



CRITFC

TECHNICAL REPORT 13-10

Columbia River Inter-Tribal Fish Commission
503.238.0667
www.critfc.org
700 NE Multnomah, Suite 1200
Portland, OR 97232

Steelhead Kelt Reconditioning and Reproductive Success 2012 Annual Report



**Douglas R. Hatch, Ryan
Branstetter, Jeff Stephenson,
Andrew Pierce, Andrew Matala,
and Jeremiah Newell**

June 29, 2013

Steelhead Kelt Reconditioning and Reproductive Success 2012 Annual Report

Edited by:
Douglas R. Hatch
Ryan Branstetter
Jeff Stephenson
Andrew Pierce Ph. D.
Andrew Matala
Jeremiah Newell

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

Prepared for:
U.S. Department of Energy
Bonneville Power Administration
Division of Fish and Wildlife
P.O. Box 3621
Portland, OR 97283-3621
Project Number: 2007-401-00
Contract Number: 0030769
June 17, 2013

Contents

Steelhead Kelt Reconditioning and Reproductive Success	1
Executive Summary	1
Chapter 1: Management Scenario Evaluation	3
Chapter 2: Kelt Reconditioning Physiology Studies:.....	3
Chapter 3: Snake River Basin kelt steelhead evaluations and management development	4
Chapter 4: Reproductive Success Evaluation:	6
Acknowledgements	7
Chapter 1: Management Scenario Evaluation.....	8
Introduction	9
Section A: Kelt Collection and In-River Release.....	11
Section B. Long-term Reconditioning Treatment	37
Section C. Management Scenario Evaluation	62
Section D: Published Work	67
Chapter 2: Kelt Reconditioning Physiology Studies	106
Introduction	108
References:	109
Section A: Metabolic endocrine factors involved in spawning recovery and rematuration of iteroparous female rainbow trout (<i>Oncorhynchus mykiss</i>)	111
Section B: Reproductive endocrine factors involved in spawning recovery and rematuration of iteroparous female rainbow trout (<i>Oncorhynchus mykiss</i>)	139
Section C: Reproductive development in kelt steelhead	161
Section D: Proteomic Analysis of Female Steelhead Plasma: Progress Report 2012	173
Section E: Effects of a supplemented diet on kelt steelhead	175

Section F: Comparison of reconditioned kelt steelhead and spawning steelhead sampled during upstream migration at Prosser dam.....	185
Section G: Safety and efficacy of ivermectin gavage versus emamectin benzoate injection for control of parasitic copepods <i>Salmincola californiensis</i> in kelt steelhead	189
Section H: Egg quality and reproductive parameters in air spawned hatchery origin maiden spawning female steelhead at Dworshak National Fish Hatchery.....	196
Chapter 3. Snake River Kelt Strategies and Management	203
Section A: 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	204
Section B: Developing Strategies to Improve Survival and Return Recruitment of Steelhead Kelts from Snake River Stocks	ccxxxiv
Section C: Developing Strategies to Improve Survival and Return Recruitment of Steelhead Kelts from Snake River Stocks	260
Section D: Developing Strategies to Improve Survival and Return Recruitment of Steelhead Kelts from Snake River Stocks	286
Section E: Master Plan Progress.....	292
Chapter 4. Reproductive Success Evaluation	298
Section A: Steelhead Kelt Gamete and Progeny Viability In a Hatchery Setting ...	299
Section B: Omak Genetics Report.....	321
Section C. Yakima Genetics Report.....	329

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 (N_e ; Waples 2002), retention of genetic diversity and persistence of the species may be favored due to these compensating life histories (Narum et al. 2008; Seamons and Quinn 2010). 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 is the ability to repeat spawn and is a natural life history strategy expressed by *O. Mykiss*. Rates of iteroparity are estimated to be as high as 79% for populations in the Utkholok River of Kamchatka, Russia (Savvaitova et al. 1996), and as high as 31% for British Columbia winter-run populations (Withler 1966). Historical rates for the interior 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), 45% of the Snake River upstream run (Jay Hesse personal communication), and 70% of the Yakima River upstream run (Chris Fredrickson personal communication) in recent years. Current iteroparity rates for interior Columbia River Basin steelhead are considerably smaller than lower-Columbia River populations, 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 4.0% of the Yakima River wild run and recent tagging data shows average return rates to Bonneville Dam of 3.1%.

Post spawn steelhead that successfully migrate to the sea or are artificially reconditioned, generally follow one of two strategies. Kelt steelhead that return to the river as repeat spawners during the fall of the same year that they migrated to the sea are termed consecutive or sequential spawners (Burgner et al. 1992). The remaining fish that spend the winter in the ocean and return as repeat spawners the following year are termed alternate or skip spawners (Burgner et al. 1992). The proportion of skip spawners varies across populations and appears to positively correlate with distance from the river mouth.

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 (Evans et al. 2008), 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 others applicable to steelhead kelts specifically 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) that will aid in determining the extent to which reconditioned kelts are contributing to subsequent generations. The overall success of kelt reconditioning, when in full production, can be assessed

most accurately 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

Evaluation of various management strategies are described 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).

Long-term reconditioning survival at Prosser Hatchery, Omak Creek, Parkdale Hatchery and Dworshak National Fish Hatchery was 62%, 20%, 62%, and 5% respectively. Survival was above average for Prosser and Parkdale and below average for Omak Creek (where survival was low for many males that were collected and held for reconditioning). Long-term reconditioning appears to provide a substantial benefit over leaving the kelts in the river. Prosser steelhead had a survival benefit 17.5 times higher than fish left in the Yakima River. We published a paper documenting the Yakima River reconditioning program (Hatch et al. 2013).

Chapter 2: Kelt Reconditioning 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. We continued to study the post-spawning physiology in rainbow trout. We have been constructing a profile of growth and reproductive endocrine physiology in post-spawning female rainbow trout which has been submitted for publication. 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 2012, we continued a study on the physiology of post-spawning rainbow trout, which is presented in Section A and B.

We are collecting blood samples at release and at intake to determine important thresholds for kelt reconditioning endocrinology and physiology at Prosser in Sections C and D. This data will be helpful to understanding similar issues at other locations. In 2012, we continued to study on a supplemented diets (Section E), collection of maiden spawning hormone profiles to compare with kelt steelhead (Section F), and a new treatment for freshwater parasitic copepods (Section G). Invasive or manipulative studies and studies involving lethal sampling are difficult with wild endangered fish. The use of hatchery fish can alleviate those concerns and should provide useful data that can be used to infer about wild kelts (Section H).

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. The results of these studies will increase the success of kelt reconditioning throughout the Columbia River Basin.

Chapter 3: Snake River Basin kelt steelhead evaluations and management development

The Columbia River Inter-Tribal Fish Commission's Hagerman lab continue to build baseline information on the kelt steelhead stocks that are represented in the juvenile bypass facility at Lower Granite Dam to better direct kelt management in the basin. The University of Idaho and the Nez Perce Tribe are conducting studies on kelt steelhead. The University of Idaho 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. The Nez Perce Tribe is developing a Kelt Master Plan for the Snake River.

The objective of the GSI study was to estimate stock proportions in a mixed stock sample, providing a better understanding of the origins of post-spawn steelhead among the major subbasins (e.g., Clearwater River, Salmon River, Grande Ronde) and major population groups (MPG's) within the Snake River Basin. Results will also relate information about the behavior and population characteristics for genetically assigned kelt stocks. We found that stocks (Middle Fork Salmon River) captured at the juvenile bypass late in the season tend to be the earliest migrators while the later migrators (Clearwater River groups) tend to be the earliest arriving kelts to the bypass. The largest proportions of kelts moving through the juvenile bypass were from the upper Snake River followed by the Grande Ronde and Middle Fork Salmon rivers.

The University of Idaho (Idaho Cooperative Fish and Wildlife Research Unit) continued to monitor for tagged kelts from Snake Basin tributaries to determine the fate of these fish as they returned to the hydrosystem utilizing PIT and acoustic tags and correlate these movements to hormone profiles.

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 years 2009-2011 provided blood plasma for nearly every month of the reproductive cycle. Organ samples were also collected to determine differences in pre-spawners and kelts. Dworshak National Fish Hatchery was particularly important in providing blood plasma and organ tissue, which was sampled from October 2009 to April 2011 and provided a physiological representation of plasma during early to mid-migration, overwintering, and at the time of spawning. We determined that no significant difference existed between the organ tissue of pre-spawners versus kelt spawners and that tissues go into stasis and not cellular degeneration (Section A).

Nutritional factors measured in the plasma change by stage of development and time in steelhead trout sampled during the spawning years 2009- 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 month of April at Dworshak National Fish Hatchery. We observed that fish in good condition showed higher values of cholesterol, triglycerides and protein (Sections A, B, and C) and similarly that the highest values that corresponded with kelts traveling the furthest distance downriver.

We compared the blood plasma metrics of the good condition kelts tagged in the Potlatch River and Fish Creek in 2010 and 2011 and 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 2010 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. This may reflect the annual difference in the quality of kelts as a result of differing environmental conditions that they experience.

Two manuscripts are currently being produced on the energy depletion of steelhead and the physiological indices for seawater readiness in steelhead kelts. Zach Penney a student on these projects will be defending his dissertation on these subjects this year.

Scott Everett and the Nez Perce Tribe continue to make progress on the Master Plan for kelt management in the Snake Basin. Site selection has been narrowed but is still ongoing and we anticipate that the plan will be nearing completion at the end of 2013.

Chapter 4: Reproductive Success Evaluation:

This chapter evaluates gamete and progeny viability of maiden and repeat spawner steelhead in the Hood River as well as field studies to describe 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. This portion of the project is wrapping up and will have a comprehensive review in the 2013 report.

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. The collection of post-spawn kelts suggests that they have successfully spawned. Alternatively, kelts that are released following reconditioning are still exposed to over-wintering and pre-spawn mortality which may reduce the number of successful spawners. 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.

Acknowledgements

We thank the following individuals and organizations for providing time and expertise toward this project: The Bonneville Power Administration for providing funding for this research project and to Tracy Hauser for project assistance. Hatchery staff for the care provided to this important species: Parkdale Fish Facility (Kieth Moody and Doug McMillian), Prosser Fish Hatchery (Bill Fiander, Mark Johnston, Michael Fiander, Carrie Skahan, and OJ Davis), Colville Staff (Darren Hathaway, Brian Miller, and Oliver Pakootas), and Nez Perce Tribal Staff (Paul Enos, Shavon Bullock, Edward Noah Jackson). The trapping crews for working the long hours and carefully capturing steelhead kelts: Chandler Juvenile Monitoring Facility, Omak and Bonaparte Creeks, ODFW (Jeremy Stahler and Michelle) and Parkdale Fisheries Staff at the East Fork Weir. Thanks to the virology staff at Oregon Department of Fish and Wildlife (Tony Amandi, John Kauffman, Jerry Jones, and Sarah Bjork), Washington Department of Fish and Wildlife (Bob Rodgers), and the staff of the Lower Columbia Fish Health Center. We also thank the following individuals for providing valuable expertise, information and supplies: Dan Green and Greg Davis at the Bonneville Captive Brood Program. Thanks to the following Columbia River Inter-Tribal Fish Staff for their technical expertise, suggestions, and assistance: Bobby Begay, Shawn Narum, Phil Roger, David Graves, Rob Lothrop, Christine Golightly, Peter Galbreath, Jeff Fryer, David Liberty, Gabe Sheoships, Agnus Strong, Nicole Tursich, and Joe Nowiski.

Chapter 1: Management Scenario Evaluation

Written by

Doug Hatch (PI)
Ryan Branstetter
Jeff Stephenson
Andrew L. Pierce Ph. D.
John Whiteaker
Jeremiah Newell
Neil Graham

Columbia River Inter-Tribal Fish Commission

William J. Bosch
Joe Blodgett
David E. Fast Ph. D.
Yakama Nation Fisheries

Scott Everett
Nez Perce Tribe

Rhonda Dasher
Colville Confederated Tribes

Albert Santos
Jim Gidley
**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 benefit steelhead productivity. 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, Transport Fed, and Long term reconditioning. These studies attempt to include measures that span from low to high intensity and low to high 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.

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 toward 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 strategies is based on two fundamental hypotheses aimed at comparing the relative survival and rematuration rates of program fish.

H₀: Iteroparity rates are similar among all treatments including: in-river release and long-term recondition and release.

H₀: Rematuration rates are similar among all treatments including: in-river release 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.

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 representative portion (10%) 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

Prosser, WA: Yakima River Basin

The Yakima River is approximately 344 km in length and enters the Columbia River at RK 539. The basin is 15,928 km² and average discharge is 99 m³/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).

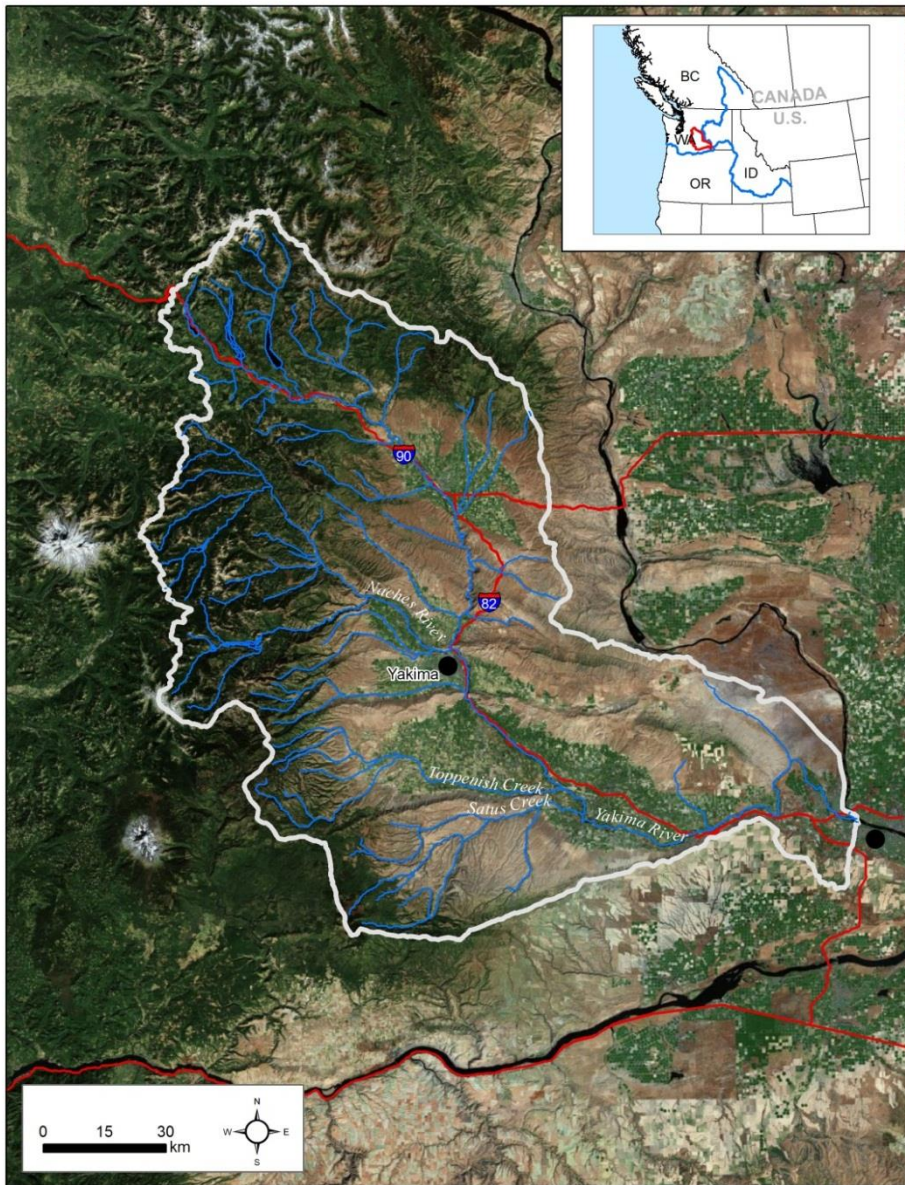


Figure 1: Map of the Yakima River Subbasin.

Lower Granite, WA: Snake River Basin

The Snake River watershed is the tenth largest among North American rivers, and covers almost 280,000 km² 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 RK 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 m³/s. At Anatone, Washington, downstream of the

confluences with the Salmon and Grand Ronde, but upstream of the Clearwater, the mean discharge is $979 \text{ m}^3/\text{s}$ (Figure 2). Steelhead spawn naturally throughout the basin with the vast amount of “b-run” steelhead produced at the Dworshak National Fish Hatchery found on the Clearwater River.

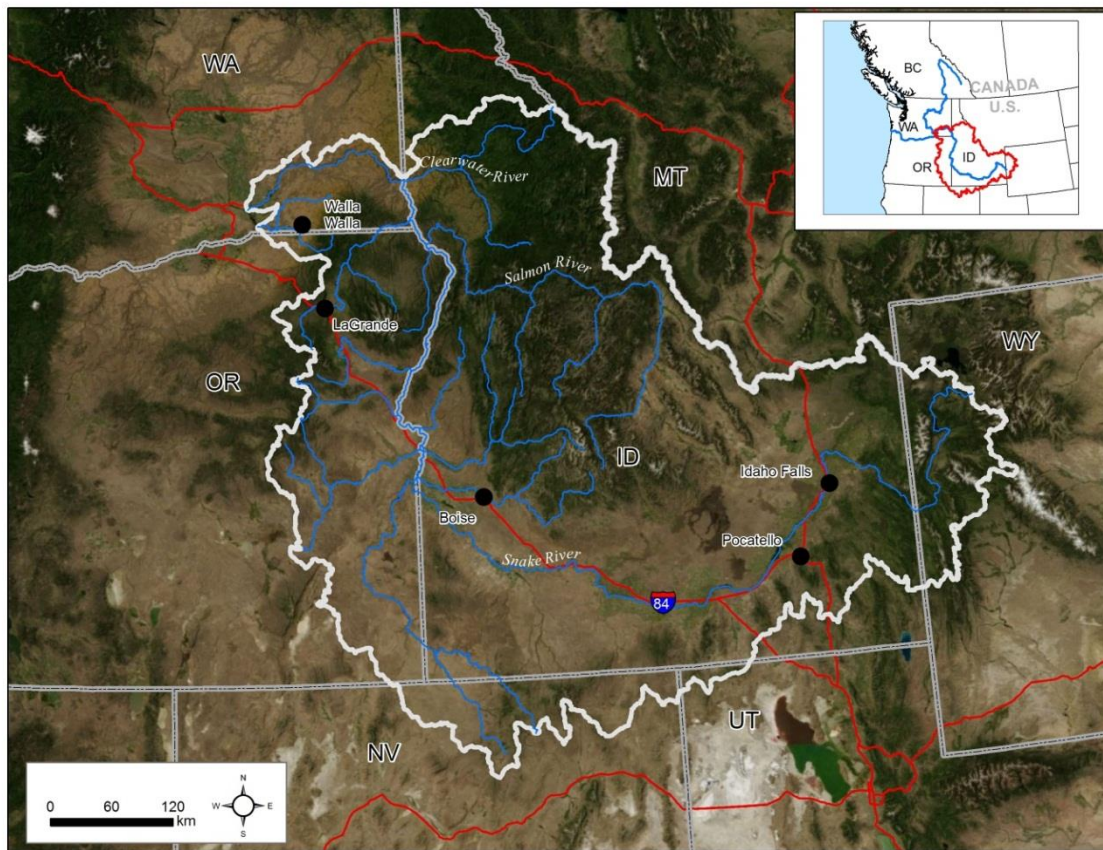


Figure 2: Map of the Snake River Basin.

Omak, WA: Okanogan River Subbasin

The Okanogan River is a tributary of the Columbia River and the confluence is located at RK 858 of the Columbia River. The Okanogan drainage area is $21,238 \text{ km}^2$ with an average discharge rate of $86 \text{ m}^3/\text{s}$. 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 RK 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.

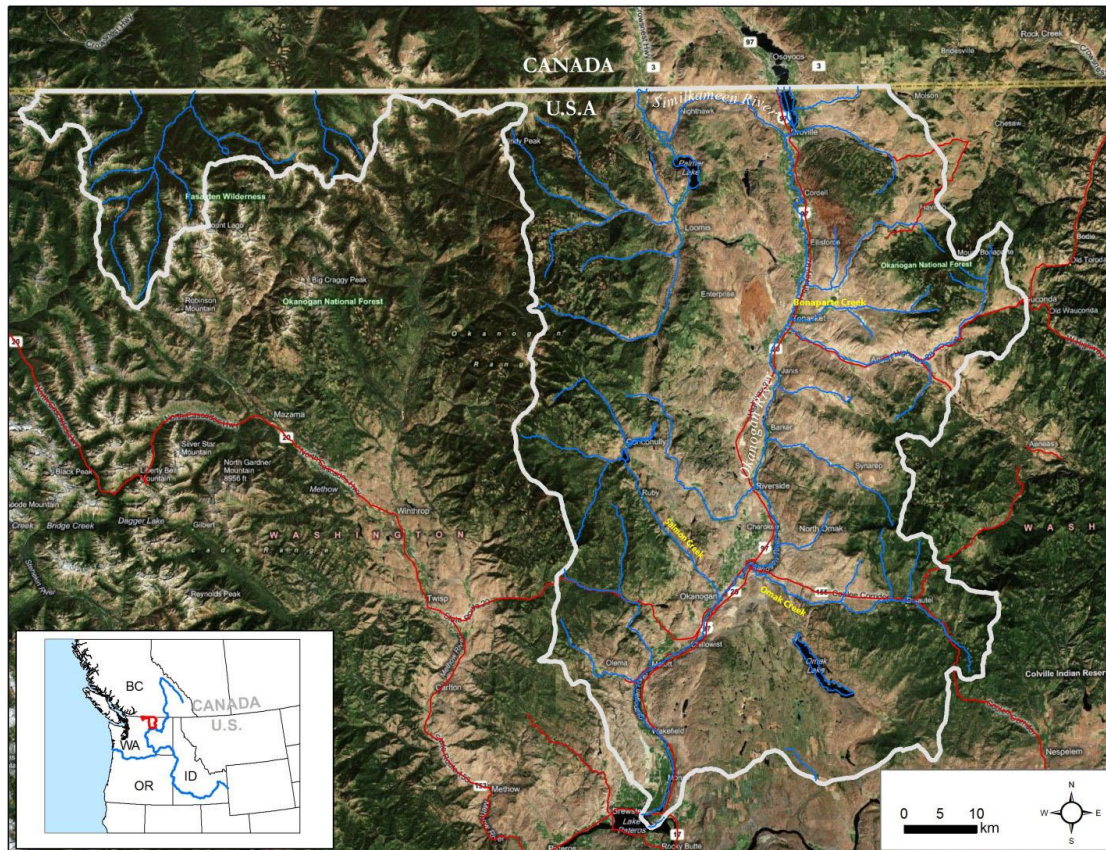


Figure 3: Map of the Okanogan River Subbasin. Omak Creek, Bonaparte Creek, and Salmon Creek in yellow font.

Parkdale, OR: Hood River Subbasin

The Hood River is a tributary of the Columbia River (at RK 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 723² km with an average discharge of 28 m³/s (Figure 4). Currently there is a winter steelhead supplementation program that spawns a small number of hatchery and wild steelhead that are typically reared at the Oak Springs hatchery and acclimated at Parkdale Fish Facility and another site on the East Fork Hood River Sand Trap (RK 16)

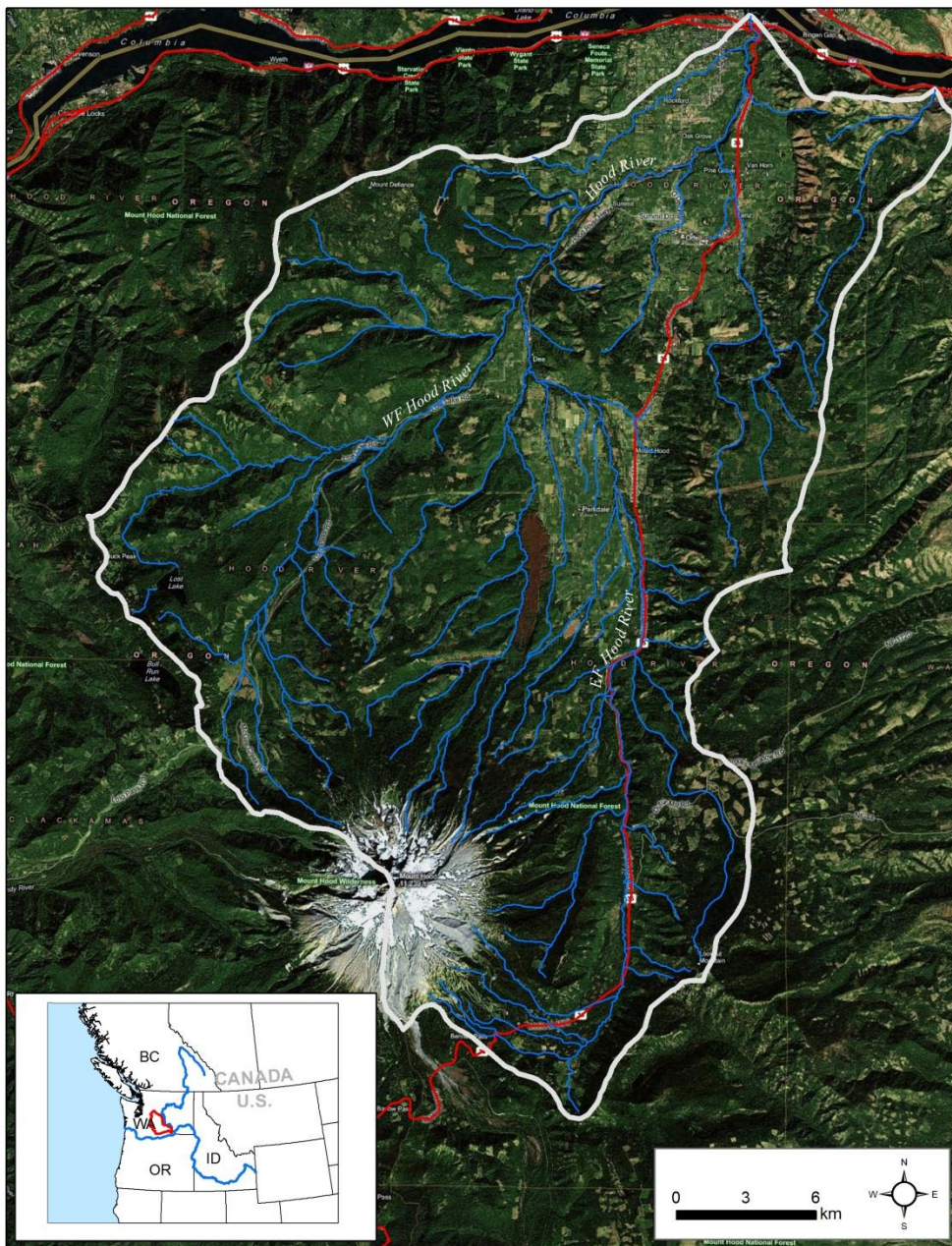


Figure 4: Map of the Hood River Subbasin.

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. 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 Juvenile 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°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 steelhead were processed for a control and experimental group (in-river release and long-term reconditioning).

A feed trail comparing diet additives was conducted as well as a comparison of two treatments for the gill parasite *Salmincola*. The long-term reconditioning had a feeding trial that compared an “orange” diet that was comprised of a mix of biovita brood pellets top coated with a mix of cyclopeeze and Alaskan fish oil versus the standard diet (customized Bio-oregon diet and krill (Hatch et al 2003a). Both groups received krill at the start of reconditioning for the same duration of time. Additionally, ivermectin vs. emamectin benzoate treatments for gill parasites were compared.



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

Following kelt identification, we sexed, weighed (collected in pounds but converted to kg for this report), measured fork and mid-eye to hypural length (cm), assigned condition rating (good- lack of any wounds or descaling, fair- lack of any major wounds and/or descaling, poor- major wounds and/or descaling), coloration rating (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 (JFF) at Lower Granite Dam (LGR) (RK 173) where Army Corps of Engineer (COE) staff. Kelts are collected off the adult fish separator bars and moved to a fish hopper that lead into the kelt

receiving tank (Figure 7). Both B-run (≥ 70 cm) and A-run (<70 cm) steelhead are selected. In 2012, 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.



Figure 7: Lower Granite Dam Juvenile Fish Facility 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 that 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 8: Tanks designed by the University of Idaho 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 (Good=1, Fair=2, Poor=3). 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 (RK 0.8) is utilized to collect broodstock and steelhead kelts for reconditioning (Figure 9). This stock is being used 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 RK) (Figure 10).



Figure 9: Resistance board weir and fish trap 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)

Steelhead in the Hood River are not collected post-spawn, instead hatchery maiden fish are air-spawned and then reconditioned as kelts. Oregon Department of Fish and Wildlife Employees capture anadromous winter run steelhead “maiden” 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. Hatchery winter steelhead are typically retained for brood or terminated and land-filled. 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 February/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 (every 10th kelt) of kelts suitable for reconditioning, were PIT-tagged and immediately released back into the Yakima River (Prosser, WA RK 75.6) to monitor the rate of natural iteroparity. This 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 Snake River (RKM 173) 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 653 live kelts were captured between March 16 and July 3, 2012 at the CJMF. There was one kelt individual discovered dead upon arrival in the bypass. There were 28 kelts in poor condition and 15 prespawn (maiden) steelhead that were released immediately back to the Yakima River on site. A total of 59 good condition kelts were diverted back to the Yakima River for the in-river release. Collection was mostly continuous throughout the outward migration, with peak collection occurring on April 16, 2012 (Figure 12). The bypass was shutdown from April 26 through May 9 for the removal of flood transported flotsam. The total number of kelts captured represented 10.3% (653 of 6,359) of the Yakima River spawning migration based on fish ladder counts obtained from Prosser Dam for the period July 1, 2011 through June 30, 2012. This collection of Yakima River steelhead kelts represents only the portions that volunteer into the Chandler bypass facility while others migrate over Prosser Dam (presentation by Chris Frederiksen, YKFP 2012).

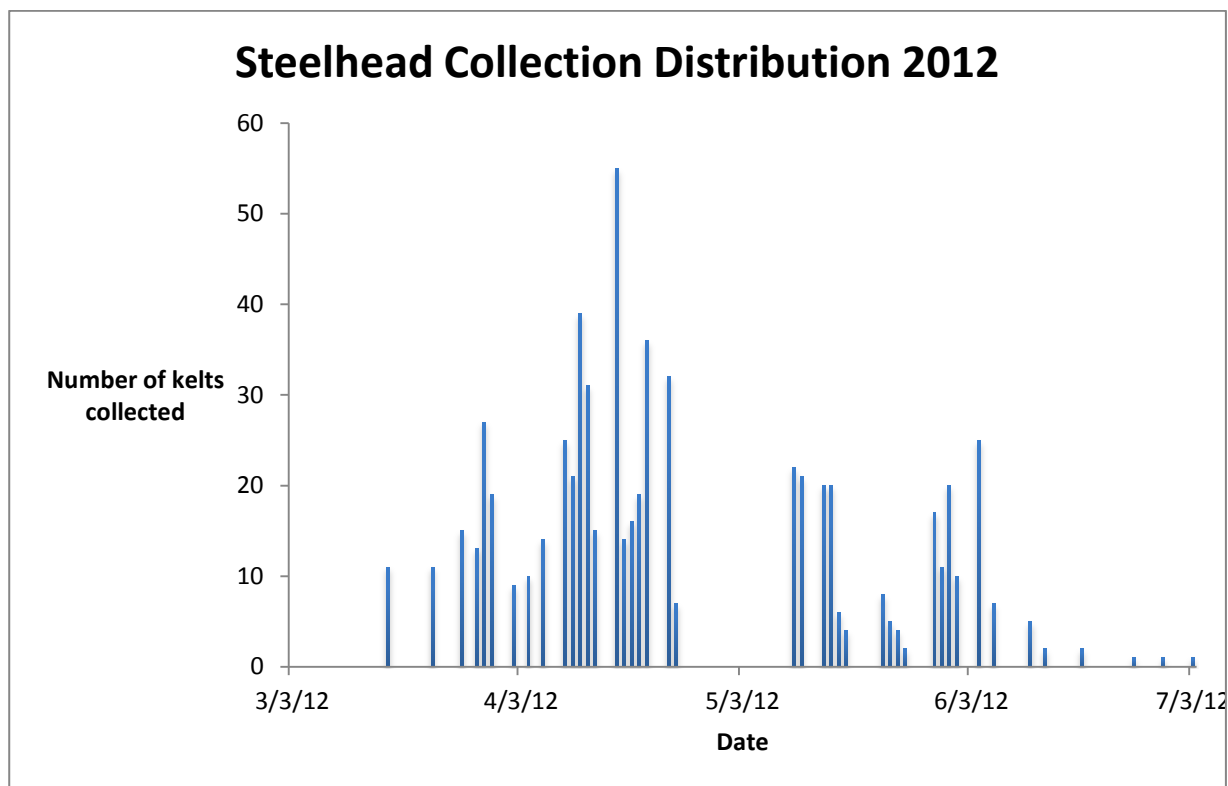


Figure 12: Yakima River kelt Steelhead collection at Prosser, WA 2012.

The majority of kelts captured in 2012 were female which is consistent with previous findings (Hatch et al. 2012). Based on visual observations, 582 of 653 (89%) of the kelts were female, n=71 (11%) were male. Most kelts were classified as fair (n=417, 64%) condition followed by good condition (n=206, 31.5) and finally as poor condition (n=30, 4.5%). Coloration was predominately intermediate (n=370, 57%) or bright (n=247, 38%) with a small percentage that were dark (n=36, 5%).

Snake River

A total of 2,276 kelts were intercepted by the LGR JFF between April 4 and June 28, 2012. 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 (105 fish) occurred on April 24, 2012 (Figure 13). In addition, Pacific Northwest National Laboratory (PNNL) outfitted a subsample of 186 fish with juvenile salmon acoustic telemetry system (JSATS) tags. These fish were held for one day and released (Colotelo et al. 2013). Table 1 summarizes the final disposition of kelts collected by the LGR JFF. There were 46 collection/handling mortalities.

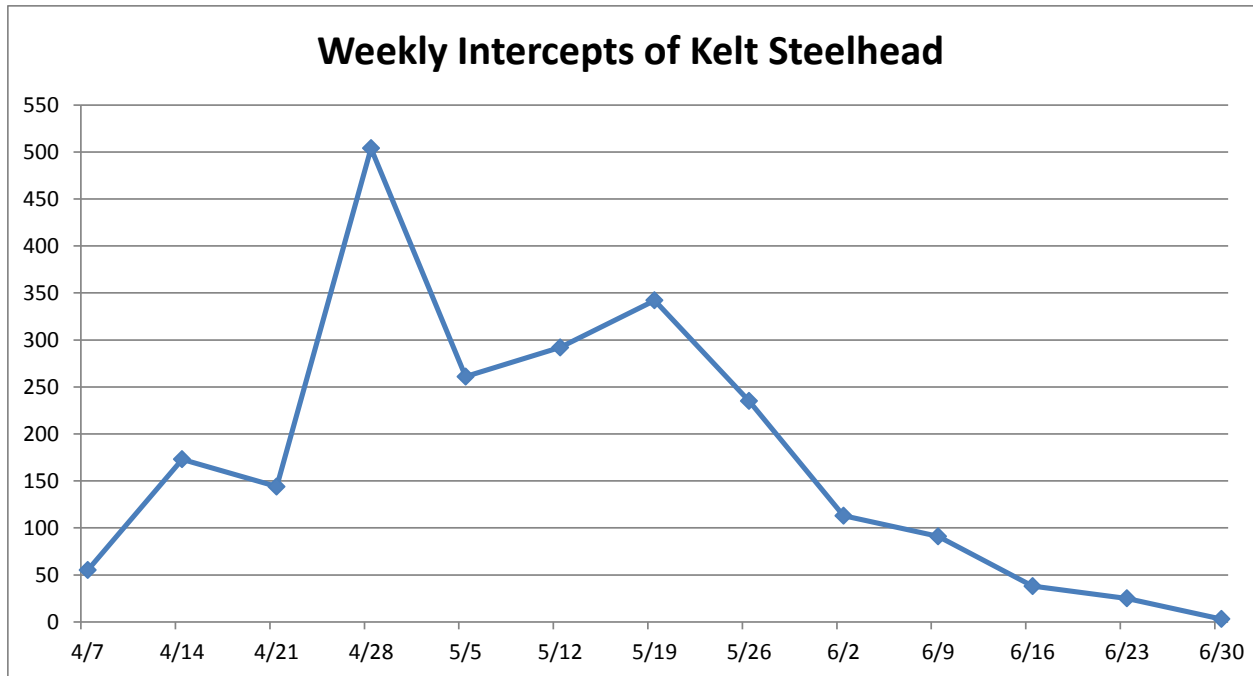


Figure 13: Weekly steelhead kelt interceptions at Lower Granite Juvenile Fish Facility in 2012.

Table 1: Summary of final disposition of fish collected at Lower Granite Juvenile Fish Facility in 2012.

Final Disposition	A-run	B-run	Total
Returned to River	1610	314	1,924
JSAT tagged and released	172	14	186
Transported to DNFH for reconditioning	59	65	124
Mortality	36	10	46
Total	1,874	402	2,276

The majority of the fish collected from the Snake River at LGR JFF in 2012 were A-run females in fair 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). Females greater than or equal to 70 cm comprised 16% of the kelts intercepted at the LGR JFF in 2012 (Table 3). Of these, the proportion rated as being in good condition has decreased since 2010 (Figure 14).

Table 2: Condition of Snake River Kelts collected at the Lower Granite Juvenile Fish Facility in 2012.

	April	May	June	Total
Good	276	200	28	504
Fair	431	546	77	1,054
Poor	222	419	77	718

Table 3: Condition of steelhead kelts by sex and size at the Lower Granite Juvenile Fish Facility in 2012.

Female	Good (22.1)	Fair (46.3%)	Poor (31.6%)	% of collection
< 70 cm	340	644	369	59.6
≥ 70cm	99	191	73	16.0
			Total	75.6
Male				
< 70 cm	58	208	252	22.8
≥ 70cm	6	8	24	1.7
			Total	24.5
Total*	503	1,051	718	

* The gender of four fish was undetermined.

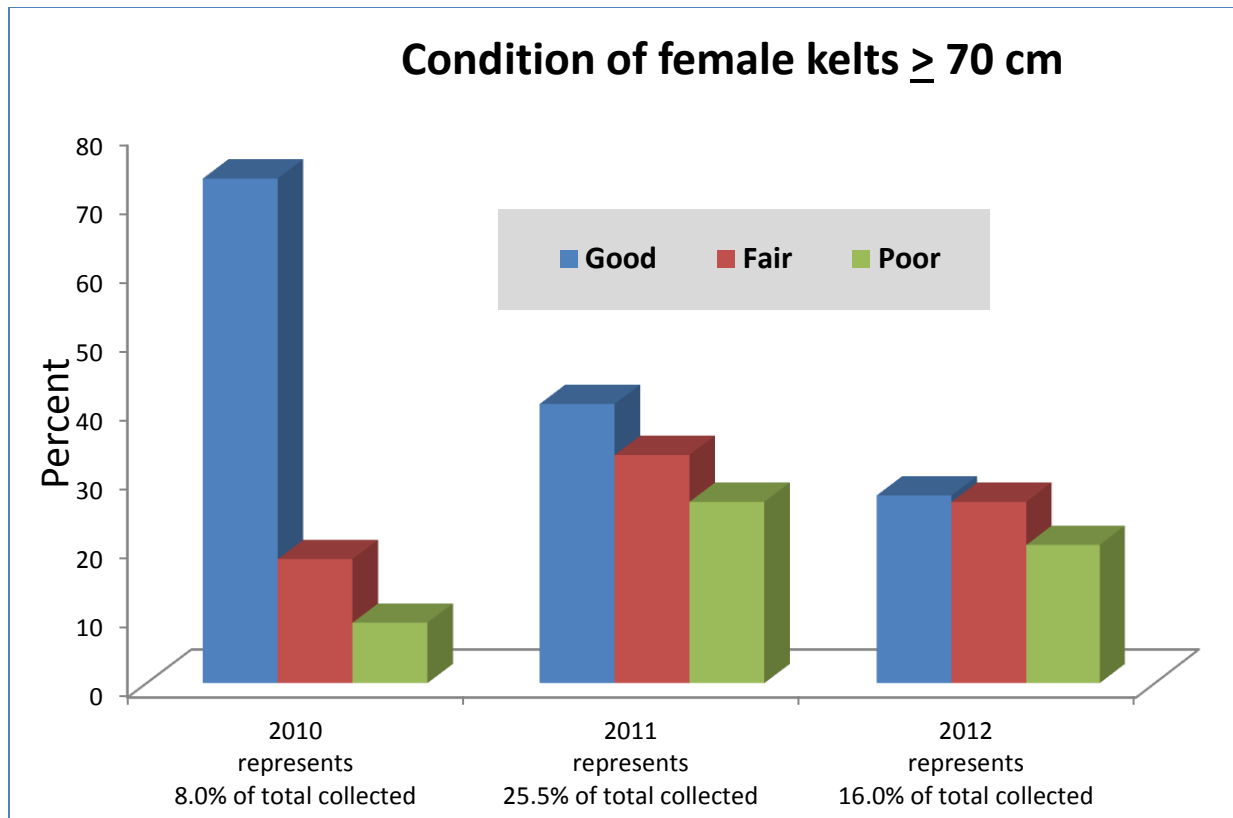


Figure 14: Percent comparison of female kelt steelhead > 70 cm at Lower Granite Juvenile Fish Facility in 2012 by condition and collection year.

Similar to 2010 and 2011, fish were observed with recent head injuries (Hatch et al. 2012). 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 15). 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 51.2% (Table 4). The weekly proportion varied between 24.4% (N=31) and 100% (N=3) throughout the collection season; however, no apparent pattern was observed with discharge (Figure 16).



Figure 15: Typical head injury observed on steelheads kelts at Lower Granite Juvenile Fish Facility.

Table 4: Percent of head injuries on steelhead kelts at the Lower Granite Juvenile Fish Facility in 2012.

	A-run	B-run	Total
No	35.7 (N=813)	6.1 (N=139)	41.8 (N=952)
Yes	46.6 (N=1,061)	11.6 (N=263)	58.2 (N=1,324)
Total	82.3 (N=1,874)	17.7 (N=402)	100 (N=2,276)

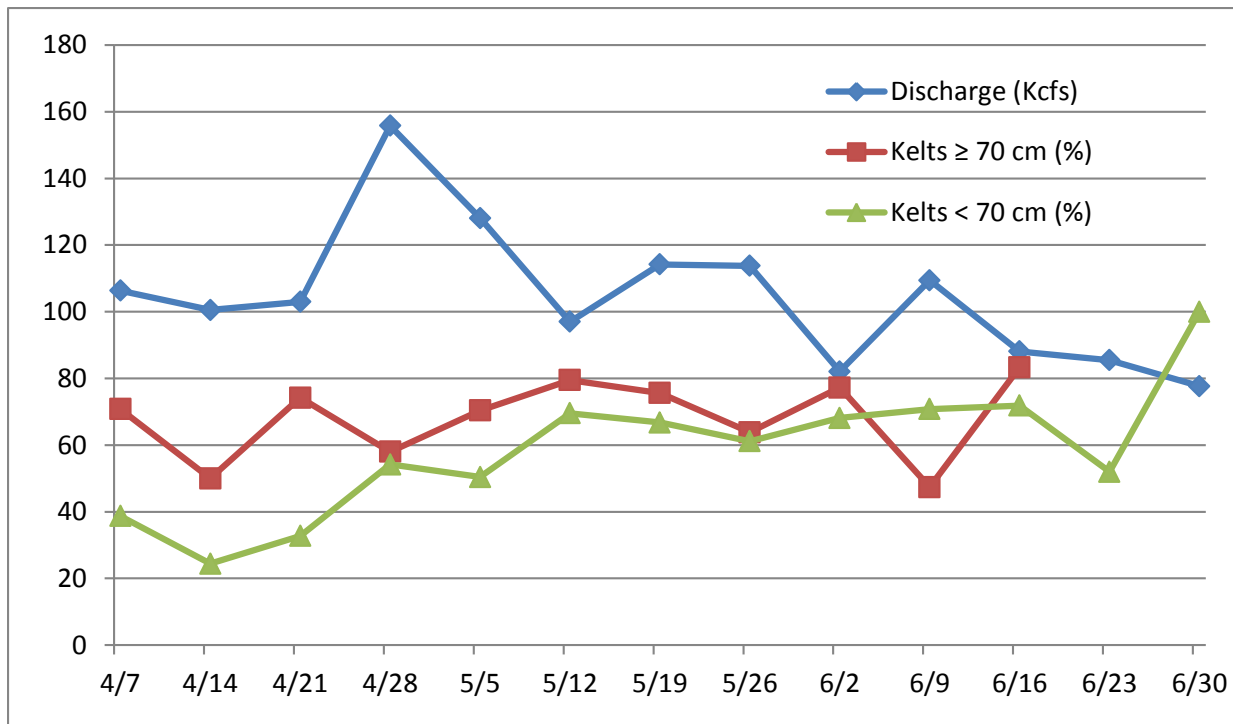


Figure 16: Mean weekly discharge (Kcfs) and percent head injuries observed on steelhead kelts at the Lower Granite Juvenile Fish Facility in 2012.

Omak Creek

The trap was in operation from February 29 through July 29, 2012 and a total of 158 adult summer steelhead were captured and sampled (includes first-time spawners and kelts). Six fish were retained for the Colville captive broodstock program housed at Winthrop National Fish Hatchery (Table 5). There were 41 males and 11 females processed at the trap migrating upriver (Table 5). Weather conditions remained mostly consistent cool with consistent cold river water temperatures throughout the trapping season with couple of high flow events negatively impacting the weir twice (approximately a 2-week shutdown total).

In the downstream trap a total of 106 kelts were collected, which consisted of 65 fish which were retained for reconditioning and 41 kelts, which were dead upon arrival (DOA) at the weir. Seventy-three percent were male (Table 5), which differs from all other collection locations.

Table 5: Percentage and totals of male, female and wild summer steelhead passed above the Omak Creek weir, 2012.

Upstream Sample			Downstream Kelt Sample			
	Total Sample (N)	Non-clipped (N)		Total Sample (N)	DOA Sample (N)	Retained for Reconditioning (N)
Males	41	4	Males	77	32	45
Females	11	6	Females	29	9	20
Total	52	10	Total	106	41	65
All Steelhead Sampled Total:			158			

Bonaparte Creek

No trapping was conducted on Bonaparte Creek in 2012.

Hood River

A total of 10 male and 22 female winter steelhead were collected for gamete and progeny viability studies. This was the second year of collection at the East Fork of the Hood River. Fish captured in 2012 were in much better condition than in 2011 likely due to trap placement improvements.

In-River Release and Return Detection Results

Yakima River

2012 In River Release

There was a total of 59 kelts released as in-river treatment fish into the Yakima River in 2012. There were no detections of these fish attempting to return in 2012 or early 2013. Skip spawning results will be reported in 2013.

2011 In-River Release

Three in-river treatment fish were detected out of the 85 that were released in the Yakima River in 2011. All of the fish were detected passing Bonneville Dam in early to mid-July in 2012 exhibiting a skip spawner life history (Table 6). In the past, we typically observe 60% sequential spawners and 40% skip spawners from in-river release groups in the Yakima River. Three fish successfully passed McNary Dam in mid to late July 2012. Two of these fish have been detected passing upriver at Prosser Dam, with nothing detected passing Priest Rapids Dam or Ice Harbor Dam (Table 6).

Table 6: Yakima in-River releases in 2011 (85 kelts) with return detections by year (2011 sequential spawners and 2012 skip spawners) and dam.

2011-12 Yakima In- River				
Detection Year	Bonneville Dam	McNary Dam	Prosser Dam	% return of total release to Yakima R.
2011	0	0	0	0
2012	3	3	2	2.4%
Total	3	3	2	2.4%

Snake River

2011 In-River Release Detected in 2012

We detected 5 of the 1,613 Snake River kelts that were PIT-tagged and released in-river in the spring of 2011 (Table 10). Two of these kelts were not detected migrating upstream during the fall of 2011; however, they were detected emigrating downstream during the spring of 2012. This indicates they may be expressing a potamodromous type life history and also sequentially spawned. The remaining three kelts are considered skip spawners. Initial detections were first recorded at Bonneville Dam with the first one occurring in early-mid-July 2012 and the other individual passing in early September 2012. Two of the three fish quickly moved through past McNary Dam and held there for about 2 months before resuming migration in the fall (one was detected the following spring in the Imnaha River). The third fish quickly moved from Bonneville to Lower Granite Dam in a matter of weeks (September-October) and was detected the following spring in the Grande Ronde River.

2012 In- River Release

There were a total of 2,093 PIT tagged kelts released as in-river fish into the Snake River in 2012. No kelts were trucked or barged from Lower Granite Dam. All fish were direct released into the tailrace.

2012 Downstream Detection After Release in 2012

There were a total of 18.8% detected (Table 7) migrating downriver. The number of PIT tag 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 2012 separated by gender, length and 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	Tagged and Released			Detected Migrating Downriver			Total
	< 70 cm	≥ 70 cm	Total	< 70 cm	≥ 70 cm	Total	% Detected
Males	504	32	536	104	0	104	19.4
Unclipped	342	26	368	72	0	72	19.6
Clipped	162	6	168	32	0	32	19.0
Females	1259	294	1553	255	42	297	19.1
Unclipped	663	220	883	140	29	169	19.1
Clipped	596	74	670	115	13	128	19.1
2012 Total	1767	326	2093	359	42	401	19.2

Table 8. First (bold), second, and third and subsequent (in parentheses) detections of kelts collected and released with PIT tags at Lower Granite Dam in 2012 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 were 18 steelhead that were tagged and identified as pre-spawn adults and later detected ascending the Lower Granite adult fish ladder after release. These 18 steelhead adults were possibly fish that fell back through the juvenile fish bypass.

2012	Little Goose	Lower Monumental	Ice Harbor	McNary	John Day	Bonneville	Towed array
Little Goose	199	17	6 (1)	7 (3)	4 (5)	2	1
Lower Mon.		89	3	3 (1)	2	5 (1)	0
Ice Harbor			36	4	1	0	0
McNary				41	1	0	0
John Day					27	2	0
Bonneville						23	0
Towed Array							3

2012 Return Detection

One kelt sequential spawner was detected returning to Bonneville on August 12, 2012 (Table 9). This fish was tagged and released into the Snake River below Lower Granite Dam on April 15, 2012. On August 27, 2012 this fish successfully navigated past Lower Granite Dam and was later detected in Joseph Creek of the Grande Ronde River basin in early March 2013.

Skip spawning for this year class will be determined in the 2013 annual report.

Table 9: 2011 Snake In-River release with return year and detection site (2011 and 2012) and the number detected at each dam.

2011 Snake In-River Releases							
Total Tagged and Released: 1,613							
Return Year	Bonneville Dam	McNary Dam	Ice Harbor Dam	Lower Monumental Dam	Little Goose Dam	Lower Granite Dam	% return of total release to Snake R.
2011	0	0	1 (spring 2012)*	1 (spring 2012)*	1 (spring 2012)*	0	0.12%
2012	3	3	0	0	0	3	0.19%
Total	3	3	1	1	1	3	0.31%

***Detection occurring (season and year)**

Section B. 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 coincident with steelhead migratory return 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 four locations with fish collected from the Prosser, Lower Granite Dam, Parkdale, and Omak. This details results of long-term reconditioning studies at each of these locations in 2012.

Study Area

Prosser Hatchery

Prosser Hatchery is located on the Yakima River just downstream of Prosser Dam (RK 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 the primary function of rearing, acclimating, and releasing fall chinook salmon (*O. tshawytscha*). It is also used for rearing coho salmon (*O. kisutch*) prior to acclimation and release in the upper Yakima River Basin (Figure 1) as well as experimental rearing of white sturgeon (*Acipenser transmontanus*) and Pacific lamprey (*Lampetra tridentate*).

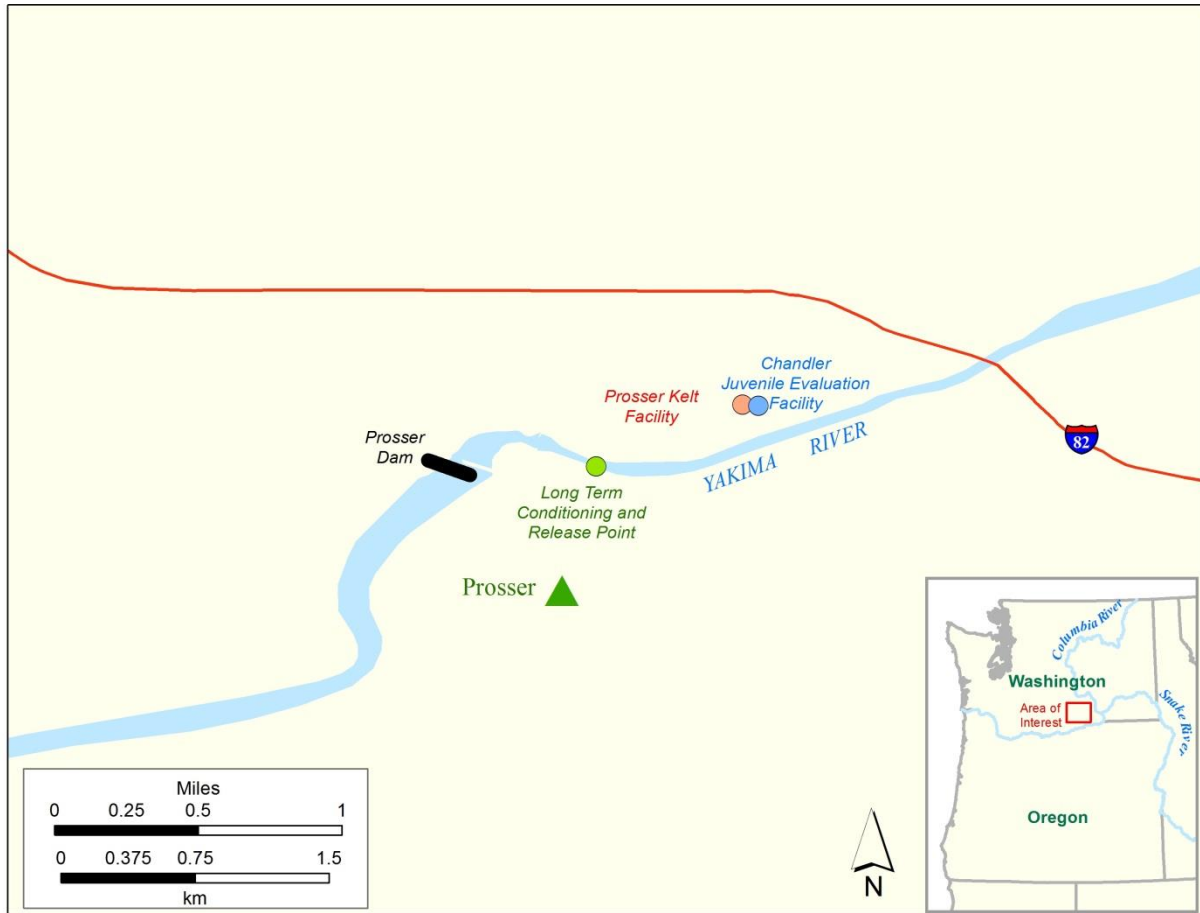


Figure 1: Map showing the location of Prosser Dam and the kelt reconditioning facility at Prosser, WA.

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 (RK 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).

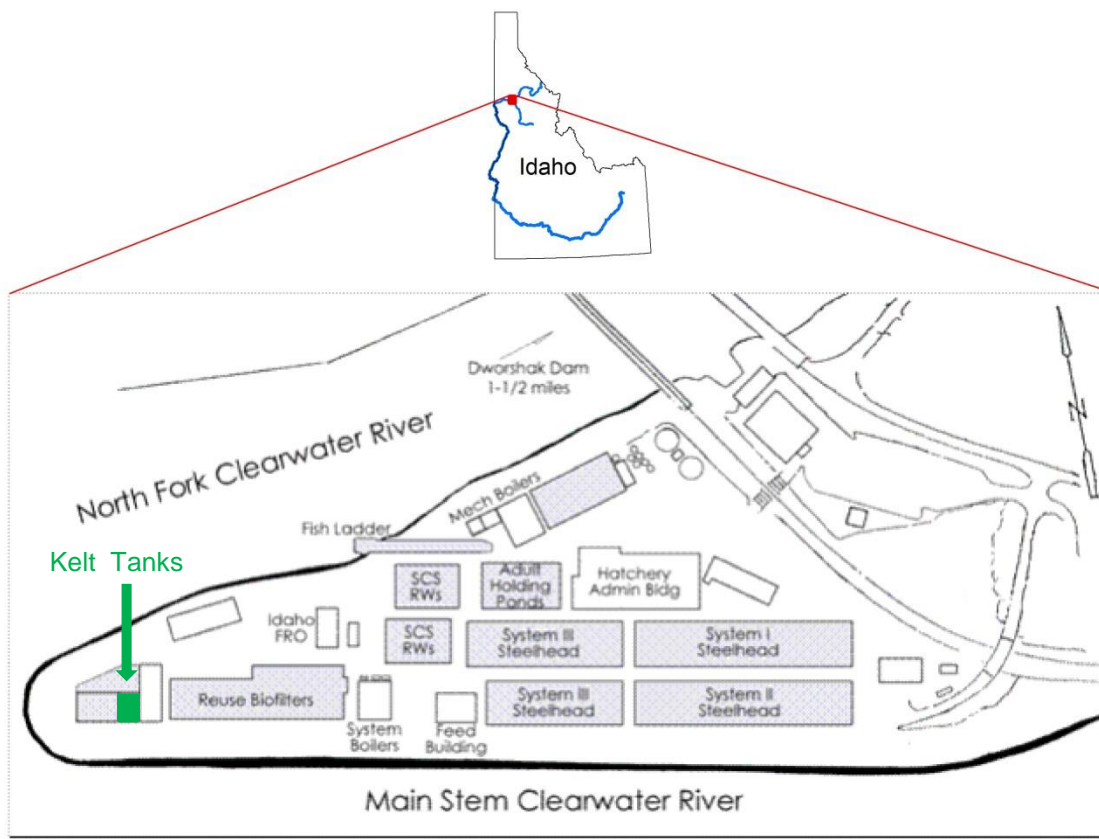


Figure 2: Map showing the location of experimental kelt reconditioning tanks at Dworshak National Fish Hatchery. Figure modified from USFWS 2009.

St. Maries Mission Acclimation Pond

Omak Creek kelt steelhead were reconditioned at the St. Maries acclimation pond located at RK 8.0 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.

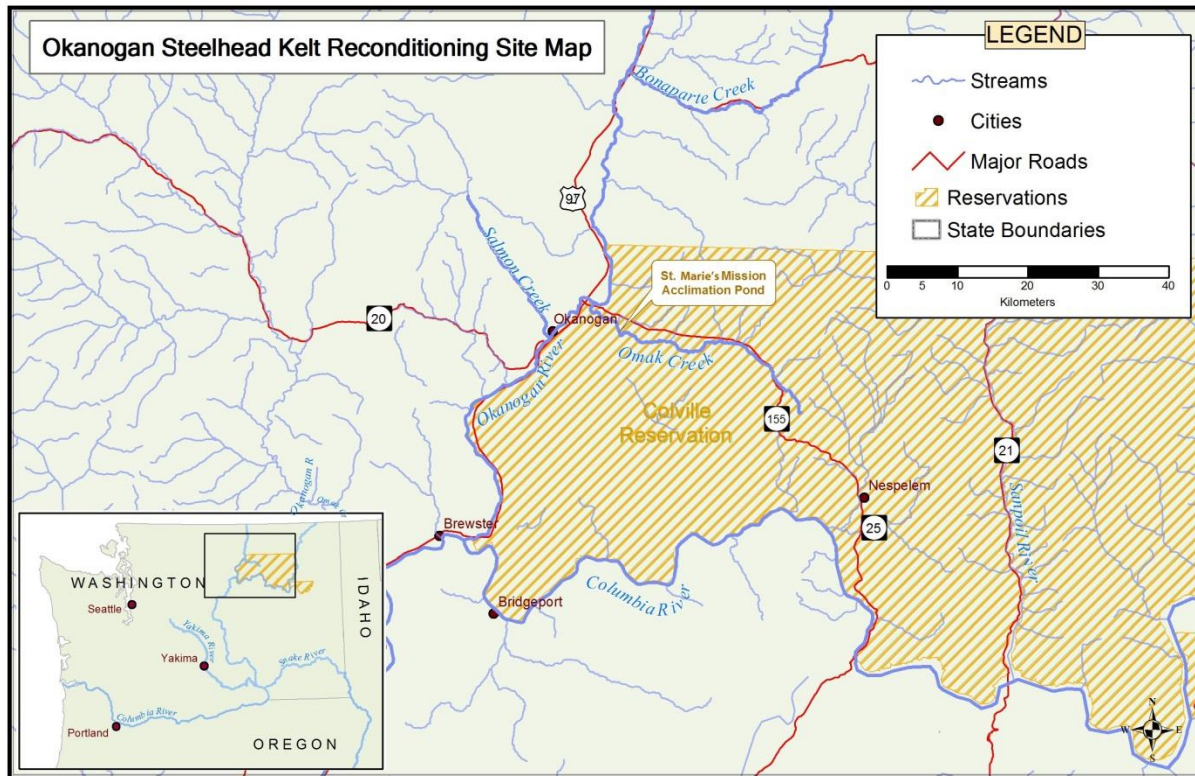


Figure 3: Map showing the locations of Omak Creek, the St. Marie's Mission Acclimation Pond, and the Confederated Tribes of the Colville Reservation.

Parkdale Fish Facility

Steelhead kelt reconditioning for the Hood River was performed at the Parkdale Fish Facility located at RK 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.

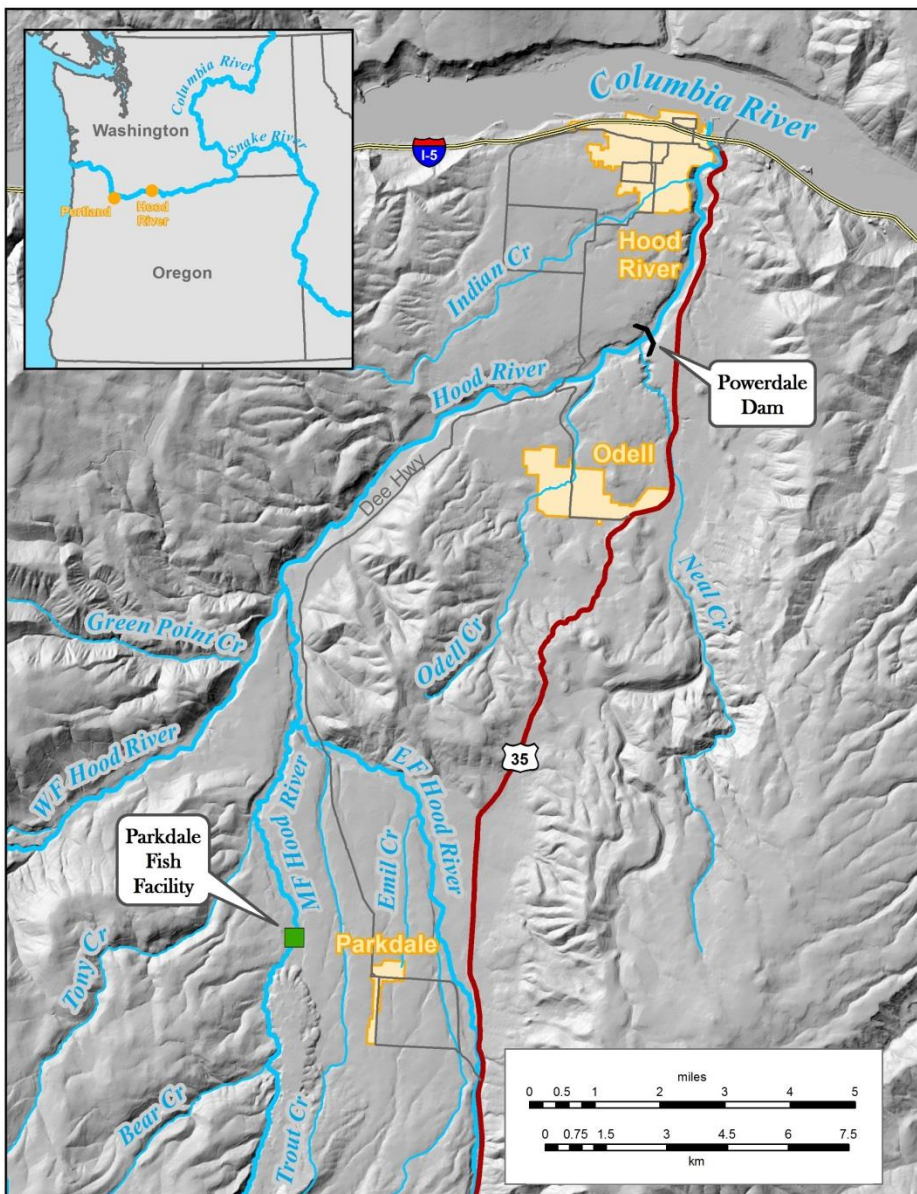


Figure 4: Map showing the location of Parkdale Fish Facility and East Fork Hood River weir.

Methods

Long-term Reconditioning Facilities

Prosser Hatchery

Transport to Prosser Fish Hatchery

Kelts were 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. 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°C well water at 757 liters/minute (l/m).



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 IvermectinTM (Johnson and Heindal 2000) or emamectin benzoate. IvermectinTM 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. In 2012 the CRITFC and Yakama Nation expanded the trial of emamectin benzoate to the majority of kelts with approximately 10% still receiving Ivermectin. The drug was administered via injection to the peritoneal cavity for the treatment of copepods (Glover et al 2010). Study results are presented in Chapter 2, Section G. 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 1.5-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). 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. Low-pressure compressed air was injected into the fish using a 20-gauge needle. Eggs were allowed to flow freely with some gentle massage to obtain the remainder. 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, 38mm 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 12 mm PIT tag was inserted with a sterile 8-gauge trocar midway between the pelvic fins (Figure 8). Length and weight were recorded. Fish received an injection of oxy-tetracycline. After sampling, each fish was placed in a recovery tank for observation prior to transfer to the kelt reconditioning tanks.

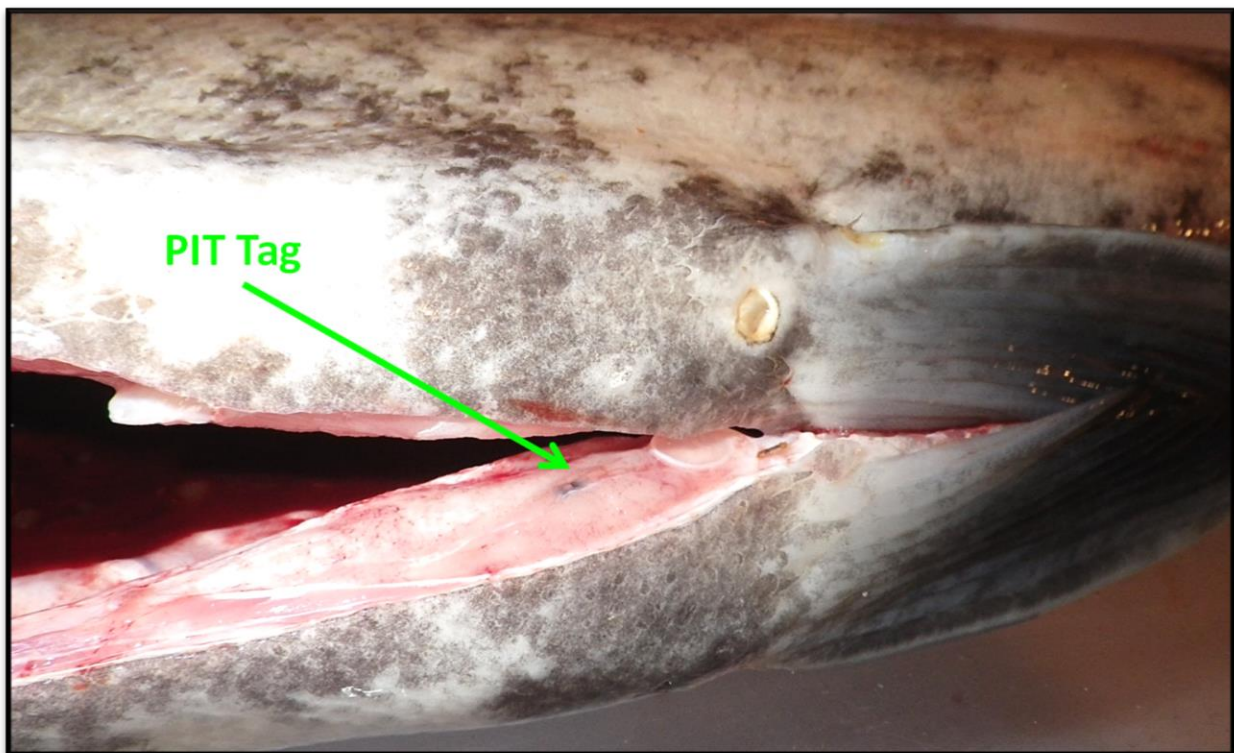


Figure 8: Necropsied steelhead kelt showing pelvic girdle and proper PIT tag location.

Reconditioning Facility and Treatment

Four 4.5m diameter tanks are located at DNFH (Figure 9). River water was provided from a fire maintenance supply line at a flow rate of 3.78 l/m 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 assembly supported by external posts is installed on the inflow to each kelt tank. Each tank has four diffusers connected to a

continually operating aeration pump. Flow, temperature, and dissolved gas levels were constantly monitored and logged using a hydrolab. In June, an emergency monitoring system was installed on each tank. Dissolved oxygen probes and flow meters were connected to an alarm system and data logger. This system allowed real time access to flow and dissolved oxygen data via a remote Internet connection. In the event of an emergency water loss, oxygen and two back-up water sources are available.

As 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 9: Experimental kelt reconditioning tanks at DNFH with anti-jump containment curtains and four bucket Koch ring packed columns.

St. Maries 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 1.5 kL tank truck to St. Maries acclimation pond 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 110C water up to 2.082 kl/m (Figure 10). 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 alga growth in the pond. An initial injection of liquamycin was administered based on recommended WDFW rates. Every 2-months, fish were checked for copepods, and given emamectin benzoate 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.

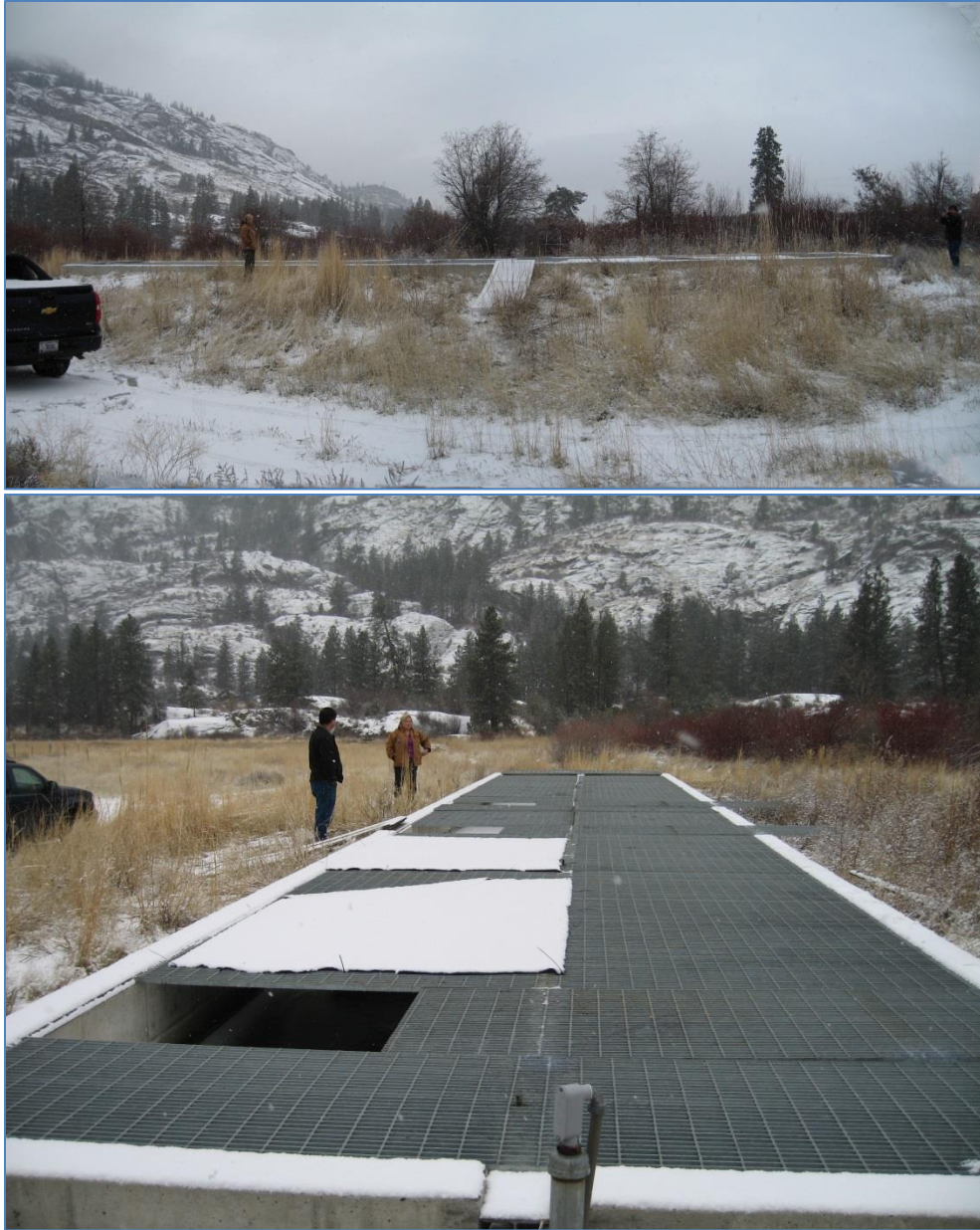


Figure 10: St. Maries Acclimation Pond Omak, WA. Top (side profile), bottom (above)

Parkdale Fish Facility

Transport to Parkdale Fish Facility

Adult steelhead were collected at the East Fork of the Hood River located at RK 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 11). 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 kL/m for reconditioning (Figure 12). Post spawn females were administered another dosage of Ivermectin after completion of air spawning. All kelts were checked in June and again in September for the presence of copepods and administered additional emamectin benzoate treatment if copepods were present. The antibiotics (oxytetracycline and erythromycin) were also administered prophylactically to prevent against cold-water disease in June and reapplied when bacterial related mortality occurred.



Figure 11: Parkdale Fish Facility raceways where kelts are held from late fall to early spring.



Figure 12: Circular tanks at Parkdale Fish Facility used seasonally (late spring into 2013) 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 on the timing of incoming kelts. Kelts then are transitioned to a pellet based diet. In 2012, the Moore-Clark broodstock pellet manufactured by bio-Oregon was discontinued and Prosser and Dworshak transitioned to the bio-brood pellet manufactured by bio-Oregon while Parkdale kelts have been on this diet since 2007. 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 (krill, cod liver oil, herring, squid) is the primary food type fed to kelts, while at Parkdale, natural feed (krill, Alaska fish oil, cyclopeeze, is offered throughout the reconditioning process.

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. PIT tags were removed from mortalities and identification numbers recorded onto computer databases along with growth measurement data. 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. Maries are released approximately at RK 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 hatchery fish reconditioned at Dworshak, which were retained or terminated for important physiological data for future indices or fish health reasons. Please See Chapter 2 (Section H) for further details.

Results

Long-Term Reconditioning and Survival to Release or Spawning

Prosser Fish Hatchery

A total of 572 kelt steelhead were collected and placed in the long-term reconditioning tanks. Survival to pre-release processing on October 10, 2012 was 60.8% (Table 1)(Figure 13). A total of 333 long-term reconditioned fish were released to the Yakima River on October 29, 2012. Steelhead kelts were released, approximately 1-month later than usual, due to the presence of a WDFW sport fishery near the release site. This release site is useful for determining parentage analysis that is based on detections of fish migrating through Prosser Dam (See Ch 4. Section C). Most migratory movements occurred in late October through November of 2012 but there were additional kelts detected migrating upriver in February/March of 2013. As of May 2013, 231 (66%) fish from the long-term release were detected by PIT tag presence migrating past Prosser Dam.

Table 1: Long-term reconditioning results by tank 2012 at Prosser Hatchery.

Long-term Reconditioning									
Tank									Long-term
	C1	C2	C3	C4	S1	S2	S3	S4	Total
Held for Reconditioning	122	122	123	121	21	21	21	21	572
Surviving fish on 10/10/2012	64	86	62	73	16	15	18	14	348
Survival Rate	52.5%	70.5%	50.4%	60.3%	76.2%	71.4%	85.7%	66.7%	60.8%



Figure 13. Long term reconditioned kelt steelhead from the Yakima River just prior to release in 2012 (Joe Blodgett pictured).

2011 Skip Spawning Long-Term Reconditioned Kelts through 2012

There were a total of 32 kelts from the 2011 long-term reconditioning program that were retained to determine how well they would recondition through 2012. Nine of these fish survived to release in the fall of 2012. As of May 2013, 6 of these fish have been detected migrating upriver over the Prosser Dam, presumably to spawn. Blood samples are currently being analyzed to determine sex hormones in these fish and will be published in the 2013 report.

2011 Long-term Reconditioned kelt PIT-tag detections in the Columbia River

A skip spawning migration pattern was observed in 1 previously long-term reconditioned kelts from the 2011 release that had a PIT-tag detection at Bonneville Dam in late summer (August) of 2012. No further upstream detection was made of this fish. We presume that this fish spawned in 2012 and was attempting to return from the ocean to attempt a third spawning in 2013.

Dworshak National Fish Hatchery.

Reconditioning

A total of 124 fish were transferred from the LGR JFF to DNFH for reconditioning (Table 2). Fish survival averaged 24.9 days after transfer to the reconditioning tanks (Figure 14). We experienced two periods of increased daily mortalities in the transferred fish (Figure 15). The first occurred approximately three weeks after the first fish transfer and lasted three weeks. We recorded significant fungal development (25-50% external coverage) on the majority of these fish (Figure 16). This spike in mortalities coincides with an emergency fire maintenance supply line shutdown. From May 2 to May 18, 2012, the water supply to the kept tanks changed to a 4 inch pump submerged into the hatchery settling pond. This water was warmer and contained various concentrations of hatchery effluent. The second period occurred approximately two months after the first fish transfer and lasted two weeks. Again, we recorded significant fungal development (25-50% external coverage) on the majority of these fish. We attribute these mortalities to poorer condition fish being transferred into the facility, as well as lingering effects of exposure to the settling pond's effluent water. There were a total of 9 (unclipped) fish, which were released to the Snake River in the fall of 2012 to coincide with the returning steelhead run.

Table 2: Snake River steelhead kelts collected at Lower Granite Juvenile Fish Facility and transferred to Dworshak National Fish Hatchery for reconditioning in 2012.

	A-run	B-run	Total
Adipose Clipped	4	5	8
Un-Clipped	37	65	103
Total	72	52	124

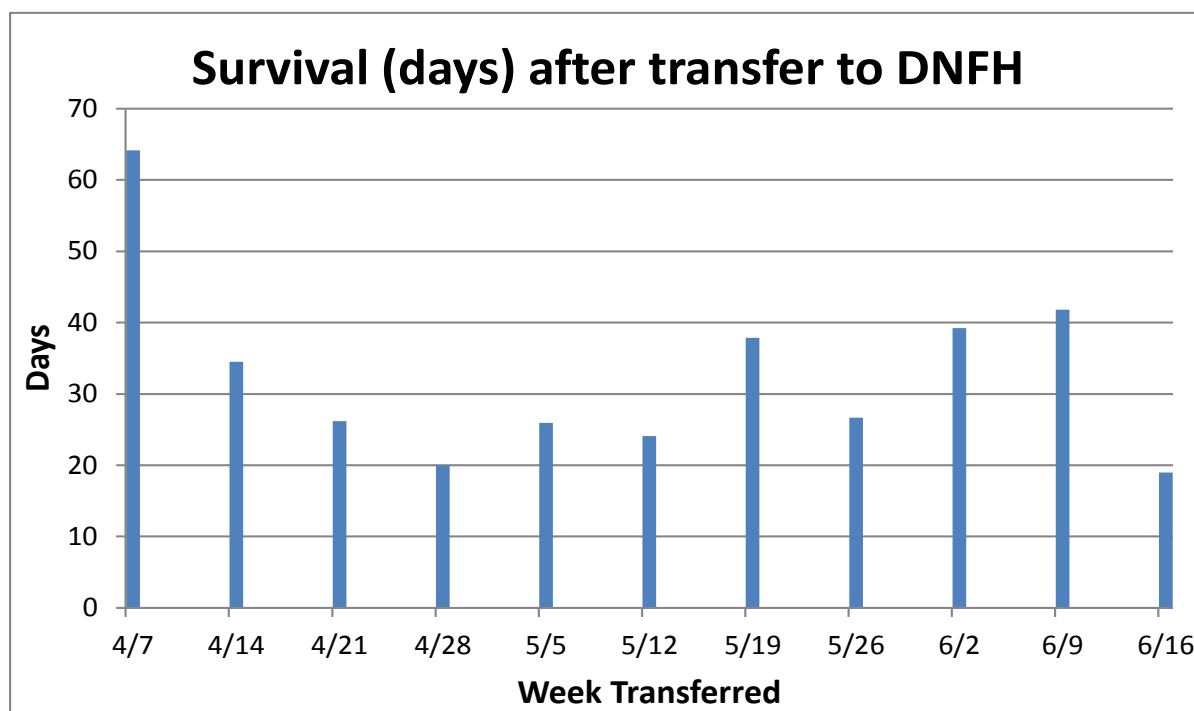


Figure 14: Mean weekly survival (days) of steelhead kelts transferred from Lower Granite Juvenile Fish Facility to Dworshak National Fish Hatchery for reconditioning in 2012.

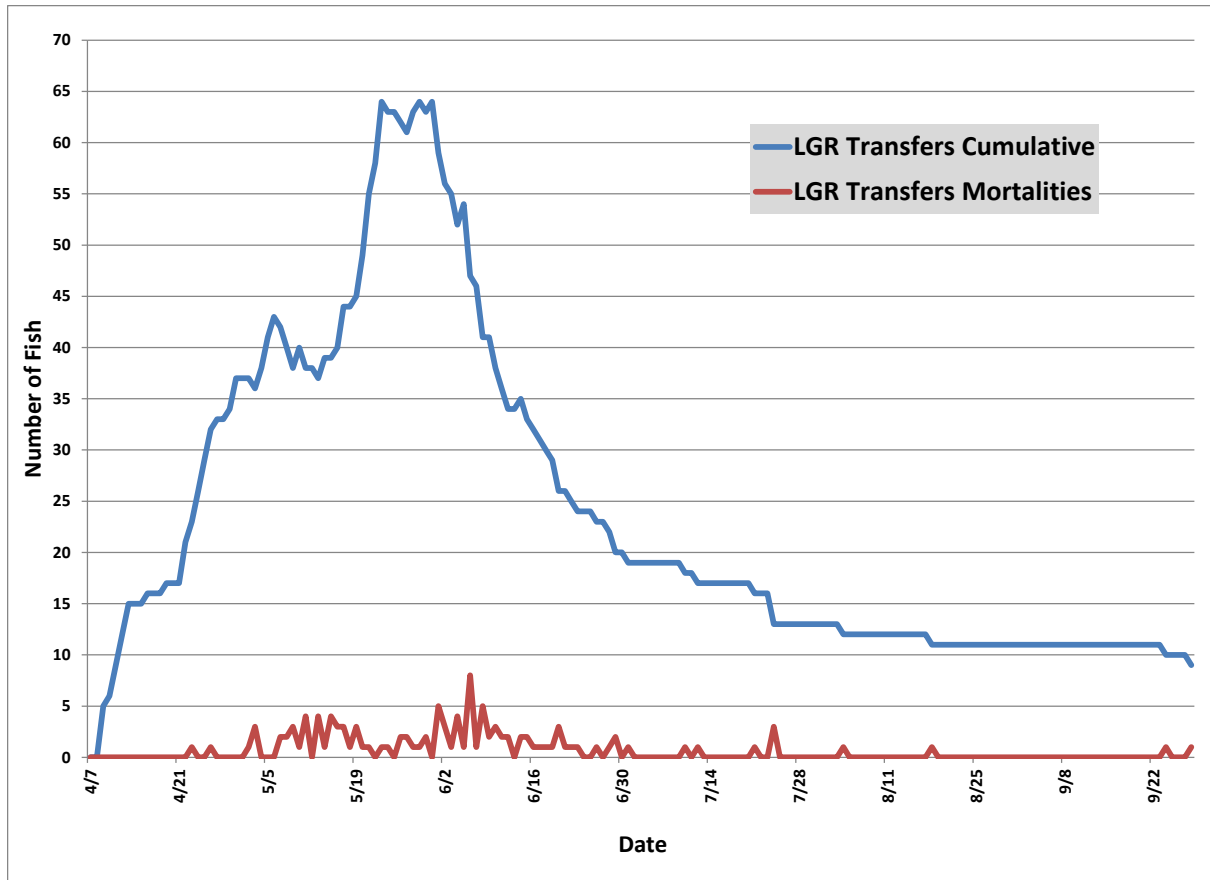


Figure 15: Cumulative on-station holding and daily mortality of steelhead kelts transferred from Lower Granite Juvenile Fish Facility to Dworshak National Fish Hatchery for reconditioning in 2012.



Figure 16: Common fungus development on mortalities of steelhead kelts transferred from Lower Granite Juvenile Fish Facility to Dworshak National Fish Hatchery for reconditioning in 2012.

Air-Spawned Steelhead

A total of 143-steelhead were air-spawned on February 7 and 8, 2012. Approximately three weeks after spawning, we experienced a substantial increase in daily mortalities (Figure 17). These mortalities continued for four weeks with a peak of 11 on March 7, 2012. We recorded significant fungal development (25-50% external coverage) on the majority of these fish. We attribute this increase partially to a 3 week delay in the beginning of the formalin bath treatments. Interestingly, staff at DNFH noted a higher than normal degree of fungus on their adults in holding during the fall of 2011. A total of 5 of the remaining fish were successfully reconditioned. These fish were lethally sampled on September 25, 2012 (see Ch 2, Sec. H).

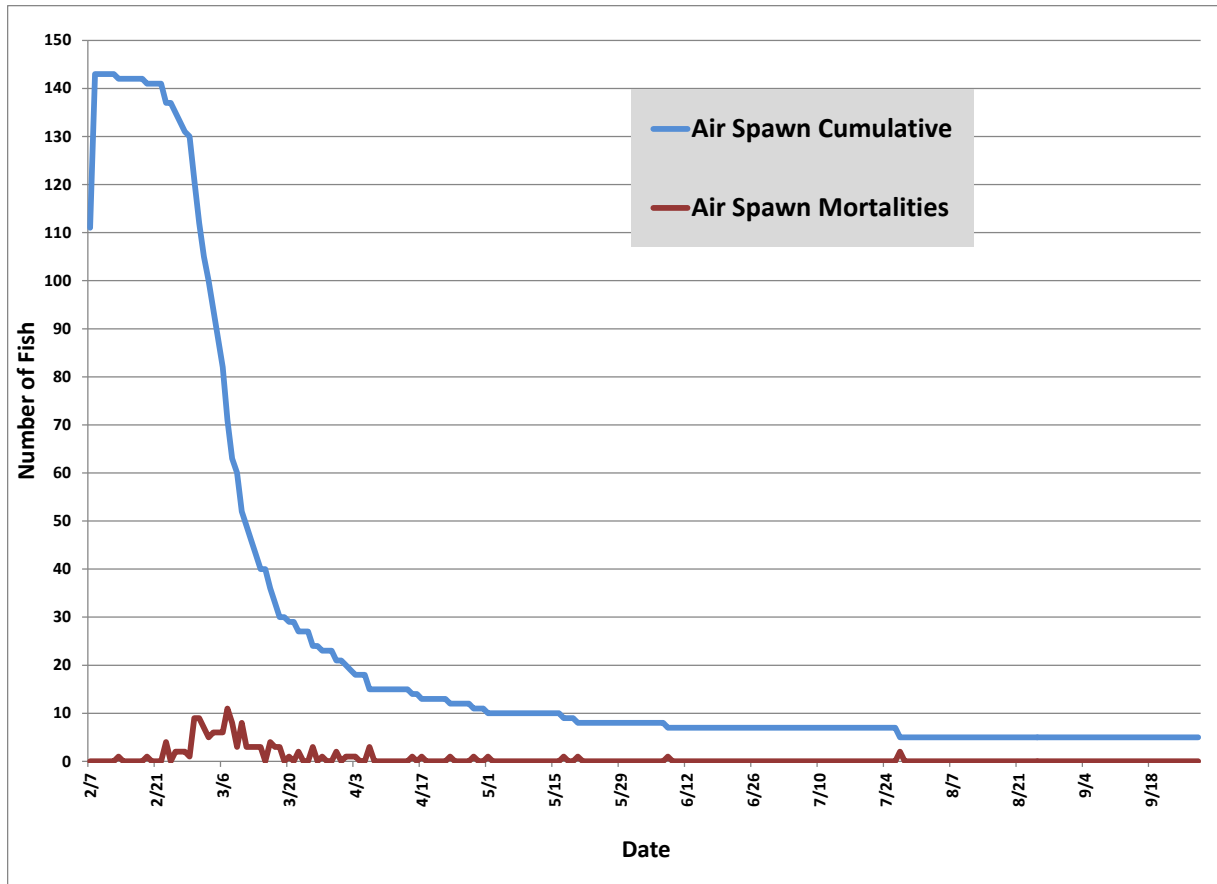


Figure 17: Cumulative on-station holding and daily mortality of steelhead kelts air-spawned at Dworshak National Fish Hatchery for reconditioning in 2012.

Reconditioned Kelts Released in 2012

We detected 5 of the 9 reconditioned kelts that were PIT-tagged and released into the Snake River in the fall 2012 (Table 3). Fish were released 3.5 km below Lower Granite Dam on October 2, 2012. There were 4 fish which were considered A-run and 5 B-run fish (Table 3). Four of these kelts were detected ascending the adult ladder at Lower Granite Dam between 2 and 5 days after release (2 A run and 2 B-run kelts). This indicates they may have sequentially spawned. Of these, one was detected in the upper Salmon River (Big Creek) the following spring. The remaining kelt (B-run) was detected moving downstream during the winter of 2012. This fish may be expressing a potamodromous type life history.

Table 3: Number of reconditioned kelts detected after release in 2012 separated by length and detected at least once in the Snake or Columbia rivers.

	Reconditioned and Released			Detected Migrating Downriver			Detected Migrating Upstream		
	< 70 cm	≥ 70 cm	Total	< 70 cm	≥ 70 cm	Total	< 70 cm	≥ 70 cm	Total
Unclipped Females	4	5	9	0	1	1	2	2	4

Table 10: 2012 Reconditioned kelts released with detection year and site and the number detected at each dam.

2012 Reconditioned Kelt Releases			
Total Tagged and Released: 9			
Detection Year	Lower Monumental Dam	Lower Granite Dam	% detected
2012	1 (winter)	4 (fall)	55.6%

St. Maries Fish Acclimation Site

Reconditioning

There were 65 post spawn kelts that were trucked to St. Marie's Acclimation Pond of which 54 were hatchery fish and 11 were natural origin. In May of 2012 we had a vandalism incident that resulted in the immediate mortality of 2 fish and subsequent mortality of 4 additional fish. It should be noted that the majority of kelts that were attempted to be reconditioned were males 45 versus the small amount of females 20. Typically, we usually avoid reconditioning male kelts due to them being in poor condition and having low survival rates but the project biologist attempted to recondition them based on their large numbers hoping that at least a sizeable portion would recondition. There will be no additional attempts to recondition large numbers of male kelts in the future at St. Maries in the near future.

There were 4 female steelhead kelts released into the mouth of the Okanagon River (2 hatchery, 2 natural origin) in late September. Upon visual inspection 3 of these fish were in good to excellent condition (Figure 18).



Figure 18: Long-term reconditioned Omak Creek kelt just prior to release in 2012.

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 2 remaining Skamania female steelhead by the beginning of 2012. Both of these fish were spawned in 2012 (Table 4). One fish remained at the beginning of 2013.

2009 Brood

One steelhead kelt successfully reconditioned for the 3rd time and was spawned in 2012 (Table 4). This brood year was the only kelt group which had all skip spawners. One fish remained at the beginning of 2013.

Table 4. Summer (Skamania) steelhead kelt reconditioning success and spawning by year 2006-2012. 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%	33%	13%
Skip Spawner kelt	0	1	3	2	2
% skip spawner of reconditioned fish	0%	8%	21%	22%	13%
2nd sequential spawning	0	0	2	1	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 were initially captured at the Powerdale trap in 2010. The removal of Powerdale dam changed the capture location in 2011 to the East Fork Hood River resistance board weir trap.

2012 Brood

This was the second year for capture of kelts at the East Fork of the Hood River weir. Fish were in better condition than in 2011 and a more proactive approach to disease treatment was used. There were 14 kelts at the beginning of 2013 (Table 5).

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 1/3rd of the fish to fungal infections in 2011.

The rest were lost either to cold-water disease or *C. shasta* infections. This left only 3 fish to attempt to recondition in 2012, one of which was successfully reconditioned (Table 5).

2010 Brood

This was the first year of reconditioning winter steelhead at Parkdale. These fish were captured at the Powerdale trap before it was dismantled. In 2012 a total of 2 female kelts were spawned in the spring. By the end of the 2011 we had 2 remaining fish (Table 5).

Table 5: Winter Kelt Reconditioning 2010-2012. TBD=To Be Determined. IHN= Infectious hematopoietic necrosis.

Brood Year	2010	2011	2012
Alive as of 1/2013	0	0	14
Maiden spawn	22 (3 culled IHN)	22	22
1st sequential spawning	5	1	TBD
Succ. Recon Rate %	32%	4%	TBD
Skip Spawner	0	0	TBD
% kelt skip spawner of reconditioned fish	0	0	TBD
2nd sequential spawning	1	0	TBD

Section C. 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, and in 2011 (Hatch et al. 2012) we primarily compare results for just that year and means across years. This year we have complete return history for transported groups since skip spawners from the 2011 release returned to the Columbia River in the summer of 2012, so we compare all years in this report.

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 $2\times (4/2=2)$ versus leaving the kelts in the river. Comparisons were made within each year and across years using weighted means to account for different sample sizes among years.

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

Results and Discussion

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

Comparison groups

Our comparison or control groups consisted of 1. The proportion of repeat spawners in the run at large at Bonneville Dam; 2. The return rate to Bonneville Dam of fish PIT tagged and released at Prosser Hatchery; 3. The return rate to Bonneville Dam of fish PIT tagged and released at John Day Dam; The return rate to Bonneville Dam of fish PIT tagged and released at Lower Granite; and, the reported proportion of repeat spawners in the run at Prosser Dam based on scale pattern interpretation (Hockersmith et al. 1995) (Table 1). The proportion of repeat spawners in the run at large at Bonneville Dam is based on scale pattern interpretation of 9 years of data collected from 14,758 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, Kelsey et al. 2011a, Kelsey et al. 2011b, Nowinski et al. 2013a, Nowinski et al. 2013b). The weighted mean composition of repeat spawners in the run at large at Bonneville Dam is 0.49%. This indicates that iteroparity is very low in steelhead populations above Bonneville Dam and in 2012 the return rate was 0.38%. The return rate to Bonneville Dam of kelts tagged and released in-river at Prosser Hatchery in 2011 (the last year with complete returns sequential and skip spawners) was 3.53% and the 8 year average of 3.08% 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 0.18%. The 7 year mean return rate to Bonneville Dam for kelts tagged and released at Lower Granite Dam is 0.38%. This is quite low and not statistically different (pooled variance t test; $t=0.407$; $p=0.691$) from the run at large at Bonneville Dam.

	Table 1. The return rate in 2011 (last year with complete returns of sequential and skip spawners) 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*	John Day	Prosser	Lower Granite	Hockersmith*
2011	0.38	-	3.53	0.18	-
mean	0.49	9.76	2.87	0.38	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 Westport, OR. The 6 year mean return rate to Bonneville Dam for fish collected at Lower Granite Dam and transported is 1.12. Four kelts were detected returning to Bonneville Dam from fish collected at Prosser Dam and transported to Hamilton Island (1 fish) and Westport (3 fish). Return rates of Prosser collected fish to Bonneville Dam were 1.00% for the Hamilton Island release and 3.33% for the Westport release. Both of these return rates are lower than the 9 year mean return rate of 4.16%.

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 higher treatment benefits of 2.58, 8.77, and 2.01 relative to in-river, the steelhead run at large at Bonneville Dam, and the Hockersmith value of 1.66, respectively. This increase is due to 2 skip spawner returns in 2012. As previously noted, any number greater than 1 is a positive benefit and any number less than 1 is a negative benefit. Nine years of transport data from Prosser origin kelts shows some benefits to transported kelts relative to control groups: 1.35, 8.55, and 2.64 relative to in-river, the steelhead run at large at Bonneville Dam, and the Hockersmith value of 1.66, respectively.

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, and increased returns to Bonneville Dam with 2 skip spawners returning in 2012.

In 2012, survival of long-term reconditioned groups was 61.8% for Prosser, 20 % (6.2% counting males too) for Omak, and 61.9% for Parkdale (Figure 1). Survival for the Prosser and Parkdale fish was above average, and survival for Omak fish was below average. This data 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 17.5 times survival advantage over the 8 year average return rate to Bonneville Dam for fish left in the river (Figure 2). We used the 8 year mean for comparison since the 2012 in-river 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 162.7 times survival advantage, those from Omak Creek had a 52.6 times advantage, and steelhead from Parkdale had a 162.9 times advantage (Figure 2). Long-term reconditioning shows great promise as a tool for restoration based on this data.

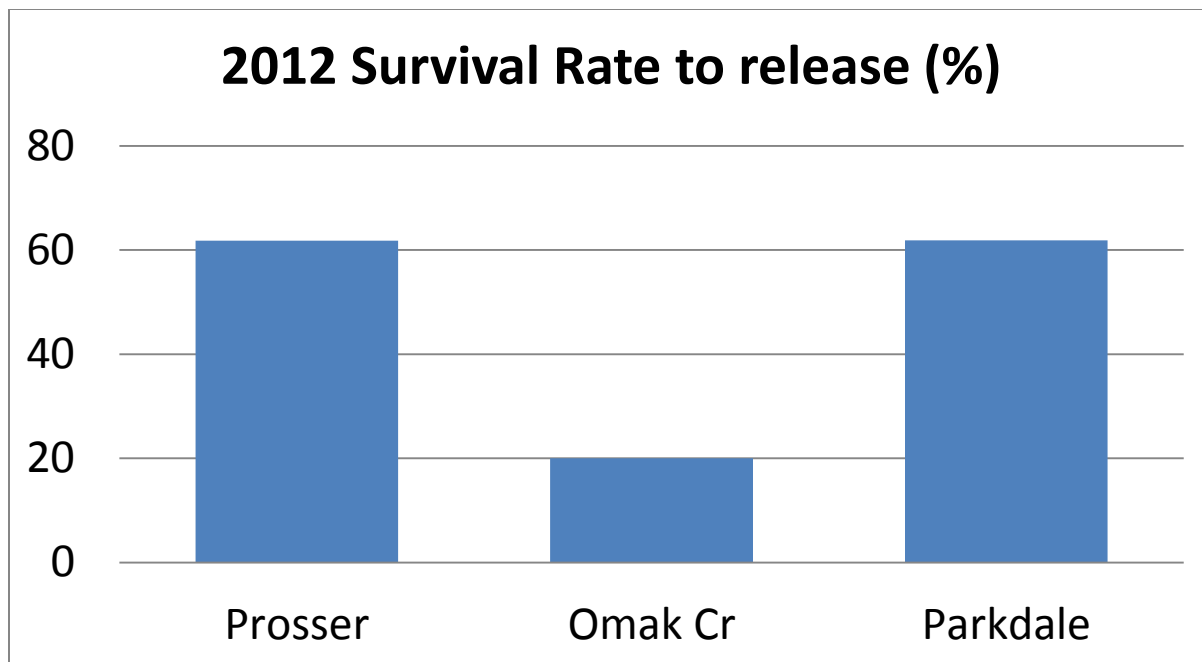


Figure 1: Survival rate (%) of long-term reconditioned kelt steelhead at 3 locations in 2012.

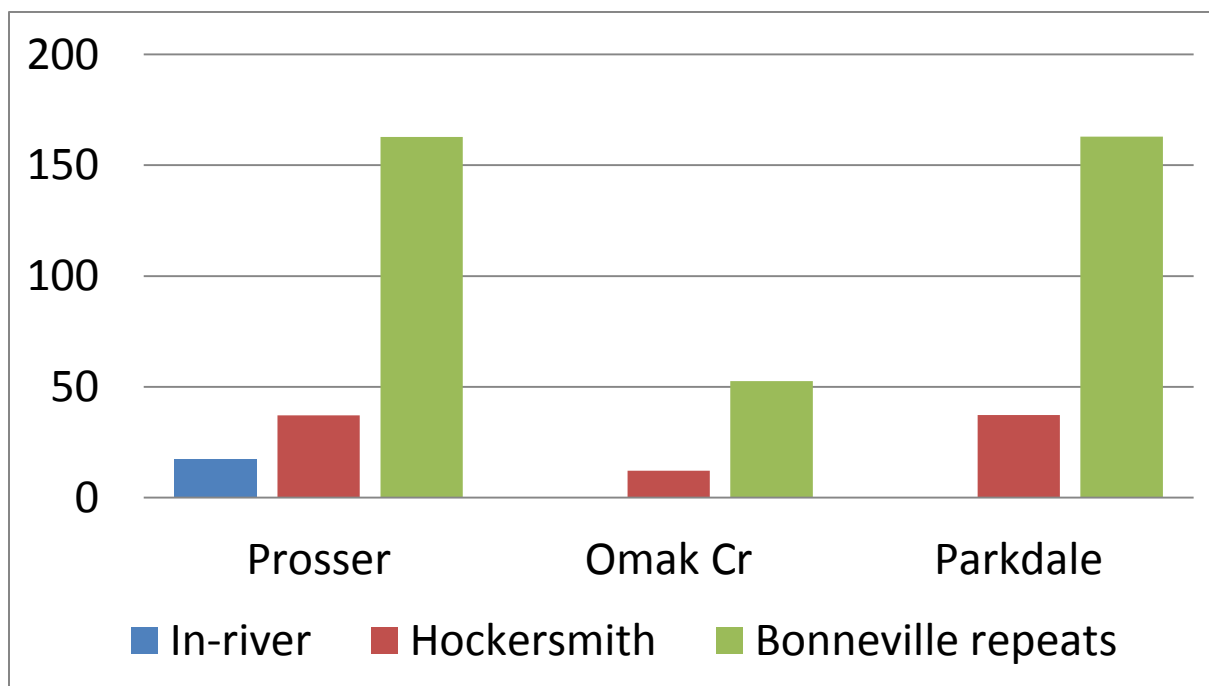


Figure 2: Long-term reconditioning benefits for 2012, calculated by dividing long-term survival rates by control group metrics.

Section D: Published Work

As part of Bonneville Power Administration work element S the following publication was submitted and accepted to the North American Journal of Fisheries Management. The following contains the citation and submitted draft of that work.

Hatch, D.R., D.E. Fast, W.J. Bosch, J.W. Blodgett, J.M. Whiteaker, R. Branstetter, and A.L. Pierce. 2013. Survival and traits of reconditioned kelt steelhead *Oncorhynchus mykiss* in the Yakima River, Washington. North American Journal of Fisheries Management 33(3):615-625.

*Survival and Traits of Reconditioned Kelt Steelhead Oncorhynchus mykiss
in the Yakima River, Washington*

Douglas R. Hatch¹, David E. Fast², William J. Bosch², Joseph W. Blodgett², John M. Whiteaker¹ Ryan
Branstetter¹, and Andrew L. Pierce¹

Running title: Yakima River Steelhead

¹Columbia River Inter-Tribal Fish Commission, 729 NE. Oregon St. Suite 200, Portland, OR 97232

²Yakama Nation Fisheries, P.O. Box 151, Toppenish, WA 98948

Corresponding Author: hatd@critfc.org, phone 503-238-0667, fax 503-235-4228

Abstract

We evaluated the traits and survival to release of reconditioned kelt steelhead *Oncorhynchus mykiss* in the Yakima River (Washington State, USA). From 2001-2011 we captured a total of 9,738 downstream migrating kelts at an irrigation diversion facility, on average about 27% of each annual wild steelhead return. Captured kelts were reared for 4.5-10 months in an artificial environment, treated for diseases and parasites, and fed both krill and pellets. Surviving reconditioned fish were released into the Yakima River coincident with the peak of upstream pre-spawn steelhead migration. Reconditioned steelhead kelts were predominantly (>92%) female. Annual survival to release ranged from 20-62% and averaged 38% over the course of the study with surviving reconditioned kelts showing increases in fork length, weight, and Fulton's K condition factor. Kelts in good condition and those with bright coloration at the time of collection were more likely to survive. Post-release upstream migration timing of reconditioned kelts was spread out over several months and correlated well with run timing of upstream pre-spawn migrants. The empirical results we observed demonstrate the potential of kelt reconditioning to provide recovery benefits for imperiled wild repeat spawning populations in highly developed river systems.

Introduction

Populations of wild steelhead *Oncorhynchus mykiss* in the Columbia River Basin (CRB) have declined dramatically from historical levels (Nehlsen et al. 1991; NRC 1996; Williams et al. 1999) and are now listed under the Endangered Species Act. The average abundance of wild steelhead (anadromous summer run/in-river maturing; there are no winter run/ocean maturing steelhead) in the Yakima River Subbasin over the last two decades is only 2% of pre-1890 abundance levels (Howell et al. 1985). Causes for these declines include a host of environmental and human-induced factors (Raymond 1988; NRC 1996; Williams et al. 1999). Common tools for increasing abundance of imperiled species in the wild include: nature reserves (Margules and Pressey 2000), restoration ecology (Dobson et al. 1997), artificial propagation and release of progeny from wild parents (Cuenco et al. 1993), captive rearing (Berejikian et al. 2001; Carmona-Catot et al. 2012), and using cryopreserved wild gametes to artificially produce offspring (Cloud et al. 1990). Historically there have been no artificial breeding programs for Yakima River steelhead and annual adult returns to the Yakima River average 98% wild with some hatchery strays from other CRB tributaries (Yakama Nation unpublished data). Some measures to restore habitats have recently been implemented to assist population rebuilding efforts (YSFWPB 2004). Due to the inherently long-term nature of habitat related recovery efforts, more immediate strategies to increase abundance are being evaluated which capitalize on the iteroparous life history steelhead exhibit.

Iteroparity 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) and extending as far inland as the Snake River Basin (Evermann 1895). Successful iteroparity appears to have been a component of all steelhead inhabiting the CRB to some degree. Although mortality occurs during secondary migrations, iteroparous fish are thought to contribute substantially to the genetic and demographic structure of salmonid populations (Ward and Slaney 1988; Fleming and Reynolds 2004; Keefer et al. 2008). Given the multiple migratory and reproductive phenotypes that contribute to reproductive success and population structure in steelhead, preserving as many of these reproductive phenotypes as possible is critical for this complex species (Nielsen et al. 2011).

Iteroparity rates vary among populations and have likely decreased in recent years, especially in upper watersheds, due to anthropogenic factors like 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 main stem hydropower facilities, and relied on scale analysis, which may have resulted in an underestimate of kelt (post-

spawned, downstream migrant) abundance due to scale reabsorption (Seamons et al. 2009). More recently, iteroparity rates for Klickitat River steelhead were reported to be 3.3% from 1979 to 1981 (Howell et al. 1985). Repeat spawners composed 1.6% of the Yakima River wild run (from data in Hockersmith et al. 1995). Keefer et al. (2008) sampled steelhead kelts at main stem hydropower dams from 2001-2004 and estimated that between 0.5-1.2% and 2.9-9.0% of those fish from the Snake River and tributaries to the Lower Columbia, respectively, exhibited an iteroparous life history. This is in contrast to the Kalama River, an un-impounded tributary of the lower CRB, where iteroparity rates in excess of 17% have been documented for the winter run steelhead (Busby et al. 1996).

While successful iteroparity rates are low for many reaches of the CRB relative to *O. mykiss* throughout their range, kelts tend to be quite abundant. Emigrating steelhead kelts averaged 46.1% of annual pre-spawn upstream runs in the Clackamas River from 1960 - 1964 (Gunsolus and Eicher 1970). Evans et al. (2004) estimated that 17% of the Snake River steelhead population was observed as kelts in the Lower Granite Dam juvenile bypass facility during a 10-week monitoring period in the spring of 2000. Though kelt abundance appears to be quite high, poor emigration survival of steelhead from upper Columbia River watersheds to the ocean appears to be the underlying limiting factor inhibiting iteroparity (Wertheimer and Evans 2005; Wertheimer 2007). Improving downstream migrating kelt survival could be a valuable restoration strategy for increasing abundance and productivity in CRB steelhead populations, especially in upper watersheds such as the Yakima River. Transportation of downstream migrating kelts around hydropower dams is a potential method to improve kelt survival that is presently being evaluated in the CRB (Evans et al. 2008).

Another method to increase the iteroparity rate of CRB steelhead is by artificially reconditioning kelts. Reconditioning is the practice of capturing, holding, and feeding post-spawned salmon or steelhead in an artificial rearing environment for the purpose of improving survival and regeneration of gonads for repeat spawning. This concept has been applied to Atlantic salmon *Salmo salar* (Gray et al. 1987; Crim et al. 1992; Johnston et al 1992), brown trout *Salmo trutta* L. (Poole et al. 1994), and Arctic charr *Salvelinus alpinus* L. (Boyer and Van Toever 1993). Null et al. (2012) reconditioned post-spawned hatchery-origin *O. mykiss* at Coleman National Fish Hatchery, California, for a short period then released these fish into the Sacramento River and used acoustic telemetry to study post-spawn migration patterns. The study we describe in this paper is the first to evaluate *O. mykiss* survival and traits of wild steelhead reconditioned in an artificial environment for several months.

Our objectives were:

1. To describe the abundance of adult steelhead migrating upstream and the abundance of kelts migrating downstream in the Yakima River;
2. To describe the traits of kelt steelhead in the Yakima River including sex ratio, condition, and timing;
3. To briefly describe the “reconditioning” process that we have developed and employed; and,
4. To report survival of reconditioning kelt steelhead to release and to assess survival relative to a number of independent variables.

Methods

Study Area and Monitoring Facilities

The Yakima River Basin is located in south central Washington State. From its headwaters near the crest of the Cascade Range, the Yakima River flows 344 km southeastward to its confluence with the Columbia River (Rkm 540; Figure 1). Steelhead populations primarily spawn upstream from Prosser Dam (Rkm 76) in Satus Creek, Toppenish Creek, the Naches River, and other tributaries (Conley et al. 2009). The Prosser Diversion Dam (Rkm 76; Figure 1) has three fish ladders each equipped with PIT detectors and fish count windows. Video camera and recording systems were deployed at each of the three fishway count windows where they recorded migrating fish 24 hours per day year round. The video was reviewed daily to produce real time counts of fish migrating past Prosser Dam. We used these counts to represent estimated annual steelhead spawner abundance.

We evaluated river flow with respect to steelhead using three different time periods. We queried the U.S. Bureau of Reclamation hydromet database to obtain average daily stream flow data (cubic meters per second – cms) for the Yakima River near Prosser Dam (see <http://www.usbr.gov/pn/hydromet/yakima/>). We then calculated mean monthly flows and standardized these by dividing by the month's 10-year average. The first time period was simply the standardized monthly flow during a calendar year. The second time period was the standardized monthly flow data during the kelt migration (March – May), and the third time period was standardized to the steelhead run (September – April).

After spawning in tributaries, a portion of the steelhead kelts migrating downstream are inadvertently diverted into an irrigation canal that has a maximum flow of 42.5 cms near Prosser Dam (BOR 2006). The canal is equipped with a fish bypass system that guides fish through the Chandler Juvenile Monitoring Facility (CJMF; Figure 1) where a separator is used to separate large and small fish from the bypass. This separator is monitored daily from mid-March through late-June annually. At Prosser Dam, the 2001-2011 mean Yakima River flow during the kelt migration (March-May) was 88.0 cms.

Kelt Collection, Holding, and Release

All kelt steelhead were dipnetted off the separator at the CJMF and transferred to a temporary holding tank containing oxygenated well water (13.8° C). Each fish was anesthetized in a buffered solution of tricaine methanesulfonate (MS-222) at 60 ppm, weighed, measured (FL), and judged by experienced fish culture staff as to maturation status, sex, condition (good=1; fair=2; poor=3) and coloration (bright=1; intermediate=2; dark=3). Methods similar to those employed by Keefer et al. (2008) were used to assess fish condition and coloration. Condition was based on the degree of visible external damage (e.g., abrasions, lesions, fungal infections; see Evans 2003), while coloration (an indicator of physiological state) was based on the degree of the fish's silvery, ocean-like external appearance. Kelts received a uniquely coded passive integrated transponder (PIT) tag that was injected into the pelvic girdle (Prentice et al. 1990) for individual fish identification during reconditioning and post-release tracking. Our evaluation was limited to kelts retained for reconditioning as described here.

Due to its success in treating the parasitic freshwater copepod *Salmincola californiensis* in this project's pilot studies conducted in 2000 (Evans and Beaty 2000), IvermectinTM was diluted with saline (1:30) and 1 to 3ccs was injected into the posterior end of the fish's esophagus using a plastic syringe (Johnson and Heindel 2000). Fish were also given an initial injection of *Oxytetracycline* and the dietary supplement hw-wiegandt multi vitTM was given in the feed (Wiegandt GmbH - Aquaristics Products, Germany). Kelts were retained in one of four 6.1 m diameter by 1.2 m high circular tanks. Water flow to the four tanks ranged from 570-950 liters per minute of 13.8° C well water, subjecting kelts to continual current. Individual tank carrying capacity was set at a maximum of 200 fish based on standard aquaculture guidelines (Piper et al. 1982) and goals established for this program. Formalin (37% concentration) was administered five times weekly at 1:6000 for 1 hour in all reconditioning tanks to prevent fungal outbreaks. Using information gained from a literature review (Evans et al. 2001) and our 2001 feed study (Hatch et al. 2002), reconditioned kelts were fed a combination of frozen krill and 6.0 mm pellet feed manufactured by Bio-Oregon. Krill was used initially to enhance the feeding response and after 4-6 weeks the pellets were introduced. Feed was administered 3-5 times daily at a rate of 1-2% body weight or until fish seemed satiated. Tanks were covered to provide shelter from sun and relieve stress from outside movement. Any mortalities were removed daily and the tanks were swept and flushed every 10-14 days as needed. The tank walls were painted white and the centers dark to discourage the kelts from rubbing the walls. We found eye damage to be prevalent when fish excessively rubbed the

walls. Aerators were placed in the tanks to break up the surface and introduce oxygen. The aerators appeared to reduce stress by providing added security for the fish.

Prior to release from the reconditioning facility, surviving steelhead were again weighed, measured (FL), sexed and scanned for PIT tags. From 2006-2011, kelt tanks were transitioned from well water to river over a 3 to 5 d period prior to release. Prior to 2006, kelts were tempered with river water in the tanker truck prior to release. Fish were released from mid-October to early December concurrent with the peak return of the natural spawning run. Releases occurred upstream of Prosser Dam from 2001-2007 and a few kilometers downstream of Prosser Dam from 2008-2011. The release location and timing allowed reconditioned kelts to naturally select their migration timing, spawning location, spawn timing, and mates. Upstream migration timing of reconditioned kelts was determined using post-release detections of PIT tags at Prosser Dam in 2008-2011.

Statistical Methods

To evaluate the change in fish form as a result of reconditioning, we calculated Fulton's condition factor (K) at collection and at release (Ricker 1975). This was calculated by:

$$K = w/l^3$$

where w = fish weight (kg), and
 L = fish length (cm).

One-way analysis of variance (ANOVA) was used to determine differences, at collection, in kelt length, weight, condition, and color across years (Sokal and Rohlf 2000). We used ANOVA tests to evaluate the association of survival (0, 1) and several fish trait variables including: fork length, weight, K, condition, and color. Additionally, we calculated correlation coefficients among these fish variables along with kelt and pre-spawner annual abundance, the proportion that kelt steelhead comprised of the previous run, length and weight change, and standardized flow periods (Sokal and Rohlf 2000).

Results

Pre-spawn Steelhead

From 2001 through 2011 the mean annual steelhead return to the Yakima River was 3,577 fish (Table 1). Fish migrated over Prosser Dam during all months of the year and the run was defined from July 1 of one year through June 30 of the following year. The majority of fish passed upstream from September through April with the peak in October (Figure 2). The median date of passage at Prosser Dam was highly variable occurring as early as October 18 and as late as December 26 (Table 1). Spawning in upstream tributaries generally occurred between February and June, with the peak occurring between early March and early May depending on stream elevation.

Kelt Steelhead

The mean annual Yakima River kelt emigration through the CJMF near Prosser Dam was 885 fish (Table 1). This collection represented an unknown portion of the total Yakima River kelt abundance, as some fish passed over the dam instead of through the irrigation canal where they could be collected. Assuming that all upstream pre-spawn migrants survived to emigrate downstream as kelts, the average annual collection would represent about 27% of the total kelt population (Table 1); if only half of upstream pre-spawn steelhead survived as kelts, the average collection would represent about 54% of the total population. Kelt emigration occurred from March through July, peaking in April. The median date of passage at Prosser Dam for kelt steelhead occurred as early as April 13, and as late as April 30, with a mean passage date of April 24 (Table 1). The abundance of emigrating kelt steelhead was strongly correlated with the abundance of the immigrating pre-spawn steelhead run of the same spawning population ($R^2=0.66$; Figure 3).

We found significant differences across years (Table 1) for kelt fork length (Mean 63.7; ANOVA: $F=65.96$; $df=10$, $P<0.001$), weight (Mean 1.97; ANOVA: $F=68.33$; $df=10$; $P<0.001$), condition at the time of collection (ANOVA: $F=2.77$; $df=10$; $P=0.002$), and color at the time of collection (ANOVA: $F=20.88$; $df=10$; $P<0.001$). Kelts from 2003 were in the best condition and those from 2011 were in the poorest overall condition. Kelts from 2007 were the brightest and those from 2001 were the darkest. Collections from all years were dominated by females (Table 1; mean=92.9% female). This

compares to a mean of about 70% female for the annual upstream pre-spawn migration (Yakama Nation unpublished data).

Kelt Reconditioning

Mean survival of kelt steelhead reconditioned for 4.5 to 10 months was 38.0% (range 20.1% to 62.4%; Table 2). Fish that survived the reconditioning treatment showed increases in fork length in 10 of 11 years and weight in all years with mean increases in fork length and weight of 0.61 cm and 0.50 kg respectively (Table 2). Survival was positively correlated with the portion of the run that is seen as kelts ($r = 0.67$; Table 3). Kelts in good and fair condition on arrival survived reconditioning at higher rates ($\chi^2 = 52.59$, $P < 0.001$), survival was 45%, 36%, and 0% for fish in good, fair, and poor condition, respectively (Figure 4). Bright and intermediate colored kelts survived reconditioning better than darker fish ($\chi^2 = 30.98$, $P < 0.001$), and survival was 44%, 36%, and 32% for bright, intermediate, and dark fish, respectively (Figure 4), although few dark fish were collected (Table 4). Migration timing of PIT-tagged, reconditioned kelts was well correlated ($r = 0.87$) with run timing of upstream pre-spawn migrants at Prosser Dam (Figure 2). Kelt survival was not associated with Fulton's K factor at intake (ANOVA: $F=1.05$; $df=1,061$; $P = 0.166$). Kelt survival decreased with length at collection (ANOVA: $F=2.77$; $df=42$; $P < 0.001$) (Figure 5). Kelt survival was not associated with fish weight at collection (ANOVA: $F=1.14$; $df=217$; $P = 0.078$).

We found significant differences in survival for both year and statistical week that kelts were collected (Year association; $\chi^2 = 395.13$, $P < 0.001$; and, Week effect; ANOVA: $F=5.09$, $df=18$, $P < 0.001$). The year association was mainly due to low survival of reconditioned fish in 2001 and 2005 (Table 2), and the week association was primarily due to low survival of individuals collected over the last few weeks of the season (Figure 6). To further investigate this, we divided weekly collections into two groups. The first group encompassed weeks 10-23 and the second group weeks 24-on and compared the association of survival and collection group. Survival rates were 39.2% for the early arriving and 29.3% for the later arriving group ($\chi^2 = 3.72$, $P = 0.054$).

Discussion

We found pre-spawn steelhead abundance to be positively correlated with kelt abundance. While this was expected, it differs somewhat from what Narum et al. (2008) found in the Snake River where kelt steelhead proportions were not significantly correlated with escapement proportions of reporting groups. The strong correlation we observed may be a result of the geographic proximity of Prosser Dam to steelhead spawning aggregates, relative to the Snake River study. In the Yakima River Basin, the majority of returning steelhead spawn in Toppenish and Satus Creeks (Yakama Nation, unpublished data), the upper reaches of which are at most 80-100km upstream from Prosser Dam (Figure 1). The furthest upstream spawning activity occurs in the upper reaches of the Yakima and Naches Rivers, approximately 200-240km upstream from Prosser Dam. In contrast, the upper reaches of Snake River steelhead spawning habitats are located over 700km upstream of Lower Granite Dam on the Snake River (ICTRT 2003). Other than one population, Narum et al. (2008) did not find that population distance influenced kelt composition but the Yakima River is a considerably smaller system and may demonstrate the effect. Alternatively, steelhead returning to the Snake River are generally larger, driven by populations that spend an additional year in the ocean relative to other CRB populations (IDFG 1994 cited in Busby et al. 1996), and these older steelhead may be less likely to be successfully iteroparous (Keefer et al. 2008). We did find that reconditioning survival decreases with increasing fish length (Figure 5) which supports this notion; however, the mean fork length of kelts in our collection was 63cm with few fish in the collection greater than 78cm, which is a size class more commonly found in the Snake River, and the decrease in survival rate with increasing length was slight. We did find that an increase in spawner density negatively affected the proportion of kelts collected in the Yakima River; this could also explain why the largest populations in Narum et al. (2008) had the lowest kelt proportions collected at Lower Granite Dam.

While the overall relationship of spawner abundance and kelt abundance we observed was positive, two key pieces of data we collected support the idea that spawner abundance may have a negative effect on kelt proportion and their survival. First, we found a negative relationship between the portion of the run seen as kelts as a function of pre-spawn fish abundance ($r = -0.64$; Table 3). This is likely not a function of river environment and collection efficiency since flow was not correlated with the proportion of kelts collected (a measure of collection efficiency) even though low flow years result in a greater proportion of the overall flow diverted into the irrigation canal relative to high flow years (Table 3). The second piece of evidence is the positive correlation we observed between the proportion of kelts

collected and kelt reconditioning survival ($r = 0.68$; Table 3). A possible explanation for these findings is density dependent competition on the spawning grounds. High spawner abundance results in more time on the spawning grounds for both male and female fish, with greater competition for redd sites among females and greater competition for access to females among males (Quinn 2005). The end result would be greater energy expenditure, more injuries, and ultimately fewer kelts, in poorer condition, emigrating through the system. Evidence supporting this was the greater proportion of kelts collected correlated with fish in better condition ($r = -0.66$; Table 3) and kelts in better condition survived the reconditioning process at a higher rate ($r = -0.70$). Stearns (1992) documented an evolutionary life history trade-off between energy investment in current reproduction and survival to breed again. The physical competition among males consumes energy (Jonsson et al. 1997) and often results in physical injury and likely resulted in the sex ratio of kelt steelhead being skewed in favor of females. The high proportion of females among the emigrating kelts may be indicative of an evolutionary advantage of female iteroparity. A trend toward higher post-spawn female survival, relative to males, is consistent with data from other iteroparous salmonid populations (Keefer et al. 2008; Seamons and Quinn 2010). Kelts collected earlier in the season survived better than those collected late in the season ($\chi^2 = 3.72$, $P < 0.054$), also most likely a result of greater energy expenditure.

Survival rates through the reconditioning process were as expected for the various condition factors we examined. While the proportion of kelts in good condition entering our reconditioning program (>40%) was comparable to the proportion of good-condition emigrating kelts observed by Keefer et al. (2008), we observed better survival-to-release rates for fair condition and darker colored kelts than the survival-to-return rates in the wild observed by Keefer et al. (2008) who found that fair fish were 2.5-5.7 times less likely to return relative to good condition kelts. Similarly, Evans et al. (2008) reported return rates for fair condition kelts at 0.8% across all treatments, sites, and years. This suggests that the reconditioning program may provide a relatively greater benefit for fair condition fish versus good condition fish.

The survival rates (20-62%) we observed for captive, reconditioned steelhead kelts were lower than reported survival rates of approximately 80-95% for Atlantic salmon kelts reared in fresh- or seawater (Gray et al. 1987; Johnston et al. 1987, 1990; Dumas et al. 1991). However, our results were consistent with kelt survival rates of 28-55% reported by Moffet et al. (1996) for Atlantic salmon artificially reconditioned in freshwater over two successive years, and reconditioned steelhead kelt return rates of 26% reported by Null et al. (2012). The annual variation we found in survival to release was

correlated with condition suggesting that environmental conditions (i.e., temperature and flow) steelhead experience throughout the winter and spring prior to collection (during holding, spawning, and repeat outmigration) along with pre-spawn steelhead densities may play a substantial role in whether or not these fish survive as captive kelts. Similar condition-dependent mortality in kelt steelhead returns was reported by Keefer et al. (2008), for fish that remained in the river.

Seamons and Quinn (2010) reported repeat spawning adults have life time reproductive success more than twice that of one-time spawners, and the average number of offspring produced by both male and female repeat spawners is much higher (1.9 times higher for females and 2.7 times higher for males). As the cumulative number of progeny gained by surviving to spawn in multiple subsequent years outweighs the number of progeny lost by not spawning in a given single year, occasional skip spawning may constitute an adaptive trait in long-lived iteroparous fish (Rideout et al. 2005). Seamons and Quinn (2010) further reported that repeat spawners grew substantially between their first and second breeding seasons (female mean growth of 41mm; males 71 mm) and estimated this additional female growth would result in an average increase in fecundity of about 400 eggs, or about 10%. Surviving captive kelts grew a comparable amount during reconditioning (Table 2), suggesting that an increase in fecundity could be expected. The hypothesis that larger fish are more productive has been tested at the population level with equivocal results including evidence supporting the notion (Helle 1989; Garant et al. 2001) and against (Holtby and Healey 1986).

In summary, we demonstrate that threatened wild steelhead kelts can be collected and reconditioned to attain survival rates that are considerably higher than if no action was taken. Fish condition, collection date, and pre-spawn abundance influenced reconditioning survival, suggesting that selection of fish at intake and the number of fish collected for reconditioning can be tailored to achieve program goals. Achieving reasonable survival rates by reconditioning wild kelt steelhead is a first step toward the development and implementation of this new stock recovery tool. However, to provide demographic and genetic benefits to the population, reconditioned kelts must migrate and spawn successfully after release. Surviving fish increased in weight and length during reconditioning, and most resumed upstream migration upon release, suggesting that these fish did likely spawn. However, additional studies of the reproductive success of reconditioned kelts are required to quantify the benefit of the reconditioning program. Studies of the reproductive success of reconditioned kelts using genetic parentage analysis, and studies of the reproductive status of reconditioned kelts at release using physiological indicators are underway. Additionally, studies of kelt migration success, spawn timing and

location, gamete quality, and progeny viability are in progress and will provide quantification of the potential of this management tool. Potential risks such as residualism of reconditioned kelts (Null et al. 2012) leading to negative ecological effects to fish in the river should be studied. These risks should be weighed against benefits including increased abundance, maintenance of the iteroparous phenotype, maintenance of the genetic diversity of the population (Crespi and Teo 2002), increased population productivity (Fleming and Reynolds 2004; Seamans and Quinn 2010), and protection against cohort failure (Fleming and Reynolds 2004; Wilbur and Rudolf 2006). The empirical results we observed demonstrate the potential of kelt reconditioning to provide recovery benefits for imperiled wild repeat spawning populations in highly developed river systems.

Acknowledgments

Monitoring and evaluation efforts for the steelhead kelt reconditioning project are the result of a cooperative effort by many individuals from a variety of agencies including the Yakama Nation Fisheries, the Columbia River Inter-Tribal Fish Commission, the United States Bureau of Reclamation, the University of Idaho, the Pacific States Marine Fisheries Commission, and the National Marine Fisheries Service. We would especially like to thank the many managers, biologists, technicians and staff who have contributed to the protection of Yakima Basin steelhead and their habitats through the years. Reviews by Matthew Keefer, and 3 anonymous reviewers improved this paper. This work was funded by the Bonneville Power Administration (BPA) through the NPCC's Fish and Wildlife Program.

References

- Berejikian, B. A., E. P. Tezak, and S. L. Schroder. 2001. Reproductive Behavior and Breeding Success of Captively Reared Chinook Salmon. *North American Journal of Fisheries Management* 21:255-260.
- BOR (U.S. Bureau of Reclamation). 2006. Fish protection at water diversions: A guide for planning and designing fish exclusion facilities. Water Resources Technical Publication, U.S. Department of the Interior, Bureau of Reclamation, Denver.
http://www.usbr.gov/pmts/hydraulics_lab/pubs/manuals/fishprotection/Fish%20Protection%20at%20Water%20Diversions.pdf
- Boyer, J.N. and W. Van Toever. 1993. Reconditioning of Arctic char (*Salvelinus alpinus*) after spawning. *Aquaculture* 110: 279–284.
- Busby, P. J., T. C. Wainwright, E. J. Bryant, L. J. Lierheimer, R. S. Waples, F. W. Waknitz, and I. V. Lagomarsino. 1996. Status review of west coast steelhead from Washington, Idaho, Oregon, and California. NOAA Tech. Memo. NMFS-NWFSC-27.
- Carmona-Catot, G, P.B. Moyle, and R.E. Simmons. 2012. Long-term captive breeding does not necessarily prevent reestablishment: lessons learned from Eagle Lake rainbow trout. *Reviews in Fish Biology and Fisheries*, 2012, Volume 22, Number 1, Pages 325-342.
- Cloud, J. G., W. H. Miller, and M. J. Levenduski. 1990. Cryopreservation of sperm as a means to store salmonid germ plasm and to transfer genes from wild fish to hatchery populations. *The Progressive Fish Culturist* 52:51-53.
- Conley, A., J. Freudenthal, D. Lind, P. Mees, and R. Visser (compilers). 2009. Yakima Steelhead Recovery Plan, Extracted from the 2005 Yakima Subbasin Salmon Recovery Plan with Updates, September 30, 2009. Yakima Basin Fish and Wildlife Recovery Board, Yakima, WA.
- Crespi, B.J., and R. Teo. 2002. Comparative phylogenetic analysis of the evolution of semelparity and life history in salmonid fishes. *Evolution* 56:1008-1020.
- Crim, L.W., C.E. Wilson, Y.P. So, D.R. Idler, and C.E. Johnston. 1992. Feeding, reconditioning, and rematuration responses of captive Atlantic salmon (*Salmo salar*) kelt. *Canadian Journal of Fisheries and Aquatic Sciences* 49:1835–1842.
- Cuenco, M. L., T. W. H. Backman and P. R. Mundy. 1993. The use of supplementation to aid in natural stock restoration. Pages 269-293 in J. G. Cloud and G. H. Thorgaard, editors. *Genetic conservation of salmonid fishes*. Plenum Press, New York.

- Dobson, A. P., A. D. Bradshaw, and A. J. M. Baker. 1997. Hopes for the Future: Restoration Ecology and Conservation Biology. *Science* 277(5325):515-522.
- Dumas, J., L. Barriere, D. Blanc, J. Godard, and S.J. Kaushik. 1991. Reconditioning of Atlantic salmon (*Salmo salar*) kelts with silage-based diets: growth and reproductive performance. *Aquaculture* 96:43-56.
- Evans, A.F. 2003. Development and application of steelhead (*Oncorhynchus mykiss*) kelt identification techniques. M.S. thesis, Oregon State University, Corvallis, OR.
- Evans, A. F. and R. E. Beaty. 2000. Identification and enumeration of steelhead (*Oncorhynchus mykiss*) kelts at Little Goose Dam Juvenile Bypass Separator, 1999. Annual Report to U.S. Army Corps of Engineers, Walla Walla District, for Contract No. DACW68-99-M-3102. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.
- Evans, A. F., R. E. Beaty, D. R. Hatch, J. Blodgett, and D. Fast. 2001. Kelt reconditioning: A research project to enhance iteroparity in Columbia Basin steelhead (*Oncorhynchus mykiss*). 2000 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 200001700, 34 electronic pages, (BPA Report DOE/BP-00004185-1).
- Evans, A.F., R.E. Beaty, M.S. Fitzpatrick, and K. Collis. 2004. Identification and enumeration of steelhead kelts at Lower Granite Dam. *Transactions of the American Fisheries Society* 133:1089-1099.
- Evans, A.F., R.H. Wertheimer, M.L. Keefer, C.T. Boggs, C.A. Peery, and K. Collis. 2008. Transportation of steelhead kelts to increase iteroparity in the Columbia and Snake rivers. *North American Journal of Fisheries Management* 28:1818-1827.
- Evermann, B.W. 1895. A preliminary report upon salmon investigations in Idaho in 1894. *Bulletin U.S. Fish Commission* 15:253-284.
- Fleming, I.A. and J.D. Reynolds. 2004. Salmon breeding systems. In *Evolution illuminated: salmon and their relatives*. Edited by A.P. Hendry and S.C. Stearns. Oxford University Press, Oxford, UK. pp.264–294.
- Garant, D., J.J. Dodson, and L. Bernatchez. 2001. A genetic evaluation of mating system and determinants of individual reproductive success in Atlantic salmon (*Salmo salar* L.). *Journal of Heredity* 92:137–145.
- Gray, R.W., J.D. Cameron, and A.D. McLennan. 1987. Artificial reconditioning, spawning and survival of Atlantic salmon, *Salmo salar* L., kelts in salt water and survival of their F1 progeny. *Aquaculture and Fisheries Management* 18:309-326.

- Gunsolus, R.T. and G. J. Eicher. 1970. Evaluation of fish-passage facilities at the North Fork project on the Clackamas River in Oregon. Research report to the Fish Commission of Oregon, Oregon Game Commission, United States Bureau of Commercial Fisheries, United States Bureau of Sport Fisheries and Wildlife, and Portland General Electric.
- Hatch, D., P. Anders, A. Evans, J. Blodgett, B. Bosch, D. Fast, and T. Newsome. 2002. Kelt Reconditioning: A Research Project to Enhance Iteroparity in Columbia Basin Steelhead (*Oncorhynchus mykiss*), 2001 Annual Report, Project No. 200001700, 89 electronic pages, (BPA Report DOE/BP-00004185-2). Bonneville Power Administration, Portland, OR.
- Helle, J.H. 1989. Relation between size-at-maturity and survival of progeny in chum salmon, *Oncorhynchus keta* (Walbaum). Journal of Fish Biology 35: 99–107.
- Hockersmith, E., J.Vella, L. Stuehrenberg, R.N. Iwamoto, and G. Swan. 1995. Yakima River radio-telemetry study: Steelhead, 1989-93. Report to US Dept. Energy, Bonneville Power Administration, for Proj. No. 89-089, Contract No. DE-AI79- 89BP00276, by Northwest Fisheries Science Center, National Marine Fisheries Service, Seattle, WA.
- Holtby, L.B. and M.C. Healey. 1986. Selection for adult size in female coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Fisheries and Aquatic Sciences 43: 1946–1959.
- Howell, P., K. Jones, D. Scarnecchia, L. Lavoy, W. Kendra, and D. Ortman. 1985. Stock assessment of Columbia River anadromous salmonids. Volume II: Steelhead stock summaries stock transfer guidelines – information needs. Report to the U.S. Department of Energy, Bonneville Power Administration, Division of Fish and Wildlife, Contract No. DE-AI79-84BP12737, Project No. 83-335. (http://www.fishlib.org/Documents/Subbasins/howell_vol2_part2.pdf).
- ICTRT (Interior Columbia Technical Recovery Team). 2003. Independent populations of chinook, steelhead and sockeye for listed evolutionarily significant units within the interior Columbia River domain. Interior Columbia Basin Technical Recovery Team Technical Review Draft. July 2003. http://www.nwfsc.noaa.gov/trt/col_docs/independentpopchinsteelsock.pdf.
- Idaho Department of Fish and Game (IDFG). 1994. Documents submitted to the ESA Administrative Record for west coast steelhead by E. Leitzinger, 18 October 1994. (Available from Environmental and Technical Services Division, Natl. Mar. Fish. Serv., 525 N.E. Oregon Street, Suite 500, Portland, OR 97232.)
- Johnson, K.A., and J.A. Heindel. 2000. Efficacy of manual removal and ivermectin gavage for control of *Salmincola californiensis* (Wilson) infestation of chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), captive broodstocks. Eagle Fish Health Laboratory and Hatchery report, Idaho Department of Fish and Game, Boise.

- Johnston, C. E., R. W. Gray, A. McLennan and A. Paterson. 1987. Effects of photoperiod, temperature, and diet on the reconditioning response, blood chemistry, and gonad maturation of Atlantic salmon kelts (*Salmo salar*) held in freshwater. Canadian Journal of Fisheries and Aquatic Sciences 44: 702-711.
- Johnston, C.E., S.R. Farmer, R.W. Gray, and M. Hambrook. 1990. Reconditioning and reproductive responses of Atlantic salmon kelts (*Salmo salar*) to photoperiod and temperature manipulation. Canadian Journal of Fisheries and Aquatic Sciences 47: 701-710.
- Johnston, C.E., M.J. Hambrook, R.W. Gray, and K.G. Davidson. 1992. Manipulation of reproductive function in Atlantic salmon (*Salmo salar*) kelts with controlled photoperiod and temperature. Canadian Journal of Fisheries and Aquatic Sciences 49: 2055–2061.
- Jonsson N., B. Jonsson, and L.P. Hansen. 1997. Changes in proximate composition and estimates of energetic costs during upstream migration and spawning in Atlantic salmon *Salmo salar*. Journal of Animal Ecology 66:425–436.
- Keefer, M.L., R.H. Wertheimer, A.F. Evans, C.T. Boggs, and C.A. Peery. 2008. Iteroparity in Columbia River summer-run steelhead (*Oncorhynchus mykiss*): implications for conservation. Canadian Journal of Fisheries and Aquatic Science 65:2592-2605.
- Long, J. B. and L. E. Griffin. 1937. Spawning and migratory habits of the Columbia River steelhead trout as determined by scale studies. Copeia 1937:62
- Margules, C. R., and R. L. Pressey. 2000. Systematic conservation planning. Nature 405:243-253.
- Moffet, I. J. J., G. J. A. Kennedy, and W. W. Crozier. 1996. Freshwater reconditioning and ranching of Atlantic salmon, *Salmo salar* L., kelts: growth and reproductive performance. Fisheries Management and Ecology 3:35-44.
- Narum, S.R., D. Hatch, A.J. Talbot, P. Moran, and M.S. Powell. 2008. Iteroparity in complex mating systems of steelhead *Oncorhynchus mykiss* (Walbaum). Journal of Fish Biology 72:45-60.
- Nehlsen, W., J. E. Williams, and J. A. Lichatowich. 1991. Pacific salmon at the crossroads: Stocks at risk from California, Oregon, Washington, and Idaho. Fisheries 16: 4-21.
- Nielsen, J.L., S.M. Turner, and C.E. Zimmerman. 2011. Electronic tags and genetics explore variation in migrating steelhead kelts (*Oncorhynchus mykiss*), Ninilchik River, Alaska. Canadian Journal of Fisheries and Aquatic Sciences 68:1-16.
- NMFS (National Marine Fisheries Service). 2010. Endangered Species Act Section 7(a)(2) Consultation Supplemental Biological Opinion Supplemental Consultation on Remand for Operation of the Federal Columbia River Power System, 11 Bureau of Reclamation Projects in the Columbia

523 Basin and ESA Section 10(a)(I)(A) Permit for Juvenile Fish Transportation Program. NOAA's
 524 National Marine Fisheries Service, Northwest Region, Seattle, WA.
 525 NRC (National Research Council). 1996. Upstream: Salmon and society in the Pacific Northwest.
 526 National Academy Press, Washington D.C.
 527 Null, R.E., K.S. Niemela, and S.F. Hamelberg. 2012. Post-spawn migrations of hatchery-origin
 528 *Oncorhynchus mykiss* kelts in the Central Valley of California. Environmental Biology of Fishes.
 529 Published online 09 Aug 2012.
 530 Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard. 1982. Fish
 531 hatchery management. U.S. Fish and Wildlife Service, Washington, D.C.
 532 Poole, W.R., M.G. Dillane, and K.F. Whelan. 1994. Artificial reconditioning of wild sea trout, *Salmo*
 533 *trutta* L., as an enhancement option: initial results on growth and spawning success. Pages 179-
 534 92 in Fisheries Management and Ecology 1:179-192.
 535 Prentice, E., T. A. Flagg, and C. S. Clinton. 1990. Equipment, methods, and an automated data-entry
 536 station for PIT tagging. Pages 335–340 in N. C. Parker, A. E. Giorgi, R. C. Heidinger, D. B.
 537 Jester, Jr., E. D. Prince, and G. A. Winans, editors. Fish-marking techniques. American Fisheries
 538 Society, Symposium Number 7, Bethesda, Maryland.
 539 Quinn, T.P. 2005. The behavior and ecology of Pacific salmon and trout. University of Washington
 540 Press, Seattle, WA.
 541 Raymond, H.L. 1988. Effects of hydroelectric development and fisheries enhancement on Spring and
 542 Summer Chinook salmon and steelhead in the Columbia River Basin. North American Journal of
 543 Fisheries Management 8:1-24.
 544 Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Fisheries
 545 Research Board of Canada Bulletin 191.
 546 Rideout, R.M., G.A. Rose, and M.P.M. Burton. 2005. Skipped spawning in female iteroparous fishes.
 547 Fish and Fisheries 6:50-72.
 548 Seamons, T.R., M.B. Dauer, J. Sneva, and T.P. Quinn. 2009. Use of parentage assignment and DNS
 549 genotyping to validate scale analysis for estimating steelhead age and spawning history. North
 550 American Journal of Fisheries Management 29(2): 396-403.
 551 Seamons, T.R. and T.P. Quinn. 2010. Sex-specific patterns of lifetime reproductive success in single and
 552 repeat breeding steelhead trout (*Oncorhynchus mykiss*). Behavioral Ecology and Sociobiology
 553 64:505-513.
 554 Sokal, R.R., and F.J. Rohlf. 2000. Biometry. W.H. Freeman and Company, New York.
 555 Stearns, S.C. 1992. The evolution of life histories. Oxford University Press, New York, NY.

556 Ward, B.R., and P.A. Slaney. 1988. Life history and smolt-to-adult survival of Keogh River steelhead
557 trout (*Salmo gairdneri*) and the relationship to smolt size. Canadian Journal of Fisheries and
558 Aquatic Sciences 45:1110–1122.

559 Wertheimer, R. H. 2007. Evaluation of a surface flow bypass system for steelhead kelt passage at
560 Bonneville Dam, Washington. North American Journal of Fisheries Management 27:21–29.

561 Wertheimer, R. H. and A. F. Evans. 2005. Downstream Passage of Steelhead Kelts through
562 Hydroelectric Dams on the Lower Snake and Columbia Rivers. Transactions of the American
563 Fisheries Society 134(4):853–865.

564 Whitt, C. R. 1954. The age, growth, and migration of steelhead trout in the Clearwater River, Idaho.
565 Master's thesis, University of Idaho, Moscow.

566 Wilbur, H.M., and H.W. Rudolf. 2006. Life-history evolution in uncertain environments: bet hedging in
567 time. American Naturalist 168:398-411.

568 Williams, R. N., W. E. McConnaha, P. R. Mundy, J. A. Stanford, R. R. Whitney, P. A. Bisson, D. L.
569 Bottom, L. D. Calvin, C. C. Coutant, M. W. Erho Jr., C. A. Frissell, J. A. Lichatowich, and W. J.
570 Liss. 1999. Return to the River: Scientific issues in the restoration of salmonid fishes in the
571 Columbia River. Fisheries 24(3):10-19.

572 YSFWPB (Yakima Subbasin Fish and Wildlife Planning Board). 2004. Yakima Subbasin Plan.
573 Northwest Power and Conservation Council, Portland, Oregon.

574 Table 1. Abundance and kelt trait data for upstream migrating (pre-spawn) steelhead at Prosser Dam and downstream migrating (kelt) steelhead at
575 the Chandler Juvenile Monitoring Facility (CJMF) for 2000-01 through 2010-11 steelhead run years (July 1 – June 30). Kelt fork length, weight,
576 condition, and color are mean values for annual collections.
577

Pre-spawn Steelhead										
At Prosser Dam			Kelt Steelhead at Collection, Chandler Juvenile Monitoring Facility (CJMF)							
Year	Abundance	Median	Abundance	Median	Proportion of Pre- spawn run	Fork length (cm)	Weight (kg)	Condition	Color	Percent female
		date of passage		date of passage						
2000-01	3,089	26-Dec	727	19-Apr	0.24	64.8	2.02	1.636	1.786	97.2
2001-02	4,525	19-Nov	1,157	24-Apr	0.26	63.4	1.96	1.561	1.603	92.6
2002-03	2,235	14-Dec	826	13-Apr	0.37	68.0	2.43	1.548	1.703	96.4
2003-04	2,755	26-Oct	998	25-Apr	0.36	60.3	1.67	1.603	1.732	93.4
2004-05	3,451	21-Oct	803	21-Apr	0.23	64.0	1.94	1.657	1.761	96.8
2005-06	2,005	18-Oct	520	13-Apr	0.26	66.5	2.10	1.648	1.660	95.2
2006-07	1,537	12-Nov	587	29-Apr	0.38	64.3	2.09	1.592	1.500	91.9
2007-08	3,310	10-Nov	847	30-Apr	0.26	62.0	1.84	1.597	1.587	92.4
2008-09	3,450	18-Nov	622	28-Apr	0.18	64.2	2.01	1.617	1.543	93.2
2009-10	6,796	3-Nov	1,659	21-Apr	0.24	62.0	1.80	1.621	1.509	89.7
2010-11	6,196	8-Nov	992	28-Apr	0.16	64.8	2.15	1.672	1.523	89.7
Average	3,577	9-Nov	885	24-Apr	0.27	63.7	1.97	1.615	1.620	92.9

578
579

580 Table 2. Abundance and kelt trait data for reconditioned kelt steelhead that survived to release, 2001-2011.

581

Year	Number	Number	Survival	Mean Fork Length (cm)			Mean Weight (kg)			Mean Fulton's K		Means At Collection	
	Reconditioned	Released	(%)	Collect	Release	Change	Collect	Release	Change	Collect	Release	Condition	Color
2001	508	108	21.3	64.18	66.82	2.65	2.00	3.22	1.22	0.031	0.048	1.56	1.81
2002	420	142	33.8	62.68	63.39	0.71	1.92	2.41	0.49	0.030	0.037	1.48	1.50
2003	482	301	62.4	67.35	68.03	0.68	2.37	3.04	0.67	0.035	0.044	1.51	1.72
2004	694	288	41.5	59.29	60.05	0.77	1.59	2.04	0.45	0.027	0.034	1.56	1.65
2005	427	86	20.1	62.15	62.28	0.13	1.82	2.26	0.44	0.029	0.036	1.62	1.68
2006	279	85	30.5	65.78	65.86	0.09	2.08	2.59	0.51	0.031	0.039	1.63	1.63
2007	422	221	52.4	63.14	63.28	0.15	1.98	2.31	0.33	0.031	0.036	1.56	1.44
2008	472	266	56.4	61.49	62.93	1.44	1.83	2.34	0.52	0.029	0.037	1.53	1.50
2009	510	141	27.6	63.22	64.82	1.61	1.96	2.61	0.65	0.030	0.040	1.54	1.55
2010	1100	426	38.7	60.91	60.45	-0.46	1.70	2.00	0.30	0.028	0.033	1.58	1.51
2011	680	223	32.8	63.30	64.13	0.83	2.04	2.50	0.47	0.032	0.038	1.61	1.49
Average	545	208	38.0	63.05	63.35	0.61	1.92	2.41	0.50	0.030	0.037	1.56	1.57

582

583

584 Table 3. Pearson correlation matrix *r* values (shaded) and associated unadjusted *P*- values (unshaded) for mean annual variables from Tables 1 and
585 2. Additionally three standardized flow variables measured near Prosser Dam are presented. The standardized monthly flow is the mean monthly
586 flow for a year divided by the 10 year average. The standardized spring monthly flow includes only March through May and standardized
587 steelhead run flow includes September through April. Significant correlations and their associated *P*-values are bold.

	Pre-spawn steelhead abundance	Kelt abundance	Proportion of kelts	Kelt survival	Δ FL	Δ Weight	K Factor at collection	K Factor at release	Condition at collection	Color at collection	Standardized monthly flow	Standardized spring monthly flow	Standardized steelhead run monthly flow
Pre-spawn steelhead abundance	1.000	0.814	-0.637	-0.265	-0.176	-0.231	-0.256	-0.330	0.313	-0.416	0.194	0.021	-0.127
Kelt abundance	0.002	1.000	-0.130	0.053	-0.367	-0.350	-0.388	-0.474	-0.106	-0.262	-0.151	-0.249	-0.324
Proportion of kelts	0.035	0.704	1.000	0.677	-0.240	-0.155	0.125	-0.065	-0.664	0.204	-0.237	-0.044	0.128
Kelt survival	0.430	0.876	0.022	1.000	-0.192	-0.304	0.297	-0.120	-0.697	-0.285	0.185	0.307	0.324
Δ FL	0.605	0.267	0.478	0.571	1.000	0.862	0.142	0.723	-0.001	0.335	-0.198	-0.159	-0.222
Δ Weight	0.493	0.292	0.650	0.363	0.001	1.000	0.335	0.919	0.044	0.568	-0.371	-0.373	-0.387

K Factor at collection	0.447	0.238	0.713	0.374	0.676	0.314	1.000	0.671	-0.224	0.027	0.375	0.355	0.376
K Factor at release	0.322	0.141	0.849	0.725	0.012	0.001	0.024	1.000	-0.058	0.462	-0.154	-0.163	-0.160
Condition at collection	0.348	0.756	0.026	0.017	0.997	0.897	0.507	0.865	1.000	0.045	0.213	0.085	0.015
Color at collection	0.203	0.437	0.547	0.396	0.314	0.068	0.937	0.152	0.895	1.000	-0.709	-0.710	-0.659
Standardized monthly flow	0.568	0.658	0.483	0.585	0.559	0.261	0.256	0.650	0.530	0.015	1.000	0.965	0.859
Standardized spring monthly flow	0.952	0.461	0.898	0.358	0.641	0.259	0.284	0.632	0.803	0.014	0.001	1.00	0.945
Standardized steelhead run monthly flow	0.709	0.331	0.707	0.330	0.512	0.240	0.255	0.639	0.964	0.028	0.001	0.001	1.00

589 Table 4. Composition of surviving reconditioned kelt steelhead separated into condition and color
590 categories at the time of collection, % (n).
591

Condition at collection	Color at collection			
	Bright	Intermediate	Dark	Total
Good	25.9 (525)	17.9 (362)	0.6 (13)	44.5 (900)
Fair	19.1 (387)	35.1 (711)	1.3 (26)	55.5 (1124)
Poor	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Total	45.1 (912)	53.0 (1073)	1.9 (39)	100 (2024)

Figure Captions

Figure 1. The Yakima River Basin, steelhead kelt reconditioning facilities, and monitoring locations.

Figure 2. Average Upstream Pre-spawn Steelhead (2000-01 through 2010-11 steelhead run years) and reconditioned kelt steelhead (PIT detections, 2008-2011) migration timing at Prosser Dam. Y-axes denote number of fish counted per day; right axis is for reconditioned fish.

Figure 3. Kelt abundance at Chandler Juvenile Monitoring Facility as a function of upstream pre-spawn steelhead count at Prosser Dam for 2000-01 through 2010-11 steelhead run years. The least squares linear regression line was: Downstream kelt collection = $0.057 * \text{upstream pre-spawn abundance} + 323.66$, $R^2 = 0.663$ and $P < 0.001$.

Figure 4. Mean survival for reconditioned kelts by condition rating (1=Good, 2=Fair, 3=Poor) (panel a) and color rating (1=Bright, 2= Intermediate, 3=Dark) (panel b) at collection, replicated by years 2001-2011, fish were captured at CJMF, Yakima River. Error bars = 95% confidence interval.

Figure 5. Mean survival for reconditioned kelts by fork length at collection, replicated by years 2001-2011, fish were captured at CJMF, Yakima River. The least squares linear regression line was: survival =

$0.979 - 0.009 * FL$, $R^2 = 0.014$ and $P < 0.001$. Error bars = 95% confidence interval. Fish in length group 50cm includes all fish smaller than 50cm and length group 85cm includes all fish greater than 85cm.

Figure 6. Mean survival for reconditioned kelts by collection year (panel a) and by statistical week (panel b), replicated by years, fish were captured at CJMF, Yakima River, 2001-2011. Error bars = 95% confidence interval. Statistical week 10 includes fish arriving prior to week 10 in some years and statistical week 26 includes fish arriving later than week 26 in some years.

Figure 1.

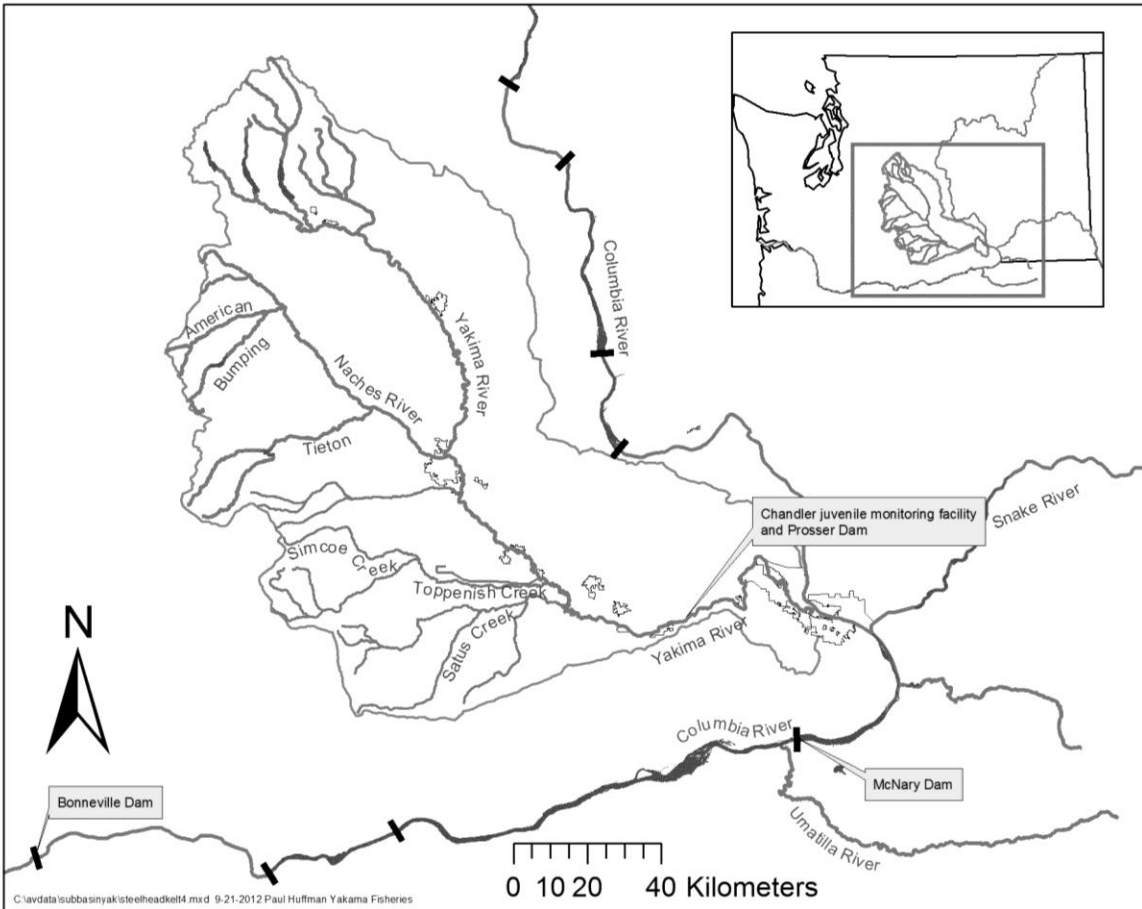


Figure 2.

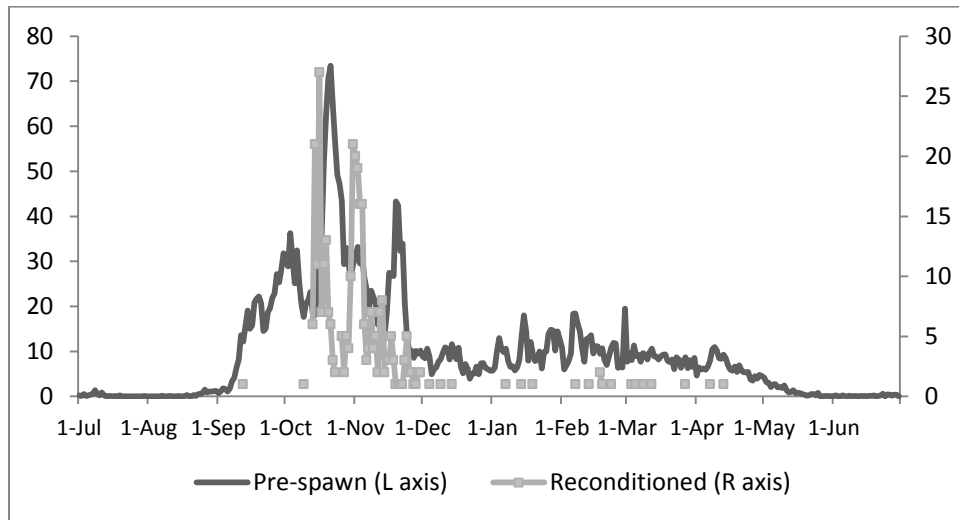


Figure 3.

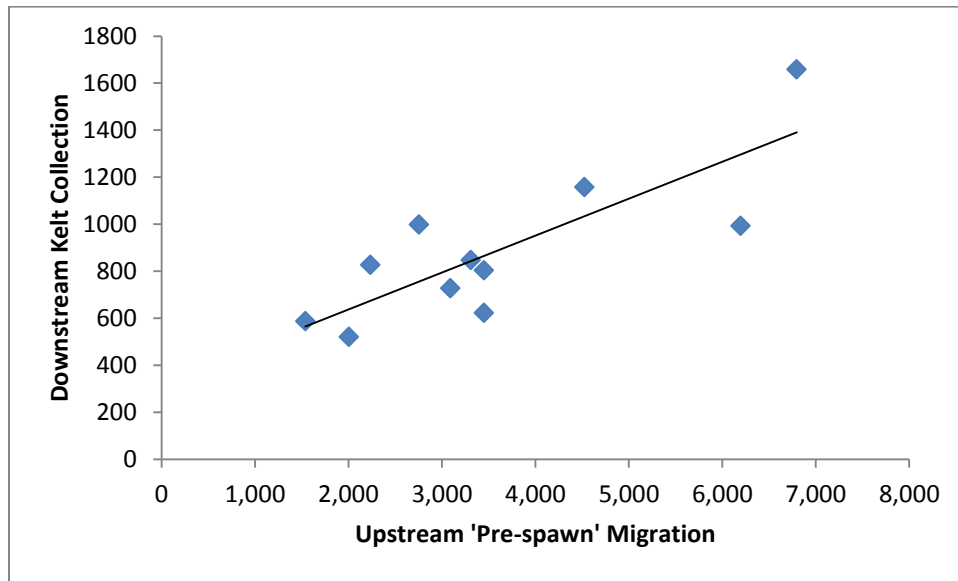


Figure 4.

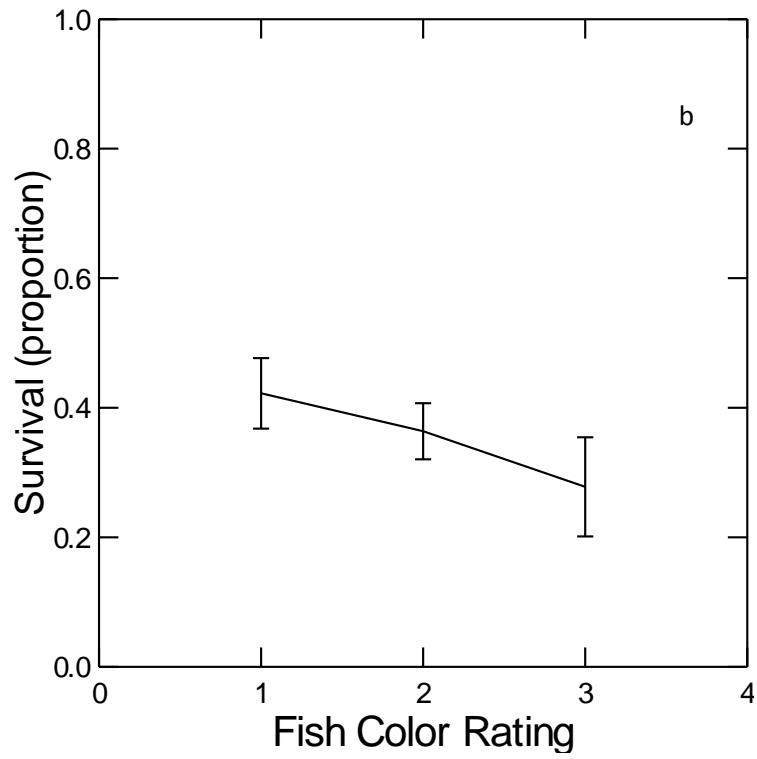
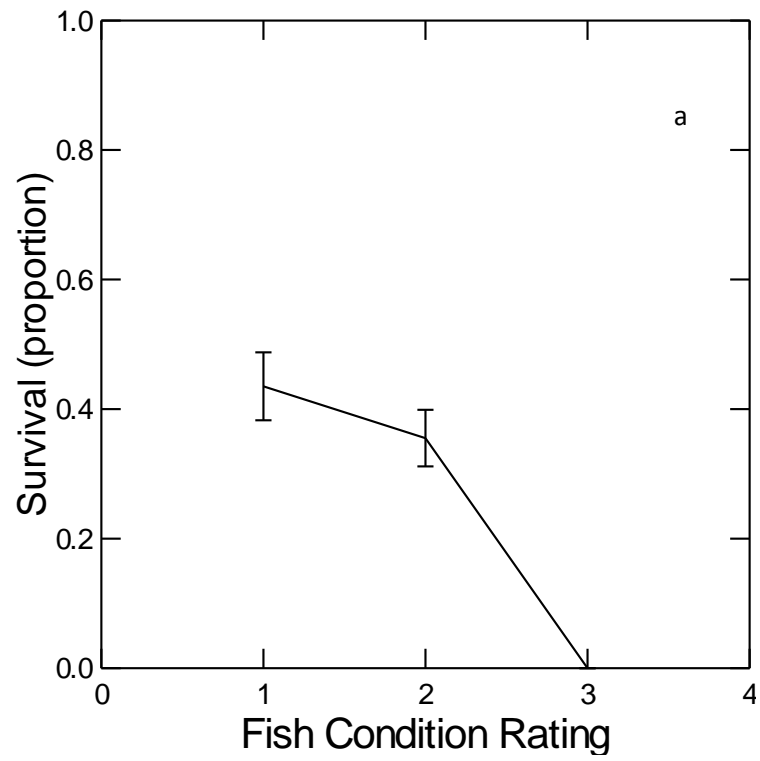


Figure 5.

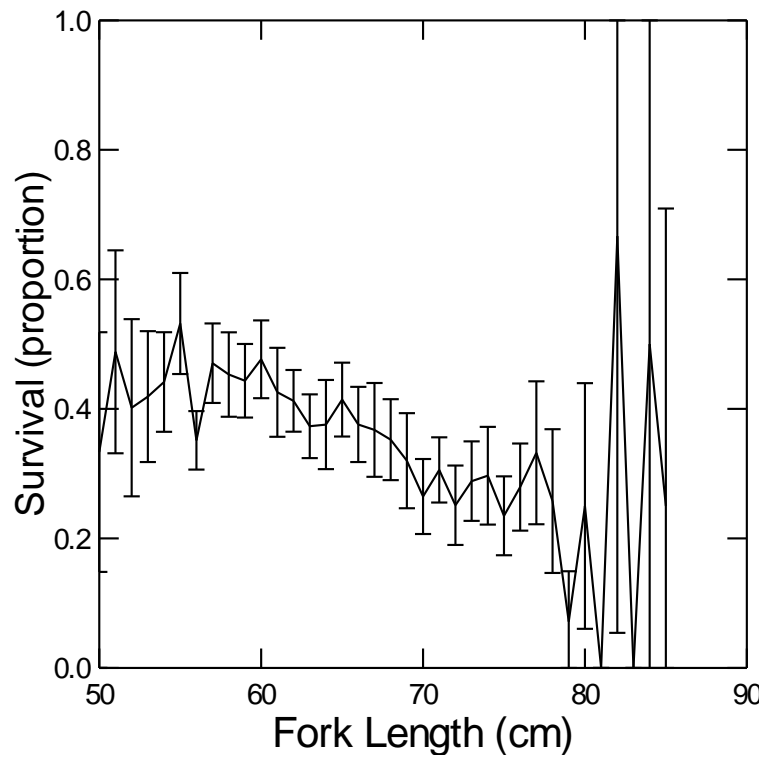
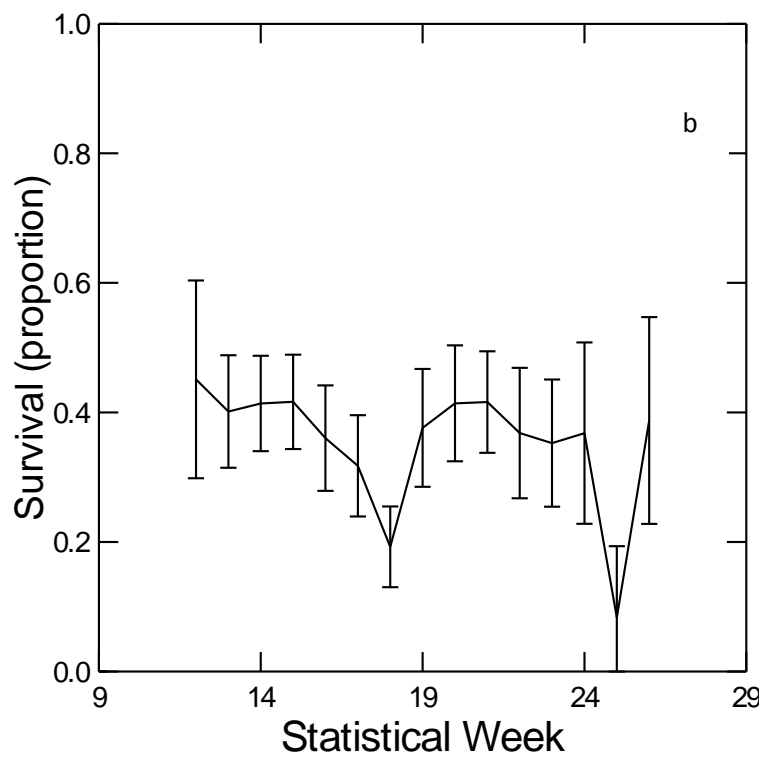
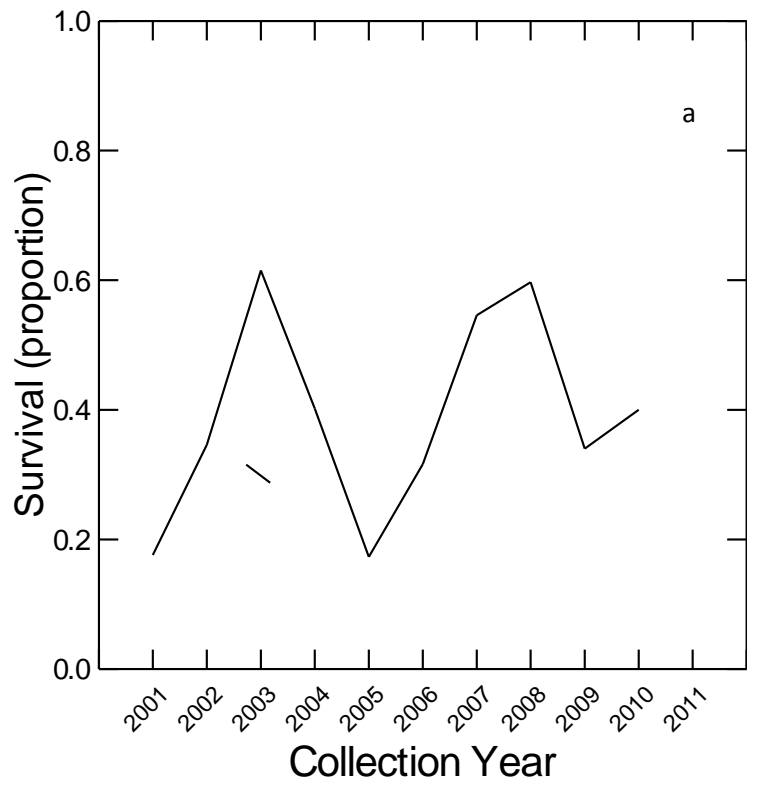


Figure 6.



References

- Colotelo AH, Jones BW, Harnish RA, McMichael GA, Ham KD, Deng ZD, Squeochs GM, Brown RS, Weiland MA, Ploskey GR, Li X, Fu T. 2013. Passage Distribution and Federal Columbia River Power System Survival for Steelhead Kelts Tagged Above and at Lower Granite Dam. PNPL-22101. Pacific Northwest National Laboratory, Richland, WA.
- Branstetter R., J. Stephenson, D. Hatch, A. Pierce. B. Bosch, D. Fast, J. Blodgett, J. Lyman, J. Graham., L. Holliday, A. Santos, J. Gidley, R. Dasher, C. Moffitt, S. Young, J. Buelow, Z. L. Penney, A. Pape, K. Hamilton, B. Sun, E. Marchio, A. Eckhart, J. Megl, B. Jones. 2010. Steelhead Kelt Reconditioning and Reproductive Success. 2009 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-00. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.
- Branstetter R., J. Stephenson, A. Pierce, D. Hatch, B. Bosch, D. Fast, J. Blodgett, M. Johnston, T. Resseguie, R. Dasher, C. Baker, A. Santos, J. Gidley, C. Brun, J. Lyman, J. Graham., L. Holliday, A. Santos, C. Gehling, C. Moffitt, J. Nagler, J. Buelow, Z. L. Penney, J. Boyce, L. K. Caldwell, T. Caileer, B. Jones, B. Sun, J. Egan, .2011. Steelhead Kelt Reconditioning and Reproductive Success. 2010 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-00. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.
- Evans, A.F. 2002. Steelhead (*Oncorhynchus mykiss*) kelt outmigration from Lower Granite Dam to Bonneville Dam: Abundance, downstream conversion rates, routes of passage, and travel times. Annual Report to U.S. Army Corps of Engineers, Walla Walla District, for Contract No. DACW68-01-0016. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.
- Evans, A.F., R.E. Beaty, D.R. Hatch, J. Blodgett, and D. Fast. 2001. Kelt reconditioning: A research project to enhance iteroparity in Columbia Basin steelhead (*Oncorhynchus mykiss*). 2000 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2000-017. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.
- Evans, A.F., R.H. Wertheimer, M.L. Keefer, C.T. Boggs, and C.A. Peery. 2008. Transportation of steelhead kelts to increase iteroparity in the Columbia and Snake rivers. *North American Journal of Fisheries Management* 28:1818-1827.
- Frederiksen, C., Steelhead Distribution in the Yakima River. Powerpoint presentation September 11, 2013.

- Glover KA, Samuelson OB, Skilbrei OT, Boxaspen K & Lunestad BT 2010 Pharmacokinetics of emamectin benzoate administered to Atlantic salmon, *Salmo salar* L., by intra-peritoneal injection. *Journal of Fish Diseases* 33 183-186.
- Hatch, D.R., R. Branstetter, and S. Narum. 2003a. Evaluate steelhead (*Oncorhynchus mykiss*) kelt outmigration from Lower Granite Dam to Bonneville Dam and test the use of transportation to increase returns of repeat spawners. Annual Report to US Army Corps of Engineers, Walla Walla District, for Contract No. DACW68-00-C-0027. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland OR.
- Hatch, D.R., P.J. Anders, A.F. Evans, J. Blodgett, B. Bosch, D. Fast., and T. Newsome. 2002. Kelt reconditioning: A research project to enhance iteroparity in Columbia Basin steelhead (*Oncorhynchus mykiss*). 2001 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2000-017. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.
- Hatch, D.R., R. Branstetter, J. Blodgett, B. Bosch, D. Fast, and T. Newsome. 2003b. Kelt reconditioning: A research project to enhance iteroparity in Columbia Basin steelhead (*Oncorhynchus mykiss*). Annual Report to the Bonneville Power Administration for Contract No. 00004185. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland OR.
- Hatch, D.R., R.D. Branstetter, J. Whiteaker, J. Blodgett, B. Bosch, D. Fast, and T. Newsome. 2004. Kelt reconditioning: A research project to enhance iteroparity in Columbia Basin steelhead (*Oncorhynchus mykiss*). 2004 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2000-017. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.
- Hatch D.R., R. Branstetter, J. Stephenson, A. Pierce, J. Whiteaker, and B. Bosch. 2012. Steelhead Kelt Reconditioning and Reproductive Success. 2011 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-000. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.
- Hockersmith, E., J. Vella, L. Stuehrenberg, R.N. Iwamoto, and G. Swan. 1995. Yakima River radio-telemetry study: steelhead, 1989-93. Project Number 89-089, Contract Number DE-AI79-89BP00276, Technical Report to U.S. Department of Energy, Bonneville Power Administration, Division of Fish and Wildlife, Portland, Oregon.
- Johnson, K.A., and J.A. Heindel. 2000. Efficacy of manual removal and ivermectin lavage for control of *Salmincola californiensis* (Wilson) infestation of chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), captive broodstocks. Eagle Fish Health Laboratory and Hatchery report, Idaho Department of Fish and Game, Boise.
- Kelsey, D., H. Ballantyne, J. Whiteaker, and J.K. Fryer. 2011a. Age and length composition of Columbia Basin Chinook and sockeye salmon and steelhead at Bonneville Dam in 2009. Columbia River Inter-Tribal Fish Commission Technical Report 11_08report. Portland, Oregon.

Kelsey, D., H. Ballantyne, J. Whiteaker, and J.K. Fryer. 2011b. Age and length composition of Columbia Basin Chinook and sockeye salmon and steelhead at Bonneville Dam in 2010. Columbia River Inter-Tribal Fish Commission Technical Report 11_09report. Portland, Oregon.

- McCann, J.A., D.W. Rondorf, H.L. Burge, and W.P. Connor. 1994. Evaluation of PIT tagging subyearling fall Chinook salmon during 1991 and 1992. Pages 63 to 91, in D.W. Rondorf and W.H. Miller, editors. Identification of the spawning, rearing, and migratory requirements of fall Chinook salmon in the Columbia River basin. 1992 Annual report to the Bonneville Power Administration, Contract Number DE-AI79-91BP21708.
- Miranda D.P., J. Whiteaker, and J. K. Fryer. 2004. Age and Length Composition of Columbia Basin Chinook, Sockeye, and Coho Salmon at Bonneville Dam in 2003. Technical Report for the Columbia River Inter-Tribal Fish Commission, Portland, Oregon.
- Miranda, D., J. Whiteaker, and J. Fryer. 2005. Age and Length Composition of Columbia Basin Chinook, Sockeye, and Coho Salmon at Bonneville Dam in 2004. Technical Report for the Columbia River Inter-Tribal Fish Commission, Portland, Oregon.
- Nowinski, J., Kelsey, D., Whiteaker, J., and Fryer, J.K. 2013a. Age and length composition of Columbia Basin Chinook and sockeye salmon and steelhead at Bonneville Dam in 2011. Columbia River Inter-Tribal Fish Commission Technical Report 13_01. Portland, Oregon.
- Nowinski, J., Kelsey, D., Whiteaker, J., and Fryer, J.K. 2013b. Age and length composition of Columbia Basin Chinook and sockeye salmon and steelhead at Bonneville Dam in 2012. Columbia River Inter-Tribal Fish Commission Technical Report 13-03. Portland, Oregon.
- Torbek, C., J. Mainord, and J. Whiteaker. 2009. Age and Length Composition of Columbia Basin Chinook and Sockeye Salmon and Steelhead at Bonneville Dam in 2008. Technical Report for the Columbia River Inter-Tribal Fish Commission, Portland, Oregon.
- U.S. Fish and Wildlife Service (USFWS). 2009. Dworshak, Kooskia, and Hagerman National Fish Hatcheries: Assessments and Recommendations Appendix B: Briefing Document; Summary of Background Information. Final Report, June 2009. Hatchery Review Team, Pacific Region. U.S. Fish and Wildlife Service, Portland, Oregon. Available at: <http://www.fws.gov/Pacific/fisheries/Hatcheryreview/reports.html>.
- Whiteaker, J., J. Fryer, and J. Doyle. 2006. Age And Length Composition Of Columbia Basin Chinook And Sockeye Salmon And Steelhead At Bonneville Dam In 2005. Technical Report for the Columbia River Inter-Tribal Fish Commission, Portland, Oregon.
- Whiteaker, J., and J. Fryer. 2007. Age and Length Composition of Columbia Basin Chinook and Sockeye Salmon and Steelhead at Bonneville Dam in 2006. Technical Report for the Columbia River Inter-Tribal Fish Commission, Portland, Oregon.
- Whiteaker, J., and J. Fryer. 2008. Age and Length Composition of Columbia Basin Chinook and Sockeye Salmon and Steelhead at Bonneville Dam in 2007. Technical Report for the Columbia River Inter-Tribal Fish Commission, Portland, Oregon.

Chapter 2: Kelt Reconditioning Physiology Studies

Andrew L. Pierce, Ph.D.

Neil D. Graham

Ryan Branstetter

Bobby Begay

Douglas R. Hatch

Columbia River Inter-Tribal Fish Commission

Lucius K. Caldwell, Ph.D. Candidate

Tim Cavileer, Ph.D., Research Scientist

Lea R. Medeiros, Ph.D., Postdoctoral Fellow

Kali Turner, Undergraduate Researcher

James J. Nagler, Ph.D., Professor

Department of Biological Sciences and Center for Reproductive Biology

University of Idaho, Moscow, ID

Joseph W. Blodgett

Michael Fiander

Chris Frederiksen

William J. Bosch

David E. Fast, Ph.D.

Yakama Nation Fisheries

Scott R. Everett

Rebecca L. Johnson

Department of Fisheries Resources Management

Nez Perce Tribe

Introduction

In 2009, studies were initiated to apply tools from fish physiology and endocrinology to issues in kelt reconditioning. These studies were continued in 2012. 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 modifications to culture conditions and physiological manipulations to improve survival and increase rematuration rate 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. The genetic stock and physiological condition of kelts arriving at reconditioning facilities varies widely between individuals, sites, and years, complicating experimental design. 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 improve rematuration and reproductive performance can be tested in rainbow trout. Studies on post-spawning rainbow trout have been conducted by Lucius K. Caldwell, a Ph.D. student in the Nagler lab at the University of Idaho. Luke has submitted a manuscript for publication on the effect of energy restriction on metabolic and growth factors during recovery from spawning (Section A), and is currently preparing a manuscript on energy restriction and plasma levels of reproductive hormones (Section B). These studies suggest that the decision to remature occurs within 10 weeks after spawning, and that this decision is strongly influenced by energetic status.

Steelhead Kelt Physiology Studies

Columbia basin steelhead have highly complex and plastic life histories at every stage of their life history up through the first spawning (Brannon *et al.* 2004). Thus, it is not surprising to find variation in post-reproductive life history. Natural repeat spawning fish may return after a single

summer in the ocean (consecutive spawners), or after two or more summers (skip spawners) (Keefer *et al.* 2008). Skip spawning is common in seasonally reproducing teleosts, and is thought to be largely driven by energetics (Rideout & Tomkiewicz 2011). In 2012, we completed laboratory analysis of blood hormone levels in samples taken from kelts at Prosser during the 2009-2011 seasons (Section C). This study focuses on evaluating the proportion of consecutive and skip spawning females produced by the reconditioning project at Prosser, establishing indicators of maturation status, and exploring factors associated with rematuration. We began another study employing a proteomics approach to determine whether an indicator of rematuration can be found in plasma samples taken at intake (Section D). We conducted the first year of a study evaluating the effect of a supplemented diet on growth, survival, and rematuration in female Prosser kelts (Section E). During the fall of 2012, we began a collaboration with a Yakama Nation Fisheries project sampling and radio tagging upriver migrating steelhead at Prosser dam (Section F). This study will allow a direct comparison of the reproductive status of reconditioned kelts at the time of release with maiden spawners migrating upstream during the same time period.

Based on treatment protocols established in Columbia River Basin captive broodstock programs, kelt steelhead have been treated with ivermectin to control parasitic copepods since 1999 (Evans *et al.* 2001; Johnson & Heindl 2001). During the 2011 season, we found that a replacement treatment was more effective than ivermectin, and strongly reduced mortality. In 2012, we repeated this study and confirmed the reduction in mortality (Section G).

Reconditioning studies employing hatchery origin kelts at Dworshak National Fish Hatchery during the 2012 season were impacted by water quality issues. Due to a shut off of the water supply, kelts were exposed to water from an effluent pond for several weeks. However, we did collect a large amount of data on egg quality and other reproductive parameters in hatchery kelts at the time of spawning, and some data on fish after reconditioning (Section H).

References:

- Brannon EL, Powell MS, Quinn TP & Talbot AJ 2004 Population Structure of Columbia River Basin Chinook Salmon and Steelhead Trout. *Reviews in Fisheries Science* **12** 99–232
- Evans AF, Beatty RA & Hatch DR 2001 Kelt Reconditioning: A Research Project to Enhance Iteroparity in Columbia Basin Steelhead (*Oncorhynchus mykiss*). 2000 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2000-017. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.

- Johnson KA & Heindl JA 2001 Efficacy of manual removal and ivermectin gavage for control of *Salmincola californiensis* (Wilson) infestation of chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), captive broodstocks. *Journal of Fish Diseases* **24** 197-203.
- Keefer ML, Wertheimer RH, Evans AF, Boggs CT & Peery CA 2008 Iteroparity in Columbia river summer-run steelhead (*Oncorhynchus mykiss*): implications for conservation. *Canadian Journal of Fishery and Aquatic Sciences* **65** 2592-2605.
- Rideout RM & Tomkiewicz J 2011 Skipped spawning in fishes: more common than you might think. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* **3** 176-189.

Section A: Metabolic endocrine factors involved in spawning recovery and rematuration of iteroparous female rainbow trout (*Oncorhynchus mykiss*)

Lucius K Caldwell^{1, *}

Andrew L Pierce^{1, 2}

Larry G Riley³

Christine A Duncan³

James J Nagler¹

1: University of Idaho, Department of Biological Sciences & Center for Reproductive Biology

2: Columbia River Intertribal Fish Commission

3: California State University, Fresno

*: Corresponding author, telephone: (208) 885-6280, email: cald0653@vandals.uidaho.edu, fax: (208) 885-7905; Postal Address: Life Sciences South 252; P.O. Box 443051; Moscow, ID 83844-3051

Abstract

To determine how energy balance affects metabolic hormones hypothesized to play a role in the onset of a new reproductive cycle in iteroparous salmonids, food availability after spawning was restricted in female rainbow trout. These fish were compared with a control group that was fed a standard brood stock ration. Bodyweight, length, and muscle lipid content were determined, and blood was collected from fish at regular intervals; a subset of fish from each group was sacrificed at each sampling time for the collection of liver tissue. Plasma hormone levels were quantified by radioimmunoassay, and tissue gene expression levels were analyzed using q-RT-PCR. The experiment was conducted twice, using two-year-old and three-year-old post-spawned fish. Food-restriction reduced Fulton's condition factor (k), muscle lipid content, and specific growth rate from one month onward, and reduced the hepatosomatic index after three months. In the liver, insulin-like growth factor (*igf1* and *igf2*) gene expression was reduced in three-year-old food-restricted fish within two months; however, no effect of ration on *igf1* or *igf2* expression was detected in two-year-old fish. In both years, IGF binding protein-1 (*igfbp1*) gene expression decreased over time in both treatment groups. Liver leptin (*slpA1*) gene expression was lower in two-year-old food-restricted fish at four months. These results show that this feed restriction regime affected factors associated with energy balance purported to play a role in initiating reproductive development within two to four months.

1. Introduction

In salmonid fishes, the physiological decision whether or not to mature is made during photoperiodically defined critical periods, which occur up to a year in advance of spawning [63]. During these critical periods, an individual fish physiologically assesses lipid stores, growth rate, and total body size to determine whether it will mature or not [59, 60]. In general, sexual maturation proceeds normally unless certain genetically determined thresholds are not exceeded during these critical periods, in which case development is arrested or inhibited [63].

While a large literature has explored the physiological regulation and endocrine signaling involved in the initial maturation (puberty) of juvenile fishes [8, 11, 26, 43, 58, 61, 65, 66], much less work has examined the regulation and signaling involved in the rematuration process among adult iteroparous fishes. Some salmonids invest heavily in both reproductive tissue and spawning behavior [22, 23, 32, 36, 37]; thus, post-spawned individuals are energetically depleted. After spawning, individuals must assess available energetic resources and make a commitment to remature or skip spawning (“resting”, *sensu* [51]) in the upcoming year. It is unclear how this rematuration process differs from puberty, or how energetic thresholds and endocrine signals involved in initiating rematuration change with reproductive age.

The peripheral endocrine factors that communicate growth and nutritional status to the reproductive endocrine axis (hypothalamus-pituitary-gonads-liver) include the various components of the insulin-like growth factor (IGF) system [15]. The IGFs are peripheral mediators of the growth effects associated with pituitary growth hormone (GH). In response to elevated plasma GH, the liver synthesizes and secretes IGFs (IGF-I and IGF-II), which then circulate systemically *via* plasma [16]. While IGF-I is the primary post-natal growth factor in mammals, IGF-I and IGF-II both appear to be important post-natal growth factors in fishes [24, 40], and have effects on reproduction [49, 61]. A class of at least six IGF-binding proteins (IGFBPs), also synthesized primarily in the liver, modulates IGF activities [14]. The main inhibitory binding protein, IGFBP-1 diminishes IGF-I activity by modulating the interaction of IGF with the IGF-receptor; IGFBP-1 is produced during a wide variety of catabolic states [34, 35]. Thus, we suspect that, in rainbow trout, the IGFs may act as peripheral signals of growth status that inform the reproductive endocrine axis, and that IGFBP-1 may regulate the activity of IGFs and the IGF response to systemic GH.

Among mammals, the peptide hormone leptin is synthesized by adipose tissue constitutively, so that leptin synthesis is proportional to lipid reserves [2, 54]. In this way, leptin functions as an adipostat, signaling the brain about the quantity of peripheral lipid reserves. Leptin also has a demonstrated role permissively gating the onset of puberty in mammals [9, 19]. Among fishes, leptin is generally produced in the liver (also an energy storage organ), but the role of leptin is unclear [33]. Some researchers have reported that fish leptin acts similarly to mammalian leptin [38], while others have reported an inverse role for leptin in fishes [41]. Opposing the actions of leptin, the peptide hormone ghrelin is synthesized and secreted from the stomach during times of energy deficit [45]. Ghrelin is involved in coordinating metabolism during fasting, as well as stimulating appetite [25, 31, 56]. Ghrelin may have a role inhibiting both puberty and reproduction in humans and other mammals

[reviewed in 50]. Thus, leptin could be a positive and ghrelin a negative peripheral metabolic signal that regulates reproductive development among iteroparous fishes such as rainbow trout.

The purpose of this study was to investigate the endocrine mechanisms that play a role in signaling energy status and growth rate to the central nervous system and reproductive endocrine axis during the hypothesized critical period immediately after spawning among iteroparous female rainbow trout. We hypothesized that liver igf1, igf2, and slepA1 expression would increase during refeeding after spawning, liver igfbp1 expression and plasma ghrelin would decrease during refeeding after spawning, and some or all of these effects would be inhibited in fish fed a restricted ration. We further hypothesized that the overall pattern would be similar among two-year-old and three-year-old fish, although energetic status of the two age classes after spawning might differ.

2. Material and Methods

2.1. Animals

Post-spawning female rainbow trout *Oncorhynchus mykiss* were purchased from Troutlodge (Sumner, WA) and transported to the University of Idaho (Moscow, ID). Fish had been manually strip-spawned 2 d prior to transport, and were fasted for one month (in the case of two-year-old trout) or two months (in the case of three-year-old trout) prior to spawning. Fish were held in 1,130 L tanks, in a recirculating system (flow rate 14 L min⁻¹ per tank, temperature 12 to 15°C following a seasonal profile). Experiments were conducted under approved protocols in accordance with the principles and procedures of the Animal Care and Use Committee, University of Idaho.

2.2. Experiments

For both experiments, treatments consisted of a control group, which was fed 0.5% total fish mass per day, and a restricted group, which was fed of 0.1% fish mass per day. Fish were fed a commercial trout broodstock diet (6.4 mm pellets, Rangen, Inc., Buhl, ID). Rations were adjusted to compensate for a 24-h pre-sampling fast, fish numbers, and fish weight based on sampling data and mortalities. Fish were individually identified by PIT tags.

2.2.1. Experiment 1: Three-year-old fish

Three-year-old fish (post 2nd spawning) were stocked into 6 tanks (25 February 2010, 26-27 fish per tank, average weight 1.35 kg). Tanks were randomly assigned control or restricted feeding treatments (n=3 tanks per treatment), and fish were sampled every 4 weeks. During sampling, all fish were anesthetized (60 mg L⁻¹ tricaine methanesulfonate, buffered). Fish were weighed, fork length was measured, muscle lipid content

was measured (Fish Fatmeter, Distell, Fauldhouse, UK), and blood (2 ml) was collected from the caudal vein using syringes that were pre-coated with heparin by aspirating and then dispensing 3.0 mL of 10 mg mL⁻¹ ammonium heparin (Sigma-Aldrich) suspended in ultrafiltered H₂O. Plasma was separated by centrifugation and stored at -80 °C. At each sampling, ten fish (n=5 fish per treatment group) were lethally sampled. Livers were dissected and weighed, and a liver sample was collected and snap-frozen in liquid N₂ for tissue gene expression analyses using q-RT-PCR. To reduce post-spawning mortality, fish were stripped of residual eggs and injected with oxytetracycline (Liquamycin®, Pfizer, Inc., New York, NY, 20 mg kg⁻¹) at the second sampling date. To control Gyrodactylus sp. gill parasites, fish were treated twice with praziquantel (Medisca, Plattsburgh, NY, water borne, 2.3 - 3.4 mg L⁻¹).

2.2.2. Experiment 2: Two-year-old fish

Two-year-old fish (post 1st spawning) were stocked into 12 tanks (3 March 2011, 26-27 fish per tank, average weight 1.13 kg). Tanks were randomly assigned control or restricted feeding treatments (n=6 tanks per treatment). Sampling was similar to Experiment 1, except that fish were sampled every 5 weeks, 12 fish were lethally sampled at each time point (n=6 fish per treatment group), and liver samples were collected in RNAlater (QIAGEN, Hilden, Germany) before being snap-frozen in liquid N₂. At the time of stocking, fish were stripped of residual eggs, injected with oxytetracycline, and treated with praziquantel as described above. In addition, fish were treated to control Saprolegnia (formalin 150 ppm and NaCl 2%, 1 hour static baths, repeated 3 times).

2.3. RNA Extractions & cDNA Synthesis

Liver samples were homogenized in 1.0 mL TRIzol® (Invitrogen™, Life Technologies, Carlsbad, CA), and RNA was isolated following the TRIzol protocol, using three chloroform:isoamyl alcohol extractions and three 70% ethanol washes. Resuspended nucleic acid fractions were treated with DNase (TURBO™ DNA-free, Ambion®, Life Technologies, Carlsbad, CA), RNA purity was assessed by spectrophotometric absorbance (NanoDrop ND-1000, Thermo Fisher), and RNA concentration was measured using the RiboGreen RNA assay kit (Invitrogen) with a fluorometer. 1 µg total RNA was reversed transcribed with the SuperScript III First-Strand Synthesis Kit (Invitrogen) using random hexamer primers. cDNA was diluted 1:5 in 1x Tris-EDTA.

2.4. q-RT-PCR

Quantitative real-time reverse transcriptase PCR (q-RT-PCR) primer sets were adapted from published sequences (Table 1). Specificity was confirmed by bioinformatic analysis, agarose gel electrophoresis of PCR products, and melting curve analysis of PCR products. To carry out the q-RT-PCR, sample cDNA was amplified in 96-well optical reaction plates (Invitrogen) containing 20 µL PCR reactions made up of 2µL cDNA, 10 µL Power SYBR® Green PCR Master Mix (Life Technologies), 6 µL H₂O, and 2 µL of a mix of forward and reverse primers at 2 pM each, in an Applied

Biosystems™ ABI 7900HT real-time PCR system (Life Technologies) (2 min @ 50°C; 10 min @ 95°C; 40 cycles of 15 sec @ 95°C and 1 min @ 60°C). Copy numbers in samples were quantified using standard curves of PCR amplicons. Three replicate PCRs were completed for each sample. Expression levels of target genes were normalized by dividing the copy number of the target gene by the copy number of the reference gene (EF1-α). The mean of the normalized expression level for the replicate PCRs is the value reported. q-PCR results were log2-transformed prior to statistical analysis.

2.5. Ghrelin RIA

Plasma ghrelin concentrations were measured using a ghrelin radioimmunoassay established by Riley et al. [52]. One hundred µL of rat ghrelin standards and plasma samples were incubated with 200 µL anti-rat ghrelin (from H. Hosada) at a dilution of 1:750,000. The anti-rat ghrelin [1-11] recognizes the octanoylated epitope (biologically active region) of ghrelin [27] and detects only the biologically active forms of ghrelin (ghrelin-C8 and ghrelin-C10). After incubation at 4°C overnight, 100 µL of ¹²⁵I-human ghrelin (Millipore, St. Charles, MO) was added before an additional overnight incubation at 4°C. Finally, 100 µL anti-rabbit IgG goat serum at 1:35 (with 10 % polyethylene glycol) was added, incubated for 2 h at room temperature, and then centrifuged at 3000 x g for 30 min to separate free and bound tracers. Radioactivity of aspirated pellet was then quantified using a gamma counter (Packard, Palo Alto, CA). Intra- and inter-assay CV's were 6.2 and 9.8%, respectively.

2.6. Data Analysis

Specific growth rate for mass (mass SGR) was calculated as
$$\ln \left[\frac{\text{mass at time 2 (g)}}{\text{mass at time 1 (g)}} \right] \times 100 \text{ interval (days)}$$
 [19]. Fulton's condition factor (k)

was calculated as
$$\frac{\text{body mass (g)}}{\text{fork length (cm)}^3} \times 100$$
 [23, 58]. Fatmeter readings were validated by chemical analyses of muscle lipid content (Fig. 1); the correlation between the two measurements was high (linear regression, $r^2 = 0.58$; Tukey mean-difference plot, matched pairs correlation =

0.76), as previously found in other studies on salmonids [14, 15]. Hepatosomatic index (HSI) was calculated as
$$\frac{\text{liver mass}}{\text{body mass}} \times 100$$
 [9]. Only data from fish that survived until being terminally sampled were included in statistical analyses for SGR, k, Fatmeter readings, and HSI. For SGR, k, Fatmeter readings, and HSI, among two-year-old trout at week 0, n=84; week 5, n=36; week 10, n=30; week 15, n=24; week 20, n=18 per treatment. For SGR, k, Fatmeter readings, and HSI, among three-year-old trout at week 0, n=46; week 4, n=18; week 8, n=13; week 12, n=8;

week 16 n=3 per treatment). For q-RT-PCR data, among two-year-old trout, n=6 at all time point, and among three-year-old trout, n=5 at all time points except week 16, when n=3 per treatment.

Systematic tank differences were not detected within treatment for any variable (ANOVA, $p > 0.05$). Therefore, tank replicates were pooled and analyzed together. Two-way ANOVA was used to detect main and interaction effects (time, treatment, time x treatment). When ANOVA indicated a significant time effect, Tukey-Kramer Honestly Significant Difference tests (Tukey-Kramer HSD) were used to compare values at all time points within a given treatment. Within each time point, two-tailed t-tests were used to detect treatment differences. Statistical analyses were performed within JMP® (Version 9, SAS Institute Inc., Cary, NC). Differences are reported as significant when $p < 0.05$.

3. Results

The feeding regime used here affected metrics of growth and metabolism in both two- and three-year-old fish. SGR was greater among control-ration fish than among restricted-ration fish at all time points in both two-year-old and three-year-old trout (Fig. 2). Fish fed the control-ration generally exhibited positive growth, while fish that were fed the restricted-ration generally exhibited negative growth. Between week zero and week five, control-ration three-year-old trout grew at nearly double the growth rate of control-ration two-year-old trout (three-year-old trout $SGR=0.33\pm0.04$, two-year-old trout $SGR=0.17\pm0.03$, $p=0.004$). Fulton's condition factor (k) decreased over time in restricted-ration fish, which had lower k values than control-ration fish at all time points after the initial sampling in both two-year-old and three-year-old trout (Fig. 3). At week zero, k was significantly greater among two-year-old trout than among three-year-old trout. Feed-restriction also affected muscle lipid content. Muscle lipid percentage increased over time among control-ration fish and decreased (two-year-old trout) or remained static (three-year-old trout) over time among restricted-ration fish. This led to greater muscle lipid level among control-ration fish than among restricted-ration fish, in both two-year-old and three-year-old trout (Fig. 4). At week zero, muscle lipid level was significantly greater among two-year-old trout than among three-year-old trout. For both two-year-old and three-year-old trout, HSI was consistently greater among full-ration fish than among restricted-ration fish, although this difference was only significant at later time-points (Fig. 5). When two-year-old trout from weeks 5-20 or three-year-old trout from weeks 4-16 were pooled and analyzed together, control-ration fish exhibited significantly greater HSI than did restricted-ration fish. Among two-year-old fish, control-ration fish HSI did not change between the beginning and end of the experiment, but restricted-ration fish showed a decrease in HSI over time. Among three-year-old fish, control-ration fish showed an increase in HSI over time, while restricted-ration fish HSI did not change between the beginning and end of the experiment. At week zero, HSI was marginally greater among two-year-old trout than among three-year-old trout ($p=0.05$).

Among two-year-old trout, between week zero and week five, control-ration fish exhibited a slight decrease, and restricted-ration fish exhibited a slight increase, in hepatic igf1 expression, leading to a difference between the two treatment groups at week five (Fig 6). In contrast, among three-year-old trout, hepatic igf1 expression was elevated in control-ration fish relative to restricted-ration fish at week eight. No difference was detected in hepatic igf1 expression among time-points within either treatment group among either two-year-old or three-year-old trout.

Among two-year-old trout, hepatic igf2 expression was elevated at week 15 compared to all other time points within control-ration group of fish; no difference in igf2 expression was detected among time-points within the restricted-ration group of fish (Fig. 7). Among two-year-old fish, no difference in igf2 expression levels was detected between the two treatment groups at any time point. Among three-year-old trout, hepatic igf2 expression was higher among control-ration fish than among restricted-ration fish at week four. Although differences were not detected at any other sampling point, there was a trend for higher igf2 expression in livers of control-ration fish across the entire experiment: when pooled, control-ration fish sampled at weeks 8-16 exhibited significantly higher igf2 expression than did restricted-ration fish. No difference in hepatic igf2 expression was detected among time-points within either treatment group.

Hepatic igfbp1 expression tended to decrease over time among both age classes and treatment groups (Fig. 8). Among two-year-old trout, hepatic igfbp1 expression decreased significantly over time within both treatment groups. Within control-ration fish, igfbp1 expression was significantly lower at week 15 compared to week zero. Within both treatment groups, igfbp1 expression was significantly lower at week 20 compared to week zero. Among three-year-old trout, hepatic igfbp1 expression decreased significantly over time within control-ration but not restricted-ration fish. Control-ration hepatic igfbp1 expression was significantly reduced versus week zero at 12 and 16 weeks. No difference in hepatic igfbp1 expression was detected between the two treatment groups at any time-point in either year.

No difference in hepatic slepA1 expression was detected among time points for either treatment group within either age class (Fig. 9). Among two-year-old trout, during weeks 10-20, there was a trend of higher hepatic slepA1 expression among control-ration fish, a difference that became significant at week 20. A similar trend was observed among three-year-old trout, although no difference was detected between treatment groups in hepatic slepA1 expression at any time-point.

No difference in plasma levels of acylated-ghrelin (ghrelin) was detected either among time points within treatment groups for a given age class, or between treatment groups at any time-point for a given age class (Fig. 10).

4. Discussion

The feeding regime selected in this study impacted fish growth and metabolism rapidly and dramatically. For both two-year-old and three-year-old post spawned female rainbow trout, the control ration induced weight gain, while the restricted ration induced weight loss; SGR was higher among control-ration fish than among restricted-ration fish at nearly every time point. Moreover, among both two-year-old and three-year-old trout, k among restricted-ration fish decreased over time, while k among control-ration fish remained stable, leading to rapid divergence in body shape between rations. Muscle lipid content and HSI tended to increase among control-ration fish and remain stable or decrease among restricted-ration fish. These results suggest that control-ration fish were accumulating excess food energy and storing it in muscle and liver tissue, while restricted-ration fish were utilizing stored energy to support metabolism, but were not growing.

Both two-year-old and three-year-old trout began the experiment in an energy-depleted state, due to the energetic demands of fasting and spawning. However, several observations suggest that the two-year-old trout were less energy-depleted than the three-year-olds at the beginning of the experiment. Two-year-old trout had greater k , higher muscle lipid content, and greater HSI at the initial sampling than did three-year-old trout. This was likely at least partially due to the difference in the duration of pre-spawning fasting imposed by the production facility from which the fish were obtained. Interestingly, in the control-ration treatment over the first 10-week period post spawning, the three-year-old trout grew at nearly double the rate of two-year-old trout. This suggests a compensatory growth response [3] that was greater among three-year-old than among two-year-old trout, consistent with the greater state of energy depletion at intake observed among three-year-old than among two-year-old fish. Compensatory growth has been previously shown to vary in proportion to degree of feed restriction in rainbow trout [28, 42]. Further, although control-ration fish were fed the same ration in both years, three-year-old fish were able to grow significantly faster, suggesting greater growth efficiency. There was an age effect with respect to HSI only, in that 2-year old control-ration fish exhibited a trend of decreasing HSI after week five while the HSI of three-year old fish increased. This may be due to greater metabolism of liver lipids in the 2-year-old fish.

Among control-ration fish, the different age classes of fish exhibited different hepatic igf expression responses to refeeding. Among three-year-old trout hepatic igf1 expression in control-ration fish exhibited a significant increase leading to levels elevated above those observed in restricted-ration fish, whereas no changes over time or between treatments were found in two-year-old fish. A similar expression pattern was observed with hepatic igf2 expression: among two-year-old trout, there was no difference detected in hepatic igf2 expression between control-ration and restricted-ration fish. However, among three-year-old trout, there was a consistent trend of higher hepatic igf2 expression among control-ration fish compared to restricted-ration fish. The difference in pattern of hepatic igf expression between the age classes may be due to differences in initial condition and subsequent compensatory growth between the two age classes after spawning. Given that plasma IGF-I levels reflect growth rate in *O. mykiss* [62] and other teleosts [46], it is likely that the elevated hepatic igf1 mRNA observed in control-ration three-year-old fish over the first eight weeks is associated with the greater compensatory growth observed among this age class, as has been

previously described in *O. mykiss* [10, 42] and other salmonid fishes [29]. The observation that hepatic igf2 expression remained elevated among 3-year-old control-ration fish supports the hypothesis that IGF-II in rainbow trout is regulated by nutritional status and involved in coordinating compensatory growth during refeeding [18]. Work in Atlantic salmon has demonstrated that IGF-II is more sensitive than IGF-I to food nutrient content [25].

Among both two-year-old and three-year-old trout, no change over time in hepatic igf1 or igf2 expression occurred among restricted-ration fish. As all fish were fasted prior to spawning, it may be the case that liver igf expression was already reduced to basal levels among incoming fish, and the restricted-ration was not sufficient to increase hepatic igf expression above basal levels. During fasting, plasma IGF-I decreased to ~40% of fed levels over two weeks and then remained constant in juvenile Chinook salmon (*Oncorhynchus tshawytscha*), suggesting that a basal plasma IGF-I level exists in salmonid fishes [47]. In the present study, restricted fish were fed a less than maintenance ration, suggesting that a greater than maintenance ration may be required to increase liver igf expression from basal levels during refeeding. Under conditions where anabolic growth is not occurring, the relationship between plasma IGF-I levels or hepatic igf expression and growth may become discordant [6].

Both two-year-old and three-year-old control-ration fish were fed a standard broodstock ration sufficient to support rematuration, which appears to have proceeded unchecked among this group (Caldwell et al., in prep). Current models suggest that these fish would have initiated rematuration during the time period covered in the present study [5, 57], and that a metabolic indicator such as IGF-I may play a permissive role in the initiation of maturation or rematuration [4, 44]. Thus, it is interesting that no post-spawning increase in hepatic igf1 mRNA was found in two-year-old fish. This suggests that an increase in liver igf1 transcription after spawning is not required to initiate rematuration in rainbow trout. Further, a significant increase in hepatic igf2 mRNA did occur 15 weeks after spawning in two-year-old control-ration fish. A trend toward elevation in hepatic igf2 mRNA occurred over this time period in three-year-old control-ration fish. Therefore, our data are consistent with the possibility that IGF-II rather than IGF-I plays a permissive role in the initiation of rematuration in rainbow trout. Alternatively, it is possible that fish may initiate rematuration without an increase in either IGF.

The hepatic igfbp1 expression patterns observed were similar in both age groups and feeding treatments, with high hepatic igfbp1 expression at week 0 followed by decreasing hepatic igfbp1 expression over the course of the experiment, suggesting that hepatic igfbp1 was increased during spawning. To our knowledge, this is the first report of a spawning elevation in hepatic igfbp1 expression. In teleost fishes, hepatic igfbp1 expression is rapidly upregulated in response to a variety of catabolic conditions including fasting [reviewed in 34], and expression is stimulated by plasma corticosteroids [48], which are elevated in salmonids during spawning [39, 53]. The lack of difference in hepatic igfbp1 expression between treatment groups may be due to regulation of IGFBP-1 at a level other than transcription. Work with Atlantic salmon [24], demonstrated that fish fasted for 14-days exhibited lower plasma IGFBP-1 compared to control fish, with no difference in hepatic igfbp1

expression between the groups. Regardless, our results suggest that decreasing hepatic igfbp1 expression or plasma IGFBP-1 levels may provide an indicator of recovery from spawning, as has been found for other stressors [35].

In both age classes, no difference in hepatic slepA1 expression was detected among time points for either treatment group. However, among two-year-old trout, hepatic slepA1 expression was elevated in control-ration versus restricted-ration fish at the final time point. Among three-year-old trout, no obvious pattern in slepA1 was apparent. Leptin physiology differs substantially between fishes and mammals. The role of leptin in salmonids is unclear [1], but hepatic leptin expression appears to be upregulated in response to various stressors, including food restriction [20, 55, 64] and temperature challenge [25]. Our results do not support regulation of hepatic slepA1 expression by either recovery from spawning or nutritional status in post-spawning rainbow trout.

Plasma ghrelin levels were not different between treatment groups of either age class. Ghrelin physiology also differs substantially between fishes and mammals. Ghrelin administration does not increase feed intake, and plasma ghrelin levels are reduced during fasting in rainbow trout [30, 31]. Our results do not support regulation of plasma ghrelin levels by either recovery from spawning or nutritional status in post-spawning rainbow trout. Further research is required to understand leptin and ghrelin physiology in rainbow trout and other teleosts.

In conclusion, a feeding regime that significantly impacted the growth and lipid levels of post spawning rainbow trout was utilized to look for age-related effects manifested in metabolic endocrine indicators. Most nutrient restriction studies in salmonids have employed rapidly growing juvenile fish. This is the first study we are aware of to examine nutritional endocrinology in post-spawning salmonids. Intriguingly, the IGFs emerged as being influenced by age, with the older three-year-old fish displaying a greater response to treatment than did the two-year-old fish.

5. References

- [1] A.J. Aguilar, M. Conde-Sieira, S. Polakof, J.M. Míguez, J.L. Soengas, Central leptin treatment modulates brain glucosensing function and peripheral energy metabolism of rainbow trout. *Peptides*. 31 (2010) 1044-1054.
- [2] R.S. Ahima, Adipose tissue as an endocrine organ. *Obesity*. 14 (2006) 242S-249S.
- [3] M. Ali, A. Nicieza, R.J. Wootton, Compensatory growth in fishes: a response to growth depression. *Fish Fish*. 4 (2003) 147-190.
- [4] H. Ando, Q. Luo, N. Koide, H. Okada, A. Urano, Effects of insulin-like growth factor I on GnRH-induced gonadotropin subunit gene expressions in masu salmon pituitary cells at different stages of sexual maturation. *Gen. Comp. Endocrinol*. 149 (2006) 21-29.
- [5] M.P. Beakes, W.H. Satterthwaite, E.M. Collins, D.R. Swank, J.E. Merz, R.G. Titus, et al., Smolt transformation in two California steelhead populations: effects of temporal variability in growth. *Trans. Am. Fish. Soc*. 139 (2010) 1263-1275.

- [6] B.R. Beckman, Perspectives on concordant and discordant relations between insulin-like growth factor 1 (IGF1) and growth in fishes. *Gen. Comp. Endocrinol.* 170 (2011) 233-252.
- [7] J.F. Carragher, N.W. Pankhurst, Plasma levels of sex steroids during sexual maturation of snapper, *Pagrus auratus* (Sparidae), caught from the wild. *Aquaculture.* 109 (1993) 375-388.
- [8] M. Carrillo, S. Zanuy, A. Felip, M.J. Bayarri, G. Molès, A. Gûmez, Hormonal and environmental control of puberty in perciform fish. *Ann. N. Y. Acad. Sci.* 1163 (2009) 49-59.
- [9] J. Cerdà, F. Chauvigne, M.J. Agulleiro, E. Marin, S. Halm, G. Martìnez-Rodrìguez, et al., Molecular cloning of Senegalese sole (*Solea senegalensis*) follicle-stimulating hormone and luteinizing hormone subunits and expression pattern during spermatogenesis. *Gen. Comp. Endocrinol.* 156 (2008) 470-481.
- [10] F. Chauvigné, J.C. Gabillard, C. Weil, P.Y. Rescan, Effect of refeeding on IGF1, IGFII, IGF receptors, FGF2, FGF6, and myostatin mRNA expression in rainbow trout myotomal muscle. *Gen. Comp. Endocrinol.* 132 (2003) 209-215.
- [11] W. Chen, W. Ge, Ontogenic expression profiles of gonadotropins (*fshb* and *lhb*) and growth hormone (*gh*) during sexual differentiation and puberty onset in female zebrafish. *Biol. Reprod.* 86 (2012) 73.
- [12] J. Colt, K.D. Shearer, Evaluation of the use of the Torry fish fatmeter to non-lethally estimate lipid in adult salmon. *Evaluation Of Migrational Delays On The Reproductive Success Of Adult Hatchery Spring Chinook Salmon In The Columbia And Snake Rivers*, Northwest Fisheries Science Center National Marine Fisheries Service, Seattle, WA, 2001.
- [13] G.T. Crossin, S.G. Hinch, A nonlethal, rapid method for assessing the somatic energy content of migrating adult Pacific salmon. *Transactions of the American Fisheries Society.* 134 (2005) 184-191.
- [14] D.O. Daza, G. Sundström, C.A. Bergqvist, C. Duan, D. Larhammar, Evolution of the insulin-like growth factor binding protein (IGFBP) family. *Endocrinology.* 152 (2011) 2278-2289.
- [15] C. Duan, Nutritional and developmental regulation of insulin-like growth factors in fish. *J. Nutr.* 128 (1998) 306S.
- [16] C. Duan, H. Ren, S. Gao, Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins: roles in skeletal muscle growth and differentiation. *Gen. Comp. Endocrinol.* 167 (2010) 344-351.
- [17] K.D. Fausch, Profitable stream positions for salmonids: relating specific growth rate to net energy gain. *Can. J. Zool./Rev. Can. Zool.* 62 (1984) 441-451.
- [18] B.K. Fox, J.P. Breves, L.K. Davis, A.L. Pierce, T. Hirano, E.G. Grau, Tissue-specific regulation of the growth hormone/insulin-like growth factor axis during fasting and re-feeding: importance of muscle expression of IGF-I and IGF-II mRNA in the tilapia. *Gen. Comp. Endocrinol.* 166 (2010) 573-580.

- [19] S.S. French, M.D. Dearing, G.E. Demas, Leptin as a physiological mediator of energetic trade-offs in ecoimmunology: implications for disease. *Integr. Comp. Biol.* 51 (2011) 505-513.
- [20] E. Frøiland, M. Jobling, B.T. Björnsson, P. Kling, C.S. Ravuri, E.H. Jørgensen, Seasonal appetite regulation in the anadromous Arctic charr: Evidence for a role of adiposity in the regulation of appetite but not for leptin in signalling adiposity. *Gen. Comp. Endocrinol.* 178 (2012) 330-337.
- [21] F. Heincke, Bericht über die untersuchungen der biologischen anstalt auf Helgoland zur naturgeschichte der nutzfische. Die Beteiligung Deutschlands an der Internationalen Meeresforschung, Wissenschaftliche Kommission, Berlin, 1908, pp. 67-155.
- [22] A. Hendry, E. Beall, Energy use in spawning Atlantic salmon. *Ecology of Freshwater Fish.* 13 (2004) 185-196.
- [23] A.P. Hendry, O.K. Berg, Secondary sexual characters, energy use, senescence, and the cost of reproduction in sockeye salmon. *Can. J. Zool./Rev. Can. Zool.* 77 (1999) 1663-1675.
- [24] E. Hevrøy, C. Azpeleta, M. Shimizu, A. Lanzén, H. Kaiya, M. Espe, et al., Effects of short-term starvation on ghrelin, GH-IGF system, and IGF-binding proteins in Atlantic salmon. *Fish Physiol. Biochem.* (2010) 1-16.
- [25] E.M. Hevrøy, R. Waagbø, B.E. Torstensen, H. Takle, I. Stubhaug, S.M. Jørgensen, et al., Ghrelin is involved in voluntary anorexia in Atlantic salmon raised at elevated sea temperatures. *Gen. Comp. Endocrinol.* 175 (2012) 118-134.
- [26] J. Hildahl, G.K. Sandvik, R.B. Edvardsen, C. Fagernes, B. Norberg, T.M. Haug, et al., Identification and gene expression analysis of three GnRH genes in female Atlantic cod during puberty provides insight into GnRH variant gene loss in fish. *Gen. Comp. Endocrinol.* 172 (2011) 458-467.
- [27] H. Hosoda, M. Kojima, H. Matsuo, K. Kangawa, Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. *Biochem. Biophys. Res. Commun.* 279 (2000) 909-913.
- [28] M. Jobling, J. Koskela, Interindividual variations in feeding and growth in rainbow trout during restricted feeding and in a subsequent period of compensatory growth. *J. Fish Biol.* 49 (1996) 658-667.
- [29] S.J.S. Johansen, M. Ekli, B. Stangnes, M. Jobling, Weight gain and lipid deposition in Atlantic salmon, *Salmo salar*, during compensatory growth: evidence for lipostatic regulation? *Aquacult. Res.* 32 (2001) 963-974.
- [30] E. Jönsson, A. Forsman, I.E. Einarsdottir, H. Kaiya, K. Ruohonen, B.T. Björnsson, Plasma ghrelin levels in rainbow trout in response to fasting, feeding and food composition, and effects of ghrelin on voluntary food intake. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* 147 (2007) 1116-1124.
- [31] E. Jönsson, H. Kaiya, B.T. Björnsson, Ghrelin decreases food intake in juvenile rainbow trout (*Oncorhynchus mykiss*) through the central anorexigenic corticotropin-releasing factor system. *Gen. Comp. Endocrinol.* 166 (2010) 39-46.

- [32] N. Jonsson, B. Jonsson, L.P. Hansen, Energetic cost of spawning in male and female Atlantic salmon (*Salmo salar* L.). J. Fish Biol. 39 (1991) 739-744.
- [33] O. Kah, Chapter 2: Endocrine targets of the hypothalamus and pituitary. In: N.J. Bernier, G.V.D. Kraak, A.P. Farrell, C.J. Brauner, (Eds.), Fish Physiology, Academic Press 2009, pp. 75-112.
- [34] S. Kajimura, C. Duan, Insulin-like growth factor-binding protein-1: an evolutionarily conserved fine tuner of insulin-like growth factor action under catabolic and stressful conditions. J. Fish Biol. 71 (2007) 309-325.
- [35] K.M. Kelley, J.T. Haigwood, M. Perez, M.M. Galima, Serum insulin-like growth factor binding proteins (IGFBPs) as markers for anabolic/catabolic condition in fishes. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 129 (2001) 229-236.
- [36] M.T. Kinnison, M.J. Unwin, A.P. Hendry, T.P. Quinn, Migratory costs and the evolution of egg size and number in introduced and indigenous salmon populations. Evolution. 55 (2001) 1656-1667.
- [37] M.T. Kinnison, M.J. Unwin, T.P. Quinn, Migratory costs and contemporary evolution of reproductive allocation in male chinook salmon. J. Evol. Biol. 16 (2003) 1257-1269.
- [38] P. Kling, I. Rønnestad, S.O. Stefansson, K. Murashita, T. Kurokawa, B.T. Björnsson, A homologous salmonid leptin radioimmunoassay indicates elevated plasma leptin levels during fasting of rainbow trout. Gen. Comp. Endocrinol. 162 (2009) 307-312.
- [39] D.A. Larsen, M. Shimizu, K.A. Cooper, P. Swanson, W.W. Dickhoff, Androgen effects on plasma GH, IGF-I, and 41-kDa IGFBP in coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol. 139 (2004) 29-37.
- [40] K. Link, G. Berishvili, N. Shved, H. D'Cotta, J.-F. Baroiller, M. Reinecke, et al., Seawater and freshwater challenges affect the insulin-like growth factors IGF-I and IGF-II in liver and osmoregulatory organs of the tilapia. Mol. Cell. Endocrinol. 327 (2010) 40-46.
- [41] A.-G.G. Moen, R.N. Finn, Short-term, but not long-term feed restriction causes differential expression of leptins in Atlantic salmon. Gen. Comp. Endocrinol. 183 (2013) 83-88.
- [42] N. Montserrat, J.C. Gabillard, E. Capilla, M.I. Navarro, J. Gutiérrez, Role of insulin, insulin-like growth factors, and muscle regulatory factors in the compensatory growth of the trout (*Oncorhynchus mykiss*). Gen. Comp. Endocrinol. 150 (2007) 462-472.
- [43] J.N. Nocillado, A. Elizur, Neuroendocrine regulation of puberty in fish: insights from the grey mullet (*Mugil cephalus*) model. Mol. Reprod. Dev. 75 (2008) 355-361.
- [44] T.A. Onuma, K. Makino, H. Katsumata, B.R. Beckman, M. Ban, H. Ando, et al., Changes in the plasma levels of insulin-like growth factor-I from the onset of spawning migration through upstream migration in chum salmon. Gen. Comp. Endocrinol. 165 (2009) 237-243.
- [45] M. Picha, C. Strom, L. Riley, A. Walker, E. Won, W. Johnstone, et al., Plasma ghrelin and growth hormone regulation in response to metabolic state in hybrid striped bass: Effects of feeding, ghrelin and insulin-like growth factor-I on *in vivo* and *in vitro* GH secretion. Gen. Comp. Endocrinol. 161 (2009) 365-372.

- [46] M.E. Picha, M.J. Turano, B.R. Beckman, R.J. Borski, Endocrine biomarkers of growth and applications to aquaculture: a minireview of growth hormone, insulin-like growth factor (IGF)-I, and IGF-Binding proteins as potential growth indicators in fish. *N. Am. J. Aquacult.* 70 (2008) 196-211.
- [47] A.L. Pierce, H. Fukada, W.W. Dickhoff, Metabolic hormones modulate the effect of growth hormone (GH) on insulin-like growth factor-I (IGF-I) mRNA level in primary culture of salmon hepatocytes. *J. Endocrinol.* 184 (2005) 341-349.
- [48] A.L. Pierce, M. Shimizu, L. Felli, P. Swanson, W.W. Dickhoff, Metabolic hormones regulate insulin-like growth factor binding protein-1 mRNA levels in primary cultured salmon hepatocytes; lack of inhibition by insulin. *J. Endocrinol.* 191 (2006) 379-386.
- [49] M. Reinecke, Insulin-like growth factors and fish reproduction. *Biol. Reprod.* 82 (2010) 656-661.
- [50] A. Repaci, A. Gambineri, U. Pagotto, R. Pasquali, Ghrelin and reproductive disorders. *Mol. Cell. Endocrinol.* 340 (2011) 70-79.
- [51] R.M. Rideout, G.A. Rose, M.P.M. Burton, Skipped spawning in female iteroparous fishes. *Fish Fish.* 6 (2005) 50-72.
- [52] L.G. Riley, B.K. Fox, J.P. Breves, H. Kaiya, C.P. Dorough, T. Hirano, et al., Absence of effects of short-term fasting on plasma ghrelin and brain expression of ghrelin receptors in the tilapia, *Oreochromis mossambicus*. *Zool. Sci.* 25 (2008) 821-827.
- [53] L.G. Riley, T. Hirano, E.G. Grau, Estradiol-17 β and dihydrotestosterone differentially regulate vitellogenin and insulin-like growth factor-I production in primary hepatocytes of the tilapia *Oreochromis mossambicus*. *Comp. Biochem. Physiol. C: Toxicol. Pharmacol.* 138 (2004) 177-186.
- [54] S.A. Robertson, G.M. Leininger, M.G. Myers Jr, Molecular and neural mediators of leptin action. *Physiol. Behav.* 94 (2008) 637-642.
- [55] I. Rønnestad, T.O. Nilsen, K. Murashita, A.R. Angotzi, A.-G. Gamst Moen, S.O. Stefansson, et al., Leptin and leptin receptor genes in Atlantic salmon: cloning, phylogeny, tissue distribution and expression correlated to long-term feeding status. *Gen. Comp. Endocrinol.* 168 (2010) 55-70.
- [56] I. Rønnestad, R. Valen, K. Murashita, A.-E.O. Jordal, Postprandial changes in GI-tract peptide hormones in the teleost Atlantic salmon. *FASEB J.* 24 (2010) 1b620.
- [57] W.H. Satterthwaite, M.P. Beakes, E.M. Collins, D.R. Swank, J.E. Merz, R.G. Titus, et al., Steelhead life history on California's central coast: insights from a state-dependent model. *Trans. Am. Fish. Soc.* 138 (2009) 532-548.
- [58] R. Schiavone, L. Zilli, C. Storelli, S. Vilella, Changes in hormonal profile, gonads and sperm quality of *Argyrosomus regius* (Pisces, Scianidae) during the first sexual differentiation and maturation. *Theriogenology.* 77 (2012) 888-898.
- [59] K. Shearer, P. Parkins, B. Gadberry, B. Beckman, P. Swanson, Effects of growth rate/body size and a low lipid diet on the incidence of early sexual maturation in juvenile male spring Chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture.* 252 (2006) 545-556.
- [60] J.T. Silverstein, K.D. Shearer, W.W. Dickhoff, E.M. Plisetskaya, Effects of growth and fatness on sexual development of chinook salmon (*Oncorhynchus tshawytscha*) parr. *Can. J. Fish. Aquat. Sci.* 55 (1998) 2376-2382.

- [61] G.L. Taranger, M. Carrillo, R.W. Schulz, P. Fontaine, S. Zanuy, A. Felip, et al., Control of puberty in farmed fish. *Gen. Comp. Endocrinol.* 165 (2010) 483-515
- [62] J.F. Taylor, H. Migaud, M.J.R. Porter, N.R. Bromage, Photoperiod influences growth rate and plasma insulin-like growth factor-I levels in juvenile rainbow trout, *Oncorhynchus mykiss*. *Gen. Comp. Endocrinol.* 142 (2005) 169-185.
- [63] J.E. Thorpe, Maturation responses of salmonids to changing developmental opportunities. *Mar. Ecol. Prog. Ser.* 335 (2007) 285-288.
- [64] S. Trombley, G. Maugars, P. Kling, B.T. Björnsson, M. Schmitz, Effects of long-term restricted feeding on plasma leptin, hepatic leptin expression and leptin receptor expression in juvenile Atlantic salmon (*Salmo salar* L.). *Gen. Comp. Endocrinol.* 175 (2012) 92-99.
- [65] F.-A. Weltzien, E. Andersson, Ø. Andersen, K. Shalchian-Tabrizi, B. Norberg, The brain-pituitary-gonad axis in male teleosts, with special emphasis on flatfish (*Pleuronectiformes*). *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* 137 (2004) 447-477.
- [66] Y. Zohar, J.A. Muñoz-Cueto, A. Elizur, O. Kah, Neuroendocrinology of reproduction in teleost fish. *Gen. Comp. Endocrinol.* 165 (2010) 438-455.

Table 1 – Primer sequence data for q-RT-PCRs.

Gene	Accession Number	Direction	Sequence	Product Size	Reference
<i>igf1</i>	NM_001124696.1	Fwd	GATGTCTTCAAGAGTGCATGTG	84 bp	[53]
		Rev	CGCCGAAGTCAGGGTTAGG		
<i>igf2</i>	NM_001124697.1	Fwd	GAAAAGACACGAA <u>T</u> ACCACTCAGT	74 bp	[7]
		Rev	TCATCTTGACCTTCATTCTTGTTT		
<i>igfbp1</i>	NM_001124561.1	Fwd	AACACCATCCGCAAGAACTG	68 bp	[55]
		Rev	TTGTCCAGAGCTGCATGCA		
<i>slepa1</i>	NM_001145890.1	Fwd	CCGCCA <u>A</u> CAGAAACAGACA	80 bp	[64]
		Rev	CCCACACTCAGACCATACTTCCT		
<i>ef1α</i>	NM_001124339.1	Fwd	GAGATGGGCAAGGGCTCTTT	74 bp	[44]
		Rev	GTGATACCACGCTCCCTCTCA		

Figure Legends

Figure 1. Relationship between Fatmeter readings and biochemical assay of wet muscle lipid content in female rainbow trout. The line shows least squares linear regression ($r^2 = 0.58$, $p < 0.0001$).

Figure 2. Mean (\pm SEM) fish mass specific growth rates (SGR) over time in female rainbow trout (A: two-year-old; B: three-year-old) fed a control-ration or restricted-ration. Growth rate over the preceding interval of four (B) or five (A) weeks is shown at each time-point. Treatment means differ significantly ($p < 0.05$) at time-points marked “*”. Within each age class, time-points within a treatment group sharing the same letter or letters are not significantly different ($p \geq 0.05$).

Figure 3. Mean (\pm SEM) condition factor (k) over time in female rainbow trout (A: two-year-old; B: three-year-old) fed a control-ration or restricted-ration. See Figure 1 for an explanation of what each symbol signifies.

Figure 4. Mean (\pm SEM) Fatmeter readings over time in female rainbow trout (A: two-year-old; B: three-year-old) fed a control-ration or restricted-ration. Data are not present for four-week sampling point in panel B. See Figure 1 for an explanation of what each symbol signifies.

Figure 5. Mean (\pm SEM) hepatosomatic index (HSI) over time in female rainbow trout (A: two-year-old; B: three-year-old) fed a control-ration or restricted-ration. See Figure 1 for an explanation of what each symbol signifies.

Figure 6. Mean (\pm SEM) liver insulin-like growth factor 1 (*igf1*) mRNA levels over time in female rainbow trout (A: two-year-old; B: three-year-old) fed a control-ration or restricted-ration. See Figure 1 for an explanation of what each symbol signifies.

Figure 7. Mean (\pm SEM) liver insulin-like growth factor 2 (*igf2*) mRNA levels over time in female rainbow trout (A: two-year-old; B: three-year-old) fed a control-ration or restricted-ration. See Figure 1 for an explanation of what each symbol signifies.

Figure 8. Mean (\pm SEM) liver insulin-like growth factor binding protein 1 (*igfbp1*) mRNA levels over time in female rainbow trout (A: two-year-old; B: three-year-old) fed a control-ration or restricted-ration. See Figure 1 for an explanation of what each symbol signifies.

Figure 9. Mean (\pm SEM) liver salmon leptin A1 (*slpA1*) mRNA levels over time in female rainbow trout (A: two-year-old; B: three-year-old) fed a control-ration or restricted-ration. See Figure 1 for an explanation of what each symbol signifies.

Figure 10. Mean (\pm SEM) plasma acylated ghrelin levels over time in female rainbow trout (A: two-year-old; B: three-year-old) fed a control-ration or restricted-ration. See Figure 1 for an explanation of what each symbol signifies.

Figure 1:

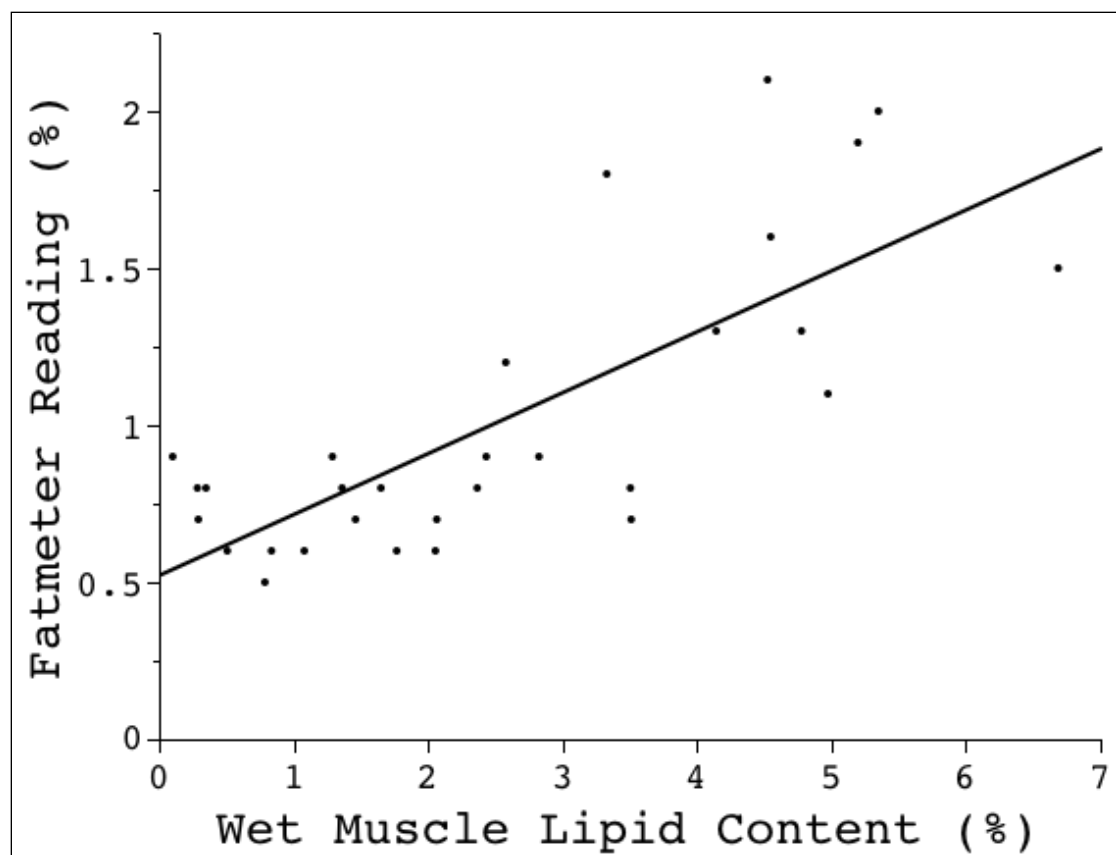


Figure 2:

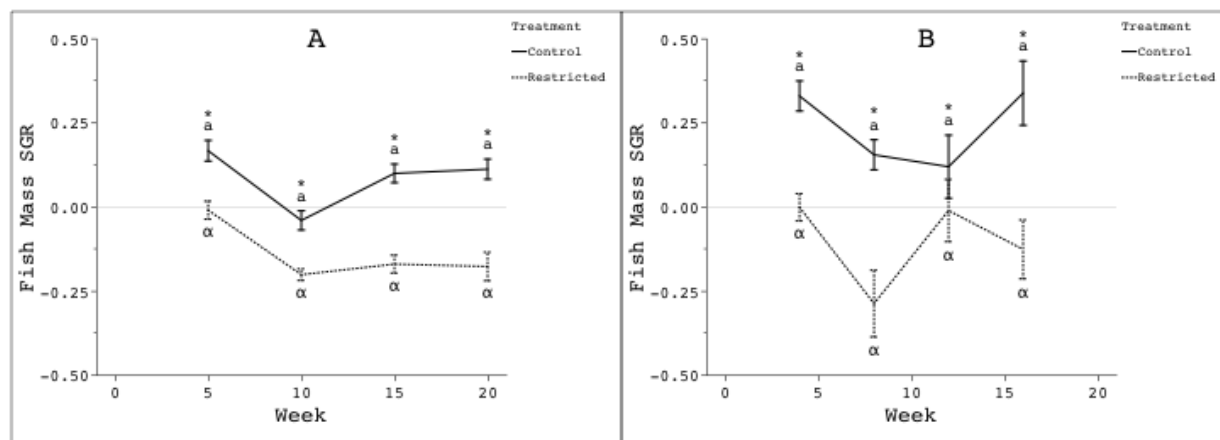


Figure 3:

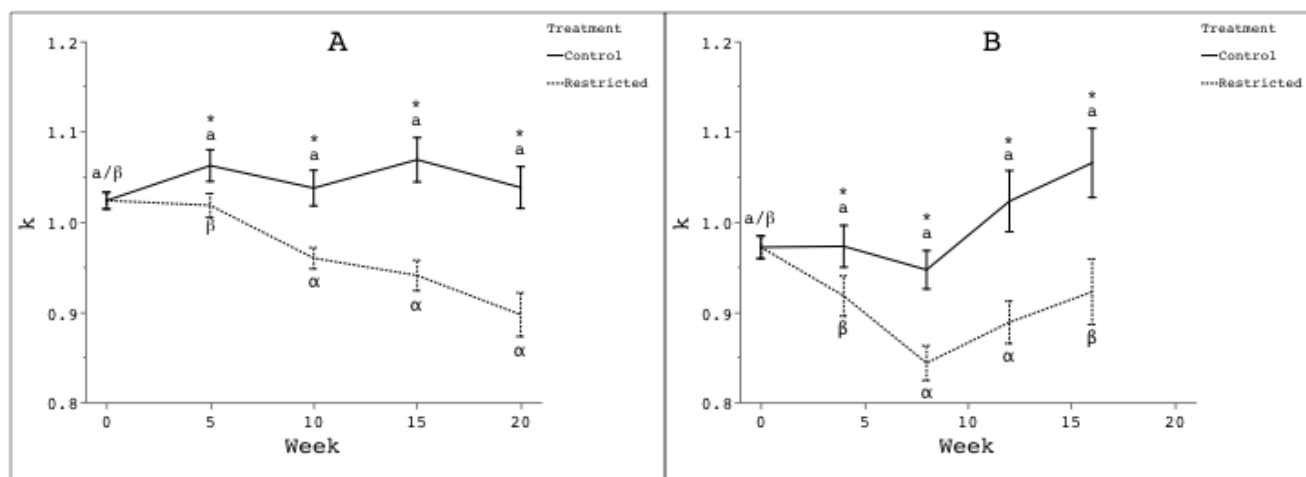


Figure 4:

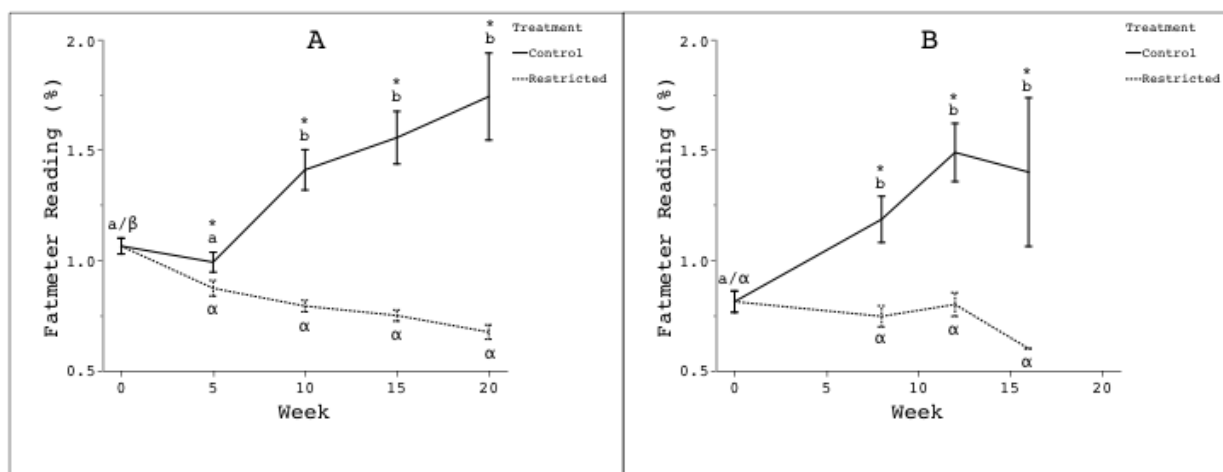


Figure 5:

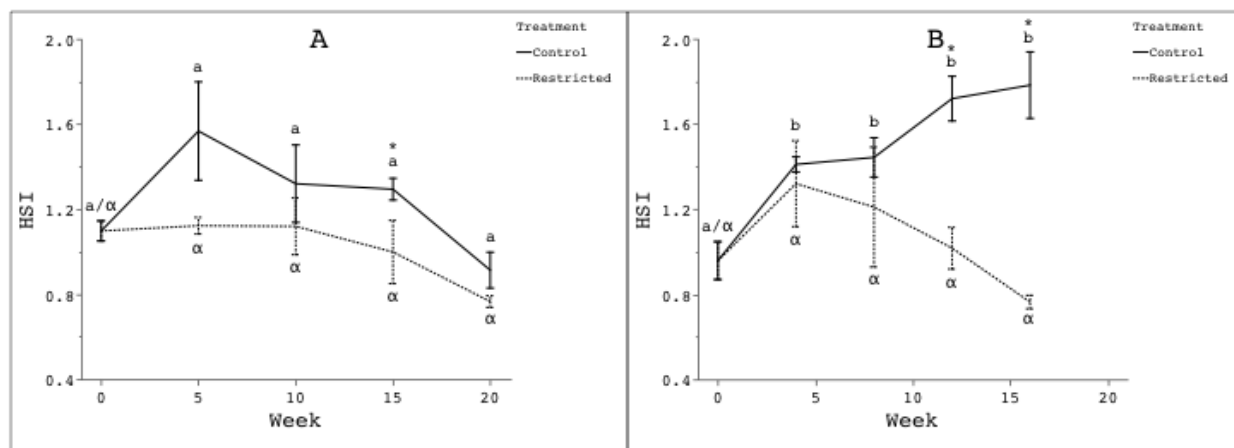


Figure 6:

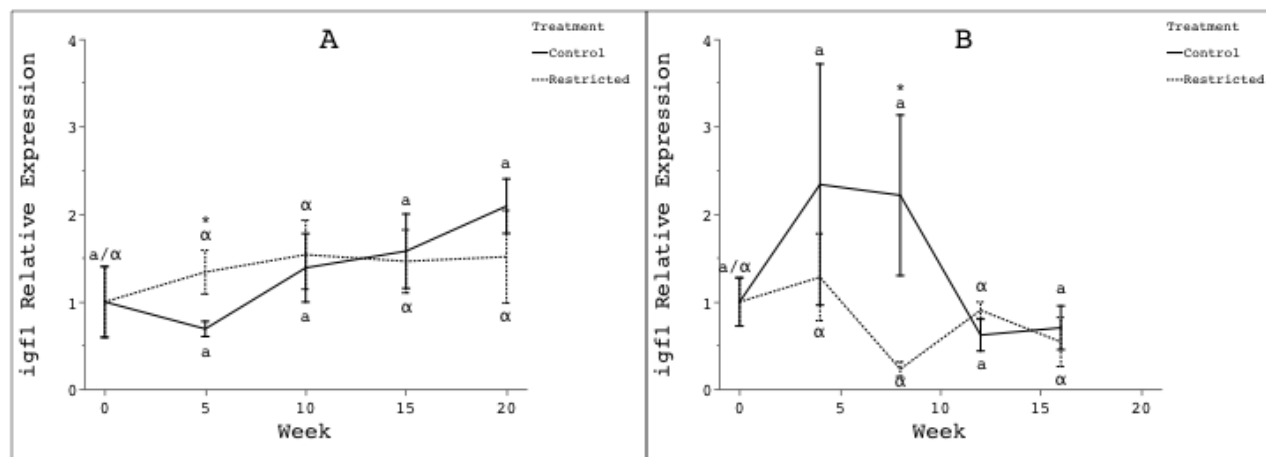


Figure 7:

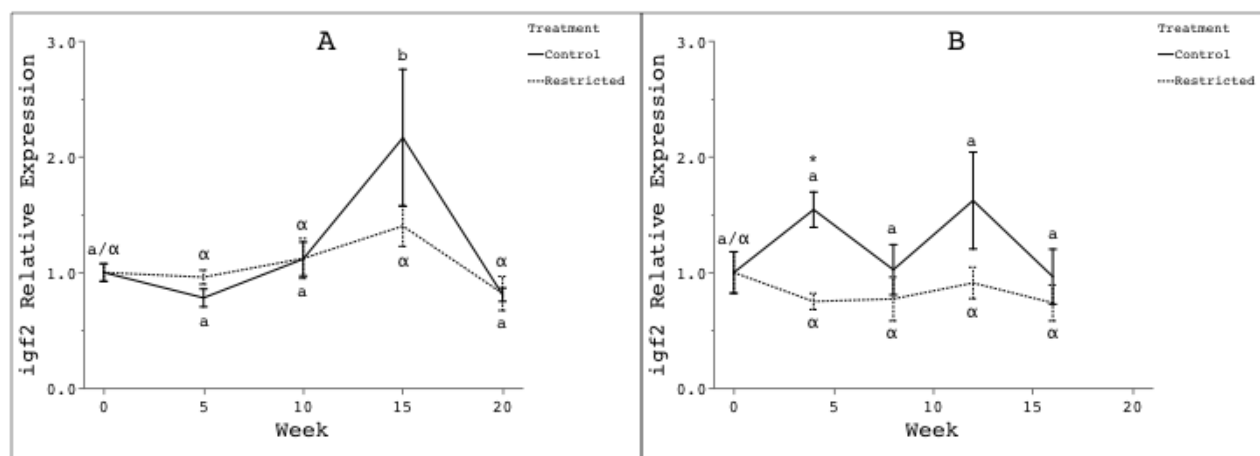


Figure 8:

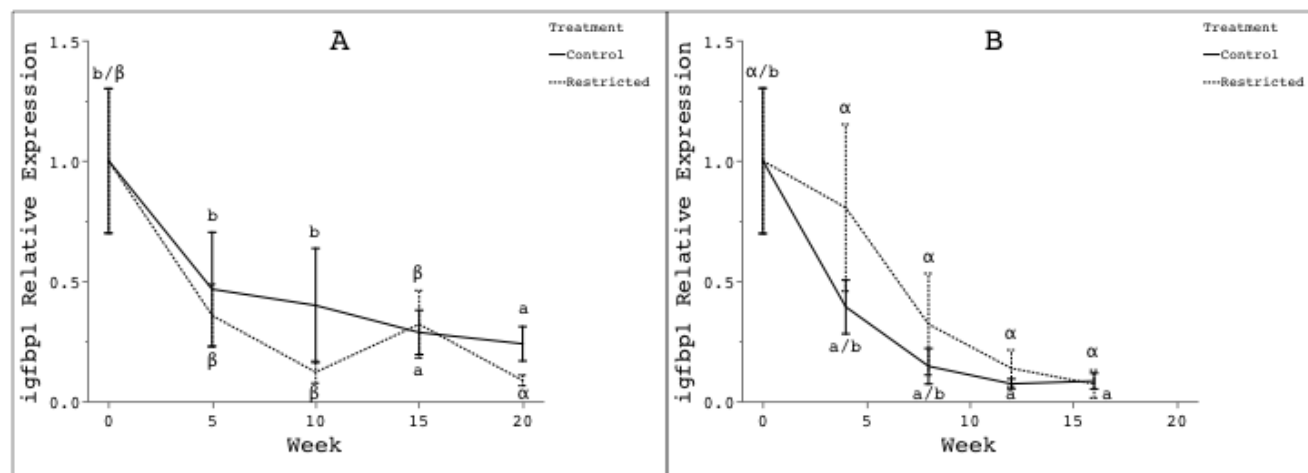


Figure 9:

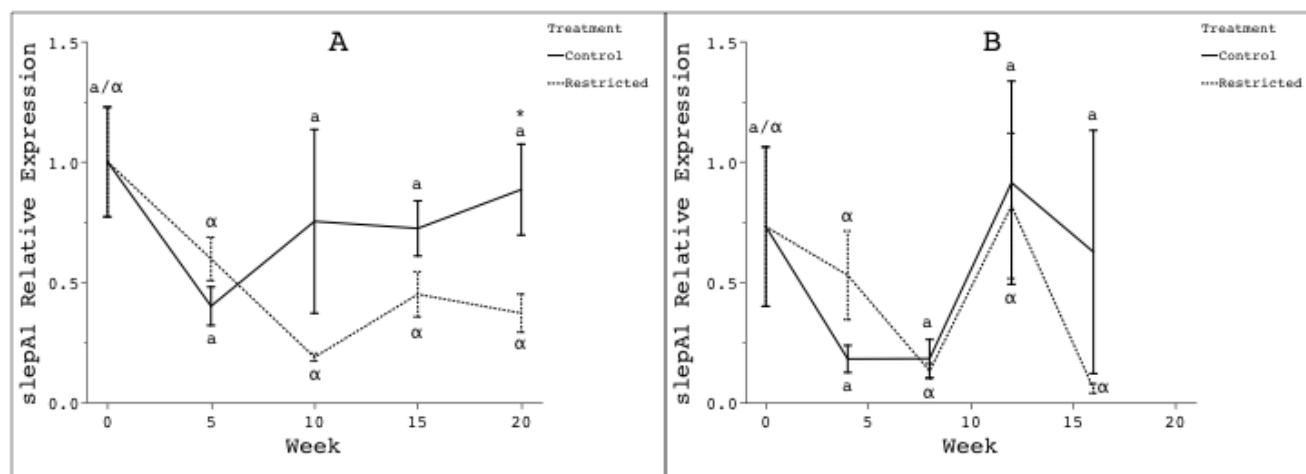
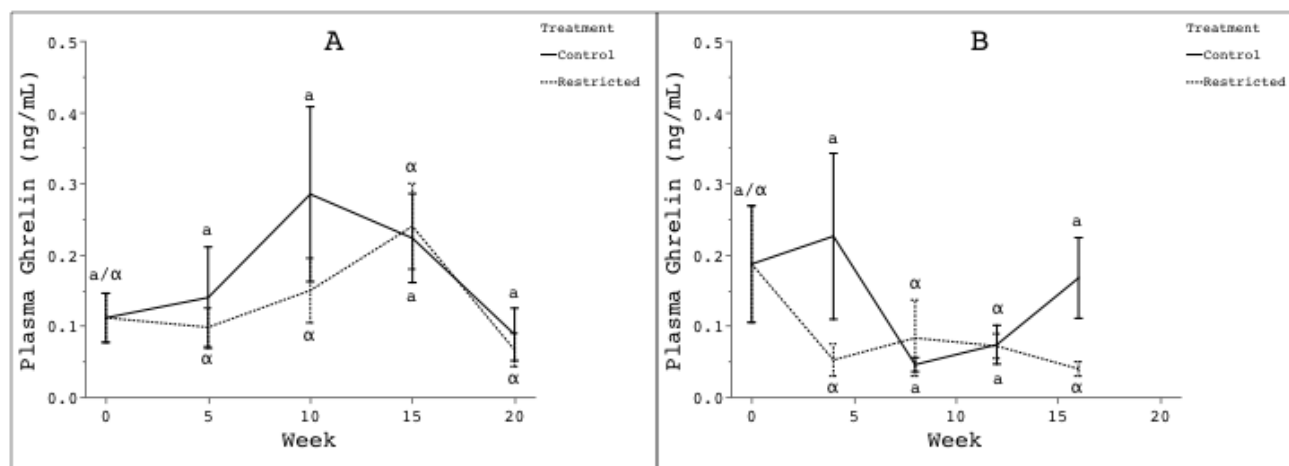


Figure 10:



Section B: Reproductive endocrine factors involved in spawning recovery and rematuration of iteroparous female rainbow trout (*Oncorhynchus mykiss*)

Lucius K Caldwell^{1, *}

Andrew L Pierce^{1, 2}

James J Nagler¹

1: University of Idaho, Department of Biological Sciences & Center for Reproductive Biology

2: Columbia River Intertribal Fish Commission

*: Corresponding author, telephone: (208) 885-6280, email: cald0653@vandals.uidaho.edu, fax: (208) 885-7905; Postal Address: Life Sciences South 252; P.O. Box 443051; Moscow, ID 83844-3051

Abstract

The endocrine factors and sequence of events that coordinate the process of initial maturation (puberty) in juvenile salmonid fishes is well described. However, the regulation of successive reproductive cycles (rematuration) and the role of nutritional stores in the rematuration process, are less understood. The purpose of this study was to determine how energy availability affects rematuration in a group of female rainbow trout (*Oncorhynchus mykiss*), and to profile changes in plasma levels and tissue expression of reproductive endocrine factors during this process. Food availability was restricted after spawning in a group of female trout, and these fish were compared with a control group that was fed a standard brood stock ration. The experiment was conducted twice, using two-year-old and three-year-old post-spawned fish. Bodyweight, length, and muscle lipid content were determined, and blood was collected from fish at regular intervals; a subset of fish from each group was sacrificed at each sampling time for the collection of ovary and pituitary tissue. Tissue gene expression was analyzed with q-RT-PCR; plasma hormone levels were quantified by radioimmunoassay (E_2) and enzyme-linked immunosorbent assay (11-KT). Food-restriction reduced Fulton's condition factor, muscle lipid content, and specific growth rate from one month onward. Food restriction arrested ovarian development by 15-20 weeks, as evidenced by lower plasma E_2 , lower ovarian mass, and smaller ovarian follicles in food-restricted fish. Pituitary *fsh- β* was elevated among control ration fish starting at 20 weeks, but not becoming significantly different until 30 weeks after spawning. Plasma 11-KT was elevated immediately after spawning, then rapidly decreased to and persisted at low levels in both control- and restricted-ration fish. Plasma E_2 levels began to increase in the control ration group between 10-16 weeks after spawning, compared to the restricted ration group, well in advance of when plasma 11-KT levels diverged at 25-30 weeks after spawning. These results show that this feed restriction regime arrested reproductive development and identified increasing E_2 , but not 11-KT, as an early indicator of advancing female reproductive development among repeat spawning *O. mykiss*.

1. Introduction

While the basic reproductive endocrine axis (brain-pituitary-gonad, BPG) is well described in many fishes [7, 48, 50], including salmonids [13, 19], the precise sequence of events that coordinate the onset of successive reproductive cycles in fishes has been less studied. Among the salmonid fishes, the mechanisms by which the BPG axis secretes endocrine factors to regulate initial maturation (puberty) are well understood [49], but there is a dearth of information concerning endocrine regulation and coordination of gonadal recrudescence (“rematuration”). While it seems reasonable to hypothesize that puberty and rematuration are essentially regulated identically, this hypothesis has largely gone untested.

The purpose of this study was to investigate the role of various reproductive endocrine factors involved in rematuration of iteroparous female rainbow trout, and to determine how energy limitation affects rematuration in a population of these fish. It was hypothesized that by restricting food availability in a group of female trout, these animals could be induced to adopt a skip-spawner life history that has been described in numerous teleost fishes [18, 27, 28] and other vertebrates [3], and that this life history decision would be reflected in both circulating levels of and tissue mRNA levels encoding message for reproductive endocrine factors.

According to our current understanding, puberty is initiated by endocrine factors—primarily the gonadotropin releasing hormones (GnRHs) – originating in the hypothalamus and other brain regions, which act hierarchically to stimulate pituitary release of the gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH), which in turn stimulate gonadal growth and development, along with steroidogenic pathways culminating in the secretion of the sex steroids 17 β -estradiol (E2) and 11-keto-testosterone (11-KT) [19, 26, 41, 48]. Although the role of these factors and the sequence of events that set about the onset of puberty in fishes have been well characterized, what is less clear is the role of these endocrine factors in rematuration of iteroparous species.

2. Material and Methods

2.1. Animals

Post-spawning female rainbow trout *Oncorhynchus mykiss* were purchased from Troutlodge (Sumner, WA) and transported to the University of Idaho (Moscow, ID). Fish had been manually strip-spawned 2 d prior to transport, and were fasted for one month (in the case of two-year-old trout) or two months (in the case of three-year-old trout) prior to spawning. Fish were held in 1,130 L tanks, in a recirculating system (flow rate 14 L min⁻¹ per tank, temperature 12 to 15°C following a seasonal profile). Experiments were conducted under approved protocols in accordance with the principles and procedures of the Animal Care and Use Committee, University of Idaho.

2.2. Experiments

For both experiments, treatments consisted of a control group, which was fed 0.5% total fish mass per day, and a restricted group, which was fed 0.1% fish mass per day. Fish were fed a commercial trout broodstock diet (6.4 mm pellets, Rangen, Inc., Buhl, ID). Rations were adjusted to compensate for a 24-h pre-sampling fast, fish numbers, and fish weight based on sampling data and mortalities. Fish were individually identified by PIT tags.

2.2.1. Experiment 1: Three-year-old fish

Three-year-old fish (post 2nd spawning) were stocked into 6 tanks (25 February 2010, 26-27 fish per tank, average weight 1.35 kg). Tanks were randomly assigned control or restricted feeding treatments ($n=3$ tanks per treatment), and fish were sampled every 4 weeks. During sampling, all fish were anesthetized (60 mg L⁻¹ tricaine methanesulfonate, buffered). Fish were weighed, fork length was measured, muscle lipid content was measured (Fish Fatmeter, Distell, Fauldhouse, UK), and blood (2 ml) was collected from the caudal vein using syringes that were pre-coated with heparin by aspirating and then dispensing 3.0 mL of 10 mg mL⁻¹ ammonium heparin (Sigma-Aldrich) suspended in ultrafiltered H₂O. Plasma was separated by centrifugation and stored at -80 °C. At each sampling, ten fish ($n=5$ fish per treatment group) were lethally sampled. Livers were dissected and weighed, and a liver sample was collected and snap-frozen in liquid N₂ for tissue gene expression analyses using q-RT-PCR. To reduce post-spawning mortality, fish were stripped of residual eggs and injected with oxytetracycline (Liquamycin®, Pfizer, Inc., New York, NY, 20 mg kg⁻¹) at the second sampling date. To control *Gyrodactylus* sp. gill parasites, fish were treated twice with praziquantel (Medisca, Plattsburgh, NY, water borne, 2.3 - 3.4 mg L⁻¹).

2.2.2. Experiment 2: Two-year-old fish

Two-year-old fish (post 1st spawning) were stocked into 12 tanks (3 March 2011, 26-27 fish per tank, average weight 1.13 kg). Tanks were randomly assigned control or restricted feeding treatments ($n=6$ tanks per treatment). Sampling was similar to Experiment 1, except that fish were sampled every 5 weeks, 12 fish were lethally sampled at each time point ($n=6$ fish per treatment group), and liver samples were collected in RNAlater (QIAGEN, Hilden, Germany) before being snap-frozen in liquid N₂. At the time of stocking, fish were stripped of residual eggs, injected with oxytetracycline, and treated with praziquantel as described above. In addition, fish were treated to control Saprolegnia (formalin 150 ppm and NaCl 2%, 1 hour static baths, repeated 3 times).

2.3. RNA Extractions & cDNA Synthesis

Pituitary samples were homogenized in 1.0 mL TRIzol® (Invitrogen™, Life Technologies, Carlsbad, CA), and RNA was isolated following the TRIzol protocol, using three chloroform:isoamyl alcohol extractions and three 70% ethanol washes. Resuspended nucleic acid fractions were treated with DNase (TURBO™ DNA-free, Ambion®, Life Technologies, Carlsbad, CA). RNA purity was assessed by spectrophotometric absorbance

(NanoDrop ND-1000, Thermo Fisher), and RNA concentration was measured using the RiboGreen RNA assay kit (Invitrogen) with a fluorometer. 1 µg total RNA was reversed transcribed with the SuperScript III First-Strand Synthesis Kit (Invitrogen) using random hexamer primers. cDNA was diluted 1:5 in 1x Tris-EDTA.

2.4. q-RT-PCR

Quantitative real-time reverse transcriptase PCR (q-RT-PCR) primer sets were adapted from published sequences (Table 1). Specificity was confirmed by bioinformatic analysis, agarose gel electrophoresis of PCR products, and melting curve analysis of PCR products. To carry out the q-RT-PCR, sample cDNA was amplified in 96-well optical reaction plates (Invitrogen) containing 20 µL PCR reactions made up of 2 µL cDNA, 10 µL Power SYBR® Green PCR Master Mix (Life Technologies), 6 µL H₂O, and 2 µL of a mix of forward and reverse primers at 2 pM each, in an Applied Biosystems™ ABI 7900HT real-time PCR system (Life Technologies) (2 min @ 50°C; 10 min @ 95°C; 40 cycles of 15 sec @ 95°C and 1 min @ 60°C). Copy numbers in samples were quantified using standard curves of PCR amplicons. Three technical replicate PCRs were completed for each sample. Expression levels of target genes were normalized by dividing the copy number of the target gene by the copy number of the reference gene (β-actin). The mean of the normalized expression level for the replicate PCRs is the value reported. q-PCR results were log₂-transformed prior to statistical analysis.

2.5. 17β-Estradiol RIA

2.5.1. Solvent Extraction

Individual aliquots of plasma were extracted twice 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 culture tube (Fisher Scientific, Hampton, NH), 4.0 mL MTBE was added to each tube, samples were vortexed for 1 m, and then samples were given 7 m for phase separation to occur. After phase separation was visually observed, a small amount of liquid N₂ 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 N₂. 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 extract 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 of the remaining aqueous fraction from each plasma sample 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.

2.5.2. RIA

Resuspended plasma extracts were analyzed for 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 is reported to be 8 pg mL⁻¹.

2.6 11-keto testosterone EIA

2.6.1. Solvent Extraction

Individual aliquots of plasma were extracted twice consecutively with anhydrous diethyl ether (JT Baker, Avantor Performance Materials, Inc.; Center Valley, PA, USA). Briefly, 100 µL plasma from each sample was pipetted into a 10 mL glass culture tube (Fisher Scientific, Hampton, NH), 2.0 mL diethyl ether was added to each tube, samples were vortexed for 1 m, and then frozen on dry ice (solid CO₂). After 6-8 m, the aqueous phase was visually inspected to ensure that it was frozen solid, and the solvent fraction was then poured off into a 5 mL glass tube. A second plasma extraction of the remaining aqueous fraction from each plasma sample was then performed, again using 2.0 mL diethyl ether, as described above; this second extract was pooled with the first extract. The pooled diethyl ether extracts were then placed in a 49°C water bath (OA-SYS™ Heating System; Organomation Associates, Inc; Berlin, MA) and dried down under a gentle stream of N₂ directed *via* a nitrogen evaporator manifold (N-EVAP™ 112; Organomation Associates, Inc; Berlin, MA). Once the solvent had completely volatilized, the residue from the pooled extracts of 100 µL plasma was resuspended in 1000 µL EIA buffer from the 11-KT EIA kit (described below), yielding a 1:10 dilution factor for the resuspended solvent-extracted plasma.

2.6.2 EIA

Resuspended plasma extracts were analyzed for 11-KT concentration using an antibody-coated 96-well plate based enzyme-linked immunosorbent assay (EIA) kit (Cayman Chemical Company; Ann Arbor, MI). Sensitivity for the assay is reported to be 1 pg mL⁻¹.

2.7. Data Analysis

Specific growth rate for mass (mass SGR) was calculated as
$$\ln \left[\left(\frac{\text{mass at time 2 (g)}}{\text{mass at time 1 (g)}} \right)^{\frac{1}{\text{interval (days)}}} \right] \times 100$$
 [6]. Fulton's condition factor (k)

was calculated as $\frac{\text{body mass (g)}}{\text{fork length (cm)}^3} \times 100$ [8]. Fatmeter readings were validated by chemical analyses of muscle lipid content (Fig. 1); the correlation between the two measurements was high (linear regression, $r^2 = 0.58$; Tukey mean-difference plot, matched pairs correlation = 0.76), as previously found in other studies on salmonids [3, 4]. Gonadosomatic index (GSI) was calculated as

$\frac{\text{ovarian mass (g)}}{\text{body mass (g)} - \text{ovarian mass (g)}} \times 100$. Only data from fish that survived until being terminally sampled were included in statistical analyses for SGR, k, Fatmeter readings, and GSI. For SGR, k, Fatmeter readings, and GSI, among two-year-old trout at week 0, n=84; week 5, n=36; week 10, n=30; week 15, n=24; week 20, n=18; week 25, n=12; week 30, n=6 per treatment. For SGR, k, Fatmeter readings, and HSI, among three-year-old trout at week 0, n=46; week 4, n=18; week 8, n=13; week 12, n=8; week 16 n=3 per treatment.

Systematic tank differences were not detected within treatment for any variable (ANOVA, $p > 0.05$). Therefore, tank replicates were pooled and analyzed together. For normality and homoscedasticity requirements of ANOVA and post-hoc tests, data were log-transformed prior to analysis; for clarity, figures depict un-transformed data. Two-way ANOVA was used to detect main and interaction effects (time, treatment, time x treatment). When ANOVA indicated a significant time effect, Tukey-Kramer Honestly Significant Difference tests (Tukey-Kramer HSD) were used to compare values at all time points within a given treatment. Within each time point, two-tailed t-tests were used to detect treatment differences. Statistical analyses were performed within JMP® (Version 9, SAS Institute Inc., Cary, NC). Differences are reported as significant when $p < 0.05$.

3. Results

As described previously (Caldwell et al. in press), the feeding regime used here affected metrics of growth and metabolism in both two- and three-year-old rematuring female rainbow trout. Additionally, the treatments produced a difference in reproductive development during the period of recrudescence, as indicated by plasma hormone levels and relative ovarian mass (i.e., GSI). SGR was greater among control-ration fish than among restricted-ration fish at all time points in both two-year-old and three-year-old trout (Fig. 1). Fish fed the control-ration generally exhibited positive growth, while fish that were fed the restricted-ration generally exhibited negative growth. Fulton's condition factor (k) decreased over time in restricted-ration fish, while remaining the same or slightly trending toward an increase in control-ration fish. Restricted ration fish had lower k values than control-ration fish at all time points after the initial sampling in two-year-old and at all time points after week four in three-year-old trout (Fig. 2). Feed-restriction also affected muscle lipid content. Muscle lipid percentage increased over time among control-ration fish and remained static over time among restricted-ration fish. This led to greater muscle lipid level among control-ration fish

than among restricted-ration fish, in both two-year-old and three-year-old trout (Fig. 3). GSI diverged between full-ration fish and restricted-ration fish toward the end of the experiment with two-year-old fish, but the experiment with three-year-old fish did not continue long enough to capture this effect (Fig. 4). Among two-year-old trout, control-ration fish exhibited significantly elevated GSI at week 30 compared to all previous weeks.

Among two-year-old trout, from weeks 15-30, pituitary fsh- β expression decreased over time within restricted-ration fish and increased over time within control-ration fish (Fig. 5). This trend lead to a significant difference between the treatment groups at week 30. Among three-year-old trout, pituitary fsh- β expression did not change over time within either treatment group, and the two groups did not differ at any time point. [FOR DISCUSSION: It may be the case that the experiment with three-year-old trout simply did not last long enough to capture the difference observed among the two-year-old trout.]

Plasma concentration of E2 (Fig. 6) showed the strongest signal of the effect of feeding regime on reproductive development, diverging between control- and restricted-ration fish by week ten among two-year-old trout, and showing a similar but insignificant trend among three-year-old trout starting at week eight. Interestingly, at week zero, three-year-old trout exhibited elevated levels of E2—an order of magnitude greater than those observed among two-year-old trout—that significantly decreased as the experiment progressed. Among two-year-old trout fed the control ration, plasma E2 levels continued to dramatically increase over the course of the experiment.

Plasma concentration of 11-KT (Fig. 7) showed a similar trend within both year classes, of elevated levels at week zero before a precipitous drop that persisted through the entire experiment. Among two-year-old trout, plasma 11-KT significantly diverged between control-ration and restricted-ration fish at week 25; the experiment with three-year-old trout was presumably not long enough to capture this effect, as no difference was detected.

4. Discussion

The restricted-ration employed in these experiments affected nutrition sufficiently to arrest reproductive development among two-year-old trout; the experiment using three-year-old trout did not continue long enough to capture this arrest. However, it is clear from the experiment using two-year-old trout that between 15 and 20 weeks post-spawn, the control-ration fish began to increasingly partition energy to the ovary for developing oocytes. Previous work (Caldwell et al. in review) described how this feeding regime induced remodeling of organs and a general redistribution of energy stores, with control ration fish accumulating lipid in muscle tissue and increasing the size of their livers. In light of these

observations, we hypothesize that the restricted-ration fish make a physiological effort to preserve their ovarian investment at the expense of muscle and liver stores of both lipid and glycogen [2, 16, 36, 37]. However, some nutritional tipping point exists—reached at 20-25 weeks in this experiment—when reduced food availability causes these animals to trade off between continuing reproductive development and survival [12], a decision that is presumably associated with a strong selective pressure to arrest rematuration [20, 21].

Interestingly, although relative ovarian size (GSI) does not reflect this divergence between the two treatment groups until 25 weeks, differences in plasma E2 between the two treatment groups are already significant at week ten, and are presumably present sometime between weeks five and ten of the experiment. This would indicate that a trajectory of reproductive rematuration for the following year's spawning effort has been at least partially determined approximately six weeks after spawning (as fish were obtained approximately one week after spawning), similar to the current understanding of the progression of puberty in salmonid fishes.

According to the model of reproductive development described by Thorpe and others [1, 29, 43, 45, 46] for Atlantic salmon (*Salmo salar*) and subsequently supported in other salmonid fishes [4, 35, 39, 40], sexual maturation begins immediately after fertilization, is arrested during the juvenile phase, and then progresses following release of inhibition dependent on physiological state during proposed critical periods. In other words, maturation is regulated by inhibition, rather than stimulation. Sexual maturity proceeds when an animal exceeds some physiological threshold during decision windows in the year prior to maturation. For example, a fall-spawning Atlantic salmon makes a physiological commitment to initiate the process of maturation (or not) sometime during November in the year prior to spawning [24]. For a spring-spawning rainbow trout, this decision presumably takes place in the late winter to early summer months, a year before spawning [32]. There is uncertainty regarding the nature of the physiological thresholds that gate the progression of maturation, but a leading current hypothesis holds that a fish assesses its physiological status, including growth rate and lipid reserves, and matures if it exceeds some genetically determined minima for critical criteria [30, 31, 44]. It seems likely that a similar situation holds for rematuring fish, and our results suggest this is the case.

Classic reproductive endocrine axis theory [e.g., 13, 48] would predict that hypothalamic (i.e., GnRH) and pituitary (i.e., FSH) factors should be elevated in advance of the observed increase in plasma E2. However, measuring plasma levels of the GnRH peptide in fishes is logistically hampered by anatomical constraints associated with fishes' lack of a hypothalamic-pituitary portal system [22]. This means that in fishes, hypothalamic factors are communicated directly from the hypothalamus to the pituitary via nerve fibers and never enter systemic circulation in appreciable amounts. Regarding the measurement of FSH, there is no currently available RIA or EIA for the measurement of plasma FSH in rainbow trout. However, we hypothesize that if plasma levels of FSH were measured, an early peak in plasma FSH may be present among those fish for which an increase in plasma E2 starting at week 10 was observed [5, 25, 47]. We could detect no such pattern using q-RT-PCR to measure pituitary mRNA levels for *fsh-β* (Fig. 5). Alternatively, adult trout that are rematuring may already exhibit FSH levels that are sufficiently elevated

to stimulate ovarian E2 synthesis, such that no increasing FSH signal is observed when collecting fish immediately after spawning. At least in mammals [34], the ovaries themselves may synthesize FSH, which then acts in an autocrine or paracrine fashion to contribute to the regulation of gonadal development. It could also be the case that during rematuration the ovary is sensitized to the effects of FSH via some other endocrine mechanism [23, 33, 49]. Finally, it must be emphasized that the relationship between FSH and E2 is not necessarily linear or simple, but may be the outcome of the interplay of numerous endocrine and paracrine factors, including activins, inhibins, and various growth factors [8, 9, 38].

The feeding regime selected for this study had a rapid and dramatic impact on fish growth and nutritional status, as evidenced by growth rate (SGR), condition factor (k), and muscle lipid content. For both two-year-old and three-year-old post-spawned female rainbow trout, control-ration fish generally maintained a state of positive growth rate for the duration of the experiment, while restricted-ration fish maintained a state of negative growth rate for the duration of the experiment. While it may be intuitive that fish that are fed more will grow more, it is important to note that the feeding regime selected for these experiments did not simply lead to a gradation of response, but rather a categorical difference in growth status (i.e., anabolic versus catabolic states) between the treatment groups.

Condition factor (k) is a metric of body shape that has been shown to be strongly correlated with total body lipid in rainbow trout [42]; k also responded to the feeding treatment within both age classes, tending to remain steady among control-ration fish, while decreasing over time among restricted-ration fish. This suggests that the reduction in growth rate observed among the control-ration fish was enough to induce organismal remodeling that was measurable by a change in body shape.

Muscle lipid content—as measured with the Distell fish fatmeter—was also affected by the feeding treatment within both age classes. Among control-ration fish, muscle lipid content increased over time; among restricted-ration fish, muscle lipid content decreased or insignificantly trended downward over time. This rapidly led to persistent differences in muscle lipid content between the treatment groups.

Finally, this study addressed recent work [15] suggesting that 11-KT—the primary androgen found in most fishes included salmonids—plays a role in stimulating early ovarian development in salmonids. While both age classes of trout did start the experiment with elevated plasma 11-KT, concentrations dropped rapidly between week zero and week five, and remained near the limit of detection for the duration of the experiment. Between weeks 25 and 30, treatment groups within the two-year-old trout diverged subtly but significantly, with control ration fish exhibiting slightly elevated plasma levels of 11-KT. Based on these results, it appears that 11-KT may be involved in spawning physiology, but we find no support for the hypothesis that 11-KT is involved in the process of rematuration during the period immediately following spawning.

In conclusion, by restricting food availability in a group of female rainbow trout, growth and energy partitioning were both affected, with restricted-ration fish generally existing in a catabolic state and control-ration fish existing in an anabolic state for the duration of the experiment.

This lead to predictable changes in body morphology and energy stores: restricted-ration fish became skinnier and mobilized muscle lipid, while control-ration fish grew rounder and accumulated muscle lipid content. The treatments used here were sufficient to induce differences in gonadal recrudescence between the two groups: while restricted-ration fish arrested ovarian growth beginning at approximately 20 weeks, control ration fish continued to rapidly accumulate ovarian tissue until the end of the experiment. Differences in ovarian size were preceded by differences in circulating levels of E2, which diverged between one and two months after the start of the experiment. Although pituitary secretion of FSH presumably drives the increase in ovarian steroidogenesis underlying elevated plasma E2, this work suggests that FSH is not regulated at the level of transcription in the months immediately after spawning. Finally, we see no evidence that 11-KT is involved in early maturation of recrudescing female rainbow trout.

Table 1 – Primer sequence data for q-RT-PCRs.

Gene	Accession Number	Direction	Sequence	Product Size	Reference
<i>fsh-b</i>	AB050835	Fwd	AGAGCTGCGATTGCATCAAA	61 bp	??
		Rev	GCCATGCTTATGCGATCACA		
<i>b-actin</i>	AJ438158	Fwd	CCAACAGATGTGGATCAGCAA	118 bp	[1]
		Rev	GGTGGCAGAGCTGAAGTGGTA		

[1] P. Koldkjær, T.G. Pottinger, S.F. Perry, A.R. Cossins, Seasonality of the red blood cell stress response in rainbow trout (*Oncorhynchus mykiss*). J Exp Biol. 207 (2004) 357-367.

5. References

- [1] C.E. Adams, J.E. Thorpe, Photoperiod and temperature effects on early development and reproductive investment in Atlantic salmon (*Salmo salar* L.). Aquaculture. 79 (1989) 403-409.
- [2] H.E. Bergan, J.D. Kittilson, M.A. Sheridan, Nutrition-regulated lipolysis in rainbow trout (*Oncorhynchus mykiss*) is associated with alterations in the ERK, PI3K-Akt, JAK-STAT, and PKC signaling pathways. Gen. Comp. Endocrinol. 176 (2012) 367-376.
- [3] J.J. Bull, R. Shine, Iteroparous animals that skip opportunities for reproduction. Am. Nat. 114 (1979) 296-303.

- [4] B. Campbell, J. Dickey, B. Beckman, G. Young, A. Pierce, H. Fukada, et al., Previtellogenic oocyte growth in salmon: relationships among body growth, plasma insulin-like growth factor-1, estradiol-17beta, follicle-stimulating hormone and expression of ovarian genes for insulin-like growth factors, steroidogenic-acute regulatory protein and receptors for gonadotropins, growth hormone, and somatolactin. *Biol. Reprod.* 75 (2006) 34-44.
- [5] B. Campbell, J. Dickey, B. Beckman, G. Young, A. Pierce, P. Swanson, Endocrine changes associated with the growth of pre-vitellogenic oocytes in coho salmon, *Oncorhynchus kisutch*. *Fish Physiol. Biochem.* 28 (2003) 287-289.
- [6] J.F. Carragher, N.W. Pankhurst, Plasma levels of sex steroids during sexual maturation of snapper, *Pagrus auratus* (Sparidae), caught from the wild. *Aquaculture.* 109 (1993) 375-388.
- [7] M. Carrillo, S. Zanuy, A. Felip, M.J. Bayarri, G. Molès, A. Gûmez, Hormonal and environmental control of puberty in perciform fish. *Ann. N. Y. Acad. Sci.* 1163 (2009) 49-59.
- [8] J. Chyb, T. Mikolajczyk, B. Breton, Post-ovulatory secretion of pituitary gonadotropins GtH I and GtH II in the rainbow trout (*Oncorhynchus mykiss*): regulation by steroids and possible role of non-steroidal gonadal factors. *J. Endocrinol.* 163 (1999) 87-97.
- [9] E. Clelland, C. Peng, Endocrine/paracrine control of zebrafish ovarian development. *Mol. Cell. Endocrinol.* 312 (2009) 42-52.
- [10] J. Colt, K.D. Shearer, Evaluation of the use of the Torry fish fatmeter to non-lethally estimate lipid in adult salmon. Evaluation Of Migrational Delays On The Reproductive Success Of Adult Hatchery Spring Chinook Salmon In The Columbia And Snake Rivers, Northwest Fisheries Science Center National Marine Fisheries Service, Seattle, WA, 2001.
- [11] G.T. Crossin, S.G. Hinch, A nonlethal, rapid method for assessing the somatic energy content of migrating adult Pacific salmon. *Transactions of the American Fisheries Society.* 134 (2005) 184-191.
- [12] B. Dantzer, E.M. Swanson, Mediation of vertebrate life histories via insulin-like growth factor-1. *Biol. Rev.* 87 (2012) 414-429.
- [13] B. Davies, N. Bromage, P. Swanson, The brain-pituitary-gonadal axis of female rainbow trout *Oncorhynchus mykiss*: effects of photoperiod manipulation. *Gen. Comp. Endocrinol.* 115 (1999) 155-166.
- [14] K.D. Fausch, Profitable stream positions for salmonids: relating specific growth rate to net energy gain. *Can. J. Zool./Rev. Can. Zool.* 62 (1984) 441-451.
- [15] K.L. Forsgren, G. Young, Stage-specific effects of androgens and estradiol-17beta on the development of late primary and early secondary ovarian follicles of coho salmon (*Oncorhynchus kisutch*) in vitro. *Biol. Reprod.* 87 (2012) 64.
- [16] K.J. Harmon, M.T. Bolinger, K.J. Rodnick, Carbohydrate energy reserves and effects of food deprivation in male and female rainbow trout. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* 158 (2011) 423-431.
- [17] F. Heincke, Bericht über die untersuchungen der biologischen anstalt auf Helgoland zur naturgeschichte der nutzfische. Die Beteiligung Deutschlands an der Internationalen Meeresforschung, Wissenschaftliche Kommission, Berlin, 1908, pp. 67-155.

- [18] J. Kennedy, P.R. Witthames, R.D.M. Nash, C.J. Fox, Is fecundity in plaice (*Pleuronectes platessa* L.) down-regulated in response to reduced food intake during autumn? J. Fish Biol. 72 (2008) 78-92.
- [19] J. Kim, W.L. Hayton, I.R. Schultz, Modeling the brain-pituitary-gonad axis in salmon. Mar. Environ. Res. 62 (2006) S426-S432.
- [20] M.T. Kinnison, M.J. Unwin, A.P. Hendry, T.P. Quinn, Migratory costs and the evolution of egg size and number in introduced and indigenous salmon populations. Evolution. 55 (2001) 1656-1667.
- [21] M.T. Kinnison, M.J. Unwin, T.P. Quinn, Migratory costs and contemporary evolution of reproductive allocation in male chinook salmon. J. Evol. Biol. 16 (2003) 1257-1269.
- [22] B. Levavi-Sivan, J. Bogerd, E.L. Mañanós, A. Gómez, J.J. Lareyre, Perspectives on fish gonadotropins and their receptors. Gen. Comp. Endocrinol. 165 (2010) 412-437.
- [23] K.-C. Liu, S.-W. Lin, W. Ge, Differential regulation of gonadotropin receptors (*fshr* and *lhcg*) by estradiol in the zebrafish ovary involves nuclear estrogen receptors that are likely located on the plasma membrane. Endocrinology. 152 (2011) 4418-4430.
- [24] M. Mangel, W.H. Satterthwaite, Combining proximate and ultimate approaches to understand life history variation in salmonids with application to fisheries, conservation, and aquaculture. Bulletin of Marine Science. 83 (2008) 107-130.
- [25] G. Maugars, M. Schmitz, Gene expression profiling during spermatogenesis in early maturing male Atlantic salmon parr testes. Gen. Comp. Endocrinol. 159 (2009) 178-187.
- [26] J.N. Nocillado, A. Elizur, Neuroendocrine regulation of puberty in fish: insights from the grey mullet (*Mugil cephalus*) model. Mol. Reprod. Dev. 75 (2008) 355-361.
- [27] R.M. Rideout, G.A. Rose, M.P.M. Burton, Skipped spawning in female iteroparous fishes. Fish Fish. 6 (2005) 50-72.
- [28] A.D. Rijnsdorp, The mechanism of energy allocation over reproduction and somatic growth in female North Sea plaice, *Pleuronectes platessa* L. Neth. J. Sea Res. 25 (1990) 279-289.
- [29] D.K. Rowe, J.E. Thorpe, A.M. Shanks, Role of fat stores in the maturation of male Atlantic salmon (*Salmo salar*) parr. Can. J. Fish. Aquat. Sci. 48 (1991) 405-413.
- [30] W.H. Satterthwaite, M.P. Beakes, E.M. Collins, D.R. Swank, J.E. Merz, R.G. Titus, et al., Steelhead life history on California's central coast: insights from a state-dependent model. Trans. Am. Fish. Soc. 138 (2009) 532-548.
- [31] W.H. Satterthwaite, M.P. Beakes, E.M. Collins, D.R. Swank, J.E. Merz, R.G. Titus, et al., State-dependent life history models in a changing (and regulated) environment: steelhead in the California Central Valley. Evolutionary Applications. 3 (2010) 221-243.
- [32] D.P. Scott, Effect of food quantity on fecundity of rainbow trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 19 (1962) 715-731.
- [33] A.N. Setiawan, Y. Ozaki, A. Shoaie, Y. Kazeto, P.M. Lokman, Androgen-specific regulation of FSH signalling in the previtellogenic ovary and pituitary of the New Zealand shortfinned eel, *Anguilla australis*. Gen. Comp. Endocrinol. 176 (2012) 132-143.

- [34] A. Shahed, K.A. Young, Intraovarian expression of GnRH-1 and gonadotropin mRNA and protein levels in Siberian hamsters during the estrus cycle and photoperiod induced regression/recrudescence. *Gen. Comp. Endocrinol.* 170 (2011) 356-364.
- [35] K. Shearer, P. Parkins, B. Gadberry, B. Beckman, P. Swanson, Effects of growth rate/body size and a low lipid diet on the incidence of early sexual maturation in juvenile male spring Chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture*. 252 (2006) 545-556.
- [36] M.A. Sheridan, Lipid dynamics in fish: aspects of absorption, transportation, deposition and mobilization. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*. 90 (1988) 679-690.
- [37] M.A. Sheridan, T.P. Mommsen, Effects of nutritional state on *in vivo* lipid and carbohydrate metabolism of coho salmon, *Oncorhynchus kisutch*. *Gen. Comp. Endocrinol.* 81 (1991) 473-483.
- [38] M.A. Shupnik, Gonadal hormone feedback on pituitary gonadotropin genes. *Trends Endocrinol. Metab.* 7 (1996) 272-276.
- [39] J.T. Silverstein, H. Shimma, H. Ogata, Early maturity in amago salmon (*Oncorhynchus masu ishikawai*): an association with energy storage. *Can. J. Fish. Aquat. Sci.* 54 (1997) 444-451.
- [40] S.M. Sogard, J.E. Merz, W.H. Satterthwaite, M.P. Beakes, D.R. Swank, E.M. Collins, et al., Contrasts in habitat characteristics and life history patterns of *Oncorhynchus mykiss* in California's central coast and central valley. *Transactions of the American Fisheries Society*. 141 (2012) 747-760.
- [41] G.L. Taranger, M. Carrillo, R.W. Schulz, P. Fontaine, S. Zanuy, A. Felip, et al., Control of puberty in farmed fish. *Gen. Comp. Endocrinol.* 165 (2010) 483-515
- [42] J.F. Taylor, M.J.R. Porter, N.R. Bromage, H. Migaud, Relationships between environmental changes, maturity, growth rate and plasma insulin-like growth factor-I (IGF-I) in female rainbow trout. *Gen. Comp. Endocrinol.* 155 (2008) 257-270.
- [43] J.E. Thorpe, Reproductive strategies in Atlantic salmon, *Salmo salar* L. *Aquacult. Fish. Manage.* 25 (1994) 77-87.
- [44] J.E. Thorpe, Maturation responses of salmonids to changing developmental opportunities. *Mar. Ecol. Prog. Ser.* 335 (2007) 285-288.
- [45] J.E. Thorpe, C.E. Adams, M.S. Miles, D.S. Keay, Some influences of photoperiod and temperature on opportunity for growth in juvenile Atlantic salmon, *Salmo salar* L. *Aquaculture*. 82 (1989) 119-126.
- [46] J.E. Thorpe, M. Mangel, N.B. Metcalfe, F.A. Huntingford, Modelling the proximate basis of salmonid life-history variation, with application to Atlantic salmon, *Salmo salar*. *Evol. Ecol.* 12 (1998) 581-599.
- [47] K.R. von Schalburg, M.L. Rise, G.D. Brown, W.S. Davidson, B.F. Koop, A comprehensive survey of the genes involved in maturation and development of the rainbow trout ovary. *Biol. Reprod.* 72 (2005) 687-699.
- [48] F.-A. Weltzien, E. Andersson, Ø. Andersen, K. Shalchian-Tabrizi, B. Norberg, The brain-pituitary-gonad axis in male teleosts, with special emphasis on flatfish (*Pleuronectiformes*). *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* 137 (2004) 447-477.

- [49] Y. Yamamoto, J.A. Luckenbach, F.W. Goetz, G. Young, P. Swanson, Disruption of the salmon reproductive endocrine axis through prolonged nutritional stress: Changes in circulating hormone levels and transcripts for ovarian genes involved in steroidogenesis and apoptosis. *Gen. Comp. Endocrinol.* 172 (2011) 331-343.
- [50] Y. Zohar, J.A. Muñoz-Cueto, A. Elizur, O. Kah, Neuroendocrinology of reproduction in teleost fish. *Gen. Comp. Endocrinol.* 165 (2010) 438-455

Figure Legends

Figure 1. Mean (\pm SEM) fish mass specific growth rates (SGR) over time in female rainbow trout (A: two-year-old; B: three-year-old) fed a control-ration or restricted-ration. Growth rate over the preceding interval of four (B) or five (A) weeks is shown at each time-point. Treatment means differ significantly (two-tailed t-test, $p < 0.05$) at time-points marked “*”. Within each age class, time-points within a treatment group sharing the same letter or letters are not significantly different (Tukey’s HSD, $p \geq 0.05$).

Figure 2. Mean (\pm SEM) condition factor (k) over time in female rainbow trout (A: two-year-old; B: three-year-old) fed a control-ration or restricted-ration. See Figure 1 for an explanation of what each symbol signifies.

Figure 3. Mean (\pm SEM) Muscle lipid content (i.e., fatmeter readings) over time in female rainbow trout (A: two-year-old; B: three-year-old) fed a control-ration or restricted-ration. Data are not present for four-week sampling point in panel B. See Figure 1 for an explanation of what each symbol signifies.

Figure 4. Mean (\pm SEM) gonadosomatic index (GSI) over time in female rainbow trout (A: two-year-old; B: three-year-old) fed a control-ration or restricted-ration. See Figure 1 for an explanation of what each symbol signifies.

Figure 5. Mean (\pm SEM) normalized ratio of pituitary fsh- β to β -actin mRNA levels over time in female rainbow trout (A: two-year-old; B: three-year-old) fed a control-ration or restricted-ration. See Figure 1 for an explanation of what each symbol signifies.

Figure 6. Mean (\pm SEM) plasma 17 β -estradiol concentration over time in female rainbow trout (A: two-year-old; B: three-year-old) fed a control-ration or restricted-ration. See Figure 1 for an explanation of what each symbol signifies.

Figure 7. Mean (\pm SEM) plasma 11-keto testosterone concentration over time in female rainbow trout (A: two-year-old; B: three-year-old) fed a control-ration or restricted-ration. See Figure 1 for an explanation of what each symbol signifies.

Figure 1

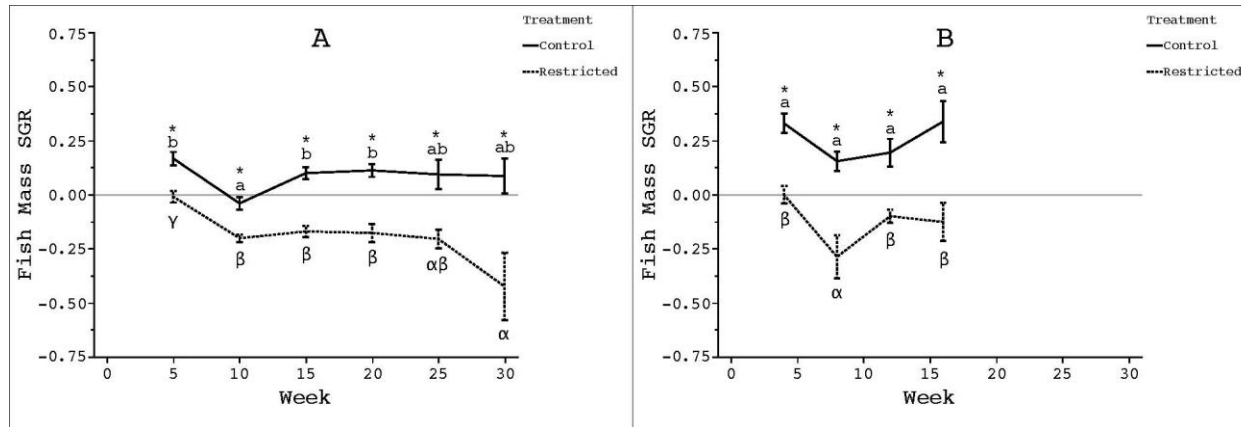


Figure 2

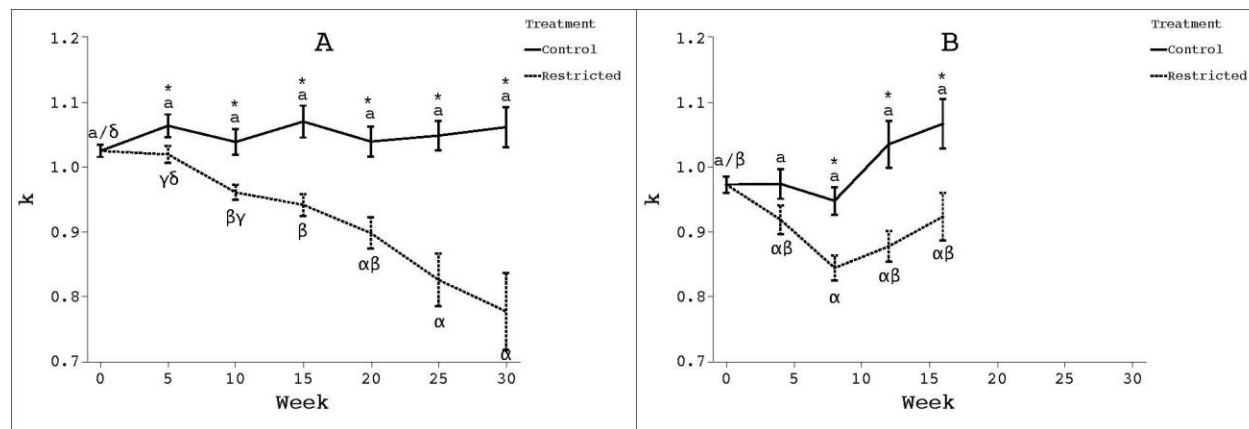


Figure 3

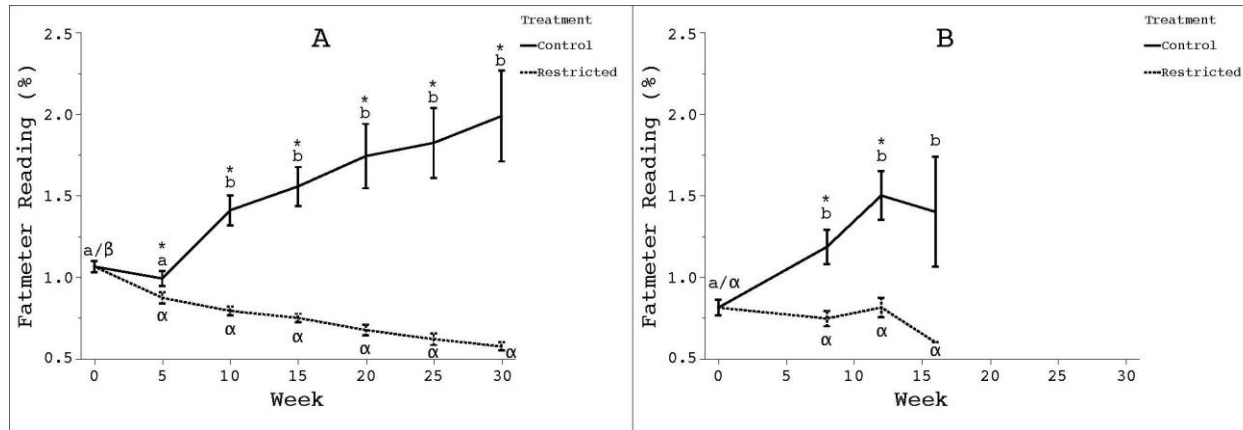


Figure 4

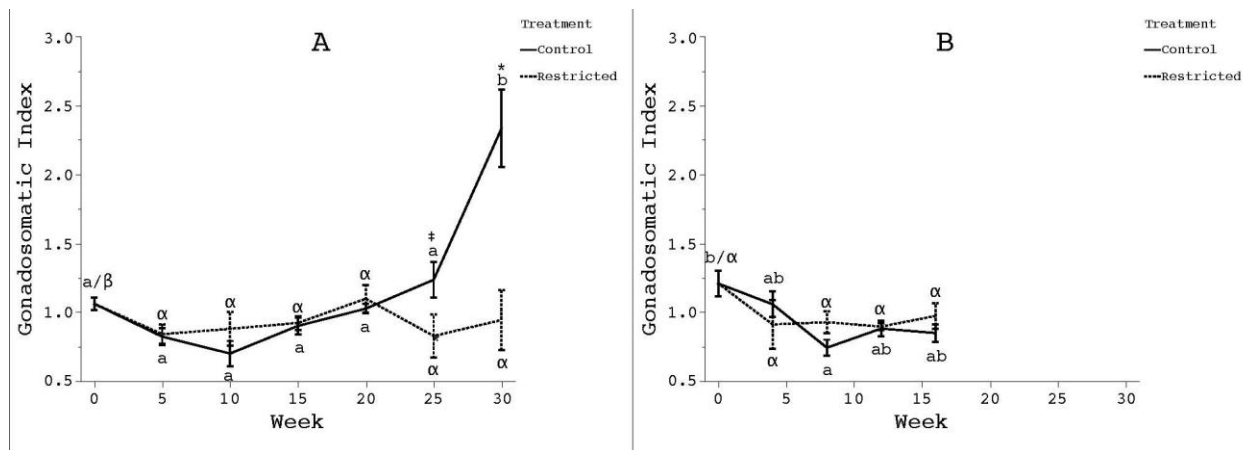


Figure 5

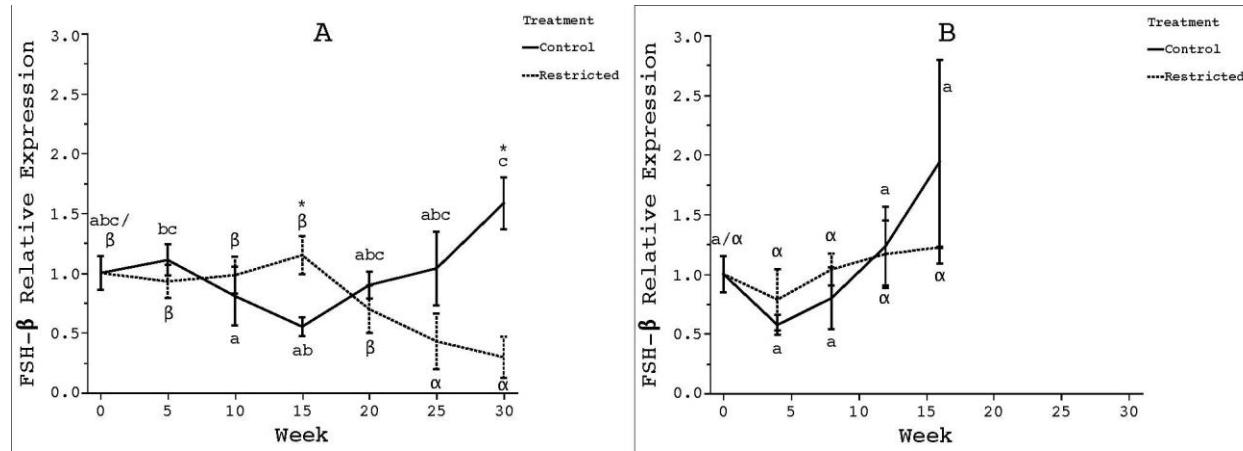


Figure 6

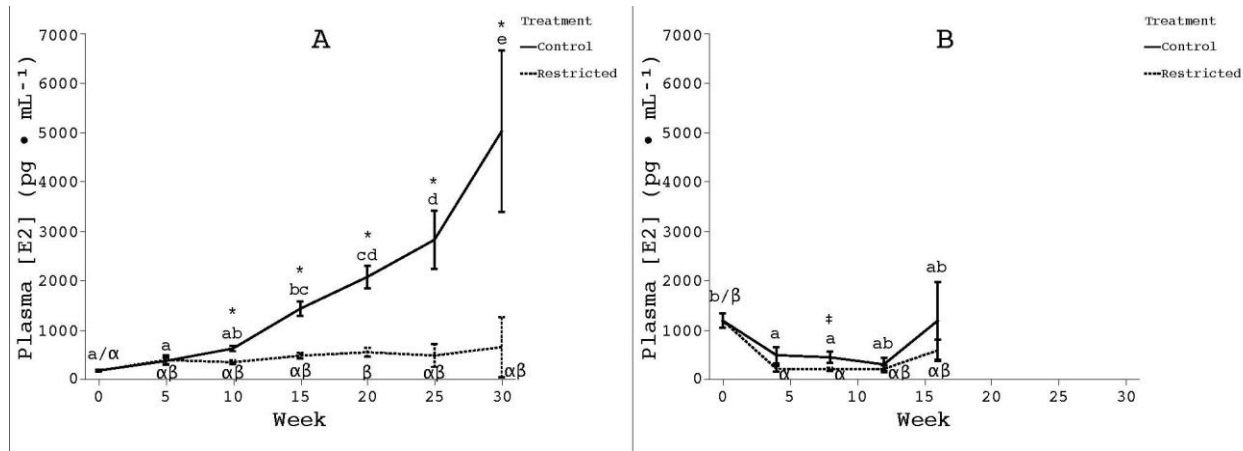
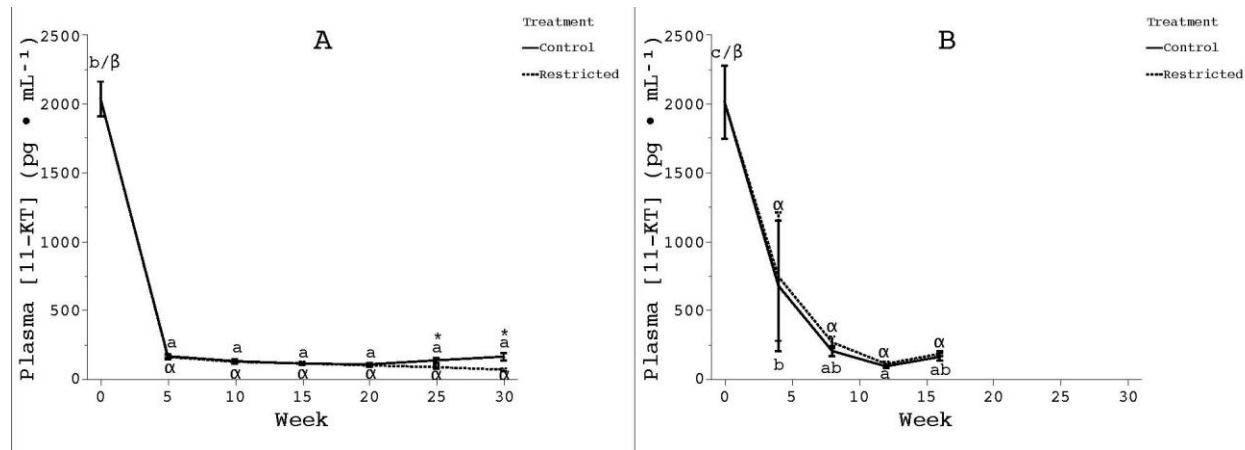


Figure 7



Section C: Reproductive development in kelt steelhead

Introduction

An understanding of the reproductive status of female kelt steelhead during reconditioning and at release is required to maximize the success of Columbia River Basin kelt reconditioning projects. Natural steelhead production is limited by the number of female spawners. In order to contribute to ESA-listed steelhead populations, female kelts must not only survive reconditioning but also remature and produce viable eggs. Questions regarding reproductive performance of reconditioned fish underlie issues raised regarding kelt reconditioning projects during ISRP review (ISRP 2011). We believe these issues can be best addressed by research aimed at an improved understanding of post-reproductive life history and physiology in steelhead.

Iteroparous female salmonids have two major post-reproductive life history trajectories (Rideout *et al.* 2005; Chaput & Jones 2006; Keefer *et al.* 2008; Rideout & Tomkiewicz 2011). After a spawning event, some fish are able to restore energy lost during migration and spawning, redevelop a mature ovary, and spawn the next year. These fish are termed consecutive spawners. Other fish do not initiate redevelopment of the ovary for the next spawning season, but instead skip a year. These fish are termed skip spawners. We hypothesize that these life history trajectories are the result of the effect of energy balance on maturation decisions made during seasonally defined critical periods. The influential critical period model of the first reproductive maturation (puberty) in salmonids posits that maturation is initiated during a decision window approximately one year prior to spawning (Shearer & Swanson 2000; Campbell *et al.* 2006; Thorpe 2007; Satterthwaite *et al.* 2009). This decision is made based on energy reserves. If maturation is initiated during this critical period, it may be arrested at a second critical period before the onset of exogenous vitellogenesis, if energy reserves are not sufficient (Yamamoto *et al.* 2011). We hypothesize that a similar decision mechanism regulates rematuration in post-spawning steelhead. In post-spawning fish, energy driven decisions take place in the context of the extreme energy deficit incurred by migration and spawning. Threshold energy levels for rematuration selected before anthropogenic changes occurred in the freshwater and marine environment may no longer be adaptive under current conditions.

In order to establish methods for monitoring the reproductive status of steelhead kelts, we sampled blood from female kelts at release and during reconditioning at Prosser, WA, and measured blood levels of estradiol and vitellogenin. Estradiol is the principal female gonadal steroid in fishes, which regulates many aspects of reproductive development, and vitellogenin is a phospholipoprotein produced by the liver under regulation by estradiol which provides most of the material for ovarian development. Our goals were to determine the proportion of consecutive and skip spawning fish under captive conditions, to develop methods for screening fish for reproductive status during reconditioning, and to explore factors associated with rematuration.

Methods

Fish Collection and Husbandry

Yakima River steelhead kelts were collected and reconditioned at Prosser, WA using established methods (Branstetter *et al.* 2011; Hatch *et al.* 2013). In 2009 and 2010, all fish were administered ivermectin by gavage at intake to reduce parasitic copepods. In 2011, all fish were administered ivermectin except for tank C2, which was used for a trial of emamectin, a new copepod treatment, and in 2012, all fish were administered emamectin except for tank C2, which was used for a repeat of the trial (See Section G). In addition, in 2012, several tanks of fish were fed an experimental diet (See Section E). Survival to release was 27.6% in 2009, 38.7% in 2010, 32.8% in 2011, and 60.8% in 2012 (Chapter 1 Section C) (Hatch *et al.* 2013).

Sampling

In 2009 – 2011, female fish were blood sampled at intake and release (October). Release samples were a random sub-sample in 2009 and 2010 (71 of 128 and 97 of 381 female fish, respectively), whereas in 2011 almost all fish were successfully blood sampled (208 of 212 female fish). In 2012 two of four large tanks and four small tanks of fish were blood sampled on 8/16/12 (152 female fish). These fish were re-sampled at release, however, blood samples from the release sample have not yet been assayed for plasma hormone levels. In 2010, one small tank of fish was serially sampled (intake, 7/21/10, 8/18/10, release 10/13/10), and in 2011, one large tank of fish was serially sampled (intake, 7/19/11, 9/8/11, release 10/13/11). During blood sampling, blood (2 mL) was drawn from the caudal vein using heparinized syringes (ammonium heparin, 10 mg/ml) and centrifuged (5 min, 1000 g). Plasma was collected and frozen on dry ice in the field prior to storage at -80°C. In addition to blood sampling, the length, weight and sex of fish was recorded, and a reading of muscle lipid levels was taken with a Distell Fish Fatmeter (Distell Inc, West Lothian, Scotland), using the rainbow trout muscle lipid setting (Trout-1) at the two most anterior measurement sites recommended by the manufacturer (Colt & Shearer 2001; Crossin & Hinch 2005).

Vitellogenin and Estradiol Assays

Fish plasma levels of vitellogenin (VG) and estradiol-17 β (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. Samples from the 8/16/2012

sampling were assayed for E2 but not VG because data from 2009-2011 established that plasma E2 level indicates maturation status as well as or better than plasma VG level, and the E2 assay is considerably less expensive to run.

Statistical Analysis

Analysis was restricted to female fish. Only fish positively identified by PIT tag code were included for analysis of intake and serial samples. VG and E2 levels were log₁₀ transformed prior to analysis. Release blood samples were divided into categories based on VG and E2 values using hierarchical cluster analysis by distance. Distance was calculated by single linkage, input data were standardized, and two clusters were specified. Maturation status was assigned based on the results of the cluster analysis and used in subsequent analyses. VG and E2 levels in rematuring and non-rematuring females were compared over time using t-tests (two tailed). Factors measured at release were compared between rematuring and non-rematuring females using t-tests (two tailed). The effect of intake date on maturation status at release was analyzed using logistic regression. Statistical analyses were conducted using JMP 10.0 (SAS Institute Inc., Cary NC) and PRISM 6.0 (GraphPad Software, San Diego CA).

Results

Cluster analysis of VG and E2 values revealed a clear division of female kelts into two groups at the time of release in October in 2009-2011 (Fig. 1). Threshold values of 0.1 mg/ml VG and 500 pg/ml E2 divided fish at the time of release. Fish in the group with high E2 and VG levels were classified as rematuring, and fish in the group with low E2 and VG levels were classified as non-rematuring. Plasma E2 values in a pre-release sample taken 8/16/12 were bimodally distributed, with values in the higher mode above 500 pg/ml E2 (Fig. 2). These samples were run in a high-range E2 assay that has a lower detection limit of 100 pg/ml. Samples from non-rematuring fish were below the detection limit of the assay and were assigned the assay minimum, which is why the lower part of the bimodal distribution is not evident on the figure. Based on available plasma hormone levels (Figs. 1 and 2), rematuration percentage was 65% in 2009, 25% in 2010, 54% in 2011, and 63% in 2012.

In fish serially sampled in 2010 and 2011, plasma E2 increased in rematuring fish, whereas VG decreased in non-rematuring fish (Fig. 3). Rematuring and non-rematuring fish did not differ in plasma VG or E2 level at intake. Plasma E2 was significantly elevated in rematuring fish by mid-July, and VG by mid-August. Rematuring and non-rematuring fish overlapped somewhat in plasma E2 levels at the July samplings. However, by the 8/18/2010 and 9/8/2011 samplings, separation by both plasma E2 and VG levels was virtually complete.

Rematuring fish grew faster than non-rematuring fish over the reconditioning period, and had higher muscle lipid levels at release (Fig. 4), indicating an association between rematuration and positive energetic status. Rematuring fish tended to arrive earlier at Prosser than non-rematuring fish (Fig. 5). However, this relationship was relatively weak, showing considerable overlap, and was significant in 2009 and 2010 but

not 2011.

Discussion

Measurement of plasma E2 and VG levels at release enabled clear division of female fish into maturing and non-maturing groups. Increases in E2 and VG by orders of magnitude at approximately six months prior to spawning in salmonids are extensively documented (Tyler & Sumpter 1996; Patino & Sullivan 2002; Lubzens *et al.* 2010). We are confident in concluding that the group of fish with high plasma E2 and VG levels at release are rematuring. The low plasma E2 and VG levels in the other group of fish indicates that ovarian development has not yet begun. Ovarian development in salmonids proceeds through a series of stages which must be initiated approximately one year before spawning (Campbell *et al.* 2006; Yamamoto *et al.* 2011). Therefore, fish in the group with low E2 and VG will not be able to spawn during the coming season and are classified as non-rematuring. A few samples had inconsistent E2 and VG levels. This could be due to assay errors, or to unusual physiological circumstances in individual fish, such as fish that may have initiated rematuration and later arrested. These samples are being reassayed to confirm results.

These results indicate that both rematuring and non-rematuring females are produced by the reconditioning project at Prosser. The low rematuration percentage in 2010 appears to be an anomaly. Excluding this year, the average rematuration rate at Prosser is approximately 60%. We hypothesize that rematuring fish are following a consecutive spawning and non-rematuring fish are following a skip spawning life history trajectory. The reasons for the variation in rematuration percentage between years are not yet known. Environmental conditions before intake into reconditioning influence kelt survival, likely by affecting the energy reserves of the fish at intake into reconditioning (Hatch *et al.* 2013). It is reasonable to hypothesize that environmental conditions before intake may influence rematuration. However, rematuration may also be influenced by culture conditions after intake into reconditioning. Interestingly, 2010 was the year with the highest number of kelts collected and released during 2001-2011. It is possible that higher rearing densities played a role in the low maturation percentage in 2010. Further research is required to determine whether this is the case. Research is underway to investigate potential strategies to increase rematuration rate, to determine if it is possible to screen fish for maturation status at intake, and to manage non-rematuring fish so as to enable them to survive and spawn in future years.

Both E2 and VG diverged between rematuring and non-rematuring fish during reconditioning. E2 appears to be a better indicator of reproductive status, partly because VG levels are very high at intake into reconditioning, and partly because E2 drives liver vitellogenin production during oogenesis. VG is highly elevated at the time of spawning in salmonids. Uptake by the developing ovary is the main mechanism for clearance of VG from the plasma. However, after spawning, the ovary stops taking up vitellogenin. VG is a large phospholipoprotein which is cleared slowly by other clearance mechanisms. This explains the relatively slow 100 to 1000 fold decrease in VG

over time in non-rematuring fish. Unlike VG, plasma E2 decreases to basal levels during the three months prior to spawning (Tyler & Sumpter 1996). The initial stages in the initiation of maturation or rematuration occur at kisspeptin and gonadotropin releasing hormone (GnRH) neurons in the brain ((Zohar *et al.* 2010). GnRH stimulation of pituitary gonadotrophs leads to an increase in secretion of follicle stimulating hormone (FSH), which in turn stimulates the ovary to produce E2, which then stimulates the liver to produce VG (Tyler & Sumpter 1996; Patino & Sullivan 2002; Lubzens *et al.* 2010). Thus, the significant increase in E2 in rematuring fish in mid-July suggests that the earlier stages in the initiation of rematuration occurred earlier, probably by at least a month earlier. Based on these results, we hypothesize that the initial maturation decision occurs between intake and mid-June. Thus, energy balance early on in reconditioning may be key to determining whether fish will remature.

Both E2 and VG enabled nearly complete separation of rematuring and non-rematuring fish by late August to September. In addition, the high and low modes in plasma E2 levels showed almost no overlap in fish sampled in mid-August of 2012 (Fig. 2). This suggests that maturation status can be established from a blood sample taken at this time by measurement of plasma E2 levels. Plasma VG levels can be used as a secondary screen for fish with borderline E2 levels. However, the cutoff values used for classifying fish as rematuring for both VG and E2 will need to be adjusted downward based on how long before release blood samples are taken. At least one fish with a vitellogenin level in the 0.01 to 0.1 mg ml⁻¹ range at the 9/8/2011 sampling was found to have developed eggs in spring of 2012 (J. Blodgett, personal communication).

Rematuring fish grew faster over the reconditioning period, had higher muscle lipid levels at release, and tended to be captured at Prosser and taken in for reconditioning earlier than non-rematuring fish. The effect of intake date is consistent with findings that late outmigration timing is associated with the skip spawning life history in ocean-reconditioned repeat spawning steelhead (Keefer *et al.* 2008). All of these findings are consistent with our working hypothesis that rematuration is plastic and depends on energetic status during seasonal critical periods (Shearer & Swanson 2000; Campbell *et al.* 2006; Thorpe 2007; Satterthwaite *et al.* 2009). Fish that arrive later have less time to restore lost energy to the critical level before the seasonal window closes. However, it is also possible that the increased growth and lipid reserves in rematuring fish were caused by the influence of rematuration on feeding and growth. Maturing fish consume more feed and increase more in weight than non-maturing fish, due in part to growth stimulation by increased levels of reproductive steroids. The degree to which reproductive trajectory is determined at intake versus subject to influence during reconditioning is an important question for further research.

References

- Branstetter R, Stephenson J, Pierce AL, Hatch DR, Bosch B, Fast D, Blodgett J, Everett SR, Paddlety J, Dasher R, et al. 2011 Steelhead Kelt Reconditioning and Reproductive Success. 2011 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-000. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.
- Campbell B, Dickey J, Beckman B, Young G, Pierce A, Fukada H & Swanson P 2006 Previtellogenic oocyte growth in salmon: relationships among body growth, plasma insulin-like growth factor-1, estradiol-17beta, follicle-stimulating hormone and expression of ovarian genes for insulin-like growth factors, steroidogenic-acute regulatory protein and receptors for gonadotropins, growth hormone, and somatolactin. *Biology of Reproduction* **75** 34-44.
- Chaput G & Jones R 2006 Reproductive rates and rebuilding potential for two multi-sea-winter Atlantic salmon (*Salmo salar* L.) stocks of the Maritime provinces. Ed FaO Canada: Canadian Science Advisory Secretariat.
- Colt J & Shearer KD 2001 Evaluation of migrational delays on the reproductive success of adult hatchery spring Chinook salmon in the Columbia and Snake Rivers (objective 2 only). 2001 Report to the U.S. Army Corps of Engineers, Contract W66QKZ00805700. Seattle: Northwest Fisheries Science Center, National Marine Fisheries Service.
- Crossin GT & Hinch SG 2005 A nonlethal, rapid method for assessing the somatic energy content of migrating adult pacific salmon. *Transactions of the American Fisheries Society* **134** 184-191.
- Hatch DR, Fast DE, Bosch WJ, Branstetter R, Blodgett JW, Whiteacre JM & Pierce AL 2013 Survival and traits of reconditioned kelt steelhead (*Oncorhynchus mykiss*) in the Yakima River, Washington. *North American Journal of Aquaculture* **33** 615–625.
- ISRP 2011 Retrospective Report 2011. Independent Scientific Review Panel.
- Keefer ML, Wertheimer RH, Evans AF, Boggs CT & Peery CA 2008 Iteroparity in Columbia river summer-run steelhead (*Oncorhynchus mykiss*): implications for conservation. *Canadian Journal of Fishery and Aquatic Sciences* **65** 2592-2605.
- Lubzens E, Young G, Bobe J & Cerda J 2010 Oogenesis in teleosts: how eggs are formed. *Gen Comp Endocrinol* **165** 367-389.
- Patino R & Sullivan CV 2002 Ovarian follicle growth, maturation, and ovulation in teleost fish. *Fish Physiology and Biochemistry* **26** 57-70.
- Rideout RM, Rose GA & Burton MPM 2005 Skipped spawning in female iteroparous fishes. *Fish and Fisheries* **6** 50-72.

Rideout RM & Tomkiewicz J 2011 Skipped spawning in fishes: more common than you might think. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* **3** 176-189.

Satterthwaite WH, Beakes MP, Collins EM, Swank DR, Merz JE, Titus RG, Sogard SM & Mangel M 2009 Steelhead life history on California's central coast: insights from a state dependent model. *Transactions of the American Fisheries Society* **138** 532-548.

Shearer KD & Swanson P 2000 The effect of whole body lipid on early sexual maturation of 1+ age male Chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture* **190** 343-367.

Thorpe JE 2007 Maturation responses of salmonids to changing developmental opportunities. *Marine Ecology Progress Series* **335** 285-288.

Tyler CR & Sumpter JP 1996 Oocyte growth and development in teleosts. *Reviews in Fish Biology and Fisheries* **6** 287-318.

Yamamoto Y, Adam Luckenbach J, Goetz FW, Young G & Swanson P 2011 Disruption of the salmon reproductive endocrine axis through prolonged nutritional stress: changes in circulating hormone levels and transcripts for ovarian genes involved in steroidogenesis and apoptosis. *Gen Comp Endocrinol* **172** 331-343.

Zohar Y, Munoz-Cueto JA, Elizur A & Kah O 2010 Neuroendocrinology of reproduction in teleost fish. *Gen Comp Endocrinol* **165** 438-455.

Figure 1: Cluster analysis of release plasma E2 and VG levels in reconditioned female kelts. Fish were blood sampled on 10/29/2009, 10/13/2010, and 10/13/2011. Groups assigned by cluster analysis are indicated by red circles and blue triangles. Threshold VG (0.1 mg/ml) and E2 (500 ng/ml) levels are indicated by lines.

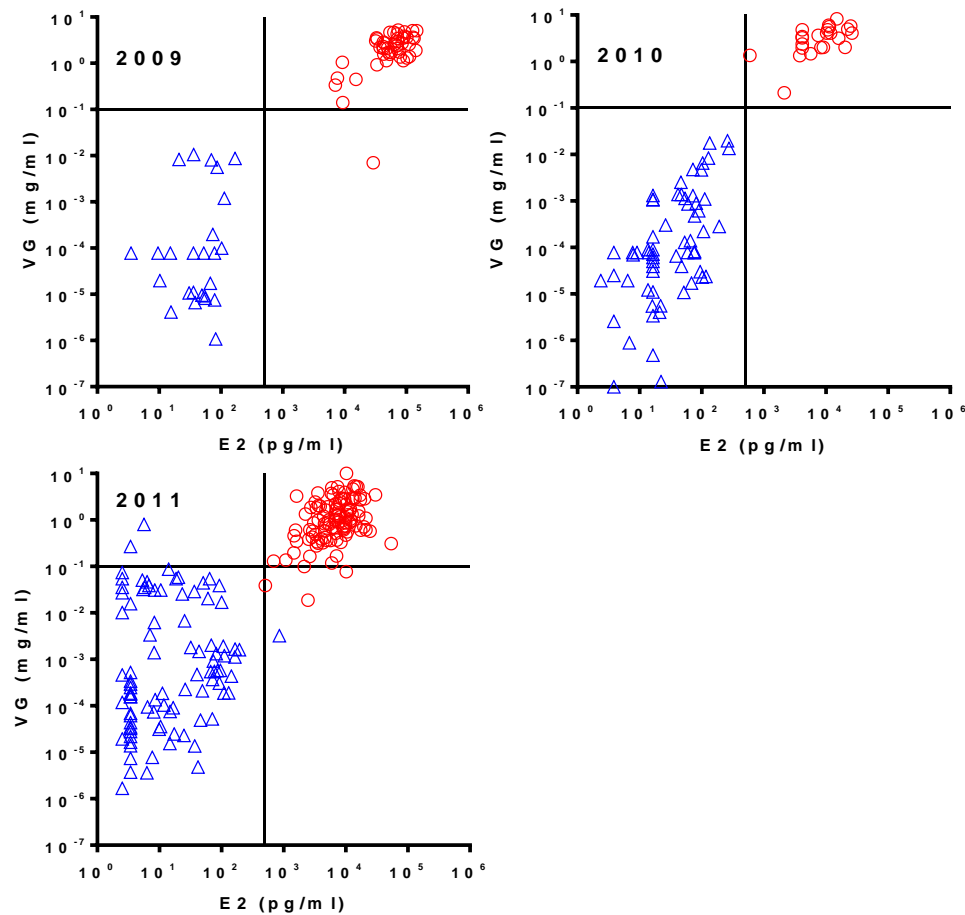


Figure 2: Frequency distribution of plasma E2 values in blood samples taken from reconditioned female kelts on 8/16/2012 showing bimodal distribution. The high mode is indicated by red and the low mode by blue bars.

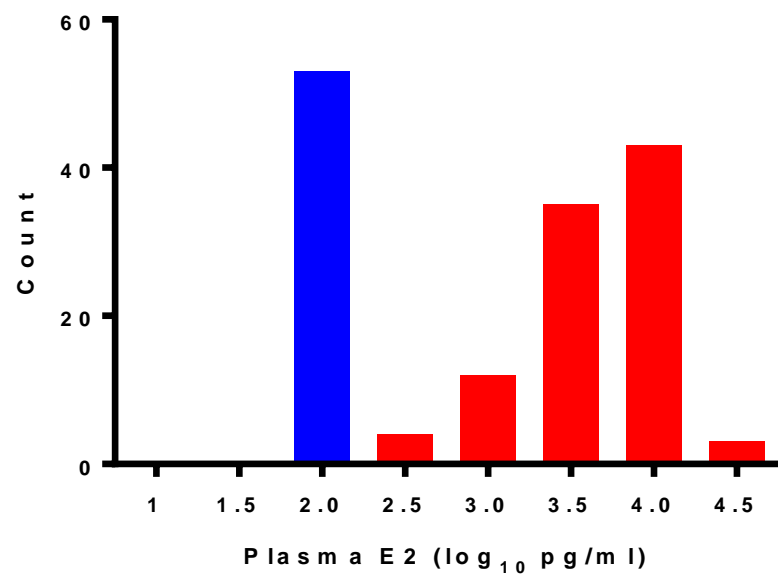


Figure 3: Plasma E2 and VG levels in female kelts serially sampled during reconditioning in 2010 (A, B) and 2011 (C, D). Individual values are shown to illustrate the degree of separation. The median and interquartile range are indicated by the central and error bars. Significant differences (t-test, $p < 0.05$) are indicated by asterisks.

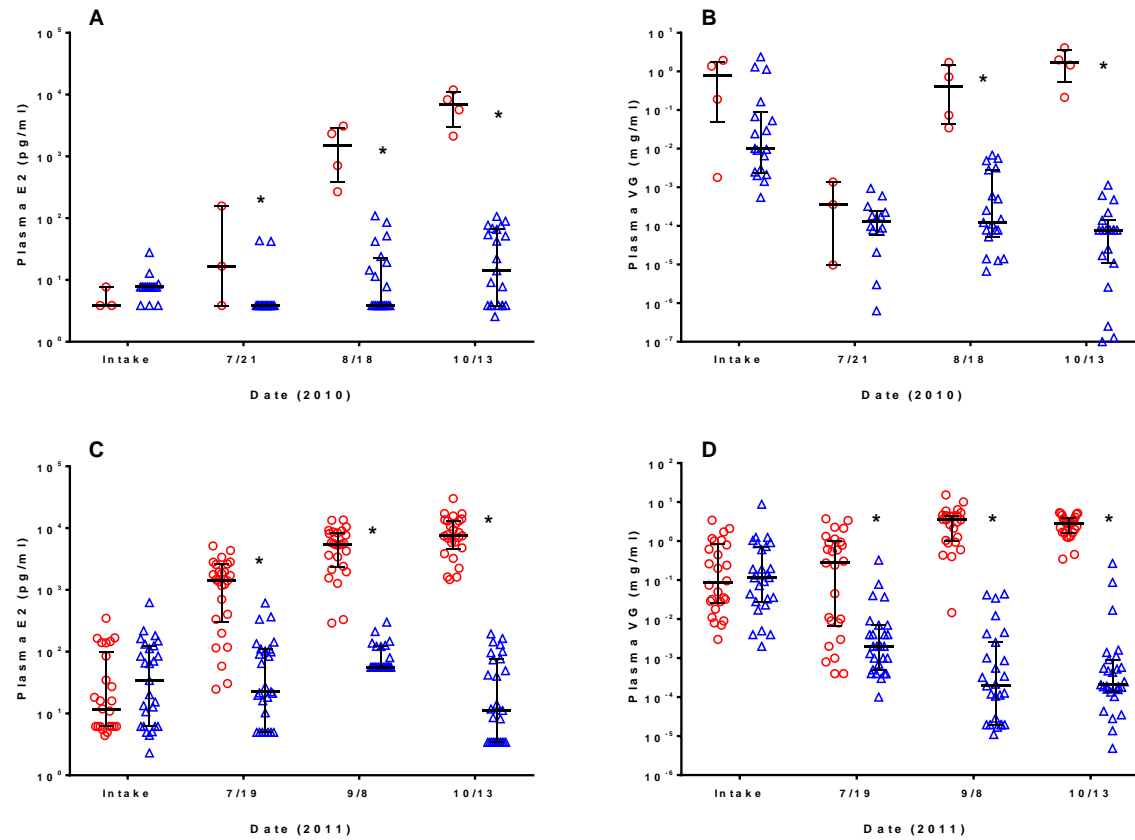


Figure 4: Specific growth rate in weight (A) and release muscle lipid levels as measured with the Fatmeter (B) in rematuring and non-rematuring reconditioned female kelts. Specific growth rate was calculated over the entire reconditioning period. Error bars show standard deviation, and significant differences (t-test, $p < 0.05$) are indicated by asterisks.

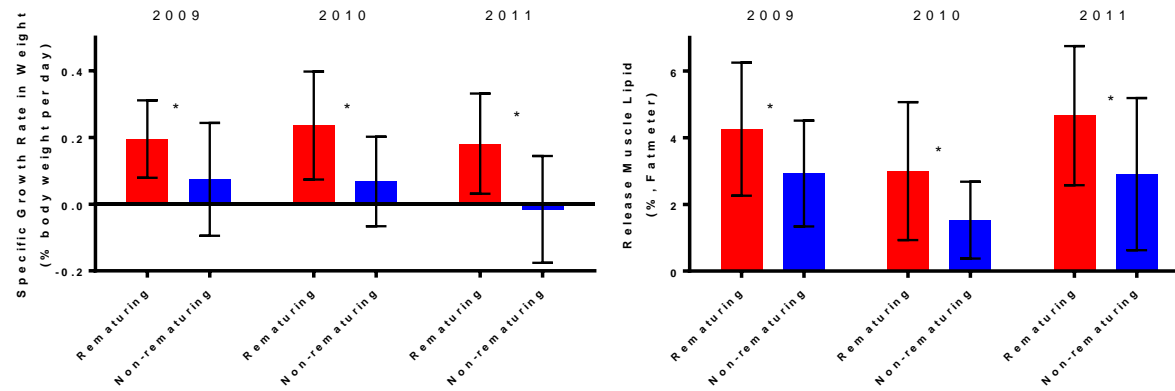
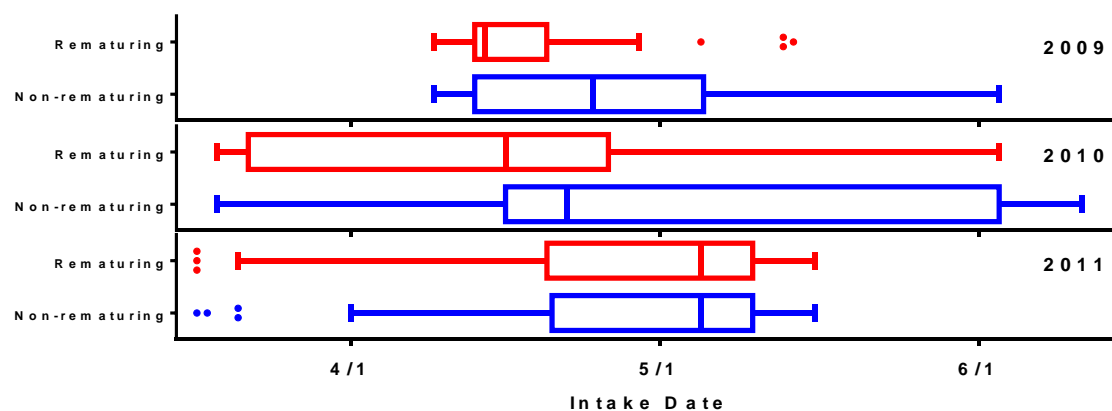


Figure 5: Relationship between intake date and maturation status at release in female kelts for 2009-2011. Box and whisker plots show median, interquartile range, and outliers by the method of Tukey. Logistic regression of maturation status on intake date: 2009 $p = 0.0014$; 2010 $p = 0.0331$; 2011 $p = 0.2229$.



Section D: Proteomic Analysis of Female Steelhead Plasma: Progress Report 2012

Purpose:

To determine whether differences exist in the plasma proteome of post-spawned female (kelt) steelhead that might predict their subsequent ovarian recrudescence.

Rationale:

Steelhead kelt have the capability to be iteroparous. Presently, there is no means available to know whether post-spawned steelhead kelt will immediately enter another reproductive cycle. A biomarker that indicates this physiological capability (intent) would be a valuable tool for managing captive fish for re-conditioning programs. Our premise is that there is a plasma protein(s) that could fill the role as a biomarker. This might be a protein that is present at higher levels (metabolic indicator) in kelts that will enter a consecutive reproductive cycle, as opposed to fish with much lower or negligible levels of this protein. To discover this protein(s) a plasma proteome approach was employed to identify a biomarker, by comparing the plasma proteomic patterns in kelt that went on to consecutively reproduce with some that did not.

Method:

Eight plasma samples were selected from fish collected at the Prosser site in April 2009 (intake collection). These samples consisted of four fish that had high levels of estradiol/vitellogenin at release in the fall (maturing; indicative of reproductive resumption) and four that had very low levels (non-maturing).

To prepare the plasma samples for mass spectrometry they were affinity purified by passing them over GlycoLink Immobilization columns that had a rainbow trout vitellogenin antibody coupling to the column. This was done to reduce the amount of vitellogenin in the samples, a high molecular weight protein present in large quantities in the samples and potentially problematic for subsequent analysis. At this point samples were frozen and shipped to the Proteomics Centre, University of Victoria, BC, Canada, for mass spectrometry analysis.

Protein concentrations were determined using a bicinchonic acid protein assay (Sigma). Samples (100 µg of each) were precipitated overnight in acetone at 4°C followed by resolubilization in 0.5M TEAB, 0.2% SDS. Proteins were reduced with TCEP and alkylated with MMTS. Proteins were then in solution digested with trypsin (Promega) and labeled with the appropriate iTRAQ label. iTRAQ labeled peptides were then combined and separated by high pH reverse phase HPLC. HPLC fractions containing peptides were then reduced in volume by speed-vac and analyzed by LC-

MS/MS. The length of the reverse gradient used was 2 hours per HPLC fraction. Samples were analyzed by reversed phase nanoflow (300 nL/min) HPLC with nano-electrospray ionization using a LTQ-Orbitrap mass spectrometer (LTQ-Orbitrap Velos, Thermo-Fisher) operated in positive ion mode.

All data was analyzed using Proteome Discoverer 1.3 (Thermo-Fisher) and MASCOT v2.3 (Matrix Science) software. Raw data files were searched against the Uniprot-SwissProt database with allspecies filter. At the time of this report the proteomic analysis was not complete. Approximately 75 proteins have been identified in the plasma samples but the analysis to make comparisons between the four maturing fish and the four non-maturing fish has not been undertaken.

Section E: Effects of a supplemented diet on kelt steelhead

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 2011). 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. Tests during 2010 suggested that coating feed items with cyclopeeze increased feeding activity in kelt steelhead. A preliminary trial in 2011 found that fish fed a cyclopeeze and fish oil supplemented 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 (Hatch *et al.* 2012). Fish fed the supplemented diet were observed to be in exceptionally good shape at release time in terms of eye injuries, fin injuries, and skin coloration and health. Fish fed the supplemented diet were detected migrating upriver after release at an exceptionally high rate (80%), much higher than that of fish fed a standard diet (30%), although the sample number was low, suggesting that a high proportion of the supplemented diet fish were reproductively maturing at release time. Based on these results, we conducted a larger scale study in 2012. The goal of this study was to determine whether a fish oil and cyclopeeze supplemented diet has beneficial effects on survival, growth, and rematuration in kelt steelhead.

Methods

Kelt diet pellets were purchased from BioOregon. Pellets were topcoated with Alaskan fish oil and freeze dried cyclopeeze to produce the “orange” diet. Cyclopeeze and fish oil were purchased from Argent Laboratories. The percentage of ingredients and calculated nutritional

composition of the diets is listed in Table 1. The percentage of cyclopeeze was reduced from the pilot study in 2011 due to the cost of this ingredient.

Table 1. Composition of experimental diets.

	Ingredients (%)			Composition (% dry wt)			
	Pellets	Cyclopeeze	Oil	Protein	Lipid	Carbohydrate	Ash
Standard	100	0	0	51.1	21.3	14.9	12.8
Orange	86.1	6.5	7.4	47.2	28.0	13.6	11.2

Kelt steelhead arriving at Prosser during the spring of 2012 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 were stocked into four small tanks (tanks S1-S4, 12' diameter, 19-21 first time reconditioned female fish per tank), and four large tanks (tanks C1-C4, 20' diameter, 102-105 first time reconditioned female fish per tank). Tanks S3, S4, and C4 were randomly assigned to the orange diet, and the rest of the tanks were fed the standard diet. All fish were treated with oxytetracycline and emamectin at intake, except tank C2. Half of the fish in tank C2 were treated with ivermectin at intake as part of our study on copepod control (Section G). Therefore, tank C2 was not included in analysis of the effect of feeding treatment. 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 female fish being reconditioned for the first time were included in the analysis (i.e. no males, fish held over the winter, or recaptured fish). 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 $\frac{\ln(\text{mass}_2/\text{mass}_1)}{\text{days between measurements}} \times 100$. Detections of fish after release were obtained by queries of the PTAGIS database.

Results

Mortality appeared to be lower in the smaller S tanks than in the larger C tanks (Fig. 1). Within each tank type, mortality appeared to be lower in fish fed the orange diet. Fish fed the orange diet had higher muscle lipid levels at release and specific growth rates over the reconditioning period than fish fed the standard diet (Fig. 2), although the difference in muscle lipid levels was marginally nonsignificant (t-test, $p = 0.0664$). As

previously found, there was a significant positive relationship between muscle lipid levels at release and growth rate (Fig. 3). There was a significant relationship between diet treatment and detection of fish post release (Fig. 4; Chi-Squared test $p = 0.0177$). Fish detected migrating upriver after release comprised 64.4 % of standard diet fish, and 80.1 % of orange diet fish. Plasma samples were taken at release from fish in a subset of the fish in the diet study (Table 2). Plasma estradiol and vitellogenin levels have not yet been measured in these samples to determine maturation status of the fish.

Table 2. Plasma samples taken at release in the diet study.

Tank	Diet	Samples
C1	Standard	45
S1	Standard	10
S2	Standard	9
C4	Orange	47
S3	Orange	15
S4	Orange	11
Total		137

Discussion

The overall mortality rate at Prosser was low in 2012. It is somewhat surprising that mortality in the small S tanks was lower than in the larger C tanks. 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, due to rubbing and collisions with tank walls (J. Blodgett, personal communication). The S tanks were stocked with fish during the peak of the run in late April and early May. The condition of these fish may have been better on average than that of fish stocked into the large tanks. In addition, the S tanks were stocked at a lower density than the C tanks. Either or both of these factors may account for the difference in mortality rate. Orange diet fish seemed to have a slightly lower mortality rate than standard diet fish. However, this difference was not large in the C tanks, and was based on only a few mortalities in the S tanks. Additional study is required to determine if the orange diet affects mortality rate.

Fish fed the orange diet had higher release muscle lipid levels and growth rates over the reconditioning period than fish fed the standard diet. The increase in growth rate is most likely due to increased feed consumption by orange diet fed fish, and/or the greater energy content of the orange diet. 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 significant regression between growth rate over the reconditioning period and muscle lipid level measured at release is similar to results from 2011 (Fig. 3; (Hatch *et al.* 2012). Increases in body weight and muscle lipid levels are both presumably driven by feed intake. This provides additional evidence that Fatmeter readings are informative about the growth status of fish at release time, further validating the use of Fatmeter readings as an indicator of fish performance in reconditioning programs.

Orange diet fish were detected migrating upriver after release at an exceptionally high rate (Fig. 4). The detection rate of 80% was similar to results from a pilot study in 2011 (Hatch *et al.* 2012), and was much higher than detection rates in previous years, which were typically about 30%. However, standard diet fish also migrated at more than double the typical rate in 2012. 2012 was a very good year for kelt survival and post-release migration, and the orange diet appears to have provided an additional benefit on top of this. The high migration rate suggests that a very high proportion of orange diet fish were reproductively maturing at release time, particularly considering that the detection efficiency for steelhead migrating upriver at Prosser dam is approximately 85% (CRITFC and Yakama Nation Fisheries, unpublished data). The reproductive status of these fish will be determined by measurement of blood estradiol and vitellogenin levels, which will provide conclusive evidence about any effect of the orange diet on rematuration (Section C).

In conclusion, this first year of a multi-year diet study showed that a diet supplemented with cyclopeeze and fish oil is beneficial for kelt steelhead. Cyclopeeze is quite expensive; however, these benefits may be large enough to justify the cost of the supplemented diet. Fish performance was high in 2012, likely due at least in part to the condition of the fish at entry into reconditioning (Hatch *et al.* 2013), and possibly also due in part to switching from ivermectin to emamectin for copepod control (Section G). Additional data in a more typical year will help to quantify the benefits of the supplemented diet.

References

Argent 2011 Argent Laboratories Cyclop-eeze Product Information. Redmond.

Branstetter R, Stephenson J, Pierce AL, Hatch DR, Bosch B, Fast D, Blodgett J, Everett SR, Paddlety J, Dasher R, et al. 2010 Steelhead Kelt Reconditioning and Reproductive Success. 2010 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2010-401. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.

Halver JE & Hardy RW 2002 *Fish Nutrition*. San Diego: Academic Press.

Hatch DR, Branstetter R, Stephenson J, Pierce AL, Whiteacre JM & Bosch B 2012 Steelhead Kelt Reconditioning and Reproductive Success, 2011 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-000. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.

Hatch DR, Fast DE, Bosch WJ, Branstetter R, Blodgett JW, Whiteacre JM & Pierce AL 2013 Survival and traits of reconditioned kelt steelhead (*Oncorhynchus mykiss*) in the Yakima River, Washington. *North American Journal of Aquaculture* **33** 615–625.

Keefer ML, Wertheimer RH, Evans AF, Boggs CT & Peery CA 2008 Iteroparity in Columbia river summer-run steelhead (*Oncorhynchus mykiss*): implications for conservation. *Canadian Journal of Fishery and Aquatic Sciences* **65** 2592-2605.

Figure Legends

Figure 1. Mortality of first time reconditioned female fish fed standard and orange diets at Prosser during 2012.

Figure 2. Release muscle lipid levels measured with the Fatmeter and specific growth rate over the reconditioning period (intake to release) in fish fed the standard and orange diets at Prosser in 2012. P values are for two tailed t-tests.

Figure 3. Relationship between specific growth rate over the reconditioning period and muscle lipid level measured with the Fatmeter. Linear regression: $p < 0.0001$, $r^2 = 0.4898$.

Figure 4. Detection percentages for fish fed standard and orange diets at Prosser in 2012. The number of first time reconditioned females released from each tank is indicated inside the bar. The detection rate of fish fed the orange diet was significantly higher in a Chi-Squared test (Detected vs Not detected x diet type, $p = 0.0177$).

Figure 1

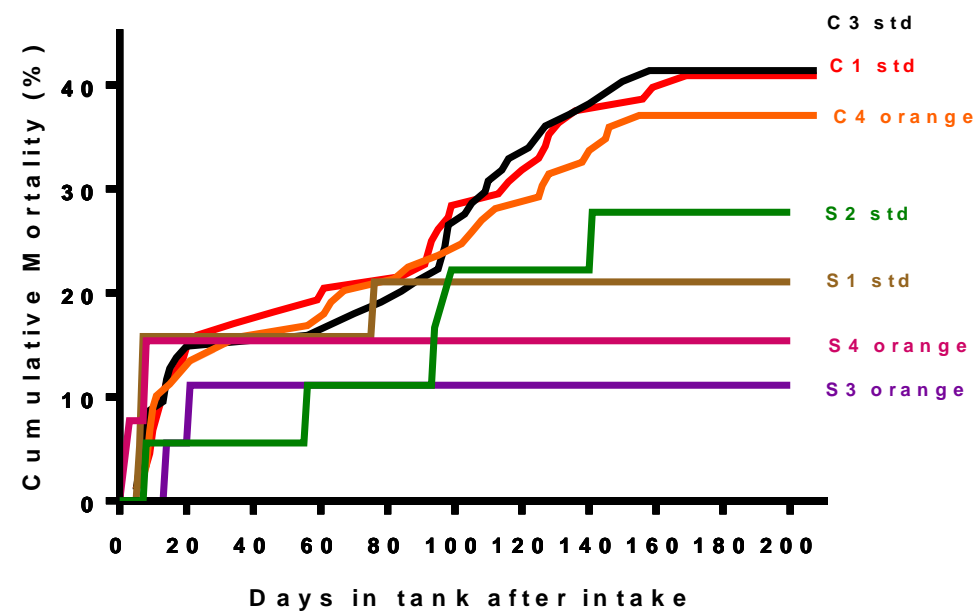


Figure 2

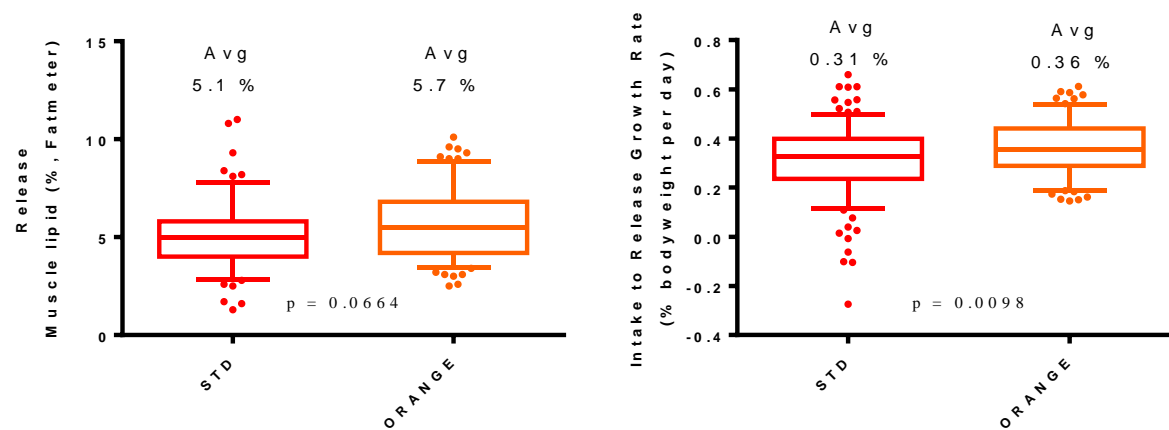


Figure 3

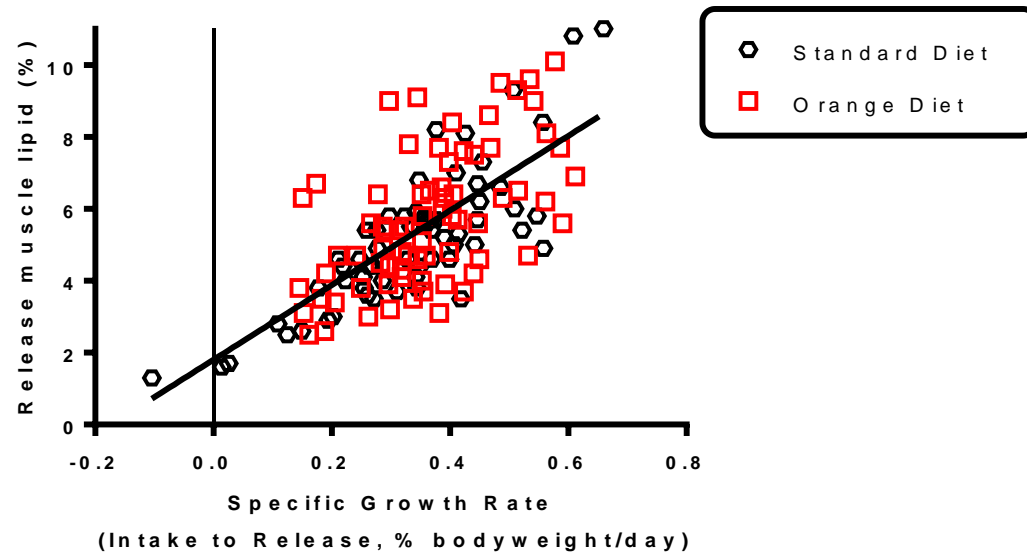
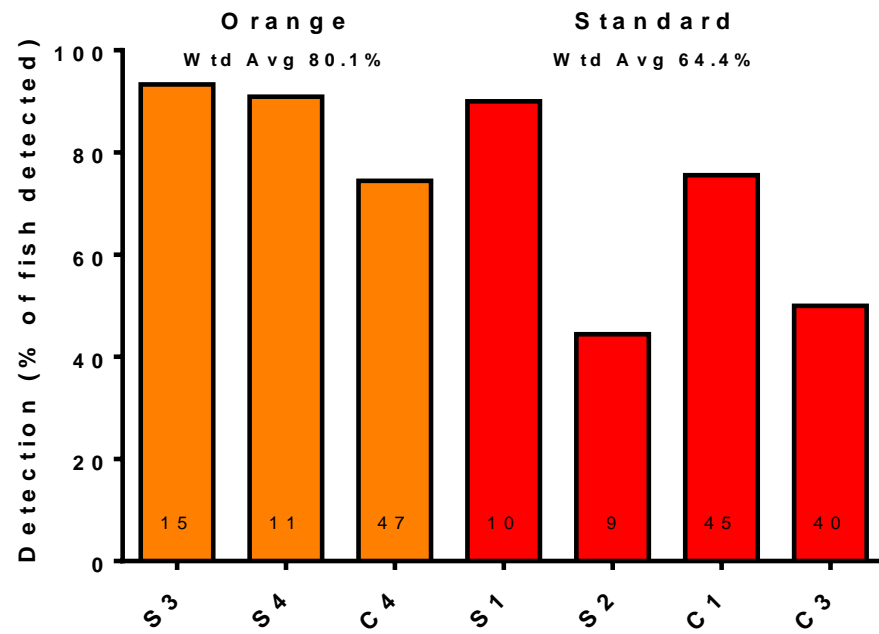


Figure 4



Section F: Comparison of reconditioned kelt steelhead and spawning steelhead sampled during upstream migration at Prosser dam

Introduction

For the last several years, we have been monitoring the reproductive status of female kelts in the reconditioning project at Prosser, WA, using measurement of blood hormone levels. The results of these studies have been interpreted based on existing data on reproductive development in captive rainbow trout. To our knowledge, no information is available on plasma hormone levels during reproductive development and spawning migration in wild naturally spawning steelhead.

In 2011, a three year radio telemetry study was begun to obtain basic information on steelhead VSP units in the Yakima Basin (Frederiksen *et al.* 2012). In this study, upstream migrating steelhead are radio tagged and sampled at the denil ladder on river right at Prosser dam. Prosser dam is less than 1 km upstream from the site where reconditioned kelts are released each fall, and PIT tag detections at Prosser dam are the principal means by which we monitor whether reconditioned kelts actively migrate toward spawning grounds. During the fall of 2012, we began a collaboration with the radio tracking study in which we are obtaining blood samples and Fatmeter readings from upstream migrating female steelhead. Our goals are to compare spawners from the ocean to fish reconditioned at Prosser in terms of: 1) reproductive status using plasma levels of estradiol and vitellogenin; 2) energy reserves using muscle lipid levels measured with the Fatmeter; and 3) migration and spawning success using radio tracking. In addition, measurement of blood hormone levels in samples from spawners from the ocean will contribute information on the stage of reproductive development of these fish for use in the radio telemetry study. Blood hormone levels can be analyzed for relationships to parameters to be estimated in the telemetry study, including pre-spawn mortality, migration patterns, spawn timing, genetic stock, and VSP population assignment.

It is important to note that reconditioned kelts are not expected to be identical to maiden spawners from the ocean in any of these measures. Our goal is not to show that the performance of reconditioned kelts is identical to that of maiden spawners. Instead, it is to obtain information which will help us to quantify the benefit of the kelt reconditioning program. In terms of the effect of captive reconditioning on fish performance, the relevant comparison is between reconditioned kelts and natural repeat spawning steelhead. Scales are being taken for the radio tracking study, so it should be possible to identify natural repeat spawners. However, the expected number of fish is low (Hockersmith *et al.* 1995).

Methods

Fish are trapped, sedated, sampled, PIT tagged, and radio tagged as described for the radio tracking study (Frederiksen *et al.* 2012). Sampling effort and radio tags are distributed throughout the run, with many more fish handled than are radio tagged. We are sampling blood and obtaining Fatmeter readings from both tagged and non-tagged fish. Effort is focused on the fall period when reconditioned kelts are released; however, we hope to obtain samples throughout the run at Prosser. Reconditioned kelts are handled identically to other fish, except that if the fish had blood drawn at release, a second blood sample is not taken.

Results and Discussion

Blood samples were collected during the period immediately before and during kelt migration through Prosser dam (Table 1). Kelts were released on 10/29/12. Additional blood samples (approximately 60) have been collected during winter and spring migration, which may include additional reconditioned kelts. Blood samples have not yet been assayed for estradiol and vitellogenin.

Table 1. Blood samples collected during fall of 2012. This does not include all of the fish that were sampled and radio tagged for the radio tagging study. "Maidens" includes all of the non-reconditioned fish sampled at the Prosser denil, which may include a small number of natural repeat spawners.

Week	Number of samples		Number of radio tags	
	maidens	kelts	maidens	kelts
9/23/2012	2	0	0	0
9/30/2012	5	0	3	0
10/7/2012	13	0	4	0
10/14/2012	20	0	20	0
10/21/2012	24	0	20	0
10/28/2012	41	29	7	10
11/4/2012	26	3	7	0
11/11/2012	3	0	3	0
11/18/2012	2	0	2	0
Total	136	32	66	10

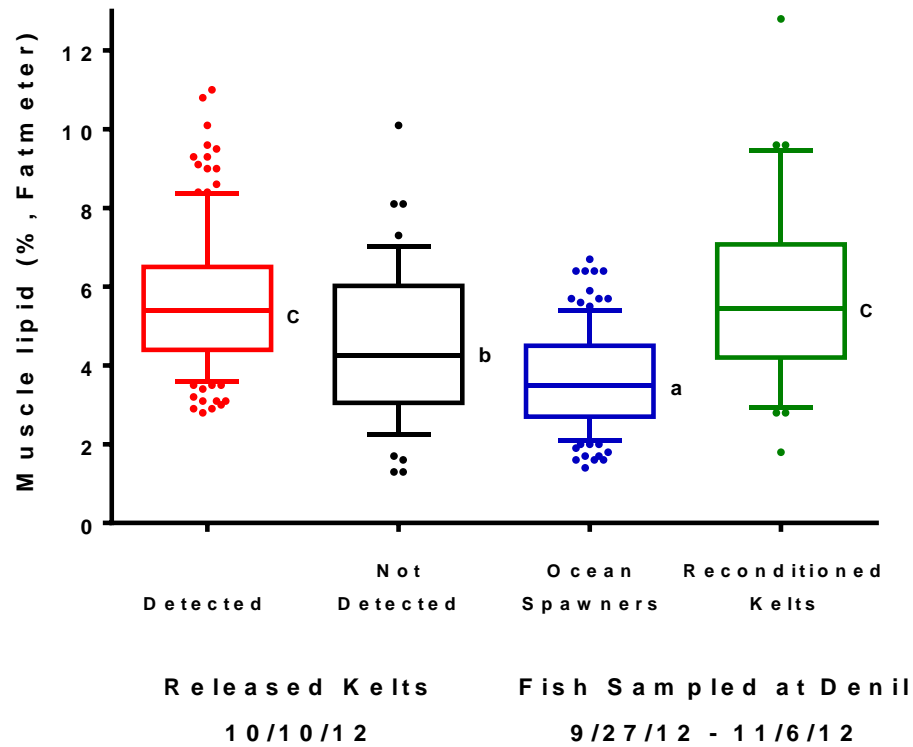
Fatmeter readings were taken at the pre-release sampling of kelts on 10/10/12 and during sampling of fish in the radio tagging study (Fig. 1). As previously found, muscle lipid levels in reconditioned kelts were higher in fish actively detected migrating through Prosser dam after release than in fish that were not detected (Branstetter *et al.* 2010, 2011; Hatch *et al.* 2012). Muscle lipid levels in reconditioned kelts were significantly

higher than those in spawners from the ocean. Yakima steelhead have presumably been selected to store sufficient energy reserves for upstream migration, completion of ovarian development, spawning, and kelt migration. Therefore, the energy reserves of ocean spawners at Prosser dam are presumably sufficient for the fish to complete these tasks successfully. Reconditioned fish do not have to migrate from the ocean to Prosser dam, which likely accounts for their higher muscle lipid levels at this point. The greater energy reserves in reconditioned kelts suggests that they have more than sufficient energy reserves to spawn successfully. Muscle lipid levels in reconditioned kelts sampled at the denil were not different from those in fish Fatmetered at release and subsequently detected migrating through Prosser dam. This suggests that fish did not use significant energy reserves during the short period of time from the pre-release sampling until when they migrated through the dam.

References

- Branstetter R, Stephenson J, Pierce AL, Hatch DR, Bosch B, Fast D, Blodgett J, Everett SR, Paddlety J, Dasher R, et al. 2010 Steelhead Kelt Reconditioning and Reproductive Success. 2010 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2010-401. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.
- Branstetter R, Stephenson J, Pierce AL, Hatch DR, Bosch B, Fast D, Blodgett J, Everett SR, Paddlety J, Dasher R, et al. 2011 Steelhead Kelt Reconditioning and Reproductive Success. 2011 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-000. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.
- Frederiksen DR, Fast D & Temple G 2012 Yakima Steelhead Viable Salmonid Population (VSP) Status & Trends Monitoring. Yakima Steelhead VSP Project Annual Report 2011, Project No. 201003000. Toppenish, WA: Prepared by Yakama Nation Fisheries and Washington Department of Fish and Wildlife.
- Hatch DR, Branstetter R, Stephenson J, Pierce AL, Whiteacre JM & Bosch B 2012 Steelhead Kelt Reconditioning and Reproductive Success, 2011 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-000. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.
- Hockersmith E, Vella J, Stuehrenberg L, Iwamoto R & Swan G 1995 Yakima River radio telemetry study: steelhead, 1989-93. Prepared for Bonneville Power Administration, P.O. Box 3621, Portland, OR. Project Number 89-089. Seattle, WA: Coastal Zone and Estuarine Studies Division, Northwest Fisheries Science Center, National Marine Fisheries Service, NOAA.

Figure 1: Muscle lipid levels in reconditioned kelts and spawners from the ocean. Bars with different letters are significantly different (ANOVA followed by Bonferroni's multiple comparison test, $p < 0.05$). Boxes represent the interquartile range, the median is indicated by a line, and the data range and outliers are indicated by whiskers and points following the method of Tukey. Please note that not all reconditioned kelts were Fatmetered at release.



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

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 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 protocols 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).

Emamectin benzoate (emamectin) is an alternative to ivermectin that has been developed for control of sea lice (marine copepod ectoparasites) 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 Government 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; Gunn *et al.* 2012). Emamectin is much less toxic to salmonids than ivermectin. The 96 hour LC₅₀ 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 emamectin by intraperitoneal injection is well tolerated, and reduces variability in dosage rate due to variation in feed consumption by fish (Glover *et al.* 2010).

During the 2011 season, we experienced heavy mortality in hatchery origin kelts held at Dworshak National Fish Hatchery (DNFH) after ivermectin gavage. Due to this problem, 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 (Hatch *et al.* 2012). Results indicated that emamectin treated fish experienced substantially lower mortality rates than ivermectin treated fish, and that emamectin injection was more effective than ivermectin gavage for copepod control. To confirm the reduction in mortality rate, we repeated the experiment during the 2012 season at Prosser. In addition, we completed the formal statistical analysis of the main results from the 2011 season.

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 $907 \text{ } \mu\text{g/ml}$ 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 $200 \text{ } \mu\text{g}$ per kg of fish.

Fish Husbandry, Copepod Treatment, and Mortality

Wild Yakima River steelhead kelts were captured and reconditioned at Prosser, WA, during the 2012 season following established protocols (Evans *et al.* 2001; Branstetter *et al.* 2007; Hatch *et al.* 2013). Fish in the general population of kelts were housed in four 20' diameter tanks. Fish stocked into tank C2 (20 ft diameter, 100 gpm) were alternately given ivermectin by gavage or emamectin by injection at intake. Ivermectin was given by gavage at a dose of $100 \text{ } \mu\text{g kg}^{-1}$, based on the experience of project personnel (J. Blodgett, personal communication). Emamectin was injected at a dose of $200 \text{ } \mu\text{g kg}^{-1}$. Mortality was recorded daily. Copepod loads were not quantified during the 2012 experiment. Experimental procedures for the 2011 experiment have already been described (Hatch *et al.* 2012).

Data Analysis

Only female fish positively identified by PIT tag code from intake to exit (mortality or release), were included in the analysis. Male kelts tend to be in poorer condition at arrival and experience higher mortality rates. Fish held over the winter after the 2011 season were not included in the

analysis. Mortality rates were compared over the first 90 days of reconditioning, because in 2011 fish were sampled and retreated after approximately 90 days. Mortality rates were compared using the Log-rank (Mantel-Cox) test.

Results and Discussion

Prosser kelts treated at intake with emamectin had significantly lower mortality than fish treated with ivermectin in during 2011 and 2012 (Fig. 1). In both years, elevated mortality in ivermectin treated fish occurred over the first 30 days of reconditioning. In 2011, an additional period of elevated mortality in ivermectin treated fish appeared to occur from 60 to 90 days post-intake; in 2012, differential mortality was less pronounced during this time period. The reduction in mortality was substantial and consistent between years: after 90 days, mortality in emamectin treated fish was 33.2% that of ivermectin treated fish in 2011, versus 34.8% in 2012. These results indicate that ivermectin treatment causes elevated mortality in kelt steelhead at intake, most likely due to toxic effects on the fish at this particularly sensitive life stage.

The toxicity of avermectins to both invertebrate parasites and their vertebrate hosts is due to central nervous system depression. Avermectins depress 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. Avermectins are transported of the central nervous system by the blood-brain barrier in most higher vertebrates, resulting in low toxicity to the host and high effectiveness at killing invertebrate parasites. In fish, ivermectin does not appear to be effectively transported out of the central nervous system, accounting for the much greater toxicity of ivermectin to fishes than to higher vertebrates (Katharios *et al.* 2004; Horsberg 2012). Recent studies in rainbow trout show that emamectin is actively transported out of the central nervous system by the ATP-binding cassette protein transporter P-glycoprotein (ABCB1), a key component of the blood-brain barrier present in brain endothelial cells. However, ivermectin is not transported out of the CNS by this transporter, resulting in accumulation in the brain (Kennedy & Mittlestadt 2012). Kelt steelhead may be particularly sensitive to the toxic effects of ivermectin at intake because of the chemical properties of the drug. Ivermectin is lipophylic, 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. These considerations explain the increased mortality found in kelt steelhead treated with ivermectin at intake into reconditioning.

In our 2012 experiment, we did not attempt to quantify levels of copepod infestation in emamectin versus ivermectin treated fish. However, very few fish were observed with significant numbers of copepods at release, suggesting that both ivermectin and emamectin treatment controlled copepods adequately. 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). Therefore, an untreated control could not be employed in these studies, due to the ESA-listed status of Yakima River steelhead.

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). 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). Fortunately, an INAD for Slice, a formulation of emamectin given in the feed, has recently become available. Based on our experiences with kelt steelhead, we recommend that project managers consider switching to Slice. Projects working with non-actively feeding adult fish may wish to consider adopting the injection protocol we have described here.

References

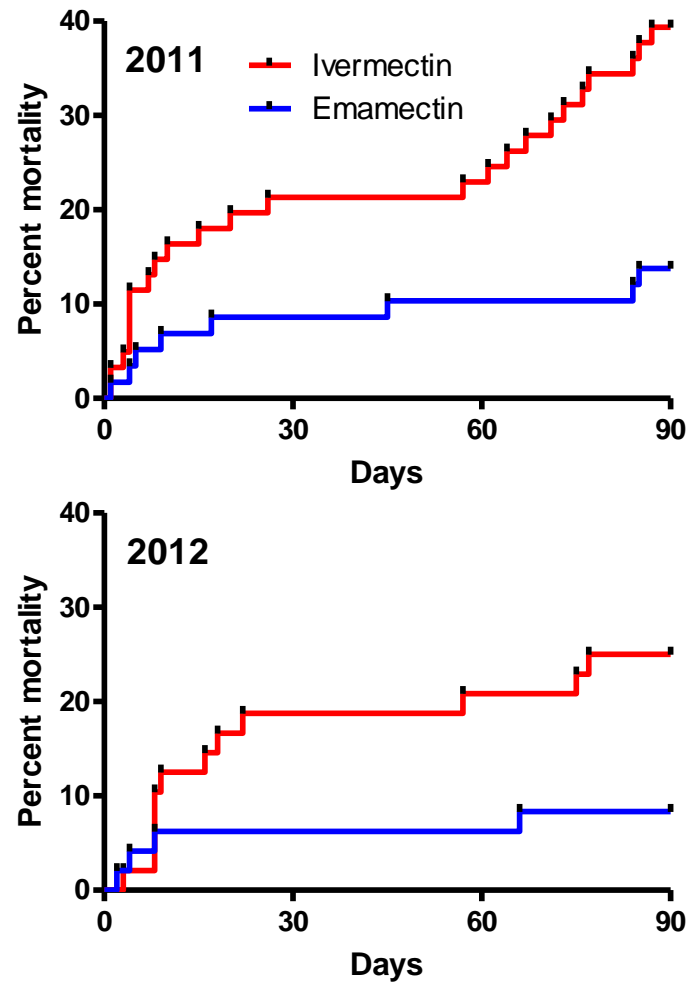
- Branstetter R, Stephenson J, Hatch DR, Whiteaker J, Hyun S-Y, Bosch B, Fast D, Blodgett J, Newsome T, Hewlet LM, et al. 2007 Steelhead Kelt Reconditioning and Reproductive Success. 2007 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.
- Duston J & Cusack RR 2002 Emamectin benzoate: an effective in-feed treatment against the gill parasite *Salmincola edwardsii* on brook trout. *Aquaculture* **207** 1-9.
- Evans AF, Beatty RA & Hatch DR 2001 Kelt Reconditioning: A Research Project to Enhance Iteroparity in Columbia Basin Steelhead (*Oncorhynchus mykiss*). 2000 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2000-017. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.
- Glover KA, Samuelsen OB, Skilbrei OT, Boxaspen K & Lunestad BT 2010 Pharmacokinetics of emamectin benzoate administered to Atlantic salmon, *Salmo salar* L., by intra-peritoneal injection. *Journal of fish diseases* **33** 183-186.
- Government C 2007 Slice: Action, Use, and Effects. Prepared by the British Columbia Centre for Aquatic Health Sciences.
- Gunn C, Carty D, Walker PG, Colburn PA & Bowker JD 2012 Pilot Field Trial to Evaluate SLICE (0.2% Emamectin Benzoate)—Medicated Feed to Reduce a Natural Infestation of *Salmincola californiensis* in Freshwater-Reared Rainbow Trout. *North American Journal of Aquaculture* **74** 424-427.

- Halley BA, Nessel RJ & Lu AYH 1989 Environmental aspects of ivermectin usage in livestock: general considerations. In *Ivermectin and Abamectin*, pp 162-172. Ed WC Campbell. New York: Springer-Verlag.
- Hatch DR, Branstetter R, Stephenson J, Pierce AL, Whiteacre JM & Bosch B 2012 Steelhead Kelt Reconditioning and Reproductive Success, 2011 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-000. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.
- Hatch DR, Fast DE, Bosch WJ, Branstetter R, Blodgett JW, Whiteacre JM & Pierce AL 2013 Survival and traits of reconditioned kelt steelhead (*Oncorhynchus mykiss*) in the Yakima River, Washington. *North American Journal of Aquaculture*.
- Horsberg TE 2012 Avermectin use in Aquaculture. *Current Pharmaceutical Biotechnology* **13** 1095-1102.
- Johnson KA & Heindl JA 2001 Efficacy of manual removal and ivermectin gavage for control of *Salmincola californiensis* (Wilson) infestation of chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), captive broodstocks. *Journal of Fish Diseases* **24** 197-203.
- Johnson S, Kent M, Whitaker D & Margolis L 1993 Toxicity and pathological effects of orally administered ivermectin in Atlantic, chinook, and coho salmon and steelhead trout. *Diseases of Aquatic Organisms* **17** 107-112.
- Katharios P, Pavlidis M & Iliopoulou-Georgudaki J 2004 Accumulation of ivermectin in the brain of sea bream, *Sparus aurata* after intraperitoneal administration. *Environmental toxicology and pharmacology* **17** 9-12.
- Kennedy CJ & Mittlestadt M 2012 Inhibition of p-glycoprotein in the blood-brain barrier of salmonids affects ivermectin-induced swimming dysfunction. In *10th International Congress on the Biology of Fish*, p 65. Ed D MacInlay. Madison, WI.
- McGladdery SE & Johnston CE 1988 Egg development and control of the gill parasite, *Salmincola salmoneus*, on Atlantic salmon kelts (*Salmo salar*) exposed to 4 different regimes of temperature and photoperiod. *Aquaculture* **68** 193-202.
- O'Halloran J, Ogden CD & Hogans WE 1992 *Ergasilus labracis* on Atlantic salmon. *Can. vet. J.* **33** 75.
- Palmer R, Rodger H, Drinan E, Dwyer C & Smith PR 1987 Preliminary trials on the efficacy of ivermectin against parasitic copepods of Atlantic salmon. *Bulletin of the European Association of Fish Pathologists* **7** 47-54.

Roberts RJ, Johnson KA & Casten MT 2004 Control of *Salmincola californiensis* (Copepoda: Lernaeapodidae) in rainbow trout, *Oncorhynchus mykiss* (Walbaum): a clinical and histopathological study. *Journal of fish diseases* **27** 73-79.

Roy WJ, Sutherland IH, Rodger HDM & Varma KJ 2000 Tolerance of Atlantic salmon, *Salmo salar*, and rainbow trout, *Oncorhynchus mykiss*, to emamectin benzoate, a new orally administered treatment for sea lice. *Aquaculture* **184** 19-29.

Figure 1. Mortality of Prosser kelts after ivermectin or emamectin treatment at intake. Log-rank test mortality curve comparison, 2011: $p = 0.0019$; 2012: $p = 0.0340$.



Section H: Egg quality and reproductive parameters in air spawned hatchery origin maiden spawning female steelhead at Dworshak National Fish Hatchery

Introduction

The objective of this experiment was to assess egg quality in reconditioned female kelt steelhead. Production of high quality eggs is necessary for reconditioned kelts to contribute to listed Snake River steelhead populations. If issues with egg quality are identified, they will need to be addressed in order for the project to succeed. It is difficult or impossible to directly to assess egg quality in wild fish, because wild fish spawn naturally before we collect them, and reconditioned wild fish must be released to spawn naturally. The DNFH hatchery origin kelt model provides a unique opportunity to directly assess egg quality in a large number of maiden spawners. If these fish can be successfully reconditioned, egg quality in eggs obtained during the first spawning can be directly compared to egg quality in eggs obtained during the second spawning. In this experiment, we aimed to compare the egg quality of DNFH hatchery-origin female steelhead at their maiden spawning with egg quality of kelts which survived and rematured at their second spawning. Due to water quality problems during reconditioning and subsequent low survival of hatchery origin kelts, we were unable to obtain data from reconditioned fish to make this comparison. However, we did collect a large amount of data on egg quality and other reproductive parameters in artificially spawned DNFH hatchery-origin maiden spawners. In addition, we were able to collect data on five reconditioned hatchery kelts which were lethally sampled at the end of September. This data will provide a baseline for comparison in future studies.

Methods

On 2/7/12 and 2/8/12, 143 hatchery origin maiden female steelhead were air spawned at DNFH. Air spawning methods are described in Chapter 1 Section B. The total weight of eggs collected from each female was recorded, and a subsample of approximately 200 eggs from each female was taken for transport to the Nagler lab at the University of Idaho. The weight of eggs was used as ovary weight for calculation of gonadosomatic index. After air spawning, lengths and weights of fish were recorded, and a non-lethal measure of muscle lipid content was taken using a Fish Fatmeter (Distell Inc., Midlothian, UK). Milt from several males remaining from DNFH production spawning was also collected and transported to the University of Idaho. Milt samples were not pooled. At the University of Idaho, the motility of milt from each male was assessed, and a male was selected with confirmed motility and sufficient volume to fertilize all of the eggs collected. The weight of a random subsample of 25 eggs from each female was recorded. Eggs were fertilized and incubated for 12 h. After 12 h, approximately 25 eggs from each female were fixed in Stockard's solution and stored (Stoddard *et al.* 2005). The percentage of eggs successfully fertilized was measured as the percentage of fixed eggs showing cleavage (cell division) in the embryo by examination under a dissecting microscope. This method is less

variable than assessments of egg quality further along in development, and eggs lots with reduced viability are clearly evident at the 12 hour time point (Stoddard *et al.* 2005).

Fish were reconditioned as described in Chapter 1 Section B. On 9/25/12, remaining hatchery origin kelts were lethally sampled. Length, weight, and muscle lipid levels were recorded, blood was drawn for hormone assays, and the weight of the ovary was measured. Gonadosomatic Index (GSI) was calculated as $100 * \text{ovary weight} / \text{carcass weight without ovary}$.

Results and Discussion

The size, K Factor, and muscle lipid stores of DNFH fish at spawning were similar to previous years (Fig. 1). Muscle lipid levels were higher than those typically observed in kelts captured during downstream migration. This is consistent with lower energy expenditures in artificially spawned hatchery fish, which do not use energy in spawning behavior or post-spawning migration. The GSI of DNFH fish was slightly lower than values reported for steelhead (median 13.5 % versus 14.9 %, (Crespi & Teo 2002)). This is at least partially due to incomplete spawning of a fraction of the fish with unovulated eggs at the time of air spawning. Fish have been kill spawned at DNFH for many years, and consequently some of the fish selected as ripe were not ripe enough to expel all of the eggs using air spawning. This is likely also the explanation for why the median fecundity of air spawned fish was 81.7 % of the 6578 eggs per female for kill spawned fish (USFWS 2012). This is of concern in that it both reduces egg take that can be integrated in to hatchery production, and residual eggs may interfere with recovery from spawning and reconditioning. However, this problem can be easily remedied by selecting fully ripe fish for air spawning. Egg size was similar to previous values for steelhead (Crespi & Teo 2002). Median fertilization success percentage was 85%. Fertilization percentages of 80% and greater are considered to indicate good egg quality in commercial rainbow trout egg production for aquaculture, and egg lots with less than 80% fertilization are considered to be sub-fertile (Stoddard *et al.* 2005). Thus, some of the maiden DNFH steelhead would be considered sub-fertile. It is not yet clear whether this is typical of DNFH production fish, and what factors may be causing sub-fertility. Many factors including stress, temperature, post-ovulatory aging, and physical disturbance of eggs may reduce egg quality (Bobe & Labbe 2010). In our experiment, fertilization was delayed by approximately 6 to 8 hours after eggs were collected, which may have reduced fertilization percentage. Eye-up percentage assessed after immediate fertilization is typically 90-95% at DNFH (Nez Perce Tribe, unpublished data, USFWS 2012).

Correlation analysis of parameters measured at spawning showed the expected positive correlations between length and weight (Table 1). Fecundity and egg size were positively correlated with fish size, although the correlation was relatively weak, as has previously been reported for salmonids (Quinn 2005). Negative correlations between GSI and K factor and GSI and weight are likely due to weighing of the fish after air spawning was complete. Retained eggs would decrease the measured GSI and increase relative fish weight and consequently increase K factor. Muscle lipid content as measured with the Fatmeter was significantly positively correlated with K factor as has been reported for Atlantic herring

(McPherson *et al.* 2011). Both of these indices are measures of energy stores, which is thought to be a key variable in the successful expression of iteroparity. However, neither muscle lipid content nor K factor was significantly correlated with survival during reconditioning, suggesting that mortality under hatchery conditions was not primarily due to the exhaustion of energy reserves. Survival (days lived) was not significantly correlated with fertilization percentage, fecundity, GSI, or egg weight. Therefore, our current results do not support the existence of a tradeoff between reproductive investment in the maiden spawning with the ability to survive after spawning, at least when fish are held under hatchery conditions. However, the collection of additional years of data will be required before any conclusions can be drawn on this topic.

Parameters measured at intake and release for five surviving air spawned hatchery kelts lethally sampled on 9/25/12 are presented in Table 2. Surviving fish were typical in terms of intake size, muscle lipid level, and egg size. GSI and fertilization percentage ranged widely, with several of the fish showing a relatively low fertilization percentage. The sample size was not sufficient to draw any conclusions regarding potential relationships between egg quality and survival over the entire reconditioning period. Surviving fish increased substantially in weight but did not increase in length during reconditioning. Muscle lipid levels increased, and were similar to levels measured at release in Prosser kelts. Four of the five fish were rematuring at release, with a GSI of 2 to 5.3 %, indicating that ovarian development was approximately 16 to 34 % complete. One fish was immature at release, with a GSI of 0.1 %. Photos of representative fish and ovaries are shown in Figure 2. Blood samples were taken from these fish on 7/24/12 and at release on 9/25/12; however, plasma estradiol and vitellogenin levels have not yet been measured. The four rematuring fish were fed the Cyclopeeze and fish oil supplemented “orange” diet, whereas the non- rematuring fish was fed standard pellets (see Section E for diet compositions). This is not a sufficient sample size to analyze the data statistically; however, it does provide preliminary evidence that the orange diet may promote rematuration. Further, these data demonstrate that fish with a B-run life history can remature as consecutive spawners over a single summer at a high rate (80%). This suggests that captive culture conditions may substantially increase the consecutive spawner rate over the 35% consecutive 65% skip spawning rate found in natural repeat spawners at Lower Granite dam (Keefer *et al.* 2008).

References

- Bobbe J & Labbe C 2010 Egg and sperm quality in fish. *Gen Comp Endocrinol* **165** 535-548.
- Crespi BJ & Teo R 2002 Comparative phylogenetic analysis of the evolution of semelparity and life history in salmonid fishes. *Evolution* **56** 1008-1020.
- Keefer ML, Wertheimer RH, Evans AF, Boggs CT & Peery CA 2008 Iteroparity in Columbia river summer-run steelhead (*Oncorhynchus mykiss*): implications for conservation. *Canadian Journal of Fishery and Aquatic Sciences* **65** 2592-2605.

McPherson LR, Slotte A, Kvamme C, Meier S & Marshall CT 2011 Inconsistencies in measurement of fish condition: a comparison of four indices of fat reserves for Atlantic herring (*Clupea harengus*). *ICES Journal of Marine Science* **68** 52-60.

Quinn TP 2005 *The Behavior and Ecology of Pacific Salmon and Trout*. Seattle: University of Washington Press.

Stoddard JW, Parsons JE & Nagler JJ 2005 Early onset of embryonic mortality in sub-fertile families of rainbow trout (*Oncorhynchus mykiss*). *Reproduction Fertility and Development* **17** 785-790.

USFWS 2012 Dworshak National Fish Hatchery Annual Report.

Table 1: Correlation analysis of parameters measured at intake and during the first 59 days of reconditioning. Pearson correlation unadjusted p-values are shown below the diagonal and r values above. Correlations with $p < 0.01$ are shown in bold.

	Length	Weight	K Factor	Muscle Lipid	Weight per egg	GSI	Fecundity	Fert %	Days Lived
Length	1.0000	0.8778	-0.2113	0.0127	0.3005	-0.0978	0.2773	-0.0813	-0.0199
Weight	<.0001	1.0000	0.2756	0.1683	0.2990	-0.3105	0.1036	0.0027	-0.0183
K Factor	0.0119	0.0009	1.0000	0.3136	-0.0051	-0.4714	-0.3594	0.1712	0.0081
Muscle Lipid	0.8815	0.0460	0.0002	1.0000	0.0611	-0.1966	-0.1485	0.0524	-0.0425
Weight per egg	0.0003	0.0003	0.9521	0.4715	1.0000	0.0860	-0.1231	-0.0696	0.0233
GSI	0.2485	0.0002	<.0001	0.0194	0.3106	1.0000	0.8323	-0.1694	0.0457
Fecundity	0.0009	0.2215	<.0001	0.0788	0.1459	<.0001	1.0000	-0.1632	0.0485
Fertilization %	0.3377	0.9746	0.0423	0.5368	0.4124	0.0446	0.0532	1.0000	0.0625
Days Lived	0.8149	0.8295	0.9240	0.6167	0.7836	0.5905	0.5683	0.4619	1.0000

Table 2: Parameters measured at intake and at lethal sampling at release in surviving hatchery origin artificially spawned female DNFH kelts.

	Intake (2/7/12)						Diet	Release (9/25/12)				Intake to Release	
Fish ID	Len cm	Wt kg	Fat %	GSI %	Wt per egg (g)	Fert %		Len cm	Wt kg	Fat %	GSI %	SGRW %BW/day	GSI % initial
3D1.2E9142AFFD	84	4.4	1	16.5	0.098	73.1	Orange	83	6.2	2.1	4.7	0.148	28
3D1.2E91447725	80	3.8	1	16.7	0.098	80.8	Orange	82	6.04	5	5.3	0.201	31
3D1.2E9144D407	82	4.3	1	8.9	0.113	93.3	Orange	82	6.38	4.4	3.0	0.171	34
3D1.2E914409B0	82	4.1	0.7	12.4	0.081	56.7	Orange	82	6.03	5.1	2.0	0.167	16
3D1.2E9144EF55	79	3.8	1	12.6	0.097	64.3	Regular	79	5.31	4.4	0.1	0.145	1

Figure 1: Body characteristics and reproductive parameters measured in hatchery origin artificially spawned female maiden DNFH steelhead at air spawning (2/7-2/8/12). Boxes represent the interquartile range, the median is indicated by a line, and the data range and outliers are indicated by whiskers and points following the method of Tukey.

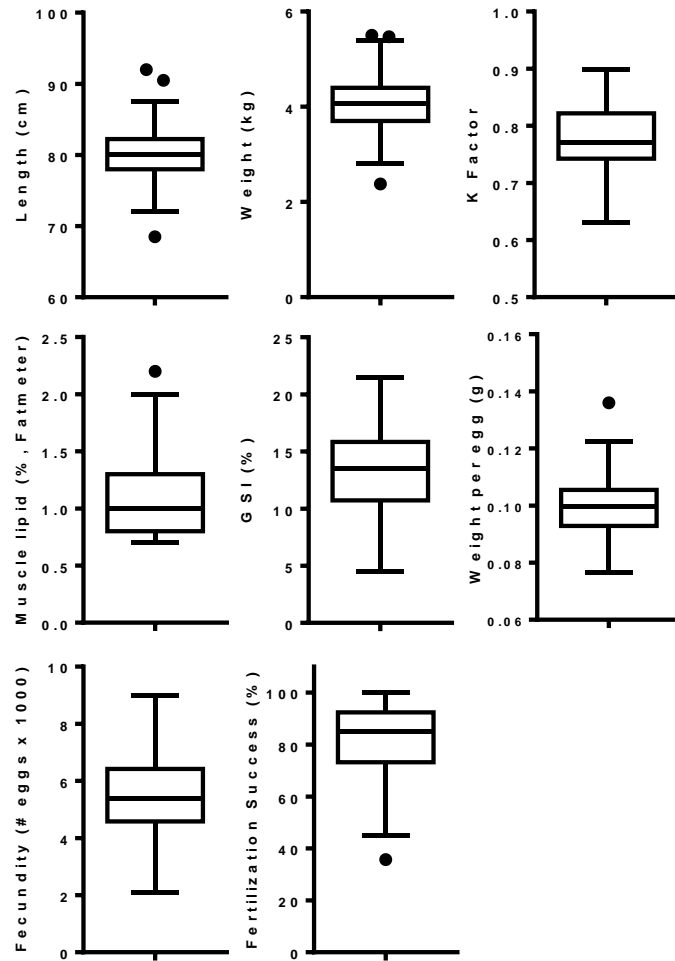
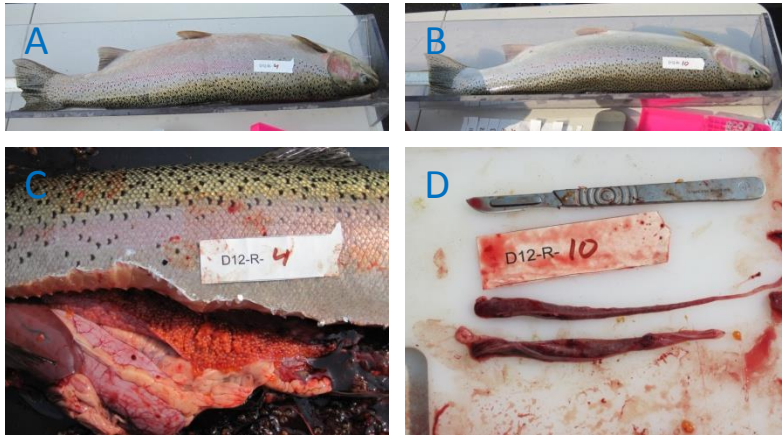


Figure 2: Photos of representative rematuring (A, C), and non-rematuring (B, D) fish and ovaries at the 9/25/12 sampling.



Chapter 3. Snake River Kelt Strategies and Management

Andrew P. Matala

Columbia River Inter-Tribal Fish Commission

Christine Moffitt, Principal Investigator

Bryan Jones

Zachary Penney

Boling Sun

Heath Hewett

Idaho Cooperative Fish and Wildlife Research Unit

Scott Everett

Nez Perce Tribe

Section A: 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

2012 Draft Annual Report

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 and 2012. The objective of this study was primarily to estimate stock proportions in a mixed stock sample, providing a better understanding of the origins of post-spawn steelhead among the major subbasins (e.g., Clearwater River, Salmon River, Grande Ronde) and major population groups (MPG's) within the Snake River Basin. Results will also 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 to evaluate assignment power and to conduct GSI analyses are described in detail in Hess et al. (2012) and Ackerman 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 successfully genotyped (n=4,138) from across the downstream migration seasons in 2009 through 2012, in coordination with other ongoing monitoring and evaluation efforts at LGD (pers. comm. Scott Everett and Zach Penny of the Nez Perce Tribe). Biological field data for kelts was recorded and provided by Nez Perce Tribe and University of Idaho biologists, including sample date, fork length, gender, disposition and overall condition. Overall condition ratings included “poor”, “fair” and “good”. Briefly, the rating is based on fish appearance

, fungal load, and presence of injuries (e.g., head wounds; pers. comm. Scott Everett, Nez Perce Tribe).

In addition to analysis of natural origin kelts, a total of n=1,434 hatchery kelts were sampled and genotyped. Only hatchery kelts in 2009 (n=41), 2010 (n=51), and 2012 (n=814) were available for GSI and parentage based tagging (PBT) analysis (total n=906). As a quality assurance/ quality control measure, hatchery kelts (excluding 2011 due to missing data) were evaluated using GSI to determine stock proportions for comparison with natural origin kelts. Further, all natural origin kelts were tested against a PBT baseline (Steele et al. 2012) to determine which if any were

actually mis-identified hatchery fish. For all natural origin kelts that proved to be hatchery fish based on PBT, a concordance test was conducted from which GSI reporting group assignment would be compared to the region of hatchery-of-origin.

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. 2012, Ackerman et al. 2012). It is comprised of 65 discrete collections, providing a genetic characterization of all major subbasins in the Snake River Basin, and typically includes multiple collections (watersheds) per subbasin such that all potential contributing stocks or discrete populations are represented (Table 1; Ackerman 2012). 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. 2012). 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 often 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, reporting group modifications or alternative groupings of collections were constructed to evaluate changes or increases in assignment accuracy for the different combinations of populations 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 the 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, the baseline has been independently evaluated in detail using several methods including 'leave-one-out' (LOO) individual assignment procedures, performed by Ackerman et al. (2012), and with complementary outcome.

Following power analysis, an assignment test was performed to estimate the origin of each kelt of "unknown" origin in the sample (e.g. a fishery mixture) in ONCOR. Individual kelts were assigned to baseline populations and designated reporting groups on the basis of information from both genotype frequencies and mixture proportions. ONCOR performs these calculations and provides probability estimates using the method of Rannala and Mountain (1997). Output of assignment test results includes the identity of the population that would most likely have produced an individual's genotype (and its probability), as well as a list of the second, third, and fourth most likely populations and their probabilities as necessary until the sum of all probabilities is greater than 0.99.

Results

Baseline Power Analysis

Based on previously described criteria, the baseline populations were partitioned into 10 reporting groups (RG) for analysis (Figure 1; Table 1). Reporting groups are consistently color coded in figures throughout this document. Baseline assignment accuracy using the 100% simulation method was variable across stream collections and RG's, but was generally at or above an 80% accuracy threshold. Population estimates ranged from 0.36 in the Tucannon River collection (Lower Snake River RG) to 1.00 in upper Big Creek (Middle Salmon River RG). At the population level, there were 37 baseline collections with average population estimates that exceeded 80%, 21 of which were greater than 90%. Estimates were generally lowest in the Lower Salmon (LS) and Lower Snake River (LSN) RG's, and highest in the South Fork Salmon and Upper Clearwater River RG's. At the reporting group level, 61 of 65 collections had average RG estimates greater than 80%, of which 51 exceeded 90%. Again, the LS and LSN reporting groups were generally lowest, but the Middle Fork Salmon River RG was the highest. Mean reporting group accuracy ranged from 81% to 99% (Table 1).

Kelt Mixture Assignments

Of the 4,138 kelt samples evaluated, 2,108 (51%) assigned with at least 80% probability (robust assignments; Table 2). Ordering of the 10 reporting groups by average probability of assignment was generally consistent from year to year. Regions believed to be dominated by A-run steelhead (Grande Ronde - GR, Imnaha - IM, lower Salmon - LS, and lower Snake River) ranked lowest as did the lower Clearwater River RG, while regions believed to be dominated by B-run fish were highest, particularly South Fork Salmon River - SFS, South Fork Clearwater River - SFC and upper Clearwater River - UCL reporting groups. Mean assignment probabilities ranged from 62% in LS to 92% in UPC. Note that the upper Salmon River RG typically scored better than the other A-run counterparts (Table 2). For RG's that ranked lowest in proportion of individuals that assigned with at least 80% probability (GR, IM, LS, or LCL), second ranked assignments were dominated by LSN and UPS reporting group assignments. Middle Fork Salmon River (MFS) and SFS reporting groups had second ranked assignments dominated by alternate Salmon River groups, as did SFC and UPC respective of Clearwater River alternate RG's (Table 3).

Stock proportions estimated using the entire sample (n=4,138) were similar but slightly skewed from stock proportions based on an 80% threshold (n=2,108; Figure 2). The largest differences occurred in UPS and MFC (greater proportion at strict criteria) and LSN and GR (both with

lower proportion at strict criteria). Greater proportional assignment to UPS presumably made up much of the differences in GR and LSN proportions (Figure 2; Table 4). Stock proportions of kelts determined by GSI analysis were compared to the stock proportions determined by GSI of returning steelhead sampled at LGD during spawn years 2009-2011 (extrapolated to estimate total escapement to LGD). Both analyses included only natural origin adult steelhead, defined as having no detectable marks or tags. Stock proportions for estimated escapement were evaluated, and data contributed by Mike Ackerman (IDFG); based on an 80% probability threshold only. Note that results for kelts varied widely from estimates from total escapement in the same years, where stock proportions among the returns were far more uniform across reporting groups (Figure 2; Table 4). The difference was particularly poignant in regions with relatively few kelts (UPC, SFC, SFS).

Demographic Correlations

For these exploratory analyses, all assignments were considered regardless of probability. Sex ratios were largely consistent across RG's that had sufficient numbers of assigned fish from each gender for comparison. The RG mean proportion of female kelts ranged from 74.2% in SFC to 85.5% in IM (Figure 3; Appendix 1). Across RG's, the mean proportion of kelts identified as female ranged from 71% in 2010 to 87% in 2011 (Figure 3). The mean across RG's was 77.0% female, and results were generally consistent with Keefer et al. (2008).

Capture date at Lower Granite Dam served as a proxy for outmigration date, which was enumerated as ordinal day (January 1st = day 1). Generally, male kelts migrated downstream later than females, and the trend was consistent across years and RG's; exceptions occurred when too few males were assigned to an RG in a given year. The mean outmigration days for females and males were day 134 and day 141 respectively (Figure 4; Appendix 1). Among RG's the overall average outmigration day ranged from 115 in SFC to 153 in MFS for females, and from 129 in SFC to 165 in SFS for males. The Lower Clearwater River (LCL) and South Fork Clearwater River (SFC) groups were consistently the earliest outmigrants, and often the difference in mean day of outmigration between males and females was greatest in these two RG's. Interestingly and in direct contrast to kelt outmigration timing, the South Fork Clearwater River was the latest returning (LCL was also among the latest) and the Middle Fork Salmon and South Fork Salmon rivers were the earliest returning based on GSI for steelhead returns at LGD from 2009 through 2011 (pers. comm. Mike Ackerman, IDFG).

Kelt size (fork length) significantly trended toward larger fish in regions generally considered to support predominantly B-run steelhead populations (e. g., South Fork Salmon River and Clearwater River), and the trend was apparent for both sexes (Figure 5). In each year, kelts assigning to the SFS, SFC and UPC reporting groups were the largest observed. Among RG's the mean female kelt length ranged from 65.1cm in 2010 to 69.1cm in 2011 (Figure 5, Appendix 1). Within each year (2009-2012) and on average, male kelts were smaller in length than females (62.1cm and 67.6cm respectively). These results are 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 better than male kelts, but there was no clear correlation or relationship between reporting group and overall condition (e.g. % good) for either gender (Figure 6). To further explore possible correlations between kelt condition and demographic variables, condition was plotted against fork length and against outmigration time using the entire kelt sample (n=4,138), across gender and reporting groups. There was no relationship observed between kelt length and condition. However, the relationship between outmigration date and overall condition rating of individuals (Figure 7) was significant, where earlier outmigration showed a smaller proportion of kelts in "good" condition.

Hatchery origin fish would presumably not assign to reporting groups as accurately as natural origin fish using a GSI baseline comprised exclusively of putative natural origin collections. Stock transfers and outplanting, and phases of the rearing cycles that occur in non-local facilities are factors also likely to further confound the ability to confidently assign hatchery fish to regions of origin based on GSI reporting group assignment. The GSI stock proportions for hatchery kelts varied greatly from those observed for natural origin kelts. For example, UPS reporting group made up as much as 66% of all sampled hatchery kelts. Results shifted using an 80% criteria for assignment probability in which proportions from GR and LSN shrank and conversely grew in UPS and SFC (Figure 8). There were n=331 natural origin kelts sampled between 2009 and 2012 (8% of the total) that were assigned as hatchery progeny using the PBT method. Note that this procedure followed primary GSI analyses, and therefore these fish were included in previously described analysis and results for natural origin kelts. Nevertheless, there was a high degree of concordance between GSI reporting group assignment and the RG's from which hatchery PBT assigned kelts originated (e.g. Sawtooth Hatchery located in the UPS reporting group). For example, of n=71 presumed natural origin kelts that were PBT assigned to Sawtooth Hatchery broodstock, 93% also GSI assigned to the upper Salmon River (UPS) reporting group, and all four Tucannon River hatchery fish assigned to the lower Snake River reporting group (Table 5).

Discussion

Baseline assignment accuracy, though variable provided a reasonable degree of power to differentiate the ten designated reporting groups and in some cases the specific population of origin. 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 (i.e. ecotype) 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, there was no significant correlation observed between fish size and the proportion of fish rated “good” in overall condition (contrasting previously reported results (Hatch et al. 2012). There is some concern that size selectivity at the Lower Granite Dam bypass may be a contributing factor to condition of kelts. 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). To date, the results of kelt GSI regarding physical condition vs. size of fish are inconclusive. In contrast to size, a later date of downstream migration was significantly associated with overall condition of the kelts (higher proportion “good” condition). However, the biological significance of this finding is difficult to discern on the basis of fresh water residence time. For example, some of the earliest steelhead to return to LGD during spawn years were often some of the last to be detected at LGD post-spawn (e.g.

Middle Fork Salmon River). The opposite is true for some Clearwater River reporting groups, where steelhead were among the last to return to LGD during the spawn year, but GSI assigned kelts were among the first to outmigrate. Observing the proportion of good condition larger sized kelt steelhead is similar to smaller fish is encouraging for the kelt reconditioning program targeting B-run steelhead since fish in better condition tend to survive reconditioning at higher rates (Hatch et al. 2013).

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). The GSI assignment proportions of kelts reported here verify or substantiate some information about the life history of the species *O. mykiss*. Specifically, female rates of iteroparity are higher than for males regardless of region or ecotype, and that the A-run ecotype (generally younger age) are more prone to repeat migrations across their distribution range. Those regions dominated by the A-run ecotype are somewhat less accurately differentiated than B-run using GSI, yet these results provide a reasonable level of confidence in evaluating which regions produce greater proportions of potentially iteroparous individuals. This is an important attribute contributing to population productivity, and monitoring of the relative abundances of both A-run and B-run forms will inform specific management of each with important implications for conservation. As expected, 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 2). With refined methods and more data it may be possible to confidently estimate relative rates of iteroparity among RG's based on escapement using such comparisons. Hatchery GSI results suggest this method may perform reasonably well for identifying kelt proportions from specific hatchery programs. 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 (Nez Perce, IDFG, University of Idaho) and the quality data provided. This study benefited greatly from resources provided by Christine Moffitt, Jessica Beulow and Zach Penny. Thank you to Nick Hoffman and Stephanie Harmon for diligent and reliable laboratory processing and collection of genotypic data. Data and information provided by Mike Ackerman and Craig Steele from IDFG, and Maureen Hess from CRITFC made a substantial contribution to these analyses.

References

- Ackerman, M. W., J. McCane, C. A. Steele, M. R. Campbell, A. P. Matala, J. E. Hess, and S. R. Narum. 2012. Chinook and steelhead genotyping for genetic stock identification at Lower Granite Dam. 2011 Annual Report. Submitted to: U.S. Department of Energy, Bonneville Power Administration, Division of Fish and Wildlife, P.O. Box 3621 Portland, OR 97283-3621, Contract #53239; Project #2010-026-00. Available at: <https://pisces.bpa.gov/release/documents/documentviewer.aspx?doc=P128035>
- Anderson, E. C., R. S. Waples, and S. T. Kalinowski. 2008. An improved method for predicting the accuracy of genetic stock identification. *Canadian Journal of Fisheries and Aquatic Sciences* 65(7):1475-1486.
- Busby, P. J., T. C. Wainwright, G. J. Bryant, L. J. Lierheimer, R. S. Waples, F. W. Waknitz and F. W. Lagomarsino. 1996. Status review of west coast steelhead from Washington, Idaho, Oregon, and California. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-NWFSC-27. Available at <http://www.nwfsc.noaa.gov/publications/techmemos/tm27/tm27.htm>
- Hatch, D.R., D.E. Fast, W.J. Bosch, J.W. Blodgett, J.M. Whiteaker, R. Branstetter, and A.L. Pierce. 2013. Survival and traits of reconditioned kelt steelhead *Oncorhynchus mykiss* in the Yakima River, Washington. *North American Journal of Fisheries Management* 33(3):615-625.
- Hess, J. E., N. Campbell, A. P. Matala and S. R. Narum. 2012. Genetic assessment of Columbia River Stocks: 2010 annual report. Submitted to Bonneville Power Administration. Contract #41224; Project # 2008-907-00 <https://pisces.bpa.gov/release/documents/documentviewer.aspx?doc=P120015>
- Keefer, M.L., R.H. Wertheimer, A.F. Evans, C.T. Boggs, and C.A. Peery. 2008. Iteroparity in Columbia River summer-run steelhead (*Oncorhynchus mykiss*): implications for conservation. *Canadian Journal of Fisheries and Aquatic Sciences* 65:2592-2605.
- Narum, S. R., D. Hatch, A. J. Talbot, P. Moran and M. S. Powell. 2008. Iteroparity in complex mating systems of steelhead *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Biology* 72:1-16.
- Rannala, B., and J. L. Mountain. 1997. Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences of the United States of America* 94(17):9197-9201.
- Steele, C., M. Ackerman, J. McCane, M. Campbell, M. Hess and S. Narum. 2012. Parentage based tagging of Snake River hatchery steelhead and Chinook salmon. 2011 Annual Report. Submitted to: U.S. Department of Energy, Bonneville Power Administration, Division of Fish and

Wildlife, Project Number 2010-031-00; Contract Number 53238. Available at:
<https://pisces.bpa.gov/release/documents/documentviewer.aspx?doc=P127156>

Table 1. Populations included in the Snake River *O. mykiss* SNP baseline. Collections representing baseline populations were partitioned into discrete reporting groups (RG's) for GSI based on assignment probability in leave-one-out bootstrap tests (see Ackerman 2012). 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. All collections are putative natural-origin fish and all are assumed anadromous. Baseline sample size (n) is shown. Results of the 100% simulation test of ONCOR are: the average population estimate defined as assignment to "self" for each individual stream collection, and average RG estimate defined as assignment to reporting group of origin; mean values within each RG are given. Baseline assignment exceeding 80% accuracy are filled light gray, while those exceeding 90% accuracy are filled dark gray.

Reporting Group (RG)	abbrev.	stream name	abbrev.	(n)	AVG (pop estimate)	AVG (RG estimate)	RG mean
<u>Grande Ronde River</u>	GR	Captain John	CPJN	56	0.91	0.93	---
	GR	Crooked	CROOK	95	0.81	0.89	---
	GR	Elk	ELK	45	0.97	0.99	---
	GR	Joseph	JOSE	45	0.39	0.67	---
	GR	Lostine	LOST	45	0.91	0.96	---
	GR	Little Minam	LTMN	48	0.95	0.97	---
	GR	Menatchee	MENA	68	0.81	0.85	---
	GR	Wenaha	WENA	93	0.79	0.91	0.90
<u>Imnaha River</u>	IM	Big Sheep	BGSP	61	0.87	0.93	---
	IM	Camp	CAMP	23	0.70	0.90	---
	IM	Cow	COW	44	0.74	0.78	---
	IM	Lightning	LTNG	38	0.73	0.90	0.88
<u>lower Clearwater River</u>	LCL	Big Bear	BGBEAR	98	0.68	0.91	---
	LCL	East Fork Potlatch	EFPOT	156	0.80	0.99	---
	LCL	Little Bear	LTBEAR	151	0.89	0.97	---
	LCL	Mission	MISS	49	0.77	0.80	---
	LCL	West Fork Potlatch	WFPOT	85	0.47	0.98	0.93
<u>Lower Salmon River</u>	LS	Boulder	BOUL	47	0.77	0.92	---

	LS	Hazard	HAZA	43	0.39	0.59	---
	LS	Rapid	RAPD	99	0.96	0.97	---
	LS	Slate	SLATE	46	0.63	0.70	---
	LS	Whitebird	WTBR	59	0.84	0.87	0.81
<u>Lower Snake River</u>	LSN	Alpowa	ALPO	98	0.46	0.91	---
	LSN	Asotin	ASOT	147	0.53	0.87	---
	LSN	George	GEO	95	0.39	0.87	---
	LSN	Tucannon	TUCA	105	0.36	0.92	0.89
<u>Middle Fork Salmon River</u>	MFS	Bargamin	BARG	46	0.83	0.88	---
	MFS	Camas	CAMA	56	0.86	1.00	---
	MFS	Chamberlain	CHAM	46	0.86	0.91	---
	MFS	Lower Big	LOBIG	46	0.89	1.00	---
	MFS	Loon	LOON	84	0.96	1.00	---
	MFS	Marsh	MARS	59	0.99	1.00	---
	MFS	Pistol	PIST	23	0.72	1.00	---
	MFS	Rapid	RAPD	31	0.93	1.00	---
	MFS	Sulphur	SULP	42	0.89	1.00	---
	MFS	Upper Big	UPBIG	45	1.00	1.00	0.98
<u>South Fork Clearwater River</u>	SFC	Tenmile	10MI	46	0.99	1.00	---
	SFC	Clear	CLEAR	45	0.92	0.97	---
	SFC	Crooked	CROOK	104	0.99	0.99	---
	SFC	Johns	JONH	36	0.75	0.87	0.96
<u>South Fork Salmon River</u>	SFS	East Fork South Fork Salmon	EFSFS	45	0.97	1.00	---
	SFS	Lick	LICK	39	0.96	0.99	---
	SFS	Secesh	SECH	45	0.98	0.99	---
	SFS	Stolle Meadows	STOL	45	0.96	0.99	0.99
<u>Upper Clearwater River</u>	UPC	Three Links/ Selway	3LIN	47	0.94	1.00	---
	UPC	Bear/ Selway	BEAR	35	0.67	1.00	---
	UPC	Canyon/ Lochsa	CANY	46	0.87	0.98	---
	UPC	Colt/ Lochsa	COLT	38	0.65	1.00	---

	UPC	Crooked Fork/ Lochsa	CRFK	44	0.77	0.99	---
	UPC	Fish/ Lochsa	FISH	99	0.87	1.00	---
	UPC	Gedney/ Selway	GEDN	45	0.54	0.99	---
	UPC	Lake/ Lochsa	LAKE	47	0.71	1.00	---
	UPC	Little Clearwater/ Selway	LTCL	59	0.93	1.00	---
	UPC	N. F. Moose/ Selway	MFMO	92	0.96	1.00	---
	UPC	OHara/ Selway	OHAR	47	0.73	0.92	---
	UPC	Mainstem Selway	SELW	76	0.97	1.00	---
	UPC	Storm/ Lochsa	STORM	38	0.78	1.00	---
	UPC	Whitecap/ Selway	WTCP	76	0.97	1.00	0.99
<u>Upper Salmon River</u>	UPS	Hayden	HAYD	84	0.80	0.96	---
	UPS	Morgan	MORG	37	0.68	0.97	---
	UPS	North Fork Salmon	NFSA	99	0.69	0.84	---
	UPS	Pahsimeroi Weir	PAHS	96	0.79	0.96	---
	UPS	Sawtooth Weir	SAW	105	0.82	0.97	---
	UPS	Valley	VALL	44	0.49	0.93	---
	UPS	West Fork Yankee Fork	WFYF	117	0.85	0.97	0.94

Table 2. Reporting group (RG) assignment probability summary for kelts (origin unknown) sampled at Lower Granite Dam. The total number sampled was 4,138. Only 2,108 met an individual assignment probability threshold of 80%; (%n) are sample proportions by RG that did not meet the threshold. The average assignment probability for reporting group of origin among baseline populations is shown for comparison (100% sim.).

RG	average probability of assignment				mean (2009-2012)	P<0.8 (%n)	baseline 100% sim.
	2009 (n=265)	2010 (n=1,363)	2011 (n=1,180)	2012 (n=1,330)			
GR	0.68	0.73	0.70	0.69	0.70	0.66	0.90
IM	0.68	0.70	0.70	0.75	0.71	0.60	0.88
LCL	0.59	0.57	0.72	0.65	0.63	0.70	0.93
LS	0.68	0.62	0.56	0.63	0.62	0.77	0.81
LSN	0.59	0.66	0.66	0.66	0.64	0.75	0.89
MFS	0.87	0.90	0.85	0.85	0.86	0.26	0.98
SFC	0.85	0.96	0.89	0.92	0.90	0.18	0.96
SFS	0.92	0.91	0.83	0.81	0.87	0.27	0.99
UPC	0.95	0.93	0.90	0.88	0.92	0.17	0.99
UPS	0.80	0.84	0.79	0.80	0.81	0.37	0.94

Table 3. Individual kelts were assigned to a reporting group of origin based on probability rankings (best estimate = highest probability regardless of a threshold criteria). For all “best” assignments the alternative or next more likely reporting groups of origin are identified by frequency of occurrence (gray fill indicates alternate probability > 20%). For example, among Grande Ronde (GR) best assignments, the next most likely reporting group of origin was Lower Snake River (LSN) in 56% of observations.

Best	2nd Best Estimate (% assignments)									
	GR	IM	LCL	LS	LSN	MFS	SFC	SFS	UPC	UPS
GR		0.09	0.05	0.04	0.56	0.01	0.00	0.00	0.00	0.25
IM	0.29		0.02	0.09	0.21	0.03	0.00	0.03	0.00	0.35
LCL	0.12	0.01		0.04	0.58	0.01	0.05	0.01	0.05	0.11
LS	0.11	0.06	0.02		0.25	0.05	0.00	0.03	0.01	0.48
LSN	0.39	0.05	0.08	0.04		0.02	0.00	0.00	0.00	0.41
MFS	0.15	0.13	0.00	0.15	0.10		0.00	0.09	0.00	0.39
SFC	0.00	0.04	0.25	0.00	0.04	0.00		0.00	0.58	0.08
SFS	0.10	0.05	0.00	0.20	0.30	0.35	0.00		0.00	0.00
UPC	0.00	0.11	0.26	0.00	0.11	0.00	0.47	0.00		0.05
UPS	0.18	0.10	0.03	0.18	0.47	0.04	0.00	0.00	0.00	

Table 4. Stock proportions for estimated escapement vs. Lower Granite Dam kelt samples. Individuals were sampled from across the migration/outmigration in each year (2012 data for escapement estimate not available). Results summarize individual assignments to reporting group for all sampled kelts, and kelt assignments at or above an 80% probability threshold. Stock proportions that exceed 10% absolute difference (abs.) between escapement and kelts are denoted (*), and reporting groups with kelt proportions exceeding 20% appear in bold gray fill.

year	reporting group	<u>LGD kelt (all)</u>		<u>LGD kelt ($P>0.8$)</u>		<u>escapement ($P>0.8$)</u>		abs.
		(n)	proportion	(n)	proportion	(n)	proportion	
<u>2009</u>	GR	38	0.14	10	0.08	60	0.11	0.04
	IM	19	0.07	7	0.05	31	0.06	0.01
	LCL	6	0.02	0	0.00	28	0.05	0.05
	LS	9	0.03	4	0.03	29	0.05	0.02
	LSN	32	0.12	5	0.04	28	0.05	0.02
	MFS	40	0.15	31	0.23	74	0.14	0.09
	SFC	6	0.02	4	0.03	75	0.14	*0.11
	SFS	6	0.02	5	0.04	35	0.07	0.03
	UPC	7	0.03	6	0.05	108	0.20	0.16
	UPS	102	0.38	61	0.46	61	0.12	*0.34
	Total	265		133		529		
<u>2010</u>	GR	186	0.14	75	0.10	129	0.14	0.04
	IM	104	0.08	43	0.06	56	0.06	0.01
	LCL	21	0.02	2	0.00	24	0.03	0.02
	LS	43	0.03	10	0.01	24	0.03	0.01
	LSN	178	0.13	52	0.07	78	0.09	0.02
	MFS	124	0.09	98	0.13	174	0.19	0.06
	SFC	24	0.02	22	0.03	97	0.11	0.08
	SFS	25	0.02	21	0.03	60	0.07	0.04
	UPC	34	0.02	30	0.04	112	0.12	0.08
	UPS	624	0.46	425	0.55	163	0.18	*0.37
	Total	1363		778		917		
<u>2011</u>	GR	199	0.17	72	0.13	144	0.13	0.00
	IM	79	0.07	26	0.05	57	0.05	0.00
	LCL	29	0.02	13	0.02	49	0.04	0.02
	LS	41	0.03	6	0.01	37	0.03	0.02
	LSN	201	0.17	43	0.08	64	0.06	0.02
	MFS	115	0.10	78	0.14	153	0.14	0.00
	SFC	31	0.03	22	0.04	156	0.14	0.10
	SFS	25	0.02	17	0.03	91	0.08	0.05

	UPC	41	0.03	33	0.06	192	0.17	*0.11
	UPS	419	0.36	241	0.44	168	0.15	*0.29
	Total	1180		551		1111		
<u>2012</u>	GR	256	0.19	73	0.11	na	---	---
	IM	60	0.05	30	0.05	na	---	---
	LCL	48	0.04	16	0.02	na	---	---
	LS	49	0.04	12	0.02	na	---	---
	LSN	242	0.18	64	0.10	na	---	---
	MFS	87	0.07	63	0.10	na	---	---
	SFC	71	0.05	60	0.09	na	---	---
	SFS	18	0.01	11	0.02	na	---	---
	UPC	31	0.02	25	0.04	na	---	---
	UPS	468	0.35	292	0.45	na	---	---
	Total	1330		646		na		

Table 5. Concordance between PBT assignment and GSI assignment for 331 kelts identified as putative natural-origin based on the absence of marks (i.e. adipose clip) or tags. For fish with an identified (“known”) hatchery-of-origin, the reporting group assignment that occurred with greatest frequency is shaded and bordered. Hatchery locations are: 1) Clearwater River, 2) Tucannon River – lower Snake River, 3) Snake River – Hells Canyon, 4) upper Salmon River, 5) upper Salmon River, 6) acclimation/release site, 7) acclimation/release site, 8) Tucannon River – lower Snake River, 9) Grande Ronde River.

PBT assignment	(n)	GSI reporting group									
		GR	IM	LCL	LS	LSN	MFS	SFC	SFS	UPC	UPS
1) Dworshak	34	0.00	0.00	0.00	0.00	0.03	0.00	0.88	0.00	0.06	0.03
2) Lyons Ferry, G.R. Cottonwood	4	0.25	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.25
3) Oxbow	6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
4) Pahsimeroi	81	0.01	0.01	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.93
5) Sawtooth	71	0.00	0.01	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.93
6) Sawtooth, East Fork Salmon River	61	0.05	0.00	0.02	0.02	0.10	0.00	0.02	0.00	0.00	0.80
7) Sawtooth, Yankee	69	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.94
8) Tucannon	4	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
9) Wallowa	1	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
total	331										

Figure 1. Map of GSI region and reporting groups established on the basis of 65 baseline *O. mykiss* populations (see Table 1).

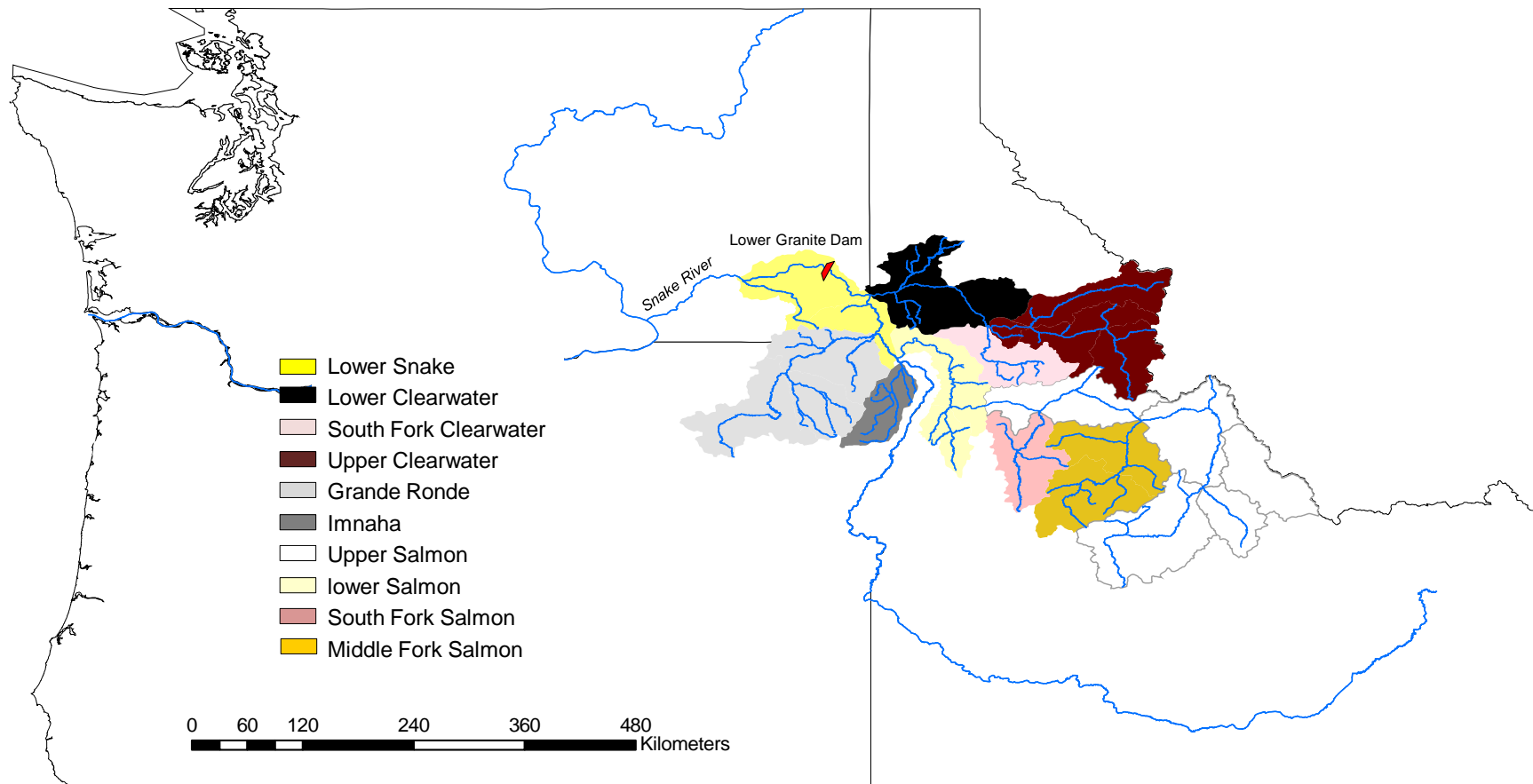


Figure 2. Stock proportions by reporting group of origin (GSI assignment) for all kelts, and for estimated escapement of steelhead sampled at LGD for spawn year 2009-2011; spawn year data provided by Mike Ackerman, IDFG.

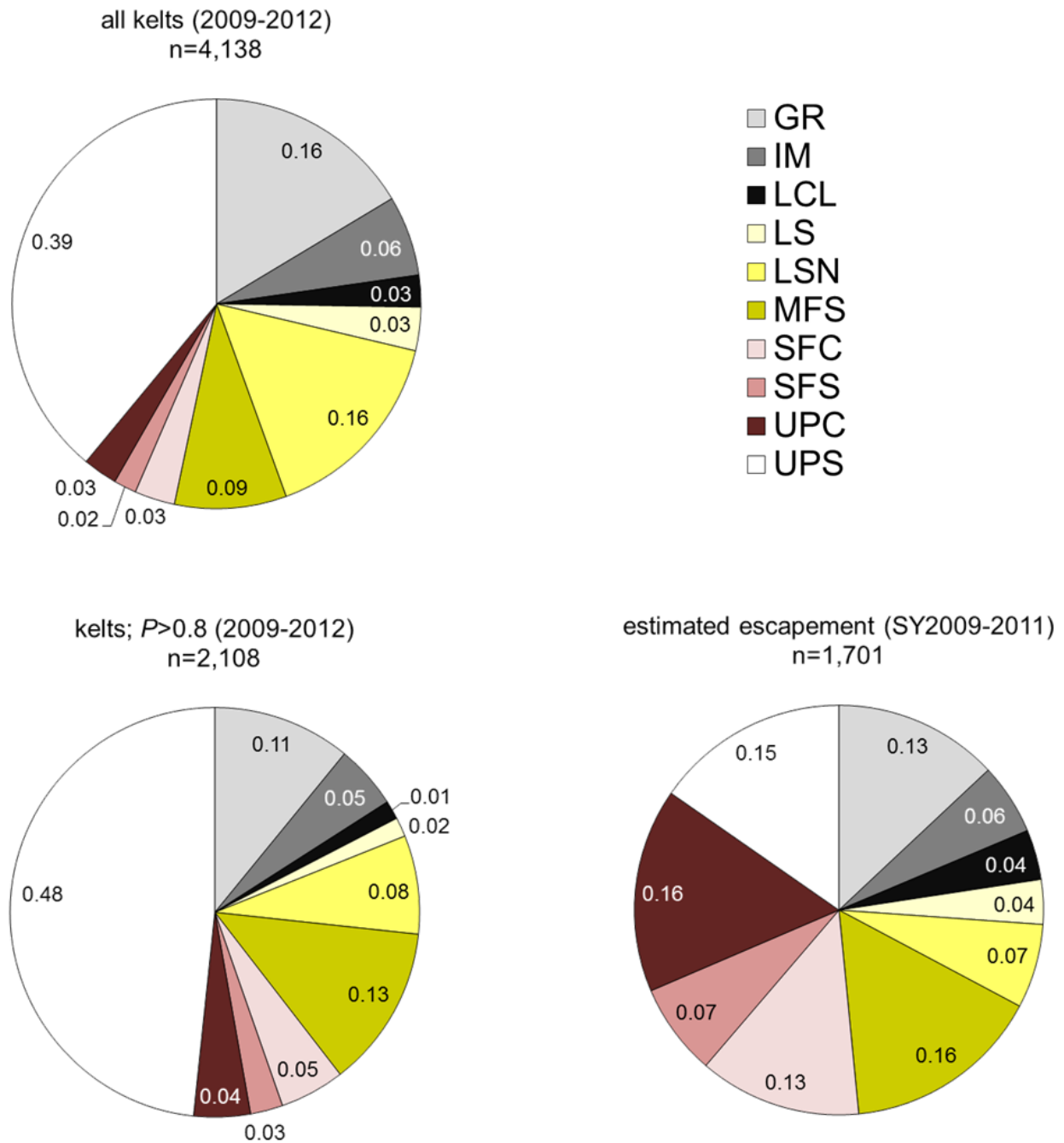


Figure 3. Proportion of male and female kelts by reporting group. The histogram depicts mean proportion across all years (2009-2012). The dashed blue lines identify the mean female proportion across reporting groups in each sample year; overall is the global mean female proportion across years and reporting groups.

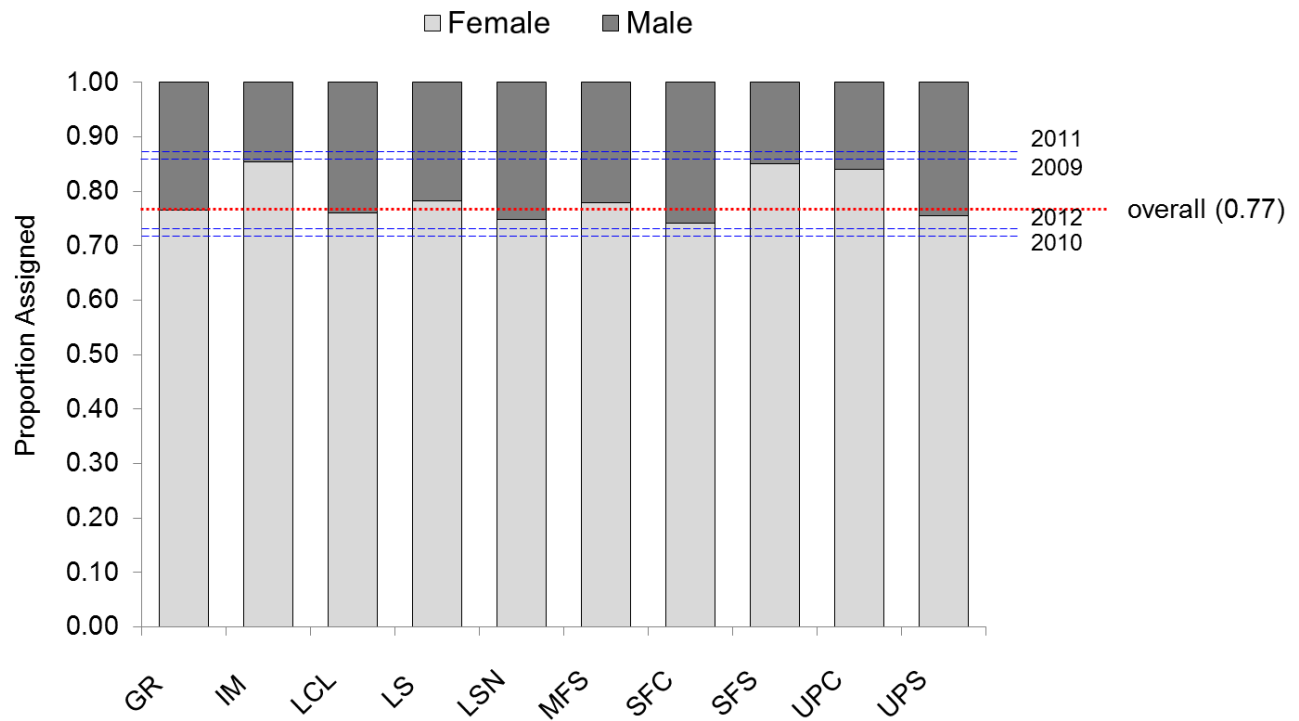
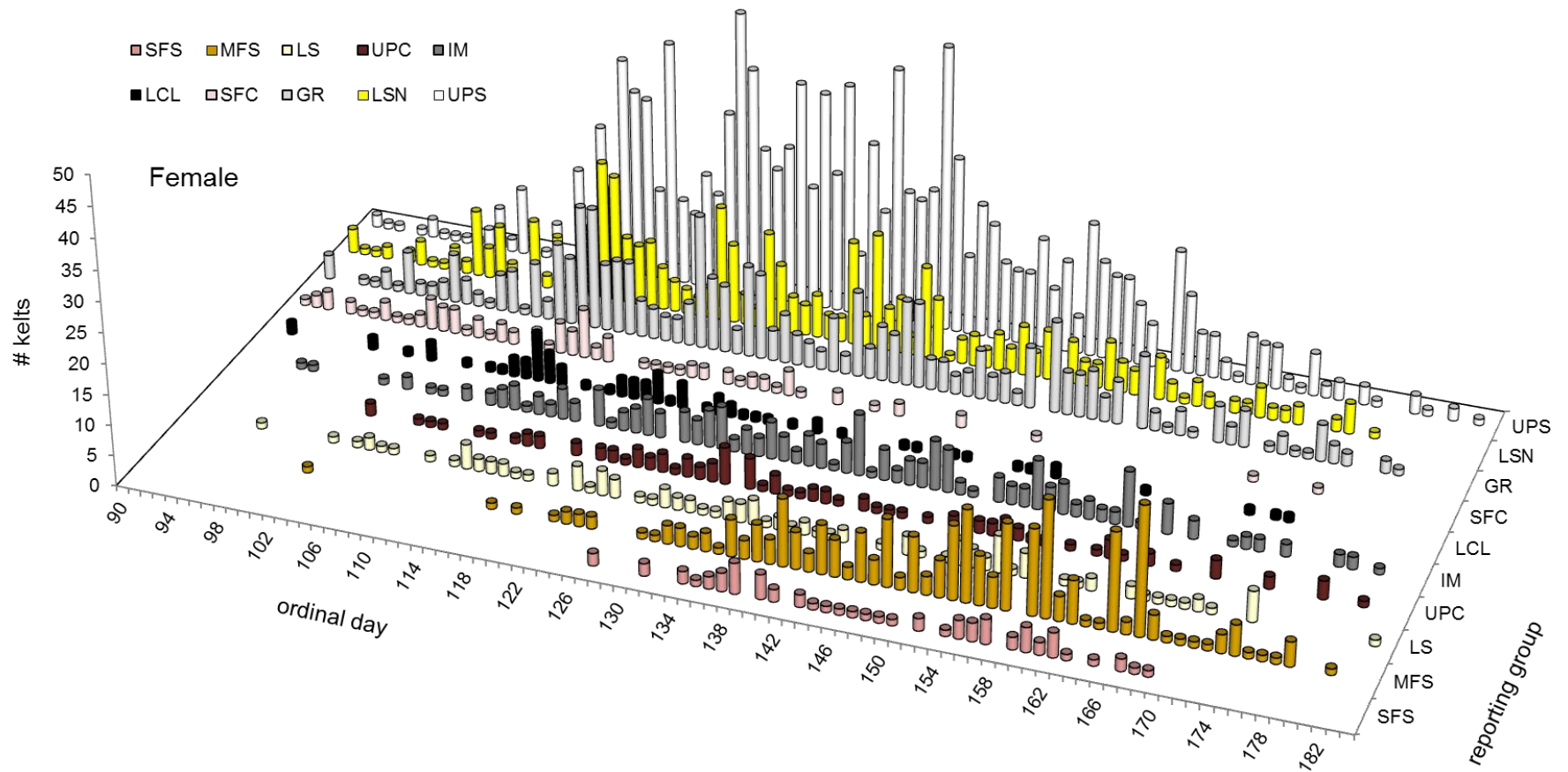


Figure 4. Sample-date distribution for kelts captured at LGD during downstream out-migration (2009-2012). Kelts are differentiated by reporting group assignment, and day is the ordinal day (January 1st = day 1).

a)



b)

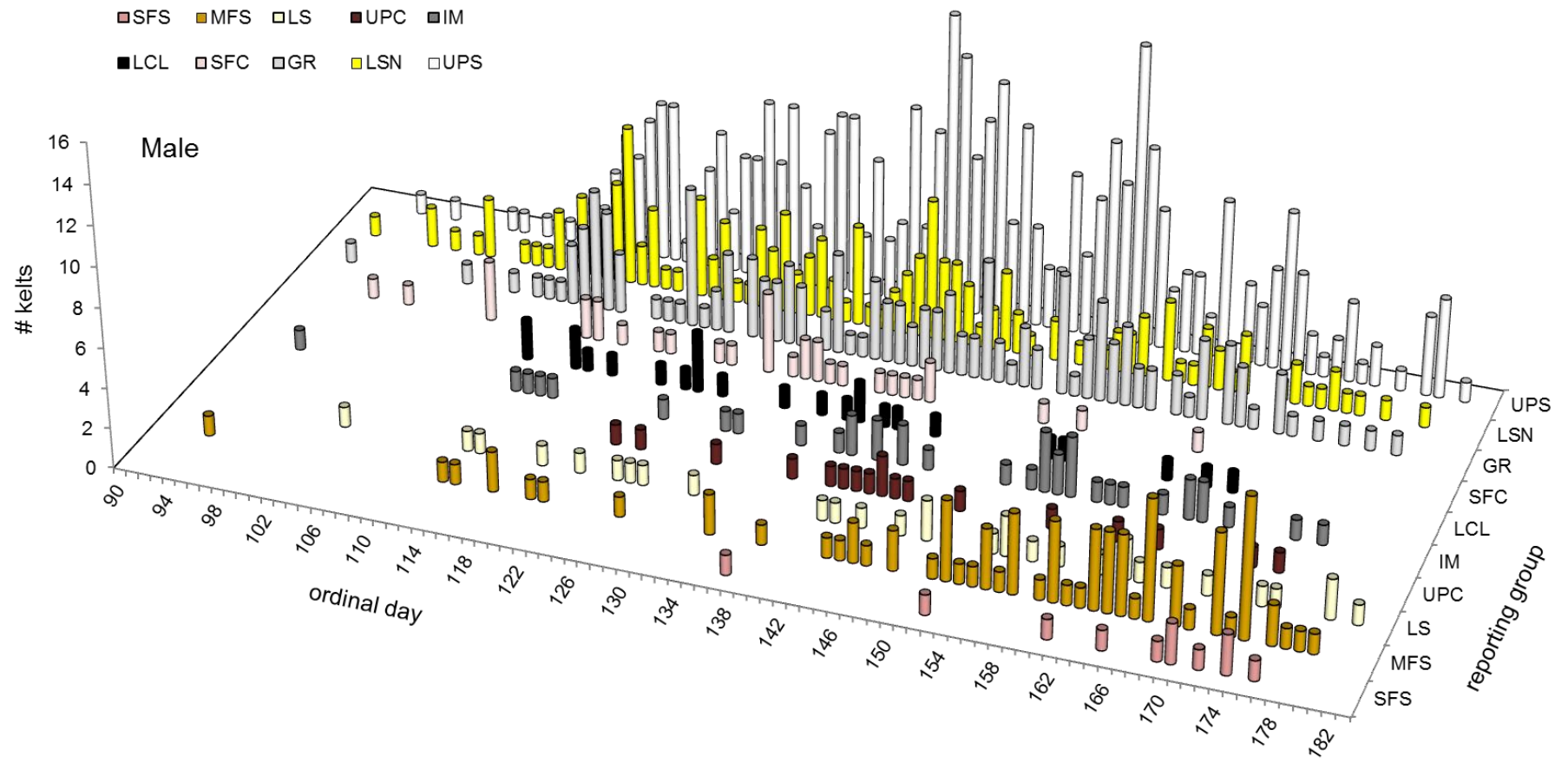


Figure 5. Mean length of kelts assigned to each reporting group. The vertical dashed lines with caps represent the range of observed lengths. The horizontal red lines are mean length across RG's (Appendix 1).

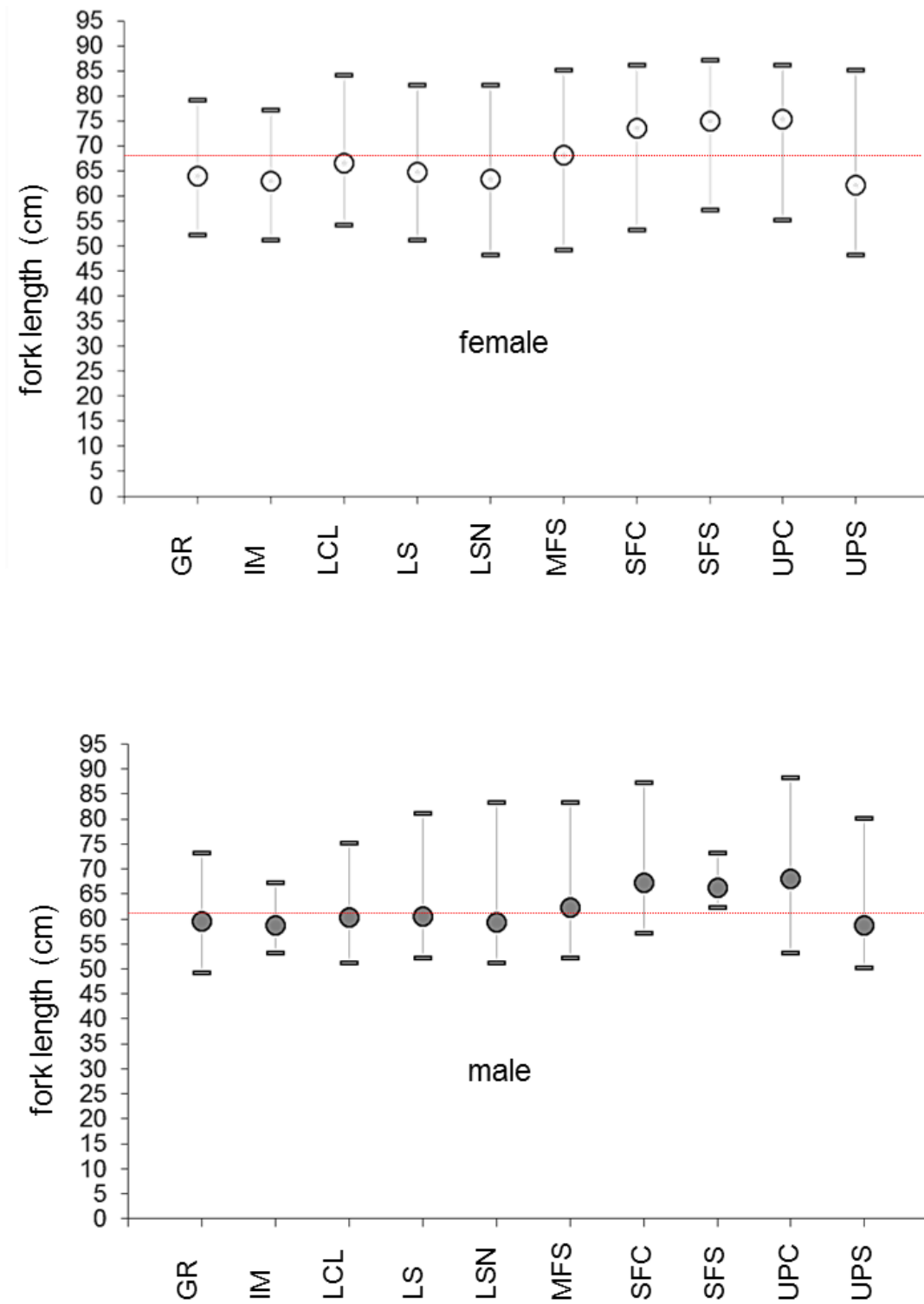


Figure 6. Overall condition of kelts sampled at LGD (2009-2012). Results are the proportion of fish in good, fair, and poor condition, partitioned by gender and reporting group assignment. Condition is contingent on presence and degree of injuries, fungus and other physiological factors. Proportions based on sample sizes less than n=10 are shown in the histograms.

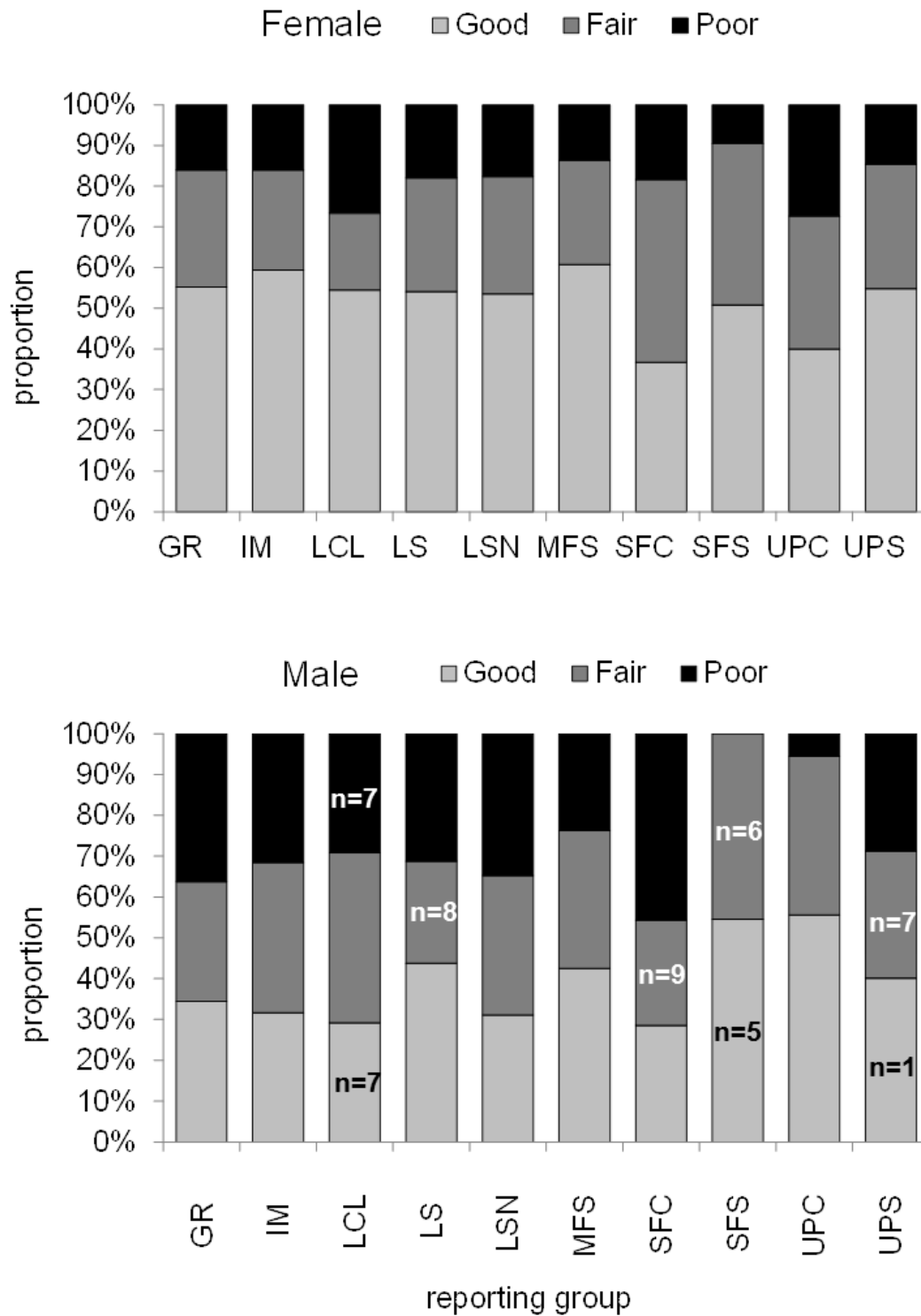


Figure 7. Correlation between life history variables. Condition of kelts ("good") is shown in association with (a) length or size of kelts, and (b) in association with sample date which is treated as a proxy for outmigration time. All sampled kelts (n=2,900) are included in the analysis, but because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

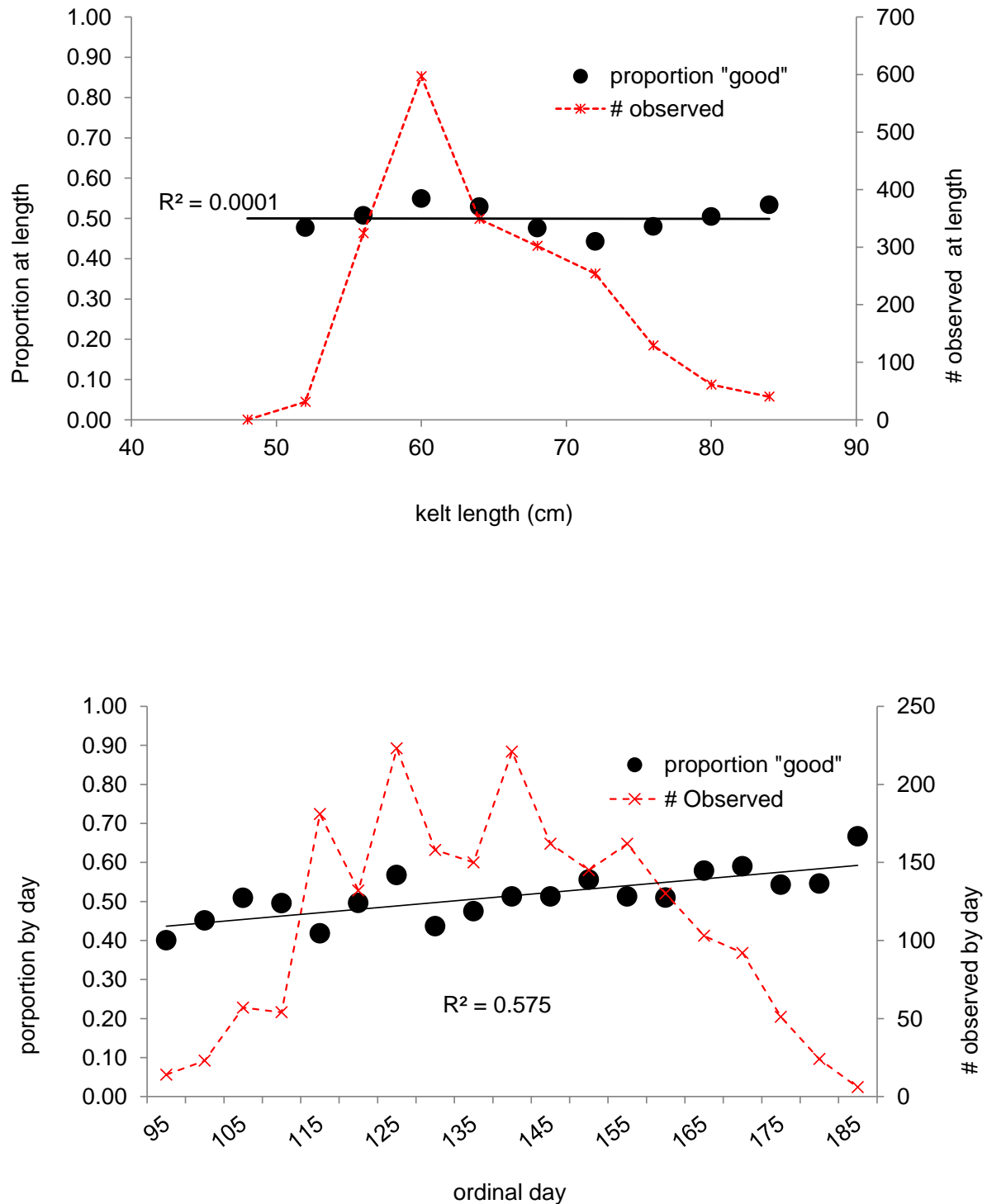
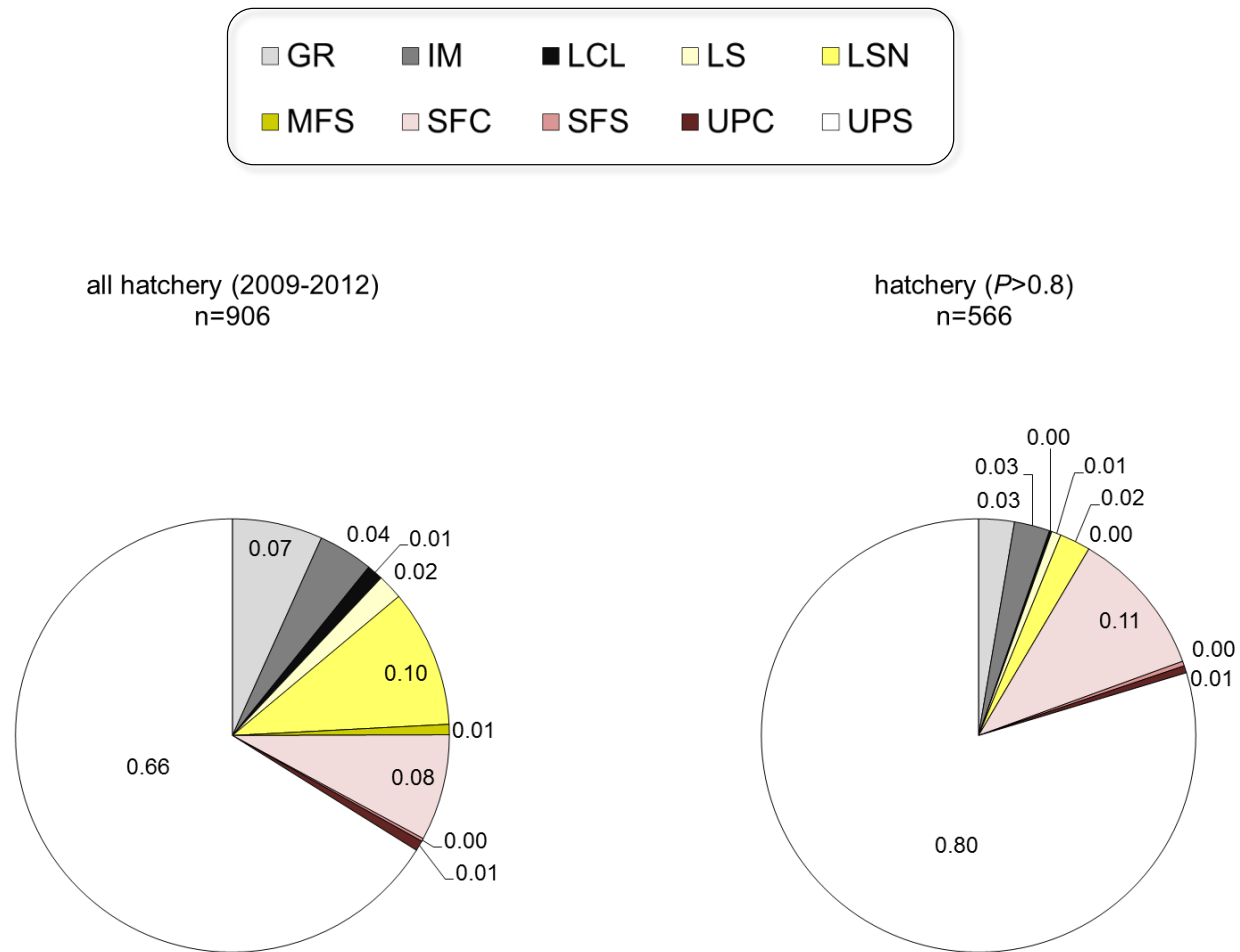


Figure 8. Hatchery GSI



Appendix 1. Summary demographic statistics by reporting group and gender, for kelts sampled at Lower Granite Dam (2009-2012). "M-F" for outmigration date (i.e. ordinal day) is the difference in mean day between genders. Under condition, G=good, F=fair, and P=poor.

Year	reporting group	Gender					ordinal day (od)			condition (F)			condition (M)			mean FL (cm)	
		F	M	total	% F	% M	F	M	M-F	G	F	P	G	F	P	F	M
200	GR	31	7	38	0.	0.	152	163	11	0.	0.	0.	0.	0.	0.	61.	59.
	IM	19	0	19	1.	0.	153	--	--	0.	0.	0.	--	--	--	63.	---
	LCL	5	1	6	0.	0.	133	166	33	1.	0.	0.	0.	0.	0.	64.	63.
	LS	8	1	9	0.	0.	159	175	16	0.	0.	0.	1.	0.	0.	69.	59.
	LSN	26	6	32	0.	0.	153	159	6	0.	0.	0.	0.	0.	0.	61.	58.
	MFS	39	1	40	0.	0.	159	177	18	0.	0.	0.	0.	0.	0.	65.	65.
	SFC	5	1	6	0.	0.	116	155	39	0.	0.	0.	1.	0.	0.	80.	61.
	SFS	6	0	6	1.	0.	160	--	--	0.	0.	0.	--	--	--	77.	---
	UPC	7	0	7	1.	0.	155	--	--	0.	0.	0.	--	--	--	73.	---
	UPS	81	2	10	0.	0.	150	157	7	0.	0.	0.	0.	0.	0.	60.	59.
	total/me	22	3	26	0.	0.	149	164	15	0.	0.	0.	0.	0.	0.	67.	60.
201	GR	13	5	18	0.	0.	130	147	17	0.	0.	0.	0.	0.	0.	62.	60.
	IM	78	2	10	0.	0.	140	149	9	0.	0.	0.	0.	0.	0.	60.	59.
	LCL	13	8	21	0.	0.	125	142	17	0.	0.	0.	0.	0.	0.	64.	59.
	LS	27	1	43	0.	0.	141	156	15	0.	0.	0.	0.	0.	0.	62.	62.
	LSN	13	4	17	0.	0.	130	141	11	0.	0.	0.	0.	0.	0.	61.	59.
	MFS	74	5	12	0.	0.	153	162	9	0.	0.	0.	0.	0.	0.	65.	61.
	SFC	15	9	24	0.	0.	123	132	9	0.	0.	0.	0.	0.	0.	69.	68.
	SFS	15	1	25	0.	0.	152	168	16	0.	0.	0.	0.	0.	0.	71.	66.
	UPC	24	1	34	0.	0.	140	150	10	0.	0.	0.	0.	0.	0.	74.	65.
	UPS	45	1	62	0.	0.	132	144	12	0.	0.	0.	0.	0.	0.	59.	58.
	total/me	96	3	13	0.	0.	137	149	12	0.	0.	0.	0.	0.	0.	65.	62.
201	GR	17	2	19	0.	0.	136	141	5	0.	0.	0.	0.	0.	0.	65.	59.
	IM	74	5	79	0.	0.	140	155	15	0.	0.	0.	0.	0.	0.	64.	56.
	LCL	27	2	29	0.	0.	125	142	17	0.	0.	0.	0.	0.	0.	67.	60.
	LS	36	5	41	0.	0.	141	146	5	0.	0.	0.	0.	0.	0.	64.	58.
	LSN	16	3	20	0.	0.	131	140	9	0.	0.	0.	0.	0.	0.	64.	59.
	MFS	10	1	11	0.	0.	155	149	-6	0.	0.	0.	0.	0.	0.	71.	61.
	SFC	28	3	31	0.	0.	122	115	-7	0.	0.	0.	0.	0.	1.	73.	66.
	SFS	25	0	25	1.	0.	148	--	--	0.	0.	0.	--	--	--	77.	---
	UPC	40	1	41	0.	0.	139	165	26	0.	0.	0.	0.	0.	0.	76.	88.
	UPS	35	6	41	0.	0.	134	145	11	0.	0.	0.	0.	0.	0.	64.	58.
	total/me	10	1	11	0.	0.	137	144	7.	0.	0.	0.	0.	0.	0.	69.	63.
201	GR	18	6	25	0.	0.	124	129	5	0.	0.	0.	0.	0.	0.	64.	58.
	IM	53	7	60	0.	0.	134	136	2	0.	0.	0.	0.	0.	0.	64.	59.
	LCL	34	1	48	0.	0.	120	127	7	0.	0.	0.	0.	0.	0.	66.	60.
	LS	40	9	49	0.	0.	132	134	2	0.	0.	0.	0.	0.	0.	65.	58.

	LSN	16	7	24	0.	0.	122	126	4	0.	0.	0.	0.	0.	0.	63.	59.
	MFS	68	1	87	0.	0.	147	148	1	0.	0.	0.	0.	0.	0.	68.	63.
	SFC	50	2	71	0.	0.	109	128	19	0.	0.	0.	0.	0.	0.	74.	67.
	SFS	17	1	18	0.	0.	145	137	-8	0.	0.	0.	0.	0.	0.	73.	64.
	UPC	24	7	31	0.	0.	135	139	4	0.	0.	0.	0.	0.	0.	74.	69.
	UPS	32	1	46	0.	0.	128	135	7	0.	0.	0.	0.	0.	0.	63.	58.
	total/me	95	3	13	0.	0.	130	133	4.	0.	0.	0.	0.	0.	0.	67.	62.
ove	GR	51	1	67	0.	0.	131	139	8	0.	0.	0.	0.	0.	0.	64.	59.
	IM	22	3	26	0.	0.	140	147	7	0.	0.	0.	0.	0.	0.	63.	58.
	LCL	79	2	10	0.	0.	123	135	12	0.	0.	0.	0.	0.	0.	66.	60.
	LS	11	3	14	0.	0.	139	149	10	0.	0.	0.	0.	0.	0.	64.	60.
	LSN	48	1	65	0.	0.	129	134	5	0.	0.	0.	0.	0.	0.	63.	59.
	MFS	28	8	36	0.	0.	153	157	4	0.	0.	0.	0.	0.	0.	68.	62.
	SFC	98	3	13	0.	0.	115	129	14	0.	0.	0.	0.	0.	0.	73.	67.
	SFS	63	1	74	0.	0.	149	165	16	0.	0.	0.	0.	0.	0.	74.	66.
	UPC	95	1	11	0.	0.	139	147	8	0.	0.	0.	0.	0.	0.	75.	68.
	UPS	12	3	16	0.	0.	133	141	8	0.	0.	0.	0.	0.	0.	62.	58.
	total/me	31	9	41	0.	0.	134	141	7.	0.	0.	0.	0.	0.	0.	67.	62.

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

Contract No C11-32

Purchase Order No C11003240

Annual Report for 2011 – 2012.

Prepared by

Christine Moffitt, Principal Investigator

Graduate Students: Bryan Jones, Zachary Penney

Staff: Boling Sun

EPSCoR student intern: Heath Hewett

Submitted to
Columbia River Inter-Tribal Fish Commission
Doug Hatch, Contract Officer

Idaho Cooperative Fish and Wildlife Research Unit
University of Idaho, Moscow, ID 83844-1141

15 October 2012

Table of Contents

Executive Summary	236
Progress by Objective	237
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.	237
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.	251
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.	255
Objective 4. Evaluate the emigration of natural origin steelhead kelts PIT tagged and released below Lower Granite Dam to migrate through the Snake and Columbia River hydrosystem.	256
References:	259
Problems Affecting Progress	259

Executive Summary

This past project year we completed all field studies and worked to complete laboratory evaluations, data analysis, and reporting requirements. We prepared presentations and posters of aspects of our studies at the American Fisheries Society annual meeting in Seattle, Washington in September 2011, and other regional venues. Students Bryan Jones and Zachary Penney complete their formal academic training, and worked to complete preparation of Master's thesis (Jones), and dissertation (Penney). Bryan Jones began employment with Pacific Northwest National Laboratory in the spring of 2012 to participate in a large scale JSATs tagging project of steelhead kelts throughout the Snake River tributaries under contract with the US Army Corps of Engineers. In the late summer, Jones returned to working on completion of his master's thesis. Student Zachary Penney continued to analyze data, and nearly completed examination of the histology of tissues collected from lethally sampled kelts during 2009 - 2011. These data are being integrated with the biochemical metrics analyzed in plasma, and proximate analysis of constituents. We anticipate completion of several manuscripts for peer review during the fall quarter of 2012.

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 data synthesis from three years of sampling steelhead trout at various phases of the reproductive cycle is nearly completed. Analysis of tissue histology is not finalized, but assessments of some tissues are completed. We are combining these profiles with the results of proximate analyses, and metrics in the blood plasma samples to provide a multi attribute profile of the changes in steelhead from the time of upstream migration through sexual maturity and downstream kelt migration. By using various assessments from lethal and non-lethal sampling, we have a substantial model of the physiological process and changes that occur in pre and post spawning Snake River steelhead stocks. We used our intensive sampling of a known stock at Dworshak National Fish Hatchery and comparisons of these fish with mixed stocks from the Snake River system to provide insight into the factors that affect survival, and the capacity for recovery following spawning.

Steelhead trout enter a period of voluntary anorexia following re-entry into freshwater to spawn. During this period of fasting, steelhead rely on lipids and protein stored in somatic and visceral tissues for energy to support basal metabolic function, upstream migration, the physical exertion of spawning, and gonadal maturation (Brannon et al. 2004). By discontinuing active consumption during spawning migrations, steelhead can curtail the energy required for digestion, which has been estimated to account for as much as 40% of basal metabolism in feeding fish (Wang et al. 2006). At the completion of spawning, it is assumed that steelhead stop fasting and can begin consuming food to replace energy. Our research provides profiles of the energy depletion, and the effects that prolonged fasting and catabolism has on the plasma physiology, muscle energy and proximate contents, and microstructure of selected organ systems in steelhead.

Gastrointestinal tract histology - For post-spawning steelhead to recover and replenish energy reserves their gastrointestinal tract must first be restored. Work in other non-mammalian vertebrates has shown that there is a gradual reduction of intestinal epithelium and nutrient transport capacities of the gastrointestinal tract during fasting, but that digestive processes are rapidly restored at the onset of feeding (Wang et al 2006). It is currently unknown how rapidly steelhead feed, digest, and assimilate food following reproduction. We examined the microstructure of cross-sections of the pyloric stomach in pre-spawning and kelt steelhead to assess differences between the two phases. In addition, we further separated our results to evaluate kelts at Lower Granite Dam observed with visible food or food residues in their GI tracts to those without food at Lower Granite Dam to determine if variations occurred with renewed feeding activity.

In spawning years 2009 and 2010, sections of the pyloric stomach were collected from steelhead at time of spawning Dworshak National Fish Hatchery, from pre spawning fish in the Clearwater River, and from

migrating kelts at Lower Granite Dam juvenile fish bypass facility (Table 1). Tissues were fixed in 10% neutral buffered formalin (10:1 formalin to tissue) for at least 10d at room temperature. Following fixation, tissue sections were trimmed and the tissues stored for processing with paraffin histology. Sections were mounted on glass slides and stained with hematoxylin and eosin (Luna 1968). We examined tissues with a compound light microscope (Leitz Laborlux) fitted with a Leica EC3 camera. Photographic software (LAS EZ 1.8.0) was used to capture images of tissues and measure various aspects of the cellular microstructure.

Table 1: Sample sizes by spawning year, site, sex, and length for pyloric stomach histological analysis.

Spawning year	Site	Phase	Sex	Histological sections (N)			
				Pyloric stomach		Anterior intestine	
				<70 cm	>70 cm	<70 cm	>70 cm
2009	Dworshak National Fish Hatchery	Spawning	Male	0	0	0	0
			Female	0	16	0	16
	Lower Granite Dam juvenile bypass facility	Kelt	Male	3	0	3	0
			Female	18	4	18	4
2010	Dworshak National Fish Hatchery	Spawning	Male	1	10	1	9
			Female	0	15	0	15
	Lower Granite Dam juvenile bypass facility	Kelt	Male	6	4	6	4
			Female	15	14	14	14
			Total	43	63	42	62

Cross sections of the pyloric stomach were scored blind for the following metrics: 1) integrity of the submucosa; 2) density of villi; 3) invagination of villi to the stratum compactum; 4) integrity of columnar epithelial cells; 5) length to width ratio of columnar epithelial cells; and 6) presence or absence of goblet (mucous) cells (Table 2). Submucosa integrity and villi density were evaluated at 4X; invagination of villi at 10X; and integrity of columnar epithelial cells, length to width ratio of columnar epithelial cells, and presence of goblet cells were evaluated at 40X (Figures 1 -4). A sum of all scores was calculated for each individual tissue. Chi-square analysis was used to compare the scores of each metric between pre-spawning and kelt steelhead and feeding and non-feeding kelts.

Table 2: Summary of criteria, scoring, and tissues evaluated in sections of the pyloric stomach of steelhead.

Tissues (magnification)	Scoring Criteria
Submucosa integrity (4X)	<p>1 = Poor integrity, severe detachment from muscularis</p> <p>2 = Fair integrity, some detachment from muscularis</p> <p>3 = Good integrity, no detachment from muscularis</p>
Density of villi (4X)	<p>1 = Low density, zero to few villi present</p> <p>2 = Moderate density, villi present</p> <p>3 = High density, high quantity of villi present</p>
Invagination of villi (10X)	<p>1 = $<1/4$ distance to stratum compactum</p> <p>2 = $1/4 \leq 1/2$ distance to stratum compactum</p> <p>3 = $1/2$ distance to stratum compactum</p>
Integrity of columnar epithelial cells (40X)	<p>1 = Poor integrity, cell membrane deteriorated and nucleation absent or poor</p> <p>2 = Fair integrity, mild to no membrane deterioration and nucleation present</p> <p>3 = Good integrity, no cell membrane deterioration and nucleus present</p>
Length:width ratio of columnar epithelial cells (40X)	<p>1 = $\leq 1/2$ length to width ratio</p> <p>2 = $>1/2$ length to width ratio</p>

Presence of goblet cells (40X)

1 = Absent

2 = Present

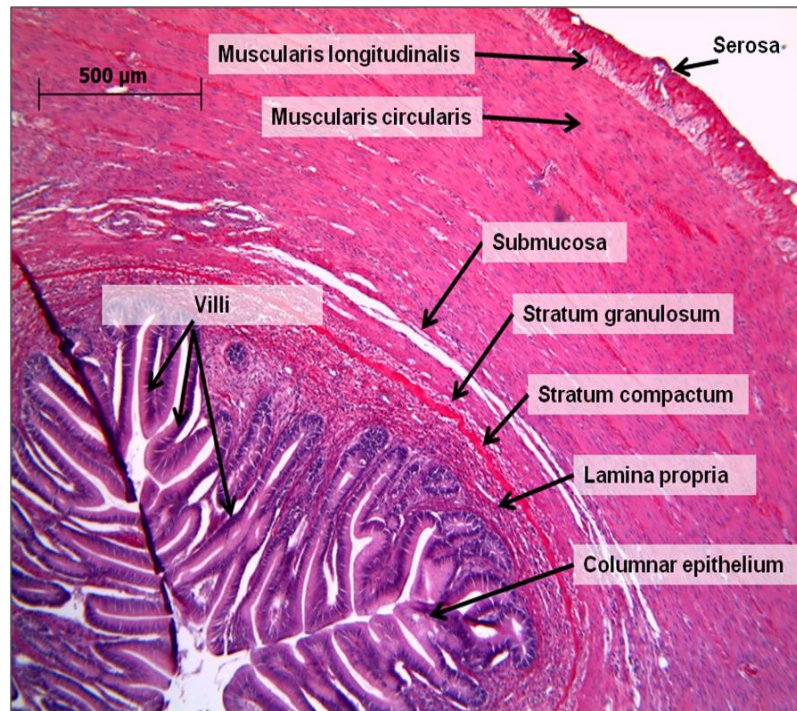


Figure 1: Cellular and muscular layers of the pyloric stomach at a magnification of 4X.

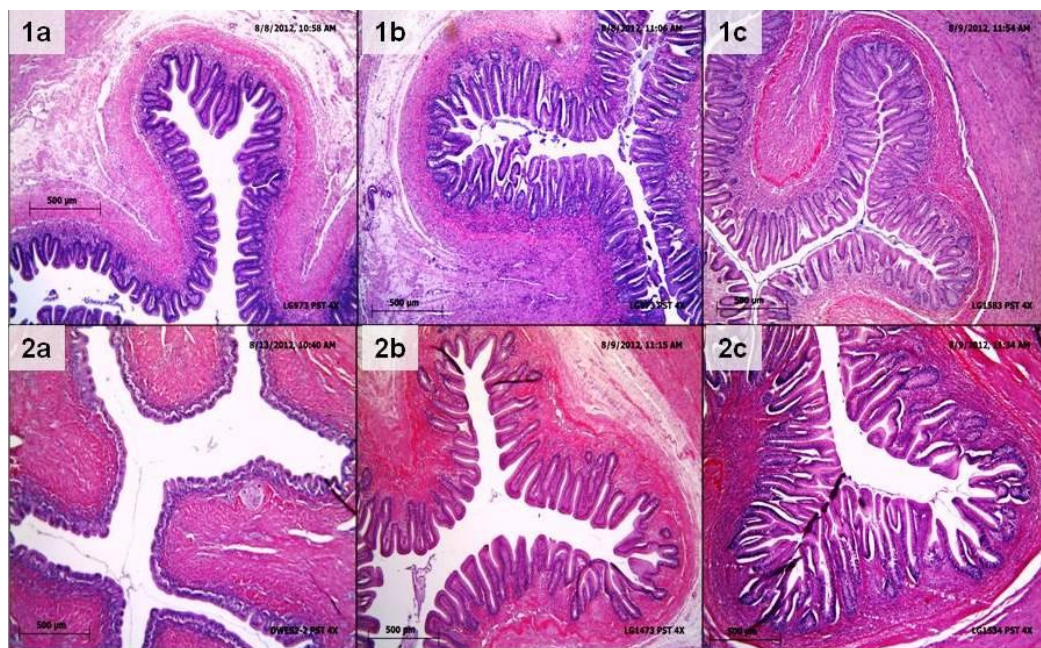


Figure 2: Examples of the scoring levels for submucosa integrity and villi density at a magnification of 4X: 1a) Poor submucosa integrity, 1b) fair submucosa integrity, 1c) good submucosa integrity, 2a) low villi density, 2b) moderate villi density, and 2c) high villi density.

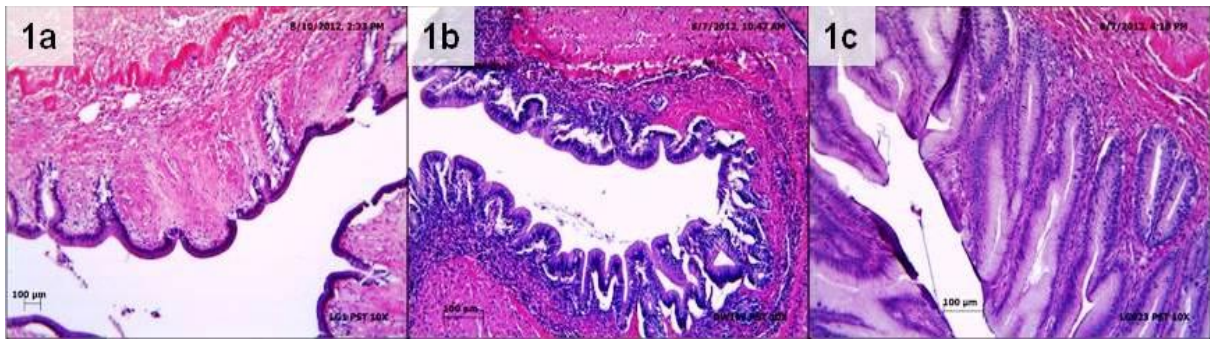


Figure 3: Examples of the scoring levels for villi invagination to the stratum compactum at a magnification of 10X. 1a) $< 1/4$ distance to stratum compactum, 1b) $1/4 \leq 1/2$ distance to stratum compactum, 1c) $> 1/2$ distance to stratum compactum.

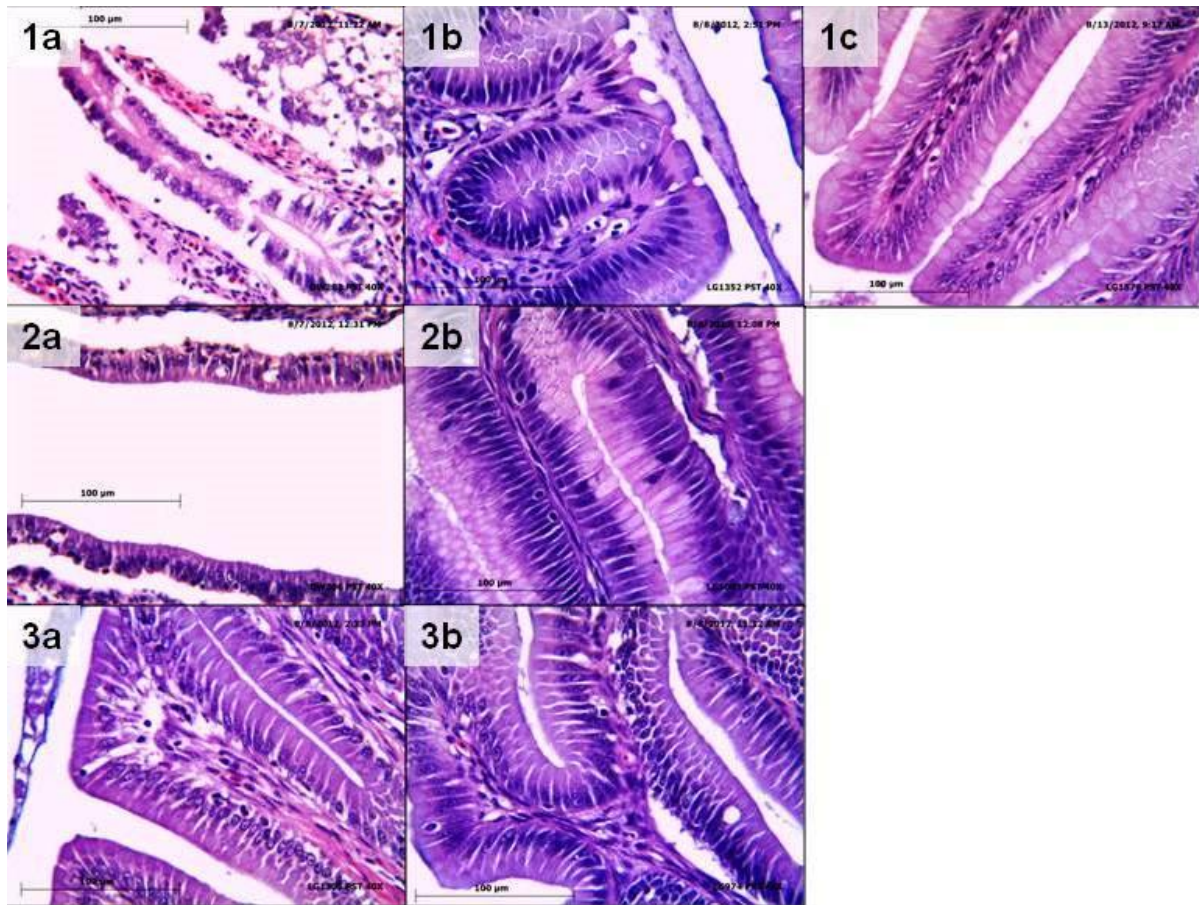


Figure 4: Examples of columnar epithelial cell integrity, length to width ratio of columnar epithelial cells, and presence goblet cells in the pyloric stomach at a magnification of 40X. 1a) Poor columnar epithelial cell integrity 1b) fair columnar epithelial cell integrity, 1c) good epithelial cell integrity, 2a) $\leq 1/2$ length to width ratio of columnar epithelial cells, 2b) $> 1/2$ length to width ratio of columnar epithelial cells, 3a) goblet cells absent, and 4) goblet cell present.

We found that villi density, villi invagination, and the total sum of scores were significantly different between the two phases (Table 3). The majority of both pre-spawning and kelt steelhead had submucosa scored as good, but some kelts were scored with poor integrity of the submucosa. Whereas all pre-spawning steelhead sampled at Dworshak National Fish Hatchery were in good external and internal condition, some kelts were in poor external and internal condition. Future work will be directed at examining the correlation between submucosa integrity and external condition.

Table 3: Chi-square results for comparisons of pyloric stomach scores between pre-spawning and kelt steelhead.

Scoring metric	Chi-square likelihood ratio (<i>P</i> -value)
Submucosa integrity	0.009
Density of villi	<.0001
Invagination of villi	<.0001
Columnar epithelial cell integrity	0.582
L:W ratio of columnar epithelial cells	0.324
Presence of goblet cells	0.562
Sum of scores	<.0001

Villi are composed of columnar epithelial cells and goblet cells, which line the stomach lumen and are important to digestion and absorption of ingested food. We found that 60% of pre-spawning steelhead had low density of villi and 60% of kelts had high density of villi (Figure 5). Over 75% of pre-spawning steelhead had villi invagination <1/4 the distance to the stratum compactum, but the majority of kelts had a longer relative ratio of the distance to the stratum compactum. Higher densities and greater invagination of the villi would provide a larger surface area for digestion and absorption. Therefore the variations in villi density and invagination between pre-spawning and kelt steelhead indicate that kelts are likely able to digest and absorb energy from food while still in freshwater. We found no significant differences between pre-spawning steelhead and kelts in the columnar epithelial cell integrity, length to width ratio of columnar epithelial cells, and presence of goblet cells, likely evidence that the pyloric stomach does not undergo complete cellular deterioration during fasting, but rather enters a period of stasis until spawning is completed. It is still unknown if ingesting food is required to begin renewal of the villi in the gastrointestinal tract.

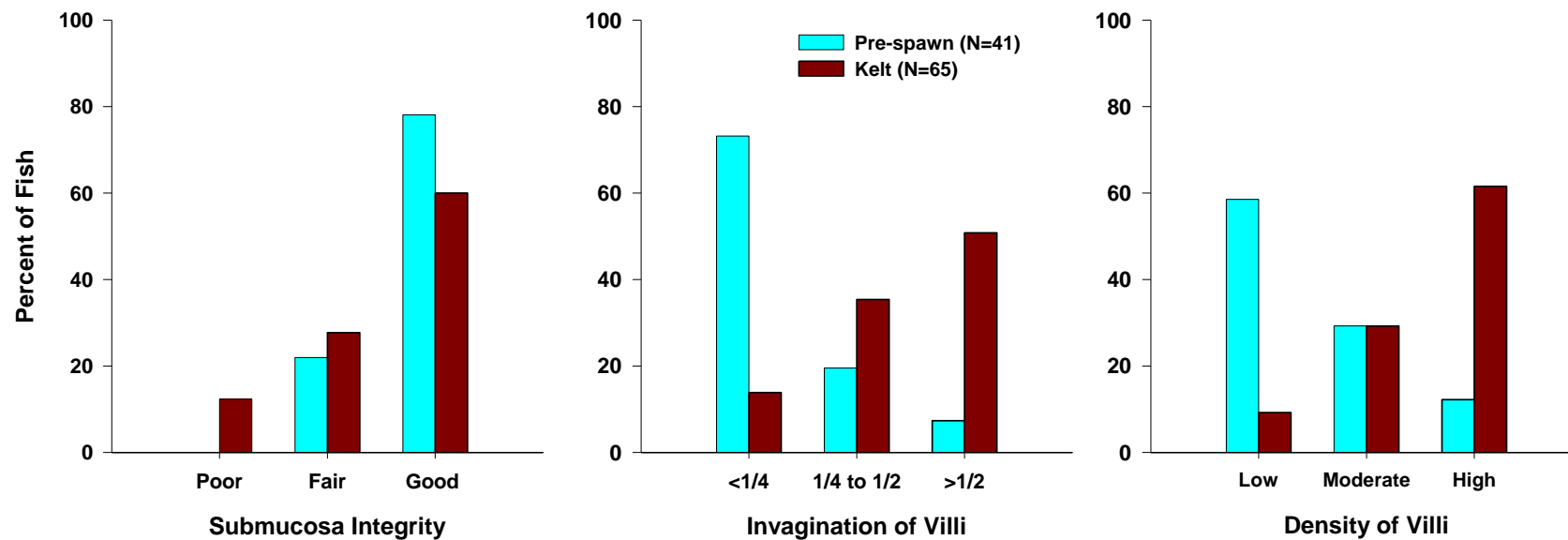


Figure 5. Summary of significant comparisons of three metrics of the pyloric stomach examined comparing pre-spawning and kelt steelhead. Samples were collected in 2009 and in 2010.

In spawning years 2009 and 2010, the proportion of kelts found with identifiable food in their gastrointestinal tracts was 32% and 47%, respectively. We found that the scores of the submucosa integrity and villi invagination were significantly different between feeding and non feeding kelts at Lower Granite dam (Table 4). The majority of feeding and non-feeding kelts had good quality submucosa, and only non-feeding kelts had poor submucosa integrity. Similarly, the majority of feeding and non-feeding kelts exhibited villi invagination $>1/4$ the distance to the stratum compactum, but only non-feeding kelts were found have villi invagination $<1/4$ the distance to the stratum compactum. Some kelts sampled at Lower Granite Dam were in poor external and internal condition, thus it is possible that kelts in poor condition were not actively feeding during emigration. Further examinations of the relationship between external fish condition and submucosa integrity and villi invagination is warranted. Regardless, it does appear that feeding kelts exhibit greater structural integrity of the submucosa and deeper invagination of villi than non-feeding kelts (Figure 6).

Table 4: Summary of Chi-square comparisons of pyloric stomach scores between known feeding and non-feeding kelt steelhead for both years combined (38 and 97 fish for 2010 and 2011, respectively).

Scoring metric	Chi-square likelihood ratio (<i>P</i> -value)
Submucosa integrity	0.013
Density of villi	0.471
Invagination of villi	0.008
Columnar epithelial cell integrity	0.496
L:W ratio of columnar epithelial cells	0.159
Presence of goblet cells	0.356
Sum of scores	0.153

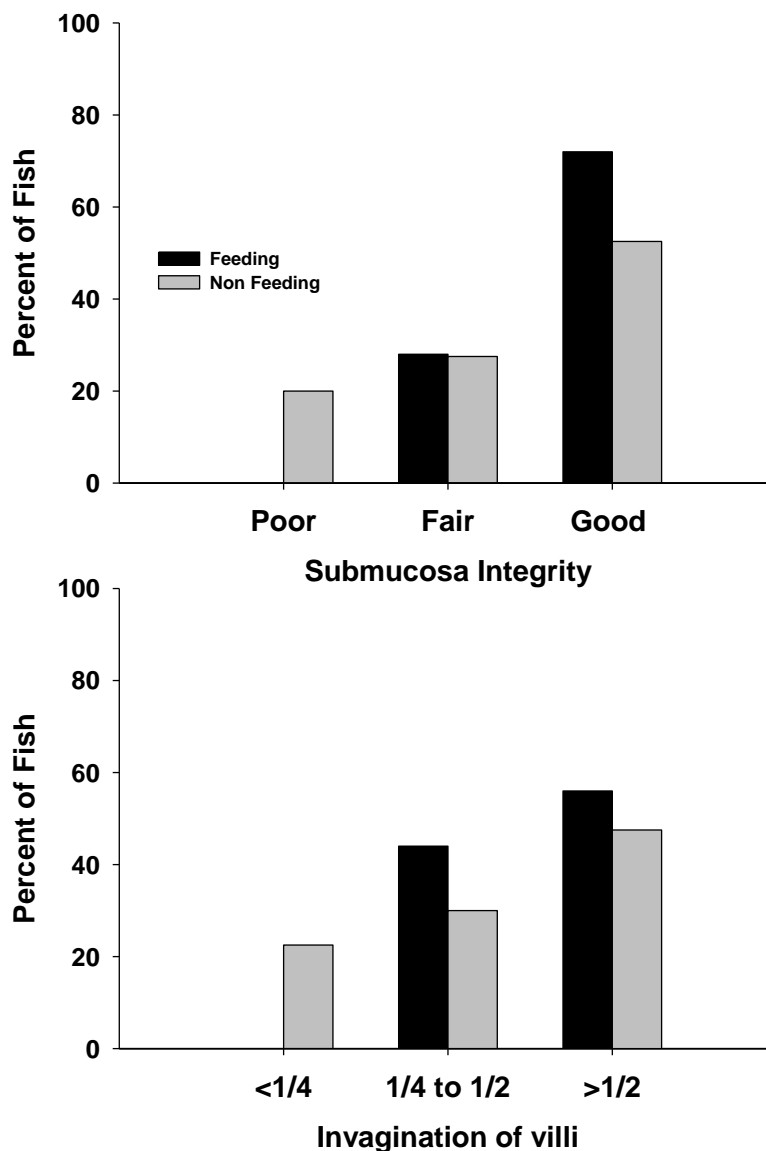


Figure 6. Percent of feeding and non-feeding steelhead trout scored for submucosa integrity and extent of villi invagination.

Comparisons with proximate tissue composition

We are synthesizing observations from three years of sampling steelhead trout at various times before, during and post spawning into models of physiological capacity. We have analyzed total energy and proximate constituents of tissue samples from lethal samples of liver, white muscle, and whole bodies from locations in the Columbia and Snake River drainage. In our determination of proximate constituents, we ignored carbohydrates because carbohydrates constitute less than 0.5% of the somatic tissues of salmonids. Measures of proximate constituents were obtained in our laboratory in Moscow (most dry weight, ash, and sample preparation), and at the Hagerman Fish Culture Experiment Station (lipid, bomb calorimetry, and proximate constituents of whole bodies).

We validated our estimates of energy estimated by bomb calorimetry with assumptions for energy of lipid and protein and used the calorimetry when available. 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 (Figure 7).

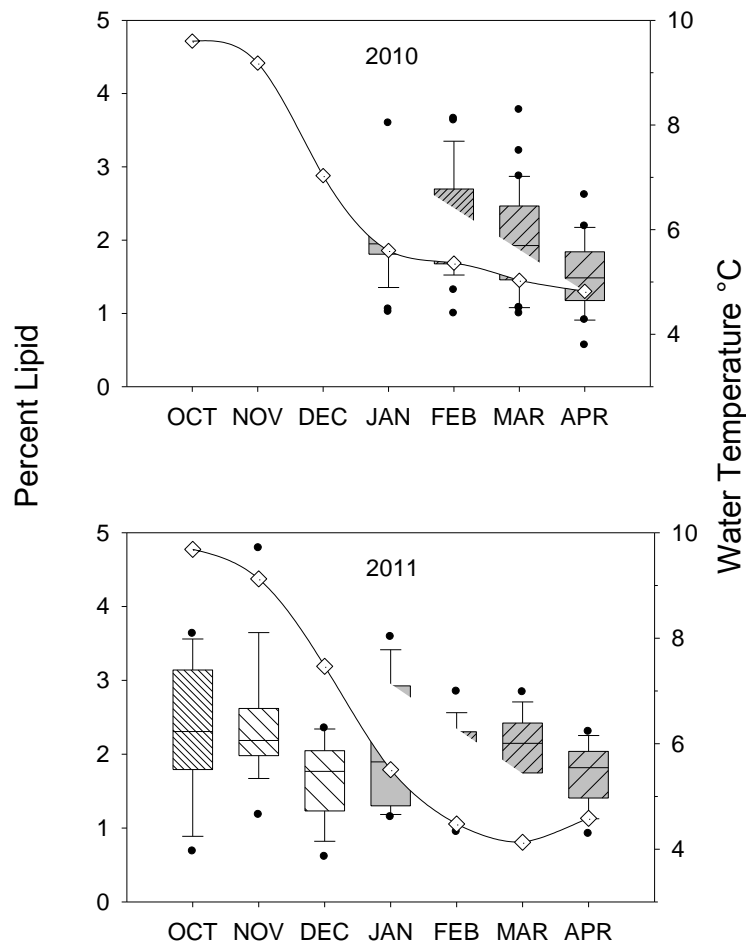


Figure 7. Proportion lipid in white muscle over time for two years of sampling at Dworshak National Fish Hatchery. Water temperatures of the hatchery are provided.

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 7 and 8). 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 9).

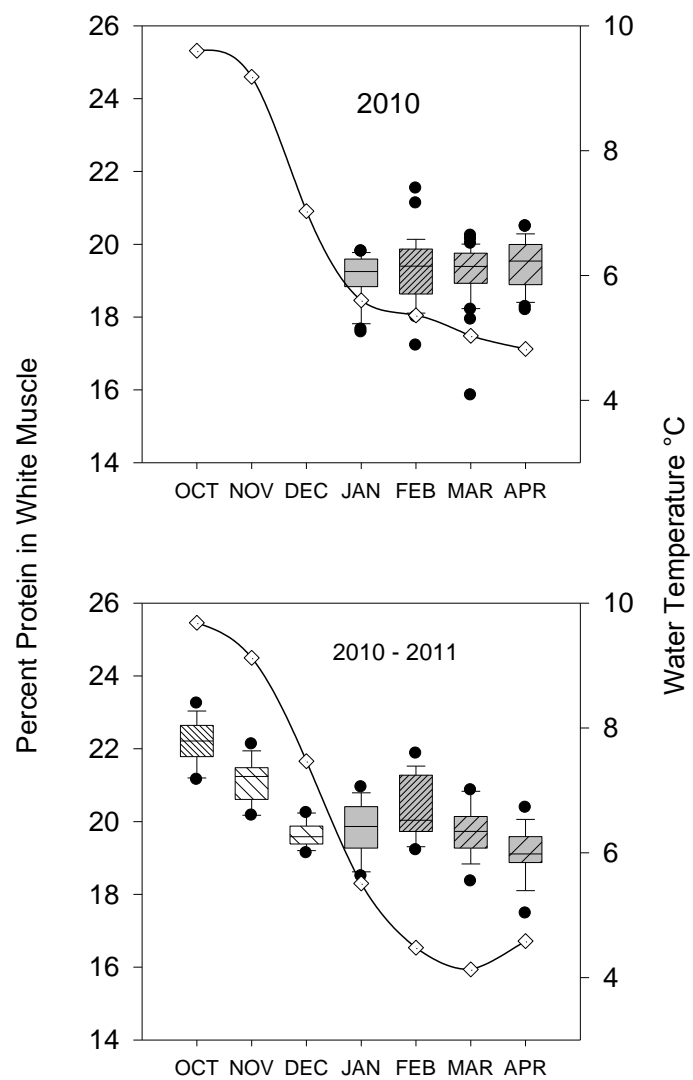


Figure 8. Profile of protein content in samples for white muscle removed from lethally sampled steelhead trout from Dworshak National Fish Hatchery, spawning years 2010 and 2011. Water temperatures are plotted on the right axis (white diamonds).

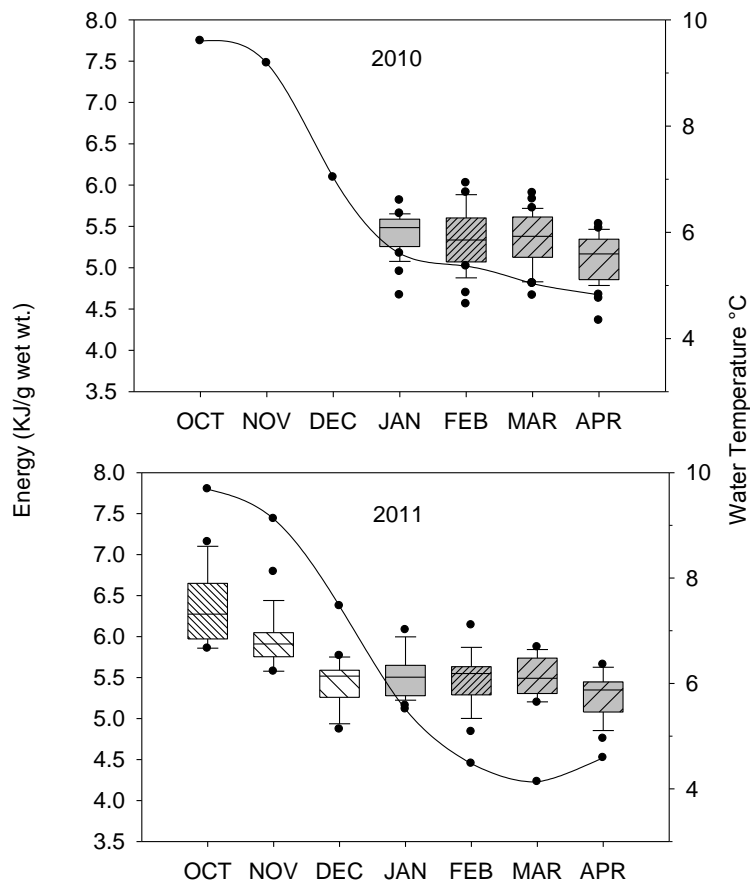


Figure 9. Profile of total energy content (determined via bomb calorimetry) in samples of white muscle removed from lethally sampled steelhead trout from Dworshak National Fish Hatchery, spawning years 2010 and 2011. Water temperatures are plotted on the right axis (white diamonds).

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.

The exact timing of migration from the weirs in the Clearwater River varied over the years, and our ability to monitor these sites varied over the years. However, in general the male steelhead were the last to migrate downstream. Sex ratios of kelts at the bypass facility at Lower Granite Dam Fish were highly in favor of female fish in April and May, and male kelts appeared in June.

From 2009 – 2011 we tagged and sampled a total of 324 kelts from the Clearwater River tributary weirs (Table 5). In 2009, we sampled 15 kelts at Fish Creek. No kelts were sampled at Crooked River in 2009 or

2011 because of the high runoff conditions rendering the weir inoperable. 2010 was the first year that kelts were sampled from the Potlatch River, and also the year with the highest total sample size (266) of kelts sampled. More kelts were sampled in 2010 largely because of the benign runoff conditions of that year which allowed operation of the weirs throughout the majority of the sampling season. Our sample of kelts in 2010 was likely the most representative sample of the steelhead kelt populations emigrating from the three tributaries. In 2011, we sampled fewer kelts (N = 43) partly because of the high runoff causing difficult or impossible sampling conditions during much of the season, and partly due to sample design, since only a sample of good condition kelts were selected for our study that year (discussed in chapter 3).

Table 1 Total kelts sampled from 2009-2011 at the Clearwater River tributary weir sites and the percent female in parentheses. *In 2009 10 kelts (not included in table) were also sampled for blood at Fish Creek that were obviously moribund or recently dead. A total of 25 kelts were sampled but only 15 live kelts were PIT-tagged and released

Total kelts sampled and (percent female)			
	2009	2010	2011
Potlatch River		156 (68)	13 (54)
Fish Creek	15* (80)	69 (74)	30 (100)
Crooked River	0	41 (15)	0
Total	15	266	43

Kelts began post spawning emigrating from the Potlatch River earlier than from the other two tributaries. In 2010 kelts were captured in the Potlatch River from mid March to mid May. In 2011 the sample timing was shorter but within the same range. This reduced time period in 2011 was not necessarily entirely representative of the kelt run timing, rather merely when the water was low enough to operate the weirs. Kelt sampling began near the first of May at Fish Creek in all three years as well as at Crooked River in 2010. Sampling continued at Crooked River in 2010 until the beginning of June. Sampling continued at Fish Creek in all three year until the end of June.

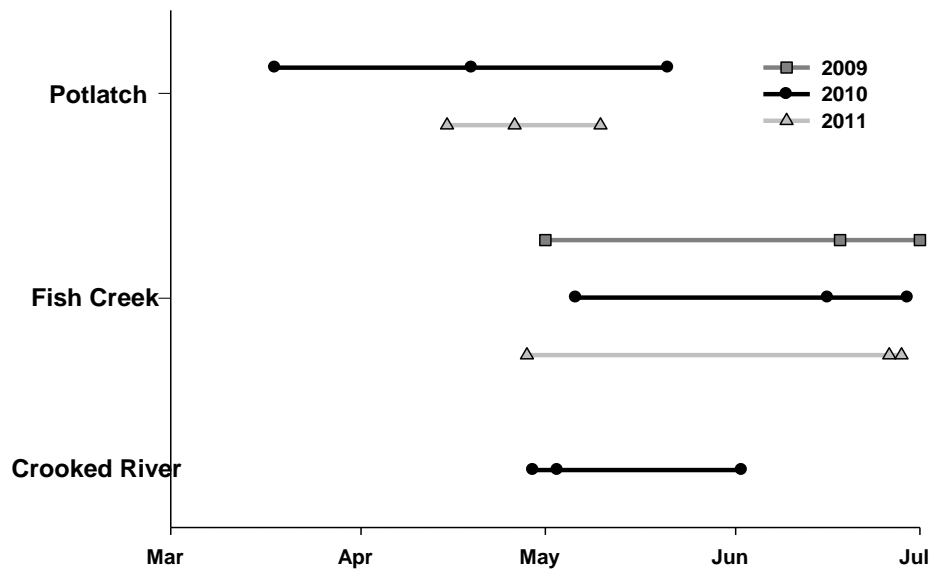


Figure 10. Distribution of sampling at three tributaries of the Clearwater River for three years.

At all sites in all three years, the majority of the kelts sampled were females, with the exception of Crooked River where only 15% of the kelts sampled were females in 2010 (Table 1). Fish Creek had the highest percentage of females in the sample, ranging from 74% in 2010 to 100% in 2011.

For the year that we sampled kelts at all tributaries those observed at the Potlatch River were on average smaller than kelts from the other two tributaries, however the range of sizes was greater there than from the other two tributaries (Figure 11). In 2010 the mean fork length of Potlatch River kelts was 67.4 cm (range: 54.2 - 87.1 cm). The mean fork length for Fish Creek kelts in 2010 was 73.1 cm (range: 61.9 - 84.0 cm). Crooked River kelts were the largest on average with a mean length of 77.8 cm (range: 63.5 – 89.0cm).

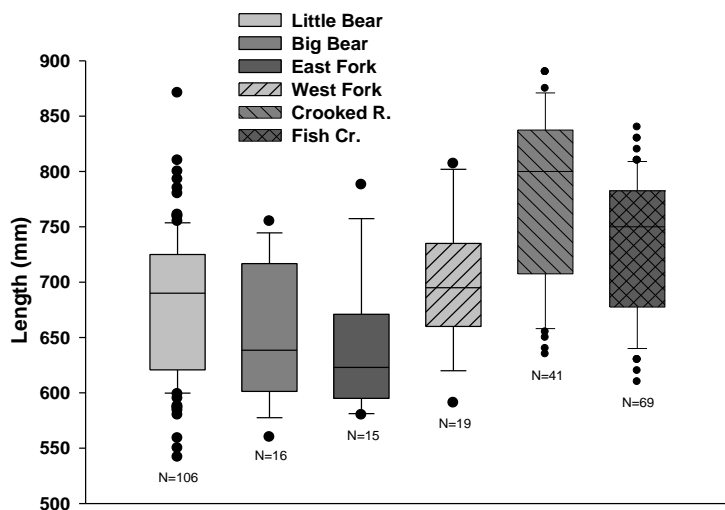


Figure 11. Summary of fork length of kelts by tributary weir in 2010.

In 2010, we found that median plasma cholesterol in kelts from the three tributaries ranged from 86 to 100 mg/dL for fish rated in good condition (Figure 12). These were obviously higher than values measured in plasma from fair or poor condition fish. The median values for fish captured at Lower Granite were lower, but followed the trend of good>fair>poor.

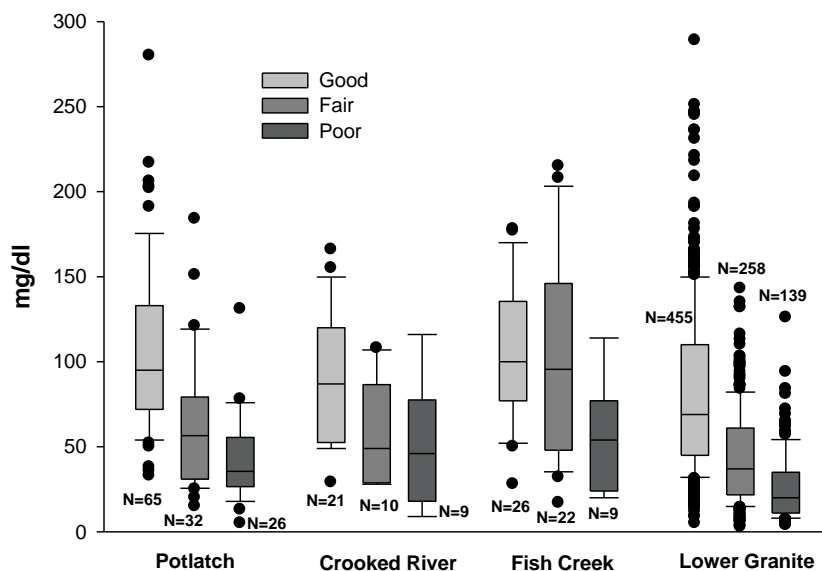


Figure 12. Plasma cholesterol for steelhead kelts captured in 2010 at tributary weirs and Lower Granite Dam separated by fish condition.

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

We have not completed analysis of these parameters, but provide the preliminary summary data from 2011. We used a series of regression models to predict water transit time through Lower Granite Reservoir, and other mainstem Snake and Columbia river reservoirs. Water transit time through Lower Granite Reservoir was predicted using the following regression model:

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

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 estimate water transit rate (km/day) that could then be compared to migration rate (km/day) of each kelt through Lower Granite Reservoir. We divided kelts sampled and tagged in 2011 into groups with high and low plasma cholesterol, and plotted kelt migration rate versus predicted water transit rate for each kelt. We found most kelts from both the Potlatch River and Fish Creek 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) (Figures 13 and 14).

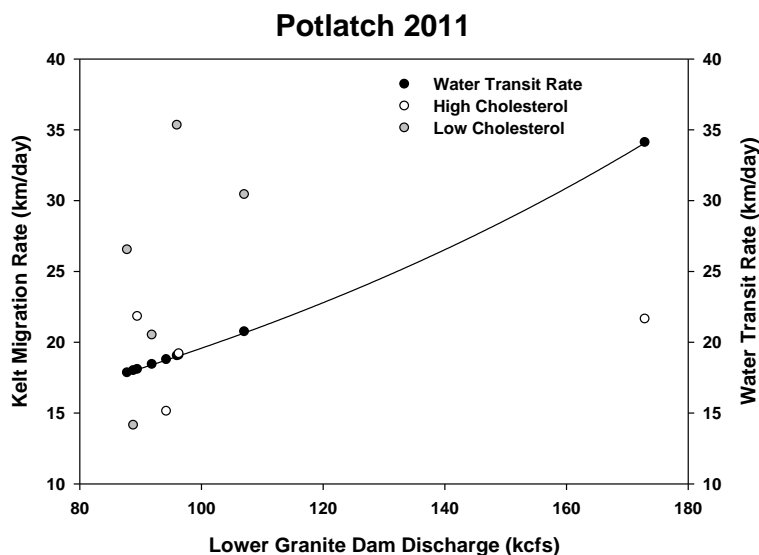


Figure 13. Plots of migration rates kelts from the Potlatch River versus discharge and water transit rate, 2011 for low and high cholesterol.

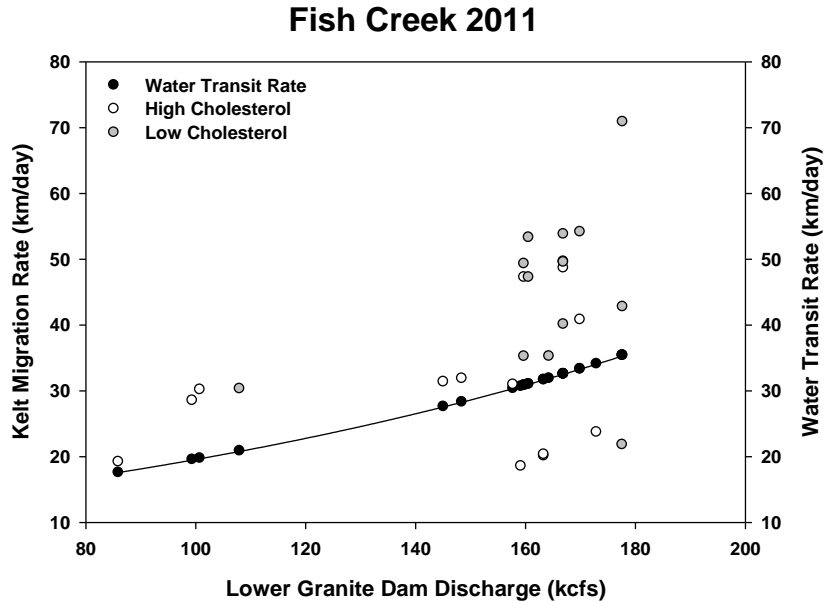


Figure 14. Plots of migration rates kelts from the Fish Creek versus discharge and water transit rate, 2011 for low and high cholesterol.

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

In 2010 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:

$$y = -2.3232 \ln(x) + 13.721 \quad (2)$$

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 15), cholesterol level (Figure 16), and sex (Figure 17). 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.

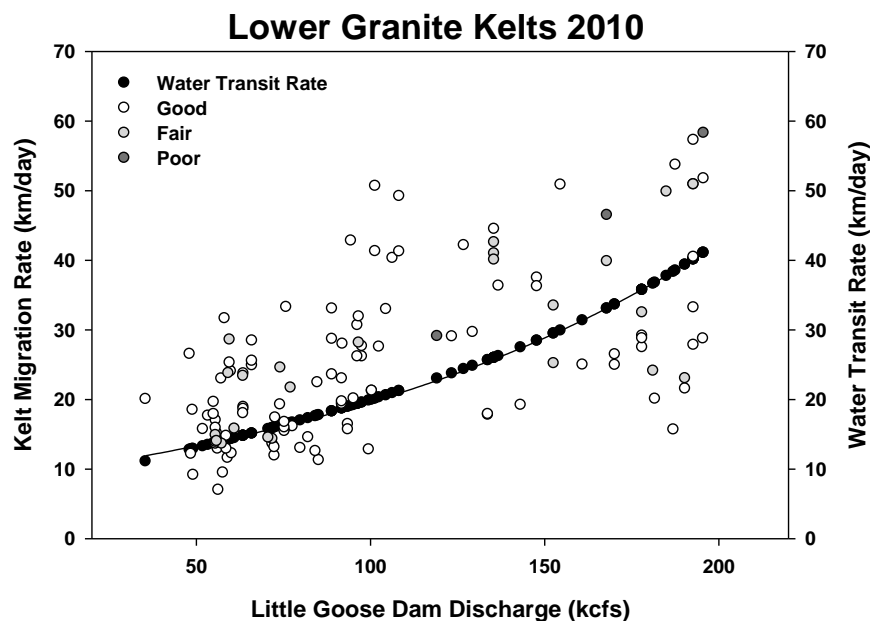


Figure 15. Migration rates of kelts tagged at Lower Granite Dam to detection at Little Goose Dam versus estimated water transit rate in the reservoir. Kelts were separated by condition.

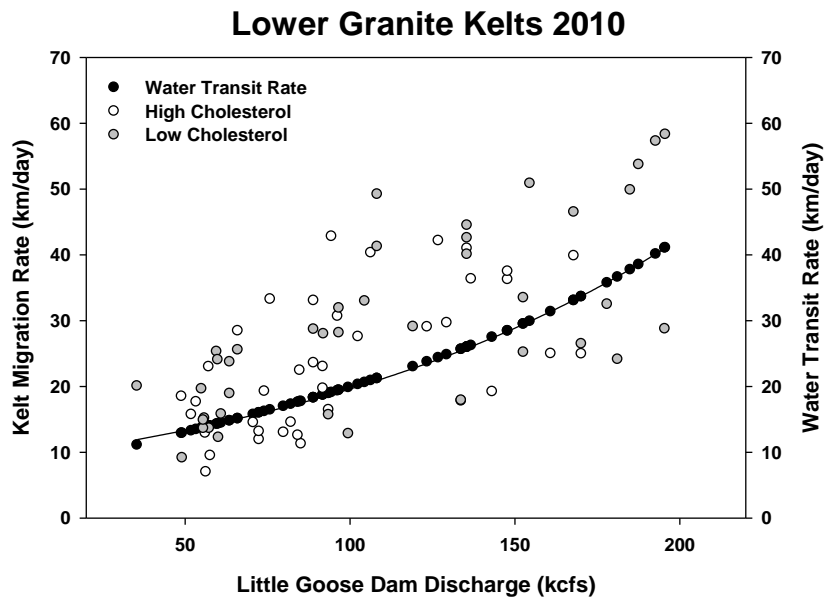


Figure 16. Migration rates of kelts tagged at Lower Granite Dam to detection at Little Goose Dam versus estimated water transit rate in the reservoir. Kelts were separated by plasma high or low cholesterol.

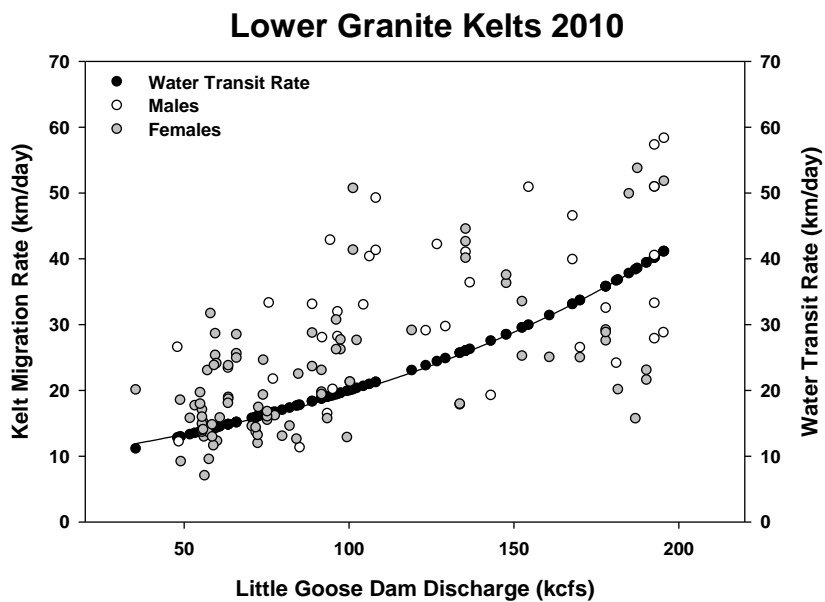


Figure 17. Migration rates of kelts tagged at Lower Granite Dam to detection at Little Goose Dam versus estimated water transit rate in the reservoir. Kelts were separated by sex.

One female kelt tagged on 24 March 2010 at the Little Bear weir in the Potlatch River drainage was detected later that year migrating back upstream at Bonneville Dam in the Columbia River on 29 August and later detected passing Lower Granite Dam 20 days later. From our 2010 Lower Granite PIT-tagging releases, we identified 5 fish repeat spawning in 2011. Four of these fish were female. One was detected repeat spawning at Hayden Creek on the Lemhi River, one at Big Creek, near the Taylor Research Station. We are continuing to analyze the data from these fish. We also identified two fish PIT-tagged at Lower Granite in 2009 detected as repeat spawning fish. One was detected in the Imnaha River, and one at the Nez Perce weir on the Lostine River, a two time repeat spawning fish. One was a skip spawning fish that was 610 mm fork length in 2009, and was recaptured in May of 2011 at 700 mm. We plan to evaluate our data to determine if any of those fish were sampled for plasma. All fish were recorded in good condition at the time of PIT-tagging.

References:

Brannon, E.L., Powell, M.S., Quinn, T.P., and Talbot, A. 2004. Population structure of Columbia River basin Chinook salmon and steelhead trout. *Reviews in Fisheries Science* 12:99-232.

Luna, L.G. 1968. *Manual of histological staining methods of the Armed Forces Institute of Pathology*, 3rd edition. McGraw-Hill Book Company, New York.

Wang, T., C.Y. Hung, D.J., Randall. 2006. The comparative physiology of food deprivation: from feast to famine. *Annual Review of Physiology* 68:223-251.

Problems Affecting Progress

We have no problems to report that are affecting progress. Student Bryan Jones anticipates he will complete his thesis and defend it in the fall semester of 2012. Student Zachary Penney continues to synthesize data, and should have a draft dissertation in the mid spring semester of 2013. We continue to be enthusiastic about the findings of our research and its implications on steelhead management.

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

Contract No C12-37

Purchase Order No C1203740

Quarterly Report for 1 October – 31 December 2012

Prepared by

Christine Moffitt, Principal Investigator

with

Graduate Students: Bryan Jones, Zachary Penney

Submitted to

Columbia River Inter-Tribal Fish Commission
Doug Hatch, Contract Officer

Idaho Cooperative Fish and Wildlife Research Unit
University of Idaho, Moscow, ID 83844-1141

25 January 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.

We are finalizing analyses and interpretations of proximate constituents and energy density of tissues collected from Snake/Columbia River steelhead at selected times during upstream migration, spawning and post-spawning kelt migrations. Our data include measures from samples of: 1) mixed stocks of pre-spawning steelhead collected in the late summer from Zone 6 Columbia River Tribal fishing area; 2) steelhead collected in the fall from tributaries of the Salmon River and from known stocks of steelhead returning to Dworshak National Fish Hatchery (DNFH); 3) samples from DNFH throughout spawning; and 4) samples from mixed stocks of kelts migrating downstream at Lower Granite Dam juvenile bypass. Median lipid content in white muscle in early migrants was > 5% lipid (per wet weight); and by the time of kelt migration, lipids was depleted to less than 0.2% (Table 1). This depletion in lipids was accompanied by a more gradual depletion in the proportion protein, and in total energy density (Table 2). Our measures for lipids in steelhead kelts were often lower than those reported in the literature for other salmonids (Table 3).

In addition to measuring the depletion of energy (lipid and protein) throughout upstream migration, maturity, spawning and kelt migration, we quantified the variation in proximate constituents and energy density in white muscle and liver tissues for known stock of B-run female steelhead in April at DNFH over two years. Lipid content of white muscle varied little between years, but we detected significant differences between years in the livers (Tables 4 and 5). We plan to correlate these findings with our histological assessments of the livers during the next quarter. We detected lower lipids, protein and energy in tissues of poor condition kelts compared with kelts in good condition at Lower Granite Dam (Tables 6 and 7). These differences were similar to trends detected previously in plasma nutritional factors associated with fish by condition at weirs and at Lower Granite Dam.

Table 1. Median percent lipid and protein content and energy density of white muscle tissues from steelhead trout over stages of maturing and sampling. The percentage change from late summer estimates to each sampling date is provided. Data were from spawning year 2011. Fall samples are separated for hatchery and natural spawning stocks, and also combined.

Maturation phase	Sample month[s]	N	Lipid (%)	Decrease from late summer migrants	Protein (%)	Decrease from late summer migrants	Total Energy (kJ/g)	Decrease from late summer migrants
Late summer	August - September	13	5.07		22.89		7.13	
Fall (Mackay Bar)	October	9	2.96	41.7	21.02	8.1	6.38	10.5
Fall (hatchery)	October	15	2.31	54.5	22.21	2.9	6.27	12.0
Combined fall	October	24	2.64	48.0	21.62	5.6	6.33	11.3
Sexual maturity (hatchery)	March	15	1.82	64.1	19.11	16.5	5.35	25.0
Kelt	April - May	20	0.13	97.5	17.27	24.6	3.98	44.1

Table 2: Proportional change in median white muscle lipid, protein, and total energy values between late summer maturation to kelt emigration in spawning year 2011. Samples at sexual maturity are from hatchery stock.

Maturational transition	Time between transitions (months)	Lipid	Protein	Energy
Late summer to fall	1	48.0%	5.6%	11.3
Fall to sexual maturity	5	16.1%	10.9%	13.7
Sexual maturity to kelt emigration	1 to 2	33.3%	8.0%	19.2
Cumulative total		97.5%	24.6%	44.8

Table 3. Average lipid, protein, and energy density for white muscle tissues in mature and post-spawning iteroparous and semelparous salmonids. Data source is provided. The sexes are combined unless listed as M or F. NA= not available.

Site	Species	Year	Phase	N	Lipid %	Protein %	Energy (kJ/g)	Source
Snake River, WA	Steelhead	2009	Kelt	37	0.19	15.82	3.67	This study
Snake River, WA	Steelhead	2010	Kelt	96	0.28	16.42	3.78	This study
Snake River, ID	Steelhead	2012	Kelt	20	0.13	17.27	3.98	This study
Nez Perce Tribal								
Hatchery, ID	Fall Chinook	2010	Mature	10	2.15	19.05	5.30	This study
South Fork Salmon River, ID	Spring Chinook	2002	Death hatchery	18	1.10	15.10	NA	Pinson (2005)
South Fork Salmon River, ID	Spring Chinook	2002	Death wild	7	3.80	16.60	NA	Pinson (2005)
Yakima River, WA	Spring Chinook	2002	Death (F)	13	0.40	13.20	2.80	Mesa and Magie (2006)
Yakima River, WA	Spring	2002	Death (M)	2	0.10	14.20	2.90	Mesa and Magie (2006)

	Chinook							
Pick Creek, AK	Sockeye	1996	Death (F)	23	0.20	14.30	2.90	Hendry and Berg (1999)
Pick Creek, AK	Sockeye	1996	Death (M)	23	0.10	14.50	3.00	Hendry and Berg (1999)
Wenatchee River, WA	Sockeye	1995	Mature (F)	20	5.50	17.10	5.40	Hendry et al. (2000)
Wenatchee River WA	Sockeye	1995	Mature (M)	21	7.50	15.30	5.80	Hendry et al. (2000)
		1991,						
River Drammen, Norway	Atlantic salmon	1992,						
		1995	Kelt (F)	26	2.10	17.10	NA	Jonsson et al. 1997
		1991,						
River Drammen, Norway	Atlantic salmon	1992,						
		1995	Kelt (M)	4	1.90	17.00	NA	Jonsson et al. 1997

Table 4. Summary of Type III ANOVA of the proportion of lipid and protein and the energy density of white muscle and liver tissues sampled in April 2009 and 2010 at Dworshak National Fish Hatchery (Model: $y_{ijk} = \mu + \alpha (\text{Spawning year})_i + \text{length} * X_j + \epsilon_{ijk}$). Energy was not measured for liver tissues.

Dependent variable	Factor	DF	Type III mean square	F	P
<i>White muscle</i>					
Lipid	Spawning year	1	0.00003117	0.68	0.415
	Length	1	0.00000066	0.01	0.905
Protein	Spawning year	1	0.00039361	3.41	0.071
	Length	1	0.00004832	0.42	0.521
Energy	Spawning year	1	0.06028357	0.73	0.398
	Length	1	0.00859786	0.1	0.749
<i>Liver</i>					
Lipid	Spawning year	1	0.00712526	221.1	<.0001
	Fork length	1	0.00004208	1.31	0.2591
Protein	Spawning year	1	0.00433126	31.64	<.0001
	Fork length	1	0.00067087	4.9	0.0319

Table 5. Summary of least squared mean proportion lipid and protein and energy density in white muscle and liver tissues sampled from female steelhead April 2009 and 2010 at Dworshak National Fish Hatchery. Means with different letters are significantly different. nd = not determined.

Spawning year	N	Lipid	Protein	Energy density
<i>White muscle</i>				
2009	30	0.018	0.202 a	5.258
2010	19	0.016	0.195 b	5.179
<i>Liver</i>				
2009	30	0.033 a	0.197 a	nd
2010	19	0.006 b	0.219 b	nd

Table 6. Type III ANOVA results for white muscle lipid, protein, and energy in poor, fair, and good condition female kelts between May of spawning years 2009 and 2010 at the Lower Granite Dam juvenile bypass facility (Model: $y_{ijk} = \mu + \alpha(\text{Spawning year})_i + \beta(\text{Condition}) + \text{length} * X_j + \epsilon_{ijk}$). *Tissue components with no significant interactions were reduced to main effects only.

Component	Factors	DF	Type III Mean square	F	P
Lipid*	Spawning Year	1	0.00000782	1.2	0.2773
	Condition	2	0.00000793	1.22	0.3028
	Length	1	0.00000677	1.04	0.3119
Protein*	Spawning Year	1	0.00059336	3.13	0.0823
	Condition	2	0.00374338	19.72	<.0001
	Length	1	0.00331043	17.44	0.0001
Energy*	Spawning Year	1	0.38211377	2.82	0.0984
	Condition	2	2.10243944	15.52	<.0001
	Length	1	1.70813106	12.61	0.0008

Table 7. Least squared mean values for white muscle lipid, protein, and energy in poor, fair, and good condition female kelts sampled in May in spawning years 2009 and 2010 at the Lower Granite Dam juvenile bypass facility.

Factor	N	Lipid	Protein	Energy
<hr/>				
Spawning year				
2009	24	0.004	0.158	3.730
2010	39	0.003	0.152	3.568
Condition				
Poor	16	0.003	0.142 a	3.347 a
Fair	15	0.003	0.156 b	3.636 b
Good	32	0.004	0.168 c	3.964 c
<hr/>				

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 tagged and sampled a total of 324 kelts from the Clearwater River tributary weirs during 2009 and 2011 (Table 8). In 2009, all were tagged and sampled at Fish Creek. No kelts were sampled at Crooked River in 2009 or 2011 because of the high river discharge rendering the weir inoperable. Kelts were sampled in 2010 and 2011 at weirs in the Potlatch River. More kelts were sampled in 2010 because river discharge that year allowed for operation of the weirs throughout most of the sampling season. Our sample of kelts in 2010 was likely the most representative sample of the steelhead kelt populations emigrating from the three tributaries. In 2011, we sampled only 43 kelts partly because high river discharge caused difficult sampling conditions, and partly due to sample design, since our target was to tag only kelts in good condition with acoustic tags.

Table 8. Total number of kelts sampled from 2009-2011 at the Clearwater River tributary weir sites and the percent female in parentheses. *In 2009 10 kelts (7 females, 3 males; not included in table) were also sampled for blood at Fish Creek that were obviously moribund or recently dead. Of the 25 kelts handled, only 15 live kelts were PIT-tagged and released.

	Total kelts sampled and (percent female)		
	2009	2010	2011
Potlatch River		156 (68)	13 (54)
Fish Creek	15* (80)	69 (74)	30 (100)
Crooked River	0	41 (15)	0
Total	15	266	43

Kelts were captured, tagged and sampled at weirs in the Potlatch River earlier than from the other two tributaries. Kelt sampling began near the first of May at Fish Creek in all three years as well as at Crooked River in 2010. Sampling continued until the beginning of June at Crooked River in 2010. Sampling continued until the end of June at Fish Creek in all three years. The majority of the kelts observed and sampled at the Potlatch River and Fish Creek weirs were female, but only 15% of the kelts at Crooked River were female. The percent female at Fish Creek ranging from 74% in 2010 to 100% in 2011. We found significant differences between tributaries in the fork lengths ($P < 0.001$; Kruskal Wallis).

Kelts in 2010 from the Potlatch River (median: 69.0 cm, range: 54.2 - 87.1 cm) were on average smaller than kelts from Fish Creek ($P < 0.001$; permutation based Tukey's HSD test) and Crooked River ($P < 0.001$) but the range of fork length was greater for kelts from the Potlatch River than from the other two tributaries. There was no difference between the fork length of Fish Creek (median: 75.0 cm, range: 61.9 - 84.0 cm) and Crooked River kelts ($P = 0.939$; median: 80.0, range: 63.5 - 89.0 cm).

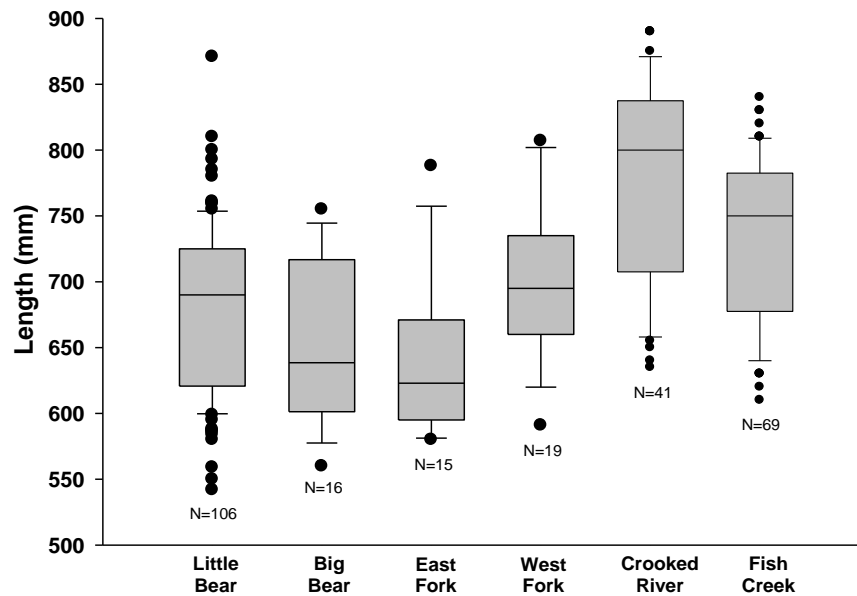


Figure 1. Fork lengths of kelts captured at the four Potlatch River weirs (Little Bear, Big Bear, East Fork, and West Fork), Crooked River and Fish Creek in 2010 for all sexes combined.

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

In 2010, we tagged 119 kelts for release at locations below Bonneville Dam. We detected 88 of the kelts (74%) at acoustic receivers at some point during their migration through the lower Columbia River. Eighteen (15%) of these kelts were detected at the farthest downstream array near the mouth of the Columbia River (Table 9).

Table 9. Kelts captured at Lower Granite Dam, implanted with acoustic transmitters, and transported to locations downstream of Bonneville Dam in 2010. The number of kelts detected at each acoustic receiver array is provided. Kelts transported by truck were released at two locations: Hamilton Island at rkm 231 just below Bonneville Dam, and Aldrich Point at rkm 50, near the estuary group of receivers.

Transportation						
method	Number	Bonneville	St. Helens	Estuary	Ocean mouth	Not detected
Truck – Hamilton	27	21	20	13	3	6
Truck – Aldrich	10	NA	NA	6	4	4
Barge	82	61	45	30	11	21
Total	119	82	65	49	18	31

Kelts were separated by location of last detection and their blood plasma metrics were summarized (Table 10). Kelts that were never detected had the lowest median values for nutritional metrics and those detected at the ocean mouth (the array farthest downstream) had the highest. This trend was true for cholesterol, triglycerides, calcium, glucose, AP, and amylase. The tissue damage factors LDH and ALT were highest in kelts never detected and lowest in those kelts detected last at the ocean mouth. The electrolytes sodium, chloride, and magnesium were highest in kelts detected last at the ocean mouth. Protein was also highest in those kelts. Cortisol median values were lowest in the kelts that were not detected; however sample sizes were low for cortisol as those assays were not performed for every fish that was sampled.

Table 10. Plasma metrics of kelts implanted with acoustic transmitters at Lower Granite Dam and transported downstream of Bonneville Dam via barge and truck in 2010 separated by location of last detection. Kelts released at Aldrich Point were excluded from this table. Values below detection were not included in this table and are distributed as follows: triglycerides = 13 and protein = 22 for the “Not Detected” group; triglycerides = 4 and protein = 20 for the “St. Helens” group; and triglycerides = 1 and protein = 6 for the “Ocean Mouth” group.

Metric	Not detected		St. Helens		Ocean mouth	
	<i>N</i>	Median (min.-max.)	<i>N</i>	Median (min.-max.)	<i>N</i>	Median (min.-max.)
Cholesterol	27	41	22	42	14	95.5
(mg/dl)		(8-247)		(17-158)		(48-236)
Triglycerides	14	25	18	24	13	57
(mg/dl)		(10-153)		(10-379)		(14-182)
Calcium	27	8.1	22	8.6	14	9.4
(mg/dl)		(6.4-12)		(6.8-9.9)		(7.6-14.2)
Glucose	27	90	22	83	14	105.5
(mg/dl)		(48-191)		(45-235)		(73-183)
AP	26	13.5	22	15	14	25.5
(u/L)		(4.0-46)		(4-49)		(9.0-84.0)
Phosphorus	27	9.8	22	9.6	14	9.4
(mg/dl)		(6.6-14.1)		(6.5-22.2)		(4.4-15)
Amylase	27	146	22	187.5	14	187.5
(u/L)		(4-807)		(26-268)		(21-1184)
LDH	27	590	22	577	14	396
(u/L)		(214-1428)		(210-3573)		(123-558)
AST	27	499	22	422	14	589
(u/L)		(114-1237)		(54-12644)		(194-992)
ALT	24	62	20	45	14	36

	(u/L)		(5-147)		(17-1624)		(11-103)
Sodium	27	151	22	153.5	14	157	
	(mmol/L)		(130-180)		(142-190)		(141-175)
Chloride	27	138	22	137.5	14	145.5	
	(mmol/L)		(119-160)		(121-173)		(128-157)
Magnesium	27	1.8	22	1.7	14	2	
	(mg/dl)		(0.8-3.29)		(1-2.51)		(1.5-2.6)
Potassium	27	2.2	22	1.8	14	1.8	
	(mmol/L)		(0.9-3.1)		(1.2-3.0)		(0.8-2.2)
Protein	5	2.9	2	2.7	8	3.2	
	(gm/dl)		(2.6-4.1)		(2.6-2.7)		(2.6-3.8)
Cortisol	4	144.2	4	179.6	2	195.3	
	(ng/ml)		(130.5-236.2)		(123.3-245.0)		(102.9-287.6)

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

In 2011, we sampled and implanted acoustic transmitters in 43 kelts from the Potlatch River and Fish Creek (Table 11). High runoff conditions prevented sampling throughout much of the season and prevented our reaching the target sample size of 40 Potlatch River kelts. Our target sample size of 30 kelts from Fish Creek was met. We detected 100% of the kelts during downstream migration and all kelts were detected at or below the mouth of the Clearwater River. Thirty eight kelts (88%) were detected at our receivers in the Lower Granite Dam forebay; however, only 4 (9%) were detected by the receivers in the lower Columbia River below Bonneville Dam.

Table 11. Number of fish implanted with acoustic transmitters in 2011 detected at each acoustic array and (km traveled since release).

Location	Number tagged	Clearwater mouth	Upper reservoir	Lower Granite forebay	Lower Columbia
Potlatch River	13	13 (46)	12 (64)	9 (98)	1 (560+)
Fish Creek	30	30 (193)	30 (211)	29 (245)	3 (700+)
Total	43	43	42	38	4

Migration rate in kelts varied from different tributaries and by river reach. Migration rates in the Clearwater River were faster for Fish Creek kelts than for Potlatch River kelts. This difference in migration rate was less evident in the Lower Granite Dam reservoir, particularly in the lower section of the reservoir. Kelts tagged at Fish Creek also showed a decrease in migration rate upon reaching the Lower Granite Dam reservoir.

The sample of Fish Creek kelts consisted of an early and a late migrating group because the weir was inoperable due to high flows and debris jams for over five weeks during the last part of May and much of June. Kelts in the early group were sampled before the weir was damaged, and those in the late group were sampled after the runoff subsided and repairs to the weir were made. Kelts tagged at Fish Creek at the end of June migrated faster than those sampled in late April and early May, though both groups showed the same trend of decreasing migration rate upon reaching the Lower Granite Dam reservoir.

Blood plasma metrics for kelts sampled at Fish Creek and the Potlatch River were separated by location of last detection of each kelt. Sample sizes were low for all groups, allowing only limited comparisons. One kelt from the Potlatch River was detected migrating in the lower Columbia River (Table 12). All plasma nutritional metric values that were measured for that fish were higher than median values of kelts last detected in the Lower Granite Dam reservoir. Tissue damage factors were lower and all electrolyte factors and protein levels were higher in that fish. We detected three kelts from Fish Creek in the lower river (Table 13). Median values of nutritional metrics (cholesterol, triglycerides, calcium, and AP) were lower in the kelts that were detected in the lower Columbia River except for glucose, phosphorus, and amylase. Median values of tissue damage factors (LDH, AST, and ALT) were lower in

kelts detected in the lower Columbia River. The electrolytes sodium and chloride were also both lower in those fish. Furthermore, all protein levels were below detection in those fish.

We divided kelts sampled and tagged at Fish Creek and the Potlatch River in 2011 into groups with high and low plasma cholesterol (as an example of nutritional status), and evaluated migration rate within the Lower Granite Dam reservoir versus estimated water transit rate for each kelt. We found that most kelts from both the Potlatch River and Fish Creek migrated faster than the estimated speed of the water within the Lower Granite Dam reservoir, but sample sizes from the Potlatch River were small ($n = 9$, versus $n = 27$ for Fish Creek;). However there was no noticeable trend in migration speed related to cholesterol level in kelts from either tributary.

Table 12. Plasma metrics of kelts implanted with acoustic transmitters in the Potlatch River in 2011 separated by location of last detection. Values below detection were not included in this table and are distributed as follows: protein = 1 and potassium = 2 for the “Mid Lower Granite Res.” group; triglycerides = 1, protein = 5 and potassium = 2 for the “Lower Granite Forebay” group.

Metric	Mid Lower Granite res.		Lower Granite forebay		Lower Columbia	
	<i>N</i>	Median (min.-max)	<i>N</i>	Median (min.-max)	<i>N</i>	Median (min.-max.)
Cholesterol	3	91	8	61	1	123
(mg/dl)		(90-113)		(23-148)		-
Triglycerides	3	30	7	112	1	171
(mg/dl)		(29-160)		(24-234)		-
Calcium	3	13.7	8	12.2	1	14.4
(mg/dl)		(12.3-13.8)		(11.4-12.6)		-
Glucose	3	73	8	92.5	1	138
(mg/dl)		(45-118)		(71-129)		-
AP	3	36	8	28	1	38
(u/L)		(25-36)		(10-74)		-
Phosphorus	3	10.4	8	11.9	1	11.7

(mg/dl)		(7.4-16.2)		(7.8-12.4)		-
Amylase	3	20	8	23	1	37
(u/L)		(19-22)		(11.0-33)		-
LDH	3	364	8	551	1	299
(u/L)		(329-552)		(308-2286)		-
AST	3	1076	8	991.5	1	693
(u/L)		(469-1836)		(474-2068)		-
ALT	3	141	8	119	1	65
(u/L)		(47-195)		(57-245)		-
Sodium	3	161	8	157	1	171
(mmol/L)		(152-162)		(153-162)		-
Chloride	3	147	8	144	1	159
(mmol/L)		(137-153)		(138-154)		-
Magnesium	3	2.3	8	2.2	1	2.4
(mg/dl)		(2-2.3)		(1.7-2.4)		-
Potassium	1	1.2	6	2.1	1	3.1
(mmol/L)		-		(1.5-2.9)		-
Protein	2	3.3	3	2.7	1	3.7
(gm/dl)		(3-3.6)		(2.6-2.7)		-

Table 13. Plasma metrics of kelts implanted with acoustic transmitters at Fish Creek in 2011 separated by location of last detection. Plasma metric values below detection were not included in this table and are distributed as follows: triglycerides = 1, protein = 7 and potassium = 7 for the “Lower Granite Forebay” group; protein = 3 for the “Lower Columbia” group.

Metric	Mid Lower Granite res.		Lower Granite forebay		Lower Columbia	
	<i>N</i>	Median (min.-max.)	<i>N</i>	Median (min.-max.)	<i>N</i>	Median (min.-max.)
Cholesterol	1	67	26	59.5	3	59
(mg/dl)		-		(30-136)		(45-98)
Triglycerides	1	53	24	34.5	3	32
(mg/dl)		-		(12-153)		(22-100)
Calcium	1	12	26	12	3	10.9
(mg/dl)		-		(10.4-13.7)		(10.7-11.2)
Glucose	1	63	26	83.5	3	91
(mg/dl)		-		(34-171)		(81-113)
AP	1	68	26	47.5	3	34
(u/L)		-		(26-80)		(19-59)
Phosphorus	1	9.1	26	9.9	3	10.4
(mg/dl)		-		(7.3-12.2)		(8.9-15.6)
Amylase	1	24	26	27	3	26
(u/L)		-		(2.0-60)		(13-39)
LDH	1	1500	26	1605	3	689
(u/L)		-		(411-3066)		(433-1368)
AST	1	2392	26	3411	3	2043
(u/L)		-		(1230-5982)		(1729-2331)
ALT	1	259	26	351.5	3	245

(u/L)		-		(2.2-2664)		(167-344)
Sodium	1	143	26	148	3	139
(mmol/L)		-		(128-167)		(125-142)
Chloride	1	123	26	129.5	3	121
(mmol/L)		-		(109-146)		(110-123)
Magnesium	1	2	26	2.2	3	2
(mg/dl)		-		(1.9-2.7)		(0.9-2.6)
Potassium	1	1.9	19	1.4	3	1.7
(mmol/L)		-		(1-2.4)		(1.4-1.8)
Protein	1	2.8	19	2.9	0	-
(gm/dl)		-		(2.4-4.2)		-

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

We sampled, PIT-tagged, and released a total of 324 kelts at the Clearwater River tributary weirs (Table 14). Few fish were detected at the PIT tag arrays at the downstream hydro dams in the Snake or Columbia Rivers, suggesting routes of migration over dams may be more common.

Table 14. Summary of tagging and PIT-tag detections of kelts from Clearwater River tributary weirs by year of tagging. There were no in-stream PIT arrays in Fish Creek or Crooked River. The mainstem Potlatch PIT array was damaged during the high river discharge early in the spring of 2011 and was not fully operational during much of the season.

	Total tagged	Detected exiting tributary (%)	Detected downstream in Snake-Columbia system (%)
2009			
Fish Creek	15		1 (7)
Annual total	15		1 (7)
2010			
Potlatch River			
Little Bear	106	56 (53)	4 (3.8)
Big Bear	16	10 (63)	1 (6.3)
East Fork	15	3 (20)	0 (0)
West Fork	19	4 (21)	1 (5.3)
Total	156	73 (47)	6 (3.8)
Fish Creek	69		1 (1.4)
Crooked River	41		0 (0)
Annual total	266		7 (2.6)
2011			

Potlatch River			
Little Bear	11	1 (9.1) ^a	1 (9.1)
East Fork	2	0 (0) ^a	0 (0)
Total	13	1 (7.7) ^a	1 (7.7)
Fish Creek	30		1 (3.3)
Annual total	43		2 (4.7)

Lower Granite Dam

In 2009, 197 kelts were PIT-tagged and released into the tailrace of Lower Granite Dam. Of these fish released, 65 (33%) of those kelts were detected during downstream migration at detection arrays. Male kelts in good condition were the group with the highest detection percentage with 67% detected. Females in good condition were the group with the second highest detection percentage with 36% detected. No kelts in poor condition were detected migrating at any sites (Table 15).

The Little Goose Dam juvenile fish bypass was the most common detection site in 2009. Bonneville Dam was the next most common detection site. Furthermore, 18 of 20 total detections at Bonneville Dam were at the 2nd powerhouse corner collector, a surface passage route. Only 2 kelts in fair condition were detected at any sites within the Columbia River, the rest were all in good condition.

The low proportion of PIT tag detections of the kelts made comparisons of blood plasma metrics of different groups difficult. However, since 20 kelts were detected at Bonneville Dam, we separated blood plasma metrics of those kelts and compared them to the remainder of the kelts sampled in 2009. Nutrition factor (e.g., cholesterol, triglycerides, calcium, and amylase) and electrolyte (e.g., sodium, chloride and magnesium) medians for these kelts were higher than measured in other kelts. Median values of the tissue damage factors LDH and AST were lower than measured in other kelts.

In 2010, 1,398 kelts were sampled, tagged, and released into the Lower Granite Dam tailrace and 252 (20%) of were later detected during their downstream migration. Good condition males again had the highest proportion detected at 36%. Good condition females again had the next highest detection percentage with 23% detected. The group with the lowest detection percentage was poor condition females with a total of just one fish detected (0.99%).

Table 15, Number of kelts tagged and released into the river at Lower Granite Dam in 2009 and 2010 by sex, and the number and percent detected at PIT-tag arrays at hydrodams or at the towed array during downstream migration at least once in the Snake or Columbia Rivers.

Group and condition	Total tagged	Number detected	Percent detected
2009			
Male			
Good	15	10	67
Fair	7	1	14
Poor	6	0	0
All male	28	11	39
Female			
Good	117	42	36
Fair	35	12	34
Poor	17	0	0
All female	169	54	32
Both sexes 2009	197	65	33
Male			
Good	174	62	36
Fair	114	13	11
Poor	86	4	5
All male	374	79	21
Female			
Good	673	152	23
Fair	250	20	8
Poor	101	1	0.99

All female	1024	173	17
Both sexes 2010	1398	252	18

Individual kelts were detected up to a total of three times at PIT-tag detection arrays during their downstream migration, but of those detected, most (81%) were only detected once; 17% were detected twice and 2% detected three times. The largest number of kelts (129) was detected in 2010 at the Little Goose Dam juvenile fish bypass. Bonneville Dam was the second most common detection site with 71 kelts detected. Of the 71 kelts detected at Bonneville Dam, 62 (87%) were detected migrating through the 2nd powerhouse corner collector. Additionally, two kelts in 2010 were detected migrating in the estuary by the trawled array. Travel rates of kelts in good condition between dams generally increased as the kelts got farther down the river. No kelts in fair or poor condition were detected at any dams in the Columbia River.

Blood samples were not collected from all kelts tagged in 2010. Of the 71 kelts detected reaching Bonneville Dam, 46 were sampled for blood. Median values of nutritional factors (e.g., cholesterol, triglycerides, calcium, glucose, and AP) and electrolytes (e.g., sodium, chloride, and magnesium) measured in those detected at Bonneville Dam were higher than measured in other kelts sampled in 2010. Additionally, median values of all tissue damage factors analyzed (LDH, AST, and ALT) in these fish were lower than other kelts sampled.

We estimated migration rates and water transit rates for the 129 kelts detected at Little Goose Dam. We separated these kelts by condition, sex, and cholesterol level (chosen as a representative of nutritional status). The majority of the kelts PIT-tagged at Lower Granite Dam and detected at Little Goose Dam in 2010 traveled faster than the estimated speed of the water in the Little Goose Dam reservoir. There were no obvious trends in migration rate of kelts related to condition, cholesterol level, or sex.

Repeat spawning fish

No kelts sampled at tributary weirs in 2009 or 2011 were detected making repeat spawning runs. However one kelt (female, 560 mm) tagged at the Little Bear Weir in the Potlatch River in April of 2010 was detected migrating back upstream at Bonneville Dam in August of that same year (consecutive spawner). This fish was last detected moving upstream in the adult fish ladder at Lower Granite Dam 20

days later. Nutritional metrics (e.g. cholesterol, triglycerides, calcium, glucose, and AP) of this fish were higher than the sample median for other Potlatch River kelts, and the tissue damage factors LDH and ALT were lower than the sample median. The electrolytes sodium, chloride, and magnesium were higher than the Potlatch River sample median.

In 2009, 197 kelts were tagged and released into the tailrace at Lower Granite Dam. Two females (580 and 610 mm; 1.02% of the 2009 sample) were later detected as upstream migrants in July and August of 2010 after having spent over a year at sea (presumably; skip spawners). Neither fish was detected during their downstream migration as kelts after the Lower Granite Dam capture. During their repeat spawning migration both of these fish were detected at tributary sites. One was detected in the Imnaha River (a Snake River tributary) on 6-May-2011, and the other was captured at a Lostine River (a Grande Ronde River tributary) weir on 5-May-2011. At the time of tagging at Lower Granite Dam in 2009, the Lostine River fish measured 61 cm fork length. At the time of recapture at the weir in 2011, her fork length was 70 cm. That fish was last detected in May of 2011 as a kelt at the Lower Monumental Dam juvenile fish bypass.

The two repeat spawning fish detected first as kelts in 2009 had median values for both cholesterol and triglycerides that were higher than median values of those kelts last detected at Bonneville Dam and higher than the median values for other kelts sampled in 2009. In 2010, 1,398 kelts were tagged and released into the tailrace of Lower Granite Dam. Four females and one male (Females: 710, 580, 570, 570 mm; Male: 570; 0.36% of the 2010 sample) were detected beginning their second spawning runs in 2011 as skip spawners. Three of these fish were detected in 2010 during their downstream kelt migration at the Bonneville 2nd Powerhouse Corner Collector (a surface passage route). During their repeat spawning runs in 2011, all five steelhead were detected migrating upstream at the Bonneville Dam fish ladders. The male was not detected again after that Bonneville Dam detection. However, the four females were all detected passing Lower Granite Dam. Furthermore, three of them were detected at tributary sites during their second spawning run. One was detected in Big Creek (a Middle Fork Salmon River tributary); another in Hayden Creek in the Lemhi River drainage (an upper Salmon River tributary) and the third was detected in the Yankee Fork Salmon River (another upper Salmon River tributary).

Blood samples were not collected from all kelts that were tagged in 2010. Of the five repeat spawning fish observed in 2011, only two were sampled for blood. Median nutritional values of these fish including cholesterol, triglycerides, calcium, glucose, and AP were higher than in kelts detected migrating downstream as kelts at Bonneville Dam in 2010 and the remainder of the 2010 kelts sampled.

Problems Affecting Progress

Student Zachary Penney continues to synthesize data, and should have completed his draft dissertation chapters by the end of the spring semester. Our progress in completing drafts for publication is slower than anticipated. We continue to be enthusiastic about the findings of our research and its implications on steelhead management. We expect to have two draft publications completed in this next quarter.

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

Contract No C12-37

Purchase Order No C1203740

Quarterly Report for 1 January – 31 March 2013

Prepared by

Christine Moffitt, Principal Investigator

with

Graduate Student: Zachary Penney

Submitted to

Columbia River Inter-Tribal Fish Commission

Doug Hatch, Contract Officer

Idaho Cooperative Fish and Wildlife Research Unit
University of Idaho, Moscow, ID 83844-1141

2 May 2013

Executive Summary

This past quarter we continued to synthesize data into peer reviewed manuscripts. We completed and submitted a paper for a special issue comparing Atlantic to Pacific salmonids in the journal *Reviews in Fish Biology and Fisheries*. A copy of the submitted manuscript is provided as an addendum to this quarterly report. In this past quarter we revised our draft manuscript on energy transformation and depletion in steelhead for submittal to *Transactions of the American Fisheries Society*. In addition, we have prepared a draft manuscript “Physiological indices of seawater readiness in postspawning steelhead kelts” for submission to *Ecology of Freshwater Fish*. Graduate student Zach Penney continues to work to complete drafts of his dissertation. The defense will be scheduled in the early fall semester, and revisions by the end of the year.

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 have drafted a manuscript that describes the transformation of energy stores in steelhead from the fall migration through spawning and kelt migration. We are making the final revisions to prepare this for submission to Transactions of the American Fisheries Society. This will serve as a chapter in Zach Penney's dissertations.

As expected, lipid, protein, and total energy decrease from late summer maturation to kelt emigration. Overall, 97% of lipid, 24% of protein, and 44% of total energy was used from late summer maturation to kelt emigration. Comparisons between spawning years showed that lipid, protein, and total energy content varied little between the years, and the median values for lipid, protein, and total energy content between years was 0.15%, 1.45%, and 0.31 kJ/g, respectively.

In our analysis of the entire cycle, we found the largest costs of reproduction/migration occurred between late summer to fall maturation and between sexual maturity to kelt emigration. Lipid content decreased 48.0% from late summer to fall maturation, 16.1% from fall maturation to sexual maturity, and 33.3% from sexual maturity to kelt emigration. Protein content decreased by 5.6% from late summer to fall maturation, 10.9% from fall maturation to sexual maturity, and 8.0% from sexual maturity to kelt emigration. Total energy decreased 16.3% from late summer to fall maturation, 13.7% from fall maturation to sexual maturity, and 19.2% from sexual maturity to kelt emigration. These changes clearly show that lipid is preferentially used over protein, especially during early sexual maturation and the act of spawning. Protein use did increase between fall maturation and sexual maturity, but never exceeded the proportion of lipid being used for energy. Only 2.6% of the entire starting quantity of lipids remained in kelts following spawning, which indicates that protein is the primary somatic energy source for kelt emigration (Table 1).

Table 1. Proportional change in median white muscle lipid, protein, and total energy values between late summer maturation to kelt emigration in spawning year 2011.

Maturational transition	Time between transitions (months)	Lipid	Protein	Energy
Late summer to fall maturation	1	48.0%	5.6%	11.3%
Fall maturation to sexual maturity	5	16.1%	10.9%	13.7%
Sexual Maturity to kelt emigration	1 to 2	33.3%	8.0%	19.2%
	Accumulative total	97.4%	24.6%	44.8%

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.

Our reporting on comparisons of upriver weirs with downstream migrating stocks will be completed with manuscripts prepared in the final quarters of this year. A manuscript on smolt characteristics is nearly complete for submission. Bryan Jones will be working on a manuscript about the weirs, and migration characteristics in July after the field season of tagging kelts for the US Army Corps funded project is completed.

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

We reported last quarter about the summary of data from kelts tagged in 2010 with acoustic tags. Of these 74% were detected at at least one location below Bonneville Dam. We found that kelts that were not detected had the lower nutritional plasma metrics than those detected.

The electrolytes sodium, chloride, and magnesium were highest in kelts detected last at the ocean mouth, as was protein. We anticipate using these comparisons in manuscripts characterizing successful kelts.

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

The focus in 2011 was to implant acoustic transmitters in kelts from the Potlatch River and Fish Creek. Although runoff conditions prevented sampling throughout the season we had considerable success in characterizing the migration. We detected 100% of the kelts during downstream migration and all kelts were detected at or below the mouth of the Clearwater River. Thirty eight kelts (88%) were detected at our receivers in the Lower Granite Dam forebay; however, only 4 (9%) were detected by the receivers in the lower Columbia River below Bonneville Dam. We will compare the blood plasma metrics using successful fish with those not detected in manuscripts prepared from these data.

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

From 2009 to 2011, we sampled, PIT-tagged, and released a total of 324 kelts at the Clearwater River tributary weirs, and learned that these fish were not likely to be detected in the juvenile bypass systems. Of the kelts sampled and tagged at Lower Granite Dam the probability of detection was higher, and was correlated with good fish condition. The corner collector at Bonneville Dam provided the most detections of fish after Little Goose Dam. As reported earlier, the proportion of fish documented as repeat spawning throughout our study has been small. No kelts sampled at tributary weirs in 2009 or 2011 were detected as repeat spawners. One kelt (female, 560 mm) tagged at the Little Bear Weir in the Potlatch River in April of 2010 was detected migrating upstream at Bonneville Dam in August of that same year (consecutive spawner). In 2009, we found two female fish (1.02% of the 2009 Lower Granite Dam samples) were detected in July and August of 2010 (skip spawners). Neither fish had been detected during downstream migration as kelts after the Lower Granite Dam capture and release. During their repeat spawning migration both of these fish were detected at tributary sites. In 2010, 1,398 kelts were tagged and released into the tailrace of Lower Granite Dam, and four females and one male were detected beginning their second spawning runs in 2011 as skip spawners. Three of these fish were detected in 2010 during their downstream kelt migration at the corner surface collector at Bonneville Dam. During their repeat spawning runs in 2011, all five steelhead were detected migrating upstream at

the Bonneville Dam fish ladders, but the male was not detected again after that Bonneville Dam. The four females were all detected passing Lower Granite Dam, and three were detected at tributary sites of the Salmon River (Big Creek in the Middle Fork Salmon River tributary; Hayden Creek in the Lemhi River drainage; and Yankee Fork of the Salmon River).

Problems Affecting Progress

Student Zachary Penney continues to synthesize data, and should have completed two publication dissertation chapters by the end of the spring semester, but has more chapters to complete. We are making progress in preparing manuscripts from these studies, but will still be delayed in completing them all before the end of June. We will be seeking ways to provide additional funds to support publications and travel for Zachary Penney.

Appendix

Histological Assessment of Organs in Sexually Mature and Post-spawning Steelhead Trout and Insights into Iteroparity by Zachary L. Penney and Christine M. Moffitt.

Section E: Master Plan Progress

MILESTONES/ACCOMPLISHMENTS

- Secured transport permits for Snake River Steelhead Kelt transfers.
- Successfully transported 124 steelhead kelts from Lower Granite Dam (LGR) to DNFH.
- Successfully air-spawned 143 steelhead at DNFH
- Outplanted 9 reconditioned steelhead kelts into the Snake River
- Assisted other steelhead kelt researchers with fish collection and telemetry tagging.
- Hired Chris Beasley of Quantitative Consultants, Inc. to assist with Master Plan development and writing (September)
- Invited researchers from various agencies to participate in Master Plan Work Group (December)
- Submitted Master Plan draft of alternatives, alternative development and the alternative selection process (essentially Chapters 4 and 5 below) to Work Group for comments and review (January)
- Received comments and review results (February-March)
- Incorporated comments into new draft which is currently undergoing an in-house (NPT) review (May-June)

COORDINATION AND MEETINGS

- Clearwater Pre-AOP Meeting, Clearwater Hatchery – January 19, 2012
- BPA Snake River Steelhead Kelt Management Plan meeting/conference calls, Portland – February 2, March 26, August 29, September 27, October 3, November 1, and December 6, 2012
- Clearwater AOP Meeting, Clearwater Hatchery – February 16, 2012
- Kelt Research Field Operations Coordination, PNNL/Battelle – March 19, 2012
- Pre-Research coordination meeting at Lower Granite Dam – March 26, 2012
- Developed working MOU with DNFH covering on-site kelt collection and kelt recondition– July 16, 2012
- DNFH kelt reconditioning tanks alternative location comparison presentation – August 29, 2012
- Steelhead Kelt Management MOU between COE and BPA conference call – September 27, 2012
- Steelhead Air-Spawn Collection Coordination, DNFH – December 5, 2012
- CRITFC and representatives from Quantitative Consultants, Inc – December XX, 2012
- BPA Snake River Steelhead Kelt Management Plan meeting/conference calls, Portland – January 24 and February 19, 2013
- Clearwater Pre-AOP Meeting, Clearwater Hatchery – January 10, 2013
- Air-spawn training and demonstration, Coleman National Fish Hatchery – January 31, 2013
- Clearwater AOP Meeting, Clearwater Hatchery – February 14, 2013

- Kelt Steelhead BiOp inquiry on B-run definition and determination of what constitutes adult return credit – February 28, 2013
- Steelhead Status and Management Review, Lapwai – March 14, 2013
- Pre-Research coordination meeting at Lower Granite Dam – March 4, 2013
- Review Master Plan comments with representatives from Idaho Department of Fish and Game – April 5, 2013

In addition to the above meetings, we conducted weekly management calls with DNFH and regular steelhead return and broodstock collection meetings.

FUTURE PLANNED ACTIVITIES

Coordination

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

Kelt Reconditioning

Develop strategies to bolster collection numbers.

Continue to experiment with air-spawning to augment specimens.

Pursue other collection sites such as tributary weirs and other mainstem dams.

Improve environment during collection and holding.

Increase primary water supply reliability.

Develop feed quality.

Minimize handling stresses.

Master Plan

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

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

Submit draft with Master Plan Work Group's comments incorporated for wider co-manager review (June)

Master Plan Outline

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. Coordination with BPA's Snake River Management Plan process with co-manager input has helped focus these research needs.

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: 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 PIT tag detection of repeat spawners, as well as added to the database of stream origin and helped identify run-portions of different life history types (skip-spawners, potamodromous/over winter, etc.).

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: Carefully determine a working definition of B-run steelhead and how this determination will apply toward adult return credit. Finalize minimum kelt reconditioning criteria. Updated 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. We currently have data from four reconditioning locations over multiple years. Dam passage route data are being collected and analyzed. The results may impact kelt collection locations and scale of collection activities.

CHAPTER 5: PROPOSED MANAGEMENT ACTIONS

Development of Alternative Management Actions

Hydrosystem Modifications and Their Effects on Kelt Survival

Description of the Criteria Used to Evaluate Actions

Evaluation of Management Actions Using Established Criteria

Alternative Action Assessment

Kelt Reconditioning Preferred Alternative

Target Population

Program Size

BiOp Credit

Facility Alternatives

Facility Specifications

Opportunities at Existing Facilities

Modification of a Fish Transport Barge

Construction of a new Facility

Management Action Implementation

Preferred Facility Alternative

Indicators of Success and Failure

Items to complete: Much of the past two 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. 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 (Chapter 4 note on B-run definition, etc). 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: 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.

Chapter 4. Reproductive Success Evaluation

Written by

Doug Hatch

Shawn Narum

Jeff Stephenson

Ryan Branstetter

Jeremiah Newell

Andrew Pierce

John Whiteaker

Neil Graham

Columbia River Inter-Tribal Fish Commission

David Fast

Bill Bosch

Todd Newsome

Tim Resseguie

Joe Blodgett

Yakama Nation Fisheries Program

Rhonda Dasher

Confederated Tribes of the Colville Indian Reservation

Albert Santos

Jim Gidley

Confederated Tribes of the Warm Springs Reservation

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

Introduction

Reproductive success is difficult to observe in the field. Steelhead, in particular, are problematic as migration and spawn timing are 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. The design of this study is to collect hatchery-origin prespawn adults and transport them to the hatchery to evaluate them in a more controlled environment. We initially began this experiment utilizing Skamania stock steelhead, a highly aggregated stock. In 2010, we also collected locally adapted winter steelhead for comparative purposes while phasing out the Skamania portion of the experiment. In 2011 and 2012, only winter steelhead were collected.

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 play an important role in 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 receives surface water from the Middle Fork of the Hood River and spring water from Rogers Creek. 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 spring Chinook.



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

Methods

For collection and reconditioning methods see Chapter 1 Sections A and B. Fish were identified primarily using PIT-tags to identify fish throughout the reconditioning and evaluation period, for this portion of the report the last 4 digits of the PIT tag (ID:xxxx) are referenced for each individual fish. 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 effect. Female gametes were collected by air spawning (Leitritz and Lewis 1980) as seen in Figure (2).



Figure 2: Air spawning 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). Approximately 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 (Figure 3). This minimized the loss of sample units as a result of positive disease tests. 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 iodophor povadine (1:100 ppm) (Argentyne) prior to placement into vertical stack incubators. Eggs were incubated at 5.5°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 they work as good rearing troughs for the fry life stage. The offspring from each female 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°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 a rate of 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

We compared the performance of artificially reconditioned repeat spawner 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 steelhead with maiden spawners that were collected and air spawned in 2012. The same individual performance metrics were used for the group comparison. A final comparison will be made of all brood years in the 2013 annual report.

For clarity it should be noted that steelhead kelts exhibit at least two strategies for repeat spawning, sequential and skip spawning (Burgner et al. 1992). In the sequential strategy a kelt stage steelhead emigrates the tributaries in the spring of the year, spends the late spring and early summer in the ocean and then returns to the Columbia River in the late summer (August through November) or, in our case, spawns the following spring. In the skip spawner strategy, the fish spend an additional year in the ocean and return in the late summer of the following year or, at Parkdale, an additional year or occasional rare two years at the hatchery. Both sequential and skip spawner strategies were exhibited by individual kelt steelhead in our study. These repeat spawning strategies provide a backdrop for understanding the reporting of individual fish in the study. Individuals were collected and spawned as maidens, then held, reconditioned and spawned as a repeat spawner either one or two years later.

Maiden Collection and Spawning

2012 Winter Steelhead Broodstock Collection

As mentioned in Chapter 1, Section A, we collected 22 female fish for spawning with milt that was cryopreserved from the same age class. We successfully spawned the 22 female kelts over a period of mid-April 2012 to early May 2012 (Chapter 1, Section B).

Eggs

The estimated average fecundity of the 22 female 2012 brood maidens spawned was 4,807 eggs with a minimum of 2,672 and maximum of 8,976. Average fertilization success based on eyed egg survival was 66%. Keel samples taken at day 15 averaged 65% which suggests that there was little difference from initial fertilization and the later eyed egg average.

Juveniles

The average starting weight was at 0.22 g per fish with an average increase of 0.66 grams for an average ending weight of 0.88 grams at week 10. The average starting length of fish was 3.0 cm with an average increase of 1.3 cm with an average final length measure of 4.4 cm over a 10-week period.

Sequential Spawners

There were two brood year groups which produced sequential spawners in 2012, the 2011 and 2010 winter broods each produced a kelt spawner. The kelt from the 2010 brood year is on the second sequential spawning event, meaning it has spawned three times consecutively since 2010. As was mentioned in Chapter 1 Section A, there were not many remaining kelts from the 2011 brood year at the beginning of 2012 (3 kelts). We believe that this low reconditioning rate may have been due to the first year of capture and hauling from the East Fork of the Hood River trap. Many fish were observed to have many lacerations or severe abrasions that later became infected with fungus which resulted in latent mortality. These types of mortality are not unusual at small weir sites as capture box placement and timing of retrieval has resulted in poor kelt reconditioning candidates (Shitike Creek, Oregon in Branstetter et al. 2010) Another additional factor was the limited prophylactic treatment in 2011 which led to increased bacterial disease related mortalities. This limitation of the prophylactic treatment was done at the behest of hatchery manager's desire to reduce handling of fish which he felt was more detrimental to fish survival.

2011 Winter Brood

Eggs

The estimated fecundity of the spawning 2011 winter brood ID:9565 was 4,563 eggs. This was a small decline (-189 eggs) from the previous year of spawning (2011) (Figure 5). Egg fertilization was extremely low for this fish in 2012 with only about 3% fertilization compared to 39% for 2011. Keel samples in 2012 indicate that there was no loss in eggs prior to eyed egg counts.

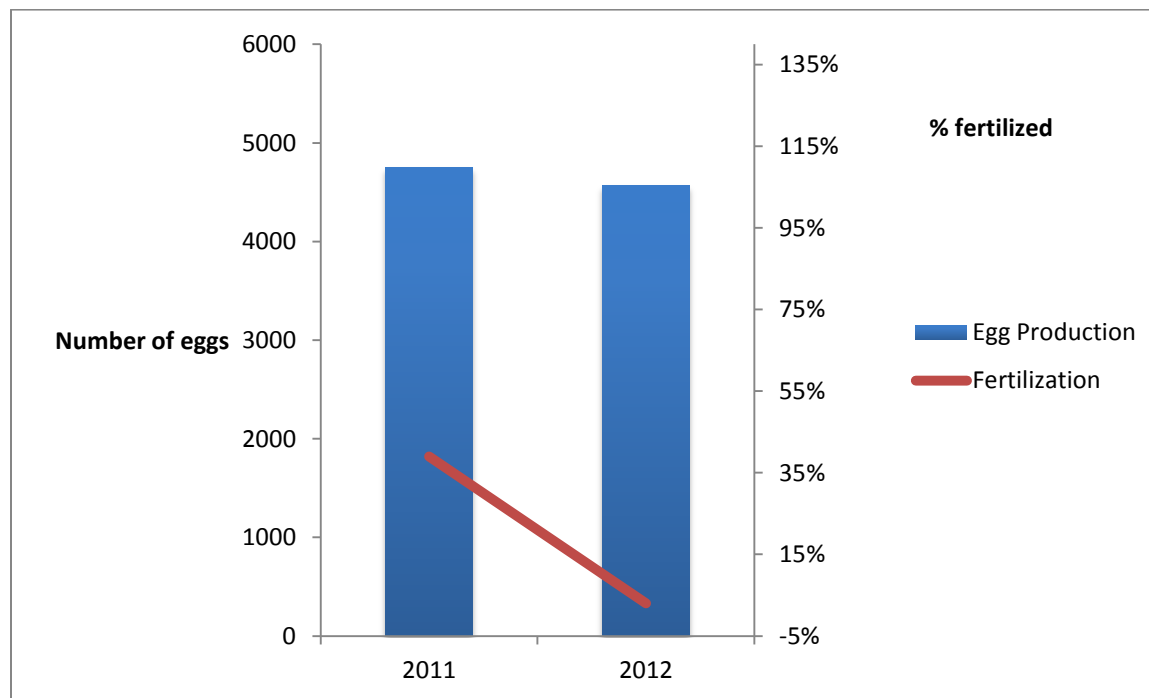


Figure 5. Average egg production (left side) and percent fertilization (right side) from maiden and sequential spawning of kelt 9565.

Juveniles

The change in weight for fry was compared at week 6 instead of the standard week 10 due to a technician error in 2011, which left weeks 7-10 unreliable. The average change in fry weight for this spawner was 0.23g while maiden spawning was 0.21g. (Figure 6). The 2012 progeny weighed less and ended smaller than in 2011 with an overall decline of .05g (Figure 6). Juvenile progeny in 2012 increased in length by 7mm, this was a millimeter of length difference for this fish's progeny in 2011 (6 mm) (Figure 7). The 2011 fish started slightly longer and ended longer than the 2012 fish (Figure 7).

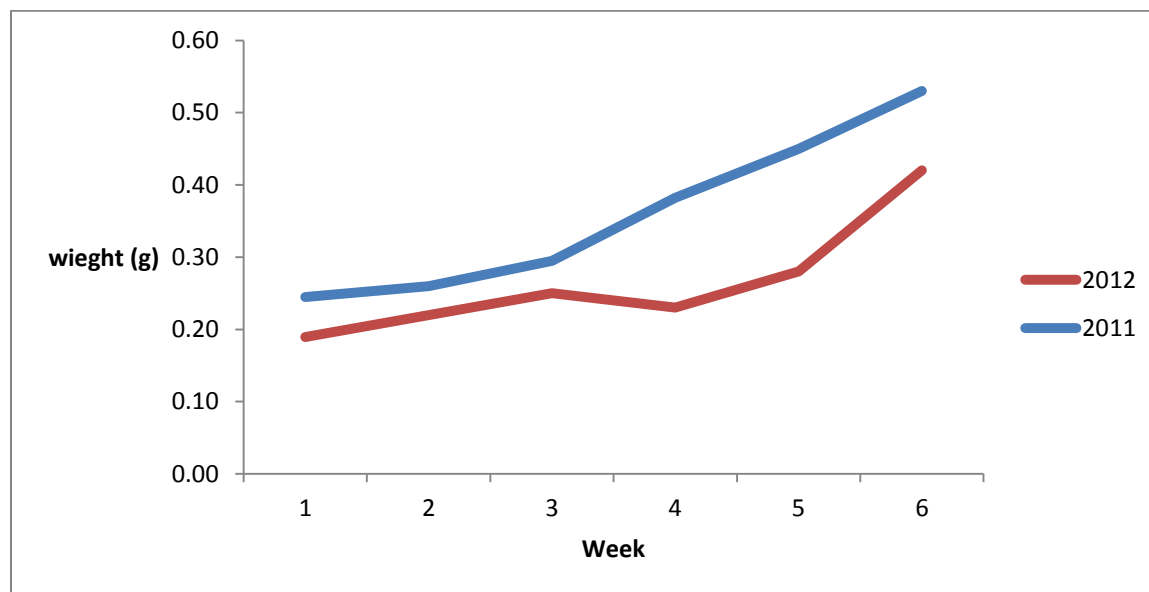


Figure 6: Juvenile weight difference of maiden (2011) and kelt spawning (2012) by sample week.

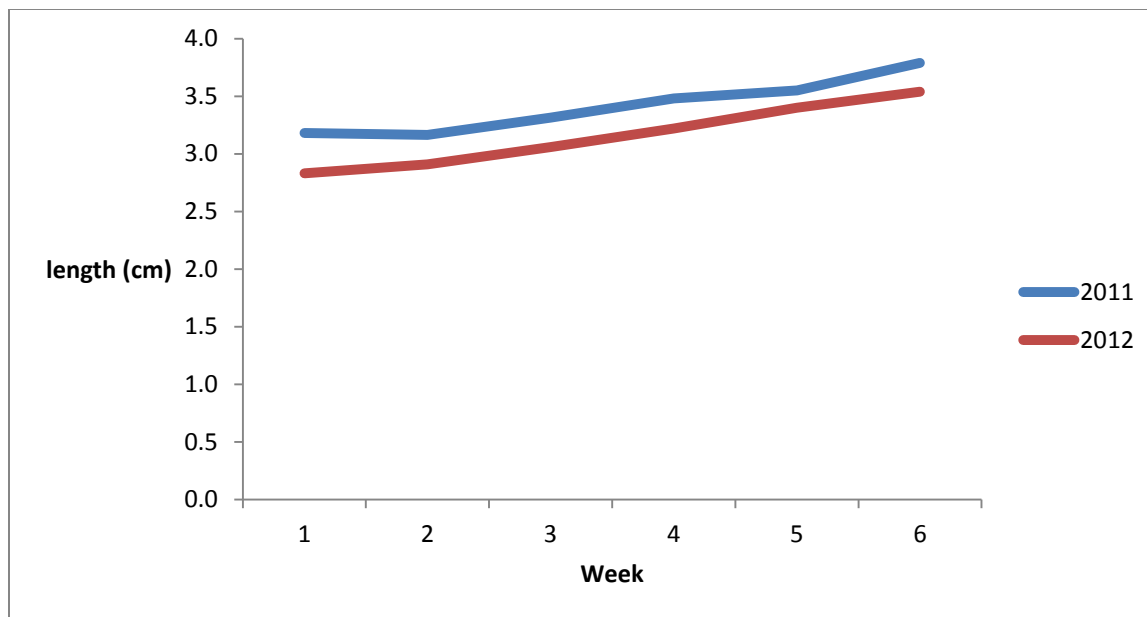


Figure 7 Juvenile length difference of maiden (2011) and kelt spawning (2012) by sample week.

2010 Winter Brood

Eggs

The estimated fecundity of the female 2010 winter brood sequential spawner was 5,904 eggs produced (Figure). During the maiden spawning this fish produced an average of 4,212 eggs (Figure 6).

Comparing the egg production of ID:2808 across spawning years; this fish had a small decrease in egg production from its first kelt spawning in 2011, but increased fertilization in the kelt spawning provided an additional 1692 viable eggs (Figure 8).

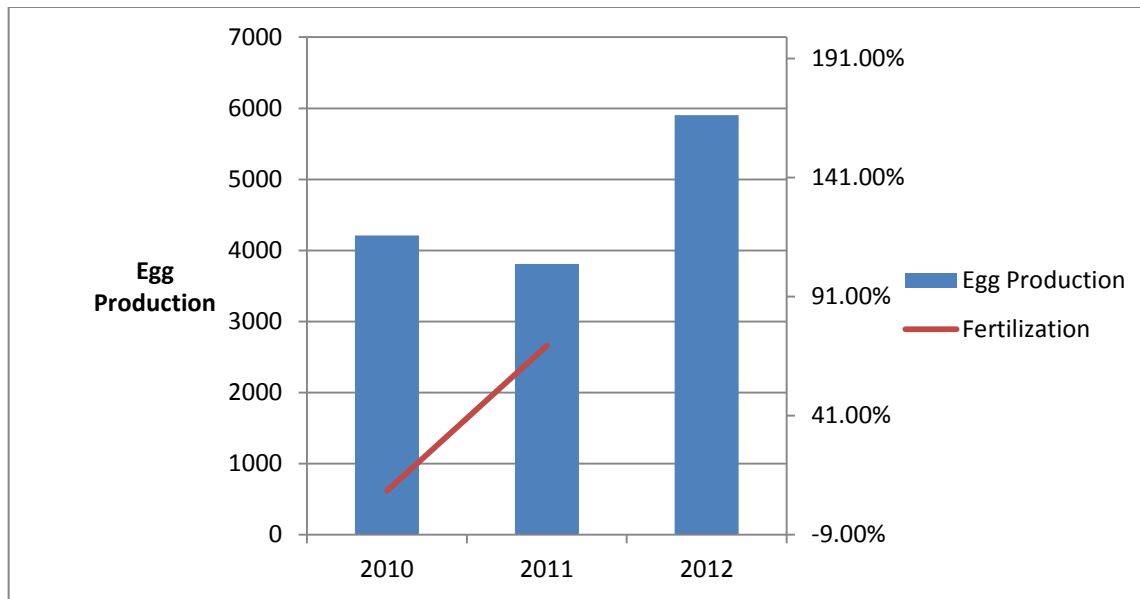


Figure 8: Average egg production (left side) and percent fertilization (right side) from maiden and sequential spawning of kelt 2808. All eggs water hardened, no fertilization to record in 2012.

Juveniles

No fertilization; fish died just prior to spawning of likely *C. shasta* infection. The eggs water hardened, but egg production was recorded.

Skip Spawners

The 2010 and 2009 Skamania broodstocks both had skip spawners in 2012. The 2009, kelt (ID:2728) was not a typical skip spawner, this fish took two years to remature before spawning for a second time. We have observed PIT-tag detections in a few Yakima River kelts that demonstrate this longer skip spawner strategy, that takes two-years at sea to remature versus the more typical single-year, but it is extremely rare.

2010 Skamania Broodstock

Two females fish ID:8738 and ID:2402 spawned as maidens in 2010 and skip spawned in 2012. Weeks 1-8 were compared for changes in growth characteristics.

Egg

The egg production in 2012 for ID:8738 was 5,540 eggs and 5,712 for ID:2402 (Figure 7 and 8). Both of these fish had an increase in their egg production from 2010. Kelt ID:8738 had the largest increase with an additional 2530 eggs, while ID:2402 was negligible with only an additional 16 eggs in 2012 (Figure 7 and 8). Fertilization for the two fish varied significantly, with ID:8738 increasing in fertilization by almost 50% while ID:2402 had a 60% decline in fecundity (Figure 9 and 10). This is likely the result of this fish being overripe as a number of eggs (1%) were water hardened (Moksness et al. 2008). Keel samples matched eyed egg counts for ID:8738 and were very similar for ID:2402 with a difference of approximately +3% precluding any pre-shock mortality.

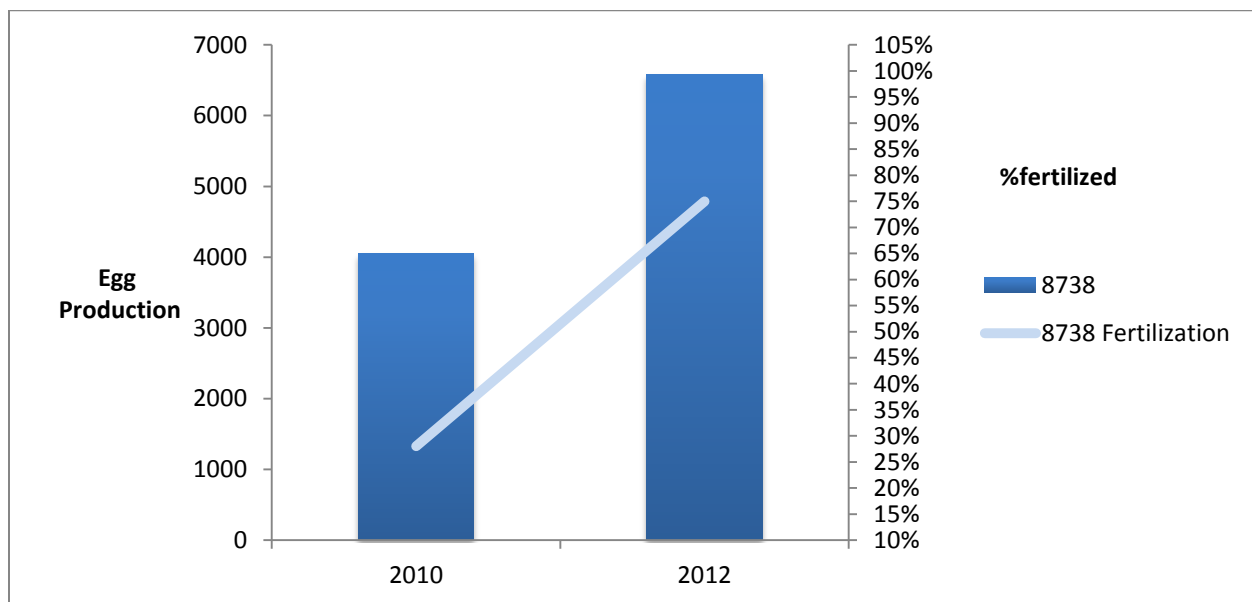


Figure 9: Maiden and skip egg production of 2010 Skamania ID:8738 (2010 and 2012) percent fertilization (right side).

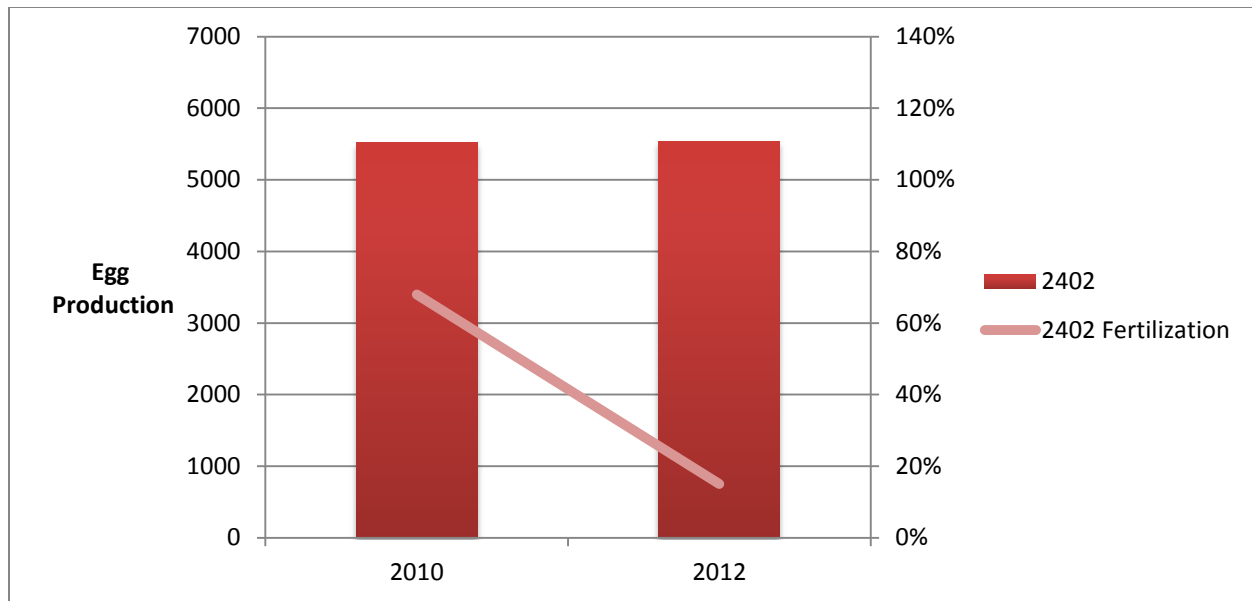


Figure 10: Maiden and skip egg production of 2010 Skamania ID: 2402 by year (2010 and 2012) percent fertilization (right side).

Juvenile

The growth (change in weight and length) of progeny from 2010 to 2012 for ID:8738 had a slight decline in weight -0.03 g and a decrease in length of -.81 in 2012 (Figure 9 & 10). The decline in weight was greater for ID: 2402 with a decrease in weight of -0.29 g and decrease in length of -1.0cm. (Figures 11 and 12).

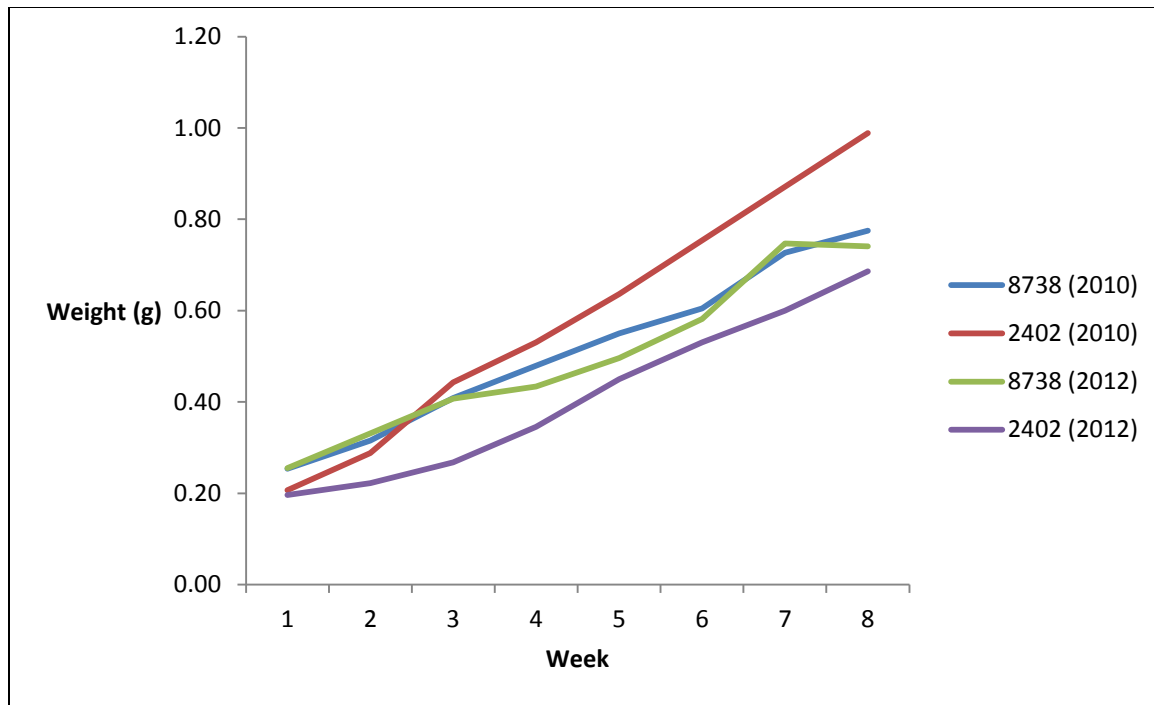


Figure 11: Juvenile weight difference of maiden (2010) and kelt spawning (2012) by sample week.

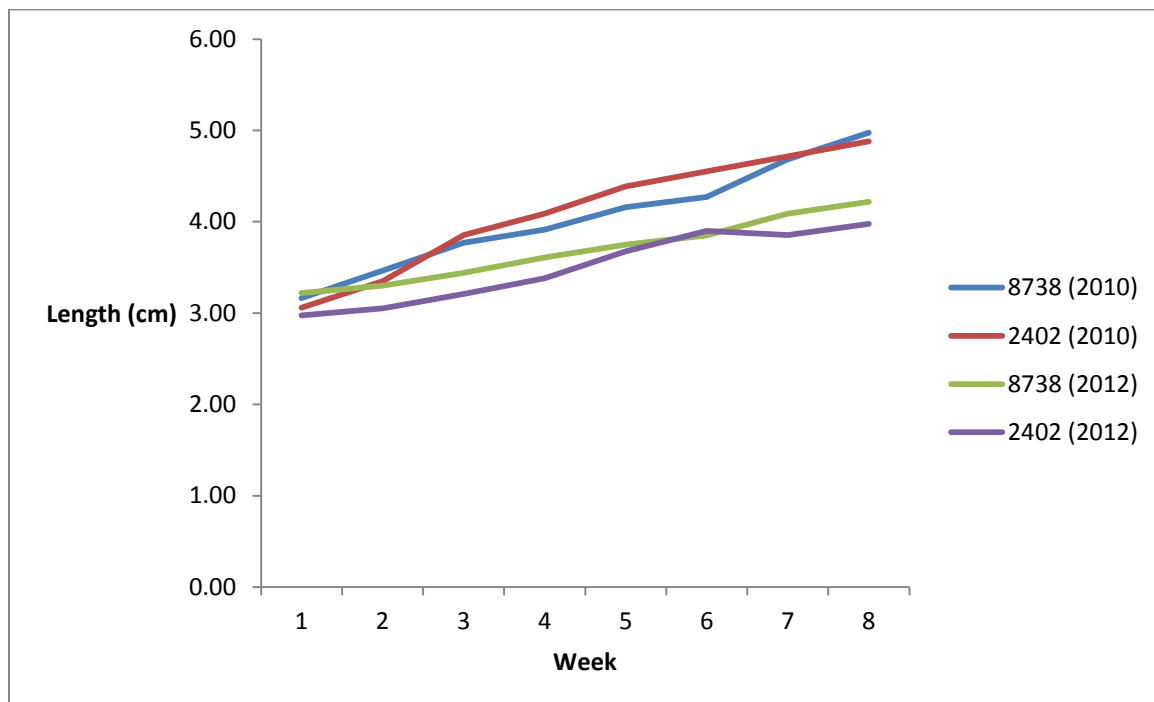


Figure 12: Juvenile length difference of maiden (2010) and kelt spawning 2012 by sample week.

2009 Skamania Broodstock

The last remaining 2009 Skamania brood fish (ID:2728) spawned in 2012. This fish skipped two years before spawning again. As previously noted, this is not a typical spawning pattern observed in kelts.

Egg

Kelt ID:2728 had an increase in egg production, with an additional 764 eggs (Figure 13). The egg fertilization rate remained unchanged at 79% (Figure). Keel rates were about 5% lower than eyed rates, demonstrating that there was little difference from fertilization to eyed egg stage. This increase in egg production and fertilization is a typical kelt response to reconditioning at Parkdale (Branstetter et al. 2011).

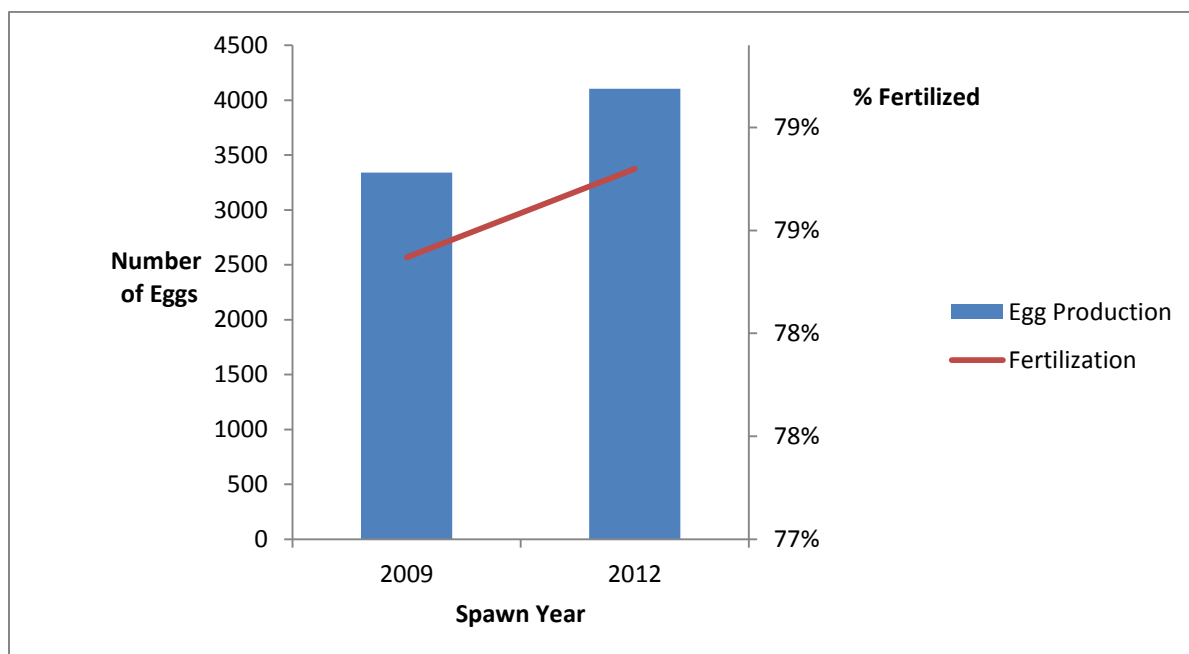


Figure 13. Average egg production (left side) and percent fertilization (red line) from maiden and sequential kelt spawning of steelhead ID 2728.

Juvenile

The growth (length and weight) of progeny from ID:2728 increased in 2012. The difference in brood juvenile weight from week one to week ten in 2012 was 0.77 grams while the difference in length was 1.5 cm (Figures 14 & 15). When comparing the temporal differences of the two spawnings of 2012 and 2009, the result is negative for both groups with a weight decrease of -0.03 g and length decrease of -

.19cm (ID:2728) (Figures 14 and 15). This decline is not untypical due to variation in individuals from year to year and the difference is not extremely large (Branstetter et al 2011 and Hatch et al 2012).

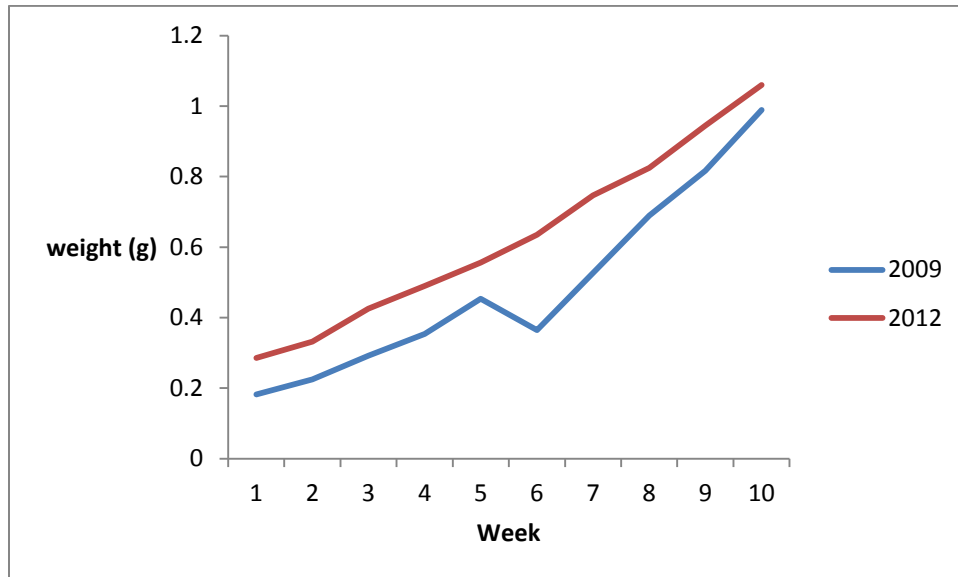


Figure 14: Juvenile weight difference of maiden (2009) and kelt spawning (2012) by sample week of steelhead ID:2728.

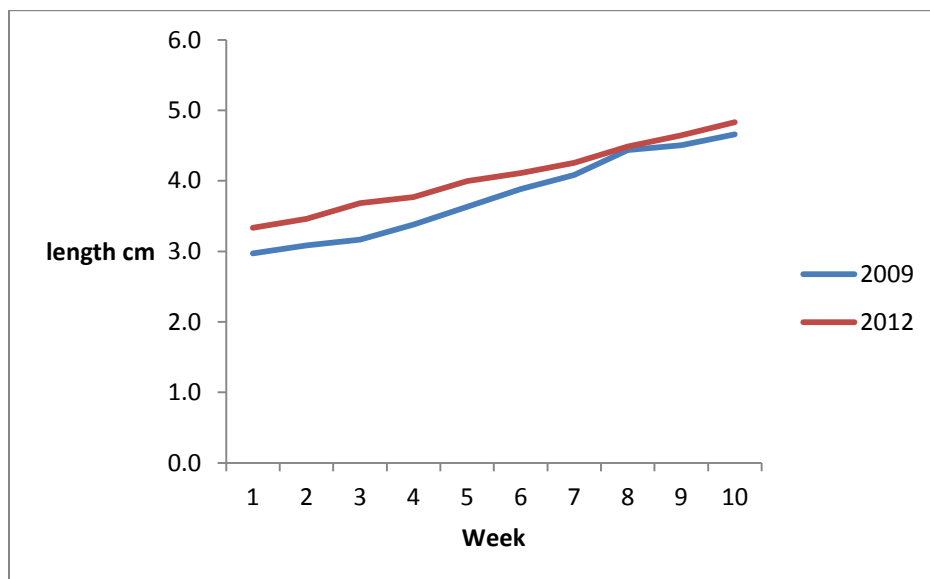


Figure 15: Juvenile length difference of maiden (2009) and kelt spawning (2012) by sample week of steelhead ID:2728.

Cumulative Skamania Maiden Spawners versus Skamania Kelt Spawners 2006-2012.

In this section we compare maiden and kelt spawners using 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 16 post maiden spawning events).

Repeated Measures General Comparison of Skamania Spawners 2006-2012

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 on average 900+ more eggs than maiden spawners (Table 1). 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 1). 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 (51%) (Table 1). 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 1 and 2).

Table 1: Mean egg production from 2006-2012 with mean for each spawning number and year. NB= No Brood for that year.

	Year							
Spawning	2006	2007	2008	2009	2010	2011	2012	Mean
1	4618	3646	4061	2745	3767	NB	NB	3767
2		3208		6160	5569	3952	5227	4823
3					5892			5892
4						6552		6552
Mean	4618	3427	4061	4453	5076	5252	4104	5259

Table 2: Mean egg fertilization based on eyed eggs from 2006-2012 with mean for each spawning number and year. NB= No Brood for that year.

	Year							
Spawning	2006	2007	2008	2009	2010	2011	2012	Mean
1	75%	64%	38%	67%	57%	NB	NB	60%
2		17%	49%	68%	50%	64%	56%	51%
3					49%			49%
4						33%		33%
Mean	75%	40%	43%	67%	52%	48%	56%	48%

In 2006 and 2007 fish were reared at the University of Idaho Aquaculture Research 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 3). The biggest year difference in weight was the skip spawning 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 3). 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 3). 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 3). Comparing the sequential and skip spawning 2008 brood fish against the maiden spawning in 2008 there is a length difference of 0.4 cm (skip) and 0.5 cm (sequential). The 2008 brood year change in length was larger than (table 9) other brood year progeny growth factors. 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 3). 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 3 and 4).

Table 3: Mean juvenile weight gain (grams) from 2006-2012 with mean for each spawning number and year. Spawning 1 is the maiden spawning year.

	Year							
Spawning	2006*	2007**	2008	2009	2010	2011	2012	Mean
1	0.6	3.33	0.73	0.77	0.81	NB	NB	1.25 (.77)***
2		3.16	0.72	0.74	1.07		0.72	1.42 (.81)***
3					0.82			0.82
4						0.78		0.78
Mean	0.6	3.24	0.73	0.76	0.9	0.78	0.72	1.07 (.80)***

NB=No Brood Reared

* Raised at University of Idaho ARI. Chilled Water Temperature at 11.1⁰C. Reared in small circular tanks.

** Raised at University of Idaho ARI. Non-chilled water temperature at 14.4⁰C. Reared in small boxes.

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

Table 4: Mean juvenile length from 2006-2012 with mean for each spawning number and year

	Year							
Spawning	2006*	2007*	2008	2009	2010	2011	2012	Mean
1	NA	NA	1.6	1.6	1.8	NB	NB	1.7
2				1.7	2.1		1.4	1.7
3					2			2
4						1.4		1.4
Mean	NA	NA	1.6	1.7	1.9	1.4	1.4	1.7

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

NB=No Brood Reared

Fry Mortality

The average fry mortality rate was 16% over 10-weeks of rearing for all groups combined. The range of mortality observed was 6-28% with the winter 2012 brood progeny individuals having both the lowest and highest mortality rates of all reared fish in 2012 (including ketl progeny). Overall, this rate is higher than in previous years at Parkdale, where average mortality was observed at 1-2%. Mortalities occurred throughout the 10-week experiment, but they were highest after transfer from vertical stack incubators to the picking troughs. Once fish were transferred, tank cleaning became the primary cause of mortality. The small fish were highly susceptible to getting caught under the cleaning brush or sucked through the vacuum. CRITFC technicians cleaned the troughs during the week and for the first year Parkdale staff assisted on the weekends. Inexperienced weekend staff and an aggressive effort to keep the troughs thoroughly clean, at the behest of the hatchery manager, also played a role in the higher mortality rates.

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 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 looked positive in early 2011. We had a good number of repeat spawners (5 kelts) in 2011 from the 2010 brood year with progeny showing little difference from the 2010 spawning. Most of the 2011 brood fish died shortly after spawning by late summer and early fall and by the time spawning time came around there were only 3 kelts left. We suspect that fish quality has declined with the new trap as successful reconditioning has also decreased. The 2011 brood year kelts suffered numerous fungal infections and numerous stress related diseases that were not observed in other brood years. The hatchery manager has also noted that many of the other brood fish (chinook and steelhead) have also been in poorer shape than in his prior years (>20) of experience at the facility (pers. conv. Gidley, J., 2013).

To date we have observed at Parkdale that steelhead kelts may be foregoing active growth and instead directing the majority of energy into egg production (Quinn et al. 2010). This observation is consistent with the short life spans of the 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 only 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). This would 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. Currently we have observed natural reproduction in both Omak Creek and Yakima River systems. Following progeny throughout the rearing and release would be ideal but there are several logistical hurdles to accomplish this goal. The first and biggest hurdle is to get a multi-agency (ODFW and Warm Springs Tribe) agreement in place. This is unlikely, since the ODFW district biologist (Rod French) stated he does not want a kelt reconditioning program in the basin. Additionally, another agreement would be needed with the ODFW hatchery (Oak Springs) to rear juveniles since Parkdale has limited rearing space and rearing a large number of fish is necessary to do an effective smolt to adult comparison. So far, our data suggests that a summer kelt program seems to have more potential than a winter one, especially since there is evidence of a distinct summer population (Matala 2009) which is extremely depressed in the Hood River basin and would benefit from a kelt reconditioning program.

References:

- Araki, H., W.R. Ardren, E. Olsen, B. Cooper, and M.S. Blouin. 2007. Reproductive success of captive-bred steelhead trout in the wild: evaluation of three hatchery programs in the Hood River. *Conservation Biology*. 21(1): 181–190.
- Beckman, B. R., D. A. Larsen, B. Lee-Pawlak, and W. W. Dickhoff. 1998. Relation of fish size and growth rate to migration of spring chinook salmon smolts. *North American Journal of Fisheries Management* 18:537-546.
- Branstetter R., J. Stephenson, A. Pierce, D. Hatch, B. Bosch, D. Fast, J. Blodgett, M. Johnston, T. Resseguie, R. Dasher, C. Baker, A. Santos, J. Gidley, C. Brun, J. Lyman, J. Graham., L. Holliday, A. Santos, C. Gehling, C. Moffitt, J. Nagler, J. Buelow, Z. L. Penney, J. Boyce, L. K. Caldwell, T. Caileer, B. Jones, B. Sun, and J. Egan. 2011. Steelhead Kelt Reconditioning and Reproductive Success. 2010 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-00. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.
- Gidley, J., personal communication May 1, 2013.
- Hatch D.R., R. Branstetter, J. Stephenson, A. Pierce, J. Whiteaker, and B. Bosch. 2012. Steelhead Kelt Reconditioning and Reproductive Success. 2011 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-000. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.
- Leitritz, E. and R.C. Lewis. 1980. Trout and Salmon Culture. Division of Agricultural Sciences. University of California.
- Matala AP, French R, Olsen E, Ardren WR (2009) Ecotype distinctions among steelhead in Hood River, Oregon, allow real-time genetic assignment of conservation broodstocks. *Transactions of the American Fisheries Society*, 138, 1490–1509.
- Moksness, E., E. Kjorsvik, Y. Olsen. 2008. Culture of Cold-Water Marine Fish. John Wiley & Sons.
- Oregon Department of Fish and Wildlife. 2008. Grande Ronde Basin Spring Chinook Salmon Captive Broodstock Program. 2008 Annual Operating Plan. Northeast Region Fish Research and Development, Oregon Department of Fish and Wildlife, La Grande.
- Pennel W. and Barton B.A., (Eds.) 1996. Principles of Salmonid Culture. Elsevier. Amsterdam, Netherlands.

- Quinn, T. P. 2005. The behavior and ecology of Pacific salmon and trout. American Fisheries Society, Bethesda, Maryland.
- Quinn T.P., T. R. Seamons, L. A. Vøllestad, E. Duffy. 2011. Effects of Growth and Reproductive History on the Egg Size-Fecundity Trade-off in Steelhead. 15 February 2011. Transactions of the American Fisheries Society
- Seamons T.R., T. P. Quinn. 2010. Sex-specific patterns of lifetime reproductive success in single and repeat breeding steelhead trout (*Oncorhynchus mykiss*). 2010. Behavioral Ecology and Sociobiology 64:505–513.
- Wedemeyer G.A., (Ed). 2002. Fish Hatchery Management Second Edition. North American Journal of Fisheries. Bethesda, Maryland.
- Zabel R.W., A. Achord. 2004. Relating Size of Juveniles to Survival within and Among Populations of Chinook Salmon. Ecology 85(3), pp. 795-806.

Section B: Omak Genetics Report

Introduction

The reproductive success of long-term reconditioned kelts needs to be explored to assess the net benefit of the kelt reconditioning 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 artificially reconditioned kelt reproductive success compare with natural repeat spawner success, and 3) How does artificially reconditioned kelt reproductive success compare with first time spawner success? 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.

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. Adults were collected at the weir in 2012 (n=137), and two individuals that were collected in 2011 were resampled or detected by PIT array in 2012.

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 2012, electrofishing started at the mouth of Omak Creek, and went as high as the barrier falls. Six of the sections are described in Miller (2013). The other eight sections are all located between the mouth of Omak Creek and the upper end of Moomah Road.

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. In 2012 two of the fish reconditioned in 2011 were detected by the PIT tag antennae. One fish was not detected again, while the second was captured at the adult weir following spawning.

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

Genetic Analysis

Fin tissue samples were collected and stored dry on whatman paper, or paper slips in coin envelopes 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. Current genotyping efforts utilize the 192 Single Nucleotide Polymorphism (SNP) markers and methods described in Hess et al. (2012). Statistical analysis used 188 of the 192 markers. Of the four not used, three are diagnostic for cutthroat, and one marker (OmyY1_2SEX) is a sex-determining marker that was used only to determine fish gender for the study. Significant linkage disequilibrium was previously observed in one pair of loci: Omy_GHSR-121 and Omy_mapK3-103 by Hess et al. (2012). Because linked loci are still informative for parentage analysis, both linked markers were included in this study.

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). 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, had a minimum of 180 loci comparisons, and zero mismatches.

Results

A total of 352 samples were successfully genotyped. Numbers by life stage can be seen in Table (2). Of the 137 adult samples genotyped in 2012, 135 were putative first time spawners. Two fish were from

the 2011 capture, reconditioning and release efforts. There were 100 genotypic males, 35 genotypic females, and 2 undetermined individuals. Departures from Hardy-Weinberg equilibrium (critical level $=0.05 / 191 \text{ loci} = 0.000266$) were seen in both adults and juveniles. 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 unless the cause is allelic dropout.

Table 2. Population Statistics. Each collection is reported in terms of sample size (n), expected heterozygosity (H_E) and observed heterozygosity (H_O).

	n	H_E	H_O	HW
Omak Anadromous Adults	137	0.3115	0.3233	6
Omak juveniles	215	0.3123	0.3162	2
Total	352			

A total of 103 juveniles were successfully assigned back to at least one adult parent. Of the 103, 17 were assigned to a parent pair. Table (3) shows the number of age 0 offspring genotyped and successfully assigned by each site. Sites are listed starting at the mouth (Site 1) moving upstream to the barrier falls (site14). Overall, 48.4% of the juveniles genotyped were assigned to at least one parent. Section 1 had a single one of the 16 genotyped juveniles successfully assigned back to at least one parent.

Table 3. Parentage Assignments. Each section is reported in numbers of genotyped fish (n), and the number and percentage of successful assignments.

Section	n	Successful Assignments	
Section 1	16	1	6.3%
Section 2	18	6	33.3%
Section 3	19	10	52.6%
Section 4	13	7	53.8%
Section 5	14	3	21.4%
Section 6	12	3	25.0%
Section 7	16	7	43.8%
Section 8	14	8	57.1%
Section 9	19	11	57.9%
Section 10	12	6	50.0%
Section 11	12	8	66.7%
Section 12	11	7	63.6%
Section 13	17	13	76.5%
Section 14	20	13	65.0%
Summary	213	103	48.4%

A single female accounted for 11 of the 103 progeny that were assigned to at least one parent, while the majority of adults had no progeny assigned to them (Table 4 and Figure 1). The lack of reproductive success detection was more pronounced in males (73%) than females (48%). A small subset of these fish (5 males, 3 females) were likely removed prior to spawning when they were collected as hatchery broodstock .

Table 4. Progeny Per Adult. Percent of adults having between 0 and 11 offspring assigned to them

Progeny	Males	Females	Unknown
0	73.0%	48.6%	50.0%
1	10.0%	28.6%	50.0%
2	3.0%	5.7%	0.0%
3	5.0%	5.7%	0.0%
4	4.0%	2.9%	0.0%
5	3.0%	5.7%	0.0%
6	2.0%	0.0%	0.0%
7	0.0%	0.0%	0.0%
8	0.0%	0.0%	0.0%
9	0.0%	0.0%	0.0%
10	0.0%	0.0%	0.0%
11	0.0%	2.9%	0.0%

Figure 1. Progeny per adults. Number of adults having between 0 and 11 offspring assigned to them.

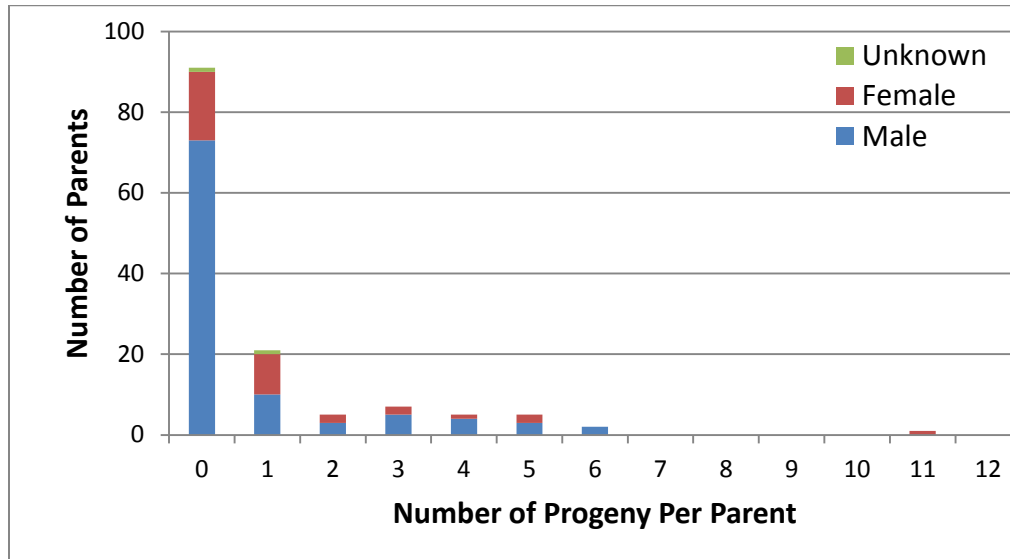


Table (5) shows a summary of reproductive success attributed to fish that went through the reconditioning process and returned to spawn again. Successful reproduction has been confirmed for four of the eleven reconditioned kelts that were detected returning to Omak Creek. Kelt progeny were not detected in 2012.

Table 5. 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

Reproductive success has been confirmed for four individuals following reconditioning efforts. 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.

The majority of adults had no detected offspring. Similar patterns have been seen in steelhead before, with very few adults accounting for most offspring (Seamons et al. 2004), and the majority of parents not having any detected offspring (Seamons and Quinn 2010). Of the juveniles, 48% were assigned to at least one parent. This is higher than 2011 when only 35% of juveniles were assigned. The remaining fish are likely progeny of fish that were not collected at the adult trap. This includes resident fish, precocial juveniles, migratory adults that spawned below the trap or in the mainstem Okanogan River, and migratory adults that bypassed the trap. This is consistent with the low parentage success seen in juveniles collected in Section 1 which was located in the first 0.3 km of Omak Creek. Previous years have shown that a high proportion of fish spawn below the trap (Arterburn et al. 2005, Fisher and Arterburn 2004, Miller et al. 2010, 2011, 2012), and additional juveniles may be actively swimming upstream following spawn events in the mainstem Okanogan River.

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 and 2012 demonstrated the ability to sample juveniles at a length that is identifiable, and will be repeated in future years.

Progeny from the two kelts potentially spawning in 2012 were not detected. However, only one of these fish was confirmed to have moved above the trap. Due to the limited number of kelts spawning, it is possible that lack of progeny detection is random, as most first time spawning fish also had zero progeny assigned to them. Additional age-0 samples were collected and are available for analysis. These samples will be added to the 2013 Genotyping effort, increasing the probability of detecting progeny of all adults.

References

- Arterburn, J., K. Kistler and R. Dasher. 2005. Okanogan Basin Steelhead Spawning Ground Surveys. Colville Confederated Tribes Fish and Wildlife, Anadromous Fish Division, Omak WA.
- Branstetter R., J. Stephenson, A. Pierce, D. Hatch, B. Bosch, D. Fast, J. Blodgett, M. Johnston, T. Resseguie, R. Dasher, C. Baker, A. Santos, J. Gidley, C. Brun, J. Lyman, J. Graham., L. Holliday, A. Santos, C. Gehling, C. Moffitt, J. Nagler, J. Buelow, Z. L. Penney, J. Boyce, L. K. Caldwell, T. Caileer, B. Jones, B. Sun, and J. Egan. 2011. Steelhead Kelt Reconditioning and Reproductive Success. 2010 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-00. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.
- Fisher, C., and J. Arterburn. 2004. Improvements of anadromous fish habitat and passage in Omak Creek; Includes 2003 steelhead surveys in Omak Creek. 2002-2003 Annual Report, Project No. 200000100, 23 electronic pages, (BPA Report DOE/BP-00005103-1).
- Guo S.W., and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics* 48:361-372.
- Haldane J.B.S. 1954. An exact test for randomness of mating. *Journal of Genetics* 52:631-635.
- Hess, J., Campbell A., Matala A., Narum S. 2012. 2011 Annual Report: Genetic Assessment of Columbia River Stocks. U.S. Dept. of Energy Bonneville Power Administration Report Project #2008-907-00.
- Kalinowski S.T., M.L. Taper, and T.C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases confidence in paternity. *Molecular Ecology* 16:1099-1106.
- Marshall, T.C.J. Slate, L. Kruuk, and J.M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7:639-655.
- Miller, B.F., J.L. Miller, S.T. Shaller, and J.E. Arterburn. 2013. Okanogan basin Monitoring and evaluation program, 2012, annual report. Colville Confederated Tribes Fish and Wildlife Department, Nespelem, WA. Project No. 2003-022-00.
- Miller, B.F., J.L. Panther, and J.E. Arterburn. 2012. 2011 Okanogan basin steelhead escapement and spawning distribution. U.S. Dept. of Energy Bonneville Power Administration Report Project #200302200.
- Miller, B.F., J.L. Panther, and J.E. Arterburn. 2011. 2010 Okanogan basin steelhead escapement and spawning distribution. U.S. Dept. of Energy Bonneville Power Administration Report Project #200302200.
- Park, S.D.E. 2001. Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection [Ph.D. thesis] University of Dublin.

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

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

Seamons TR, Bentzen P, Quinn TP. 2004. The effects of adult length and arrival date on individual reproductive success in wild steelhead trout (*Oncorhynchus mykiss*). *Can J Fish Aquat Sci* 61:185–192

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

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

Section C. Yakima Genetics Report

Introduction

The reproductive success of long-term reconditioned kelts needs to be explored to assess the net benefit of the kelt reconditioning 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 artificially reconditioned kelt reproductive success compare with natural repeat spawner success, and 3) How does artificially reconditioned kelt reproductive success compare with first time spawner success? In this study we utilize DNA markers and pedigree analysis to address these questions for kelt steelhead in tributaries of the Yakima river basin.

METHODS

Sample Collection

Anadromous adult steelhead were collected as downstream kelt migrants at the Chandler Juvenile Monitoring Facility after presumably spawning in the spring. Age-0 juveniles (juveniles collected in the same calendar year as the spawning event) were targeted using electrofishing techniques (NMFS 2000 Electrofishing Guidelines) during the fall in natal tributaries. Sampling was targeted near areas where steelhead spawning was observed. Sample numbers for each collection at the tributary level are reported in Table (1). Fork length was recorded and graphed by each collection site as dates varied within the Satus and Toppenish drainages. Age-0 length range was determined by choosing the first break in slope seen on a scatter plot.

Genetic Analysis

Fin tissue samples were collected and stored dry on whatman paper, or paper slips in coin envelopes 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. Current genotyping efforts utilize the 192 Single Nucleotide Polymorphism (SNP) markers and methods described in Hess et al. (2012). Statistical analysis used 188 of the 192 markers. Of the four not used, three are diagnostic for cutthroat, and one marker (OmyY1_2SEX) is a sex-determining marker that was used only to determine fish gender for the study. Significant linkage disequilibrium was previously observed in one pair of loci: Omy_GHSR-121 and Omy_mapK3-103 by Hess et al. (2012). Because linked loci are still informative for parentage analysis, both linked markers were included in this study.

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). 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, had a minimum of 180 loci comparisons, and zero mismatches.

Expected assignment rates of juvenile offspring were calculated using sample completion rates (S_{cr}) which were based off the percentage of genotyped adults ($n=464$) expected to spawn in 2012, as a function of the steelhead escapement estimates $n=(6,359)$ passing Prosser prior to the 2012 spawn event.

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

However, because the majority of adult samples were collected as kelts, the samples represented females in greater proportions than males. This creates higher expected probability of one versus two parent assignments.

To act as negative controls for parentage assignment, a limited number of samples from the 2010 collection were included since these fish were not expected to spawn in 2012. No juveniles assigned to negative controls.

RESULTS

Plotted lengths (figure 1- Currently at end of report) for each collection show likely break points representing different age groups. Although sometimes ambiguous, the first break in the slope of lengths varied between 50 and 90 mm in fork length.

Numbers for each collection, by location and year, can be seen in Table (1). Departures from Hardy-Weinberg equilibrium (critical level $=0.05 / 188 \text{ loci} = 0.000266$) were seen in three 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).

	n	HE	HO	HW
Cowiche Cr	49	0.3313	0.3434	0
Little Rattlesnake Cr	36	0.31347	0.31953	0
N.F. Little Naches Cr	38	0.31822	0.32517	0
Nile Cr	58	0.32951	0.32971	1
Quartz Cr	62	0.31415	0.3163	0
Satus Cr	187	0.29639	0.29919	0
Toppenish Cr	181	0.2817	0.27926	3
Chandler 2010	88	0.318	0.30312	0
Chandler 2011	56	0.33159	0.32579	0
Chandler 2012	408	0.31687	0.30986	1
Total	1163			

Across all juvenile collections, 13 offspring were successfully assigned to at least 1 adult (Table 2). Assigned offspring include 5 to Satus Cr., 4 to Toppenish Cr., 2 to Nile Cr., and 1 to Little Rattlesnake Cr. Twelve of the assignments were between 48 and 70 mm in fork length, while 1 was seen at 88mm in length. Table (3) shows the expected, observed and percent of expected parentage assignments for each of the juvenile classes. Assuming all 611 potential offspring were of age-0, 85.9 would be expected to assign back to at least one parent, while 13 assignments were found.

Table 2. Juvenile offspring assigned to at least one parent.

Individual Name	Location	Date	FL
OmyLRS12j-054	Little Rattlesnake Creek	10/4/2012	48
OmyNile12j-030	Nile Creek	9/6/2012	44
OmyNile12j-061	Nile Creek	9/6/2012	44
OmySat12-0029	Satus Creek-Below High Bridge	9/5/2012	56
OmySat12-0030	Satus Creek-Below High Bridge	9/5/2012	88
OmySat12-0127	Satus Creek-above Kushi Creek	9/20/2012	70
OmySat12-0179	Satus Creek-above Wilson Charley Creek	9/20/2012	65
OmySat12-0184	Satus Creek-above Wilson Charley Creek	9/20/2012	65
OmyTopp12-0121	Toppenish Creek-Simcoe Creek	9/27/2012	51
OmyTopp12-0222	Toppenish Creek- Above 3 way	10/3/2012	55
OmyTopp12-0229	Toppenish Creek- Above 3 way	10/3/2012	54
OmyTopp12-0232	Toppenish Creek- Above 3 way	10/3/2012	54

Table 3. Assignment probabilities. Each class of assignment is listed for the probability of assignment by individual juvenile progeny, and the expected and observed numbers for all individuals.

	Probability	Expected	Observed
probability of trio detection	0.0053	3.3	1
Probability of pair only detection	0.1353	82.7	12
Probability of no parents	0.8594	525.1	598
Probability of at least one parent	0.1406	85.9	13

DISCUSSION

The 13 observed parentage matches was pointedly lower than the 85.6 expected. However, some of this can be attributed to the inclusion of what are likely age-1 or greater samples. While field sampling was directed at age-0 in Satus and Toppenish, accurate identification at the time of sampling is difficult. Analysis of length within each collection set suggested that multiple age classes are present. Because there had been major rainfall (Ladd 2012a, 2012b) scouring events in the Yakima Drainage in 2012 that may have split the spawn times of steelhead, it was thought that age-0 fish may display multiple size classes. However, only one fish greater than 70mm in fork length was assigned back to at least one parent. The parent of the 88mm juvenile was a successfully reconditioned kelt from the 2011 spawn year that was detected moving across Prosser Dam following reconditioning at The Chandler facility. While this fish likely spawned again in 2012, the larger relatively larger length of the juvenile suggests that it from the brood year 2011.

The rainfall events of 2012 resulted in large floods and scouring of existing steelhead redds (Ressiguie 2012). These events likely occurred during the spawning period of anadromous steelhead. Low Age-0 parr densities calculated from snorkel surveys in Satus, Toppenish, and Ahtanum creek were subsequently attributed to the scour events (Ressiguie 2012). As these flood events were timed with the spawning period of anadromous steelhead, it is possible that a higher proportion than normal of the samples juvenile samples collected in 2012 were of resident origin. This may explain part of the difference between expected and observed juvenile parentage assignments, as potential resident parents were not collected.

Additional juvenile sampling techniques will be considered for all future collections. Radio-tagged kelts may allow tracking, identification of spawning, and targeted sampling of putative offspring. Targeted sampling could be extended to any steelhead redd, to minimize presence of resident offspring. Sampling swim up fry would also exclude any ambiguity of juvenile age.

The collection of Age-0 juveniles should continue in future years as the primary method of offspring collections. However, it may be necessary to collect additional length data from all size classes to

demonstrate separation of age classes. Up to 30% of the juveniles genotyped in 2012 are likely attributed to age-1 or greater fish, which explains a portion of the difference between expected and observed juvenile parentage assignments.

While reproductive success has been confirmed for four reconditioned kelts spawning in the Yakima drainage in 2011, 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 relative to overall adult escapement in the Yakima River. For the 2013 spawn year, the expected number of reconditioned kelt spawners is greater than that in 2012. Of the 333 kelts successfully reconditioned and released in the fall of 2012, 231 have been detected moving over Prosser Dam. The reproductive success of these fish will be compared to that of first time spawners captured at Prosser during a similar time frame for the 2013 report. This comparison will help isolate any issues related to differences between observed and expected offspring assignment rates.

References

- Anderson, E.C., R.S. Waples, S.T. Kalinowski. 2008. An improved method for estimating the accuracy of genetic stock identification. *Canadian Journal of Fisheries and Aquatic Sciences* 65:1475-1486.
- Arterburn, J., K. Kistler and R. Dasher. 2005. Okanogan Basin Steelhead Spawning Ground Surveys. Colville Confederated Tribes Fish and Wildlife, Anadromous Fish Division, Omak WA.
- Blankenship SM, Campbell MR, Hess JE, Hess MA, Kassler TW, Kozfkay CC, Matala AP, Narum SR, Paquin MM, Small MP, J Stephenson, K. Warheit. 2011. Major lineages and metapopulations in Columbia River *Oncorhynchus mykiss* are structured by dynamic landscape features and environments. *Trans. Am. Fish. Soc.* 140:665–684.
- Branstetter R., J. Stephenson, A. Pierce, D. Hatch, B. Bosch, D. Fast, J. Blodgett, M. Johnston, T. Resseguie, R. Dasher, C. Baker, A. Santos, J. Gidley, C. Brun, J. Lyman, J. Graham., L. Holliday, A. Santos, C. Gehling, C. Moffitt, J. Nagler, J. Buelow, Z. L. Penney, J. Boyce, L. K. Caldwell, T. Caileer, B. Jones, B. Sun, and J. Egan. 2011. Steelhead Kelt Reconditioning and Reproductive Success. 2010 Annual Report to U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-00. Prepared by the Columbia River Inter-Tribal Fish Commission, Portland, OR.
- Cavalli-Sforza, L.L., and E.W.F. Edwards. 1967. Phylogenetic analysis; models and estimation procedures. *Evolution* 32:550-570.
- Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14:2611-2620.
- Falush, D., M. Stephens and J.K. Pritchard. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* 164:1567-1587.
- Fisher, C., and J. Arterburn. 2004. Improvements of anadromous fish habitat and passage in Omak Creek; Includes 2003 steelhead surveys in Omak Creek. 2002-2003 Annual Report, Project No. 200000100, 23 electronic pages, (BPA Report DOE/BP-00005103-1).
- Guidelines for Electrofishing Waters Containing Salmonids Listed under the Endangered Species Act (Backpack Electrofishing Guidelines, NMFS, June 2000)
- Guo S.W., and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics* 48:361-372.
- Haldane J.B.S. 1954. An exact test for randomness of mating. *Journal of Genetics* 52:631-635.
- Hatch D.R., R. Branstetter, J. Stephenson, A. Pierce, J. Whiteaker, and B. Bosch. 2012. Steelhead Kelt Reconditioning and Reproductive Success. 2011 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 2007-401-000. Portland, OR: Prepared by the Columbia River Inter-Tribal Fish Commission.
- Hess, J., Campbell N., Matala A., Narum S. 2012. 2011 Annual Report: Genetic Assessment of Columbia River Stocks. U.S. Dept. of Energy Bonneville Power Administration Report Project

#2008-907-00.

- Kalinowski S.T., M.L. Taper, and T.C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases confidence in paternity. *Molecular Ecology* 16:1099-1106.
- Langella, O. 2001. Populations 1.2.24: population genetic structure (individuals or populations distances, phylogenetic trees). Available: <http://www.pge.cnrs.gif.fr/bioinfo/populations/>.
- Ladd, S. 2012a. Hydrologic conditions update. April 2012. Yakama Nation Water Resource Program Memorandum For Record.
- Ladd, S. 2012b. Hydrologic conditions update. May 2012. Yakama Nation Water Resource Program Memorandum For Record.
- Marshall, T.C.J. Slate, L. Kruuk, and J.M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7:639-655.
- McConnell, S., L. Hamilton, D. Morris, D. Cook, D. Paquet, P. Bentzen, and J. Wright. 1995. Isolation of salmonid microsatellite loci and their application to the population genetics of Canadian east coast stocks of Atlantic salmon. *Aquaculture* 137:19-30.
- Narum S., Campbell N., Matala A., Hess J. 2010. 2009 Annual Report: Genetic Assessment of Columbia River Stocks. U.S. Dept. of Energy Bonneville Power Administration Report Project #2008-907-00.
- Olsen, J.B., P. Bentzen, M.A. Banks, J.B. Shaklee, and S. Young. 2000. Microsatellites reveal population identity of individual pink salmon to allow supportive breeding of a population at risk of extinction. *Transactions of the American Fisheries Society* 129:232-242.
- Park, S.D.E. 2001. Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection [Ph.D. thesis] University of Dublin.
- Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945-959.
- Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86:248-249.
- Resseguie, T. 2013. Steelhead (*Oncorhynchus mykiss*) population and Habitat Monitoring in Lower Yakima River Tributaries 2011 and 2012. 2012 Annual Report to the U.S. Dept. of Energy, Bonneville Power Administration, Project No. 1996-035-01-Contract 52386. Portland, OR.
- Rousset, F., and M. Raymond. 1995. Testing heterozygote excess and deficiency. *Genetics* 140:1413-1419

- Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution*. 43: 223-225
- Stephenson J.J., M.R. Campbell, J.E. Hess, C. Kozfkay, A.P. Matala, M.V. McPhee, P. Moran, S.R. Narum, M.M. Paquin, O. Schlei, M.P. Small, D.M. Van Doornik, J.K. Wenburg. 2008. A centralized model for creating shared, standardized, microsatellite data that simplifies inter-laboratory collaboration. *Conservation Genetics* 10:1145-1149.
- Weir B.S. 1990. Genetic data analysis. Sinauer Publ., Sunderland, MA.

Figure 1. Plotted lengths of genotyped samples. Graphs are divided by sections within each major tributary. Lengths on the Y axis are plotted against Individuals on the X axis after sorting by length.

