to maintain a broodstock composed of 100% natural origin adults for the existing winter steelhead program and for any summer steelhead production program that might be considered. The following sections describe the potential benefits of incorporating kelts into hatchery production, logistical considerations and constraints accompanying kelt integration, a proposed alternative for their incorporation, and uncertainties accompanying kelt integration.

5.3.1 Potential Benefits of Kelt Integration

As described in sections 5.3.6 and 5.3.7 HRPP adult escapement and juvenile production goals for winter and summer steelhead have not been routinely achieved. In general, both the existing winter steelhead production program and the discontinued summer steelhead production program suffer(ed) from an inability to collect sufficient broodstock within the guidelines of retaining no greater than 25% of the total natural origin escapement. In fact,

the cessation of the summer steelhead production program was largely a function of the inability to collect broodstock without exceeding this guideline (Reagan 2010).
Given limitations placed on broodstock collection, the integration of kelts may be ideally suited to the existing winter steelhead production program and may provide a low-impact alternative to the collection of returning adults should managers choose to re-initiate a summer steelhead production program. In general, three alternatives could be pursued:

1. Short-term kelt reconditioning – this alternative would target the collection of post-spawned kelts as they emigrate from the Hood River. Collected kelts would be placed into a short-term reconditioning program at Parkdale Hatchery and either released back into the Hood River or transported below Bonneville Dam to continue their seaward emigration.
2. Long-term kelt reconditioning and release - this alternative would target the collection of post-spawned kelts as they emigrate from the Hood River. Collected kelts would be placed into a long-term reconditioning program at Parkdale Hatchery and released for natural spawning in the Hood River the subsequent year.
3. Long-term kelt reconditioning and retention for broodstock - this alternative would target the collection of post-spawned kelts as they emigrate from the Hood River. Collected kelts would be placed into a long-term reconditioning program at Parkdale Hatchery and retained for broodstock.

If successful, the first two alternatives would function to increase total escapement by ostensibly improving the survival of kelts and increasing their contribution towards natural escapement. The latter alternative would reduce the number of adults collected from natural origin escapement by utilizing surviving reconditioned kelts for broodstock. If successful, any of the proposed alternatives has the potential to reduce the impacts of broodstock collection on natural origin escapement.

5.3.2 Logistical Considerations and Constraints Accompanying Kelt Integration
Each of the alternatives described in section 5.3.1 requires the ability to collect and recondition emigrating kelts. None of the adult collection or holding facilities in the Hood River were explicitly designed to support a kelt program. The following paragraphs describe logistical considerations for adult collection and reconditioning that would accompany the proposed alternatives.

For winter steelhead the floating resistance board weir, and a downstream trap on Neal Creek are the only locations currently in operation that could potentially be used for kelt collection. Similarly for summer steelhead, the moving falls adult collection facility is the only location expected to allow kelt collection. Problematically, kelt collection was not explicitly considered in the design of either facility. Although kelts have been observed washing onto the resistance board weir, it is unclear whether these individuals are representative of the kelt emigration at large or potentially the subset of kelts that may be in the process of expiring. Similarly, it is unclear whether the number of kelts that could be

collected at the weir would be of sufficient magnitude to meaningfully support a kelt reconditioning program. Construction of the moving falls adult collection facility is anticipated to be complete in 2013. Thus, it is unclear at this point whether that facility could function as a kelt collection device.

Adult holding facilities at the Parkdale Hatchery consist of two 1,280 cubic foot raceways, providing more than sufficient space for existing winter steelhead and spring Chinook salmon production programs and a summer steelhead program similar to the recently suspended program described in the original HRPP (Table 21). Sufficient space would still be available should the spring Chinook salmon program be expanded to achieve the 150,000 smolts identified in the 2008 HRPP. In practice, initiation of a kelt reconditioning program for either winter or summer steelhead would have little impact on facility operations. Historically, summer steelhead adults were held at Parkdale year around due to their prolonged run-timing. Winter steelhead are currently held at Parkdale from January through June. Initiation of a kelt program would require year around adult holding for winter steelhead similar to the recently terminated summer steelhead production program. It is assumed that winter steelhead will continue to be acclimated in two groups at Parkdale Hatchery in one of the two 2,560 cubic foot raceways. If a summer steelhead program is reinitiated, juveniles could be either directly released above the Moving Falls adult trap or potentially acclimated in one of the four raceways constructed at that site.

Table 91. Adult holding space requirements per Integrated Hatchery Operations Team (IHOT 1994) recommendations.

Stock	Number of Adults	Cubic Feet/Adult	Space Required (Cubic Feet)
Winter Steelhead	66	3	198
Summer Steelhead	33	3	99
Near-Term Spring Chinook Salmon	66	8	528
Long-Term Spring Chinook Program	200	8	1600

5.3.3 Proposed Alternative for Kelt Integration
In the following sections we describe a phased approach for the integration of kelts into the winter steelhead broodstock. Following that discussion we explore the potential benefits of kelt reconditioning for summer steelhead.

5.3.3.1 Winter Steelhead
We propose a "phased" approach for the integration of kelts into the winter steelhead broodstock. Each phase of the integration program will test the efficacy of kelt integration while minimizing risk to the existing production program. Given the uncertainties accompanying kelt integration, we recommend that the program initiate Phase Three for a minimum of five years.

Phase One of the kelt program will focus on the collection of post-spawned kelts at the mainstem floating resistance board weir at Dee. Captured adults will either be PIT tagged and released to resume their emigration or transported to the Parkdale Hatchery for reconditioning. Ultimately, Phase One will test whether kelt collection at the resistance

board floating weir is logistically feasible and whether sufficient numbers of adults can be collected at that facility. PIT tagged adults released back to the river will provide an estimate of the adult-to-adult return rate of post-spawned steelhead. This information will be used determine the impact of post-spawned kelt collection on subsequent escapement of repeat spawners.

Phase Two of the program will focus on long-term reconditioning of adults collected at the resistance board floating weir. Ultimately, Phase Two is intended to determine survival expectations and rates of skip-spawning for adults collected at the resistance board weir, transported to Parkdale Hatchery and subsequently reconditioned. During Phase Two, hormone assays (Branstetter et al. 2009) will be performed on a regular basis to test whether this approach is useful for identification of skip-spawners.

Phase Three will focus on testing the potential to replace some fraction of broodstock with surviving reconditioned kelts. Managers can either choose to reduce production from the standard broodstock program to offset the production from reconditioned kelts, or if space at the Parkdale and Oak Springs Hatchery is available, both programs could proceed side-by-side. In the latter case there is the potential that total production from the standard and reconditioned broodstock would exceed HRPP targets. As described in the following section, we propose to representatively tag juvenile production from both groups to determine whether post-release survival and SAR differs between the two production groups.

Phase Four constitutes a program decision point. Data from at least five years of Phase Three implementation is expected to be sufficient to determine whether adult collection and reconditioning is capable of offsetting all or part of standard broodstock collection. Juvenile post-release survival comparisons between hatchery release groups will be available at this point; however SAR comparisons will be complete only for adult returns from the first release. If the SAR comparison suggests a reduced SAR for offspring of reconditioned kelts, the program should not proceed to Phase Five until at least three years of comparative SAR data are available.

Phase Five constitutes full program implementation or cessation. Based on analyses conducted during Phase Four, managers will have a detailed understanding of the potential for kelt reconditioning to replace all or part of standard broodstock collection. Phase Five will formalize the kelt reconditioning program and update the HRPP to reflect the program.

5.3.3.2 Summer Steelhead

The summer steelhead program identified in the original HRPP was terminated largely as a result of inability to collect sufficient broodstock (Reagan 2010) and concerns regarding reduced reproductive success observed for hatchery steelhead (Araki et al. 2007). The 2008 HRPP includes the potential to reinitiate a summer steelhead program after a period of two generations following its termination. As described in section 5.3.7, summer steelhead escapement and natural production appears to be depressed.

Should managers choose to reinitiate a summer steelhead production program, kelt reconditioning offers a promising alternative or supplement to broodstock collection that would otherwise target natural origin adults. Upon completion of the Moving Falls adult collection facility, we propose to implement the first two phases of the kelt integration program described for winter steelhead. Reconditioned kelts will be PIT tagged, transported to the West Fork Hood River, and released to either undertake a spawning migration or emigration in the case of skip-spawners. Interrogation of PIT tagged adults at the lower Hood River instream PIT tag array and Bonneville Dam will be used to determine the survival rate of reconditioned kelts.

Should managers choose to reinitiate a summer steelhead production program, the remaining three phases described for winter steelhead kelt integration could be pursued.

5.4 Final Recommendations

Section 5.3.3 developed a phased approach for the integration of kelts into the winter steelhead production program and identified an approach for the re-initiation of a summer steelhead production program and/or kelt reconditioning program as a potential means to boost escapement of that stock. This section summarizes potential risks to existing HRPP programs and identifies a number of research initiatives necessary to inform decisions about the utility of kelt reconditioning to support HRPP objectives.

5.4.1 Remediation of Risk Posed by Kelt Reconditioning

The proposed alternative is unlikely to adversely impact existing spring Chinook salmon and winter steelhead production programs, owing to the fact that recent renovations at the Parkdale Hatchery were designed to support full implementation spring Chinook salmon production while maintaining HRPP winter and summer steelhead production programs. Additionally, the proposed phased implementation enables a test of the efficacy of kelt collection and the quality of juveniles derived from reconditioned kelts prior to any substitution of standard broodstock with reconditioned kelts. While the potential to successfully supplement or substitute standard broodstock collection with reconditioned kelts cannot be surmised at this time, initiation of the proposed alternative poses no tangible risk to existing production programs. Phase Four of the proposed alternative provides a decision point wherein an assessment of information provided by Phases One through Three can be used to quantitatively assess the utility of kelt reconditioning. Full program implementation or cessation (Phase Five) will proceed only when the utility and reliability of kelt reconditioning can be quantitatively evaluated.

5.4.2 Research Needs

The proposed kelt integration program is accompanied by a number of uncertainties including:

- Adequacy of existing facilities to support kelt capture.
- Influence of kelt collection on the abundance of repeat spawners.

- Influence of mortality rates during reconditioning and skip-spawning on kelt collection targets.
- Performance of hatchery origin juveniles parented by reconditioned kelts versus those derived from standard broodstock.

5.4.2.1 Adult Collection

Any alternative to integrate kelt reconditioning into winter or summer steelhead propagation programs in the Hood River is dependent on the ability to collect and transport post-spawn kelts to Parkdale Hatchery or alternative holding facilities (e.g., Moving Falls). Phase One is designed to evaluate the efficacy of winter steelhead kelt collection at the West Fork resistance board weir and summer steelhead kelt collection at the Moving Falls adult trap. Insofar as possible, Phase One investigation should assess the ability of these facilities to representatively trap emigrating kelts. It is entirely possible, for example, that adult vulnerability to interception at the resistance board weir may be a function of reduced swimming ability, potentially suggesting that vulnerability is a function of poor condition. If this is true, adults collected at this location may be less likely to survive reconditioning. Additionally, condition aside, it is possible that the interception efficiency of these devices will be insufficient to collect more than a handful of adults. If kelt collection proves logistically infeasible and/or non-representative, alternative kelt collection methodologies will be developed.

5.4.2.2 Influence of Post-Spawned Kelt Collection on Subsequent Escapement of Repeat Spawners

In general, this document has assumed that the collection, reconditioning, and subsequent use of kelts for broodstock will have little impact on the contribution of repeat spawners in subsequent years. This assumption is based on two observations:

1. On average natural origin repeat spawners constitute a relatively small fraction of total adult escapement in a given calendar year; 5% and 5.9% respectively for winter and summer steelhead.
2. Little information exists to assess the adult to adult return rate for kelts, which is assumed to be vanishingly small.

Without additional information, it appears reasonable to assume that the collection of post-spawned kelts is likely to have little influence on the fraction of individuals that successfully emigrate after spawning and subsequently return to the Hood River. On the other hand, if the program is highly successful at intercepting post-spawned kelts and implements an aggressive reconditioning program, there is every reason to believe that the subsequent release of reconditioned individuals in excess of broodstock requirements would increase the prevalence of repeat spawners by reducing both immediate mortality and subsequent out of subbasin mortality.

5.4.2.3 Formulating an Appropriate Kelt Collection Target

If managers are successful at intercepting post-spawn kelts, the potential for successful implementation of the proposed alternative requires the ability to successfully recondition kelts and identify the fraction of reconditioned individuals that are destined to spawn in the following year versus out-years (skip-spawners). Phase Two is designed to determine survival rates during reconditioning and to develop methods to identify females that may be skip-spawners. Ultimately, the information developed in Phase Two will be used to determine how many post-spawned kelts must be collected to support broodstock and reconditioned kelt release goals.

5.4.2.4 Relative Juvenile Survival

We have generally assumed that the post-release survival of juveniles from reconditioned kelts will not differ from those of juveniles produced from standard broodstock. Phase Three of the implementation plan identifies the need to test this assumption. In this section we describe three approaches that could be considered to test the assumption that post-release survival of progeny from maiden spawners versus reconditioned kelts are equivalent.

5.4.2.4.1 Large Scale Paired Release to Evaluate Differential SAR

One approach for testing the assumption that post-release survival of progeny from maiden spawners versus reconditioned kelts is equivalent is a “large-scale” paired release. This approach would utilize a paired release of PIT tagged juveniles produced from the standard broodstock and those derived from reconditioned kelts, both reared using standard hatchery practices in a common environment. The paired release will test the null hypothesis that SAR does not differ between the two production groups. Assuming an average smolt to adult return rate (SAR) of 1.6% for juveniles obtained from standard broodstock (Reagan 2010) a paired release of 77,000 juveniles from each production group would be required to detect a difference in SAR of 10% or greater while maintaining a 10% probability of a type I error and 20% probability of rejecting the null hypothesis (Table 22; Hinrichsen 2011). The winter steelhead production program currently targets the release of 50,000 smolts annually. The number of years necessary to obtain the required sample size for the SAR comparison is therefore a function of how managers choose to distribute production among standard versus reconditioned kelt broodstock. Since the release is paired in time (both groups are released simultaneously), releases can be combined across years until the target sample size is achieved. In essence, this assumes that any “year” effect (e.g., difference in survival across years due to changes in migration conditions) is immaterial, since the release groups in a given year would be subject to the same conditions.

We caution that the approach described above is accompanied by substantial risk. If a large fraction of total production (e.g., 50%) is dedicated towards production of progeny from reconditioned kelts, and their subsequent post-release survival is substantially lower than that of the standard production group, subsequent hatchery origin adult escapement from those brood years would be reduced. However, this approach is also accompanied by the benefit of “quickly” evaluating (i.e., with one or two brood years of production) whether a

survival differential exists. Similarly, in contrast to the alternative studies described below, the release sizes possible with this approach enable an evaluation of total life-cycle survival (from juvenile release through adult return), which is ultimately the most useful metric for fisheries management.

Table 22. Paired release requirements to obtain a 90% probability of detecting a difference in SAR of 10% or greater between juvenile production groups.

Number Tagged	Alpha	Delta	Standard Error	CV	Power
20,000	0.1	0.10	0.08	0.79	0.35
30,000	0.1	0.10	0.06	0.65	0.46
40,000	0.1	0.10	0.06	0.56	0.56
50,000	0.1	0.10	0.05	0.50	0.64
60,000	0.1	0.10	0.05	0.46	0.70
70,000	0.1	0.10	0.04	0.42	0.76
80,000	0.1	0.10	0.04	0.40	0.81
75,000	0.1	0.10	0.04	0.41	0.79
76,000	0.1	0.10	0.04	0.41	0.79
77,000	**0.1**	**0.10**	**0.04**	**0.40**	**0.80**
78,000	0.1	0.10	0.04	0.40	0.80

5.4.2.4.2 Small Scale Paired Release to Evaluate Differential Smolt Survival with Controls for Family Effects

Ideally, as described in Section 5.4.2.4.1, a comparative study with sufficient sample sizes of progeny from reconditioned kelts and maiden spawners would be reared identically, PIT tagged, and simultaneously released within the Hood River. Subsequent detections of juveniles from each group would allow a direct comparison of post-release survival. However, a study of this size would be accompanied by substantial risk, owing to the fact that up to half of all hatchery capacity would be dedicated towards production from reconditioned kelts. Alternatively, managers could use a sequential release strategy, wherein a subset of maiden steelhead would be captured and their eggs fertilized with cryopreserved milt, reared in common with general hatchery production, PIT tagged, and released in common with general hatchery production. The maidens could be retained and reconditioned, and in the following year fertilized with cryopreserved milt from the same males utilized during their initial spawning as maidens. As with their first spawning, juveniles would be reared in common with general hatchery production, PIT tagged, and released in common with general hatchery production.

The subsequent survival of juvenile releases from both groups (maiden versus reconditioned kelt) could be compared to determine whether their post-release survival differs. A benefit of this approach is that the study would use the same females fertilized with cryopreserved milt from the same males as both maidens and reconditioned kelts, so family effects would contribute equally to survival of juveniles from maiden and reconditioned kelts. However, comparisons of post-release survival would have to account for a “year effect” that might occur if hatchery or in-river conditions (e.g., water

temperature or dam operations) exerted a different probability of survival across brood years. In order to control for a year effect, the survival of general production releases could be compared across years. This would require representatively PIT tagging a subset of general production juveniles prior to release.

A comparison of survival between the maiden and reconditioned kelt release groups could be conducted while simultaneously controlling for a year effect by fitting a binomial generalized linear model (GLM). The GLM would include an effect for maiden versus reconditioned kelts, migration year (informed by survival of the general production release in both years), and a spawning group effect (comparison of maiden versus general production and reconditioned kelt versus general production survival). The benefit of utilizing a GLM is its ability to estimate an effect size – or magnitude of survival differential between progeny of maiden versus reconditioned kelts. As with any such study a *post hoc* power analysis should be conducted to determine the minimum detectable difference in survival between groups. Similarly, managers should plan to replicate this study across years to ensure that the results are repeatable.

Notably, the sizes of the release groups would likely be limited to the progeny of a relatively small number of females. As such, the post-release survival evaluation would likely be limited to short-term survival calculated via recapture at the lower Hood River migrant trap and/or Bonneville Dam (see section 5.4.2.4.3). Therefore, this design might serve as a precursor to the large-scale design described in section 5.4.2.4.1.

5.4.2.4.3 Small Scale Paired Release to Evaluate Differential Smolt Survival

Ultimately, the potential for the substitution or supplementation of standard broodstock with reconditioned kelts will be best informed by the comparison of SARs described in section 5.4.2.4.1. However, given the risks accompanying that design, it may be worth first investigating whether short-term post-release mortality differs among progeny of maiden versus reconditioned kelt spawners. The juvenile migrant trap, located on the lower mainstem Hood River, provides an opportunity to evaluate whether short-term juvenile mortality differs between the two production groups prior to emigration from the Hood River. Regan (2010) reports that between 25.9% and 100% (mean 75.5%) of Hood River stock winter steelhead hatchery origin smolts were estimated to have survived to pass the lower Hood River migrant trap. Assuming an average survival of 75.5% to the lower river migrant trap for juveniles obtained from standard broodstock a paired release of 2,245 PIT tagged juveniles from each production group would be required to detect a difference in survival of 5% or greater while maintaining a 5% probability of a type I error and 20% probability of rejecting the null hypothesis (Hinrichsen 2011). Additionally, managers could evaluate survival to Bonneville Dam. However, given the paucity of recaptures expected below Bonneville Dam (i.e., in the Columbia River trawl), it is unlikely that precise detection efficiencies could be generated at Bonneville Dam. If it can be reasonably assumed that detection efficiencies do not differ for progeny of reconditioned versus maiden spawners, managers could simply evaluate whether total detections at Bonneville Dam differ between release groups.

This small scale paired release strategy offers the benefit of minimizing the fraction of hatchery capacity dedicated towards rearing progeny of reconditioned kelts. However, this strategy potentially suffers from the assumption that a small group of reconditioned kelts can meaningfully represent survival that could be expected from the statistical population of reconditioned kelts. In this regard the small scale study described in section 5.4.2.4.2 is superior, in that it controls for variance that might be contributed by individual and family effects. Additionally, as with the design described in section 5.4.2.4.2, this design is likely limited to short-term comparisons of survival, given that release sizes would likely be insufficient to generate useful SAR estimates. Similar to the design described in section 5.4.2.4.2, this design might serve as a precursor to the large-scale paired release design described in section 5.4.2.4.1.

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Appendix I. Repeat Spawner Composition for Selected Populations

The following table is reproduced from Busby et al. (1996).

Table 103. Repeat spawning frequency for selected steelhead populations. Data were collected from scale samples. Numbers indicate the proportion of steelhead collected in each study during a given spawning migration; for example,89% of the steelhead collected by Chapman (1958) in the Alsea River were on their first spawning migration. Populations are generally arranged from north to south.

Population	Run type[a]	Spawning migration					Sample Size	Reference
		1	2	3	4	5		
British Columbia (mainland)								
Babine River	S	**0.97**	0.03	--	--	--	121	Narver 1969
Cheakamus River	O	**0.69**	0.26	0.05	--	--	64	Withler 1966
Capilano River	S	**0.94**	0.06	--	--	--	99	Withler 1966
Seymour River	O	**0.95**	0.05	--	--	--	41	Withler 1966
Seymour River	S	**0.96**	0.04	--	--	--	45	Withler 1966
British Columbia (Fraser River Basin)								
Coquitlam River	O	**0.95**	0.03	0.02	--	--	148	Withler 1966
Coquihalla River	O	**0.94**	0.03	0.03	--	--	31	Withler 1966
Coquihalla River	S	**0.94**	0.06	<0.01	--	--	158	Withler 1966
Washington								
Skagit River	O	**0.92**	0.07	0.01	--	--	n/a[b]	WDFW 1994
Snohomish River	O	**0.92**	0.06	0.01	--	--	n/a	WDFW 1994
Green River	O	**0.93**	0.07	<0.01	--	--	n/a	WDFW 1994
Puyallup River	O	**0.89**	0.1	<0.01	--	--	n/a	WDFW 1994
Nisqually River	O	**0.93**	0.06	0.01	--	--	n/a	WDFW 1994
Quillayute River	O	**0.91**	0.07	0.01	--	--	n/a	WDFW 1994
Columbia River Basin								
Cowlitz River	O	**0.96**	0.04	--	--	--	56	Howell et al. 1985
Toutle River	O	**0.89**	0.05	0.05	--	--	37	Howell et al. 1985
Kalama River	O	**0.93**	0.06	<0.01	<0.01	--	1,363	Howell et al. 1985
Kalama River	S	**0.94**	0.06	<0.01	--	--	909	Howell et al. 1985
Klickitat River	S	**0.97**	0.02	0.01	--	--	148	Howell et al. 1985
Oregon								
Alsea River	O	**0.89**	0.09	0.02	--	--	1,223	Chapman 1958
Siuslaw River	O	**0.86**	0.11	0.02	--	0.01	125	Lindsay et al. 1991
Rogue River	S	**0.79**	0.17	0.04	--	--	4,058	ODFW 1994
California								
Mad River	O	**0.77**	0.17	0.06	--	--	35	Forsgren 1979
Jacoby Creek	O	**0.83**	0.17	--	--	--	109	Harper 1980
Sacramento River	O	**0.83**	0.14	0.02	0.01	--	n/a	Hallock 1989
Waddell Creek	O	**0.83**	0.15	0.02	<0.01	--	3,888	Shapovalov and Taft 1954

[a]O = Ocean maturing; S = Stream maturing.
[b]Sample size not indicated in reference