Kelt Reconditioning and Reproductive Success Evaluation Research:

2014 Annual Technical Report

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Abstract

The Kelt Reconditioning and Reproductive Success Evaluation Project is a research, monitoring, and evaluation (RM&E) uncertainties category project funded through the Columbia Basin Fish Accords. The objectives are to evaluate methodologies to produce viable artificially reconditioned repeat steelhead spawners and to determine the productivity of repeat spawners. Work occurs in the Yakima and Snake river basins. We focused on collecting steelhead kelts at juvenile bypass facilities in Prosser and Lower Granite dams, and additionally some fish were collected at Dworshak National Fish Hatchery, and the Fish Creek weir. These kelts were reconditioned (given prophylactic treatments and fed a specially formulated diet) at Prosser and Dworshak National fish hatcheries. Survival to fall of long-term reconditioned steelhead was 61% at Prosser and 30% at Dworskak hatcheries in 2014. Using estradiol assays, we have established that steelhead rematuration rates vary annually and spatially and range from 10.4% to 80.0%. We have also determined from our study streams kelts can remature as consecutive or skip spawners, typically returning to spawn in 5 or 6 months after kelting or 17 to 18 months later. We characterized the outmigrating Snake River kelt run as primarily composed of Salmon, Grand Ronde, and the Imnaha populations based on GSI analysis at Lower Granite Dam. A total of 34 reconditioned B-run steelhead were released below Bonneville Dam in 2014 to address Reasonable and Prudent Alternative 33 of the FCRSP Biological Opinion. We air-spawned a group of maiden Dworshak Hatchery steelhead in 2013. These fish were then reconditioned and rematuring fish were air-spawned as repeat spawners in 2014 to compare performance between maiden and repeat spawnings. Comparisons of fecundity, fertilization rate, and egg size variables revealed that repeat spawners had larger eggs and a greater abundance of eggs and no differences in fertilization rates were detected. Reproductive success of reconditioned steelhead was confirmed in the Yakima River with assignments of 23 juvenile fish to 11 unique parents. A work plan was developed and approved to use the Cle Elum Hatchery spawning channel to improve reproductive success estimates for reconditioned kelt steelhead. Fish will be stocked in the channel in 2015. We used radio telemetry in 2014 to track 19 reconditioned kelts to spawning streams to refine our estimates of production in the Yakima River and its tributaries. We developed a model to examine population recovery from the perspective of a kelt reconditioning program. The model mimics iteroparity in ways explicit to body condition, reconditioning, and release method. We have shown that repeat spawners could contribute up to 10% of spawning if sufficient kelts are captured and reconditioned, consistent with existing data on survival and maturation rates and estimates of repeat spawner fecundity. This modeling tool provides the means to examine several questions regarding potential avenues for recovery, and management options for doing so. Our team published 5 manuscripts and gave 14 professional presentations in 2014.

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Introduction

The Kelt Steelhead Reconditioning and Reproductive Success Evaluation Project is a research, monitoring, and evaluation (RM&E) category project funded through the Columbia Basin Fish Accords. The project studies and evaluates two broad topics with respect to post-spawn steelhead, first it assesses reconditioning processes and strategies, and second, it measures reproductive success of artificially reconditioned kelt steelhead. The specific Reasonable and Prudent Alternative (RPA) which this research is identified are RPAs 33 and 42 (NMFS 2008, 2010, and 2014). The RPA 33 requires the Action Agencies to develop, in cooperation with regional salmon managers, and implement a Snake River steelhead kelt management plan designed to provide at least a 6% improvement in B-run population productivity. Toward that goal, a variety of approaches are being tested and implemented including passage improvements and reconditioning kelt steelhead. The RPA 42 focuses on the reconditioning component and seeks to preserve and rebuild the genetic resources through safety-net (kelt reconditioning) and mitigation actions to reduce short-term extinction risk and promote recovery. 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. Additional information on kelt reconditioning can be found in (Hatch et al. 2013b) which provides additional support of the benefits of kelt reconditioning to address population demographic and genetic issues in steelhead recovery.

<A>Background

All populations of anadromous *Onchorynchus mykiss* in the Columbia River Basin are listed as either threatened or endangered under the Endangered Species Act (ESA). Populations of wild steelhead 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).Steelhead populations that existed pre-dam building era in the Columbia River were characterized by unimpeded migration with the exception of commercial and subsistence fisheries. The dam construction and post-dam construction era for most of the 20th century had prioritized water retention for the production of electricity, providing irrigation, navigation, and recreational opportunities. This severly impacted or even extirpated anadromous steelhead runs.

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 may average twice the reproductive success of steelhead spawning a single time (Seamons and Quinn 2010).

Iteroparity is the ability to repeat spawn and is a natural life history strategy expressed by *0. mykiss*. Steelhead kelts are defined as anadromous post spawn O. mykiss that have the potential to spawn more than once. Naturally, steelhead kelts would migrate to the ocean and spawn again the next spawning migration (sequential spawner) or a subsequent year (skip spawner). 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 iteroparity rates for the interior Columbia River are not well documented but kelts detected emigrating were 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 less 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. 1985). Summer steelhead in the South Fork Walla Walla River 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%.

With the passage of the ESA the National Oceanic and Atmospheric Administration's National Marine Fisheries Service (NOAA's NMFS) is required to develop a biological opinion of the management of the Federal Columbia River Power System (FCRPS) to institute Reasonable and Prudent Alternatives (RPA) to standard operational practices to reduce adverse conditions due to management and operations of the FCRPS. In order to mitigate for steelhead losses from operation of the FCRPS two approaches have been investigated that would use steelhead kelts. Investigations began in late 1999 to take advantage of this history strategy to reduce or stop population declines in anadromous steelhead by improving fish passage, culturing techniques,

or a combination of the two. Regional conservation plans recognize the need to protect and enhance weak upriver steelhead populations while maintaining the genetic integrity of those stocks (NPPC 1995).

One conservation approach has been to improve downstream migration passage at the mainstem hydroelectric dams for downstream migration of steelhead kelts to the ocean (RPA's 52, and 54) (NMFS 2008, 2010, and 2014). Several studies have looked at the effectiveness of transportation of kelts to bypass the dams, the usage of corner collectors, and kelt survival through the hydrostyem (Hatch et al. 2003a; Hatch et al 2003b; Hatch et al. 2004; Wertheimer and Evans 2005; Wertheimer 2007; Evans et al. 2008; Weiland et al. 2009; Branstetter et al. 2010; Kahn et al. 2010; Branstetter et al. 2011; Kahn and Royer 2012; Rayamajhi et al. 2013; Colotelo et al. 2014; Harnish et al. 2014) The survival of kelts in this approach is dependent on good migration conditions in the river (both exiting and returning), surviving predation (human and non-human), and conducive ocean conditions.

The other simultaneous approach that is being investigated, by the CRITFC and its partners, involves capturing steelhead kelts and rehabilitating them with prophylactic treatments and providing highly nutritive food. Reducing chances for mortality in the hydrosystem and ocean will provide another opportunity for fish to reproduce in the wild. This approach is titled the Kelt Steelhead Reconditioning and Reproductive Success Evaluation Project which is a research, monitoring, and evaluation (RM&E) category project funded through the Columbia Basin Fish Accords. Techniques used in kelt reconditioning were initially developed for Atlantic salmon *Salmo salar* and Brown or Sea-trout *S. trutta*, and a review of these studies and others applicable to steelhead kelts are summarized in Evans et al. (2001). The CRITFC's goals are to thoroughly assess reconditioning processes and strategies, measure reproductive success of artificially reconditioned kelt steelhead, and collaborating with the Nez Perce Tribe on the development of a kelt management plan for the Snake River.

<A>Current Research Direction

In a recent positive review of our sister project the Yakama Nation's Upper Columbia Kelt Reconditioning Program (BPA Project #2008-458-00) by the Independent Science Review Panel (ISRP) (ISRP 2014), they recommended that the kelt project focus on addressing the following uncertainties:

1. The prior recommendation, by the ISRP, to establish methods to assess how kelt reconditioning may benefit population growth, abundance, spatial structure, and diversity still needs to be addressed.
2. Some modeling and a power analysis need to be conducted to clarify how many juvenile and F1 adults should be sampled to detect meaningful differences in the breeding and reproductive success of HOR, NOR, and reconditioned NOR females.
3. Methods to assess the fat levels, maturation timing, fecundity, egg size, and gamete viability of the project's reconditioned kelts need to be developed and implemented. The fate of non-maturing or skip-repeat reconditioned fish also should be disclosed.

4. Viable plans are needed to monitor the homing and straying rates of reconditioned kelts released by the project.
5. Experiments are needed to discover the best geographic locations and times of year for release of the project's reconditioned fish.

In 2014 the CRITFC worked to answer these critical uncertainties which we address in this year's annual report. In 2013 we began developing the population growth, abundance, spatial structure, and diversity models that we initially published in last year's report under Hatch et al. 2014. This year we refined and inputted data to these models and ran the simulations. The results of these models are published under the kelt population model section. In 2014, we assessed maturation status in blood samples from kelts taken in 2013 and 2014 (Reproductive development in kelt steelhead section). These samples will allow a direct comparison of the reproductive and energetic status of reconditioned kelts with maiden spawners. We completed a study employing a proteomics approach to search for an indicator of rematuration in plasma samples taken at intake (Proteomic Analysis of Female Steelhead Plasma section). Additionally, the other metrics of fish maturation identified in question #3 primarily fat levels, are collected at Bonneville Dam, Prosser Dam and Hatchery, Lower Granite Dam and Dworshak National Fish Hatchery by the CRITFC. Following the principal of adaptive management we are currently retaining non-mature fish at both Prosser and Dworshak to hold over for rematuration to see if this is a viable option at boosting repeat spawning in those basins. All captured kelts are PIT-tagged and monitored by in-stream PIT-tag arrays. Also, in the Snake Basin, we investigated collection of kelts at Lower Granite Dam and Fish Creek a tributary of the Lochsa River. We may plan on collecting kelts at Little Goose Dam and collect South Fork Clearwater fish again as was done in 2013 (Hatch et al. 2014). The kelt diet continues to be fine tuned to increase survival and maturation. We are collaborating with a fish nutritionist at the U.S. Department of Agriculture to optimize a diet that incorporates the Cyclopeeze top coating into the pellet (Hatch et al 2014) and increases lipid levels which are important for egg development and production.

The Relative Reproductive Success portion of the project is to focus on measuring reproductive capabilities of artificially reconditioned kelts. We conducted a study using hatchery origin kelts at Dworshak National fish hatchery to assess the effect of reconditioning on egg quality and other aspects of reproductive performance. This experiment replicates a similar experiment that we conducted at Parkdale Hatchery (Hatch et al 2013a) providing us with important geographical replication component to this work. We continued to try and quantify kelt contribution to steelhead populations in the wild by collecting juvenile genetic samples and matching them to repeat spawner parents in the Yakima River and its tributaries. We also radio tagged 70 of the reconditioned kelts (termed repeat spawners) that were released in the fall of 2013 and tracked migrants to areas where they likely spawned for later juvenile DNA collections. Genetic analysis is currently being conducted on these fish and will be presented in the 2015 annual report. We have made progress towards utilizing the spawning channel at Cle Elum hatchery to monitor kelt reproductive capabilities and are intending to complete a feasibility study beginning in 2015. The kelt master plan for the Snake River is currently in draft form for internal review and will be available in 2015 for action agency reviews. We have

produced 5 publications and provided 14 presentations to increase exposure of the program to a wider audience and to inform management decions in the Columbia River Basin.

<A>Reconditioning Processes Strategies

Kelt steelhead reconditioning process evaluations involve fish culturing practices, studying alternative management strategies, and implementing research scale reconditioning programs. The evaluation of kelt steelhead restoration strategies is based on two fundamental hypotheses aimed at comparing the relative survival and rematuration rates of program fish.

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

and,

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

The goal of this group of studies is to develop and evaluate potential strategies to increase steelhead productivity by maintaining or restoring iteroparity. Providing assistance to kelts in the form of transportation, feed, captivity, and prophylactic measures (anti-fungal, antibiotics, and anti-parasitic) will increase the probability that individual steelhead repeat spawn and contribute to population growth. The group of studies includes in-river release 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. The two listed scenarios were pursued in 2013, in previous years we have also explored other methods for improving kelt survival but have focused on the low cost and high cost scenarios. Kelt steelhead reconditioning process evaluations involve fish culturing practices, studying alternative management strategies, and implementing research scale reconditioning programs.

<B>Kelt Collection

Steelhead kelts are collected from 3 main areas throughout the Columbia River Basin, Prosser, WA, Lower Granite, WA, and Fish Creek, ID. This section details the capture locations, capture methods, and biological information collected from the specimens.

Fish Creek Weir

We added an additional stream location to collect more B-run steelhead kelts at a weir on the Fish Creek a tributary of the Lochsa River in eastern Idaho. In early 2014, a considerable amount of time was spent developing the study design, determining logistical issues and obtaining Idaho Department of Fish and Game cooperation. We conducted initial capture and transport of kelts to Dworshak National Fish Hatchery from this location in 2014.

<B>Genetic stock identification (GSI) to assign individual stock-of-origin and estimate stock proportions in a mixed sample of kelt steelhead sampled at Lower Granite Dam

Kelt is the term used to describe steelhead trout (*Oncorhynchus mykiss*) that survive after spawning. This ability represents the defining stage of an iteroparous life history and is unique to *O. mykiss* among all Pacific salmon. The demographic benefit of an iteroparous life history is realized when kelts migrate to the ocean and successfully complete one or more subsequent spawning migrations. Kelts are found throughout the Snake River Basin, but their spatial distribution or occurrence among watersheds is highly variable. Rates of iteroparity or repeat spawning in the Snake River are highest among populations characterized by smaller, 1-ocean age individuals (A-run). Conversely, repeat spawning is less frequent among B-run populations typically comprised of larger, 2-ocean age individuals (Narum et al. 2008). We used multilocus genotype data at single nucleotide polymorphism (SNP) loci to conduct an analysis of genetic stock composition among kelt steelhead sampled at Lower Granite Dam (LGD) between 2009 and 2013. 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 provide managers with valuable information about the relative behaviors and population demographics exhibited by genetically assigned kelt stocks. The 2014 composition will be provided in the 2015 annual report.

<B>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 (Prosser Hatchery) and Snake rivers (Lower Granite Dam) to act as a control group and determine the baseline steelhead kelt iteroparity rate under current hydrosystem management. 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. Leaving steelhead kelts in the river also represents the lowest cost option, which is currently the status quo for the majority of the Columbia River Basin.

<B>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 additional spawning opportunities. The long-term steelhead reconditioning diet and care treatments were established from the studies conducted in 2001 and 2002 (Hatch et al. 2002a and Hatch et al. 2003b) and continue at reconditioning facilities located in Prosser Hatchery, WA, and Dworshak National Fish Hatchery, ID. These fish are typically released in the fall to over-winter and return to the spawning sites volitionally. This treatment represents the highest cost alternative.

Prosser Fish Hatchery

Prosser is where the reconditioning of steelhead kelts originated with this research endeavor (Hatch et al. 2013b). Steelhead kelts are collected at the Chandler Juvenile Monitoring Facility's (CJMF) bypass separator that is just downstream of Prosser Dam. These fish are either PIT-tagged and released back to the Yakima River or retained and held for long-term reconditioning. Steelhead kelts are reconditioned and then utilized to further our understanding of kelt maturation and enhance survival for research, conservation, and mitigation purposes. Kelts that survived the artificial reconditioning were released or retained for further additional reconditioning research (maturation assesement, gondal somatic index assesment, management strategy approaches, and innovative feeding/treatment approaches). This portion of the project addresses the RPA number 42 to "Implement Conservation Programs to Build Genetic Resources & Assist in Promoting Recovery" (NMFS 2008).

Dworshak National Fish Hatchery

Specific to Snake River B-run steelhead, our project collection locations include kelts at the Lower Granite Dam juvenile bypass separator, kelts collected from the Fish Creek weir (see Fish Creek below) and air spawned Dworshak National Fish Hatchery returns. Fish collected from Lower Granite Dam and Fish Creek were either transported to Dworshak Hatchery for reconditioning, or PIT tagged and released back into the Snake River as a representative control group. Dworshak Hatchery and Fish Creek fish were retained for experiments to further understand repeat spawner rematuration. The fish that survived reconditioning were released downstream of Lower Granite Dam in December 2014. The successful reconditioning and subsequent release of wild B-run steelhead back into the Snake River system addresses the RPA

number 33 to Develop and Implement a Kelt Management Plan to improve the productivity of interior basin B-run steelhead populations (NMFS 2008, 2010, and 2014).

Diet Enhancement

Based on results of our feeding trials in 2012 and 2013 it was decided that we needed to increase the efficiency in the delivery of lipids in the kelt diet. Currently we are collaborating with Dr. Rick Barrows from the U.S. Department of Agriculture (USDA) to formulate a diet that should boost lipid intake and in turn increase survival and rematuration in reconditioned kelts. Initial results from this experiment should be available in the 2015 annual report.

<B>Kelt Reconditioning Physiology Studies

In this objective we will study the physiology and endocrinology of steelhead kelts with a goal of evaluating the feasibility and success of several strategies for rehabilitating and handling of steelhead captured at Lower Granite Dam or at other sites during their downstream migration in the Snake River system. Our research will focus on the physiology, health and condition of both B and A stocks of steelhead trout. Through this research we will pose, develop, and test protocols that can be used to collect and transport spent spawners, rehabilitate them for the most effective period of time to maximize their ability and contribution to the next spawning generation. Our focus is to develop the background science needed for evaluating different production plans for rehabilitation of kelts. This portion of the work is a collaboration between the CRITFC, Nez Perce Tribe, and University of Idaho.

Reproductive Development in Kelt Steelhead

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 (Chaput and Jones 2006; Keefer, et al. 2008; Rideout, et al. 2005; Rideout and 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 (Campbell, et al.

2006b; Satterthwaite, et al. 2009; Shearer and Swanson 2000; Thorpe 2007). 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. Consistent with this idea, we found that energy restriction affected reproductive development within 10 weeks after spawning in female rainbow trout (Caldwell, et al. 2013; Caldwell, et al. 2014). 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 maturation or rematuration are determined by the genetic makeup of the fish and subject to selection (Carlson and Seamons 2008; Hutchings 2011).

Studies conducted in 2009-2011 established that blood levels of estradiol and vitellogenin diverge between rematuring and non-rematuring fish during reconditioning. 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. Estradiol indicates maturation earlier than vitellogenin, and the cost of the estradiol assay is about 1/4th of the cost of the vitellogenin assay.

During 2014, we measured estradiol level in a large number of blood samples, mostly from the 2013 and 2014 seasons. We collected blood from fish in the reconditioning programs at Prosser, Dworshak, and Winthrop. We provided maturation status for Prosser fish to project managers prior to release. Laboratory assays and data analysis are ongoing. Preliminary results are presented here, with the caveat that they may change as more assays and analysis are completed.

Proteomic Analysis of Female Steelhead Plasma

Female steelhead kelts 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 may be 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 identify potential biomarkers of reproductive status in steelhead kelts at intake into reconditioning, a plasma proteome approach was employed. Plasma proteomic patterns were compared between kelts that went on to consecutively reproduce similar and fish that did not.

<B>Population model

In 2013, we constructed a prototype steelhead model to examine the management implications of kelt reconditioning. The model was designed to address the factors influencing kelt condition at capture, the effect of the capture rate of kelts on the overall recovery rate, the effects of the in-river release, transport unfed, transport fed, and long term reconditioning survival on

recovery, and the proportion of kelts reconditioned, transported or released. The model was intended to be used to assess the effectiveness of alternate reconditioning strategies, and limitations of the reconditioning program. This was done by comparing the rate of achievable population increase when captured kelts are reconditioned under assumed potential capture rates and assumed survival rates. The model was intended to be a very general implementation, capable of reproducing multiple age classes of adult returns, and tracking all components of the kelt program. The model explicitly tracked groups of kelts of different conditions (fair, good, and poor) and of different release groups (in-river, fed-transported, and unfed-transported).

<A>Reproductive Success of Artificially Reconditioned Kelt Steelhead

This evaluation program is designed to investigate the reproductive success of artificially reconditioned kelt steelhead. Since direct examination of reproductive success in the field is very difficult, we are also measuring physiological and endocrinological parameters as an index to rematuration and reproductive success and at a variety of scales. These variety of scales are at the individual fish level (egg quality) and natural stream level (Yakima River Basin and Fish Creek (in-development)). We will add another dimension with the development of a controlled environment (Cle Elum spawning channel) study. This will provide direct measures of kelt reproductive capability in a semi-natural setting that can control some of the natural environmental variables (predation, conspecific competition, and high/low flow events). This project is a collaborative effort amongst two of our member tribes (Nez Perce Tribe and Yakama Nation), the University of Idaho, and the Columbia River Inter-Tribal Fish Commission.

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

<B>Egg quality and reproductive parameters in hatchery origin maiden female steelhead and reconditioned kelts at Dworshak National Fish Hatchery

In their recent review of the Upper Columbia Kelt Reconditioning Program, ISRP recommended that: “Methods to assess the fat levels, maturation timing, fecundity, egg size, and gamete viability of the project’s reconditioned kelts need to be developed and implemented…” (ISRP 2014, Qualification 3). To address ISRPs recommendation, we are conducting an experiment to assess reproductive performance in hatchery origin kelts at DNFH.

It is difficult or impossible to directly to assess egg quality and fecundity in wild fish, because wild fish spawn naturally before collection, and reconditioned wild fish are released to spawn naturally. The DNFH hatchery origin kelt model provides a unique opportunity to directly assess egg quality and fecundity in a large number of maiden spawners. If these fish can be successfully reconditioned, egg quality and fecundity in the first spawning can be directly compared to the second spawning. 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. On

the other hand, fecundity increases with body size in salmonids (Quinn 2005), suggesting that reconditioned kelts should have higher fecundity than maiden fish. The production of eggs that can be fertilized and develop successfully is a necessary but not sufficient condition for reproductive success of reconditioned kelts in the wild. However, if egg quality and spawning success are equal, then the relative fecundity of reconditioned kelts can provide an estimate of the productivity of reconditioned kelts versus maiden steelhead. Thus, assessment of egg quality and fecundity in reconditioned kelts is a step toward our goal of measuring the relative reproductive success of reconditioned kelts.

After reconditioning in the ocean, repeat spawning steelhead may spawn either in the same year, known as consecutive spawning, or in the following year, known as alternate- or skip-spawning. Consecutive repeat spawning and alternate (skip) repeat spawning are diverse life histories found within populations of successfully repeat spawning (iteroparous) post-spawn fish (kelts), which have been detected in the wild in Alaska (Nielsen, et al. 2011), and on the Snake River (Keefer et al. 2008), and in the captive kelt reconditioning project on the Yakima River (Branstetter et al. 2011; Hatch et al. 2013a; Hatch et al. 2012), and Upper Columbia (Abrahamse and Murdoch 2013). The causes and consequences of alternate reproductive behavior in post-spawning in steelhead have been little studied, although relevant information is available in Atlantic salmon. Atlantic salmon repeat spawning kelts add life history variation to populations and function as population stabilizers (Halttunen 2011). In naturally repeat spawning Atlantic salmon, egg size was decreased in consecutive spawning kelts versus skip spawning kelts, possibly due to reduced energetic reserves for ovarian development (Reid and Chaput 2012). The availability of prey in the estuary was associated with differing migration patterns and return proportions of consecutive and skip spawners (Chaput and Benoit 2012), suggesting that post-spawning life history is plastic and depends on feeding conditions in the ocean. This is supported by studies on steelhead showing that maturation is associated with growth in the marine environment (Quinn, et al. 2011).

In this experiment, we aim to compare the reproductive performance of DNFH hatchery-origin female steelhead at their maiden spawning with that of kelts which survive and remature at their second spawning. Since we anticipate that repeat spawners may follow either a consecutive or skip spawning trajectory, we will compare reproductive parameters in these two types versus maiden spawners. This experiment is ongoing, and results may change as more data is collected and additional analysis is completed.

<B>Kelt Reproduction in a Natural Setting

Yakima Parentage Analysis

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.

Repeat Spawner Post Release Tracking

We radio tagged 70 long term reconditioned kelts in October of 2013, with the goal of tracking individual fish to their prospective redds to increase the odds of sampling progeny from the reconditioned kelts. The radio tagged artificially reconditioned kelts were assumed to be rematuring based on elevated levels of estradiol that was collected in August of 2013. The radio tracking was conducted with assistance from the Yakima River Steelhead VSP (Frederiksen et al., 2014). In the fall of 2014, we electroshocked specific areas that radio tagged fish likely spawned in. We collected genetic samples from age-0 steelhead fry in an attempt at assigning parentage back to artificially reconditioned kelt repeat spawners. In addition, 21 of these radio tagged fish have presumed "maiden" spawning detection histories from the previous year (2012/13) which we can also use to rate the fidelity of repeat versus maiden spawnings. We also continue to randomly sample in the basin to find kelt parentage from non-radio tagged individuals. Even with this combined expansive and targeted sampling, finding evidence of natural spawning (observed spawning or genetic evidence of spawning) is still difficult.

<B>Cle Elum Spawning Channel

The difficulty of finding spawning in the wild results in low sample sizes that limits statistical power when analyzing effects of artificially reconditioned repeat spawners on the total population. To address this problem, we have initiated a study using the Cle Elum Spawning Channel which had previously been used for spring Chinook. That prior experiment was used to successfully observe spring chinook natural spawning capabilities and behavior (Schroder et al. 2008; Schroder et al., 2010). We will utilize the spawning channel to conduct a similar experiment to observe and determine artificially reconditioned kelt reproductive capabilities in the channel. This will help to reduce some of the variables that occur in nature, namely predation, navigation of degraded habitat, and fluctuating natural conditions (flood events and low water years),and speficic to our study, the low percentage of spawning adults genotyped.. Most of 2014 was spent on creating the study design and obtaining support and permission from the YKFP Policy Group (Yakama Nation and Washington Department of Fish and Wildlife) through the YKFP technical review process. Collaborators include: U.S. Fish and Wildlife Service, BPA, and NOAA through the Cle Elum technical team approval process. Please see Appendix 1.e. for further details on plan.

<A>Kelt Master Plan Development

The Nez Perce Tribe are currently evaluating the Kelt Master Plan document through the Nez Perce Tribe's governmental review process. We anticipate that the Steelhead Kelt Master Plan will move through the review process and be approved in mid-2015. Afterwards it should be ready for basinwide review and approval by the action agencies sometime in mid-to-late 2015.

<A>Publications

To better understand and share knowledge with the science and conservation communities on the physiological processes that influence post-spawn steelhead recovery the CRITFC and its partners published five papers to the published literature in 2014 ((Buelow and Moffitt 2014; Caldwell et al. 2014; Penney and Moffitt 2014 a.; Penney and Moffitt 2014 b.; Hernandez et al. 2014). Our team gave 14 project presentations on our research in 2014 at the basin, regional, national, and international levels. See Appendix A.2 for specifics.

Pacific Coast Steelhead Management Meeting-1
Yakima Basin Science and Management Conference, Ellensburg, WA -3
National American Fisheries Society, Quebec City, QC, CA-5
1st Annual CRB Trainee Symposium-1
Fish Culture Conference, Mission, OR-3
Department of Biological Sciences University of Idaho-1

Methods

A list of methods is provided in the **Appendix A.3**. This list provides direct hyperlinks to detailed project methods that are hosted on the **Monitoring Methods website**.

<A>Study Area

<B>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 m^3/s. Summer steelhead populations primarily spawn upstream from Prosser Dam in Satus Creek, Toppenish Creek, Naches River, and other tributaries of the Yakima River (TRP 1995) (Figure 1). The Prosser Dam in Prosser, WA is a diversion dam, which collects water for irrigation in the Yakima River valley.

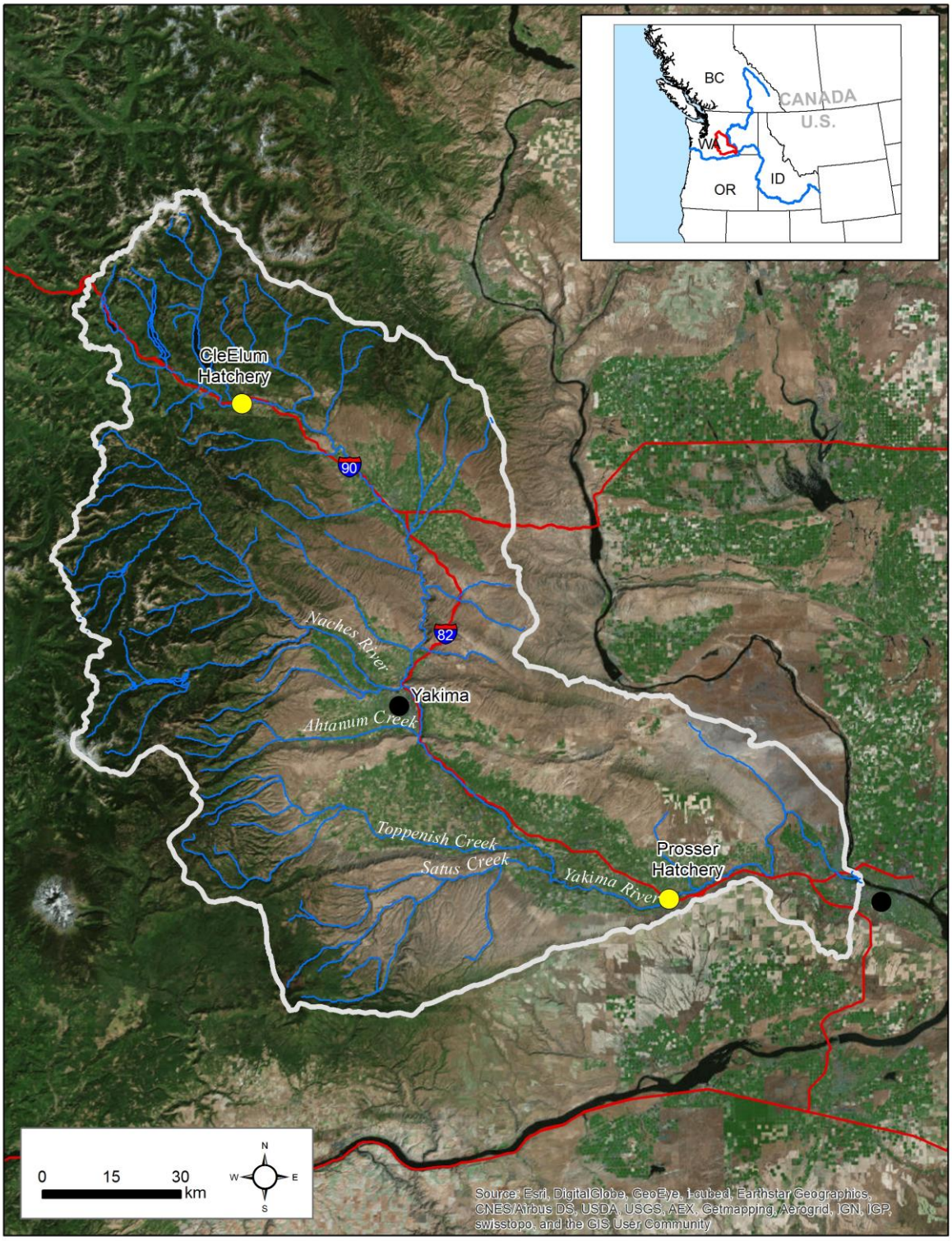


Figure 1: Map of the Yakima River Subbasin. Some of the steelhead natal spawning creeks are listed. Both the Cle Elum and Prosser hatcheries are included.

<B>Prosser Hatchery

Prosser Hatchery is located on the Yakima River just downstream of Prosser Dam (RK 75.6) (Figure 2). 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 9) as well as experimental rearing of white sturgeon *(Acipenser transmontanus)* and Pacific lamprey (*Entosphenus tridentate*).

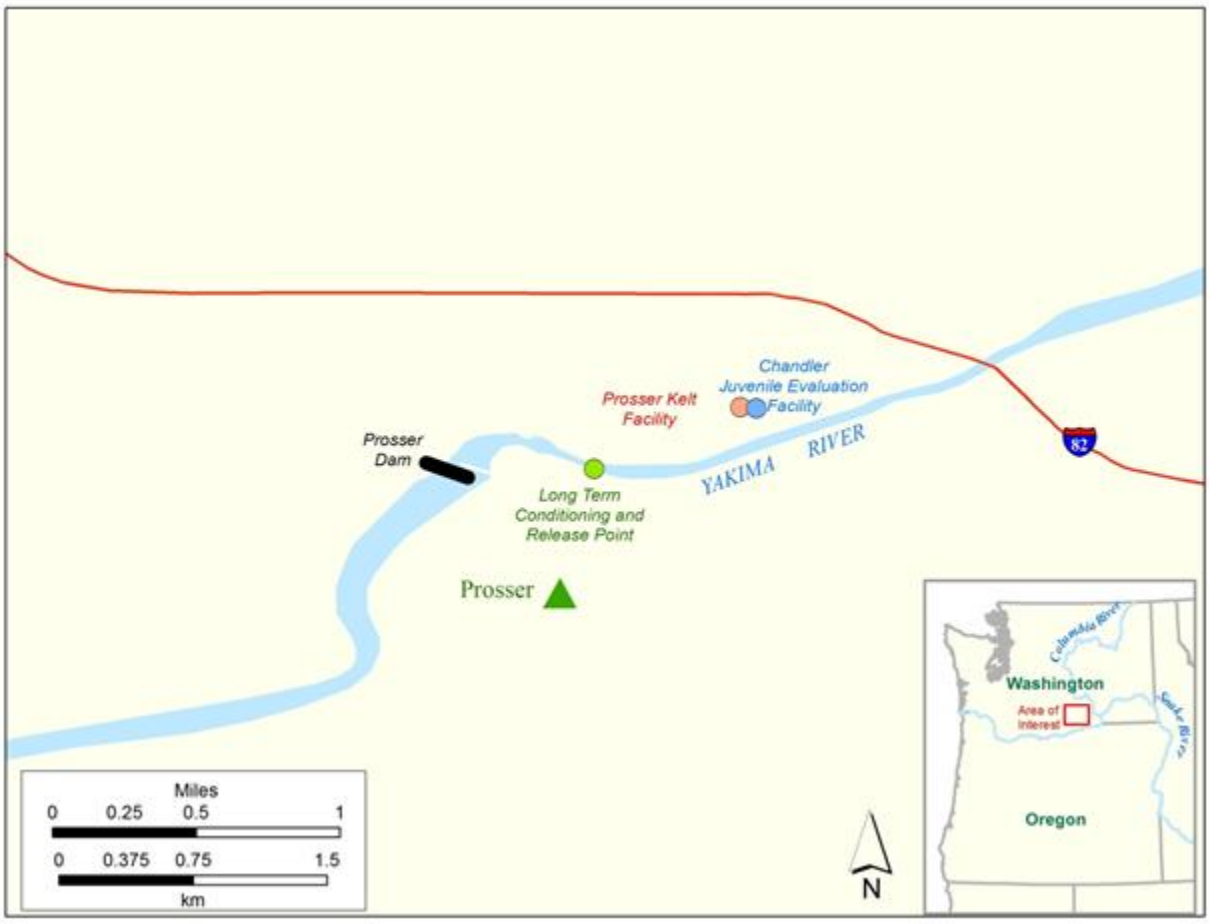


Figure 2: Map showing the location of Prosser Dam and the kelt reconditioning facility at Prosser, WA.

<B>Lower Granite, WA: Snake River Basin

The Snake River watershed is the tenth largest among North American rivers, and covers almost 280,000 km^2 in portions of six U.S. states: Wyoming, Idaho, Nevada, Utah, Oregon, and Washington, with the largest portion in Idaho. Most of the Snake River watershed lies between the Rocky Mountains on the east and the Columbia Plateau on the northwest. The largest tributary of the Columbia River, the Snake River watershed makes up about 41% of the entire Columbia River Basin. The Snake River enters the Columbia at 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 m^3/s. At Anatone, Washington, downstream of the confluences with the Salmon and Grand Ronde, but upstream of the Clearwater, the mean discharge is 979 m^3/s (Figure 3). The third dam on the Snake River Lower Granite Lock and Dam is a concrete gravity run-of-the-river dam on the Snake River, in the U.S. state of Washington. The dam is located 22 miles (35 km) south of the town of Colfax, and 35 miles (56 km) north of Pomeroy. Steelhead spawn naturally throughout the lower portion of the basin with the vast amount of “B-run” steelhead produced at the Dworshak National Fish Hatchery found on the Clearwater River.

<B>Fish Creek, ID: Lochsa River Subbasin

Fish Creek is a tributary of the Lochsa River which is part of the greater Clearwater subbasin that feeds into the Snake River basin. This stream system is primarily dominated by O. mykiss (Copeland et al. 2013).

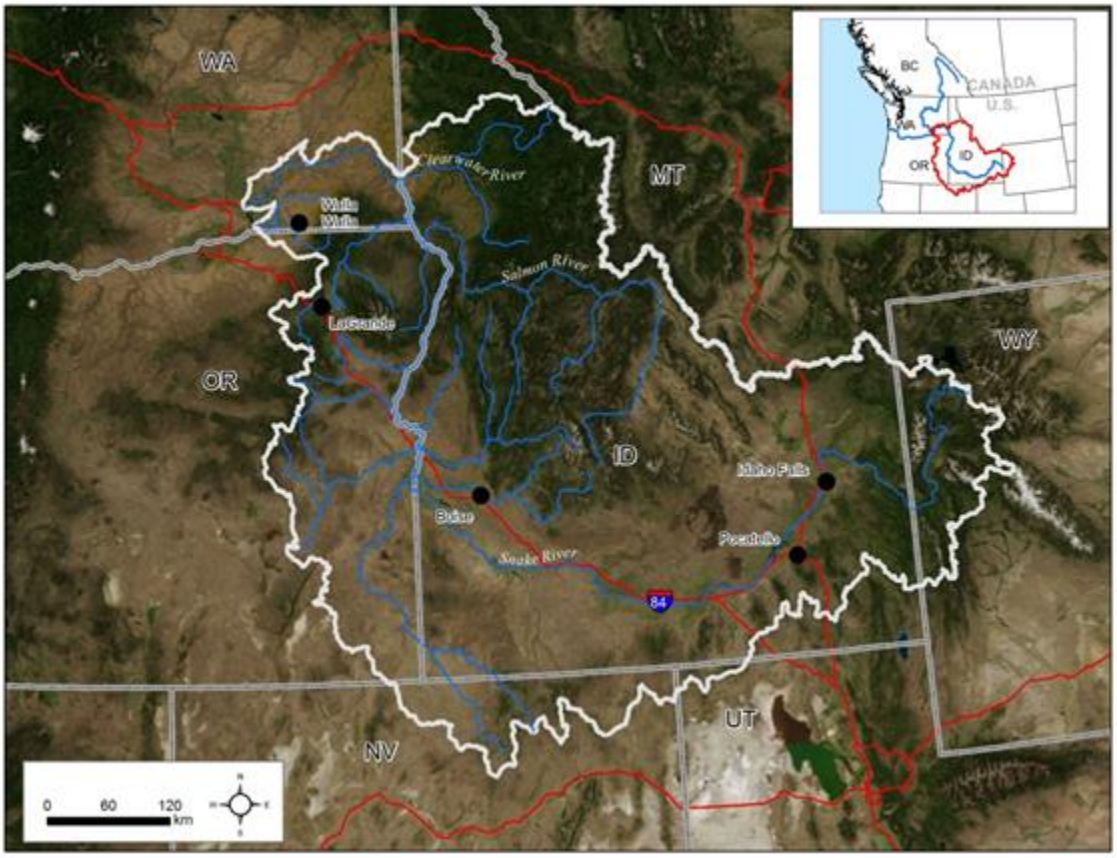


Figure 3: Map of the Snake River Basin.

<B>Dworshak National Fish Hatchery

Kelt reconditioning facilities are located at Dworshak National Fish Hatchery (DNFH) in Ahsahka, Idaho (Figure 4). 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).

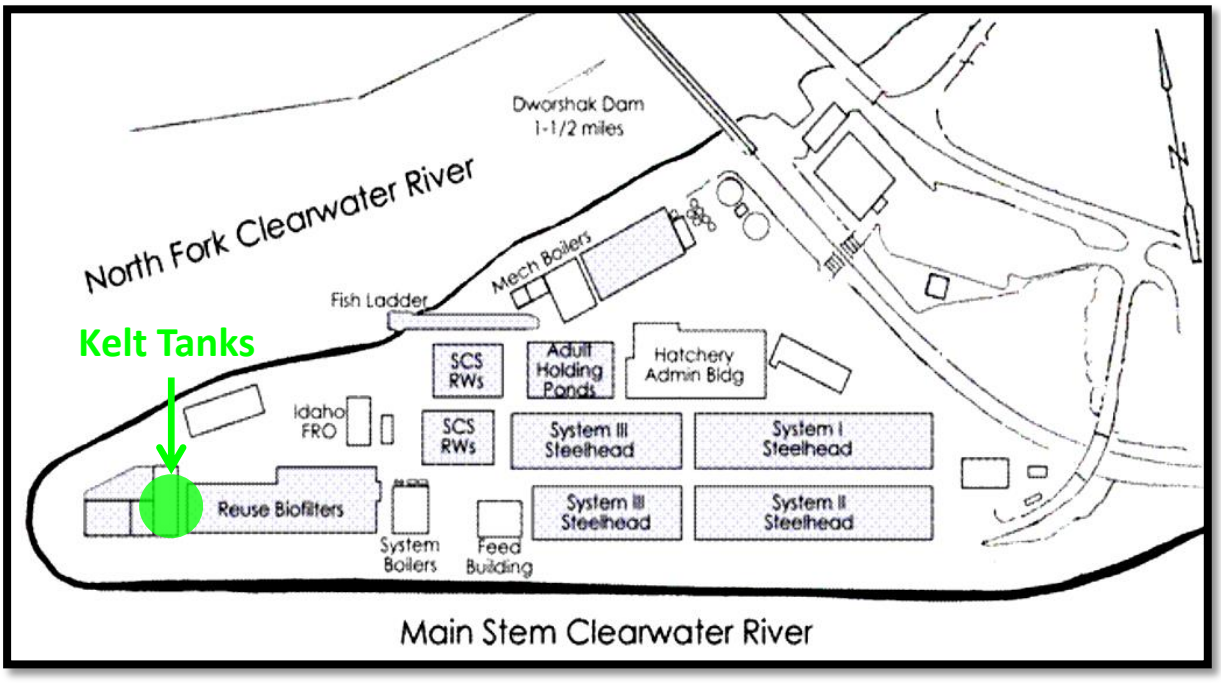


Figure 4: Map showing the location of experimental kelt reconditioning tanks at Dworshak National Fish Hatchery. Figure modified from USFWS 2009.

<B>Cle Elum Research Facility

The Cle Elum Supplementation and Research Facility (CESRF) was built in 1997 to research the effects of supplementation programs on the Upper Yakima near the town of Cle Elum, WA (figure 5). In 2000, an artificial stream 127m x 7.9 m wide was built at the CESRF. The wetted width of the stream ranges between 4.3 and 5.5m. The artificial stream has the ability to be subdivided into 7 sections (figure 6) (Schroder et al. 2008). For the purposes of our experiment we will likely only split into 3 sections.

Figure 5: Cle Elum Spawning Channel overhead photo.

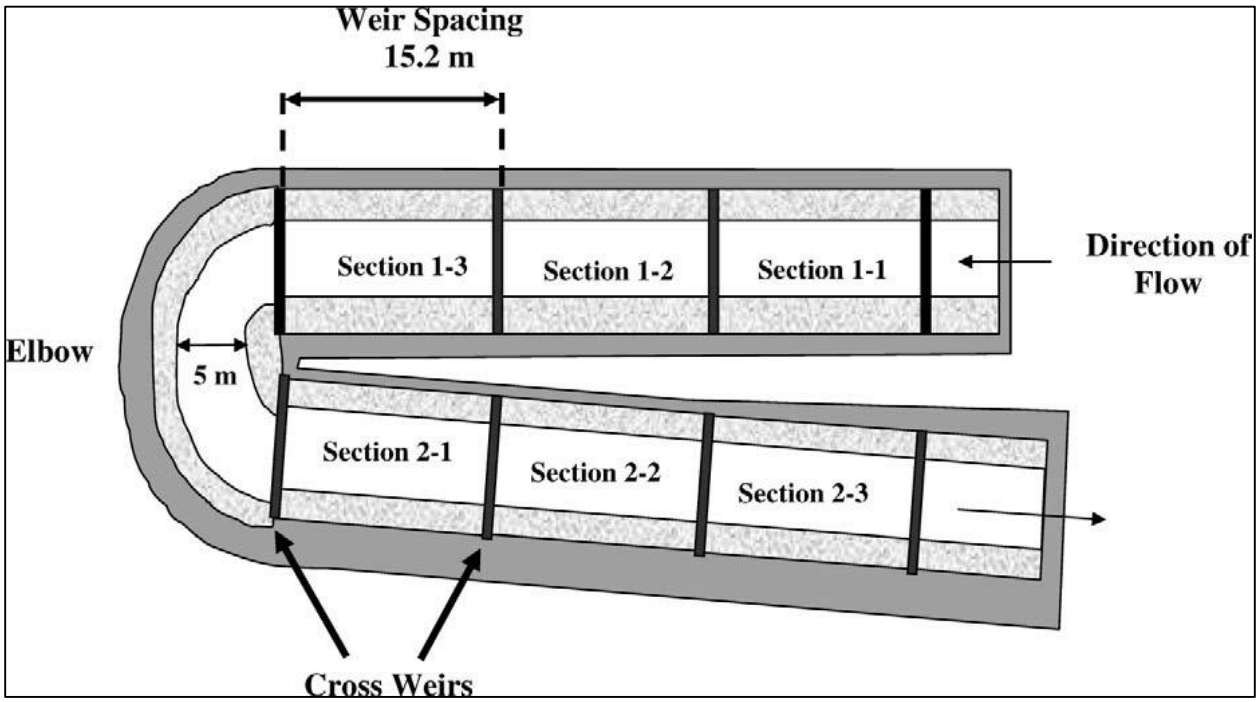


Figure 6: Cle Elum spawning channel setup. Figure used with permission from North American Journal of Fisheries Management.

<A>Reconditioning Processes Strategies

<B>Kelt Collection

Chandler Juvenile Monitoring Facility (Yakima River)

Post spawn steelhead migrating downriver are inadvertently collected by way of the Chandler Juvenile Monitoring 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 7). Yakama Nation staff monitored the Chandler bypass separator during the kelt migration.

Figure 7: Inside view of the Chandler Juvenile Monitoring Facility showing the separator rack where kelt steelhead are collected.

All adult steelhead are placed into a water-lubricated PVC pipe slide that diverts fish to a temporary holding tank 6.1 m (l) x 1.8 m (w) x 1.2 m (h) containing oxygenated well water at 13.8^0C (Figure 8). All specimens were then 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 the control and long-term reconditioning.

Following kelt identification, fish were 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.

Figure 8: Chandler Juvenile Monitoring Facility PVC slide and holding tanks.

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 they are collected by Army Corps of Engineer (COE) staff. Kelts are netted off the adult fish separator bars and moved to a fish hopper that led into the kelt receiving tank (Figure 9). Both B-run ($\geq$ 70 cm) and A-run (<70 cm) steelhead are selected. Our determination differs from the TAC cutoff at 78cm (Busby et. al., 1996) based on evidence that this size distinction does not seem to fit the size distribution of the population. This determination is reinforced, based on our own analyasis of the kelt run length data that has found that there appeared to be a bimodal size distribution in kelts at 63cm (Graham et al, 2014 poster presentation (Appendix 2). In 2014, 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 9: 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 1.8m wide by 7.6m long and 1.8m deep. The tanks have built in crowders, which move along a guided track chain. Each crowder has a lower gate panel, which can be raised mechanically. Both tanks have a release chamber with a lifting floor and an exit gate. The exit gates are connected to pipes leading directly to the river. The receiving tank (tank #1) is nearest to the river and has an additional crowder to allow separation of treatment groups. The holding tank (tank #2) has an additional exit gate, which can be connected to a large diameter hose for alternative release locations (Figure 10).

Figure 10: 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) or Aqui-S® (clove oil) buffered with standard stock solution of sodium bicarbonate to decrease stress and mortality (McCann et al. 1994). Fish were measured, weighed and graded by condition. In assessing the condition, several factors were considered. The condition rating we used referred to the fish's potential for reconditioning. This rating was based on physical appearance, texture and firmness. This rating used three criteria: color, fungus, and injury. Fish also had blood and tissue samples collected for

physiological measures and genetic profiling. All fish that were not moribund received a PIT-tag before being assigned to a treatment or released back to the river.

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.

Fish Creek Weir

The resistance board weir is used to interrogate upstream and downstream migrants (Figure 11). The trap was operated by IDFG. The weir trap box was examined several times daily for fish and kelts were kept in the trap box until processing. The Nez Perce Tribe/CRITFC processed captured female kelts then graded by condition, measures of length, weight at collection, genetic samples taken, and then retained for shipment to Dworshak National Fish Hatchery for long-term reconditioning or released downstream of the trap. The same methods used at Lower Granite Dam to transport to Dworshak were utilized at this location.

Figure 11: Fish Creek Weir. Picket weir from which kelts are collected and then transported to Dworshak National Fish Hatchery for reconditioning.

Dworshak National Fish Hatchery

Collection

Fish volitionally entered the adult ladder at DNFH. They are then crowded mechanically into collection baskets and anesthetized in tricaine methanesulfonate (MS-222) or Aqui-S® (clove oil). However, several of the air-spawned fish had been anesthetized with carbon dioxide during the previous weeks for ladder counting and fish sorting. Carbon dioxide presents sub-lethal stresses that are likely to be adverse to survival of the kelts. Sorted steelhead were emptied on to a large stainless steel table and assessed by observing several physical factors prior to being selected for air spawning and reconditioning. Fish health was evaluated by: 1) maturation level - only very ripe females and 2) morphological fitness – no physical injuries on the body surface, no obvious fungus present, no fin rot, or head burn. Fish not selected for reconditioning were air-spawned, PIT tagged and released into the mainstem Clearwater River after a three day recovery period.

Brood Air Spawning

Similar to 2010-13, steelhead were air-spawned at DNFH to augment the number of fish for reconditioning experiments. Selected fish were transferred to an area set aside for the air-spawning procedure (Lietritz and Lewis 1976). 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 (Figure 12). Each female's eggs were collected in a bucket with a distinct

identification tag. 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. A majority of the eggs were fertilized and incorporated into DNFH production. Eggs not used by DNFH were treated with iodine, rinsed and frozen. These eggs were used to supplement feed for reconditioning kelts.

Figure 12: Air-spawning steelhead at Dworshak National Fish Hatchery.

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 Distell Fish Fatmeter to the outside of the fish. Tagging needles were factory-loaded with PIT tags. A 12mm PIT tag was inserted with a sterile PIT tag implant gun midway between the pelvic fins. 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.

<B>Genetic stock identification (GSI) to assign individual stock-of-origin and estimate stock proportions in a mixed sample of kelt steelhead sampled at Lower Granite Dam

Sampling and genotyping

All kelt steelhead captured in juvenile bypass at Lower Granite Dam during downstream migrations between 2009 and 2013 were sampled for genetic analysis. Biologists from the Nez Perce Tribe and University of Idaho provided field data that was recorded at the time of sampling. Field data included PIT-tag information, sample date, fork length, gender, disposition and overall condition (Appendix 1.a). An overall condition rating of "poor", "fair" or "good" was based largely on physical appearance, fungal load, and presence of injuries (e.g., head wounds; pers. comm. Scott Everett, Nez Perce Tribe). The field identifications of all natural-origin kelts sampled between 2010 and 2013 were verified using parentage based tagging (PBT) data in a baseline that was initiated in BY2008 (Steele et al. 2012). Those natural origin kelts that were subsequently assigned as progeny of PBT broodstock adults were re-classified as hatchery-origin and excluded from GSI analysis of natural-origin kelts. Since PBT is capable of identifying the true (specific) hatchery-of-origin for each hatchery kelt individual, the assignment concordance between PBT and GSI methods was used to evaluate the accuracy of GSI assignments for all hatchery kelts sampled in 2010, 2012, and 2013 (2011 was excluded due to missing genotypes for the PBT panel of SNPs). In addition, PIT-tag detections at LGD and associated interrogation data obtained from the PTAGIS database (Pacific States Marine Fisheries Commission 2009) was used to evaluate concordance between detection sites (i.e. known mark or release sites) and corresponding GSI assigned RGs of kelts. A total of 192 unique SNP loci (Hess et al. 2012) were pared to 188 loci following exclusion of a sex determining marker, and three *O. clarkii* hybrid determining markers, to be used for genetic stock identification (GSI) analysis specific to the Snake River Basin. A description of 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).

GSI procedures

Typically GSI is a regional application drawing on the scope of demographic influences (e.g., migration) and evolutionary factors (e.g. local adaptation) to delineate groups of genetically similar populations. When evaluating a mixed stock sample using GSI, assigning individuals to a particular population of origin has proven to be routinely less accurate than assignment to a reporting group (RG) representing a larger, genetically similar aggregate (Hess et al. 2012). For these analyses, reporting groups were assembled from a Snake River reference baseline compiled by CRITFC and Idaho Department of Fish and Game (IDFG; Hess et al. 2012, Ackerman et al. 2012). The reference baseline was comprised of 73 discrete collections, representing all major subbasins in the Snake River Basin, and included multiple collections (watersheds) per subbasin in an attempt to account for all contributing stocks or discrete populations (Appendix 2). Genetic similarity among the 73 reference baseline populations was gauged on the basis of observed allele frequency variation, but ultimately the reporting groups were defined on the basis of the following prioritized sources of information: 1) the genetic similarity of populations based on structure analyses, 2) major population groups (MPG's) determined by managers, 3) geographic structure (i.e. adjacency of watersheds in the Snake River), and 4) formation of RGs using iterative combinations of populations based on the prior three criteria.

The resolving power (i.e. power to differentiate between stocks) of the steelhead reference baseline was evaluated to provide an expectation of assignment accuracy in GSI analyses. The analysis program GENECLASS2 (Piry et al. 2004) was used to estimate rate of self-assignment within each RG using a jackknife, or 'leave-one-out' (LOO) procedure, and implementing the Bayesian method of Rannala and Mountain (1997). Baseline resolving power was determined by rate of self-assignment for each reference population. Self-assignment was defined as the proportion of individuals that assigned to their respective, pre-determined RG-of-origin (not necessarily population of origin) with highest probability. The top five population assignment probability scores were ranked for each individual (rank 1 = highest probability, rank 2 = next highest probability, etc.), and an overall RG assignment probability was calculated by summing successively ranked probability scores when the corresponded populations were from the same predetermined RG. Individual kelt assignment probabilities were estimated in a process similar to the baseline power analysis. The most likely population-of-origin for each individual kelt was determined on the basis of highest observed assignment probability, corresponding to one of 73 reference populations. Similarly, the overall RG assignment probability of each kelt was calculated as the sum of successively ranked population probability scores corresponding to the same RG.

<B>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.

<B>Long-term Reconditioning

Prosser Hatchery Reconditioning Facility

Kelts were captured at the CJEF and then placed in a holding tank. 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 with a small aerated holding tote to the hatchery (Figure 13). Steelhead kelts retained for the long-term reconditioning treatments were held in one of four 6.1 m (d) x 1.2 m (h) circular tanks (Figure 5). Loading densities were approximately 2/3rd of the 300 fish carrying capacities of these tanks. Tanks were fed oxygenated 13.8^{0}C well water at 757 liters/minute (l/m).

Figure 13: 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 emamectin benzoate (200 µg kg^{-1}). The drug was administered via injection to the peritoneal cavity for the treatment of copepods (Glover et al 2010). All fish held for long-term reconditioning received an intraperitoneal injection, based on weight, of the antibiotic oxytetracycline.

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 (formaldehyde 37%) 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

<C>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.

<C>Dworshak Reconditioning Facility and Treatment

Four 4.5m diameter tanks are located at DNFH (Figure 14). Tanks have anti-jump curtains and shade covers. River water is provided from a fire maintenance supply line at a flow rate of 3.78 l/m per tank. Tank outflows are plumbed to both the DNFH settling pond and DNFH's System-III digester. 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 are constantly monitored and logged using a data logger. An emergency monitoring system is installed on each tank. Dissolved oxygen probes and flow meters are connected to an alarm system and data logger. This system allows 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, oxytetracycline, is administered to all kelts when transferred to the tanks. A programmable peristalsis pump and drip system was installed in 2013 to deliver formalin for fungus control. Feeding begins after initial sampling. Fish are first presented with

krill or eggs until the feeding response is well established. Then fish are given a higher lipid content kelt/broodstock feed.

Figure 14: Experimental kelt reconditioning tanks at DNFH with anti-jump containment curtains and four bucket Koch ring packed columns.

<B>Kelt Reconditioning Physiology Studies

Reproductive development in kelt steelhead

<C> Blood Sampling

Fish were blood sampled at the indicated dates in 2013 and 2014 (Table 1). Steelhead kelts were collected and either released or retained for reconditioning at Prosser Hatchery, Washington, Dworshak National Fish Hatchery, Idaho, and Winthrop National Fish Hatchery, Washington as described elsewhere (Kelt Collection) (Abrahamse and Murdoch 2013, 2014). 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 and Shearer 2001; Crossin and Hinch 2005). The maturity status of fish sampled on 9/10/14 at Prosser was assessed by experienced fish culture staff. Female kelts with spawning color, a rounded belly, and a protruding vent were classified as rematuring, whereas fish without these characteristics were classified as non-rematuring. Necropsies were conducted on mortalities that occurred at Prosser after the 9/10/14 sampling. The weight of the ovary was recorded and gonadosomatic index (GSI) was calculated as 100 * ovary weight/body weight.

Table 1: Steelhead sampled in 2013 and 2014. DNFH: Dworshak National Fish Hatchery, WNFH: Winthrop National Fish Hatchery, Prosser: Prosser Hatchery, Prosser Denil: Denil trap for upriver fish at Prosser dam. LGR: Lower Granite Dam adult ladder.

Location	Sample date	Fish type	# Fish	Blood samples	Notes
Prosser Denil	Spring 2013	maidens	46	46	
Prosser Denil	Fall 2013	maidens	61	61	
DNFH	2/26/2013	spawners	69	0	Fish not blood sampled
DNFH	3/5/2013	spawners	77	0	includes South Fork fish
DNFH	3/12/2013	spawners	37	0	incudes South Fork fish
DNFH	4/4/2013	kelts	170	0	strip residual eggs and emamectin inj
DNFH	8/9/2013	kelts	144	144	hatchery and wild
DNFH	9/30/13, 10/3/13	kelts	133	133	hatchery and wild
Prosser	8/14/2013	kelts	408	408	all fish
WNFH	9/26/2013	kelts	6	6	all fish
Prosser	10/22/2013	kelts	98	98	radio tagged kelts

2013 total			**1249**	**896**	
DNFH	2/3/2014	kelts	46	46	2013 spawn year kelts
DNFH	3/4/14, 3/6/14	spawners	82	82	air spawned hatchery, some released
DNFH	4/1/2014	spawners	92	92	air spawned hatchery, some released
DNFH	5/7/14, 5/8/14	kelts	122	122	hatchery kelts
DNFH	6/25/2014	kelts	70	70	hatchery kelts
DNFH	8/28/2014	kelts	105	105	all fish
DNFH	11/6/2014	kelts	79	79	all fish
LGR	Sept-Oct 2014	maidens	360	360	200 hatchery + 160 wild
Prosser	9/10/2014	kelts	382	382	all fish
WNFH	10/1/2014	kelts	58	58	all fish
2014 total			**1396**	**1396**	

<C>Estradiol Assay

Fish plasma level of estradiol-17β (E2) is an indicator of reproductive development. Fish plasma samples must be solvent extracted prior to E2 assay to remove interfering substances. Plasma samples (250 µL) were extracted twice consecutively in 10 mL glass tubes with anhydrous diethyl ether (JT Baker, Avantor Performance Materials, Inc.; Center Valley, PA, USA). 2.0 mL diethyl ether was added to each tube and samples were vortexed for 1 m, and then frozen on dry ice. After 6-8 m, the aqueous phase was inspected to ensure that it was frozen solid, and the solvent fraction was then poured off into a 5 mL glass tube. Diethyl ether extracts were then placed in a 49ºC water bath (OA-SYS™Heating System; Organomation Associates, Inc; Berlin, MA) and dried down under a gentle stream of N_2 directed *via* a nitrogen evaporator manifold (N-EVAP™ 112; Organomation Associates, Inc; Berlin, MA). A second 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. Dried extracts of fish plasma were resuspended in 250 µL assay buffer from the estradiol assay kit.

Plasma E2 concentrations were assayed by an enzyme immunoassay using an acetylcholinesterase linked estradiol tracer (Cayman Chemical, Ann Arbor, MI). Extracted plasma samples were appropriately diluted and duplicate technical replicates assayed in the EIA according to the manufacturer's instruction manual provided with the kit.

Proteomic Analysis of Female Steelhead Plasma

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 coupled 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.

<B>Population Model

Model Description

The model assumes that iteroparity rates do not differ among years, but that the iteroparity rate for virgin spawners r_N differs from that of repeat spawners r_I. The number of virgin spawners N_t in run year t is the sum of all spawners of age a coming from run year t-a. The following equations describe the life history model. All symbols are indexed by run year, so the smolts (S_t) from run year t are in fact observed in year t+3, the ocean adults $O_{a,t}$ are in fact observed in year t+a+1, and the returns $N_{a,t}$ are also in year t+a+1.

The model begins by summing all virgin returning adults $N_{a,t-a}$ and repeat spawners $I_{a,t-a}$.

$$N_t = \sum_{a=4}^{7} N_{a,t-a}$$

$$I_t = \sum_{a=1}^{4} I_{a,t-a}$$

Virgin and repeat spawners are subject to a prespawn mortality rate m_s and added to get the total number of spawners in run year t after pre spawn mortality.

$$S_t = (1 - m_s)[N_t + I_t]$$

Kelts are then calculated by a virgin and repeat kelt rate (r_N and r_I respectively).

$$K_t = (1 - m_s)[r_N N_t + r_I I_t]$$

Smolts M_t are calculated using a Ricker function with productivity a_t and capacity b_t.

$$M_t = S_t e^{(a\left(1-\frac{S_t}{b_t}\right))}$$

A condition function is calculated where the condition Ct is predicted using a logistic function with a base rate α and scaled to the normalized flow, temperature and spawner density using rates β, γ, and δ respectively.

$$C_t = 1/(1 + e^{-\alpha-\beta FLOW_t-\gamma TEMP_t-\delta S_t})$$

Depending on condition, kelts fall into the category of good (G_t), fair (F_t), poor (P_t), with the proportion of good q_g, fair q_f, and poor q_p depending on condition C_t.

$$G_t = q_g K_t$$

$$F_t = q_f K_t$$

$$P_t = q_p K_t$$

The number of in-river releases depends both on the capture rate π of good and fair kelts and the proportion of captures released θ_r .

$$R_t = \pi\theta_r (G_t + F_t) + (1 - \pi)(G_t + F_t)$$

All poor condition kelts are released in-river.

$$RP_t = P_t$$

The number of transported un-fed kelts depends both on the capture rate π of good and fair kelts and the proportion of captures transported and not fed θ_u .

$$U_t = \pi\theta_u (G_t + F_t)$$

The number of transported fed kelts depends both on the capture rate π of good and fair kelts and the proportion of captures transported and fed θ_f .

$$F_t = \pi\theta_f(G_t + F_t)$$

The number of long-term reconditioned kelts depends both on the capture rate π of good and fair kelts and the proportion of captures reconditioned fed θ_l .

$$L_t = \pi\theta_l(G_t + F_t)$$

The number of repeat spawners in year a years after the first spawning migration year is the sum of the products of survival rates and kelt classifications for each of R_t RP_t, U_t, F_t, and L_t, with respective survival rates $s_R^a, s_P^a, s_U^a, s_F^a, s_l^a$ where the superscript *a* denotes the number of years between successive spawnings.

$$I_{a,t} = R_t s_R^a + RP_t s_P^a + U_t s_U^a + F_t s_F^a + L_t s_l^a$$

The number of ocean adults $O_{4,t}$ pre spawning migration after one year in the ocean (i.e.: 3 years after spawning and four years after spawning migration) is given by at Beverton-Holt survival function with productivity p and capacity k. Note that both parameters can vary in time as a function of environmental conditions, and so may not be constant. Note also that capacity can be set to near infinity to eliminate density dependence.

$$O_{4,t} = p_{1,t} M_t \frac{p_{1,t} M_t}{1 + \frac{p_{1,t}}{k_{1,t}} M_t}$$

The number of adults returning to spawn after one year $N_{4,t}$ is the ocean adults multiplied by the maturation rate φ_1 after one year in the ocean.

$$N_{4,t} = \varphi_1 O_{4,t}$$

The ocean adults surviving a second year in the ocean is the $O_{4,t}$ that do not migrate times a Beverton-Holt survival function for survival a second ocean year.

$$O_{5,t} = (1 - \varphi_1) p_{2,t} O_{4,t} \frac{(1 - \varphi_1) p_{2,t} O_{4,t}}{1 + \frac{p_{2,t}}{k_{2,t}} = (1 - \varphi_1) O_{4,t}}$$

The number of adults returning to spawn after one year $N_{5,t}$ is the ocean adults multiplied by the maturation rate φ_2 after a second year in the ocean.

$$N_{5,t} = \varphi_2 O_{5,t}$$

The ocean adults surviving a third year in the ocean is the $O_{5,t}$ that do not migrate times a Beverton-Holt survival function for survival a third ocean year.

$$O_{6,t} = (1-\varphi_2)p_{3,t}O_{5,t}\frac{(1-\varphi_2)p_{3,t}O_{5,t}}{1+\frac{p_{3,t}}{k_{3,t}}(1-\varphi_2)O_{5,t}}$$

The number of adults returning to spawn after one year $N_{5,t}$ is the ocean adults multiplied by the maturation rate φ_3 after a third year in the ocean.

$$N_{6,t} = \varphi_3 O_{6,t}$$

After a fourth year in the ocean, all adults return to spawn, so the fraction of $O_{6,t}$that did not spawn after the third year in the ocean are predicted to survive a fourth year and return to spawn.

$$N_{7,t} = (1-\varphi_3)p_{4,t}O_{6,t}\frac{(1-\varphi_3)p_{4,t}O_{6,t}}{1+\frac{p_{4,t}}{k_{4,t}}(1-\varphi_3)p_{4,t}O_{6,t}}$$

The model will be used to examine various metrics of recovery success under assumed survival rates and capture rates. The key variables that will be assumed or estimated will be relative survival rates of the four recondition groups ($s_R^a, s_P^a, s_U^a, s_F^a, s_l^a$), the productivities and capacities in fresh and ocean stages, the kelt rates r_N and $r_{l,,.}$ The capture rate π, and the proportions $\theta_r, \theta_f, \theta_u, \theta_l$ are the quantities of interest that govern the potential recovery rate improvement that can be achieved with the kelt program. We will examine a range of possible population trajectories by setting rates for productivities and capacities, and maturation rates and calculated the relative recovery rates by changing rate π, ,$\theta_r, \theta_f, \theta_u, \theta_l$.

Data and model implementation

In 2014 we began formal parameterization of the model using known steelhead spawning abundances, smolt migration abundances, survival rates of kelt release groups, portions of captured kelts going into each release group, and the kelt capture rate itself.

We obtained spawning abundance data from upstream-migrating (prespawn) steelhead at Prosser Dam. Smolt abundances were taken from Frederiksen *et al.* (2014). Kelt success rates of each release group were obtained from summaries of tagged captures and returns. Estimates of survivals were 39.75%, 3.6%, 15%, and 2.6% respectively for long-term reconditioned fish, in-river releases, fed transported fish, and unfed transported fish. Success rates were calculated as the average success rate over all years that a cohort of kelts was available for each category. We parameterized the model such that the predicted success rate of each kelt category was the same each year. We used the average of the proportions of kelts falling into each category as evaluated from the total collections and returns.

Initializing the model requires initial spawning abundances, which were available from 1985-2013. Smolt abundance data were also available for the same period. For the model to be able to predict future generations of fish, it must be able to predict smolts from spawners, adults in the ocean from smolts, and returning spawners from ocean adults. We obtained estimates of the Ricker smolt production parameters by fitting the smolt abundances predicted by the Ricker

function to the observed smolt abundances. A Ricker function was fit to the model using a process error model with a log-normal negative log-likelihood minimization. We used migration year total smolts two years after the spawning year as the estimated smolts from a spawning group. Since the majority of outmigrating juveniles are two years old, this value would be most robust to temporal variation. The model fit estimates a Ricker productivity parameter of 3.94, and a capacity parameter of 11,278 (using the Ricker formula in the model description). Figure 15 shows the fit of the model to the data, and the predicted smolts per spawner using the parameterized model. We see that smolts per spawner have been approximately 15-20 smolts per spawner on average, requiring approximately 5-7% SAR for population stability.

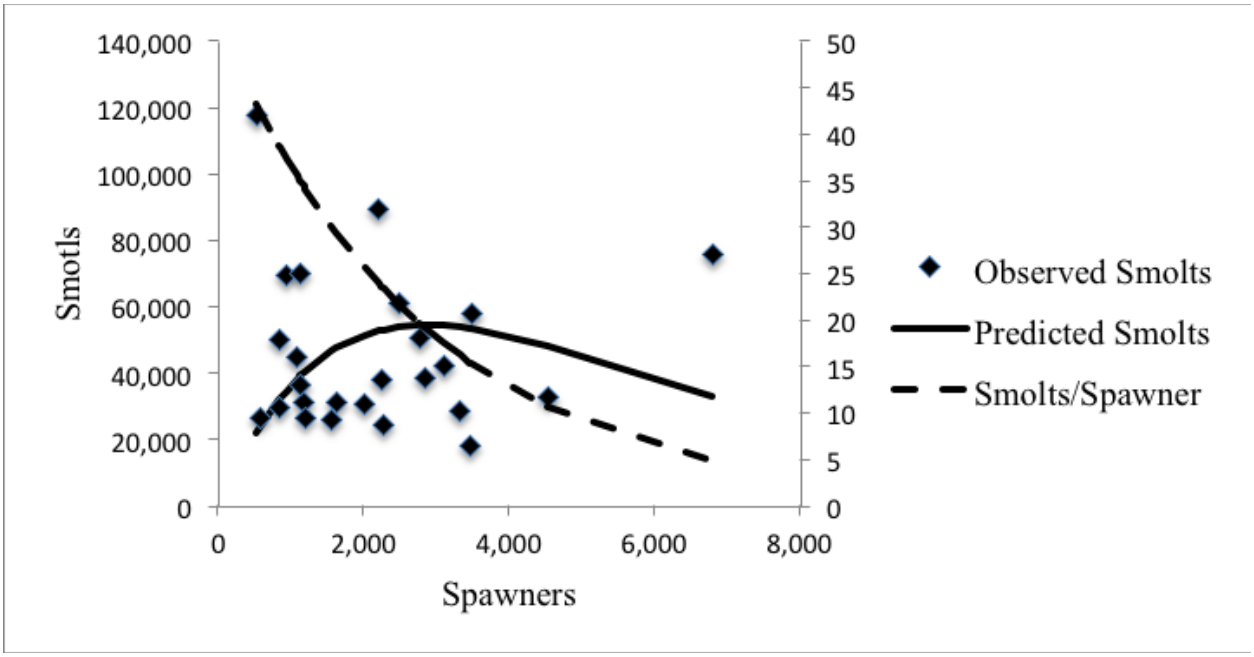


Figure 15: Ricker stock recruitment model fit of smolts to spawners.

Because we did not have age structure data of returning adults, we assumed that all returning adults spent two years in the ocean. We fixed the maturation rate of first year ocean fish to zero so that all fish would remain an additional year in the ocean, and we fixed the second year maturation rate to 1.0 so that all fish would return after the second year in the ocean. The model thus predicted only four year old returns. Additional assumptions in the life cycle parameterization included: 1. Ocean capacity terms of 1.e12 (effectively eliminating density dependence), 2. Kelt capture rates of 40%, repeat kelt rate of 25%, 6% in-river release portion of kelts, 5% transport unfed portion of captured kelts, 15% transport fed portion of kelts, and 73% long term recondition portion of kelts. Additionally, we used the Ricker productivity and capacity parameters estimated from fitting the Ricker smolt production function.

<A>Reproductive Success of Artificially Reconditioned Kelt Steelhead

<B>Egg quality and reproductive parameters in hatchery origin maiden female steelhead and reconditioned kelts at Dworshak National Fish Hatchery

In 2013 and 2014, hatchery origin maiden female steelhead were air spawned at DNFH (Table 2). Air spawning was conducted as previously described (Hatch, et al. 2014). In both years, 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). In 2014, the total weight of eggs collected from each female was recorded, and a subsample of approximately 100 eggs from each female was taken for transport to the Nagler lab at the University of Idaho. The total weight of eggs was used as ovary weight for calculation of gonadosomatic index. 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 for calculation of egg weight. 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).

Table 2: Hatchery origin female steelhead artificially spawned and reconditioned at Dworshak National Fish Hatchery in 2013 and 2014.

Spawn Year	Fish Air Spawned	8/9/2013		8/28/2014	
		Alive (%)	Rematuring (%)	Alive (%)	Rematuring (%)
2013	163	74 (45.4)	16 (21.6)	29 (50)	27 (93.1)
2014	149	-	-	32 (21.5)	2 (6.3)

Fish were reconditioned as described (Hatch et al. 2014)(Long-term Reconditioning). The 2013 spawn year hatchery origin kelts were sampled on 8/9/13, 10/3/13, and 2/3/14. After the 2/3/14 sampling, kelts were checked weekly and ripe fish were air spawned. Eggs quality and fecundity were assessed as described above. Remaining non-rematured 2013 hatchery origin kelts and 2014 spawn year hatchery origin kelts were sampled on 5/7/14-5/8/14, 6/25/14, 8/28/14, and 11/6/14. During sampling, length, weight, and muscle lipid levels were measured and blood was drawn for hormone assays. Laboratory analysis of these samples is ongoing.

Results are reported for assays that have been completed. Plasma estradiol was assayed as described (Methods:Estradiol Assay)(Hatch et al. 2014).

<B>Kelt Reproduction in a Natural Setting

Yakama Parentage Analysis

<C>Sample Collection

Anadromous adult steelheads were collected as upstream migrants at Prosser Dam or downstream migrants at the Chandler Juvenile Monitoring Facility. Samples collected as upstream migrants at Prosser Dam were treated as maidens and referred to as pre-spawn maiden collections. Post-spawn adults collected at The CJMF that survived reconditioning to release in the fall were referred to as kelts for the spawning event following their release. For the spawning event prior to their capture, they are treated as maidens and referred to as post-spawn maidens.

Age-0 juveniles (juveniles collected in the same calendar year as the spawning event) were primarily targeted using electrofishing techniques (NMFS 2000 Electrofishing Guidelines) during the fall in natal tributaries. Fork length was recorded collected. Sampling was targeted near areas where steelhead spawning was observed or a spawning redd was detected. Technicians in the field were directed to target only age-0 juveniles. A 100mm general minimum length was used in addition to the best judgment of those collecting the samples. An additional 50 samples were collected at a rotary screw trap in Toppenish Creek.

<C>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 172 of the 192 markers. Of those not used, three are diagnostic for cutthroat, one (OmyY1_2SEX) is a sex-determining marker and one (Omy_mapK3-103) was known to have significant linkage disequilibrium issues with another locus (Hess et al. 2012). An additional 15 loci with minor allele frequency less than 0.05% were also dropped from analysis (Omy_cd28-130, Omy_pad-196, Omy_UT16_2-173, Omy_97077-73, Omy_inos-97, Omy_carban1-264, OMS00095, Omy_b9-164, Omy_mcsf-268, Omy_97865-196, Omy_sSOD-1, Omy_gadd45-332, Omy_LDHB-2_i6, OMS00169, Omy_nips-299).

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). Linkage disequilibrium was tested using exact tests

(Haldane 1954, Weir 1990, Guo and Thompson 1992) implemented in GENEPOP v3.4 (Raymond and Rousset 1995).

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. Individual parentage assignments were included if they had a minimum of 164 Loci comparisons and met the critical LOD value of 3. For loci with a single locus mismatch, a more stringent LOD value of 15 was used. This accounts for the presence of minor genotyping errors, while still incorporating additional samples.

Parentage data was stratified by reporting reproductive success of three primary classes: 1) Maidens collected as pre-spawners, 2) Maidens collected as post-spawners, and 3) Reconditioned kelts. To account for differences in collection times, and potential post collection mortality, parentage results were correlated to: 1) All fish, 2) All females, and 3) All females detected at or expected to have been upstream of Prosser Dam.

Repeat Spawner Post Release Tracking

Reconditioned kelts selected for the radio tag portion of this experiment received the same treatments as kelts in the long term artificial reconditioning program. Candidates were selected from a pool of fish deemed to be maturing based on estradiol assays (see Estradiol Assay). Also, candidates had additional selection criteria applied, based on visual condition (good color, good condition, and weight gain) and the discretion of the hatchery manager based on belly firmness, presence of developing eggs. Candidates that had previous detection histories as maiden fish were also prioritized.

All radio tagged reconditioned kelts were anesthetized with AQUI-S and had condition factors recorded (condition, color, length, and weight) just prior to release. All fish were scanned for PIT-tags and those that expelled their tags during the reconditioning process, had new tags inserted into the pelvic girdle. A genetic sample was collected from every fish at intake. Lotek model MCFT2-3A transmitter, with dimensions 16 mm in diameter x 46 mm in length, with a water weight of 6.7g, burst interval of 4 seconds, and tag life of 1112 days (estimated) were utilized. The tags are outfitted with motion sensors capable of emitting both active and inactive codes. A radio tag implanted in a live swimming fish will continually transmit an active code. In the event the tag has been regurgitated or the fish has been depredated, the tag will emit an inactive code after laying motionless for a 24 hour delay period. The motion sensor feature will greatly assist in determining tag regurgitation rates and depredation events of individual fish. The tags were coated with glycerin and had a surgical rubber band attached (aid in tag retention) and then inserted with a specially crafted PVC rod that inserted the tag into the stomach (Mellas and Haynes 1985). Chris Fredericksen tagged all of the candidate fish, as he has numerous years of experience radio tagging steelhead in the Yakima River Basin.

The radio tags were on Lotek's 2000 code set, and were on one narrowband radio frequency of 149.380, Channel 55. Tracking was done primarily using a Lotek SRX 600 receiver connected to

multi directional yagi antennas mounted to the hitch of a pickup truck. These antennas were positioned at 45 degree angles to the left and right of the truck so that they were forward facing to maximize their detection efficiency. Tracking was mainly conducted along roadways adjacent to the Yakima River and its tributaries. Some areas had no road access so tracking was also done on foot and occasionally by helicopter or fixed wing aircraft (assisted by the Yakama Nation VSP project). Mobile tracking was conducted every other week during late fall/early winter, with frequency of sampling increasing to two to three times a week as water temperatures increased in late winter/early spring

PIT-tag antenna arrays at Prosser and Roza Dam, and Satus, Toppenish, Ahtanum and Cowiche creeks were used to confirm radio tag detection histories and infer likely spawning status. Naches River was the only main tributary without and array system.

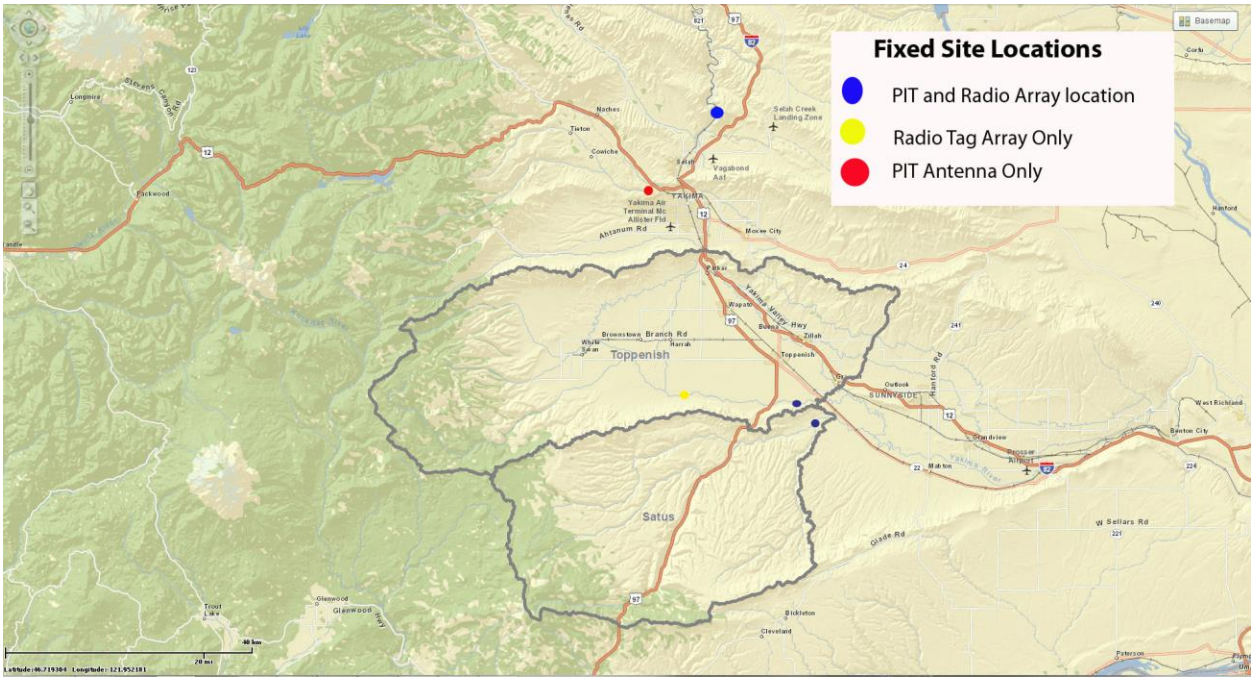


Figure 16: Location of PIT tag arrays and radio tag arrays for detections in the Yakima River Basin.

Juvenile collection

Juvenile collection was done utilizing an electroshocking backpack unit from Smith-Root (NMFS 2000) starting downsteam of suspected redds moving upstream to suspected redd location. Collection timing was determined by using temperature units (TU's) in the spawning areas subsequent to last Radio or PIT-tag detection dates for the tracked individual (Embody 1934). Effort was focused on collecting age-0 fish so that we would not get parentage from the previous year's spawning. Juveniles were anesthetized using MS-222, a non-lethal genetic sample was obtained by taking a small fin clip from the caudal fin. Fish were recovered in a bucket of fresh river water and then released in an area which was similar to the habitat from which they were collected and away from the thalweg.

Results

<A>Reconditioning Processes Strategies

<B>Kelt Collection

Yakima River

A total of 580 live kelts were captured between March 21 and June 30, 2014 at the CJMF. There were 22 steelhead discovered dead upon arrival in the bypass, 20 kelts in poor condition, and 11 prespawn (maiden) steelhead that were released immediately back to the Yakima River on site. A total of 46 good/fair condition kelts were diverted back to the Yakima River for the control. Collection was mostly continuous throughout the migration, with peak collection occurring on May 5, 2014 (Figure 17). The total number of kelts captured represented 13.7% (569 of 4,141) of the Yakima River spawning migration based on fish ladder counts obtained from Prosser Dam for the period June 31, 2013 through June 30, 2014. This collection only represents the portion of the population that volunteer into the Chandler bypass while others migrate over Prosser Dam (Frederiksen et al. 2014).

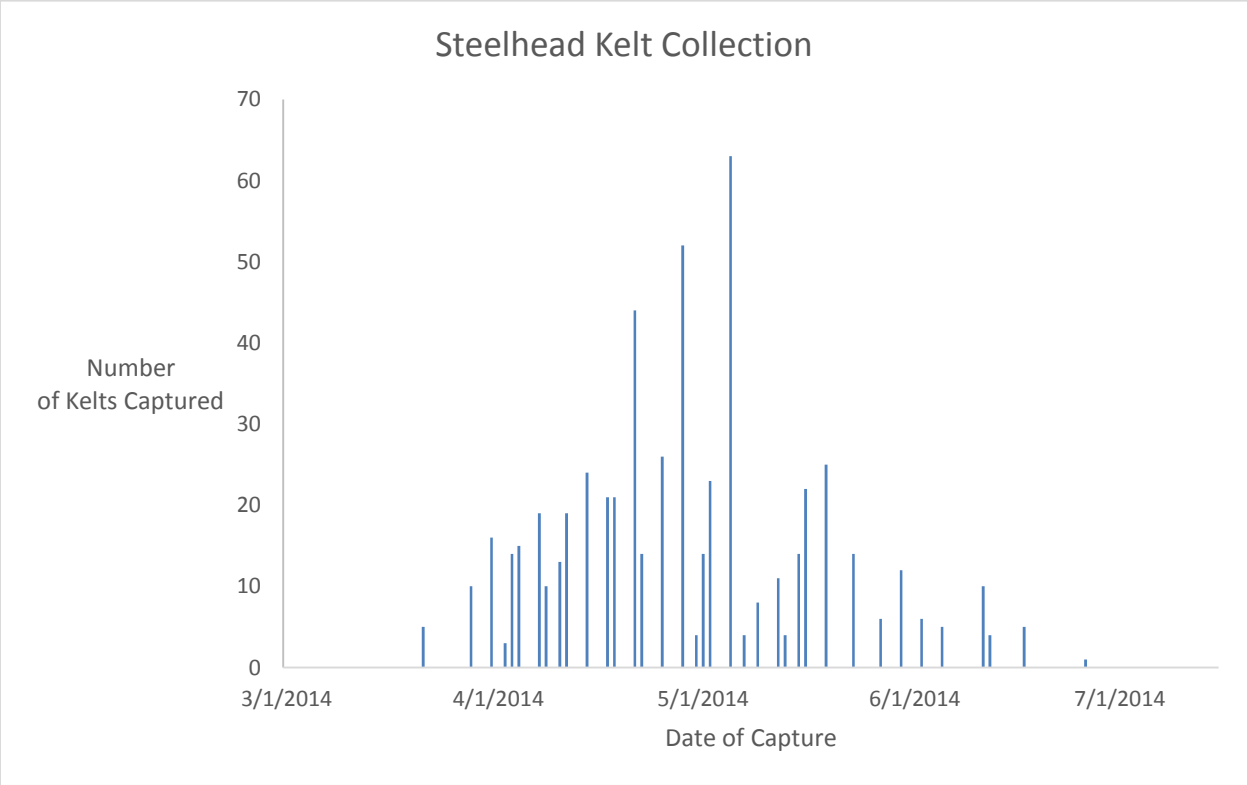


Figure 17: Yakima River kelt steelhead collection at CJMF Prosser, WA in 2014.

Based on visual observations, 469 of 573 (82%) of the kelts were female and 104 (18%) were male. This is a higher number of males than in past years (typically under 10%) (Hatch

et al. 2012). There were 7 fish which had no sex assignment. Most kelts were classified as good condition (n=360, 62%) followed by fair (n=181, 31%) condition and finally as poor condition (n=39, 7%). Coloration was predominately intermediate (n=298, 51%) or bright (n=248, 43%) with a small percentage that were dark (n=34, 6%).

Snake River

<C>Lower Granite Dam
A total of 2,695 kelts were intercepted by the LGR JFF between March 26 and June 26, 2014. Collection was mostly continuous throughout the season. The separator was shutdown on a few days for only a few hours to clear debris. The peak collection (459 fish) occurred the week of May 26, 2014 (Figure 18).

From May 2 to 21, 2014, Blue Leaf Environmental (BLE) floy tagged a subsample of 92 fish to study prototype passage structures. These fish were monitored by BLE in a 300 gallon tank on site and released within 6 hours (O'Conner et al. 2014). Table 3 summarizes the final disposition of kelts collected by the LGR JFF. There were 13 collection/handling mortalities.

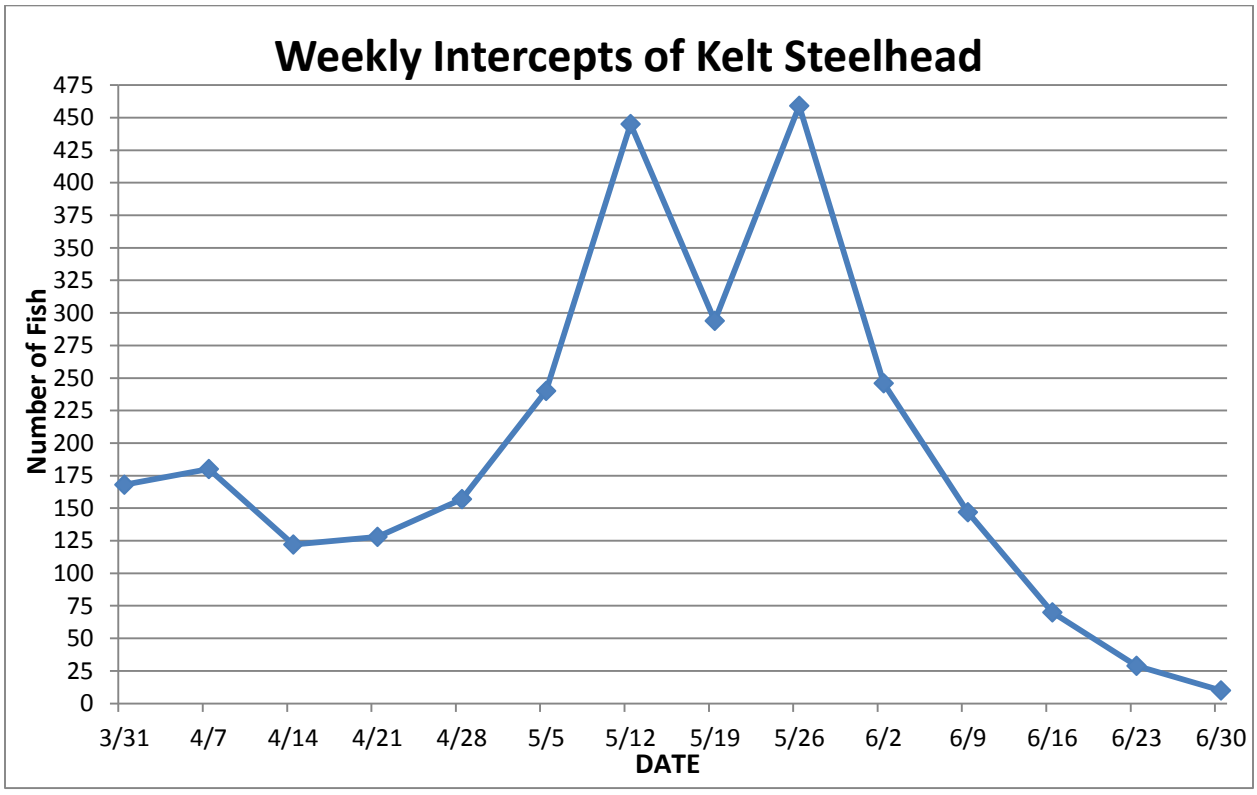


Figure 18: Weekly steelhead kelt interceptions at LGR JFF in 2014.

Table 3: Summary of final disposition of fish collected at LGR JFF in 2014.

Final Disposition	A-run	B-run	Total
Returned to River	2317	162	2479
Flogged tagged and released (BLE)	85	7	92
Transported to DNFH for reconditioning	0	111	111
Mortality	11	2	13
Total	**2413**	**282**	**2695**

The majority of the fish collected from the Snake River at LGR JFF in 2014 were A-run females in good condition. Most fish were without any major wounds (scraps, cuts, fungal infections) with the majority of them collected in the month of May (Table 4). Females ≥70 cm comprised 8.4% of the kelts intercepted at the LGR JFF in 2014 (Table 5). Of these, the proportion rated as being in good condition was 49.1% (Figure 19).

Table 4: Condition of Snake River Kelts collected at the LGR JFF in 2014.

	March	April	May	June	Total
Good	60	294	815	152	1321
Fair	58	195	405	94	752
Poor	50	156	356	60	622

Table 5: Condition of steelhead kelts by sex and size at the LGR JFF in 2014.

Female	Good (49.0%)	Fair (27.9%)	Poor (23.1%)	% of collection
< 70 cm	844	435	304	58.7
≥ 70cm	111	63	52	8.4
			Total	**67.1**
Male				
< 70 cm	361	247	258	32.1
≥ 70cm	4	7	9	0.8
			Total	**32.9**
Total	1320	752	623	**2695**

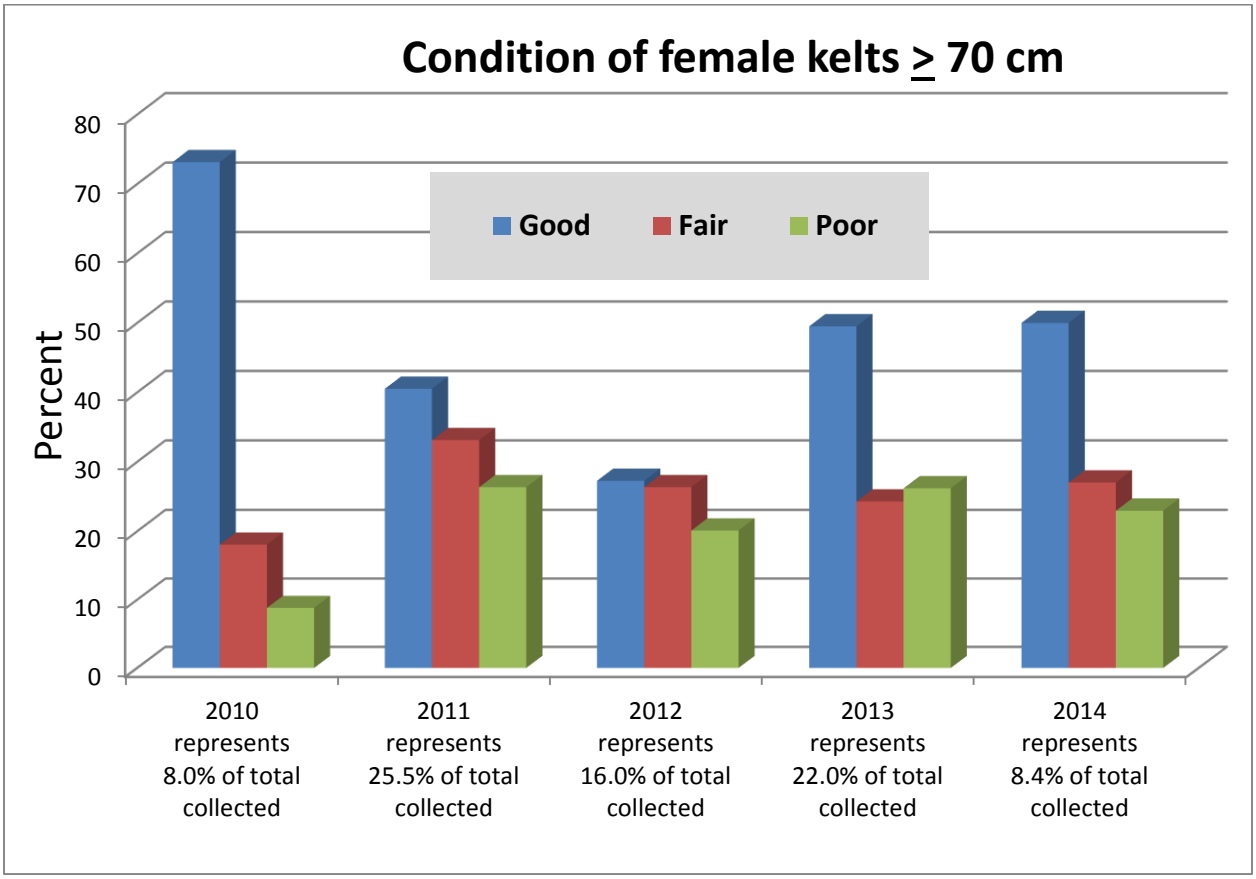


Figure 19: Percent comparison of female kelt steelhead ≥ 70 cm at LGR JFF by condition and collection year.

<C>Dworshak National Fish Hatchery

A total of 147 steelhead was air-spawned and retained for reconditioning. Air-spawning occurred on four separate days (Table 6). From the 2014 air-spawning, a total of 13 DNFH ladder fish remain on-station. In addition, there are 21 surviving 2013 air-spawned females. These fish will continue to be monitored for ripeness. Surviving mature fish will be air spawned during the spring of 2015.

Table 6: Snake River steelhead air-spawned and kept for reconditioning in 2014.

Spawn Date	Total Air-Spawned
Feb 25, 2014	20
March 4, 2014	32
March 6, 2014	20
April 1, 2014	75
Total	**147**

Fish Creek Weir

There were 91 (38 females/ 52 male) fish that were interrogated migrating upstream in 2014. Out of the 24 females that were collected as kelts we transferred 12 of them for long-term reconditioning. Fish were transfered from June 4 to July 3, 2014 (Table 7). The weir was out of operation few days due to high water. There was one male that was originally misidentified.

Table 7: Summary of kelts collected at Fish Creek weir in 2014.

Steelhead Kelts	Collected*	Transferred to DNFH
Females	24	11
Males	48	1
Total	**72**	**12**

***draft data provided by IDFG**

<B>Genetic stock identification (GSI) to assign individual stock-of-origin and estimate stock proportions in a mixed sample of kelt steelhead sampled at Lower Granite Dam

Ultimately, a total of 4,171 natural-origin kelts and 2,288 hatchery-origin kelts were successfully genotyped and evaluated using GSI to estimate stock proportions (Table 8a and 8b).

Table 8a. Estimated stock proportions for natural-origin (NOR) kelts sampled at LGD during outmigration years 2009 through 2013. Results are given in reference to all kelt assignments (all), and only the assignments that exceeded an 80% probability threshold (p>80). Assignments are the total number observed (n) and corresponding stock proportion (%) for each year as defined by reporting group.

	assigned reporting group										
	LSNAKE	LOCLWR	SFCLWR	UPCLWR	GRROND	IMNAHA	LOSALM	SFSALM	MFSALM	UPSALM	total
all (n)											
2009	18	18	5	9	46	24	23	11	49	62	265
2010	90	81	24	35	192	151	54	38	121	438	1224
2011	89	87	31	52	255	140	54	43	128	234	1113
2012	121	119	37	43	286	114	59	28	104	217	1128
2013	39	39	8	17	108	51	27	10	37	105	441
overall	357	344	105	156	887	480	217	130	439	1056	4171
all (%)											
2009	0.07	0.07	0.02	0.03	0.17	0.09	0.09	0.04	0.18	0.23	---
2010	0.07	0.07	0.02	0.03	0.16	0.12	0.04	0.03	0.10	0.36	---
2011	0.08	0.08	0.03	0.05	0.23	0.13	0.05	0.04	0.12	0.21	---
2012	0.11	0.11	0.03	0.04	0.25	0.10	0.05	0.02	0.09	0.19	---
2013	0.09	0.09	0.02	0.04	0.24	0.12	0.06	0.02	0.08	0.24	---
overall	0.09	0.08	0.03	0.04	0.21	0.12	0.05	0.03	0.11	0.25	---
***p*>80 (n)**											
2009	0	2	4	7	12	15	6	5	35	31	117
2010	12	12	19	25	55	73	12	27	96	251	582
2011	4	21	24	37	85	66	11	29	96	96	469
2012	19	32	28	24	79	48	13	15	67	79	404
2013	4	11	6	13	33	18	4	7	22	48	166
overall	39	78	81	106	264	220	46	83	316	505	1738
***p*>80 (%)**											
2009	0.00	0.02	0.03	0.06	0.10	0.13	0.05	0.04	0.30	0.26	---
2010	0.02	0.02	0.03	0.04	0.09	0.13	0.02	0.05	0.16	0.43	---
2011	0.01	0.04	0.05	0.08	0.18	0.14	0.02	0.06	0.20	0.20	---
2012	0.05	0.08	0.07	0.06	0.20	0.12	0.03	0.04	0.17	0.20	---
2013	0.02	0.07	0.04	0.08	0.20	0.11	0.02	0.04	0.13	0.29	---
overall	0.02	0.04	0.05	0.06	0.15	0.13	0.03	0.05	0.18	0.29	---

Table 8b. Estimated stock proportions for hatchery-origin (HAT) kelts sampled at LGD during outmigration years 2009 through 2013. Results are given in reference to all kelt assignments (all), and only the assignments that exceeded an 80% probability threshold (p>80). Assignments are the total number observed (n) and corresponding stock proportion (%) for each year as defined by reporting group.

	assigned reporting group										
	LSNAKE	LOCLWR	SFCLWR	UPCLWR	GRROND	IMNAHA	LOSALM	SFSALM	MFSALM	UPSALM	total
all (n)											
2009	2	2	9	0	4	2	1	0	1	20	41
2010	11	12	5	8	31	22	16	5	15	68	193
2011	42	54	45	22	64	52	30	0	21	268	598
2012	66	32	88	19	134	61	26	3	16	571	1016
2013	25	23	38	6	35	32	18	1	3	259	440
overall	146	123	185	55	268	169	91	9	56	1186	2288
all (%)											
2009	0.05	0.05	0.22	0.00	0.10	0.05	0.02	0.00	0.02	0.49	---
2010	0.06	0.06	0.03	0.04	0.16	0.11	0.08	0.03	0.08	0.35	---
2011	0.07	0.09	0.08	0.04	0.11	0.09	0.05	0.00	0.04	0.45	---
2012	0.06	0.03	0.09	0.02	0.13	0.06	0.03	0.00	0.02	0.56	---
2013	0.06	0.05	0.09	0.01	0.08	0.07	0.04	0.00	0.01	0.59	---
overall	0.06	0.05	0.08	0.02	0.12	0.07	0.04	0.00	0.02	0.52	---
***p*>80 (n)**											
2009	0	0	9	0	1	2	0	0	1	13	26
2010	0	2	2	7	10	11	6	4	13	35	90
2011	2	1	25	2	6	5	1	0	2	107	151
2012	4	6	72	6	26	34	1	2	4	332	487
2013	2	2	28	2	7	14	1	0	0	147	203
overall	8	11	136	17	50	66	9	6	20	634	957
***p*>80 (%)**											
2009	0.00	0.00	0.35	0.00	0.04	0.08	0.00	0.00	0.04	0.50	---
2010	0.00	0.02	0.02	0.08	0.11	0.12	0.07	0.04	0.14	0.39	---
2011	0.01	0.01	0.17	0.01	0.04	0.03	0.01	0.00	0.01	0.71	---
2012	0.01	0.01	0.15	0.01	0.05	0.07	0.00	0.00	0.01	0.68	---
2013	0.01	0.01	0.14	0.01	0.03	0.07	0.00	0.00	0.00	0.72	---
overall	0.01	0.01	0.14	0.02	0.05	0.07	0.01	0.01	0.02	0.66	---

Baseline Power Analysis

Based on previously described criteria, the baseline populations were partitioned into 10 reporting groups (RG) for analysis (Figure 20; Appendix 1.a.). Reporting groups are consistently

color coded in Figures throughout this document. The highest self-assignment rates and largest mean probabilities were observed in the middle and south forks of both the Clearwater River and Salmon River. Among mis-assignments for populations in these same four reporting groups, the next most likely assignments of RG-of-origin (most frequently observed) were from the same region (e.g., SFCLWR assigning to UPCLWR; Figure 21). The rates of correct self-assignment in IMNAHA and UPSALM we intermediate at 0.71 each, with average self-assignment probabilities of 83% and 80% respectively. The lowest observed assignment accuracies occurred in three lower river reporting groups (LOSALM, LOCLWR, LSNAKE).

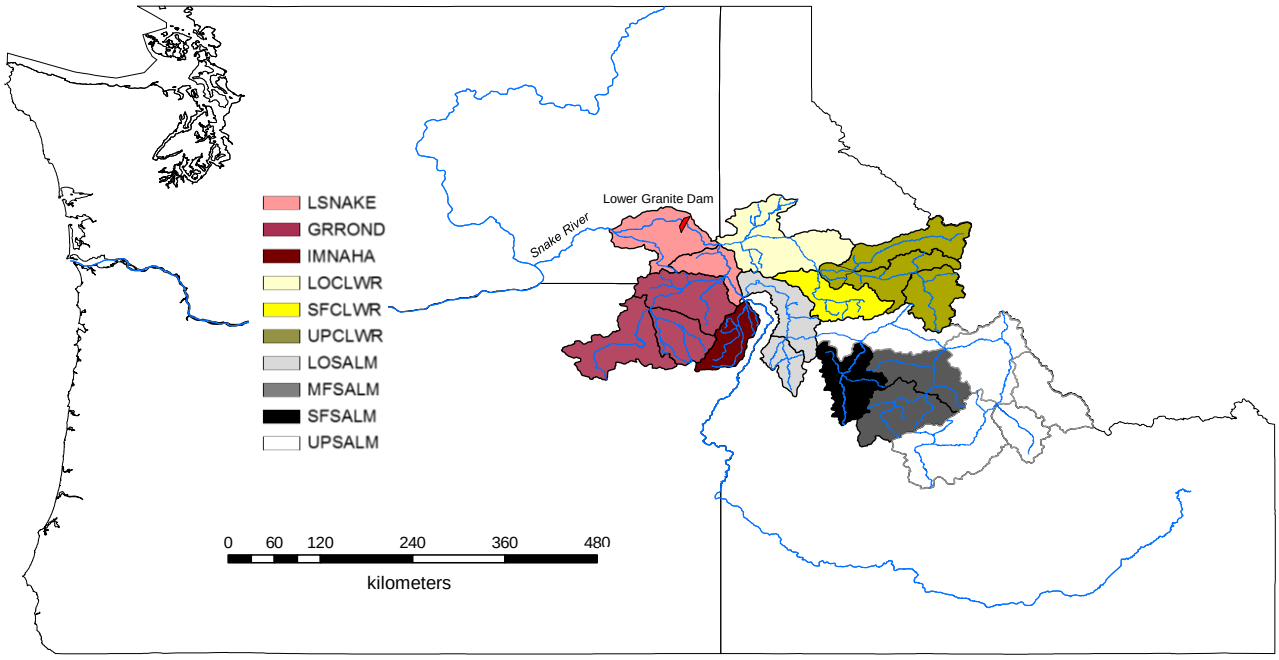


Figure 20. Map of GSI region and reporting groups established on the basis of 73 baseline O. mykiss populations (see Appendix 1.a). Lower Snake River (LSNAKE); Grande Ronde River (GRROND); Imnaha River (IMNAHA); Lower Clearwater River (LOCLWR); South Fork Clearwater River (SFCLWR); Middle Fork Clearwater River (UPCLWR); Lower Salmon River (LOSALM); Middle Fork Salmon River (MFSALM); South Fork Salmon River (SFSALM); Upper Salmon River (UPSALM).

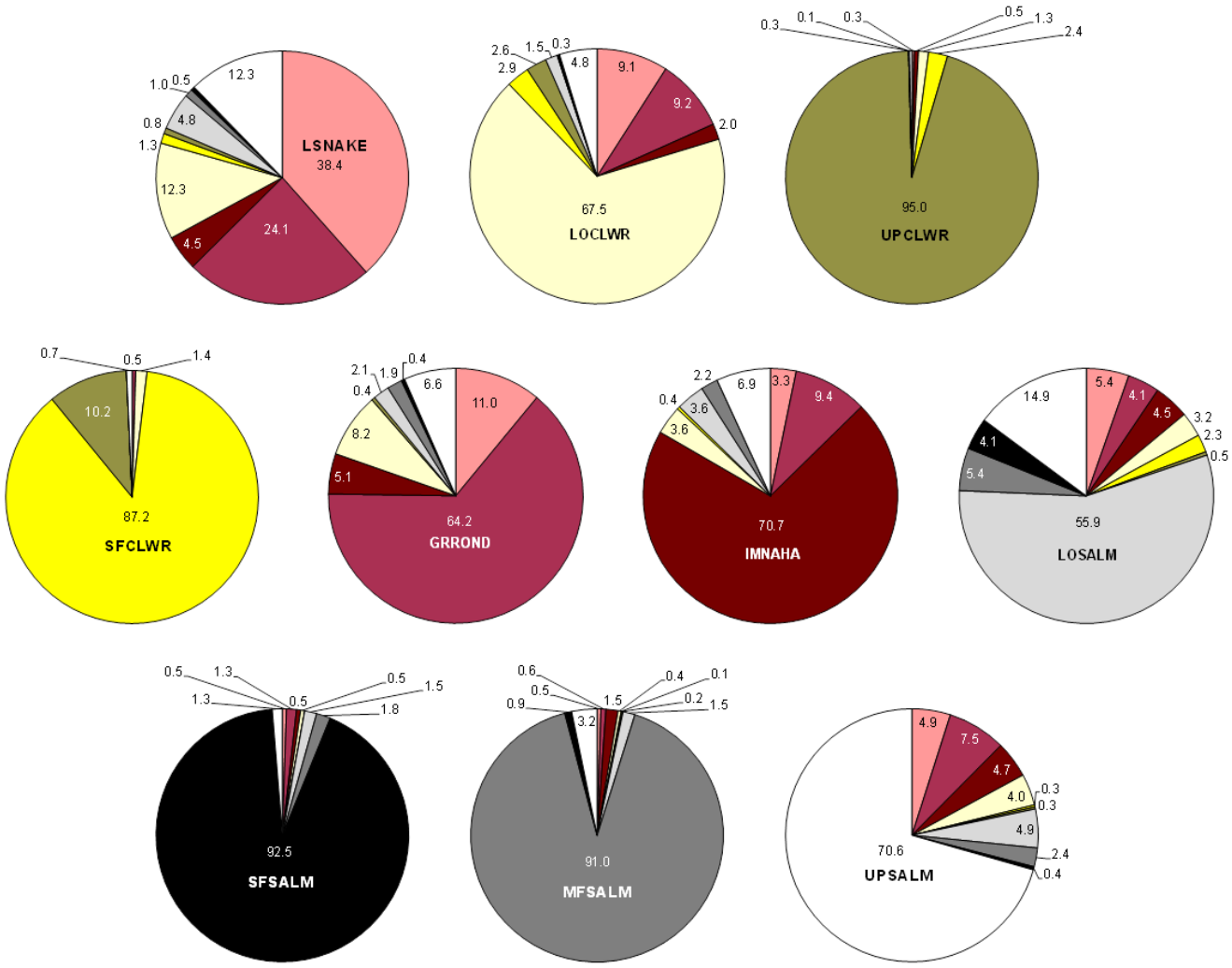


Figure 21. Baseline leave-one-out (LOO) assignment proportions by designated reporting group.

GSI assignments (kelt stock proportions)

Kelt stock-of-origin was evaluated using two approaches: 1) Identifying stock proportions from all sampled kelts, based on highest probability assignment regardless of magnitude, 2) Identifying stock proportions based on an 80% assignment probability threshold (i.e. excluding assignments below the threshold). In each sample year between 2009 and 2013 the largest assigned stock proportions were allocated to the UPSALM reporting group for both natural-origin kelts (n=1,056; 25% overall) and hatchery origin kelts (n=1,186; 52% overall). For NOR kelts the lowest assigned stock proportion (3%) was observed in both the SFCLWR and SFSALM reporting groups (Table 8a and Figure 22), while the corresponding assignment probabilities for those same two RGs were among the highest observed (88% and 81% respectively; Table 9). For HAT kelts the lowest assigned stock proportion (0%) was observed in the SFSALM reporting group (Table 8b). Only 42% of the total kelt sample (1,738 NOR and 957 HAT) was retained

when a probability threshold of $p \geq 0.80$ was applied for "correct" assignment (Table 8a & b). However, the observed stock proportion estimates based on the 80% threshold criteria differed very little from assignment proportions based on highest probability assignments. For natural-origin kelts the mean change in assignment proportions ranged from 1.7% in SFSALM to 7.7% in MFSALM (average 3.7% across RGs). For hatchery-origin kelts the results were similar (average 4.2% across RGs) except for UPSALM, which increased by 14.4% when the $p \geq 0.80$ threshold was applied (Table 8b and Figure 23). Note that in the 2013 annual report it was shown that LGD kelt stock proportions and LGD steelhead escapement proportions was significantly different. For example the UPCLWR and SFCLWR estimated stock proportions for total escapement were substantially larger than the estimated kelt stock proportions for those same areas. Similar escapement data to accompany 2013-2014 kelt data is forthcoming, but was unavailable for comparison in the current report.

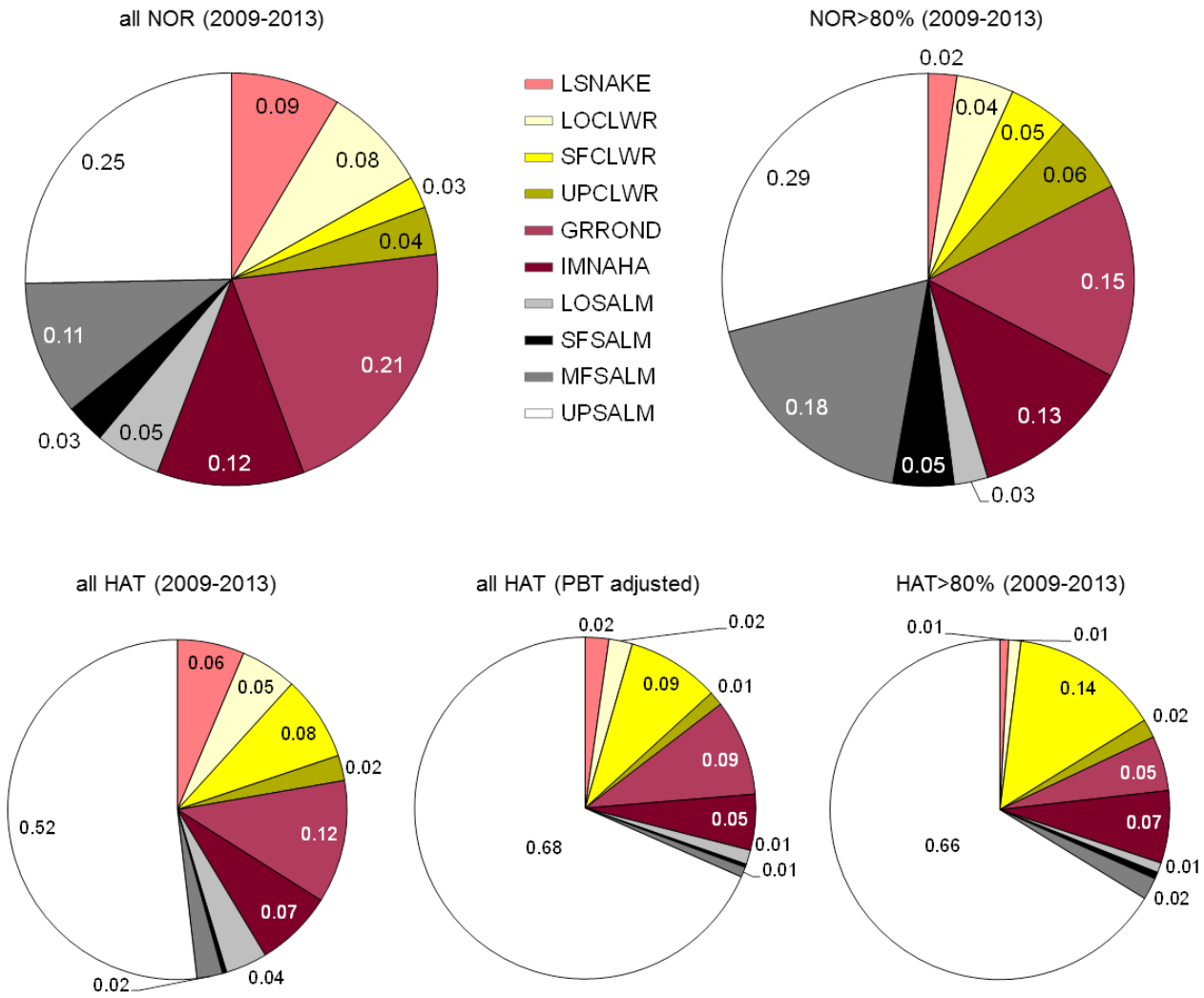


Figure 22. Overall estimated stock proportions based on GSI assignment of kelt steelhead. Results for HAT stock proportions are for all kelts based on GSI, and for all HAT kelts after correction for mis-assignments based on PBT results (see Table 3). Results using an 80% assignment threshold are also provided.

Table 9. Summary of mean assignment probability (p) to reporting group (RG) for natural-origin kelts The baseline mean self-assignment probabilities (p) and self-assignment rates (%) are shown for comparison.

	baseline LOO		All kelts (*p*)						Kelts $p \leq 0.8$	
RG	(*p*)	(%)	2009	2010	2011	2012	2013	mean	(n)	(%total)
LSNAKE	0.57	0.38	0.37	0.52	0.48	0.54	0.48	0.50	39	0.11
LOCLWR	0.84	0.68	0.55	0.55	0.58	0.61	0.62	0.59	78	0.23
SFCLWR	0.92	0.87	0.89	0.90	0.87	0.88	0.86	0.88	81	0.77
UPCLWR	0.95	0.95	0.85	0.84	0.84	0.79	0.85	0.83	106	0.68
GRROND	0.73	0.64	0.62	0.62	0.63	0.62	0.65	0.63	264	0.30
IMNAHA	0.83	0.71	0.76	0.73	0.70	0.67	0.65	0.70	220	0.46
LOSALM	0.80	0.56	0.59	0.60	0.56	0.52	0.55	0.56	46	0.21
SFSALM	0.95	0.93	0.78	0.82	0.82	0.80	0.87	0.81	83	0.64
MFSALM	0.95	0.91	0.82	0.86	0.84	0.80	0.77	0.83	316	0.72
UPSALM	0.80	0.71	0.73	0.77	0.68	0.66	0.69	0.72	505	0.48
mean	0.83	0.73	0.70	0.72	0.70	0.69	0.70	0.71	---	0.46

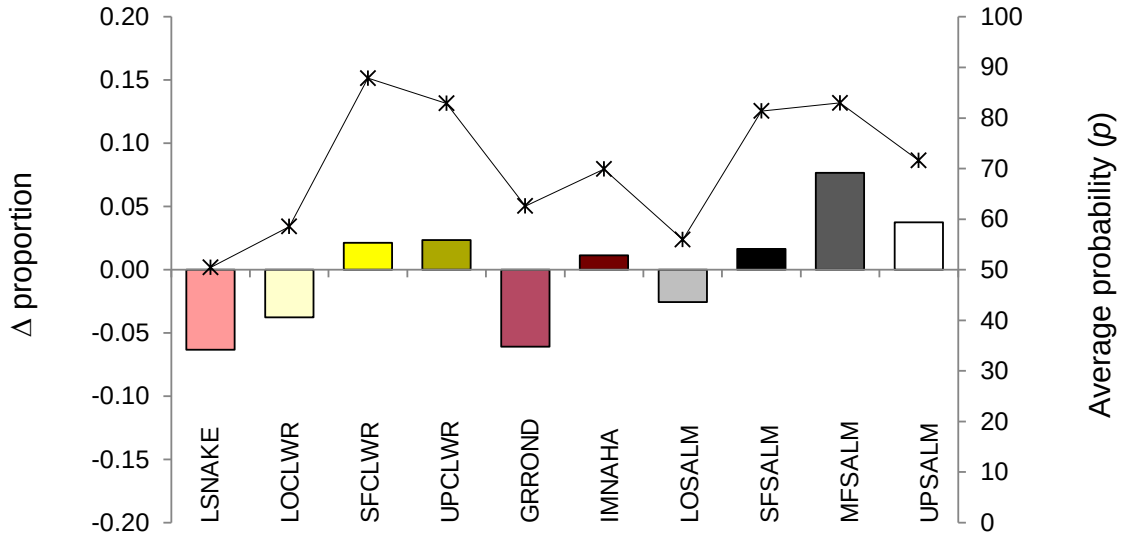


Figure 23. Overall estimated stock proportions based on GSI assignment of kelt steelhead. Results for HAT stock proportions are for all kelts based on GSI, and for all HAT kelts after correction for mis-assignments based on PBT results. Results using an 80% assignment threshold are also provided.

Demographic Correlations with assigned NOR kelt stocks

The evaluation of differences in life history attributes among assigned kelt stocks was based on all NOR kelt data (i.e. highest assignment probability with no threshold). There was no difference in average assignment probability between male and female kelts (Figure 24), and sex ratios were largely consistent across RG's, with averages ranging from 72%-85% female (overall 76%; Appendix 1.a.). The sample date at Lower Granite Dam served as a proxy for

estimating mean kelt outmigration time for each assigned stock-of-origin; date was enumerated as ordinal day (January 1st = day 1). Results for male kelts excluded 2009 samples due to insufficient sample size. The downstream migration of female kelts generally occurred earlier (mean day 134.2) than male kelts (mean day 138.7), and the trend was consistent across years and RG's. However, smaller sample sizes for males contributed to greater variation among yearly estimates (Figure 25). Kelts that were assigned to the SFSALM and MFSALM stocks were consistently the latest to outmigrate, while kelts assigned to the SFCLWR reporting group were among the earliest outmigrants. Female kelts assigned to each RG were consistently larger than their male counterparts (an average of 55.9mm larger). Kelts that were assigned to the SFCLWR, UPCLWR, SFSALM reporting groups were larger in fork length (average 97.1mm for females and 64.6mm for males) than kelts assigned to each of the remaining seven RG's (Figure 26; Appendix 1.a).

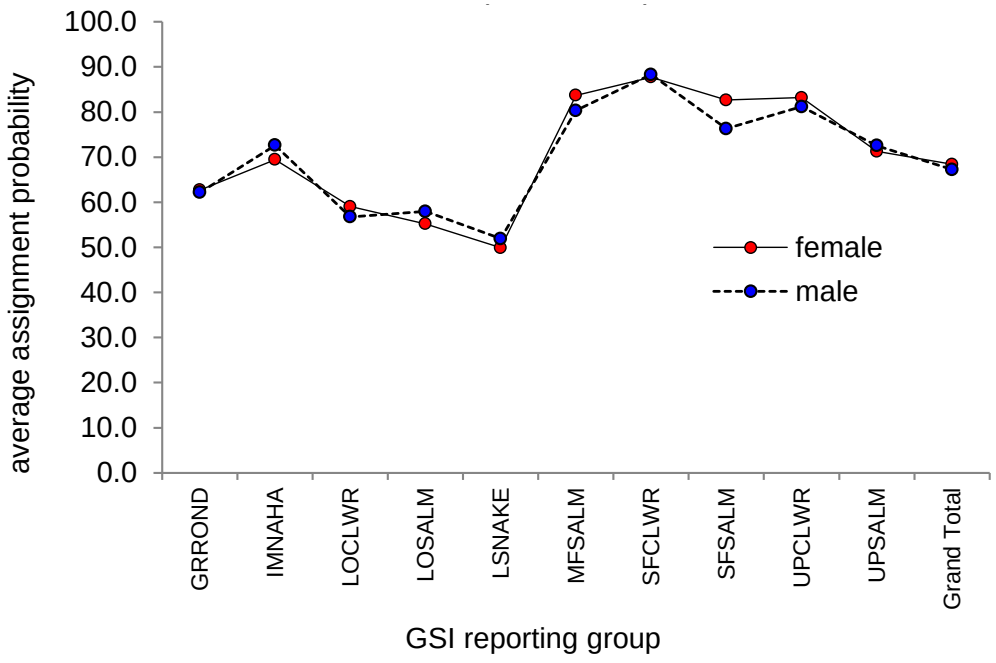


Figure 24. Average GSI assignment probability comparison between NOR male and NOR female kelts. Calculations are based on highest probability assignment for all sampled kelts.

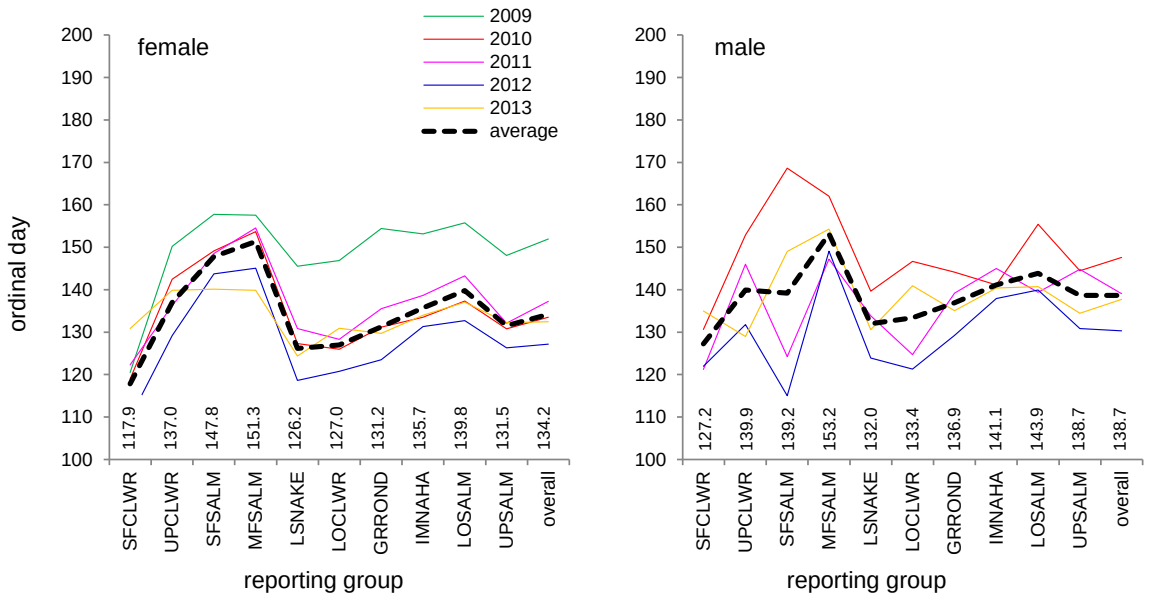


Figure 25. Average outmigration day for NOR kelt grouped by assigned RG-of-origin. The overall average (2009-2013) appears above the RG name.

Note that SFCLWR, UPCLWR, SFSALM are generally considered to support predominantly B-run steelhead populations. 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 overall than for male kelts, and there were no observed differences between reporting groups. Among RGs there was also no significant relationship observed between kelt size (fork length) and condition rating (% “good”), or outmigration timing and condition rating (Figure 27).

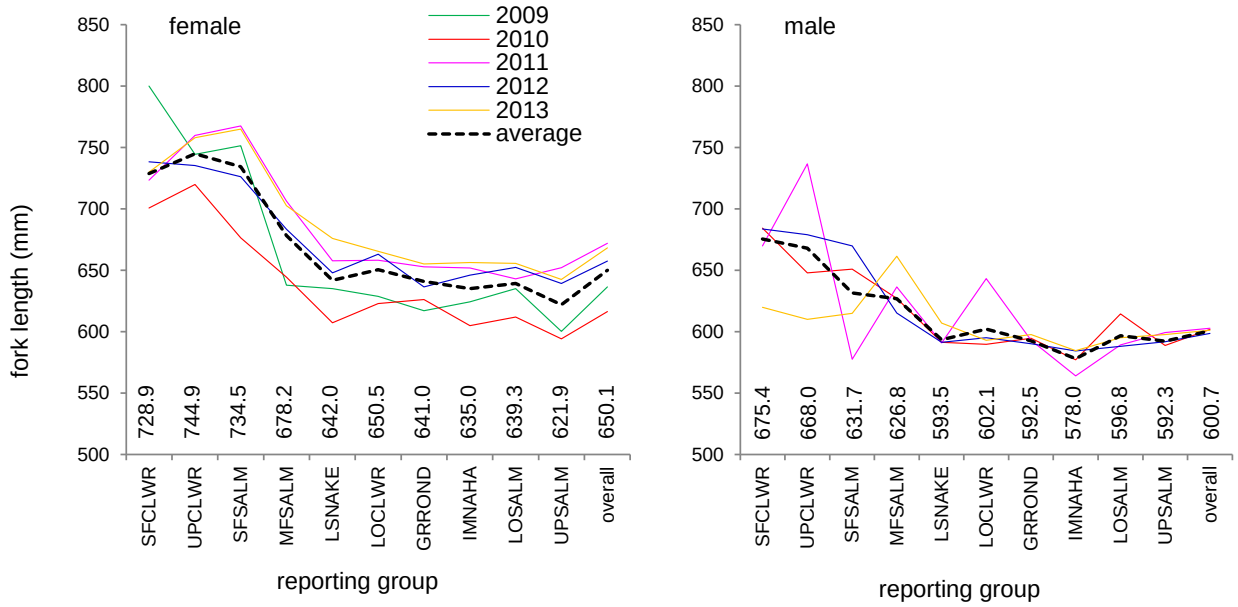


Figure 26. Average fork length for NOR kelt grouped by assigned RG-of-origin. The overall average (2009-2013) appears above the RG name.

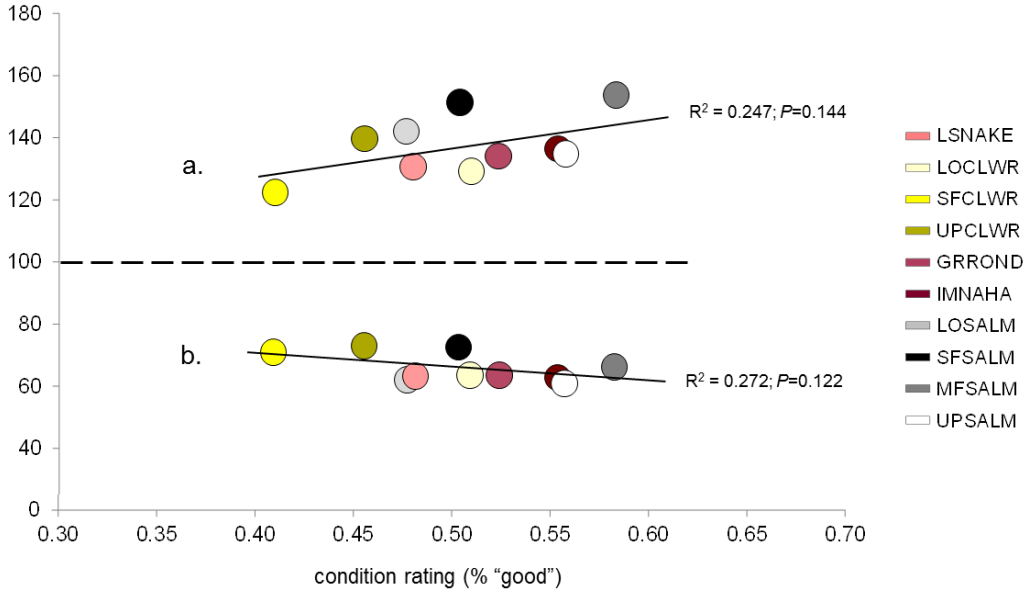


Figure 27. Correlation between condition rating and a) mean ordinal day, b) mean fork length (cm).

Concordance results: PIT-tag and PBT

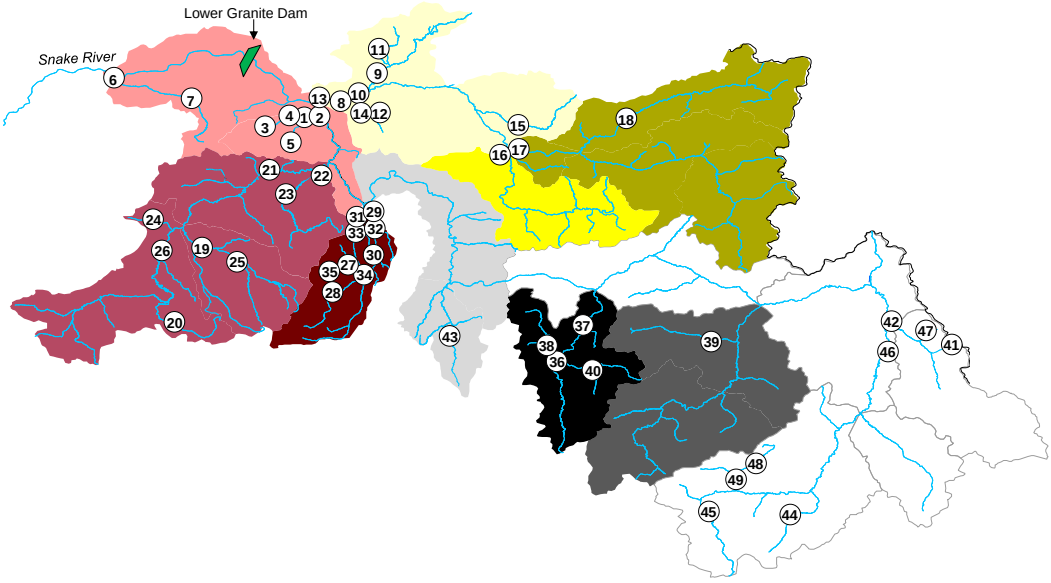
For all hatchery kelts sampled between 2010 and 2013 the Snake River PBT baseline was used to screen for hatchery origins. PBT origins were identified for 1,685 of 2,288 (74%) sampled hatchery kelts. Concordance results indicated a high rate of GSI assignment error based on PBT comparisons. Most commonly, kelts identified as broodstock progeny from hatcheries in the UPSALM reporting group mis-assigned to other RGs based on GSI analysis (rows in Table 10). For example, 78% of kelts that GSI assigned to LSNAKE were reared in hatcheries in the UPSALM reporting group according to PBT results. Misallocations of hatchery-origin kelts originating from the UPSALM reporting group ranged from 4.8% in SFCLWR to 96.7% in LOSALM. The overall rate of misallocation was 30% (n=398). Conversely, 955 of 974 kelts that GSI assigned to UPSALM (98%) had concordant PBT assignments.

Table 10. Concordance summary between GSI and PBT assignment methods for hatchery-origin kelts. Some GSI reporting groups do not have hatchery programs in operation (*); therefore, all corresponding PBT assignments indicate GSI mis-assignment. Both the SFCLWR and UPCLWR reporting groups are represented by the Dworshak-NFH in the Clearwater River subbasin. A total of 1,685 PBT and GSI assignment comparisons are allocated by GSI reporting group ("total"). Discordant results between GSI and PBT indicate an incorrect assignment based on GSI; the greatest misallocation to reporting group is shaded.

		PBT assignment									
		LSNAKE		DWOR		GRRONDE		IMNAHA		UPSALM	
GSI assigned	total	(n)	%	(n)	%	(n)	%	(n)	%	(n)	%
LSNAKE	109	4	**3.7**	1	0.9	16	14.7	3	2.8	85	78.0
LOCLWR	72	2	2.8	5	6.9	11	15.3	4	5.6	50	69.4
SFCLWR	126	0	0.0	120	**95.2**	0	0.0	0	0.0	6	4.8
UPCLWR	24	0	0.0	15	**62.5**	2	8.3	0	0.0	7	29.2
GRRONDE	168	4	2.4	1	0.6	58	**34.5**	8	4.8	97	57.7
IMNAHA	116	0	0.0	0	0.0	4	3.4	50	**43.1**	62	53.4
LOSALM	60	1	1.7	0	0.0	1	1.7	0	0.0	58	96.7
SFSALM	2	0	0.0	0	0.0	0	0.0	1	50.0	1	50.0
MFSALM	34	1	2.9	0	0.0	0	0.0	1	2.9	32	94.1
UPSALM	974	2	0.2	0	0.0	16	1.6	1	0.1	955	**98.0**
misallocation		10		7		50		18		398	
overall	1685	14	0.8	142	8.4	108	6.4	68	4.0	1353	---

Pit-tag and tag detection data among NOR kelts indicated variable levels of concordance among reporting groups. Detection data for mark and release locations were generally more concordant with GSI assignments than were tag detections corresponding to adult steelhead observed at tributary arrays (Figure 28; Appendix 1.c.). For example, all kelts with known

mark/release locations in the UPSALM (n=10) were also GSI assigned to that reporting group. Most tag detections came from Grande Ronde River releases, for which 11 of 24 failed to assign to the GRRONDE reporting group in GSI analyses. Tag detection data indicating observation and release sites for hatchery-origin kelts (Appendix 1.d.; Figure 29) were concordant with PBT assignments in 25 of 28 (90%) observations. By comparison, the rate of concordance between tag detection site and GSI assigned reporting group was 59% (26 of 44). Kelt PIT-tag detection sites were concordant with both GSI and PBT assignments in 46% (20 of 44) of the observations. Most discrepancies between GSI assignments and PIT-tag detection sites occurred for kelts that were PBT assigned to the UPSALM reporting group (Appendix 1.d.).



Sites are: 1.) ***ACB*** *- Asotin Cr. Cloverland Brdg.; 2.)* ***ACM*** *- Asotin Cr. mouth; 3.)* ***AFC*** *- No./So. Fk Asotin Cr.; 4.)* ***ASOTIC*** *- Asotin Cr. Clarkston, WA; 5.)* ***GEORGC*** *- George Cr.; 6.)* ***LTR*** *- Lower Tucannon R.; 7.)* ***UTR*** *- Upper Tucannon R.; 8.)* ***CLWTRP*** *- Clearwater Trap; 9.)* ***JUL*** *- Potlatch R. Juliaetta; 10.)* ***LAP*** *- Lapwai Cr.; 11.)* ***LBEARC*** *- Little Bear Cr.; 12.)* ***MIS*** *- Mission Cr.; 13.)* ***SNKTRP*** *- Snake Trap; 14.)* ***SWT*** *- Sweetwater Cr.; 15.)* ***LC2*** *- Upper Lolo Cr. (rkm 25); 16.)* ***SC1*** *- Lower SF Clearwater R. (rkm 1); 17.)* ***SC2*** *- Lower SF Clearwater R. (rkm 2); 18.)* ***FISTRP*** *- Fish Cr. Trap; 19.)* ***BCANF*** *- Big Canyon Facility; 20.)* ***CATHEW*** *- Catherine Cr. Weir; 21.)* ***COTP*** *- Cottonwood Acclimation Pond; 22.)* ***JOC*** *- Joseph Cr. (km 3); 23.)* ***JOSEPC*** *- Joseph Cr.; 24.)* ***LOOKGC*** *- Lookingglass Cr.; 25.)* ***LOSTIW*** *- Lostine R. Weir; 26.)* ***UGR*** *- Upper Grande Ronde (rkm 155); 27.)* ***BSC*** *- Big Sheep Cr. (km 6); 28.)* ***BSHEEC*** *- Big Sheep Cr.; 29.)* ***COC*** *- Cow Cr.; 30.)* ***HORS3C*** *- Horse Cr.; 31.)* ***IMNTRP*** *- Imnaha Trap; 32.)* ***IR1*** *- Lower Imnaha R. (km 7); 33.)* ***IR2*** *- Lower Imnaha R. (km 10); 34.)* ***IR3*** *- Upper Imnaha R. (km 41); 35.)* ***LSHEEF*** *- Little Sheep Facility; 36.)* ***KRS*** *- SF Salmon R. Krassel Cr.; 37.)* ***SFG*** *- SF Salmon Guard Station Br.; 38.)* ***ZEN*** *- Secesh R. Zena Cr. Ranch; 39.)* ***TAY*** *- Big Cr. Taylor Ranch; 40.)* ***ESS*** *- EFSF Salmon R. Parks Cr.; 41.)* ***KENYC*** *- Kenney Cr.; 42.)* ***LLR*** *- Lower Lemhi R.; 43.)* ***LSALR*** *- Little Salmon R.; 44.)* ***SALEFT*** *- East Fork Salmon R. Trap; 45.)* ***STL*** *- Sawtooth Hat. Adult Trap; 46.)* ***USE*** *- Upper Salmon R. (rkm 437); 47.)* ***WIMPYC*** *- Wimpey Cr.; 48.)* ***YANKFK*** *- Yankee Fork Salmon R.; 49.)* ***YFK*** *- Yankee Fork Salmon R.*

Figure 28. PIT-tag detection site for natural-origin kelts. Numbers correspond with map ID in Appendices 1.c. and 1.d.

<B>In-River Release and Return Detection Results

Yakima River

<C>2014 In River Control

A total of 45 kelts were released as in-river control fish in the Yakima River in 2014 with 2 sequential spawners detected to date. So far, 2 of these fish have made it past Prosser Dam. Skip spawning results will be reported in 2015.

<C>2013 In-River Control

None of the 52 in-river treatment fish released in the Yakima River in 2013 were detected moving upstream at Bonneville Dam in late 2014 that exhibited a skip spawner life history. There were no sequential returns that were detected in late 2013/early 2014 (Hatch et al 2014).

Snake River

2013 In-river Control Detections

There were 2 of the 825, 2013 in-river release kelts which returned to Bonneville Dam as skip spawners in 2014 (Hatch et al. 2014). There were no sequential spawners from this group in 2013.

2014 In-river Control Detections

There were 9 kelts from the 2,687 kelts released in 2014 in-river release which returned to Bonneville Dam as sequential spawners towards the end of 2014.

<B>Long-Term Reconditioning and Survival to Release or Spawning

Yakima River

A total of 481 kelt steelhead were collected and retained for long-term reconditioning. There were an additional 43 skip spawning kelts which were retained from 2013 that were held in tank S5. Survival to release/retentions on November 6, 2014 was 295 (61%) (Table 11, Figure 29). A total of 198 long-term reconditioned fish were available for release to the Yakima River. We retained 46 of these fish for use at the Cle Elum spawning channel. An additional 126 kelts with low estradiol levels (immature) were retained and those surviving will be released in the fall of 2015. Steelhead kelts which were released, were done so approximately two weeks later than was done in the past to avoid a WDFW sport fishery near the release site. Most migratory movements are expected to occur in November of 2014 but there is typically a small number of kelts that will migrate in February/March of 2015. These fish will be reported in the 2015 annual report. As of early December of 2014, 112 (74%) fish from the long-term release were detected by PIT tag presence migrating past Prosser Dam.

Table 11: Long-term reconditioning survival by tank 2014 at Prosser Hatchery.

Long-term Reconditioning										
	Tank									Long-term
	C1	C2	C3	C4	S1	S2	S4	S5*	S6	Total
Held for Reconditioning	100	98	96	98	25	24	14	43	26	**481**
Surviving fish on 11/4/2014	52	65	70	54	13	18	3	3	17	**292**
Survival Rate	52%	66%	73%	55%	52%	75%	21%	7%	65%	**61%**

***Retained from 2013 long-term reconditioning. Not computed towards this year's total.**

Figure 29: Long term artificially reconditioned female kelt steelhead from the Yakima River just prior to work up in October of 2014 (Joe Blodgett pictured).

<C>2013 Skip Spawning Long-Term Reconditioned Kelts through 2014

There were a total of 43 non maturing kelts from the 2013 long-term reconditioning program that were retained to determine how well they would recondition through 2014. Of these retained group, 3 fish survived to release in the fall of 2014. As of December 2014,

2 of these fish were detected migrating upriver over the Prosser Dam which we infer as successful skip spawner reconditioning.

<C>2013 Long-term Reconditioned kelt PIT-tag 2014 return detections in the Columbia River
There were no 2013 kelts which were detected trying to return from the ocean in 2014 moving through the mainstem. One long-term kelt was reinterred in spring of 2014 (assumed to have spawned in the Yakima River) to the long-term reconditioning and was released in late fall of 2014 and was detected moving upriver past Prosser Dam in November of 2014.

Snake River

<C>Lower Granite Dam
A total of 111 fish were transferred from the LGR JFF to DNFH for reconditioning (Table 12). Fish survival averaged 79 days after transfer to the reconditioning tanks (Figure 30). Fish survived an average 19 fewer days than the fish transferred in 2013. We experienced high mortalities for approximately one month from early May to early June (Figure 31).

Table 12: Snake River steelhead kelts collected at LGR JFF and transferred to DNFH for reconditioning in 2014.

	A-run	B-run	Total
Adipose Clipped	0	0	0
Un-Clipped	0	111	111
Total	**0**	**111**	**111**

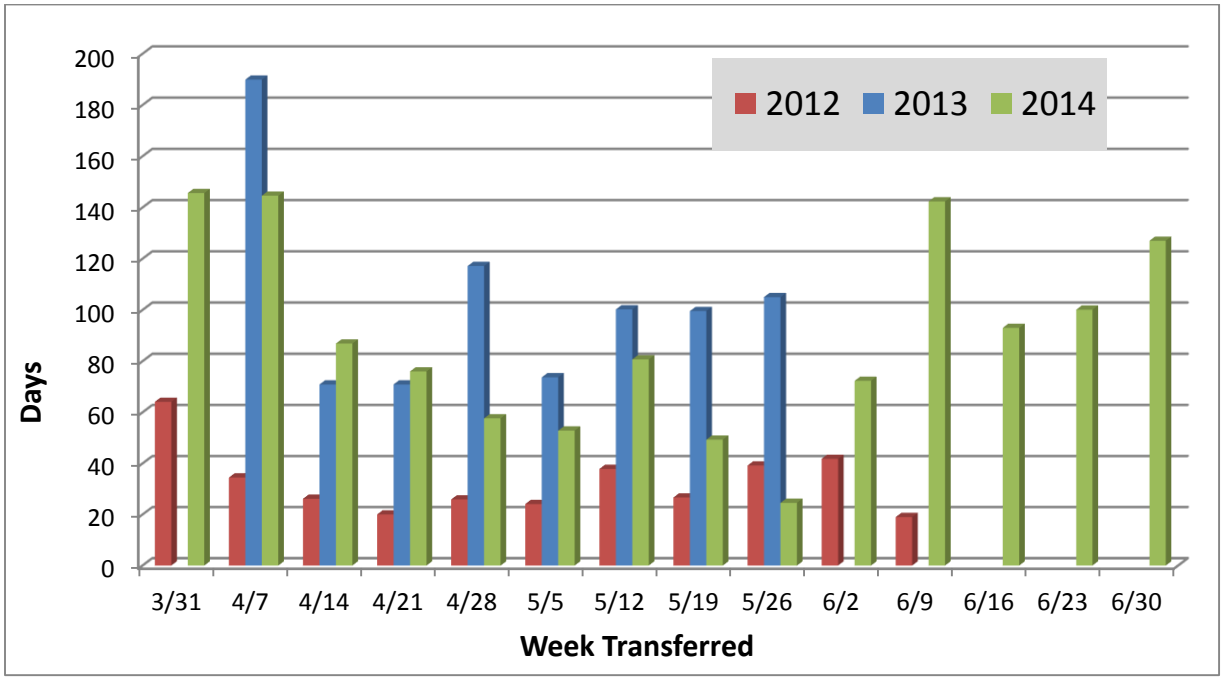


Figure 30: Mean weekly survival (days) of steelhead kelts transferred from LGR JFF to DNFH for reconditioning in 2012-14.

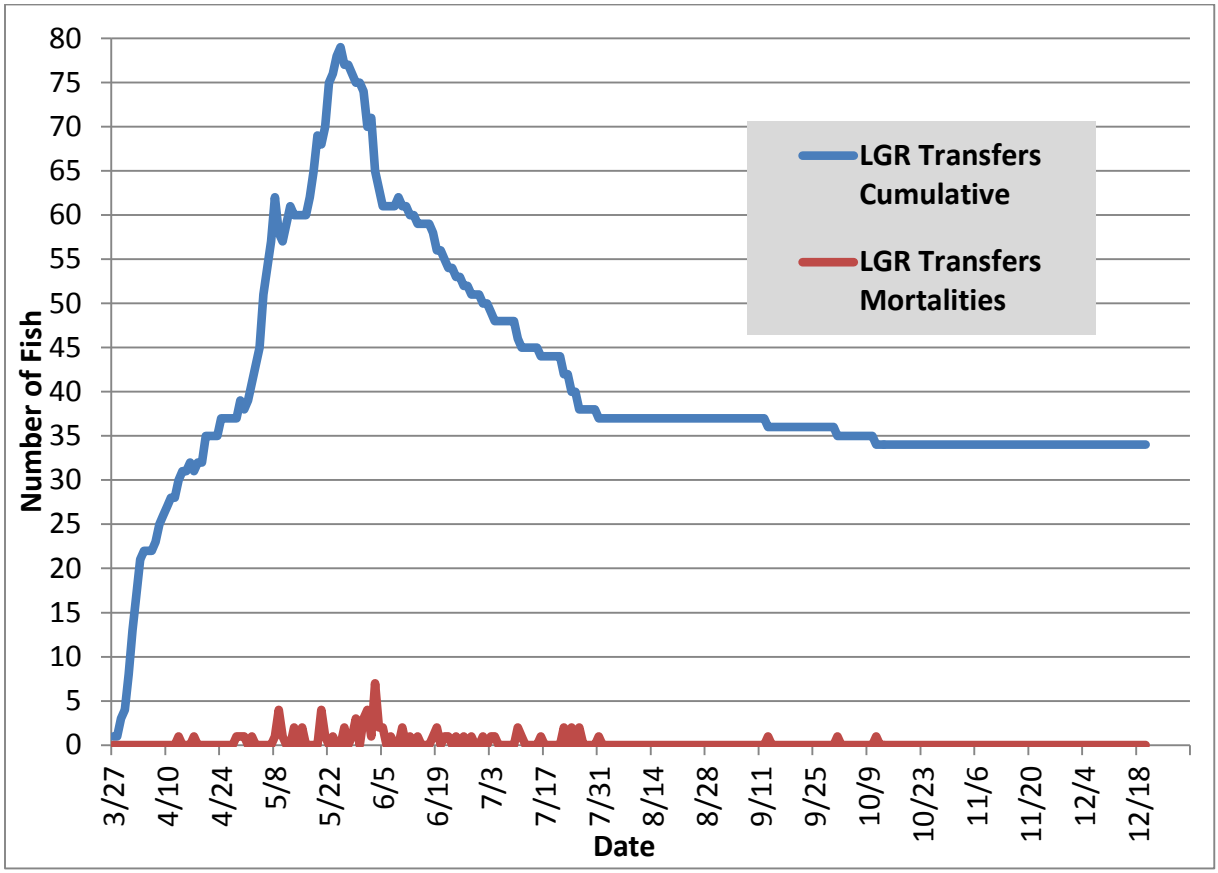


Figure 31: Cumulative on-station holding and daily mortality of steelhead kelts transferred from LGR JFF to DNFH for reconditioning in 2014.

There was a total of 34 (unclipped) fish released to the Columbia River below Bonneville on December 20, 2014 (Figure 32).

<C>Fish Creek Weir

Out of the 12 retained kelts, 1 survived the reconditioning process. This kelt will be retained due to lack of detectable maturation. She will be held and released in the late fall/winter of 2015.

<C>Dworshak National Fish Hatchery

As of December 31, 2014, fish from the DNFH ladder survived an average 110 days after transfer to the reconditioning (Figure 32 and 33).

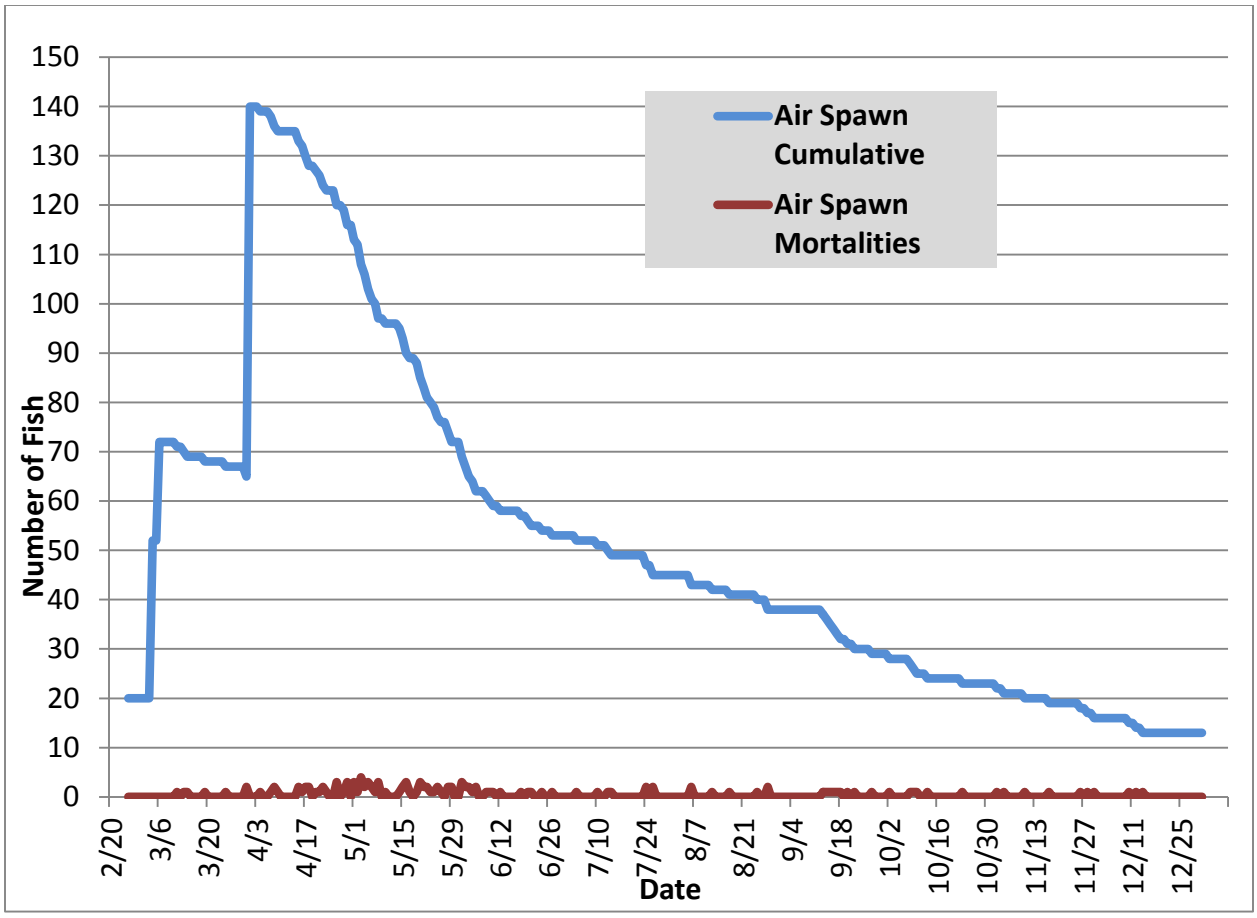


Figure 32: Cumulative on-station holding and daily mortality of steelhead kelts air-spawned at DNFH for reconditioning in 2014.

Figure 33: Successfully reconditioned Snake River steelhead 2014.

<B>Kelt Reconditioning Physiology Studies

Reproductive development in kelt steelhead

Plasma estradiol levels were bimodally distributed in blood samples taken from female kelts in all projects from August onward (Figs 34-36). As previously observed, the maturation percentage of kelts as consecutive spawners varied considerably between years and projects. The maturation percentage at Prosser and Winthrop was comparable (2013 62.8% and 66.6%, respectively; 2014 46.0% and 53.4%, respectively). Maturation rates as consecutive spawners were considerably lower at Dworshak, and varied between years and with fish source. The highest maturation percentage as consecutive spawners was obtained with air spawned kelts from the South Fork of the Clearwater River (33.3%). These are wild origin kelts used in a localized broodstock development program. Kelts collected at Lower Granite dam rematured at very low rates in both years (2013: 5.5%, 2014: 0%). Hatchery origin kelts rematured as consecutive spawners at a 22.9% rate in 2013 and at 6.3% in 2013. However, non-rematuring 2013 spawn year hatchery origin kelts held for an additional year rematured as skip spawners at a very high rate in 2014 (93.1%).

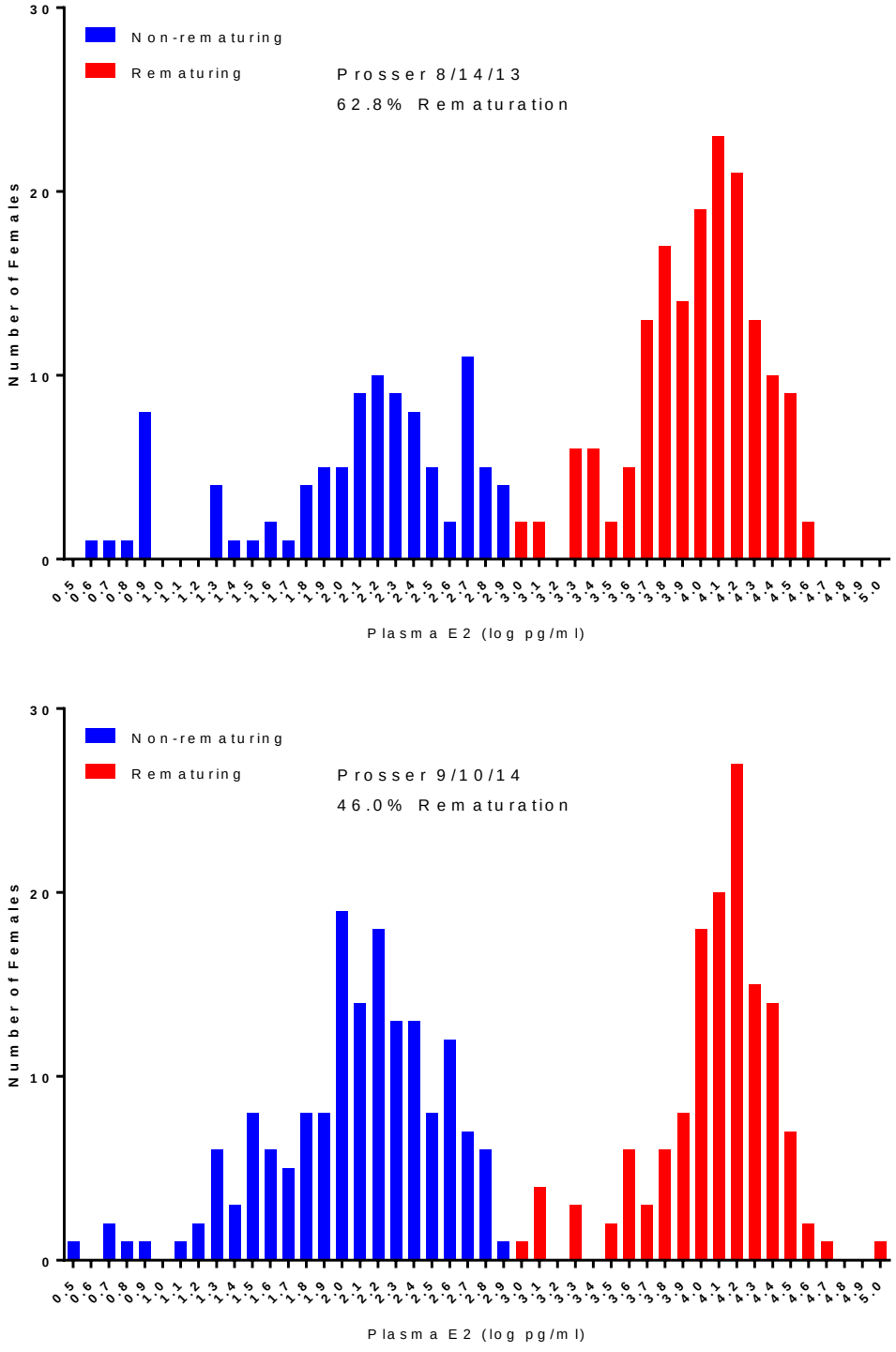


Figure 34: Plasma estradiol levels in female kelts in the reconditioning program at Prosser, Washington in 2013 and 2014.

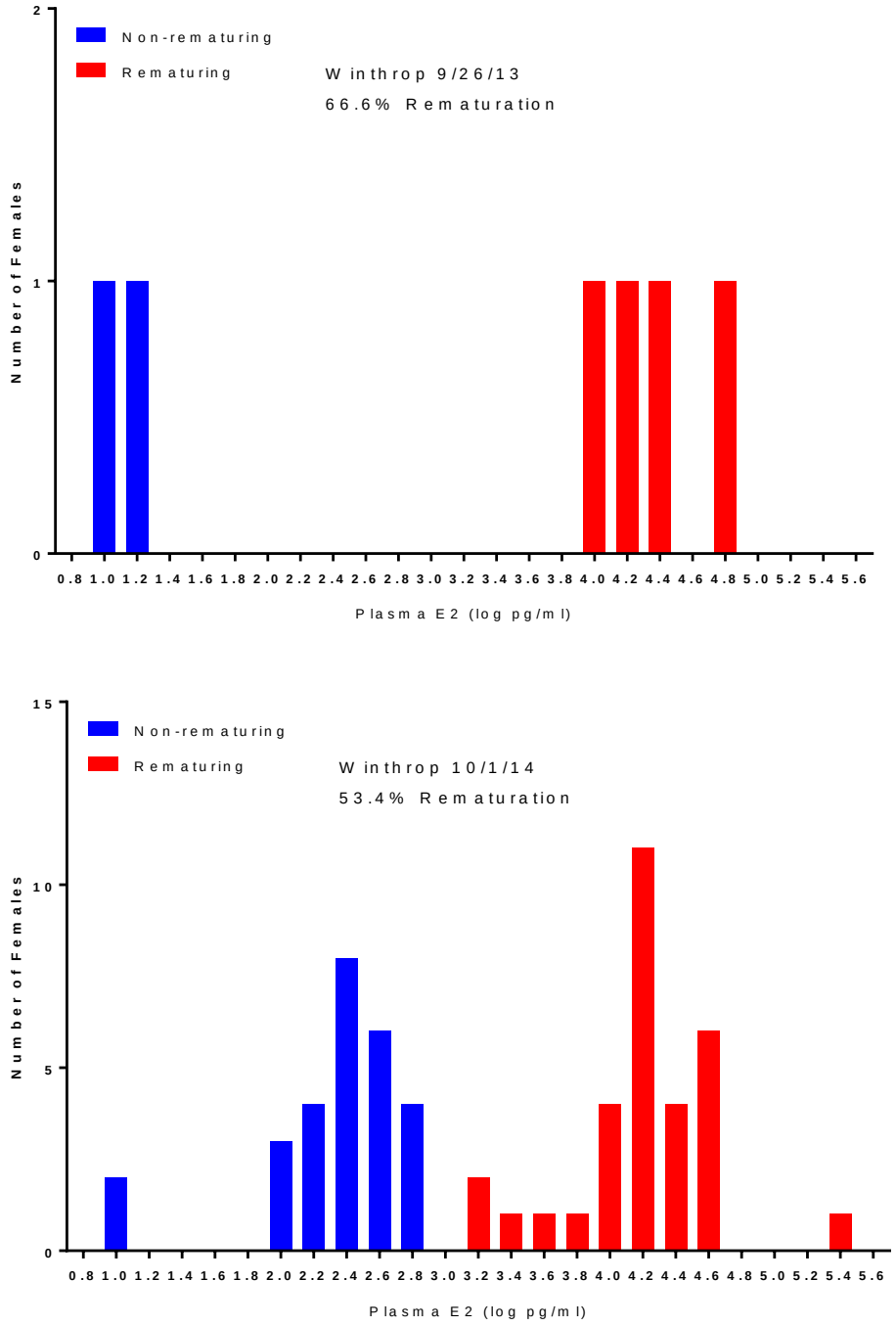
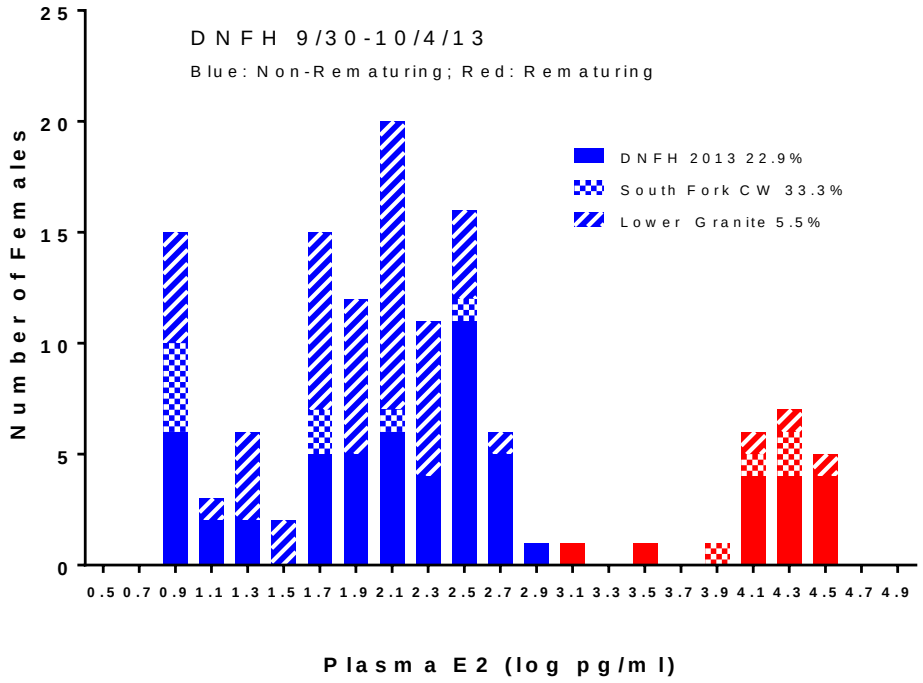


Figure 35: Plasma estradiol levels in female kelts in the reconditioning program at Winthrop, Washington in 2013 and 2014.



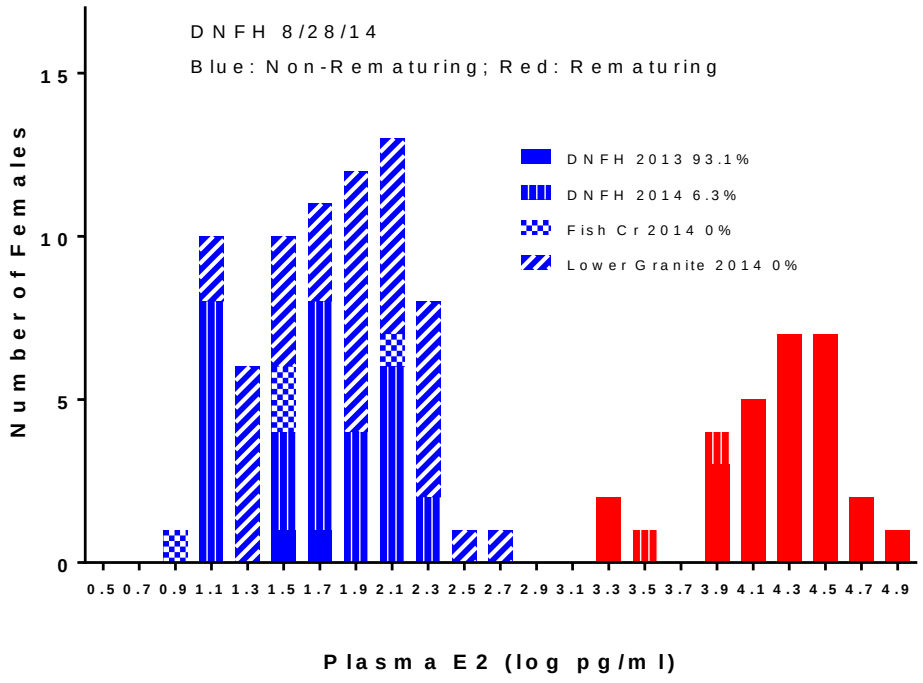


Figure 36: Plasma estradiol levels in female kelts in the reconditioning program at Dworshak National Fish Hatchery, Idaho in 2013 and 2014.

Muscle lipid levels and condition factor were consistently greater in rematuring kelts than in non-rematuring kelts in samples taken near the end of the reconditioning period (Figs 37, 38). Muscle lipid levels in rematuring fish were generally greater than 2%.
Gonadosomatic index (GSI) was assessed in mortalities that occurred after the 9/10/14 sampling at Prosser (Fig. 39). Fish with rematuring estradiol levels (> 1000 pg/ml) almost all had a GSI over 1%, whereas fish with non-rematuring estradiol levels has a GSI below 1%. Significant positive and negative correlations were observed between plasma estradiol levels and GSI in rematuring and non-rematuring kelts, respectively.

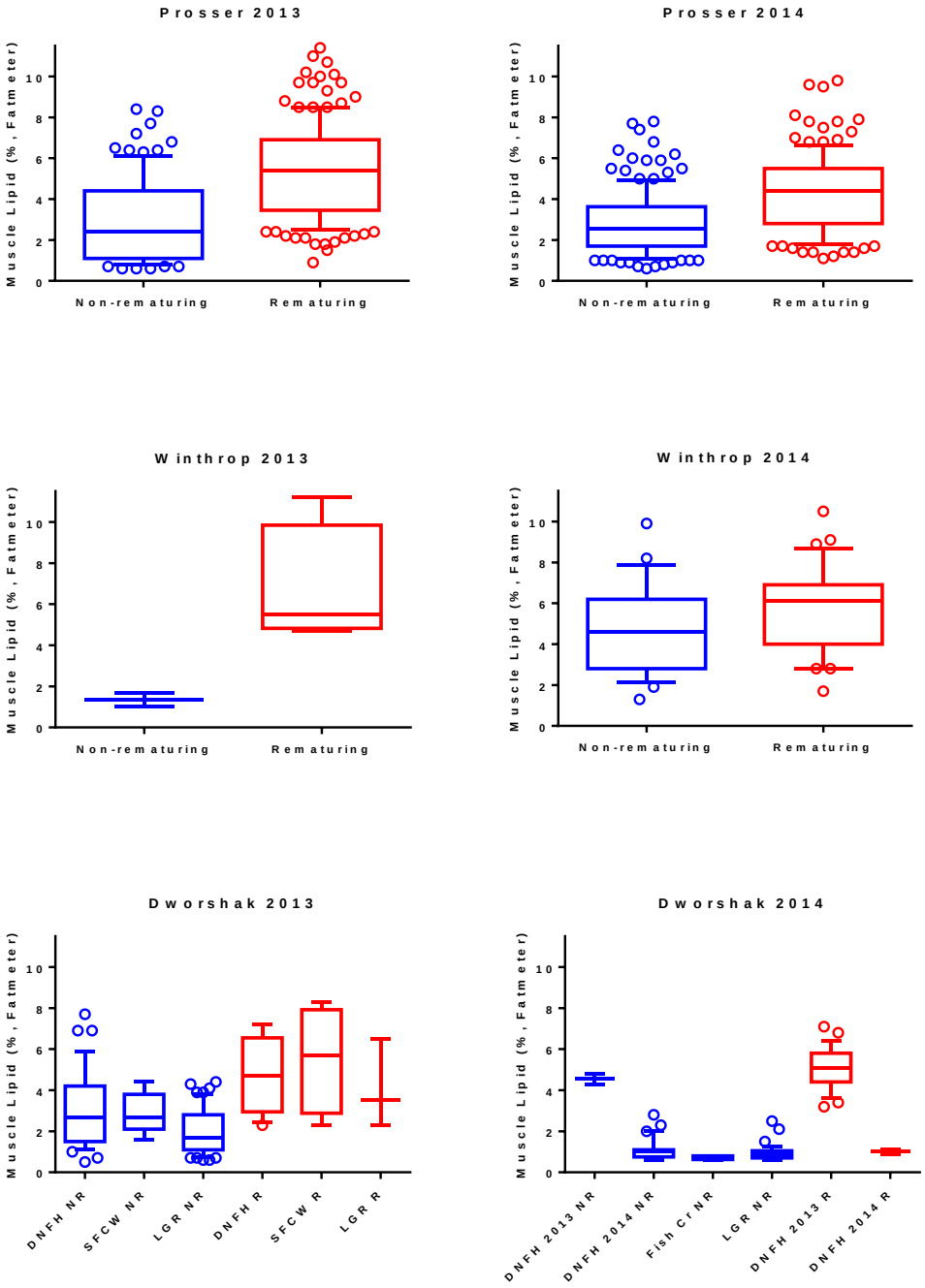


Figure 37: Muscle lipid levels in release samples from kelts in 2013 and 2014. See Figures 1-3 for sampling dates. Rematuring fish are in red and non-rematuring fish are in blue. All differences were significant in t-tests except Winthrop 2013 (p=0.0773) and Winthrop 2014 (p=0.1266). Rematuring and non-rematuring fish at Dworshak were pooled prior to t-tests. Dworshak legends DNFH: air spawned Dworshak hatchery origin fish; SFCW: air spawned South Fork of the Clearwater River fish; LGR: kelts collected at Lower Granite Dam; Fish Cr: kelts collected at the Fish Creek weir (Lochsa River).

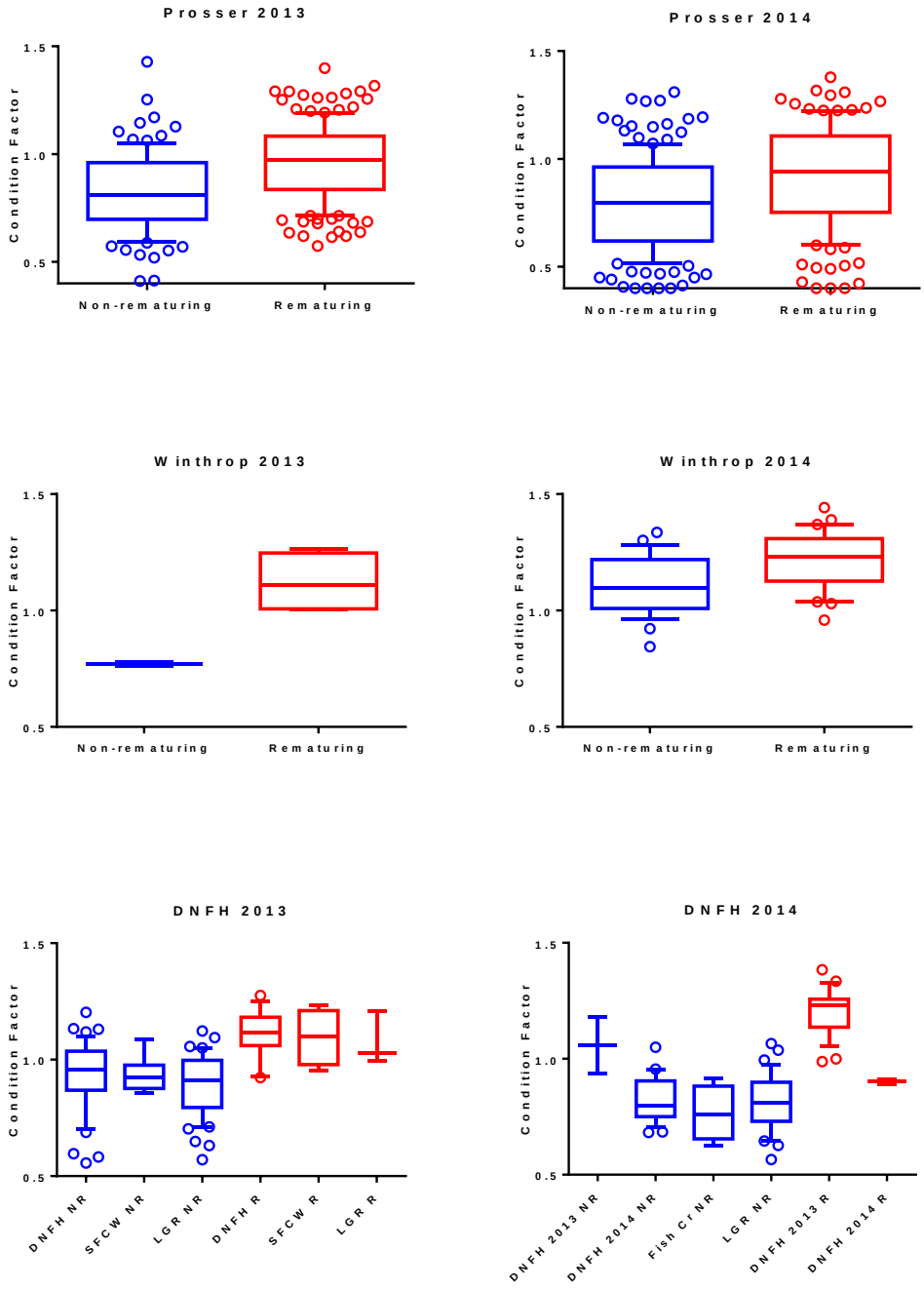


Figure 38: Fulton's condition factor at the release sampling for kelts in 2013 and 2014. See Figures 1-3 for sampling dates. Rematuring fish are in red and non-rematuring fish are in blue. All differences were significant in t-tests. Rematuring and non-rematuring fish at Dworshak were pooled prior to t-tests. See the previous figure legend for fish categories at Dworshak.

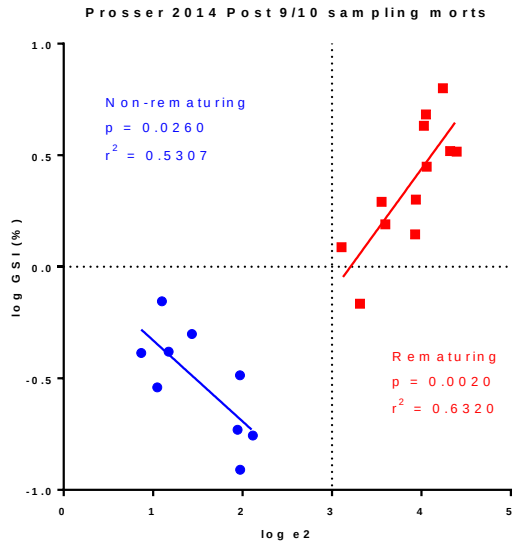


Figure 39: Estradiol levels and gonadosomatic Index (GSI) in mortalities after the 9/10/14 sampling at Prosser, Washington.

The maturity status of female kelts was assessed by visual appearance of the fish at the 9/10/14 sampling at Prosser (Fig. 40). Maturity assessment calls were substantially better than random (Chi-Squared test, $p < 0.0001$). However, approximately 30% of rematuring fish were called as non-rematuring by visual appearance, and similarly approximately 30% of non-rematuring fish were called as rematuring by visual appearance.

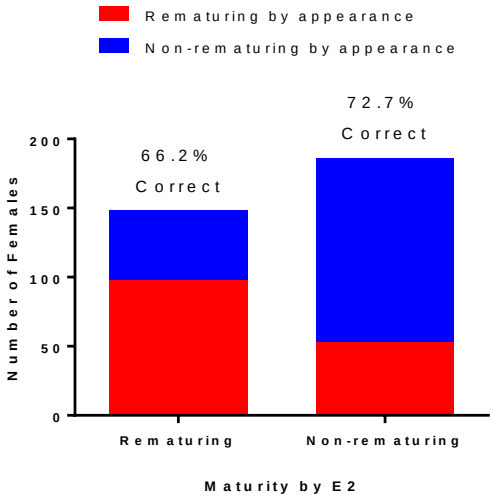


Figure 40: Assessment of maturation status by visual appearance in female kelts determined to be rematuring or non-rematuring based on plasma estradiol level at the 9/10/14 sampling at Prosser, Washington. There was a significant association between maturity by E2 level and maturity by visual appearance (Fisher's exact test, $p < 0.0001$).

The relationship between intake date and maturation status at release was assessed in samples from Prosser from 2009 to 2014. During 2009 to 2013, a significant difference in intake date was found for 3 out of 5 years, with rematuring fish arriving at Prosser earlier than non-rematuring fish (Fig. 41). In 2014, the arrival date of fish was examined in terms of VSP population segment for fish assigned to a VSP segment by detection at a PIT tag array (Fig. 42). The arrival time of fish varied widely for the VSP segments. With VSP segments, there was a trend toward earlier arrival for the Naches and Upper Yakima VSP segments. In fish that could not be assigned to a VSP segment (the majority), rematuring kelts again arrived significantly earlier than non-rematuring fish.

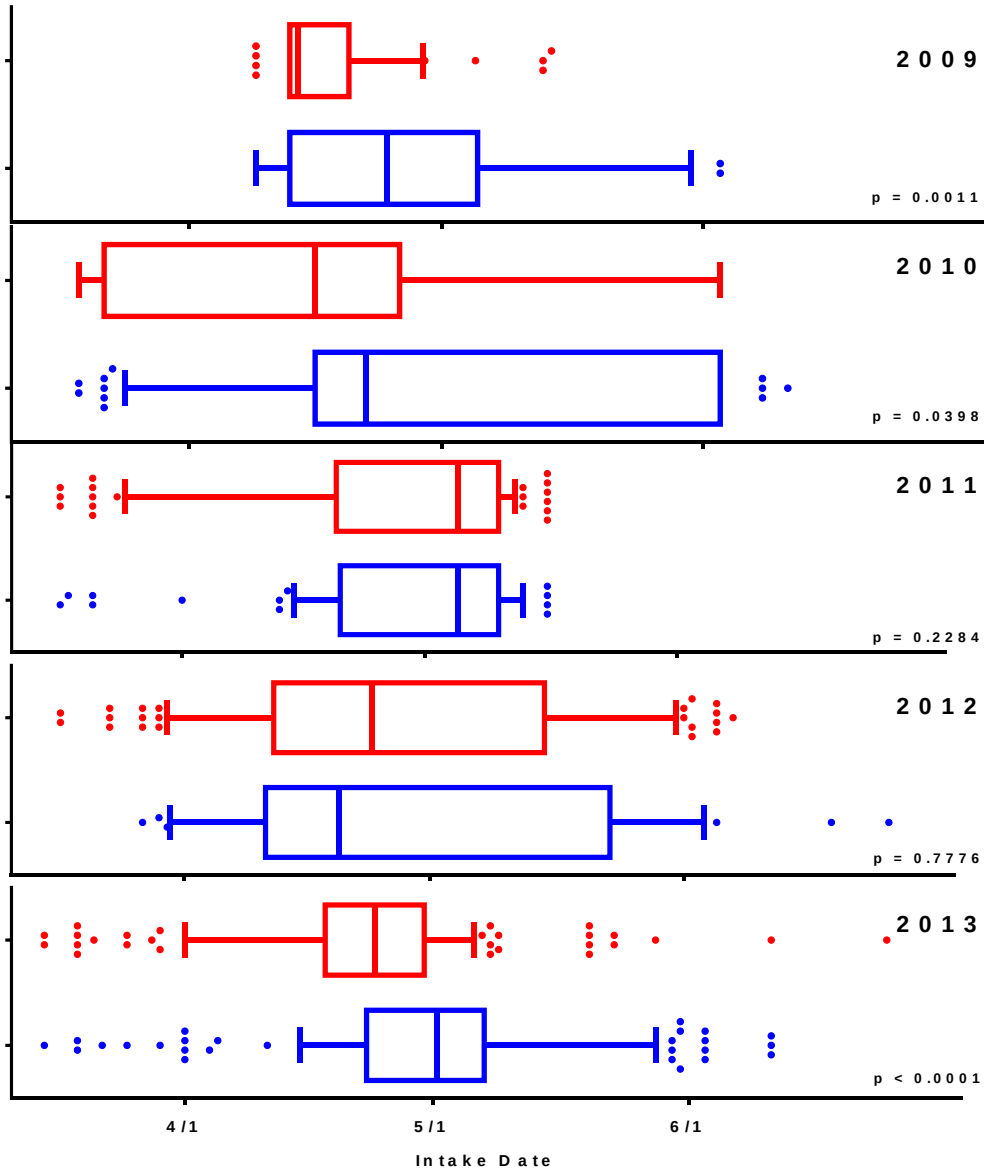


Figure 41: Intake date and maturation status at release in female kelts at Prosser 2009-2013. Rematuring fish are red, and non-rematuring fish are blue. Whiskers indicate 10-90 percentile. Differences were tested by t-tests.

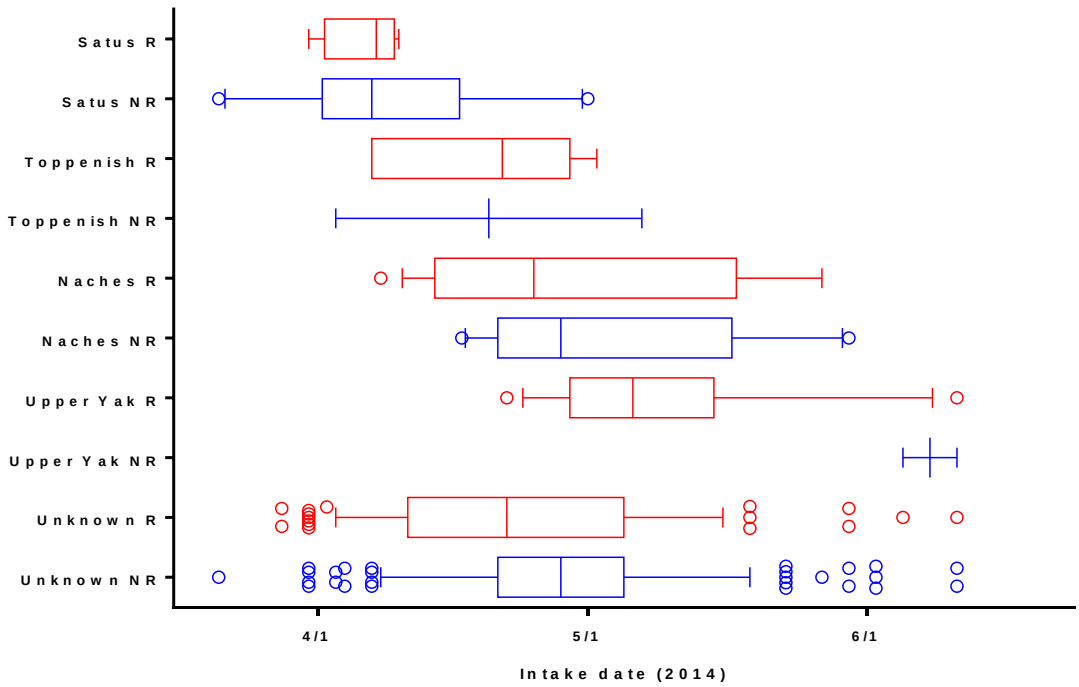


Figure 42: Intake date and maturation status at release in female kelts at Prosser 2014. Rematuring fish are red, and non-rematuring fish are blue. Whiskers indicate 10-90 percentile. Fish with pre-existing PIT tags were assigned to a VSP segment based on detections at PIT tag arrays. Fish detected at Prosser dam that were not detected at the Satus or Toppenish arrays or processed at Roza were assumed to belong to the Naches VSP segment. The difference between Unknown origin rematuring and non-rematuring fish was significant (t-test, p = 0.0093).

Proteomic Analysis of Female Steelhead Plasma

LC-MS/MS generated 113622 spectra, of which 10881 matched peptide spectra in the search database. Matched peptide spectra resolved into 1450 individual peptides, which assembled into 185 proteins (Appendix 1.f.). Of these, no proteins were identified as significantly different in abundance between kelts that subsequently rematured versus those that did not.

<B>Population Model

The parameterization above produced a prediction of future spawning abundances, smolt abundances, and adult returns based on the parameters derived from acoustic tagged kelt capture and release analysis, the rates of tagging and release groups, and the Ricker stock recruitment analysis. We further refined the model by fitting the predicted spawners to the observed spawners. We fit the model with a log-normal likelihood function, where the predicted spawners were compared to the estimated spawners. We minimized the negative log likelihood by searching for the values of first and second year ocean survival that minimized the likelihood. Since the kelt capture rates, the portions in release and recondition groups, and the survivals of those groups were all fixed to the values described, the only remaining parameters to estimated were ocean survivals, which were estimated to be 10% survival in the first year, and 49% survival in the second year in the ocean. Note that since all fish returned as two-salt fish, only the product of the two is relevant, which is about 5%. Recalling that 5% is approximately the SAR required for the population to be stable at about 20 smolts per spawner production levels, the estimate seems correct. The observed and predicted spawners from the fitting process are shown in Figure 43.

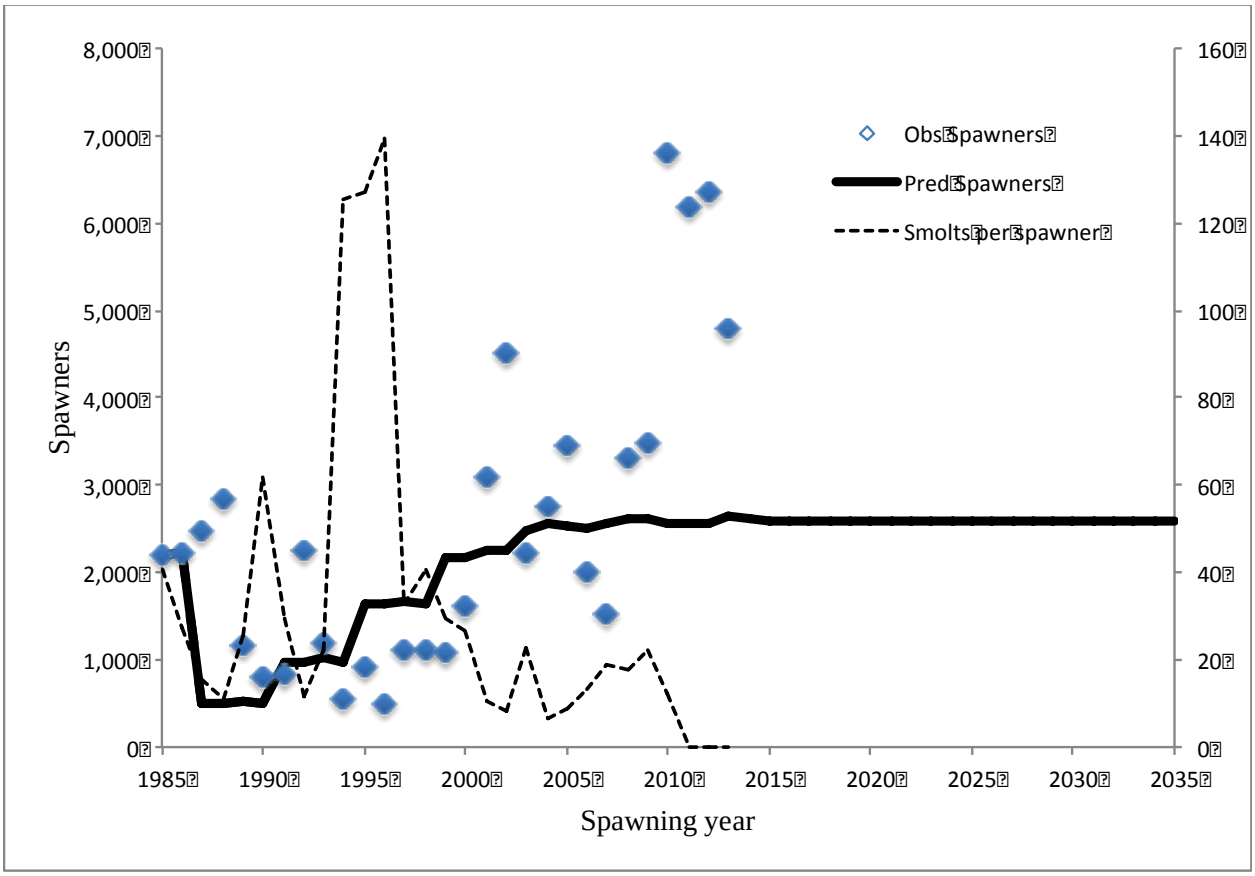


Figure 43: Observed and predicted spawners from model fit.

We can see in Figure 43 that the predicted spawners overestimate spawning in the 1990's, then underestimate spawning in the 2000's. This is because the Ricker productivities and capacities are constant across years as estimated in the fit of predicted to observed smolts. Those parameters were used to generate predicted smolts in this model, but in this fitting procedure, those predicted smolts were based on the predicted returning spawners from previous smolts. Thus the model fitting in the population model was dependent on previous years. This had the effect of propagating predictions into future predictions. As a result, the model was unable to fit every data point, and appears to underestimate recent spawning. Figure 43 also shows the empirical pattern in smolts per spawner, with effective productivity declining in recent years.

We further validated the Ricker production parameter estimates by mapping out the likelihood that the parameter values could be responsible for seeing the spawning abundances empirically observed. Figure 44 shows the negative log likelihood of the predicted versus observed spawners in relation to the value of the Ricker b parameter. We see that the population model suggests that the capacity is probably higher than the Ricker function predicted from the predicted-to-observed smolt abundance fit. It appears that to explain the pattern in spawning abundance, capacity needs to be about 14,000 vs 11,278 in the smolt fit.

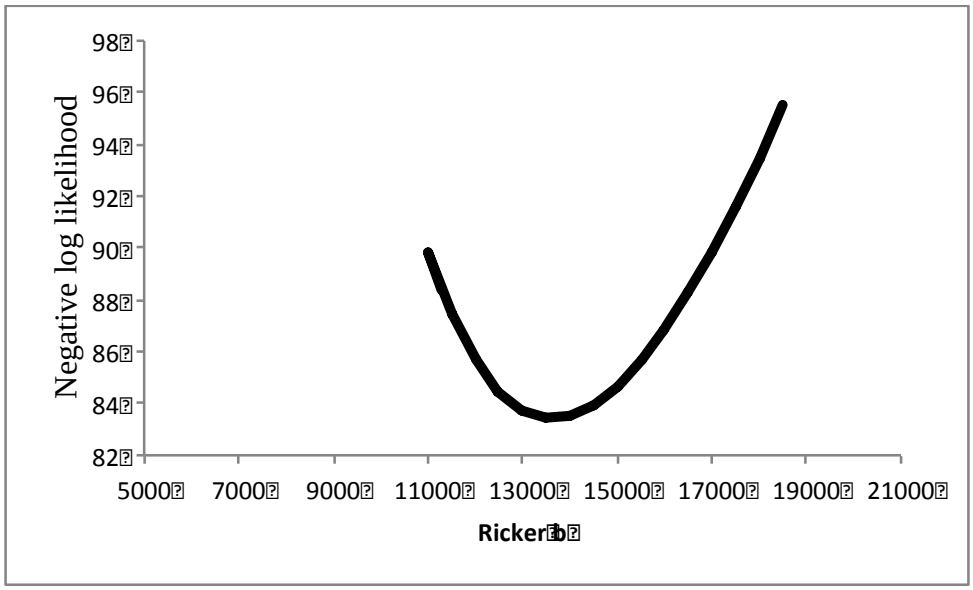


Figure 44: Likelihood profile of Ricker b parameter from spawning abundance likelihood.

We repeated this same analysis with the Ricker a productivity parameter. The likelihood profile of the Ricker a parameter from the spawning abundance likelihood is shown in Figure 45. The Ricker a productivity parameter does not appear to be higher as result of fitting to spawning abundances.

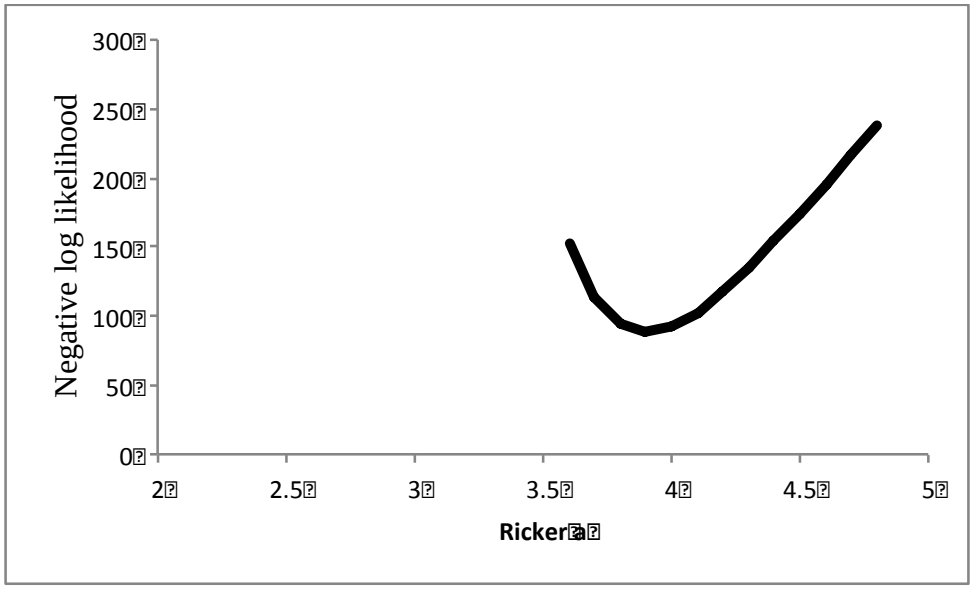


Figure 45 Likelihood profile of Ricker a parameter from spawning abundance likelihood.

The population model was forced to balance several factors: predicting spawners from ocean returns, predicting smolts from predicted spawners, and using those predictions to propagate the population forward. If you consider that the smolts being used to carry the population forward come not from data, but instead from a prediction based on previous predictions, it's not surprising that the population prediction find place somewhere in the middle of the data. It

is worth noting that the population model predictions of smolts do not differ from empirical values with any noticeable bias. Figure 46 shows the time series plot of observed and predicted smolts in the population model.

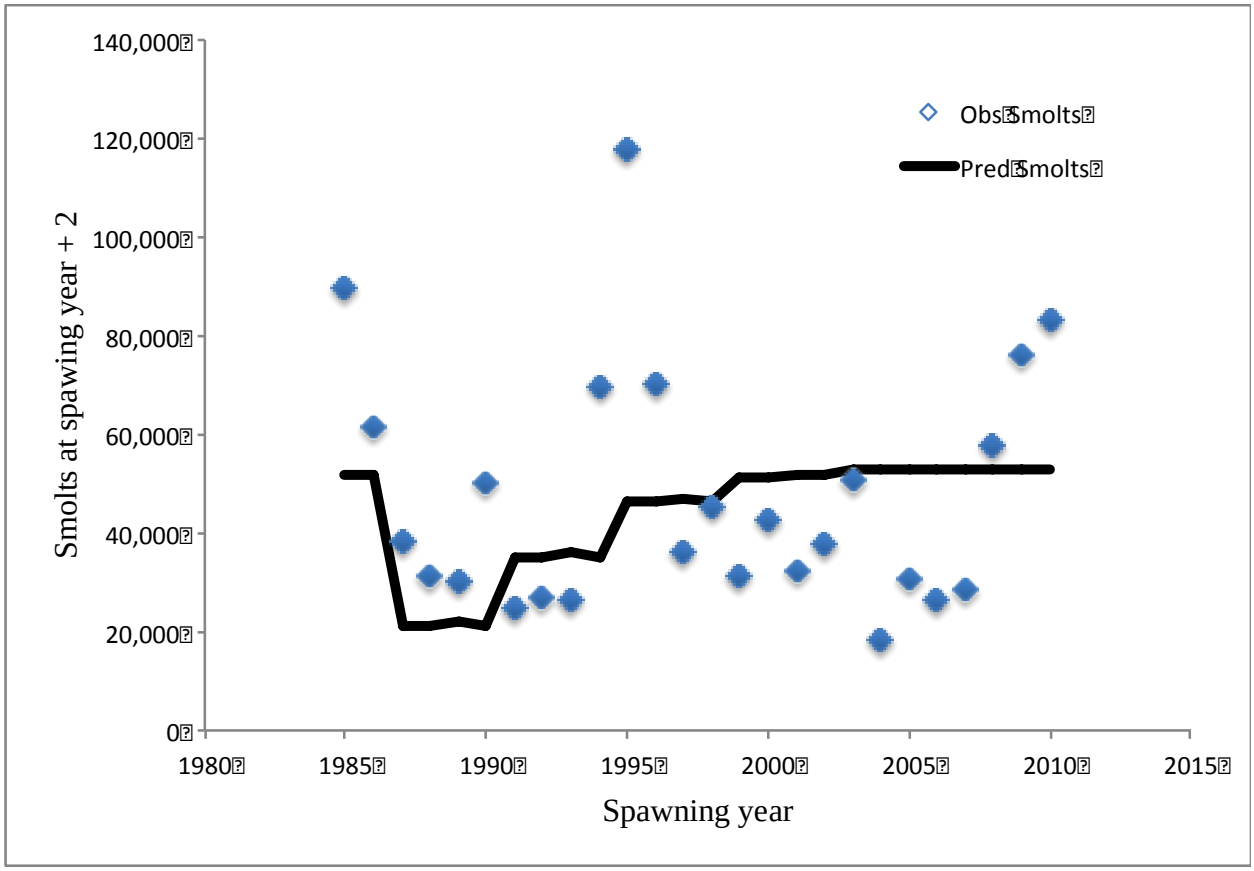


Figure 16: Observed smolts and smolts predicted from the population model.

Having been statistically fit to empirical data, the model can be used as a population prediction tool. By predicting population trends with initial spawning abundances and known parameters (fixed and estimated), we can postulated the relative effect of altering a parameter of interest. An example of this is the kelt capture rate. The assumption in the model as parameterized, was that 40% of kelt were captured, and the uncapture kelts survived as in-river fish of good, fair and poor conditions. It the assumption is that capturing more kelts would result in more ultimate spawners, and if that would lead to an increase in the overall population size, then the obvious question is “How much will the additional collection contribute to spawning?”. To answer this question, we used the predicted returning kelts and returning spawners over a fiteen year period, and calculated the average portion of spawners that were returning kelts. Figure 47 shows the relative change in the portion of kelts at different collection rates.

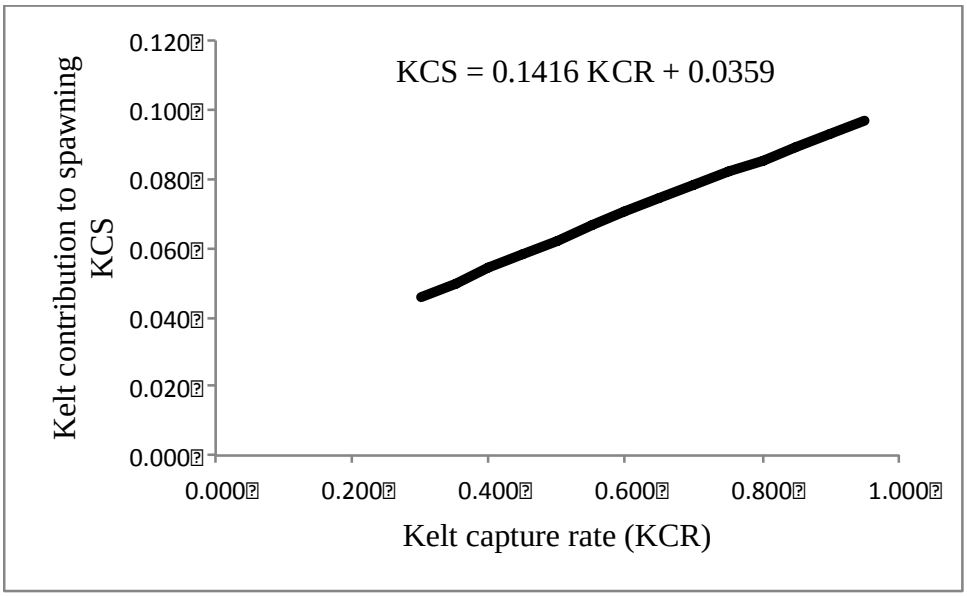


Figure 47 Relative contribution of kelts to spawning abundance.

We also wanted to look at the possible impact of kelt collection on the overall returning abundance of spawners. Figure 48 shows the returning spawning abundance in 2020 predicted by a given kelt capture rate. We see that an increase in the kelt capture rate from 40% (assumed to be current capture rate) to 80% would yield a predicted 120 more spawners. While this may not seem like much, two things must be noted: 1. The population fit to empirical trends appears to underestimate recent production, so it would be reasonable to assume the number could be higher than 120, and most importantly 2. There is no decline in the rate of production from increasing kelt capture rates, i.e., there is no apparent diminishing returns in doing so.

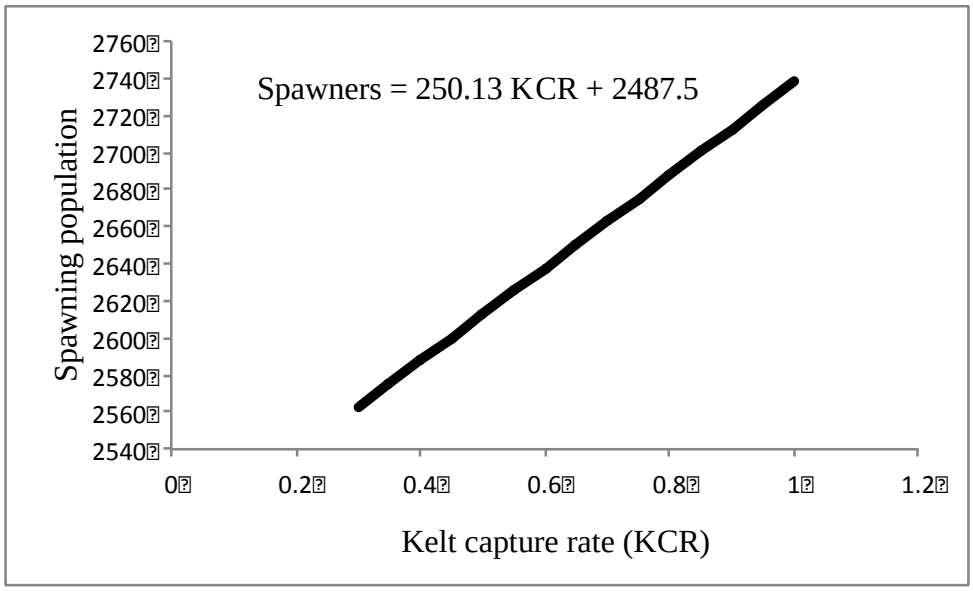


Figure 48: Predicted increase in spawning population with increase in kelt capture rate.

<A>Reproductive Success of Artificially Reconditioned Kelt Steelhead

<B>Egg quality and reproductive parameters in hatchery origin maiden female steelhead and reconditioned kelts at Dworshak National Fish Hatchery

During the summer of 2013, 21.6% of surviving kelts were identified as rematuring based on plasma estradiol level (Fig. 49). Rematuring fish had E2 levels over 1000 pg/ml by August 8th, whereas non-rematuring fish were below this level. Plasma estradiol levels increased significantly in both rematuring and non-rematuring fish from August 8 to October 3rd. Complete separation in plasma estradiol levels was maintained. Rematuring kelts had significantly higher muscle lipid levels than non-rematuring fish on 10/3/13 (Fig. 50). Muscle lipid levels decreased significantly in rematuring fish and increased significantly in non-rematuring fish from 10/3/13 to 2/3/14, resulting in significantly higher levels in non-rematuring fish on 2/3/14.

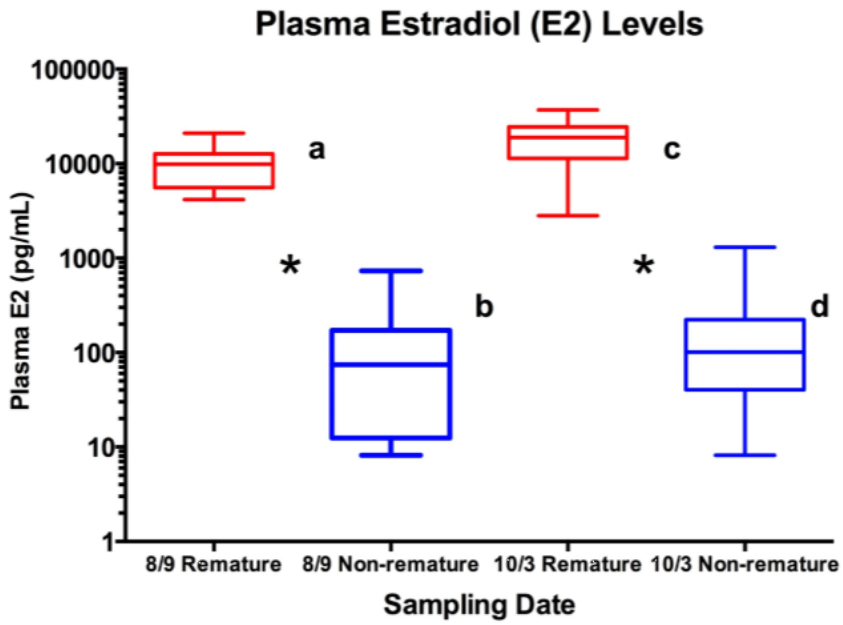


Figure 49: Plasma estradiol levels in rematuring and non-rematuring 2013 spawn year kelts during summer and fall 2013. Bars not sharing a letter are significantly different.

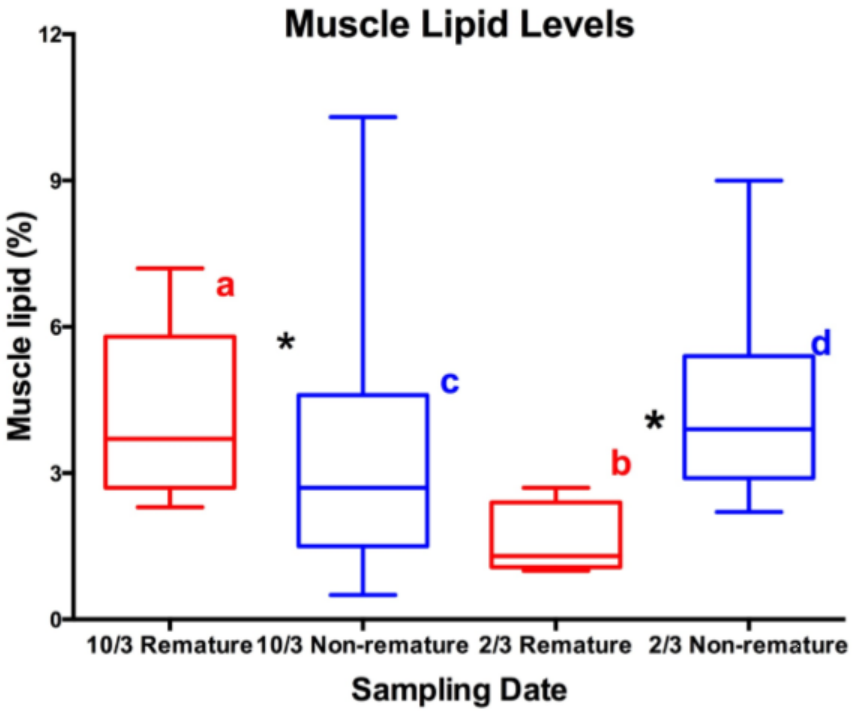


Figure 50: Muscle lipid levels in rematuring and non-rematuring 2013 spawn year kelts during summer and fall 2013. Bars not sharing a letter are significantly different.

Consecutive spawning 2013 spawn year steelhead were spawned median 1.5 weeks earlier than the date of their maiden spawning (Fig. 51). Spawn week did not differ significantly versus maiden spawning week. Fecundity increased with length in both maiden and consecutive repeat spawning steelhead (Fig. 52). The length-fecundity relationship did not differ significantly between groups. Fecundity and egg size were significantly higher in reconditioned consecutive spawning 2013 kelts than in 2014 maiden spawners (Figs. 53, 54; fecundity 1.23 fold maiden fecundity, egg size 1.19 fold maiden egg size). Fertilization success was not significantly different between maiden spawning steelhead and consecutive spawning reconditioned steelhead (Fig. 55; maiden average 92%, kelt 96%, p=0.7434).

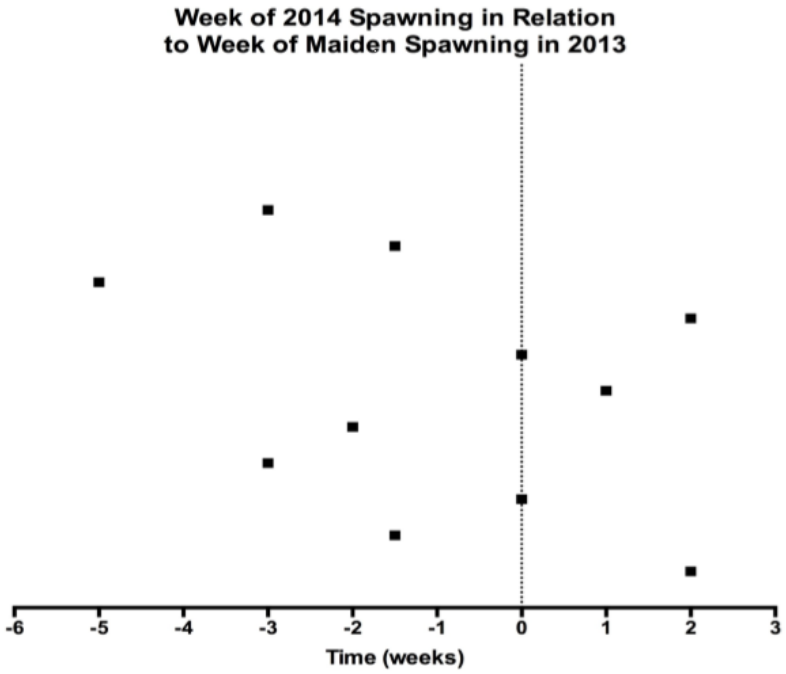


Figure 51: Spawn week in consecutive spawning 2013 steelhead versus maiden spawning week. No significant difference from maiden spawning week was detected (p= 0.1516).

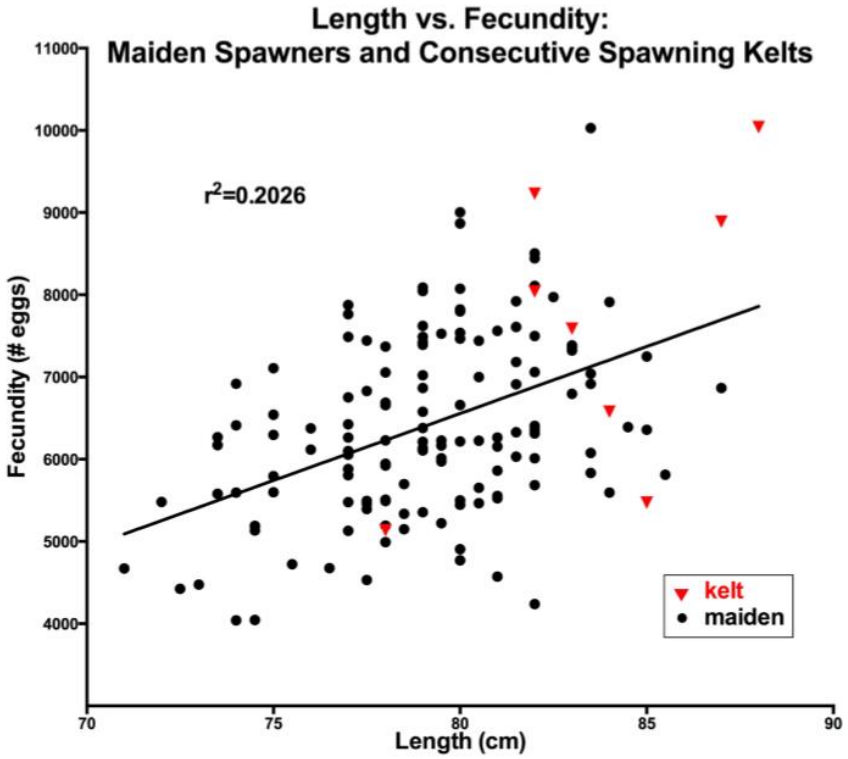


Figure 52: Length versus fecundity in 2014 maiden spawning steelhead and 2013 reconditioned consecutive spawning kelt steelhead.

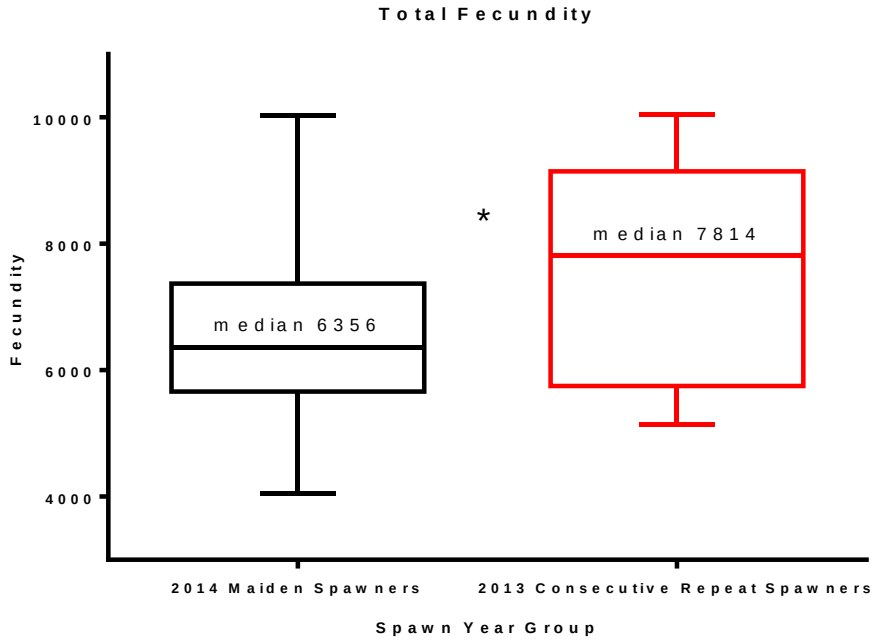


Figure 53: Fecundity in 2014 maiden spawners and reconditioned 2013 kelts spawning as consecutive spawners in spring of 2014 (t-test, p = 0.0076).

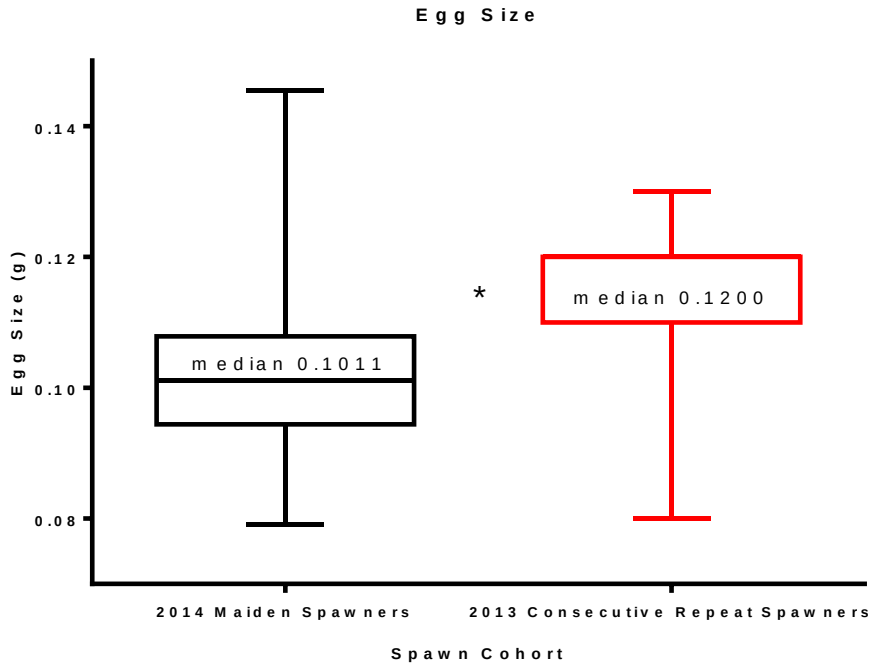


Figure 54: Egg size in 2014 maiden spawners and reconditioned 2013 kelts spawning as consecutive spawners in spring of 2014 (Mann Whitney test, p = 0.0005).

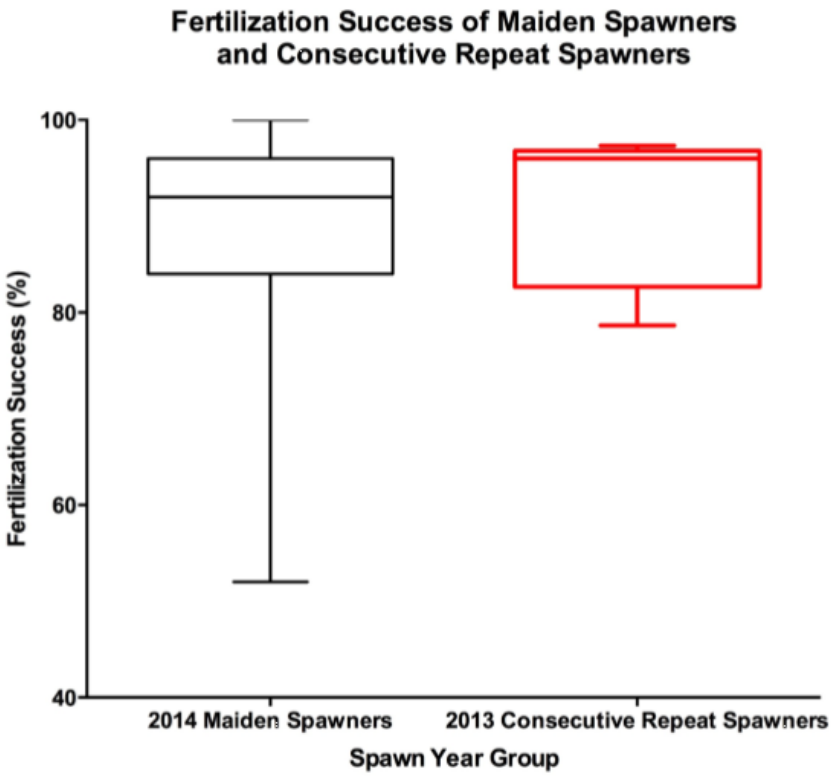


Figure 55: Fertilization success in 2014 maiden spawning and 2013 consecutive spawning reconditioned DNFH steelhead.

No differences were detected in factors measured at intake between fish that rematured as consecutive spawners versus fish that deferred maturation (Fig. 56). Rematuring kelts had significantly higher specific growth rate over the period from intake to 8/8/13, but not from 8/8/13 to 10/10/13 (Fig. 57).

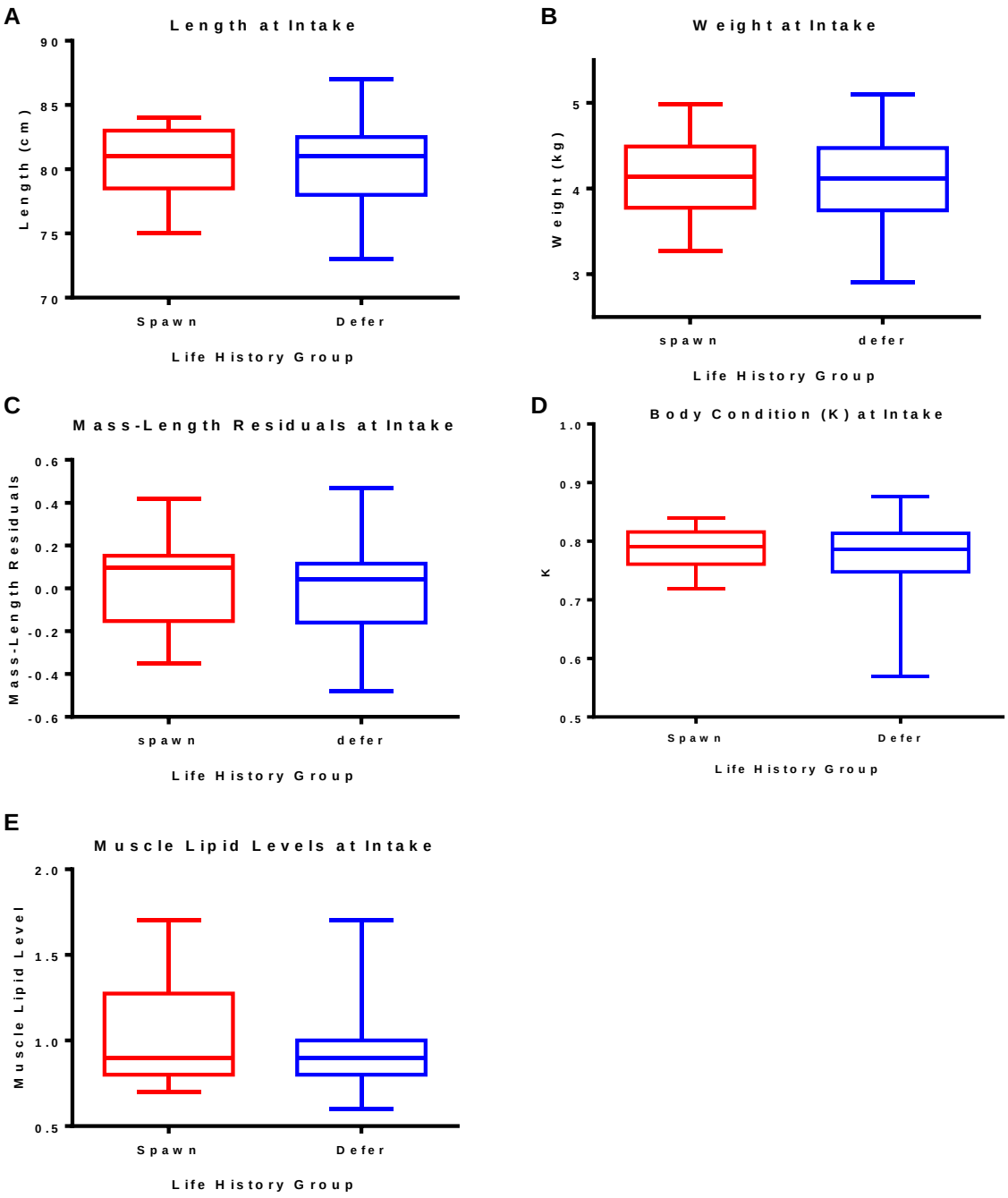


Figure 56: Factors measured at intake 2013 in kelts that rematured as consecutive spawners versus fish that deferred spawning. No significant differences were detected. A: unpaired T-test p = 0.8556; B: unpaired T-test p=0.5877; C: unpaired t test p=0.5389; D: unpaired t test p=0.4081; E: Mann Whitney test p=0.436.

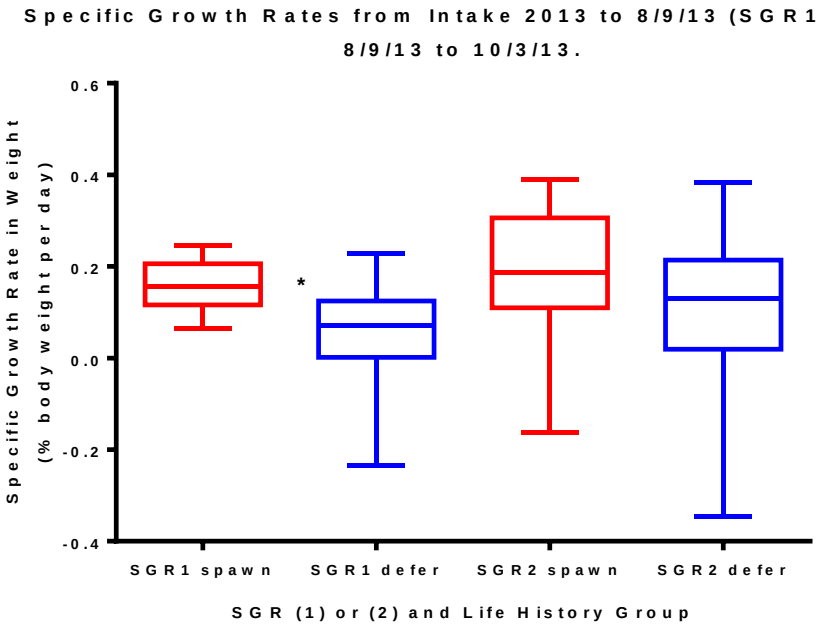


Figure 57: Specific growth rate in weight during 2013 in in kelts that rematured as consecutive spawners versus fish that deferred spawning. SGR1: unpaired t test with Welch's correction p < 0.0001; SGR2: unpaired t test p=0.1430.

Complete results for 2013 skip spawners and 2014 consecutive spawners are not yet available. Preliminary data suggests that surviving non-mature 2013 fish rematured at a high rate (93.1%, Table 13).

Table 13: Hatchery origin female steelhead artificially spawned and reconditioned at Dworshak National Fish Hatchery in 2013 and 2014.

Spawn Year	Fish Air Spawned	8/9/2013		8/28/2014	
		Alive (%)	Rematuring (%)	Alive (%)	Rematuring (%)
2013	163	74 (45.4)	16 (21.6)	29 (50)	27 (93.1)
2014	149	-	-	32 (21.5)	2 (6.3)

<B>Kelt Reproduction in a Natural Setting

Yakama Parentage Analysis

Numbers for each collection, by location and year, can be seen in Table (14). Departures from Hardy-Weinberg equilibrium are reported for both uncorrected (0.05) and corrected (0.05 /172 loci = 0.000291) critical values. Linkage disequilibrium was reported only as an uncorrected value due the large number (14706) of pairwise comparisons. For comparison purposes, the

expected number of random departures without multiple test correction is 735 (0.05 *14706 =735) for each collection. Departures from random values were seen for all collections, although this is expected due to population admixture. Adult collections included samples from multiple tributaries to the Yakima River. Juvenile collections included tributaries within both Satus and Toppenish Creek and are likely to also include a resident component. Because these departures were expected, and parentage analysis does not strictly require Hardy-Weinberg or Linkage equilibrium, analysis proceeded as normal.

Table 14. 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 0.05	HW 0.00029	LD 0.05
Pre-spawn Maiden	451	0.342	0.334	18	1	1058
Post-spawn Maiden	330	0.346	0.334	19	1	1024
Reconditioned Kelt	347	0.341	0.333	14	2	1004
Satus Age-0	248	0.321	0.316	17	0	1816
Toppenish Age-0	250	0.309	0.307	16	1	2244
Toppenish Age-0 ST	50	0.308	0.308	5	0	916

Parentage assignment was successful for 26.3% (137) of the 548 juveniles included in the parentage analysis. Single genotype mismatches were seen in 17 of these samples, but were accompanied by higher LOD scores. Three juveniles were assigned to two parents indicating both maternal and paternal parentage. Four additional samples were “re-assigned” to a second adult to account for overlap between the pre-spawn and post-spawn maiden designations. The replication of these 7 individuals resulted in 144 total assignments to single parental fish.

Parentage analysis included 451 maidens collected as pre-spawners, 330 maidens collected as post-spawners, and 347 kelts taken into the reconditioning program (Table 15). Female fish accounted for 306 of the pre-spawn maidens, all of which crossed Prosser Dam where they were sampled for genetic tissue. Females accounted for 307 of the post-spawn maidens. All of these were assumed to have spawned above Prosser and are therefore considered to have been detected at Prosser Dam. Of the 347 kelts, 209 females were detected moving upstream at Prosser Dam following reconditioning and release.

Table 15. Number of genotyped fish for all fish, all females, and all females detected moving across Prosser Dam.

	All	Females	Prosser
Pre-spawn Maiden	451	306	306
Post-spawn Maiden	330	307	307
Reconditioned Kelt	347	318	209

The number of parentage assignments to each parental class is shown in Table 16. For female parents detected at Prosser, 42 assignments were to pre-spawn maidens, 53 were to post-spawn maidens, and 17 were to reconditioned kelts. Compared to pre-spawn maidens, post-spawn maidens and reconditioned kelts had a relative reproductive success of 1.25 and 0.59 respectively (Table 17).

Table 16. Number of progeny assigning back to each class of parent

	All	Females	Prosser
Pre-spawn Maiden	58	42	42
Post-spawn Maiden	61	53	53
Reconditioned Kelt	25	20	17

Table 17. Relative Reproductive success between classes

	All	Females	Prosser
Pre-spawn Maiden	1	1	1
Post-spawn Maiden	1.44	1.26	1.26
Reconditioned Kelt	0.56	0.46	0.59

Of female fish detected at Prosser Dam, 13 pre-spawn maidens, 26 post-spawn maidens, and 7 reconditioned kelts had at least offspring match (Table 18). The average number of progeny assigning to each unique adult was 3.23 for pre-spawn maidens, 2.04 for post-spawn maidens, and 2.43 for reconditioned kelts (Table 19).

Table 18. Number unique adults with at least one juvenile assignment

	All	Females	Prosser
Pre-spawn Maiden	24	13	13
Post-spawn Maiden	29	26	26
Reconditioned Kelt	12	9	7

Table 19. Average number of progeny per unique adult

	All	Females	Prosser
Pre-spawn Maiden	2.42	3.23	3.23
Post-spawn Maiden	2.10	2.04	2.04
Reconditioned Kelt	2.08	2.22	2.43

The number of progeny collected at individual sites, and the corresponding number and percentage of samples assigned to at least one adult parent is shown in table 20. Numbers varied between zero assignments for offspring collected in Willy Dick Creek (Tributary to Toppenish Creek), and 36 assignments in Toppenish Creek above the three way.

Table 20. Sample number and parentage assignments by location

	n	Assigned to adult	
Satus Creek-Dry Creek at elbow xing	88	8	9.1%
Satus Creek-Below High bridge	75	21	28.0%
Satus Creek-Above Logy Creek	3	2	66.7%
Satus Creek-County Line	14	1	7.1%
Satus Creek-Holwengers Ranch	21	5	23.8%
Satus Creek-Dry mouth	11	8	72.7%
Satus Creek-Above High bridge	36	14	38.9%
Toppenish Creek- Above 3 way	100	36	36.0%
Toppenish Creek-Willy Dick Creek	46	0	0.0%
Toppenish Creek-Swim hole	10	4	40.0%
Toppenish Creek-Simcoe at Simcoe Creek Rd	45	16	35.6%
Toppenish Creek-Simcoe NF SF confluence	49	8	16.3%
Toppenish Creek- ScrewTrap	50	14	28.0%
Sum	548	137	25.0%

The average fork length of offspring assigning to at least one adult was 70.2 (range of 44-90) for pre-spawn maidens, 64.7 (range of 42-86) for post spawn maidens, and 72.5 (Range of 52-98) for reconditioned kelts. However, two of the offspring that assigned to a single reconditioned kelt were at lengths of 93mm and 98mm. As seen in Figure 58, this is larger than the majority of fish sampled at this area. If these two samples were removed from analysis, the average length of offspring assigning to reconditioned kelts was 70.5 with a range of 52-88.
Lengths for all fish assigning to any reconditioned kelts are seen in Table 21.

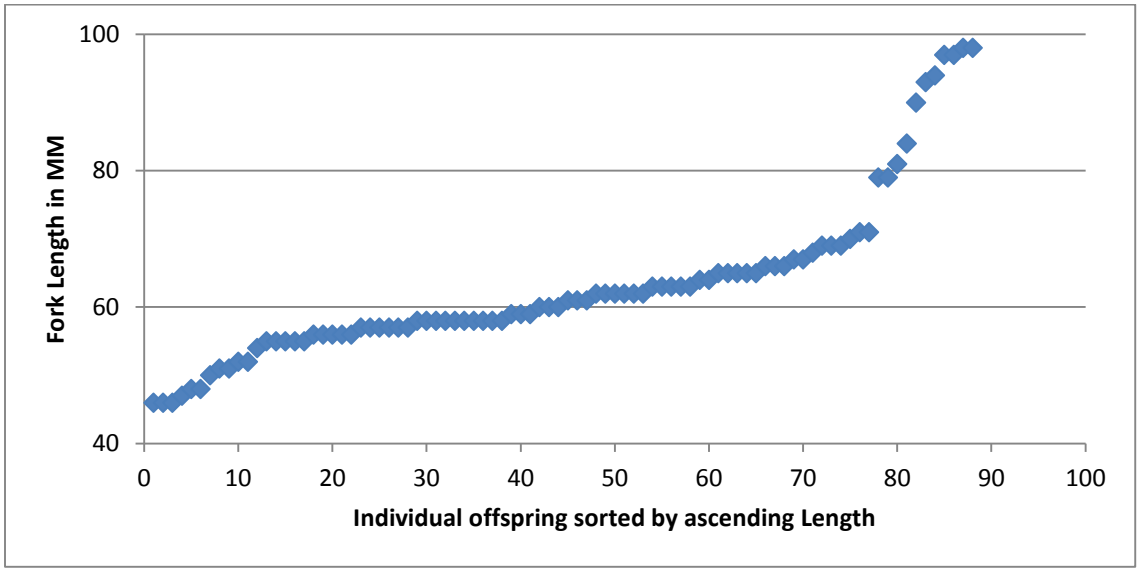


Figure 58: Fork length of all fish sampled in the Satus Creek-Dry Creek Elbow Xing location.

Table 21. List of all offspring assigning to a reconditioned adult

Juvenile	FL	Date	Parent
OmySat13_0230	76	10/2/2013	OmyYRC12k-0094
OmySat13_0093	54	9/18/2013	OmyYRC12k-0094
OmySat13_0239	84	10/2/2013	OmyYRC12k-0094
OmySat13_0123	59	9/18/2013	OmyYRC12k-0094
OmySat13_0157	70	10/2/2013	OmyYRC12k-0096
OmyTopSTBY13-41	88		OmyYRC12k-0128
OmySat13_0128	70	9/18/2013	OmyYRC12k-0201
OmySat13_0130	66	9/18/2013	OmyYRC12k-0201
OmySat13_0067	60	9/18/2013	OmyYRC12k-0276
OmySat13_0146	52	9/18/2013	OmyYRC12k-0285
OmyTop13_0055	83	9/19/2013	OmyYRC12k-0319,ProSTh204
OmyTop13_0033	72	9/19/2013	OmyYRC12k-0319
OmyTop13_0066	73	9/19/2013	OmyYRC12k-0319
OmyTop13_0089	75	9/19/2013	OmyYRC12k-0319
OmyTop13_0098	75	9/19/2013	OmyYRC12k-0319
OmyTop13_0040	76	9/19/2013	OmyYRC12k-0319
OmyTop13_0029	77	9/19/2013	OmyYRC12k-0319
OmyTop13_0027	70	9/19/2013	OmyYRC12k-0319
OmyTop13_0243	62	9/23/2013	OmyYRC12k-0332
OmySat13_0005	93	9/18/2013	OmyYRC12k-0342
OmySat13_0003	98	9/18/2013	OmyYRC12k-0342
OmyTopSTBY13-34	80		OmyYRC12k-0441
OmySat13_0063	61	9/18/2013	OmyYRC12k-0479
OmySat13_0073	55	9/18/2013	OmyYRC12k-0479
OmyTopSTBY13-42	84		OmyYRC12k-0523

Variance in reproductive success for females detected at Prosser Dam, or presumed to have crossed Prosser Dam is shown in Figure 59. Number of progeny per adult varied between 0 and 11. Zero progeny were seen for 96.1% of pre-spawn maidens, 91.5% of post-spawn maidens, and 96.7% of reconditioned kelts.

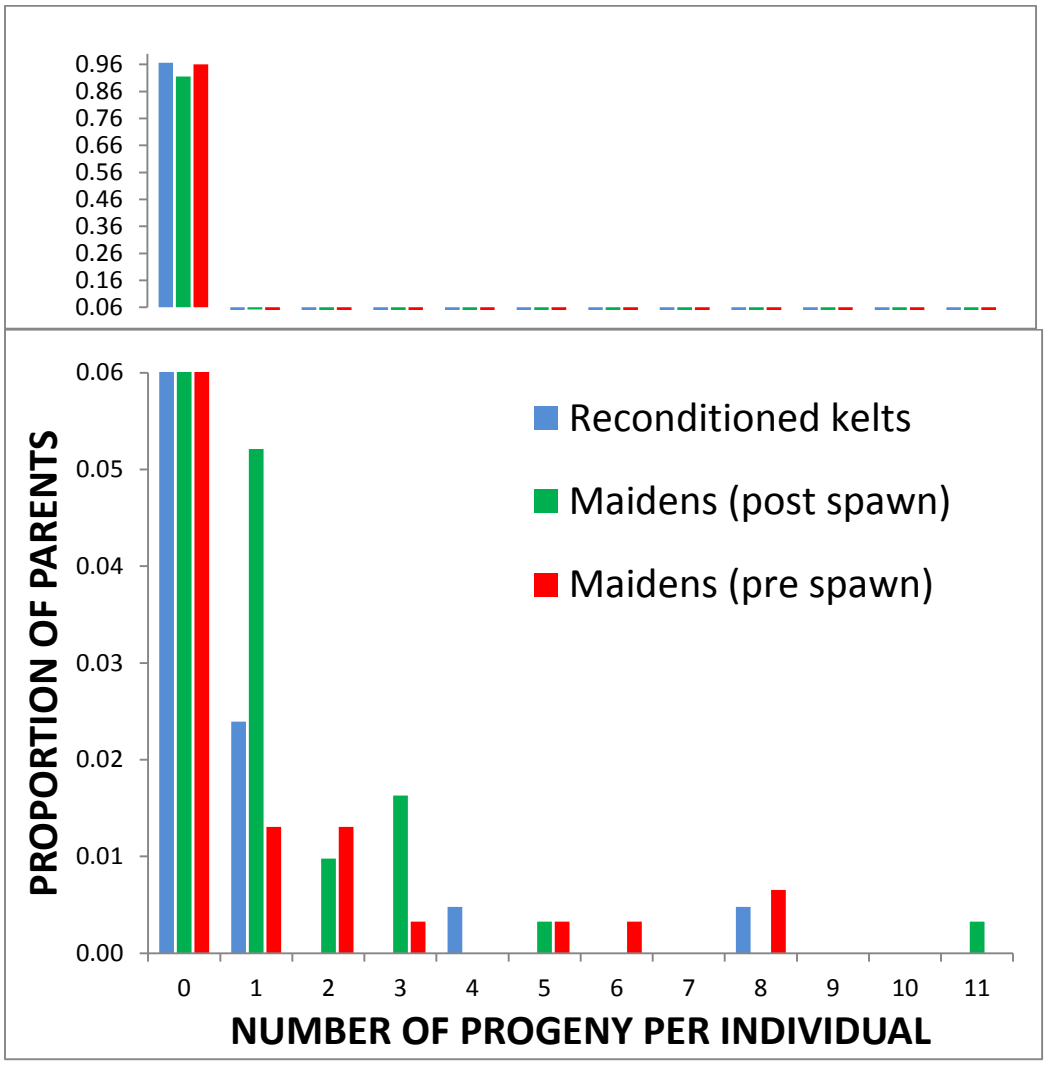


Figure 59. Average number of progeny per adult

Tracking Repeat Spawners in the Yakima River Basin

<C>Repeat spawner post release tracking
We radio tagged 70 female kelts. There were an additional 154 repeat spawners based on sex hormone data that were considered mature were PIT-tagged only. There were a total of 40 (57%) radio tagged kelts which passed Prosser Dam in late fall 2013/spring 2014. This compares with 42% (65) of the PIT tag only kelts migrating over Prosser in the late fall of 2013 with a handful of spring 2014 migrators. Of these radio tagged fish all but 3 crossed in late October/Early November of 2013. The remaining three overwintered below Prosser Dam and crossed in early March 2014. Radio tag loss or regurgitated tags was estimated at 10% (7) based on radio tag recovery or PIT-tag detection without radio tag movement. This tag regurgitation rate was higher than maiden fish (pers communication Chris Frederiksen). We

were still able to observe these fish moving in and out of spawning tributaries based on PIT-tag detections but we could not determine exact spawning location.

We observed 2 of the radio tagged fish and 13 of the PIT-tagged only group which moved downriver with no upstream detections. Most of the PIT-tag downstream migrating group (9) moved downstream almost immediately after release and were sampled moving through the McNary juvenile bypass, implying active migration to the ocean. The remaining downstream migrators (2 radio tagged and 4 PIT-tagged only fish) were detected in the McNary juvenile bypass in the late spring.

We detected 27% (19) (Table 22) of the radio tagged kelts successfully returning to spawning tributaries. Most of these fish moved into the natal streams around the freshet (early-mid March) which also coincides with warming water temperatures (Figure 60). The PIT only fish had a smaller proportion of fish that were detected moving into the natal streams 18% (27) (Table 22). It should be noted that there is no detection system in the Naches with which to confirm attempted migration so this comparison is somewhat limited. The natal populations that were most represented was Satus, Naches, and Toppenish Creeks (Table 22). Though the Naches may have had more spawners in the PIT-tag only group but we could only consider them successful spawners if they returned as kelts that appeared at Prosser Dam which does not necessarily negate successful spawning if mortality occurs after spawning or the fish passed over Prosser dam instead of volunteering into the Chandler bypass.

Table 22: Detections of artificially reconditioned repeat spawners in natal streams.

	Satus	Toppenish	Ahtanum	Cowiche	Naches/Mainstem Yakima	Upper Yakima	Total
Radio/PIT	5	3	2	1	7	1	19
PIT Only	8	10	0	1	6*	2	27
Total	13	11	2	2	13	3	46

***Based solely on return collection and condition at Prosser.**

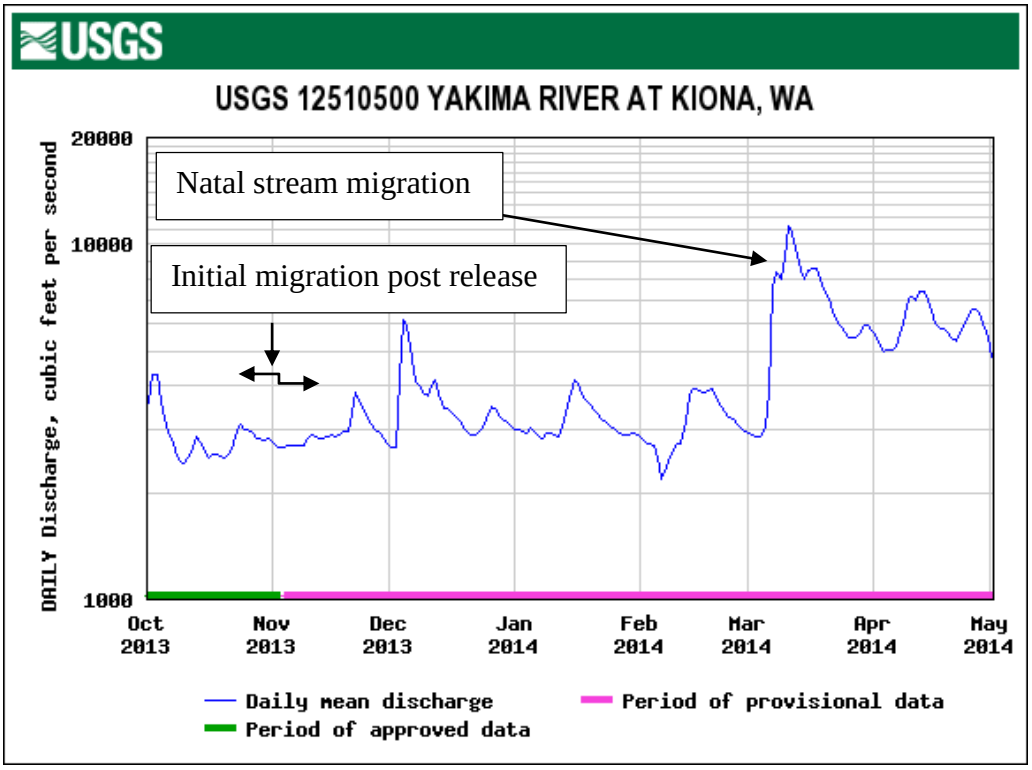


Figure 60. Fish migration from post release into mainstem Yakima River and later when majority of kelts migrated into spawning tributaries (Satus, Toppenish, Ahtanum, Cowiche, Naches, and Upper Yakima). Image courtesy of US Geological Services.

When repeat spawners moved into the entrance of the natal spawning stream it took approximately 1-3 weeks to migrate to the suspected redd locations. Spawning (time it took to construct redd and deposit eggs) was estimated to take an average of 2-5 days. Fish that survived to the completion of spawning rapidly moved out of the natal tributary streams to the mainstem Yakima River in approximately 1-3 days. We observed that the PIT-tagged only fish had similar timing (natal stream entrance to exit) as the radio tagged kelts. Although, we do not have an estimated time of spawning for the PIT-tag only group, we assume it was similar, due to similar total natal stream entry-to-exit timing.

There also were 2 PIT-tag only fish that entered Toppenish at the end of 2013 (late-November/mid- December). We had no further detection of these fish to confirm if they had spawned in Toppenish. There were 3 of the radio tagged repeat spawners which entered the natal streams (Satus and Ahtanum) in mid-January 2014. Using the river guage as a proximate for tributary flows in the area there was a decline in flow in the early part of 2014 (figure 61). This decrease in stream flow was one of the strongest that has been observed in the early winter months in at least the last 6 years. This dry spell left early migrators in streams with extremely low flows which in turn left them vulnerable to predation by eagles, osprey, river otters, and brown bears. The Satus Creek group of repeat spawners had the highest rate of suspected predation due to it being the first spawning tributary in the system. It is possible that these fish lost their radio tags but there was no further PIT-tag detections to confirm this.

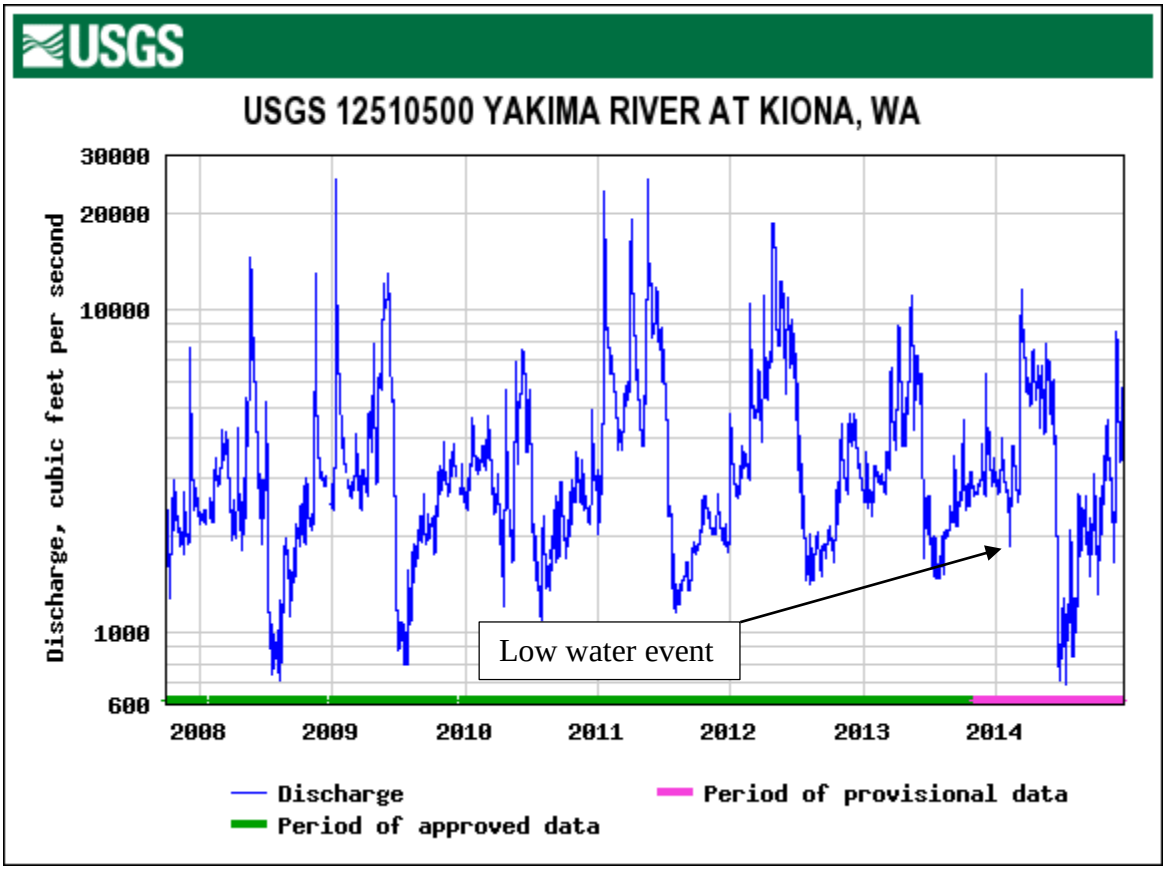


Figure 61: Streamflow discharge of the Yakima River at Kiona Rkm 48.

Some of the radio tagged (4) and PIT-only (8) repeat spawners had prior detection histories as maiden fish that we could use as a performance and fidelity comparison. All 12 of these fish returned to their natal streams as repeat spawners in 2014 at nearly the same date as the previous year (2013) as maiden spawners (within as little as a few days to a couple of weeks). These previous migration histories represent the multiple migration types that we observed in 2013/2014 which were also present in the maiden spawners (2012/2013) PIT-tag data.

High water levels during the freshet prevented us from recovering any possible carcasses to fully determine if tag regurgitation or mortality was responsible. There were 15 (21%) of the radio tagged repeat spawners which had no further movement detection histories from either radio or PIT-tag detections upon release and throughout the run-season. They may have not been able to navigate the dam, were predated on, or perished from stress/ or stress induced disease.

There were 19 of the 40 (27%) migrating repeat spawners that did not make it to the natal spawning areas after passage above Prosser Dam. A sizeable portion ceased to move about 1-10 river kilometers upriver of the dam. We were not able to retrieve these tags as they were in approximately 6-10 meters of fast moving water. We assumed that these tags were likely mortalities. While there could have been spit tags we have no further PIT-tag detections to

confirm this. The only possibility would be if they spawned in the Naches but had no further detections in the system which is possible during the freshet, but unlikely. One fish that had no additional radio detections but was recovered at Prosser Hatchery with no radio tag was captured migrating through the juvenile bypass. This would probably still constitute a small portion of the kelts as we would not expect such large tag regurgitation.

There were 13 of the 19 migrating radio tagged repeat spawners which we tracked to assumed spawning locations (Table 23) based on movement data (Figure 62). The greatest number of locations were in the Naches/Mainstem Yakima area (Table 23). The Satus spawner locations were low due to 4 possible regurgitated tag/predation mortalities. Of these regurgitated tag mortalities, 1 was a radio tag which was recovered just below areas where spawning activity was occurring (no carcass). The other tags were either regurgitated or mortalities as they did not move, which occurred during the low (2) in January and high (1) in March, stream flow events (no radio tag or carcass recoveries). There were no further PIT-tag detections or recaptures of these fish.

Table 23. Total number of proximate locations of radio tagged repeat spawners by natal tributary in 2014.

Satus	Toppenish	Ahtanum	Cowiche	Naches/Mainstem Yakima	Upper Yakima	Total
1	2	2	1	6	1	13

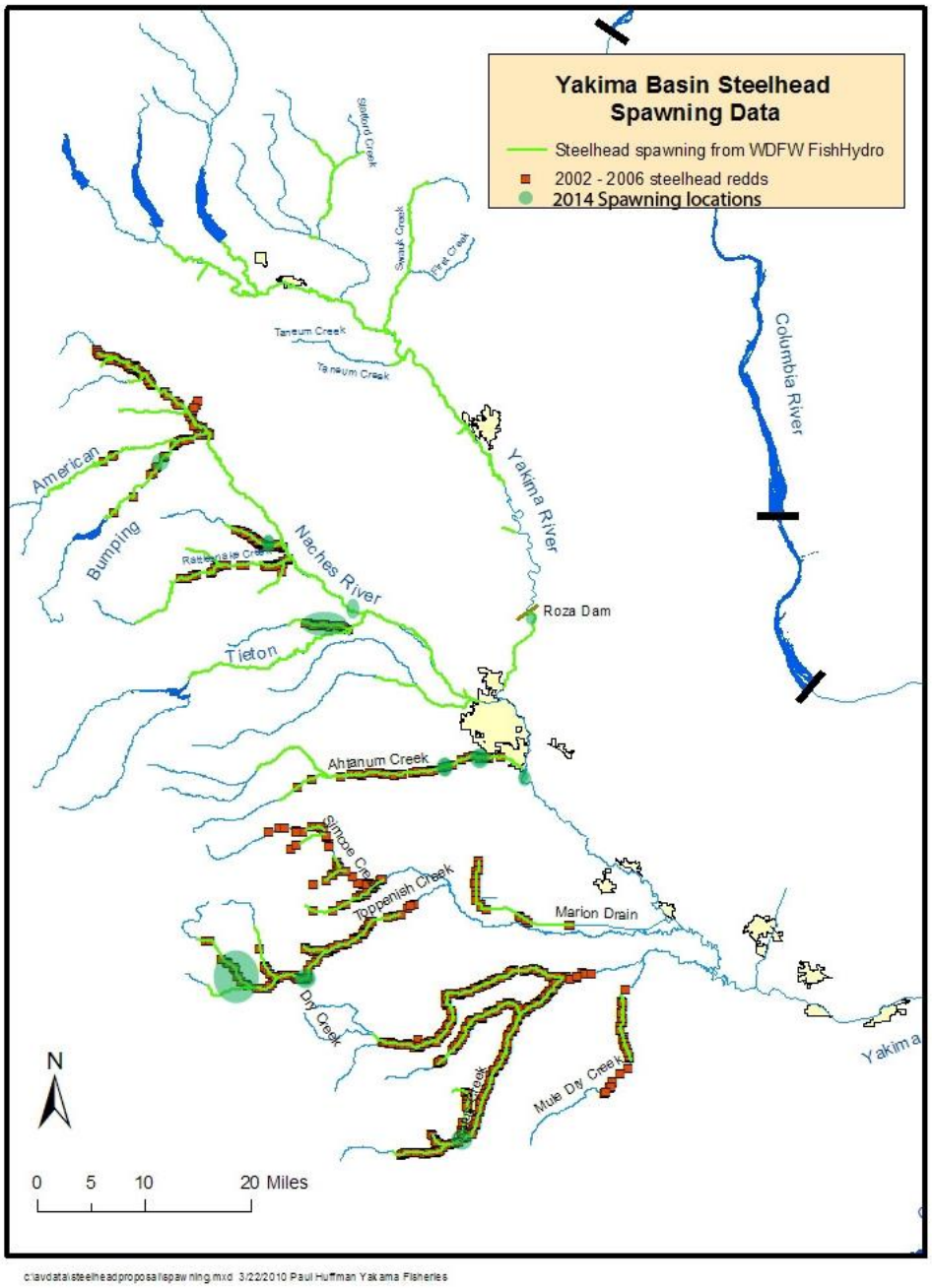


Figure 62. Areas circled with opaque dark green are suspected areas of spawning.

A portion of the radio-tagged and PIT-only tagged repeat spawners were recaptured at the Prosser juvenile bypass as kelts (Table 24). A total of 5 of these fish were taken back into the reconditioning program. There was 1 fish which was placed into the in-river release group.The rest of the kelts captured were returned to the river in poor shape or perished by the time of sampling. All known kelts conditions were recorded and a few were photographed for evidence of spawning.

Table 24. Number of radio tagged/PIT only kelts which were captured at Prosser Juvenile Bypass Facility in 2014.

Satus	Toppenish	Ahtanum	Cowiche	Naches/Mainstem Yakima	Upper Yakima	Total
1*	0	1*	1	5 (2*)	0	8

*** designates radio tagged kelt.**

There were 3 of the repeat spawners, based on entrance and timing into spawning tributaries, which were detected exiting to the ocean as kelts. Additionally, 2 were detected in the McNary juvenile bypass in late April and the other 1 at the Bradford Island ladder in the middle of May in 2014.

Juvenile Sampling

Sampling was conducted in the lower streams of Satus and Toppenish in early part of August while the other tributaries (Ahtanum, Cowiche, Nile, and Bumping creeks) were sampled in late August. We had robust samples sizes at Satus, Ahtanum, and Cowiche (Table 25). The Toppenish, Nile, and especially Bumping creeks had much lower sampling numbers (Table 25).

Table 25. Number of genetic samples collected from age-0 fry for parentage analysis and location.

Collection Site	Satus Creek	Toppenish Creek	Ahtanum Creek	Cowiche Creek	Naches River		Total
					Nile Creek	Bumping (American) Creek	
Samples Collected	105	20	150	100	17	2	394

Parentage Assignment

Parentage assignments and analysis will be concluded in the beginning of 2015. Results will be presented in the 2015 annual report.

<B>Cle Elum Spawning Channel

A copy of the Cle Elum study plan is located in Appendix A1:Products.

Discussion/Conclusion

<A>Kelt Collection

<B>Yakima River

Collection was lower at Prosser than had been in previous years and was comparable to 2006/2007 for collection. We found that kelt condition factor was not as good, which also

indicates that either river and/or ocean conditions could have been harder on fish in 2013/14 than more recent years.

<B>Snake River

Lower Granite Dam

However, unlike previous years we observed two peaks in the weekly kelt interceptions at LGR. These peaks centered on the weeks of May 5 and May 19, 2014. Furthermore, our weekly interception rate began high relative to the previous four years. During the 2010-2013 collection seasons, Lower Granite Dam spill operations began before kelt collection efforts. In 2014, we had 8 days of kelt collection prior to spill which may likely account for the higher interception rate.

Similar to 2010-13, fish were observed with recent head injuries (Branstetter et al. 2012). These head injuries look very similar in nature, which may indicate something restricting their journey to or through the bypass system (Figure 63). 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. The proportion of head injuries increased to 90.7% for 2014 (Table 26). However, the severity of those injuries was far less than observed in previous years. We are currently developing a means to more accurately quantify head injuries. For fish $\geq$70 cm, the weekly proportion varied little throughout the collection season. For fish < 70 cm, the injury rate varied; however, no apparent pattern was observed with discharge (Figure 64).

Figure 63. Typical head injury observed on steelheads kelts at LGR JFF.

Table 26. Percent of head injuries on steelhead kelts at the LGR JFF during 2014.

	A-run	B-run	Total
No	30.9	2.7	33.6
	(N=834)	(N=72)	(N=906)
Yes	59.9	6.5	66.4
	(N=1615)	(N=174)	(N=1789)
Total	90.7	9.2	100
	(N=2449)	(N=246)	(N=2695)

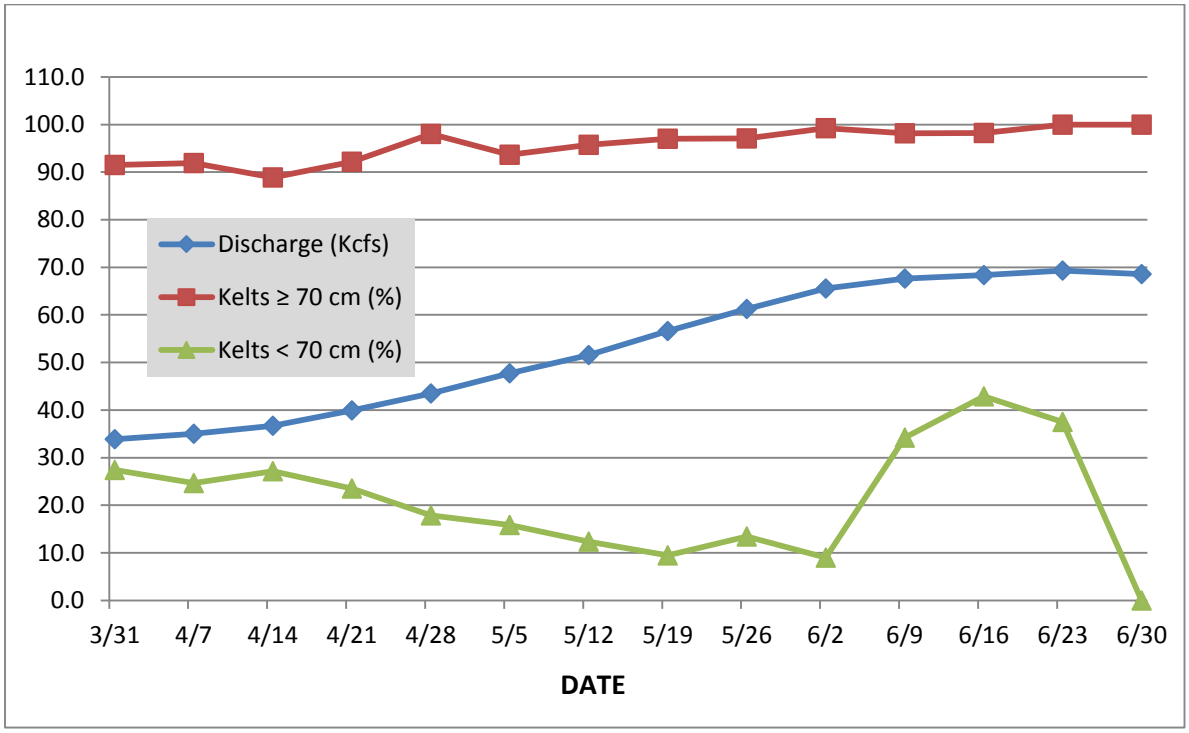


Figure 64. Mean weekly discharge (Kcfs) and percent head injuries observed on steelhead kelts at the LGR JFF in 2014.

Fish Creek Weir

Unfortunately, the 2013/2014 run saw a shortage of anadromous steelhead returning to Fish Creek, which was compounded by the weir shutdown that occurred during the peak of kelt collection, and a population that was heavily skewed male. This lead to a collection of only a small number of quality female kelts for reconditioning. IDFG personel also noted that fish size was below average which may indicate that ocean conditions may not have been as favorable to fish and with lower fat supplies may have caused an increase in mortality. We anticipate, based on PIT-tag number returns from Fish Creek in the mainstem PIT arrays, that 2015 will be a much improved year for collection and reconditioning of kelts from this location.

Dworshak National Fish Hatchery

Reconditioning survival was down at Dworshak as well. Many of the fish that were collected had been anesthesized multiple times with carbon dioxide which has been observed to have negative effects on fish (Wagner 2002). This is especially true when fish are stressed out which is compounded by spawning season and capture (Bernier and Randall 1998). Last year carbon dioxide was not used and MS-222 was instead. When we compare our previous reconditioning effort in 2013, which was at 33% survival, versus 7% in 2014 there is a demonstrable effect. We have tried to convince Dworshak unsuccessfully so far, to utilize AQUI-S instead of MS-222 or carbon dioxide. This has had the unfortunate side effect of lowering our sample size numbers for our egg quality analysis.

<A>Genetic stock identification (GSI) to assign individual stock-of-origin and estimate stock proportions in a mixed sample of kelt steelhead sampled at Lower Granite Dam

Results of reference baseline power analyses indicated an overall low level of resolving power at the individual level. For most RGs the mean individual assignment probability was greater than 70%, while the percentages of correct self-assignments were low, indicating limited confidence in the accuracy of self-assignments. However, the accuracy of estimated kelt stock proportions was relatively robust among RGs (Figures 21 & 22). In other words, proportions based on the 80% threshold criteria were generally complementary to unconstrained assignment proportions (i.e. based on highest probability). Based on GSI analyses, kelt steelhead within the Snake River Basin exhibited demographic characteristics and life history variation consistent with previous descriptions for *O. mykiss* (Busby et al. 1996, Keefer et al. 2008, Narum et al. 2008). For example, rates of iteroparity (and presumably incidence of repeat migration) were higher among female kelt steelhead, and among regions supporting A-run steelhead stocks. The largest stock proportions were observed among the UPSALM, GRROND and IMNAHA groups respectively. The south and middle forks of the Clearwater River and the South Fork Salmon River are generally considered to support productivity of B-run steelhead. Individuals among B-run populations are typically larger owing to a protracted ocean growth period. However, they are observed to less likely follow an iteroparous life history trajectory. Note that in GSI analyses the kelts that assigned to B-run regions were significantly larger but the estimated stock proportions were relatively small (~16% of all kelts). Mean individual assignment probabilities reflected a high degree of confidence in identifying individuals from each of the B-run regions, indicative of highly differentiated stocks. Among the kelts representing in this data set, the proportion of fish rated “good” in overall condition was not significantly correlated with either fish size or outmigration time. At the Lower Granite Dam bypass, where entrance orifices are undersized (12"), there has been some speculation that size selectivity may be a factor contributing to poorer condition of kelts. It was thought that entrances may by affecting the incidence of head wounds on large fish, while selecting for smaller fish and excluding larger ones. The lack of an observed relationship between size and condition of kelts is encouraging for the reconditioning program targeting B-run steelhead since fish in better condition tend to survive reconditioning at higher rates (Hatch et al. 2013b).

Estimated stock proportions among hatchery-origin kelts were markedly different from their NOR counterpart. This is somewhat intuitive given that several RGs (e.g., MFSALM, SFSALM) have no hatchery operations or directed supplementation activities. However, the PBT results indicated a large contingent of HAT kelts originating from UPSALM hatcheries that assigned to other RGs in error. After adjusting estimated stock proportions based on PBT concordance the threshold estimates (80% probability) were highly complementary with proportions based on highest probability assignments (66% UPSALM vs. 68% UPSALM respectively; Figure 21). The cause/s for an elevated incidence of post-spawn survival between HAT steelhead (66%) from UPSALM vs. NOR steelhead (29%) from UPSALM is a demographic curiosity that deserves additional scrutiny. Hatchery-origin kelts sampled at LGD during post-spawn outmigrations are presumed to have spawned naturally (did not return to the hatchery). A decreased level of genetic distinction among several RG's for both HAT an NOR kelts may be reflective of this elevated incidence of straying, and/or the prolonged influence of stock transfers and outplanting, and these behaviors may be more prevalent among certain stocks.

<A>In River Release

The in-river release in 2014 was very much improved over the 2013 group for both locations. There are many reasons that this occurred throughout the basin, from good migration conditions in the river to good ocean conditions thus fish had good stores of fat with which to migrate, spawn, and then migrate to the ocean as kelts. Some clues point to good ocean conditions based on the large number of fish that returned as sequential spawners this year. It will be interesting to see what happens in 2015 if there is a large component of skip spawners which attempt to repeat spawn.

<A>Long-term Reconditioning

In 2014, survival of long-term reconditioned groups was 61% for Prosser and 30% for Dworshak (Figure 65). Survival for the Prosser fish was above average while Dworshak was lower than last year but still slightly above the project average (28%). Specific to the Fish Creek kelt group mortalities we observed, records were reviewed for water quality, feed schedule, ration rate, as well as, formalin delivery. In addition, several fish were sent to US Fish and Wildlfie fish health for necropsy examination. However, no singular causative agent or aquaculture practice was discovered to explain the high mortalities. Similar to 2013, little mortality was observed after the first week of August.

Previous years data along with this year's data indicates that steelhead kelts can be successfully reconditioned at both locations with sufficiently trained staff and adherence to reconditioning protocols established in Hatch et al 2013b. We think that reconditioning could have been closer to Prosser in 2014 but the main culturing expert at Dworshak missed work during the initial critical period, when kelts were being brought in, due to injury. We believe that there was a decline in the number of successful kelts that were produced from this location due to lack of familiarity with equipment by some of the field staff that focus on Lower Granite Dam instead of culturing at Dworshak. Additionally, kelts in the basin were down with decline in fish size,

which could have had a negative impact on fish health in the river. We have instituted important changes in protocols and staff allocation to insure that we are back on track for a better year in 2015 at Dworshak. We are also expecting a larger run than we had observed in 2014.

We calculated the benefits of long-term reconditioning based upon survival-to-release for the long-term treated fish. Fish reconditioned at Prosser Hatchery had a 9.2 times survival while Dworshak had a very large advantage to 103.8 times advantage over the 2014 return rate to Bonneville Dam for fish left in the river (Figure 66). When kelt releases are compared against the Hockersmith number the increase is much higher for Prosser at 36.5 and 18.6 for Dworshak. The Long-term reconditioning fares even better against the proportion of repeat spawners in the run at large at Bonneville Dam. The Prosser Hatchery reconditioned kelts had a 99.2 survival advantage and those from the Dworshak reconditioning had a 50.5 times advantage (Figure 66). Long-term reconditioning shows the greatest promise as a tool for restoration based on this data since we can bypass artificially created hydrosystem conditions and years with difficult ocean conditions for fish.

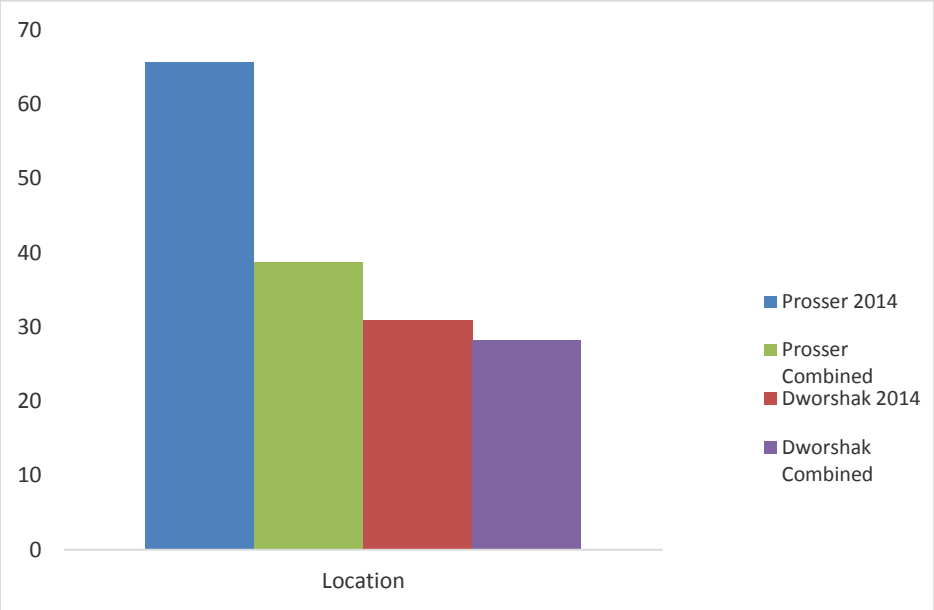


Figure 65. Survival rate (%) of long-term reconditioned kelt steelhead to release at 2 locations in 2014 and combined years.

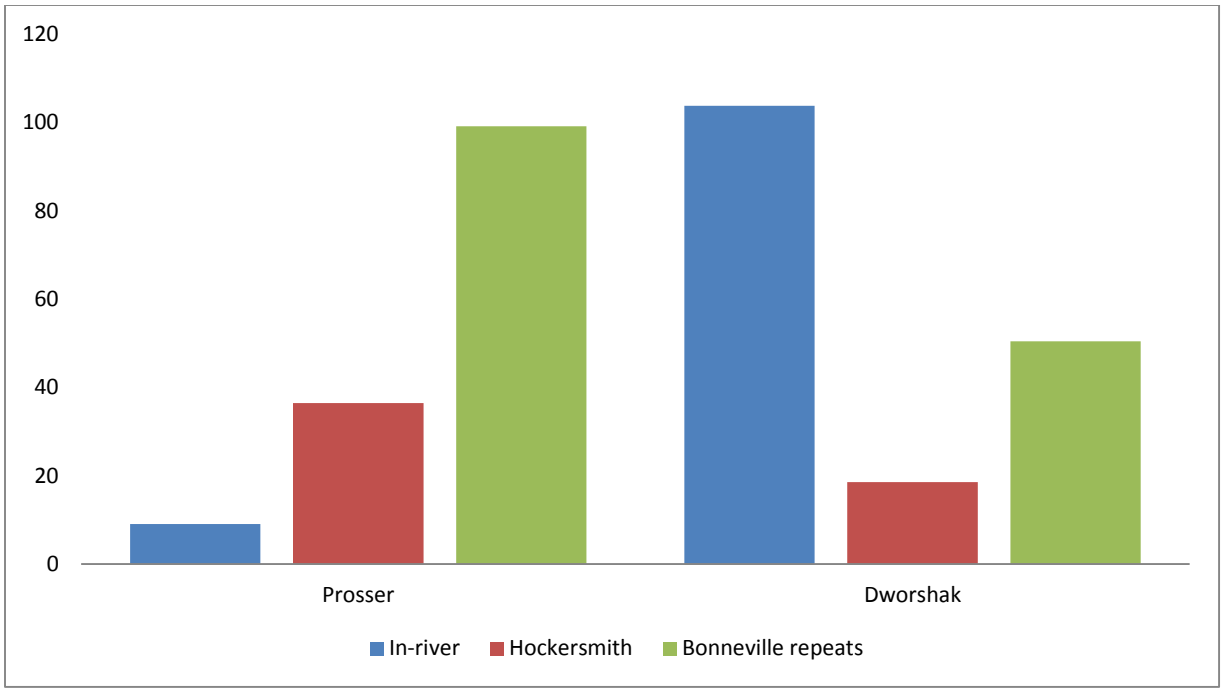


Figure 66: Long-term reconditioning benefits for 2014, calculated by dividing long-term survival rates by control group metrics.

<A>Kelt Reconditioning Physiology Studies

<B>Reproductive development in kelt steelhead

It is now well established that some female steelhead kelts remature after a summer of reconditioning, whereas other fish do not, and that plasma estradiol level from mid-August onward indicates maturation status. Evidence in both steelhead kelts and post-spawning rainbow trout suggests that the decision to remature is made early, before mid-July for kelts and during the 10 weeks after spawning in rainbow trout (Bromage, et al. 1992; Caldwell et al. 2013; Caldwell et al. 2014; Hatch, et al. 2013a). Plasma estradiol levels in rematuring and non-rematuring kelts for 2013 and 2014 at Prosser, Winthrop, and Dworshak were similar to previous years (Figs 34-36). Maturation rates at Prosser were in the range observed during previous years. Maturation rates at Prosser and Winthrop were comparable (2013 62.8% and 66.6%, respectively; 2014 46.0% and 53.4%, respectively). Maturation rates decreased from 2013 to 2014 at all three locations. This suggests that pre-capture environmental conditions common to the three projects may influence maturation rate. However, additional data is and analysis is required before conclusions can be drawn on this topic.

Maturation rates at Dworshak were lower than at the other two projects. There are a number of possible reasons for this difference. The Dworshak project reconditions both wild and hatchery origin B-run kelts, selected on the basis of body size. These fish spend two or more

winters in the ocean before their maiden spawning, indicating that initial maturation (puberty) is delayed relative to typical steelhead. It is possible that rematuration schedules are similarly delayed. Steelhead in the Skeena and Nass systems in British Columbia have a life history similar to Snake River B-run steelhead. A substantial proportion of natural repeat spawners was found in these systems, however, all of them spent at least a full year in the ocean, indicating that they all had a skip spawning life history (Moore, et al. 2014). In a previous report, only one of 25 Skeena female repeat spawners followed a consecutive spawning life history; 24 of 25 were skip spawners (Chudyk 1976). Thus, B-run steelhead may have an inherently low maturation rate as consecutive spawners. In addition, water temperatures at Dworshak are lower than at the other two projects, due to different water sources. Colder water temperatures may restrict growth and suppress rematuration. Finally, differences in feeding and disease treatment protocols may have resulted in less favorable growth conditions at Dworshak. Further investigation is required to examine these possibilities.

Among fish held at Dworshak, air spawned wild kelts from the South Fork of the Clearwater (2013) and air spawned hatchery origin kelts had the highest rematuration rates. This suggests that energy expenditure during spawning and post-spawning migration may suppress maturation. Obtaining kelts as close as possible to spawning is expected to result in increased maturation rates. Air spawned kelts from the South Fork of the Clearwater River localized broodstock program, and kelts collected from tributary weirs such as Fish Creek on the Lochsa River are highly desirable to achieve project goals.

In all three projects, a substantial number of female kelts were not rematuring at the time of release in the fall. These fish represent a major opportunity to increase the impact of kelt reconditioning projects, as illustrated by the 93% rematuration rate hatchery origin kelts held for an additional year at Dworshak. Skip spawning is a normal life history in Columbia River Basin steelhead (Keefer et al. 2008). Skip spawning Atlantic salmon contribute disproportionally to population productivity due to increased fecundity and egg size (Reid and Chaput 2012). Making adjustments to kelt reconditioning projects to allow skip spawners to contribute to target populations should be a high priority for action agencies and project managers. Rematuring kelts had consistently higher muscle lipid levels and condition factors than non-rematuring fish across years and sampling locations (Figs. 37, 38). This association has been found in every year since 2009 at Prosser (Branstetter, et al. 2010, 2011; Hatch, et al. 2012; Hatch, et al. 2013b). These findings suggest that there is a strong association between rematuration and energy reserves. Almost no kelts with muscle lipid levels below 2% at sampling points from mid-August onward were found to be rematuring. Almost all of the 2014 spawn year kelts reconditioned at Dworshak had muscle lipid levels below 2%, supporting the hypothesis that the low rematuration rates at this site in 2014 were at least partly due to insufficient nutrition. However, many non-rematuring kelts at Prosser and Winthrop had muscle lipid levels greater than 2%, and high condition factors. Thus, muscle lipid levels over 2% and high condition factors are necessary but not sufficient conditions for identifying rematuring kelts. This is probably because the decision to remature is made based on energetic and nutritional status early in reconditioning. Fish that do not have sufficient energy reserves to initiate rematuration during the critical window around spawning may feed enough afterward

to be in good condition in the fall.

Necropsy of mortalities after the 9/10/14 sampling at Prosser revealed that kelts with estradiol levels greater than 1000 pg/ml, indicating rematuration, had a GSI over 1%, with one exception (Fig. 39). Studies on rainbow trout show that the GSI increases to over 1% of body weight by 6 months prior to spawning (Bromage, et al. 1982; Tyler and Sumpter 1996; Tyler, et al. 1990). Therefore, these results validate the use of plasma estradiol levels as a screening tool for maturation status. In addition, a significant positive correlation was found between plasma estradiol level and GSI. Similar positive correlations have been found in previous years (data not shown). This indicates that, among rematuring fish, plasma estradiol level indicates not only maturation status but the size of the ovary, suggesting that plasma estradiol level may predict aspects of reproductive performance such as spawn timing, fecundity, or egg size. Further studies will be required to investigate these possibilities. The significant negative correlation between plasma estradiol level and GSI in non-rematuring fish is puzzling. It is possible that this is a spurious correlation. Alternatively, this correlation may be driven by reduced body weight in fish with low estradiol levels.

Blood sampling kelts and assaying estradiol levels is a great deal of work, and assays and data analysis must be competed in a short period of time to provide status of individual fish to project managers before fish are released. Therefore, we have begun exploring alternative methods for determining maturation status in kelts. In 2014, we tested judgment of maturation status by physical appearance (Fig. 40). There was a strong association between maturation status as determined by blood estradiol level, and maturation status by physical appearance, indicating that the two are related. However, judgment by physical appearance misclassified approximately 30% of fish. This is too high a misclassification percentage to use for sorting fish. Classification of fish by blood hormone level is still the best option. Alternatives such as ultrasound can be tested against blood hormone level in future studies.

Kelts that were rematuring at release arrived significantly earlier at Prosser in some years (Fig. 41). However, this relationship is not consistently found. Classification of 2014 spawn year fish by VSP segment based on PIT tag detections revealed that arrival date differed substantially between spawning tributaries (Fig. 42). Arrival time at Prosser appeared strongly related to spawn timing in the VSP tributaries. Steelhead spawning on the Yakima begins earliest in the lowest elevation tributaries, with higher elevation tributary fish spawning later (Frederiksen, et al. 2012; Hockersmith, et al. 1995). This suggests that the relationship between intake date and maturation status at release is probably confounded by the presence of multiple spawning populations with different timing in the kelt run at Prosser dam. Assignment of individual fish to a VSP population is an important next step in understanding the relationship between arrival date and maturation status of the fish after a summer of reconditioning. Analysis of the relationship of other factors at intake to maturation status at release is likely similarly confounded. Techniques such as genetic stock identification and PIT tag detections should be used to assign as many fish as possible to a subpopulation.

<B>Proteomic Analysis of Female Steelhead Plasma

The proteomic approach used here assigned MS spectra from peptide fragments to known proteins by searching against an existing protein database. Unfortunately, this approach resulted in peptide spectra matches for only 9.6% of the generated spectra. This is presumably due to lack of coverage of the rainbow trout plasma proteome in current protein databases. The remaining 90.4% of spectra could not be assigned to any known protein using the present approach, suggesting that a high percentage of proteins present in the plasma samples were not able to be identified. Of the 185 plasma proteins that were identified, none were detected as significantly different in abundance in intake plasma from female kelts that rematured as consecutive spawners versus fish that did not. However, given the low percentage of plasma proteins that were identified in our samples, and the sample size of the study (n=4), we cannot conclude that a plasma indicator is not present. Interestingly, a number of proteins associated with iron metabolism and oxygen transport (hemoglobins, serrotransferins), immune system function (complement proteins), and DNA packaging (histones) tended to be more abundant (not significant) in blood samples from fish which subsequently rematured. The rainbow trout genome has recently been sequenced (Berthelot, et al. 2014). Annotation of the rainbow trout genome will presumably result in the availability of a rainbow trout proteome at some point in the future. Analysis of data from this study by searching peptide spectra against a complete rainbow trout proteome could identify differentially abundant proteins. The possibility also remains that there is no biomarker of reproductive outcome at intake.

<A>Population Model

A model was developed for the purpose of examining population recovery from the perspective of the kelt reconditioning program. The model mimics iteroparity in ways explicit to body condition, reconditioning, and release method. We have shown that kelts contribute up to 10% of spawning if sufficient kelts are captured and reconditioned. This is obvious from the raw data on success and survival rates, but speculation on the relative benefit of capture and reconditioning has remained elusive. This modeling tool provides the means to examine several questions regarding potential avenues for recovery, and management options for doing so. The model was parameterized with constant rates obtained from tagging data, constant rates estimated from a Ricker spawner to smolt recruitment function model fitting, and constant survival rates obtained from fitting the population model to spawner returns. The rates being constant, the best fitting model would necessarily not fit all the data perfectly. Given that there was a systematic increase in spawning abundance during the period of time that the population model was used to fit the spawning abundance data, the best fit resulted in overestimation of spawners in the 1990's, and an underestimation of spawners between 2000 and 2013. As a result of the recent underestimation, the projected spawning abundance is potentially biased low. This could be for a number of reasons. First, it's possible that survival rate have been better than average in recent years. This seems like a likely scenario since empirical spawning numbers have increased despite smolt numbers being constant or declining since 2000. This is consistent with recent population increases in Columbia River salmonid populations, and points to improved mainstem or ocean survival conditions. If an argument can be made that model projections relying on the constant rates are underestimating spawning abundance in recent

years, and if the cause of this is that smolt to adult survival is higher than average currently, then it stands to reason that performance measures are conservative if based on this model parameterization. The obvious solution is to characterize the cause of the systematic change in survival so that the bias can be accounted for, but for the meantime it's reasonable to conclude that the benefits of kelt reconditioning are being underestimated since the returns rate is biased low.

We posit that variability in some key parameters such as Ricker productivity and smolt to adult survival can be explained by modeling the mechanisms by which survival varies with environmental variables. We built such a feature into the model for Ricker productivity, but were unable thus far to populate the environmental data fields, and as such Ricker productivity has been effectively treated as constant. Further development will take place in the area of environmental sensitivity parameterization for spawner to smolt productivity, as well as for survival rates from smolt to spawning.

The model remains in development, but efforts in 2014 have resulted in significant advancement of the quantitative validation of the model. Despite not having full age structure implemented, we have been able to demonstrate a useful comparison of production levels with simple alteration of a key parameter: the kelt capture rate. This same type of comparison can be implemented on other key variables, such as pre-spawn mortality, repeat kelt rate, sensitivity of Ricker production parameters to environmental variation, sensitivity of kelt survival to body condition, and sensitivity of body condition to environmental conditions and density.

We remain cautious about our results because the we are at a relatively early stage of statistical model validation, and we have not incorporated the full suite of empirical data that could be brought to bear on this analysis, but the model has shown promise that it can be used to evaluate kelt management strategies in conjunction with broader spawning and rearing mechanisms of production and survival.

<A>Reproductive Success of Artificially Reconditioned Kelt Steelhead

<B>Egg quality and reproductive parameters in hatchery origin maiden female steelhead and reconditioned kelts at Dworshak National Fish Hatchery

Survival and maturation of hatchery origin kelts was much lower in 2014 than 2013. The reason for this likely was the inclusion of more fish that were in poorer condition at intake. 2014 was a low run year for Clearwater steelhead. It was difficult to obtain fish for our studies due to concerns about the hatchery meeting its egg take requirements, and fish were obtained late in the season. Many of these fish had been sorted repeatedly using CO_2 anesthesia before they were air spawned. In addition, several problems with our formalin treatment system during the spring likely increased mortality. Maturation percentage as consecutive spawners for air-spawned hatchery origin kelts at DNFH has ranged from 80% (2012 spawn year, 4 of 5 fish), to 22% (2013 spawn year), to 6% (2014 spawn year). Both pre-capture and culture conditions likely play a role in determining maturation percentage. Culture conditions have been variable between years. For example, in 2012, fish were placed on effluent water due to a problem with the water line supplying the kelt tanks, resulting in high mortality. Even in the Prosser reconditioning project, where culture conditions are constant, rematuration rates range from 25% to 80%. More data is required before any conclusions can be drawn regarding typical survival and rematuration rates for hatchery origin kelts.

Encouragingly, the maturation rate for 2013 fish held for an additional year was high (93.1%). This suggests that depletion of energy reserves due to the demands of migration, ovarian development, and spawning suppresses rematuration in kelts. Consistent with this idea, muscle lipid levels in 2013 kelts that deferred spawning were higher than those of 2014 kelts at intake (Figs. C2.2, C2.8).

Plasma estradiol level indicated maturation status of female kelts by 8/9/13, indicating that reproductive trajectory is set before this date. This is similar to results from the kelt reconditioning project at Prosser, Washington, showing that reproductive trajectory is determined within the first few months after intake ((Branstetter et al. 2011; Hatch et al. 2013a; Hatch et al. 2012). Estradiol levels increased in both rematuring and non-rematuring females from 8/9/13 to 10/3/13. Estradiol level increases to a peak approximately 6 months before spawning in rainbow trout (Prat, et al. 1996; Tyler and Sumpter 1996; Tyler et al. 1990). The increase in non-rematuring fish may be due to the presence of a dummy cycle (Taylor, et al. 2008).

Muscle lipid levels were significantly higher in rematuring versus non-rematuring kelts in October, consistent with the association between high muscle lipid levels that and rematuration that has been found at Prosser (Hatch 2013, 2012). Levels increased in non-rematuring kelts and decreased in rematuring kelts as spawning time approached, resulting in significantly higher muscle lipid levels in non-rematuring kelts by February. The decrease in muscle lipid stores in rematuring fish is likely due to mobilization to support ovarian development. During exogenous vitellogenesis, which occurs during the final six months of ovarian development, stored lipids are mobilized and transported to the ovary, where they are

incorporated into the eggs (Lubzens, et al. 2010; Tyler and Sumpter 1996). Non-rematuring kelts increased lipid levels to much higher than kelts at intake in the spring, which may account for the much higher rematuration percentage of fish held for a second year.
The spawn timing of consecutive spawning reconditioned kelts was slightly earlier (1.5 weeks) than their maiden spawning, although this difference was not significant. Atlantic salmon repeat spawners have been found to ascend rivers earlier than maiden spawners (Niemela, et al. 2006b). Results of the present study suggest that spawn timing was not substantially altered by artificial reconditioning. Fecundity increased with length in both maidens and consecutive spawning kelts, as expected (Quinn 2005; Quinn et al. 2011). No difference in the length-fecundity relationship was detected between maidens and reconditioned kelts, however, the sample size for reconditioned kelts was low. The significantly greater fecundity and egg size of consecutive spawning kelts (1.23 and 1.19 fold maiden levels, respectively), suggests that reconditioned kelts have greater productivity than maidens. The greater fecundity of Atlantic salmon repeat spawners results in a disproportionate contribution to population productivity (Halttunen 2011; Moore, et al. 1995; Niemela, et al. 2006a). Alternate spawning reconditioned kelts are expected to have even higher fecundity than consecutive spawning fish. Fertilization success in consecutive repeat spawning reconditioned kelts was not significantly different from maiden spawners. Thus, there is no indication that artificial reconditioning decreases egg quality. Median fertilization success was 92% in maiden spawners and 96% in consecutive spawning reconditioned kelts. 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). No reconditioned kelts and few maiden fish were sub-fertile.

We attempted to identify factors measured at intake into reconditioning associated with reproductive trajectory. We expected size (length, weight or mass-length residuals), body condition (K), and/or muscle lipid levels to be significantly different at intake between fish that spawned and fish that deferred, based on a condition-dependent life history strategy. However, none of these factors differed significantly between spawning and deferring fish. Moreover, none of these factors were found to be significant predictors of maturity in multiple linear regression models. It is possible that this is a result of the coarse resolution of our measurements and our limited sample size. However, the possibilities also remain that 1) reproductive trajectory depends on aspects of physiological condition at intake that were not captured in our measurements, or 2) reproductive trajectory does not depend on condition at intake. Further study is required to investigate these possibilities.

In contrast to the lack of any detectable effect of factors measured at intake on reproductive trajectory, growth rate during reconditioning was elevated in rematuring fish from intake to August. This is before ovarian growth would be expected to substantially contribute to increases in weight. In a multiple linear regression model, specific growth rate from intake to August was the strongest predictor of maturation. Muscle lipid stores were also elevated in rematuring fish in October compared with non-rematuring fish. Elevated growth rates and increased late summer to fall muscle lipid levels have been consistently found in rematuring Prosser kelts. The consistent and strong association of growth rate and maturation suggests

that 1) increased growth rate stimulates maturation, and/or 2) maturation stimulates growth. Evidence exists for both of these possibilities. Growth rate has been found to greatly impact divergent maturation within populations of other salmonids, such as Chinook (Shearer, et al. 2006), and body growth has been found to influence oocyte development rate during the critical period for initiation of maturation in Coho (Campbell, et al. 2006a; Campbell et al. 2006b). In rainbow trout, elevations in growth rate and plasma levels of insulin-like growth factor-1 (Igf-1) were found in rematuring fish, and preceded increases in plasma levels of reproductive steroids (Taylor et al. 2008). Igf-1 is a growth stimulatory metabolic hormone (Picha, et al. 2008). On the other hand, reproductive steroids and other gonadal factors stimulate growth in fishes (Bhatta, et al. 2012).

<B>Yakima River Parentage Analysis

The largest number of parentage assignments to date were seen in the 2013 brood year analysis. This year's analysis also included pre-spawn maidens for the first time, helping to provide a total of 137 progeny assignments. Of these, at least 23 are attributed to a spawning event following successful reconditioning of a kelt. Two additional offspring assigned to a reconditioned kelt, but at longer (93 and 98mm) that could also be attributed to the first time spawning event for these fish.

The presence of progeny show that reconditioned kelts are able to successfully spawn in the wild. While relative reproductive success of pre-spawn maidens was greater than that of reconditioned kelts, any spawning by a reconditioned kelt is additive to the population and should be considered a success.

The vast majority of adults in this study had zero offspring assignments (figure 58), but this was not unexpected as the number of adults interrogated is a fraction of the anadromous population and does not account for the large resident component in the Yakima River Basin. We were able to assign 26.7% of the juveniles collected to at least 1 adult anadromous steelhead, indicating that we are sampling in areas where steelhead spawning has occurred. Increasing this percentage would be desirable, but may not be feasible given our sampling structure. However, 26.7% may be adequate for relative reproductive success purposes, and we are planning to increase the juvenile sample numbers in the future.

Future sampling will continue to focus on age-0 fish in areas that spawning was expected to have occurred. For 2013, at least one progeny was seen for all locations except Willy Dick Creek in the Toppenish drainage. If no progeny are detected for this site in 2014, it will likely be excluded in 2015. Sampling efforts in 2014 will include a component that targets areas that radio tagged kelts are suspected to have spawned in. Sampling efforts in 2015 will increase the number of samples taken from areas associated with anadromous sampling. Although we will still lack the ability to account for the resident population, and will still not genotype the majority of the anadromous adults, it is hoped that the increase in juvenile sampling will provide adequate data for statistical analysis.

<B>Tracking Repeat Spawners in the Yakima River Basin

Repeat spawner post release tracking

We observed that kelts followed six general migration patterns in chronological order of observation. I. No additional movement. This movement type would be analogous to a PIT-tagged adult return that was detected at McNary dam ladder but never made it to Prosser. II. Immediate Movement over Prosser Dam and then immediate movement towards a spawning tributary. This movement primarily occurred in the lower elevation Satus and Ahtanum Creeks. There were only 3 of these fish that exhibited this behavior. III. Immediate movement over Prosser Dam and then holding until spring at the Satus Bar area. IV. Immediant movement over Prosser Dam but no subsequent movement after the first 1-10 river kilometers. V. Active migration to the ocean. A group of fish (9) actively outmigrated to the ocean in the late fall of 2013 with another group (6) in the spring. VI. Lastly, holding below Prosser Dam and then migrating rapidly in the spring to a spawning tributary. This behavior was observed from the lower most (Satus)-to upper most end of the basin (Upper Yakima/Naches) although the sample size (3) was low for this group.

We assume that fish without migratory detection data, or that navigated the dam and then stopped migration, were mortalities that occurred directly following release. Causation is difficult to discern during the migration period for a number of reasons: (predation (human, avian, mammalian)), disease, stress related (tagging and handling), and environmental (successful river navigation of obstructions, high and low water events). It is possible that these fish regurgitated their tags and managed to bypass Prosser Dam or migrated downstream via spill. These scenarios are highly unlikely with the absence of any additional PIT-tag data. The other possibility is that the gastrically inserted tag was detrimental to their well being and resulted in higher than normal mortality (Corbett et al. 2012).

We also found that the rate of regurgitated tag rates were higher in the artificially reconditioned repeat spawners than the maiden kelts (pers. Comm. Chris Fredericksen). This is likely due to the artificially reconditioned repeat spawners not having the stomach atrophy that characterizes maiden fish. This is caused from a long migration period in the river with little to no food consumption.

We compared the repeat spawner movements against any previous historical detections that they may have had as maiden fish. We found that these patterns where consistent with previous years PIT-tag observations in 2012/13 for both groups of repeat spawners (radio and PIT tagged only). The sample size is small (12) but preliminary evidence would suggest that natal spawning fidelity is unaffected by artificial kelt reconditioning. The only major difference we found is that they did stay 16-49 longer in the natal spawning stream than in 2013. We believe that this is caused from environmental variability from differences in stream flow from year to year and with the additional possibility of variance in mate selection timing.

It was difficult to track fish in upper Toppenish Creek due to limited road access or trails. We suspected that there were also possible mainstem spawners but due to the lack of access (private property) it was difficult to observe exactly where these fish could be spawning.

Juvenile Collection

Juvenile collections went well for Satus, Ahtanum, and Cowiche likely due to the stability of flow in these systems and easy road access. These systems tended to warm up quickly, thus keeping emergence well on time. The progeny samples at Toppenish, Nile, and Bumping were smaller than we anticipated. It is possible that at least one repeat spawner in Toppenish was predated on by a bear as they were numerous and actively patrolling the road along the stream during peak spawning time. The other repeat spawner in Toppenish was detected in the upper reaches of Toppenish and access was limited. Also, staff time was spread throughout the basin focusing on the higher yield targets so we were not able to collect any information from this spawner. Nile Creek juvenile collections may have been low due to a large water event from a thunderstorm that dropped a large amount of precipitation in late July (pers. Comm Jeffery Trammell, Yakama Nation Biologist). This large flow event may have caused the dispersal of juvenile fish from the redd areas and pushed them into the mainstem Naches. The lowest yield for sampling, the Bumping Creek, may have had a similar situation as Nile Creek, but was man made from the summer water releases from the Bumping Lake Dam which provide irrigation water for the Yakima Valley during the warm dry time of the year. These outflows occur on a regular basis, and are quite voluminous and the water is extremely cool.

<A>Cle Elum Spawning Channel

The study plan was created and successfully endorsed by members of the Cle Elum technical committee which is comprised of Yakama Nation, Washington Department of Fish and Wildlife, the U.S. Fish and Wildlife Service. We have since processing fish in October retained 18 Naches (17 females/1 Male) and 18 Upper Yakima (13 females/ 5 males) kelts. These fish are currently being held at Prosser and will be trucked to the spawning channel in early Febuarary of 2015. We have coordinated with WDFW to obtain resident males that will be available for the spawning channel and enhancements to the spawning gravels within the channel have been made to better optimize them for steelhead spawning. The initial results of this feasibility study will be available in 2015 annual report. The decision will be made in late 2015 to expand the study or decline further research at this facility.

Adaptive Management & Lessons Learned

There are still some critical uncertainties associated with this project which we believe we are close to answering. In order to fully answer these questions we have many difficulties and much to consider.

In the Snake River Basin we are attempting to meet the goals of RPA 33 in the supplemental biop (NOAA 2014) even though we do not currently operate a production level facility we have been close to producing the number of fish designated in the RPA's. We are collecting

additional B-run kelts at areas such as Fish Creek and South Fork Clearwater River. With the much anticipated completion of the Snake River kelt master plan in 2015 by the Nez Perce Tribe the focus in the Snake will be on setting up a kelt reconditioning facility that will provide the Snake River Basin a consistent production of artificially reconditioned B and A- run repeat spawners .

Results from the genetic stock identification 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 steelhead forms, and will inform specific management of each with important implications for conservation. Interestingly, the upper Salmon River region produces a disproportionate number of Snake River kelt steelhead, and is presumably an important factor in spawner abundance for that region. This result is mirrored among hatchery-origin fish. Underlying biological and behavior factors contributing to such discrepancies are not well understood but likely warrant further investigation. With more data including escapement comparisons, it may be possible to refine the confidence in estimated rates of iteroparity among RG's. Hatchery-origin GSI results suggest this method may perform reasonably well for identifying proportions of kelts originating from specific hatchery programs. High relative rates of iterparity (kelt proportions) among UPSALM hatcheries should be monitored closely and underlying causative factors explored.

To get a better measure of kelt reproductive capabilities we have opted to discontinue utilizing the site at Omak Creek and instead focus on utilizing the Cle Elum spawning channel a semi-natural setting. We are testing the feasibility of the site this year to see if it is adequate for steelhead as it was previously used for spring Chinook to great success. If the site is feasible we propose expanding the project to observe maiden/repeat spawner and will consider resident interactions.

Providing assistance to post-spawn steelhead in the forms of feed, and prophylactic measures may increase the probability that individual steelhead repeat spawn and contribute to population growth. We feel that this approach can improve steelhead populations by increasing the number of female spawners in basins with listed populations. Not only will increased spawner production reduce extinction probability through increased productivity (Seamons and Quinn 2010) but they also act as important living genetic reservoirs (Narum 2008). Having a genetically diverse population can help act to as a buffer against manmade and natural stochastic events.

While we recognize that reconditioning may not work everywhere primarily due to following limiting factors such as flashy stream hydrology combined with poor capture capabilities and/or shortage of water, rearing capacity, or simply lack of a sufficient operating budget. We believe that it can be utilized as an important tool in the right locations. We base this belief on our current evidence that artificially reconditioned repeat spawners are contributing to ESA-listed

populations based on the parentage analysis results. To quantify exactly how much is a difficult proposition and remains to be determined, but the results of egg quality experiments at both Parkdale and Dworshak would suggest that they are doing so at rates that were similarly observed in Seamons and Quinn (2010). We have learned much about steelhead kelts since the inception of this program in 1999 and look forward to learn more from them and continue to work towards maintaining this important life history strategy of this species in the Columbia River Basin.

As mentioned earlier in this document we have provided 5 publications and 14 presentations that would be informative to management of kelts in the basin (please see Appendix A.2. for list of available resources). Work from this project and its publications has been instrumental in helping to develop the Snake River Kelt Management Plan (BPA & USACE 2014).

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Appendices

A.1: Data sets or products:

Raw Data:

Raw data files can be obtained with permission by contacting the Primary Investigator: Doug Hatch in writing to:

Doug Hatch Primary Investigator, Scientist
Fish Science Department
Columbia River Inter-Tribal Fish Commission
700 N.E. Multnomah St., Ste. 1200
Portland, OR 97232
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Appendix 1.a. Summary demographic statistics for NOR kelts sampled at Lower Granite Dam (2009-2012). Rows are organized by sample year and GSI reporting group. Gender is male (M) and female (F). "abs" is the difference in "outmigration" day between males and females. The observed physical condition (externally) was recorded as the proportion good (G), Fair, or poor (P).

		Assigned (n)			ordinal day			condition (F)			condition (M)			mean FL (cm)		
Year	reporting group	F	M	%F	F	M	abs.	G	Fair	P	G	Fair	P	F	M	all
2009	LSNAKE	14	4	0.78	145.6	158.3	12.7	0.50	0.43	0.07	0.25	0.75	0.00	635.0	606.3	628.6
	LOCLWR	15	3	0.83	146.9	165.7	18.8	0.93	0.00	0.07	1.00	0.00	0.00	628.7	621.7	627.5
	SFCLWR	4	1	0.80	120.5	155.0	34.5	0.75	0.00	0.25	1.00	0.00	0.00	800.0	610.0	762.0
	UPCLWR	9	0	1.00	150.2	---	---	0.56	0.33	0.11	---	---	---	744.4	---	744.4
	GRROND	37	9	0.80	154.4	161.0	6.6	0.86	0.14	0.00	0.22	0.44	0.33	617.2	585.6	611.0
	IMNAHA	23	1	0.96	153.1	147.0	-6.1	0.61	0.26	0.13	1.00	0.00	0.00	624.3	600.0	623.3
	LOSALM	19	4	0.83	155.7	165.0	9.3	0.53	0.37	0.11	0.75	0.00	0.25	635.0	571.3	623.9
	SFSALM	11	0	1.00	157.7	---	---	0.82	0.18	0.00	---	---	---	751.4	---	751.4
	MFSALM	45	4	0.92	157.6	163.8	6.2	0.82	0.13	0.04	0.75	0.00	0.25	637.8	608.8	635.4
	UPSALM	50	12	0.81	148.1	155.0	6.9	0.70	0.14	0.16	0.42	0.33	0.25	600.3	590.4	598.4
	total/mean	**227**	**38**	**0.86**	**152.0**	**159.4**	**7.4**	**0.73**	**0.19**	**0.08**	**0.50**	**0.29**	**0.21**	**636.4**	**594.1**	**630.3**
2010	LSNAKE	68	22	0.76	127.3	139.6	12.3	0.72	0.24	0.04	0.59	0.23	0.18	607.2	591.4	603.3
	LOCLWR	59	22	0.73	126.0	146.7	20.7	0.68	0.19	0.12	0.50	0.32	0.18	623.0	589.8	613.7
	SFCLWR	15	9	0.63	118.9	130.7	11.7	0.60	0.27	0.13	0.44	0.33	0.22	700.7	684.4	694.6
	UPCLWR	25	10	0.71	142.4	152.9	10.5	0.72	0.20	0.08	0.90	0.10	0.00	720.0	648.0	699.4
	GRROND	133	59	0.69	131.2	144.1	12.9	0.74	0.19	0.08	0.56	0.29	0.15	626.3	594.7	616.6
	IMNAHA	123	28	0.81	133.5	141.1	7.6	0.75	0.22	0.03	0.41	0.30	0.30	605.0	577.0	599.9
	LOSALM	34	20	0.63	137.3	155.5	18.1	0.74	0.21	0.06	0.50	0.30	0.20	612.1	614.5	613.0
	SFSALM	26	12	0.68	149.2	168.6	19.5	0.77	0.23	0.00	0.64	0.36	0.00	676.5	650.9	668.9
	MFSALM	75	46	0.62	153.7	162.1	8.4	0.85	0.15	0.00	0.67	0.24	0.09	644.4	627.2	637.9
	UPSALM	316	122	0.72	130.8	144.5	13.7	0.67	0.25	0.07	0.52	0.33	0.16	594.1	588.8	592.6

	total/mean	**874**	**350**	**0.71**	**133.5**	**147.6**	**14.1**	**0.72**	**0.22**	**0.06**	**0.55**	**0.29**	**0.16**	**616.4**	**601.8**	**612.2**
2011	LSNAKE	66	23	0.74	130.9	133.8	3.0	0.53	0.17	0.30	0.52	0.09	0.39	657.7	590.9	640.4
	LOCLWR	72	15	0.83	128.3	124.7	-3.6	0.64	0.11	0.25	0.47	0.20	0.33	658.2	643.3	655.6
	SFCLWR	26	5	0.84	122.3	121.2	-1.1	0.35	0.27	0.38	0.60	0.00	0.40	723.5	670.0	714.8
	UPCLWR	49	3	0.94	136.2	146.0	9.8	0.37	0.29	0.35	0.67	0.33	0.00	760.0	736.7	758.7
	GRROND	208	47	0.82	135.5	139.2	3.7	0.66	0.19	0.15	0.57	0.13	0.30	652.8	593.0	641.8
	IMNAHA	125	15	0.89	138.6	145.0	6.4	0.65	0.14	0.21	0.40	0.20	0.40	652.0	564.0	642.6
	LOSALM	40	14	0.74	143.3	139.4	-3.9	0.48	0.33	0.20	0.64	0.00	0.36	643.0	589.3	629.1
	SFSALM	39	4	0.91	148.6	124.3	-24.3	0.41	0.46	0.13	0.25	0.25	0.50	767.7	577.5	750.0
	MFSALM	115	13	0.90	154.6	147.2	-7.3	0.55	0.23	0.23	0.46	0.08	0.46	706.3	636.5	699.2
	UPSALM	189	45	0.81	132.1	144.8	12.7	0.67	0.15	0.19	0.60	0.11	0.29	652.1	599.2	641.9
	total/mean	**929**	**184**	**0.83**	**137.3**	**139.1**	**1.8**	**0.59**	**0.20**	**0.21**	**0.54**	**0.12**	**0.34**	**672.0**	**602.9**	**660.6**
2012	LSNAKE	80	41	0.66	118.7	123.9	5.3	0.33	0.45	0.23	0.10	0.51	0.39	647.9	591.5	628.8
	LOCLWR	73	46	0.61	120.8	121.3	0.5	0.38	0.38	0.23	0.11	0.50	0.39	663.2	595.2	636.9
	SFCLWR	29	8	0.78	109.8	122.0	12.2	0.28	0.62	0.10	0.25	0.50	0.25	738.3	683.8	726.5
	UPCLWR	33	10	0.77	129.2	131.8	2.6	0.27	0.42	0.30	0.10	0.70	0.20	735.5	679.0	722.3
	GRROND	208	78	0.73	123.5	129.3	5.7	0.33	0.46	0.21	0.14	0.37	0.49	636.6	590.1	623.9
	IMNAHA	96	18	0.84	131.3	137.9	6.6	0.33	0.41	0.26	0.33	0.44	0.22	646.0	584.4	636.3
	LOSALM	44	15	0.75	132.8	139.9	7.2	0.32	0.48	0.20	0.07	0.27	0.67	652.3	588.0	635.9
	SFSALM	27	1	0.96	143.7	115.0	-28.7	0.22	0.56	0.22	0.00	0.00	1.00	726.3	670.0	724.3
	MFSALM	77	27	0.74	145.1	149.1	4.0	0.30	0.48	0.22	0.11	0.33	0.56	683.5	615.2	665.8
	UPSALM	151	66	0.70	126.3	130.8	4.5	0.32	0.50	0.19	0.20	0.47	0.33	639.4	591.8	624.9
	total/mean	**818**	**310**	**0.73**	**127.2**	**130.3**	**3.1**	**0.32**	**0.46**	**0.22**	**0.15**	**0.44**	**0.41**	**657.5**	**598.7**	**641.3**
2013	LSNAKE	29	10	0.74	124.4	130.6	6.2	0.68	0.25	0.07	0.50	0.30	0.20	676.1	607.0	657.9
	LOCLWR	28	10	0.74	130.9	140.9	10.0	0.54	0.32	0.14	0.60	0.20	0.20	665.4	593.0	646.3
	SFCLWR	7	1	0.88	130.9	135.0	4.1	0.57	0.43	0.00	0.00	1.00	0.00	730.0	620.0	716.3
	UPCLWR	15	2	0.88	139.9	129.0	-10.9	0.53	0.27	0.20	0.50	0.00	0.50	758.0	610.0	740.6
	GRROND	91	17	0.84	129.7	135.1	5.3	0.53	0.24	0.23	0.47	0.47	0.06	655.2	597.6	646.1

	IMNAHA	36	15	0.71	134.0	140.4	6.4	0.53	0.33	0.14	0.27	0.53	0.20	656.4	584.7	635.3
	LOSALM	18	8	0.69	137.1	140.8	3.7	0.56	0.33	0.11	0.25	0.25	0.50	655.6	595.0	636.9
	SFSALM	8	2	0.80	140.1	149.0	8.9	0.75	0.13	0.13	0.00	0.00	1.00	765.0	615.0	735.0
	MFSALM	30	7	0.81	139.9	154.3	14.4	0.73	0.17	0.10	0.57	0.14	0.29	702.7	661.4	694.9
	UPSALM	73	31	0.70	132.3	134.5	2.2	0.63	0.19	0.18	0.39	0.29	0.32	642.5	597.7	629.1
	total/mean	**335**	**103**	**0.76**	**132.4**	**137.7**	**5.3**	**0.59**	**0.25**	**0.16**	**0.41**	**0.33**	**0.26**	**668.2**	**601.2**	**652.4**
overall	LSNAKE	257	100	0.72	126.2	131.7	5.5	0.53	0.30	0.17	0.35	0.34	0.31	642.0	593.5	628.3
	LOCLWR	247	96	0.72	127.0	131.1	4.1	0.58	0.23	0.19	0.33	0.36	0.30	650.5	602.1	636.9
	SFCLWR	81	24	0.77	117.9	127.0	9.1	0.41	0.40	0.20	0.42	0.33	0.25	728.9	675.4	716.7
	UPCLWR	131	25	0.84	137.0	141.7	4.7	0.44	0.31	0.25	0.52	0.36	0.12	744.9	668.0	732.6
	GRROND	677	210	0.76	131.2	137.5	6.3	0.57	0.28	0.16	0.39	0.30	0.31	641.0	592.5	629.5
	IMNAHA	403	77	0.84	135.7	141.1	5.3	0.59	0.25	0.16	0.37	0.36	0.28	635.0	578.0	626.0
	LOSALM	155	61	0.72	139.8	146.7	6.9	0.50	0.35	0.15	0.41	0.20	0.39	639.3	596.8	627.3
	SFSALM	111	19	0.85	147.8	153.6	5.8	0.51	0.38	0.11	0.44	0.28	0.28	734.5	631.7	720.1
	MFSALM	342	97	0.78	151.3	156.0	4.6	0.61	0.25	0.14	0.48	0.23	0.29	678.2	626.8	666.9
	UPSALM	779	276	0.74	131.5	140.6	**9.1**	0.60	0.26	0.14	0.43	0.32	0.24	621.9	592.3	614.1
	Grand Total/Mean	**3183**	**985**	**0.76**	**134.2**	**140.0**	**5.8**	**0.57**	**0.28**	**0.16**	**0.41**	**0.31**	**0.28**	**650.1**	**600.7**	**638.4**

Appendix 1.b. GSI Snake River reference baseline.

reporting group (RG)		reference population					sample size	
code	Subbasin	code	tributary	year	lat.	long.	adult	juv.
LSNAKE	Lower Snake R.	ALPW	Alpowa Cr.	2010	46.4076	-117.2198	98	
	Lower Snake R.	ASOW	Asotin Cr.	2008	46.3228	-117.1368	99	
	Lower Snake R.	GEORGE	George Cr.	2008	46.3228	-117.1368	95	
	Lower Snake R.	TUCAN	Lower Granite Dam	2010	46.6583	-117.4336	106	
LOCLWR	Lower Clearwater R.	BBER	Big Bear Cr.	2007	46.6336	-116.6552	99	
	Lower Clearwater R.	EFPOT	EF Potlatch R.	2008	46.7984	-116.4235	158	
	Lower Clearwater R.	LAPWAI	Lapwai Cr.	2013	46.3355	-116.6045		32
	Lower Clearwater R.	LBER	little Bear Cr.	2007	46.6336	-116.6552	151	
	Lower Clearwater R.	MISSION	Mission Cr.	2013	46.3355	-116.6045		60
	Lower Clearwater R.	SWEET	Sweetwater Cr.	2013	46.3355	-116.6045		50
	Lower Clearwater R.	WEBB	Webb Cr.	2013	46.3355	-116.6045		16
	Lower Clearwater R.	WFPOT	WF Potlatch R.	2009	46.8055	-116.4190	84	
SFCLWR	S. F. Clearwater R.	CLEARCR	Clear Cr.	2000	46.0486	-115.7817		45
	S. F. Clearwater R.	CROOKSF	Crooked R.	2007	45.8212	-115.5279	106	30
	S. F. Clearwater R.	LOLO	Lolo Cr.	2012	46.2905	-115.9342	9	85
	S. F. Clearwater R.	NEWSOME	Newsome Cr.	2012	45.8366	-115.6156		99
	S. F. Clearwater R.	TENMILE	Tenmile Cr.	2000	45.8053	-115.6818		47
UPCLWR	M. F. Clearwater R.	BEAR	Bear Cr.	2000	46.0191	-114.8381		70
	M. F. Clearwater R.	COLT	Colt Cr.	2000	46.4311	-114.5395		47
	M. F. Clearwater R.	CFLR	Crooked Fork Lochsa R.	2000	46.5250	-114.6777		44

	M. F. Clearwater R.	EFMOOSE	EF Moose Cr.	2012	46.1742	-114.8861		44
	M. F. Clearwater R.	FISHCLR	Fish Cr.	2010	46.3336	-115.3481	83	17
	M. F. Clearwater R.	GEDNEY	Gedney Cr.	2000	46.0583	-115.3141		45
	M. F. Clearwater R.	LAKECLR	Lake Cr.	2010	46.3336	-115.3481	17	30
	M. F. Clearwater R.	LtlCLR	Little Clearwater R.	2008	45.7534	-114.7749		59
	M. F. Clearwater R.	UPSEL	upper Selway R.	2008	45.7534	-114.7749		78
	M. F. Clearwater R.	WtCAP	White Cap Cr.	2008	45.7534	-114.7749		110
	M. F. Clearwater R.	NFMOOSE	NF Moose Cr.	2012	46.1740	-114.9001		94
	M. F. Clearwater R.	3LINK	Threelinks Cr.	2000	46.0481	-115.5169		81
	M. F. Clearwater R.	OHARA	O'Hara Cr.	2000	46.0770	-115.5168		85
	M. F. Clearwater R.	STORM	Storm Cr.	2000	46.4694	-114.5415		38
GRROND	Grande Ronde R.	CATH	Catherine Cr.	2011	45.2406	-117.9220	91	
	Grande Ronde R.	GRCROOK	Crooked Cr. Wenaha R.	2001	45.9775	-117.5548		97
	Grande Ronde R.	Wenaha	Wenaha R.	2001	45.9775	-117.5548		94
	Grande Ronde R.	Joseph	Elk Cr. Joseph Cr.	2000	45.7036	-117.1571	79	18
	Grande Ronde R.	UPGR	Grand Ronde R.	2009	45.4965	-117.9244	65	
	Grande Ronde R.	LtlMIN	Little Minam R.	2000	45.3997	-117.6731		48
	Grande Ronde R.	Wallowa	Lostine R.	2000	45.5500	-117.4886	72	45
	Grande Ronde R.	MENAT	Menatchee Cr.	1999	46.0111	-117.3664		73
IMNAHA	Imnaha R.	BIGSH	Big Sheep Cr.	2001	45.5494	-116.8442	14	77
	Imnaha R.	GUMBOOT	Gumboot Cr.	2011	45.1780	-116.8775	39	
	Imnaha R.	LIGHT	Lightning Cr.	2000	45.6556	-116.7263		39
	Imnaha R.	LtlSHEEP	Little Sheep Cr.	2011	45.4724	-116.9624	16	77
	Imnaha R.	MAHOG	Mahogany Cr.	2012	45.1780	-116.8775	14	
LOSALM	Lower Salmon R.	BOUL	Boulder Cr.	2000	45.2019	-116.3113		47
	Lower Salmon R.	RAPIDsal	Rapid R.	2000	45.3586	-116.3877	100	
	Lower Salmon R.	SLATE	Slate Cr.	2000	45.6396	-116.2732		75

SFSALM	S. F. Salmon R.	EFSF	EFSF Salmon R.	2000	45.0128	-115.7131	9	37
	S. F. Salmon R.	JOHNSON	Johnson Cr.	2010	44.9349	-115.4857		89
	S. F. Salmon R.	LAKE	Lake Cr.	2010	45.3465	-115.9457	7	43
	S. F. Salmon R.	LICK	Lick Cr.	2010	45.3465	-115.9457		63
	S. F. Salmon R.	SECESH	Secesh R.	2010	45.3465	-115.9457	4	91
	S. F. Salmon R.	SFSR	Lower Granite Dam	2010	46.6583	-117.4336	11	34
MFSALM	M. F. Salmon R.	BRVC	Bear Valley Cr.	2010	44.4146	-115.4672		81
	M. F. Salmon R.	BIGC	Big Cr.	2000	45.1509	-115.3015	40	184
	M. F. Salmon R.	CAMAS	Camas Cr.	2000	44.8918	-114.7222		97
	M. F. Salmon R.	CAPEH	Cape Horn Cr.	2009	44.3929	-115.1710		77
	M. F. Salmon R.	CHAMB	Chamberlain Cr.	2000	45.4523	-114.9310		95
	M. F. Salmon R.	WFCHAMB	W. F. Chamberlain Cr.	2011	45.4543	-114.9359		94
	M. F. Salmon R.	ELKMF	Elk Cr. MF Salmon R.	2010	44.4146	-115.4672		92
	M. F. Salmon R.	LOON	Loon Cr.	2000	44.5974	-114.8121		131
	M. F. Salmon R.	MARSH	Marsh Cr.	2009	44.4471	-115.2283		118
	M. F. Salmon R.	PISTOL	Pistol Cr.	2000	44.7214	-115.1545		58
	M. F. Salmon R.	RAPIDMF	Rapid R.	2000	44.7214	-115.1545		75
	M. F. Salmon R.	SULPHUR	Sulphur Cr.	2000	44.5451	-115.3070		94
UPSALM	Upper Salmon R.	HAYDEN	Hayden Cr.	2010	44.8616	-113.6319	7	79
	Upper Salmon R.	HERD	Herd Cr.	2010	44.1115	-114.2574		85
	Upper Salmon R.	MORG	Morgan Cr.	2000	44.6206	-114.1822		61
	Upper Salmon R.	NFSALM	NF Salmon R.	2010	45.4200	-113.9945	100	
	Upper Salmon R.	PAH	Pahsimeroi R.	2006	44.6801	-114.0353	97	
	Upper Salmon R.	SAW	Sawtooth Hatchery	2011	46.6583	-117.4336	63	45
	Upper Salmon R.	VALL	Valley Cr.	2010	46.6583	-117.4336	14	80
	Upper Salmon R.	WFYANK	W. F. Yankee Fork	2010	46.6583	-117.4336	41	76

Appendix 1.c. Mark, release, or observation sites for natural-origin kelts sampled at Lower Granite Dam and identified via PIT-tag. See Figure 9 for corresponding geographic locations in the Snake River Basin. Concordance between GSI assigned reporting group (GSI) and detection locations are shaded and bolded. PIT-tag detection type was mark & release (M/R), "release" or observation (Obs) following release at Lower Granite Dam. Detection site is identified by code and region corresponding to reporting groups. Interim is the time in months between tag detection (PTAGIS) and Lower Granite Dam kelt observation. For the interim period, a positive value indicates that the most recent tag detection preceded the kelt observation date, while a negative interim value indicates the kelt observation date preceded the most recent tag detection (detections representing return migrations are underlined).

			detection			kelt			
map ID	type	site code	year	ordinal day	region	year	ordinal day	interim (months)	GSI
21	M/R	COTP	2008	98	**GRROND**	2012	106	48.9	**GRROND**
23	M/R	JOSEPC	2012	141	**GRROND**	2012	144	0.1	**GRROND**
23	M/R	JOSEPC	2012	134	**GRROND**	2012	139	0.2	**GRROND**
23	M/R	JOSEPC	2012	132	**GRROND**	2012	135	0.1	**GRROND**
23	M/R	JOSEPC	2011	66	**GRROND**	2011	132	2.2	**GRROND**
23	M/R	JOSEPC	2012	33	**GRROND**	2012	101	2.3	**GRROND**
23	M/R	JOSEPC	2012	116	**GRROND**	2012	119	0.1	**GRROND**
23	M/R	JOSEPC	2012	122	**GRROND**	2012	126	0.1	**GRROND**
23	M/R	JOSEPC	2012	129	**GRROND**	2012	134	0.2	**GRROND**
23	M/R	JOSEPC	2012	126	**GRROND**	2012	130	0.1	**GRROND**
23	M/R	JOSEPC	2012	113	**GRROND**	2012	116	0.1	**GRROND**
23	M/R	JOSEPC	2012	114	**GRROND**	2012	119	0.2	**GRROND**
23	M/R	JOSEPC	2013	133	**GRROND**	2013	136	0.1	**GRROND**
23	M/R	JOSEPC	2013	74	GRROND	2013	103	1.0	UPSALM
23	M/R	JOSEPC	2012	121	GRROND	2012	125	0.1	LOCLWR
23	M/R	JOSEPC	2012	116	GRROND	2012	128	0.4	UPSALM
23	M/R	JOSEPC	2012	126	GRROND	2012	131	0.2	LSNAKE

23	M/R	JOSEPC	2013	90	GRROND	2013	106	0.5	UPSALM
23	M/R	JOSEPC	2012	129	GRROND	2012	133	0.1	LOCLWR
23	M/R	JOSEPC	2012	126	GRROND	2012	131	0.2	LSNAKE
23	M/R	JOSEPC	2012	124	GRROND	2012	128	0.1	IMNAHA
23	M/R	JOSEPC	2012	94	GRROND	2012	111	0.6	IMNAHA
23	M/R	JOSEPC	2012	112	GRROND	2012	116	0.1	LOCLWR
23	M/R	JOSEPC	2013	89	GRROND	2013	116	0.9	LSNAKE
28	M/R	BSHEEC	2008	99	**IMNAHA**	2010	133	25.5	**IMNAHA**
28	M/R	BSHEEC	2008	99	IMNAHA	2010	112	24.8	UPSALM
31	M/R	IMNTRP	2007	136	**IMNAHA**	2009	148	24.7	**IMNAHA**
31	M/R	IMNTRP	2007	131	**IMNAHA**	2010	118	36.1	**IMNAHA**
8	M/R	CLWTRP	2010	126	**LOCLWR**	2012	130	24.5	**LOCLWR**
11	M/R	LBEARC	2012	118	LOCLWR	2012	123	0.2	LSNAKE
13	M/R	SNKTRP	2008	108	LOCLWR	2010	141	25.4	UPSALM
13	M/R	SNKTRP	2008	115	LOCLWR	2010	136	25.0	LOSALM
4	M/R	ASOTIC	2010	107	**LSNAKE**	2010	113	0.2	**LSNAKE**
4	M/R	ASOTIC	2012	106	**LSNAKE**	2012	114	0.3	**LSNAKE**
4	M/R	ASOTIC	2012	72	**LSNAKE**	2012	115	1.4	**LSNAKE**
4	M/R	ASOTIC	2010	159	LSNAKE	2010	165	0.2	GRROND
4	M/R	ASOTIC	2013	88	LSNAKE	2013	123	1.2	GRROND
4	M/R	ASOTIC	2013	72	LSNAKE	2013	151	2.6	GRROND
18	M/R	FISTRP	2010	170	**UPCLWR**	2010	174	0.1	**UPCLWR**
18	M/R	FISTRP	2013	148	**UPCLWR**	2013	154	0.2	**UPCLWR**
41	M/R	KENYC	2012	112	**UPSALM**	2012	176	2.1	**UPSALM**
43	M/R	LSALR	2008	92	**UPSALM**	2010	161	26.6	**UPSALM**
43	M/R	LSALR	2008	92	**UPSALM**	2010	124	25.4	**UPSALM**
43	M/R	LSALR	2008	92	**UPSALM**	2010	127	25.5	**UPSALM**
43	M/R	LSALR	2008	92	**UPSALM**	2010	124	25.4	**UPSALM**
44	M/R	SALEFT	2008	115	**UPSALM**	2010	140	25.2	**UPSALM**
47	M/R	WIMPYC	2012	116	**UPSALM**	2012	137	0.7	**UPSALM**
48	M/R	YANKFK	2008	132	**UPSALM**	2010	128	24.2	**UPSALM**

48	M/R	YANKFK	2008	132	**UPSALM**	2010	143	24.7	**UPSALM**
48	M/R	YANKFK	2008	120	**UPSALM**	2010	159	25.6	**UPSALM**
19	Obs	BCANF	2012	107	GRROND	2012	142	1.2	LSNAKE
20	Obs	CATHEW	2013	114	**GRROND**	2013	135	0.7	**GRROND**
22	Obs	JOC	2012	101	**GRROND**	2012	113	0.4	**GRROND**
22	Obs	JOC	2013	146	**GRROND**	2013	150	0.1	**GRROND**
22	Obs	JOC	2012	134	GRROND	2012	137	0.1	LOCLWR
22	Obs	JOC	2012	95	GRROND	2012	99	0.1	LOCLWR
22	Obs	JOC	2012	54	GRROND	2012	114	2.0	MFSALM
22	Obs	JOC	2012	74	GRROND	2012	113	1.3	LSNAKE
22	Obs	JOC	2012	126	GRROND	2012	130	0.1	IMNAHA
22	Obs	JOC	2014	68	GRROND	2012	114	-10.6	UPSALM
23	Obs	JOSEPC	2012	104	GRROND	2012	115	0.4	LSNAKE
23	Obs	JOSEPC	2012	133	GRROND	2012	139	0.2	LOCLWR
23	Obs	JOSEPC	2013	88	GRROND	2013	101	0.4	LSNAKE
24	Obs	LOOKGC	2011	167	GRROND	2011	174	0.2	LOSALM
24	Obs	LOOKGC	2011	167	GRROND	2011	170	0.1	IMNAHA
25	Obs	LOSTIW	2012	152	**GRROND**	2012	174	0.7	**GRROND**
25	Obs	LOSTIW	2011	128	**GRROND**	2009	166	-23.1	**GRROND**
26	Obs	UGR	2013	66	**GRROND**	2013	131	2.2	**GRROND**
27	Obs	BSC	2011	94	**IMNAHA**	2011	160	2.2	**IMNAHA**
27	Obs	BSC	2012	102	**IMNAHA**	2012	131	1.0	**IMNAHA**
27	Obs	BSC	2012	143	**IMNAHA**	2012	150	0.2	**IMNAHA**
27	Obs	BSC	2012	111	**IMNAHA**	2012	137	0.9	**IMNAHA**
27	Obs	BSC	2012	101	**IMNAHA**	2012	115	0.5	**IMNAHA**
27	Obs	BSC	2012	90	**IMNAHA**	2012	136	1.5	**IMNAHA**
27	Obs	BSC	2013	129	**IMNAHA**	2013	135	0.2	**IMNAHA**
27	Obs	BSC	2014	117	**IMNAHA**	2012	144	-11.3	**IMNAHA**
27	Obs	BSC	2013	119	IMNAHA	2013	197	2.6	LOCLWR
29	Obs	COC	2012	103	IMNAHA	2012	116	0.4	GRROND

29	Obs	COC	2012	124	IMNAHA	2012	140	0.5	LOSALM
30	Obs	HORS3C	2013	140	**IMNAHA**	2013	146	0.2	**IMNAHA**
32	Obs	IR1	2011	101	**IMNAHA**	2011	162	2.0	**IMNAHA**
32	Obs	IR1	2011	130	IMNAHA	2011	159	1.0	LOCLWR
32	Obs	IR1	2012	70	IMNAHA	2012	122	1.7	LOCLWR
32	Obs	IR1	2011	126	IMNAHA	2009	159	-23.2	MFSALM
33	Obs	IR2	2012	85	IMNAHA	2012	115	1.0	GRROND
33	Obs	IR2	2012	75	IMNAHA	2012	114	1.3	LSNAKE
34	Obs	IR3	2011	130	**IMNAHA**	2011	155	0.8	**IMNAHA**
34	Obs	IR3	2012	89	**IMNAHA**	2012	133	1.5	**IMNAHA**
34	Obs	IR3	2013	119	IMNAHA	2013	137	0.6	LOSALM
34	Obs	IR3	2013	119	IMNAHA	2013	145	0.9	LOCLWR
34	Obs	IR3	2013	116	IMNAHA	2013	145	1.0	UPSALM
34	Obs	IR3	2013	115	IMNAHA	2011	125	-24.0	UPSALM
35	Obs	LSHEEF	2012	110	**IMNAHA**	2012	138	0.9	**IMNAHA**
9	Obs	JUL	2012	74	LOCLWR	2012	105	1.0	UPSALM
10	Obs	LAP	2010	101	LOCLWR	2010	108	0.2	GRROND
10	Obs	LAP	2010	103	LOCLWR	2010	108	0.2	LSNAKE
12	Obs	MIS	2012	92	LOCLWR	2012	114	0.7	LSNAKE
14	Obs	SWT	2012	115	LOCLWR	2012	122	0.2	UPSALM
1	Obs	ACB	2012	76	LSNAKE	2012	103	0.9	GRROND
2	Obs	ACM	2012	75	**LSNAKE**	2012	144	2.3	**LSNAKE**
2	Obs	ACM	2012	75	LSNAKE	2012	96	0.7	LOSALM
3	Obs	AFC	2012	122	LSNAKE	2012	126	0.1	UPSALM
5	Obs	GEORGC	2012	65	**LSNAKE**	2012	116	1.7	**LSNAKE**
5	Obs	GEORGC	2013	88	LSNAKE	2013	103	0.5	GRROND
6	Obs	LTR	2010	115	LSNAKE	2010	113	-0.1	MFSALM
6	Obs	LTR	2010	122	LSNAKE	2010	119	-0.1	LOCLWR
7	Obs	UTR	2012	113	LSNAKE	2012	98	-0.5	LOCLWR
39	Obs	TAY	2010	107	**MFSALM**	2010	154	1.6	**MFSALM**
39	Obs	TAY	2010	149	**MFSALM**	2010	157	0.3	**MFSALM**

39	Obs	TAY	2011	124	**MFSALM**	2011	167	1.4	**MFSALM**
39	Obs	TAY	2012	104	**MFSALM**	2012	139	1.2	**MFSALM**
39	Obs	TAY	2012	107	**MFSALM**	2010	160	-22.6	**MFSALM**
39	Obs	TAY	2013	112	MFSALM	2012	148	-11.0	SFSALM
15	Obs	LC2	2013	85	SFCLWR	2013	129	1.5	LOCLWR
16	Obs	SC1	2012	106	**SFCLWR**	2012	100	-0.2	**SFCLWR**
16	Obs	SC1	2013	74	**SFCLWR**	2013	141	2.2	**SFCLWR**
17	Obs	SC2	2012	77	SFCLWR	2012	125	1.6	UPCLWR
17	Obs	SC2	2012	103	SFCLWR	2012	141	1.3	UPCLWR
36	Obs	KRS	2011	102	**SFSALM**	2011	132	1.0	**SFSALM**
36	Obs	KRS	2012	105	**SFSALM**	2012	138	1.1	**SFSALM**
36	Obs	KRS	2010	109	SFSALM	2010	133	0.8	MFSALM
37	Obs	SFG	2012	109	**SFSALM**	2012	154	1.5	**SFSALM**
38	Obs	ZEN	2010	117	**SFSALM**	2010	156	1.3	**SFSALM**
40	Obs	ESS	2010	122	UPSALM	2010	165	1.4	SFSALM
40	Obs	ESS	2011	114	UPSALM	2011	160	1.5	SFSALM
40	Obs	ESS	2011	121	UPSALM	2011	150	1.0	MFSALM
40	Obs	ESS	2013	118	UPSALM	2013	130	0.4	LSNAKE
40	Obs	ESS	2013	117	UPSALM	2013	138	0.7	IMNAHA
40	Obs	ESS	2013	110	UPSALM	2013	137	0.9	SFSALM
42	Obs	LLR	2010	116	**UPSALM**	2010	162	1.5	**UPSALM**
45	Obs	STL	2012	111	UPSALM	2012	154	1.4	GRROND
46	Obs	USE	2013	88	**UPSALM**	2013	134	1.5	**UPSALM**
46	Obs	USE	2013	112	**UPSALM**	2013	141	1.0	**UPSALM**
49	Obs	YFK	2012	115	**UPSALM**	2012	135	0.7	**UPSALM**
49	Obs	YFK	2012	115	UPSALM	2012	121	0.2	IMNAHA
22	release	COLR3	2012	250	GRROND	2013	116	7.7	LOSALM
22	release	JOSEPC	2012	72	**GRROND**	2012	138	2.2	**GRROND**
22	release	JOSEPC	2012	66	**GRROND**	2012	98	1.1	**GRROND**
22	release	JOSEPC	2012	58	**GRROND**	2012	137	2.6	**GRROND**
22	release	JOSEPC	2013	126	**GRROND**	2013	130	0.1	**GRROND**

23	release	JOSEPC	2013	76	**GRROND**	2013	131	1.8	**GRROND**
23	release	JOSEPC	2013	74	**GRROND**	2013	127	1.8	**GRROND**
23	release	JOSEPC	2012	28	**GRROND**	2012	123	3.2	**GRROND**
23	release	JOSEPC	2013	86	**GRROND**	2013	113	0.9	**GRROND**
23	release	JOSEPC	2012	81	**GRROND**	2012	139	1.9	**GRROND**
23	release	JOSEPC	2013	75	**GRROND**	2013	115	1.3	**GRROND**
23	release	JOSEPC	2013	89	**GRROND**	2013	120	1.0	**GRROND**
23	release	JOSEPC	2013	90	GRROND	2013	134	1.5	LSNAKE
23	release	JOSEPC	2013	89	GRROND	2013	120	1.0	LSNAKE
23	release	JOSEPC	2013	89	GRROND	2013	129	1.3	LOCLWR
23	release	JOSEPC	2012	98	GRROND	2012	129	1.0	UPSALM
23	release	JOSEPC	2013	88	GRROND	2013	119	1.0	MFSALM
23	release	JOSEPC	2013	48	GRROND	2013	128	2.7	UPSALM
23	release	JOSEPC	2013	89	GRROND	2013	118	1.0	LOCLWR
23	release	JOSEPC	2013	46	GRROND	2013	106	2.0	UPSALM
23	release	JOSEPC	2013	74	GRROND	2013	115	1.4	MFSALM
33	release	BONN	2011	214	**IMNAHA**	2012	131	9.4	**IMNAHA**
34	release	BONN	2011	200	IMNAHA	2012	146	10.4	GRROND
30	release	BSHEEC	2008	99	**IMNAHA**	2010	131	25.4	**IMNAHA**
30	release	HORS3C	2012	111	IMNAHA	2012	155	1.5	LOCLWR
30	release	HORS3C	2012	114	IMNAHA	2012	149	1.2	UPSALM
9	release	LBEARC	2010	83	**LOCLWR**	2010	111	0.9	**LOCLWR**
3	release	ASOTIC	2012	111	LSNAKE	2012	127	0.5	GRROND
4	release	ASOTIC	2013	62	LSNAKE	2013	106	1.5	LOCLWR
18	release	FISTRP	2008	255	**UPCLWR**	2012	155	45.3	**UPCLWR**
42	release	BONN	2012	243	UPSALM	2013	117	8.0	LOSALM
42	release	PRD	2013	134	**UPSALM**	2013	139	0.2	**UPSALM**
42	release	PRD	2013	67	**UPSALM**	2013	137	2.3	**UPSALM**

Appendix 1.d. Summary of hatchery-origin kelt PIT-tag detections. Results indicate concordance between PBT and GSI methods for only those kelts that were also identified via PIT-tag at Lower Granite Dam. PIT-tag detection type was either mark or release (M/R), or observation (Obs; including recaptures). A single mortality (mort) was also observed in association with PIT-tag detections. Detections site is identified by code and region corresponding to reporting groups. Interim is the time in months between last known detection (PTAGIS) and Lower Granite Dam kelt observation. Concordance results between PBT and GSI are bolded, and concordance between each assignment method and PIT-tag information is shaded and boxed.

PIT-tag detection						PBT		GSI	
type		*site code	region	date	interim	broodstock	region	RG	*p*
M/R	‡	LSALR	LOSALM	4/8/2009	36	---	---	GRRONDD	65.4
Obs		ACB	LSNAKE	4/16/2012	8	---	---	GRRONDD	77.2
M/R		JOSEPC	GRROND	5/6/2013	0	---	---	IMNAHA	42.6
Obs		SFG	SFSALM	5/12/2010	8	---	---	IMNAHA	80.2
Obs		JUL	LOCLWR	4/21/2010	6	---	---	SFCLWR	94.9
M/R	‡	LSALR	LOSALM	3/31/2008	22	---	---	UPSALM	89.6
Mort		ASOTIC	LSNAKE	4/18/2012	7	---	---	UPSALM	42.4
M/R	†	LSALR	LOSALM	4/11/2011	25	PAHH10S	***UPSALM***	***UPSALM***	81.0
M/R	†	LSALR	LOSALM	4/9/2010	25	PAHH09S	***UPSALM***	***UPSALM***	76.7
M/R		SNAKE2	LSNAKE	10/6/2011	3	PAHH08S	***UPSALM***	***UPSALM***	74.6
Obs		JOC	GRROND	3/15/2012	7	---	---	GRRONDD	22.6
M/R		JOSEPC	GRROND	4/18/2012	0	---	---	GRRONDD	73.6
Obs		LSHEEF	IMNAHA	3/23/2012	7	---	---	IMNAHA	97.3
Obs		LBEARC	LBEARC	3/31/2010	1	---	---	LOCLWR	45.1
Obs		ASOTIC	LSNAKE	4/19/2010	1	---	---	LSNAKE	26.5
Obs		TAY	MFSALM	4/21/2010	7	---	---	MFSALM	96.3
Obs		SC2	SFCLWR	3/12/2013	6	DWOR09S	***DWOR***	***SFCLWR***	96.7
Obs		SC1	SFCLWR	3/23/2012	6	DWOR09S	***DWOR***	***SFCLWR***	92.4
M/R		GRNTRP	GRROND	4/22/2010	24	WALL0	***GRRO***	***GRRO***	95

M/R	†	SALR3	UPSALM	4/5/2011	25	9S	***ND***	***ND***	.3
						PAHH1	***UPSA***	***UPSA***	90
						0S	***LM***	***LM***	.7
Obs		YFK	UPSALM	4/20/2013	7	SAWT	***UPSA***	***UPSA***	40
						10S	***LM***	***LM***	.2
M/R	‡	SAWT	UPSALM	4/25/2011	25	SAWT	***UPSA***	***UPSA***	76
						10S	***LM***	***LM***	.7
Obs		YFK	UPSALM	4/19/2013	8	SAWT	***UPSA***	***UPSA***	98
						09S	***LM***	***LM***	.1
Obs	‡	USI	UPSALM	4/12/2013	24	SAWT	***UPSA***	***UPSA***	76
						10S	***LM***	***LM***	.4
Obs		USE	UPSALM	3/27/2013	6	EFSW	***UPSA***	***UPSA***	87
						09S	***LM***	***LM***	.4
Obs	†	SALR4	UPSALM	4/25/2011	23	SAWT	***UPSA***	***UPSA***	93
						10S	***LM***	***LM***	.3
Obs		USE	UPSALM	3/23/2013	7	PAHH1	***UPSA***	***UPSA***	78
						0S	***LM***	***LM***	.7
M/R	†	SALR4	UPSALM	4/14/2009	36	SAWT	***UPSA***	***UPSA***	97
						08S	***LM***	***LM***	.0
M/R		SNAKE4	UPSALM	3/29/2010	25	OXBO	***UPSA***	***UPSA***	33
						09S	***LM***	***LM***	.3
M/R	†	SALR4	UPSALM	4/27/2010	25	SAWT	***UPSA***	***UPSA***	99
						09S	***LM***	***LM***	.7
Obs		VC2	UPSALM	4/18/2012	7	SAWT	***UPSA***	***UPSA***	90
						08S	***LM***	***LM***	.3
M/R		SNAKE4	UPSALM	3/24/2010	26	OXBO	***UPSA***	***UPSA***	97
						09S	***LM***	***LM***	.9
M/R	‡	SALREF	UPSALM	5/3/2010	24	EFSW	***UPSA***	***UPSA***	53
						09S	***LM***	***LM***	.1
M/R	†	SALR3	UPSALM	4/7/2010	25	PAHH0	***UPSA***	***UPSA***	80
						9S	***LM***	***LM***	.5
Obs		YFK	UPSALM	4/19/2012	7	SAWT	***UPSA***	***UPSA***	94
						09S	***LM***	***LM***	.0
M/R	†	SALR4	UPSALM	4/27/2010	24	SAWT	***UPSA***	***UPSA***	99
						09S	***LM***	***LM***	.5
Obs		USE	UPSALM	4/21/2013	7	SAWT	UPSA	LSNA	45
						10S	LM	KE	.4
M/R	‡	SALEFT	UPSALM	5/3/2011	24	EFSW	UPSA	GRRO	41
						10S	LM	ND	.9
Obs		YFK	UPSALM	4/4/2013	7	SAWT	UPSA	LOCL	31
						09S	LM	WR	.0
M/R	‡	SALREF	UPSALM	5/3/2010	23	EFSW	UPSA	LOCL	71
						09S	LM	WR	.5
Obs		YFK	UPSALM	4/25/2012	7	SAWT	UPSA	GRRO	54
						09S	LM	ND	.3
Obs		SALEFT	UPSALM	4/25/2012	7	EFSW	UPSA	LOCL	90
						08S	LM	WR	.8
M/R		PAHTRP	UPSALM	4/21/2010	25	PAHH0	UPSA	GRRO	58
						9S	LM	ND	.3
M/R		PAHTRP	UPSALM	4/26/2010	26	PAHH0	UPSA	LSNA	46
						9S	LM	KE	.5

‡ - marked at HAGE; † - marked at MAVA (upper Snake River near Twin Falls Idaho)

***ACB** - Asotin Cr. at Cloverland Brdg.; **ASOTIC** - Asotin Cr., Snake R. Clarkston; BONAFF – Bonneville Dam - Adult Fish Facility; **CLWH** - Clearwater HAT; **COLR3** - Lewis R. to Bonneville Dam (km 140-234); **GRNTRP** - Grande Ronde R. Trap; **HAGE** - Hagerman NFH; **JOC** - Joseph Cr. ISA @ km 3; **JOSEPC** - Joseph Cr., Grande Ronde R.; **JUL** - Potlatch R. near Juliaetta; **KHS** - Big Bear Cr. @ Kendrick HS; **LBEARC** - Little Bear Cr., Potlatch R. ; **LGR** - Lower Granite Dam; **LGRLDR** - LGR - Adult Fish Ladder; **LGRRBR** - LGR - Release and Barge; **LGRTAL** - LGR - Release into Tailrace; **LMJ** - Lower Monumental Dam; **LSALR** - Little Salmon R.; **LSHEEF** - Little Sheep Facility; **MAVA** - Magic Valley HAT; **MCJ** - McNary Dam; **NISP** - Niagara Springs HAT; **PAHTRP** - Pahsimeroi R. Trap; **SALEFT** - East Fork Salmon R. Trap; **SALR3** - Salmon R. (km 319-489); **SALR4** - Salmon R. (km 489-650); **SALREF** - East Fork Salmon R.; **SAWT** - Sawtooth HAT; **SC1** - Lower SF Clearwater R at RK 1; **SC2** - Lower SF Clearwater R at RK 2; **SFG** - SF Salmon, Guard Station Br.; **SNAKE2** - Palouse R. to Clearwater R. (km 96-224); **SNAKE4** - Salmon R. to Hells Canyon Dam (km 303-397); **TAY** - Big Cr. at Taylor Ranch; **USE** - Upper Salmon R. at RK 437; **USI** - Upper Salmon R. (RK 460); **VC2** - Valley Cr., Downstream Site; **YFK** - Yankee Fork Salmon R.*

Appendix 1.e.: Proteins identified in intake plasma samples by proteomic analysis. No proteins were identified as significantly different in abundance between fish that later rematured and fish that did not.

Relative Abundance (log2 rematuring/non-rematuring)	Protein Hit
-2.40275917	Vitellogenin OS=Oncorhynchus mykiss GN=vtg1 PE=1 SV=1
-2.398207425	Vitellogenin-1 OS=Fundulus heteroclitus GN=vtg1 PE=1 SV=2
-1.984471894	Protein ssnA OS=Escherichia coli (strain K12) GN=ssnA PE=1 SV=2
-1.609181381	Parvalbumin beta 1 OS=Salmo salar PE=1 SV=1
-1.545531208	Parvalbumin beta 2 OS=Salmo salar PE=1 SV=3
-1.523909747	Diaminopimelate decarboxylase OS=Buchnera aphidicola subsp. Schizaphis graminum GN=lysA PE=3 SV=1
-1.347121019	Triosephosphate isomerase OS=Schistosoma japonicum GN=TPI PE=2 SV=1
-1.310990523	Triosephosphate isomerase OS=Sus scrofa GN=TPI1 PE=2 SV=4
-1.270848245	Mitogen-activated protein kinase kinase kinase 13 OS=Pongo abelii GN=MAP3K13 PE=2 SV=1
-1.268105395	Fibrinogen beta chain (Fragment) OS=Gallus gallus GN=FGB PE=1 SV=1
-0.990816678	Beta-enolase OS=Gallus gallus GN=ENO3 PE=1 SV=3
-0.990384134	Coiled-coil domain-containing protein 27 OS=Homo sapiens GN=CCDC27 PE=2 SV=2
-0.969748198	50S ribosomal protein L22 OS=Blochmannia floridanus GN=rplV PE=3 SV=1
-0.842260537	Pyruvate kinase isozymes M1/M2 OS=Mus musculus GN=Pkm2 PE=1 SV=4
-0.826106096	Creatine kinase M-type OS=Oryctolagus cuniculus GN=CKM PE=1 SV=1
-0.816687926	Uncharacterized protein C6orf58 homolog OS=Oncorhynchus mykiss PE=2 SV=1
-0.796885621	Alpha-enolase OS=Rattus norvegicus GN=Eno1 PE=1 SV=4
-0.781686448	Alpha-enolase OS=Gallus gallus GN=ENO1 PE=2 SV=2
-0.55481289	Beta-enolase OS=Oryctolagus cuniculus GN=ENO3 PE=1 SV=4
-0.528074917	Gamma-enolase OS=Homo sapiens GN=ENO2 PE=1 SV=3
-0.506341212	Uncharacterized protein C20orf135 OS=Homo sapiens GN=C20orf135 PE=2 SV=1
-0.489405492	Uncharacterized protein C2A9.13 OS=Schizosaccharomyces pombe GN=SPBC2A9.13 PE=2 SV=1
-0.479929228	Beta-lactoglobulin OS=Ovis orientalis musimon GN=LGB PE=1 SV=1
-0.475221112	Keratin, type II cytoskeletal 8 OS=Danio rerio GN=krt8 PE=1 SV=1
-0.461443698	Fibrinogen gamma-B chain OS=Bos taurus GN=FGG PE=1 SV=1
-0.45915702	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase OS=Debaryomyces hansenii GN=HIS6 PE=3 SV=2
-0.455340839	Protein kinase C, eye isozyme OS=Drosophila melanogaster GN=inaC PE=1 SV=1
-0.399057365	Intelectin-1b OS=Mus musculus GN=Itln1b PE=1 SV=1
-0.327497312	Apolipoprotein E OS=Tupaia glis GN=APOE PE=2 SV=1
-0.32166016	Protein translocase subunit secA OS=Streptococcus pneumoniae serotype 2 (strain D39 / NCTC 7466) GN=secA PE=3 SV=1
-0.311598917	Creatine kinase M-type OS=Torpedo marmorata PE=2 SV=1
-0.309557242	Arginyl-tRNA synthetase OS=Streptomyces avermitilis GN=argS PE=3 SV=1
-0.309557242	Reticulon-1 OS=Mus musculus GN=Rtn1 PE=1 SV=1
-0.308416893	Creatine kinase M-type OS=Homo sapiens GN=CKM PE=1 SV=2
-0.30252574	Creatine kinase, testis isozyme OS=Oncorhynchus mykiss GN=tck1 PE=2 SV=1
-0.286729533	Enolase 1 OS=Saccharomyces cerevisiae GN=ENO1 PE=1 SV=2
-0.268173129	Creatine kinase M-type OS=Gallus gallus GN=CKM PE=2 SV=1
-0.239329896	Ubiquitin OS=Bos taurus GN=RPS27A PE=1 SV=1

-0.237077378	Ribosomal protein S12 methylthiotransferase rimO OS=Burkholderia pseudomallei (strain 668) GN=rimO PE=3 SV=1
-0.219626429	Putative antiporter subunit mnhA2 OS=Staphylococcus epidermidis (strain ATCC 35984 / RP62A) GN=mnhA2 PE=3 SV=1
-0.218992159	Actin OS=Encephalitozoon cuniculi GN=ECU01_0460 PE=1 SV=2
-0.218992159	Actin-1 OS=Plasmodium berghei (strain Anka) GN=PB000323.01.0 PE=3 SV=1
-0.178667462	Ceruloplasmin OS=Homo sapiens GN=CP PE=1 SV=1
-0.178667462	Ceruloplasmin OS=Mus musculus GN=Cp PE=1 SV=2
-0.17071617	Beta-lactoglobulin OS=Bos taurus GN=LGB PE=1 SV=3
-0.16761336	Calcium/calmodulin-dependent protein kinase type II delta chain OS=Rattus norvegicus GN=Camk2d PE=2 SV=1
-0.165798991	Calcium/calmodulin-dependent protein kinase type II delta 1 chain OS=Danio rerio GN=camk2d1 PE=2 SV=2
-0.153008606	Galactose/methyl galactoside import ATP-binding protein mglA OS=Clostridium perfringens (strain SM101 / Type A) GN=mglA PE=3 SV=1
-0.150535403	L-lactate dehydrogenase A chain OS=Mus musculus GN=Ldha PE=1 SV=3
-0.137226995	Bifunctional purine biosynthesis protein purH OS=Clostridium botulinum (strain Alaska E43 / Type E3) GN=purH PE=3 SV=1
-0.124027786	Corticoliberin OS=Canis familiaris GN=CRH PE=3 SV=2
-0.122798983	Enolase 2 OS=Saccharomyces cerevisiae GN=ENO2 PE=1 SV=2
-0.107183347	Glutamyl-tRNA synthetase OS=Enterococcus faecalis GN=gltX PE=3 SV=1
-0.101428361	Lipoprotein-releasing system ATP-binding protein lolD OS=Haemophilus influenzae GN=lolD PE=3 SV=1
-0.10120377	Proteasome subunit alpha type-3 OS=Bos taurus GN=PSMA3 PE=1 SV=3
-0.086629689	Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6
-0.077807446	Complement component C9 (Fragment) OS=Oncorhynchus mykiss GN=c9 PE=2 SV=2
-0.071193477	Glyceraldehyde-3-phosphate dehydrogenase OS=Trionyx sinensis GN=GAPDH PE=2 SV=1
-0.069381202	Apolipoprotein A-I-1 OS=Oncorhynchus mykiss PE=2 SV=1
-0.06663137	Complement component C9 OS=Fugu rubripes GN=c9 PE=3 SV=1
-0.063694675	Complement component C8 beta chain OS=Oncorhynchus mykiss GN=c8b PE=2 SV=1
-0.061962378	Trypsin OS=Sus scrofa PE=1 SV=1
-0.055167833	Nucleoside diphosphate kinase A OS=Homo sapiens GN=NME1 PE=1 SV=1
-0.043624815	Nucleoside diphosphate kinase A2 OS=Xenopus laevis PE=2 SV=1
-0.042834433	Nucleoside diphosphate kinase 3 OS=Spinacia oleracea PE=1 SV=1
-0.042834433	Nucleoside diphosphate kinase 4, chloroplastic OS=Spinacia oleracea GN=NDK4 PE=1 SV=1
-0.042627865	Hephaestin-like protein 1 OS=Homo sapiens GN=HEPHL1 PE=2 SV=2
-0.042240358	50S ribosomal protein L20 OS=Dehalococcoides ethenogenes (strain 195) GN=rplT PE=3 SV=1
-0.03722732	Alcohol dehydrogenase 1 OS=Saccharomyces cerevisiae GN=ADH1 PE=1 SV=4
-0.027331982	Ribonuclease P protein component 1 OS=Methanosarcina acetivorans GN=rnp1 PE=3 SV=1
-0.016797319	Glyceraldehyde-3-phosphate dehydrogenase OS=Glossina morsitans morsitans GN=Gapdh PE=2 SV=1
-0.013870154	Threonyl-tRNA synthetase OS=Rickettsia canadensis (strain McKiel) GN=thrS PE=3 SV=1
0.004170043	Cingulin-like protein 1 OS=Mus musculus GN=Cgnl1 PE=1 SV=2
0.00479248	Inter-alpha-trypsin inhibitor heavy chain H3 OS=Mesocricetus auratus GN=ITIH3 PE=1 SV=1
0.00479248	Inter-alpha-trypsin inhibitor heavy chain H3 OS=Mus musculus GN=Itih3 PE=1 SV=2
0.008914019	Ornithine aminotransferase, mitochondrial OS=Drosophila ananassae GN=Oat PE=1 SV=1
0.013568217	Kininogen (Fragments) OS=Anarhichas minor PE=1 SV=1
0.014319025	Zinc finger CCHC domain-containing protein 10 OS=Homo sapiens GN=ZCCHC10 PE=2 SV=1
0.032845235	Transcriptional activator protein DAL81 OS=Saccharomyces cerevisiae GN=DAL81 PE=1 SV=3
0.034164268	Protein FIZZY-RELATED 3 OS=Arabidopsis thaliana GN=FZR3 PE=1 SV=1
0.034230515	Integrin beta-1 OS=Felis silvestris catus GN=ITGB1 PE=2 SV=1
0.034230515	Integrin beta-1-A OS=Xenopus laevis GN=itgb1-A PE=2 SV=1
0.041541043	Serum albumin 1 OS=Salmo salar GN=alb1 PE=2 SV=1
0.043390214	Histidine-rich glycoprotein (Fragment) OS=Oryctolagus cuniculus GN=HRG PE=1 SV=1
0.045768882	Serum albumin 2 OS=Salmo salar GN=alb2 PE=2 SV=1
0.054066341	Potassium-transporting ATPase A chain OS=Erwinia tasmaniensis (strain DSM 17950 / Et1/99) GN=kdpA PE=3 SV=1
0.057305204	Corticoliberin OS=Ovis aries GN=CRH PE=1 SV=1
0.060616506	Actin-17 OS=Dictyostelium discoideum GN=act17 PE=3 SV=1
0.062865589	Malate dehydrogenase (Fragment) OS=Thermoleophilum album GN=mdh PE=1 SV=1
0.074897182	Putative serine/threonine-protein phosphatase C22H10.04 OS=Schizosaccharomyces pombe GN=SPAC22H10.04 PE=2 SV=1
0.078249021	Cationic trypsin OS=Canis familiaris PE=2 SV=1
0.086958208	Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=5
0.08944619	Fructose-bisphosphate aldolase OS=Saccharomyces cerevisiae GN=FBA1 PE=1 SV=3
0.101230278	Protein SKG3 OS=Saccharomyces cerevisiae GN=SKG3 PE=1 SV=1

0.10218357	Alpha-1-antitrypsin homolog OS=Cyprinus carpio PE=2 SV=1
0.103407084	Glyceraldehyde-3-phosphate dehydrogenase OS=Panulirus versicolor PE=1 SV=1
0.110082922	Apolipoprotein A-I-2 OS=Oncorhynchus mykiss PE=2 SV=1
0.127122225	Apolipoprotein A-I OS=Salmo salar GN=apoa1 PE=2 SV=1
0.127493084	Protein hook OS=Culex quinquefasciatus GN=hk PE=3 SV=2
0.137470328	Scolopendra 20566.01 Da toxin (Fragment) OS=Scolopendra angulata PE=1 SV=1
0.13843011	Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2
0.150194697	Actin, cytoplasmic OS=Branchiostoma lanceolatum PE=2 SV=1
0.150194697	Actin-1/2 OS=Podocoryne carnea GN=ACTIA PE=2 SV=1
0.15738629	Keratin, type II cytoskeletal 1b OS=Homo sapiens GN=KRT77 PE=1 SV=2
0.158911463	Serum albumin OS=Oryctolagus cuniculus GN=ALB PE=2 SV=2
0.161243163	Actin OS=Cyanidioschyzon merolae PE=3 SV=1
0.161847419	Glycogen phosphorylase, brain form OS=Bos taurus GN=PYGB PE=2 SV=3
0.161847419	Glycogen phosphorylase, muscle form OS=Homo sapiens GN=PYGM PE=1 SV=6
0.167100865	Actin-1 OS=Daucus carota PE=2 SV=1
0.167100865	Actin-1 OS=Pisum sativum PE=2 SV=1
0.170207689	Actin-1 OS=Aedes aegypti GN=ACT-1 PE=2 SV=2
0.170454878	Actin, cytoskeletal 3B OS=Strongylocentrotus purpuratus GN=CYIIIB PE=2 SV=1
0.170840708	Actin, cytoskeletal 1A OS=Strongylocentrotus purpuratus GN=CYIA PE=3 SV=1
0.171885375	Actin-1 OS=Onchocerca volvulus GN=act-1a PE=3 SV=1
0.171885375	Actin-3 OS=Diphyllobothrium dendriticum GN=ACT3 PE=2 SV=1
0.171885375	Actin-6 (Fragment) OS=Diphyllobothrium dendriticum GN=ACT6 PE=2 SV=1
0.174894676	Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3
0.175199943	Plastin-2 OS=Danio rerio GN=lcp1 PE=2 SV=1
0.176070853	Ig kappa chain V region AH80-5 OS=Oryctolagus cuniculus PE=1 SV=1
0.178213937	Actin-like protein 53D OS=Drosophila melanogaster GN=Arp53D PE=2 SV=2
0.180792227	Keratin, type II cytoskeletal 6A OS=Rattus norvegicus GN=Krt6a PE=1 SV=1
0.181036863	Actin, cytoplasmic 1 OS=Ctenopharyngodon idella GN=actb PE=3 SV=1
0.190440133	Non-structural protein 3 OS=Rotavirus C (isolate Human/United Kingdom/Bristol/1989) PE=3 SV=1
0.19268348	Actin-3 OS=Glycine max GN=SAC3 PE=3 SV=2
0.19268348	Tyrosine-protein kinase transforming protein Fgr OS=Feline sarcoma virus (strain Gardner-Rasheed) GN=V-FGR PE=3 SV=1
0.194816177	Actin OS=Toxoplasma gondii GN=ACT1 PE=3 SV=1
0.198789619	Actin-1 OS=Naegleria fowleri PE=2 SV=2
0.21145629	Probable receptor-like protein kinase At3g46290 OS=Arabidopsis thaliana GN=At3g46290 PE=1 SV=1
0.234695534	Keratin, type II cytoskeletal 7 OS=Bos taurus GN=KRT7 PE=2 SV=1
0.234695534	Keratin, type II cytoskeletal 75 OS=Bos taurus GN=KRT75 PE=2 SV=1
0.234695534	Keratin, type II cytoskeletal 75 OS=Rattus norvegicus GN=Krt75 PE=2 SV=2
0.237226816	Ig kappa-b4 chain C region OS=Oryctolagus cuniculus PE=1 SV=1
0.243877888	EF-hand calcium-binding domain-containing protein 4B OS=Homo sapiens GN=EFCAB4B PE=2 SV=1
0.243877888	Polypeptide N-acetylgalactosaminyltransferase 5 OS=Homo sapiens GN=GALNT5 PE=1 SV=1
0.245135004	Triosephosphate isomerase OS=Nocardia farcinica GN=tpiA PE=3 SV=1
0.246384936	Ig kappa-B5 chain V region 2699 (Fragments) OS=Oryctolagus cuniculus PE=1 SV=1
0.278728696	Ig kappa-b4 chain C region OS=Oryctolagus cuniculus GN=K-BAS PE=4 SV=1
0.284729477	Ig gamma chain C region OS=Oryctolagus cuniculus PE=1 SV=1
0.30184655	Uncharacterized protein MG328 OS=Mycoplasma genitalium GN=MG328 PE=4 SV=1
0.319776186	GTP-binding protein Rit1 OS=Homo sapiens GN=RIT1 PE=1 SV=1
0.340538664	Tropomyosin alpha-3 chain OS=Bos taurus GN=TPM3 PE=2 SV=1
0.340538664	Tropomyosin alpha-4 chain OS=Equus caballus GN=TPM4 PE=1 SV=2
0.360428684	Alpha-lactalbumin OS=Bos mutus grunniens GN=LALBA PE=2 SV=1
0.362696062	Putative ankyrin repeat protein L767 OS=Acanthamoeba polyphaga mimivirus GN=MIMI_L767 PE=4 SV=1
0.364286851	Serotransferrin (Fragment) OS=Gadus morhua GN=tf PE=2 SV=1
0.365528241	Ovotransferrin OS=Anas platyrhynchos PE=1 SV=1
0.368998257	Serotransferrin OS=Oncorhynchus kisutch GN=tf PE=2 SV=1
0.372883516	Complement C4-A OS=Homo sapiens GN=C4A PE=1 SV=1
0.396990254	Plasma retinol-binding protein 2 OS=Oncorhynchus mykiss GN=rbp4b PE=1 SV=2
0.407433695	Myosin-IXa OS=Mus musculus GN=Myo9a PE=1 SV=2
0.407823201	Complement C3 (Fragment) OS=Oncorhynchus mykiss GN=c3 PE=1 SV=1
0.420136542	30S ribosomal protein S2 OS=Arthrobacter sp. (strain FB24) GN=rpsB PE=3 SV=1
0.435111754	Serotransferrin-1 OS=Salmo salar GN=tf1 PE=1 SV=1
0.447678132	Biotinidase OS=Fugu rubripes GN=btd PE=3 SV=1

0.462368288	Phosphatidylserine decarboxylase proenzyme 1, mitochondrial OS=Saccharomyces cerevisiae GN=PSD1 PE=1 SV=1
0.469567796	Cobra venom factor OS=Naja kaouthia GN=C3 PE=1 SV=1
0.469567796	Complement C3 OS=Bos taurus GN=C3 PE=1 SV=2
0.469567796	Complement C3 OS=Cavia porcellus GN=C3 PE=1 SV=2
0.469567796	Complement C3 OS=Mus musculus GN=C3 PE=1 SV=2
0.469567796	Complement C3 OS=Naja naja GN=C3 PE=2 SV=1
0.469567796	Complement C3 OS=Rattus norvegicus GN=C3 PE=1 SV=3
0.507537529	Hemoglobin subunit alpha-2 OS=Trematomus newnesi GN=hba2 PE=1 SV=1
0.551769322	UvrABC system protein B OS=Natronomonas pharaonis (strain DSM 2160 / ATCC 35678) GN=uvrB PE=3 SV=1
0.55760885	Hemoglobin subunit beta OS=Chelidonichthys kumu GN=hbb PE=1 SV=1
0.673253983	Lysozyme C II OS=Oncorhynchus mykiss PE=1 SV=2
0.6844592	Hemoglobin subunit beta OS=Pagrus major GN=hbb PE=2 SV=3
0.772153677	Histone H1 OS=Oncorhynchus mykiss PE=1 SV=2
0.83645554	Tryptophan synthase beta chain OS=Listeria innocua GN=trpB PE=3 SV=1
0.859327727	Glucose-6-phosphate 1-dehydrogenase OS=Buchnera aphidicola subsp. Schizaphis graminum GN=zwf PE=3 SV=1
0.950538913	Hemoglobin subunit beta-3 OS=Muraena helena GN=hbb3 PE=1 SV=1
0.957399519	Hemoglobin subunit beta-4 OS=Oncorhynchus mykiss GN=hbb4 PE=1 SV=3
0.963556601	Hemoglobin subunit beta OS=Leiostomus xanthurus GN=hbb PE=1 SV=1
0.96640293	Hemoglobin subunit beta OS=Oncorhynchus nerka GN=hbb PE=2 SV=3
1.020957459	Cytidylate kinase OS=Polynucleobacter necessarius (strain STIR1) GN=cmk PE=3 SV=1
1.051095565	Hemoglobin subunit alpha-4 OS=Oncorhynchus mykiss GN=hba4 PE=1 SV=1
1.06513502	Histone H4 OS=Dictyostelium discoideum GN=H4a PE=1 SV=1
1.148169118	Histone H4 OS=Acrolepiopsis assectella GN=His4 PE=3 SV=2
1.154627198	Histone H2B OS=Aspergillus clavatus GN=htb1 PE=3 SV=1
1.154627198	Histone H2B.1, embryonic OS=Psammechinus miliaris PE=3 SV=2
1.165692776	Putative ABC transporter substrate-binding protein yesO OS=Bacillus subtilis GN=yesO PE=1 SV=2
1.166362451	Hemoglobin subunit alpha OS=Salmo salar GN=hba PE=2 SV=2
1.221755189	Hemoglobin subunit beta-1 OS=Oncorhynchus mykiss GN=hbb1 PE=1 SV=1
1.22858587	Hemoglobin subunit alpha-1 OS=Oncorhynchus mykiss GN=hba1 PE=1 SV=1
1.283860054	Hemoglobin cathodic subunit beta OS=Conger conger PE=1 SV=1
1.319365221	Histone H2B 1.2 OS=Xenopus laevis PE=1 SV=2

Appendix 1.f.: Proposal for Evaluating the Reproductive Success of Artificially Reconditioned Kelt Steelhead in a Semi-Natural System

Date October 2, 2014

Collaborators Yakama Nation, Columbia River Inter-Tribal Fish Commission, Washington Department of Fish and Wildlife

Purpose The purpose of this study is to quantitatively evaluate the spawning and reproductive success of artificially reconditioned kelt steelhead relative to maiden steelhead in a semi-natural system.

Background Artificial reconditioning post spawn steelhead (kelts) shows great promise as a restoration tool. Recent studies in the Yakima River system report survival rates of nearly 40% from collection in the spring to release in the fall of the same year (Hatch et al. 2013). Additionally, Null et al. (2013) reported return rates of 26% for kelts that were artificially reconditioned and then released into the Sacramento River.

Seamons and Quinn (2010) reported that 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

males). Further, repeat spawners grew substantially between their first and second breeding seasons (female mean growth of 41 mm; males 71 mm) and estimated this additional female growth would result in an average increase in fecundity of about 400 eggs or about 10% (Seamons and Quinn 2010).

Steelhead kelt reconditioning programs are ongoing in the Yakima, Methow, and Clearwater river systems. We have demonstrated that we can collect and successfully recondition kelt steelhead in the Yakima River Basin (Hatch et al. 2013). While this is certainly an important milestone, quantifying the reproductive success of reconditioned kelts is necessary to assess the overall benefits of this recovery tool. Using the Cle Elum spawning channel to conduct these studies and comparing results for reconditioned fish with maiden spawners will allow us to control for environmental variation and identify any biologically significant differences in spawner success. We hope that the information gained can be used to further recovery efforts in other parts of the Columbia River Basin as well.

Hypotheses to be tested 1. Reconditioned kelt steelhead can build redds, find mates, and successfully spawn in an artificial spawning channel; 2. Spawning behaviors of reconditioned kelt steelhead are similar to those of maiden steelhead in an artificial spawning channel; 3. Reconditioned kelt steelhead have reproductive metrics (fry production and survival rates) similar to those of maiden steelhead in an artificial spawning channel.

Duration of study Preparation of the spawning channel for this study would commence in June of 2014 upon the completion of the present spring chinook study. Adult steelhead would first be placed in the channel early in 2015. Sexually mature adults would be stocked in the channel in February-April and fry would be collected in May-September for each year of the study.

The main objective in 2014-15 will be to address feasibility issues such as:

- Can sufficient numbers of kelts and maiden fish be collected to make the experiment viable?
- Will steelhead survive holding, transportation, and successfully use the artificial channel for spawning?

If feasible, we anticipate the study would continue for approximately 5 years.

Proposed implementation plan

<u>Spawner Collection and Holding</u>
Reconditioned Kelts: During the kelt collection season at Prosser, we propose to identify any kelts originally PIT-tagged or detected at Roza Dam or in upper Yakima tributary PIT arrays during their upstream migration. We expect to collect in the range of 5-30 (on average about 18) Upper Yakima kelts annually with 80-90% of these fish being females. Because of the risk of collecting only a few known Upper Yakima kelts, we plan to also identify Naches kelts at the Prosser Hatchery using available radio and PIT tag information from the Yakima steelhead VSP project. We expect to collect in the range of 5-30 Naches kelts annually with 80-90% of these fish also being females. These kelts would be held at Prosser Hatchery and reconditioned from collection in March-June until October. To the extent that we can, Upper Yakima and Naches kelts will be held separately at Prosser during reconditioning. We expect to achieve about 35-50% survival during reconditioning. DNA samples would be collected from these fish at Roza or Prosser Denil (during upstream

migration) and again at Prosser should their upstream-migration-planted PIT tags not be retained. Fish will be held in Yakima River water at Prosser Hatchery for over-wintering and trucked to Cle Elum to be placed in the spawning channel the following spring. Since upper Yakima steelhead typically don't migrate upstream until the spring, conditions at the Prosser facility are likely to be more consistent with natural over-wintering conditions for these fish. Water temperature profile should match the Satus bar where many Yakima Major Population Group (MPG) steelhead hold over the winter. At this time, we do not have an estimate for over-winter survival of these fish, but assuming 35-50% reconditioning survival, and 80% over-winter survival, we expect to have in the range of 5-40 reconditioned kelts available annually for the spawning channel experiment. Note that we will only use Naches kelts in the spawning channel if it is determined that the number of mature Upper Yakima kelts surviving to the spring is insufficient to make the experiment viable.

Maiden Steelhead: At a minimum, we will collect enough maiden males to assure at least one (1) male (including reconditioned males) for every female reconditioned kelt held at Prosser. These fish will be collected at Roza or, if necessary, in the Naches system (including DNA sample) during the spring migration and immediately trucked to Cle Elum for placement in the spawning channel. These males could be either resident or anadromous fish; however at this time there are no plans to collect any anadromous fish from the Naches system. We are still evaluating whether, and if so how many, maiden females (resident or anadromous) to collect at Roza, or if necessary resident females from the Naches system, in the spring and also place in the channel. Using maiden steelhead in the Channel is important as it will allow evaluation of whether or not the spawning channel will work for steelhead, and to quantify the reproductive success of reconditioned steelhead relative to maiden fish. If no steelhead successfully spawn or no fry are produced then the issue is not kelt viability but rather something else such as: suitable spawning habitat for steelhead, egg to fry survival, fry containment and sampling, or other issues. Quantifying the relative reproductive success of reconditioned steelhead compared to maiden fish will enable us to construct a model of the benefit of reconditioning programs, as requested by ISRP.

<u>In-channel replication</u>

At this time we are still evaluating whether kelts would be allowed to use the entire spawning channel or whether to use screens to restrict them to a smaller area. We may develop strategies for in-channel replication each year when it is determined approximately how many female kelts will be available for the channel experiment. If Naches fish are deemed necessary for experimental purposes, it would be best to segregate these from Upper Yakima fish in the channel; however, experience with spring Chinook experiments suggests that, while this may be feasible for adults, it is not necessarily so for emerging fry.

<u>Fry sampling</u>

Fry traps would be placed at the outlet of each channel section by the end of May each year. Sampling would begin in mid-June and continue through the end of August. The goal would be to sample 10% of the fry from each channel section or from the entire channel. These fish would be lethally sampled for the parent-progeny analysis.

<u>Genetic Analysis</u>

Genotyping efforts will be completed at the CRITFC Hagerman Fish culture Experiment Station. Funding will come from the existing kelt reproductive success project. Published manuscripts and presentations will be a collaborative effort.

<u>Channel Observation</u>

For the first year of study, observation resources will be limited. A video system will be deployed to record fish activity. We will review this record to quantify fish movement and behavior.

Disposition of unsampled fry

We are still evaluating options that include (option 2 is preferred alternative but need fish health screening – 60 fish – hold remainder 1 month until results are received; if any test positive would recommend killing them; where to hold fry- in channel – just open sample boxes long enough to get samples):

1. Allowing unsampled fry to exit volitionally to the Yakima River through the hatchery slough.
2. If Naches fish are used, separating Naches origin fry and trucking them to the Naches for release (Nelson Springs); allowing Upper Yakima fry to exit volitionally to the hatchery slough.
3. Trapping all fry at the channel exit and trucking them to an off-site release location (to be determined) within the Upper Yakima River Basin.
4. Trapping all fry at the channel exit and trucking them to a lake within the boundaries of the Yakama Nation reservation.

Disposition of surviving adults

After spawning, surviving Roza, and if necessary Naches fish (including reconditioned kelts) will be netted from the channel, trucked, and released in their respective watershed to continue their life cycle in the wild.

Processing of adult mortalities

Separate equipment (e.g., freezer, refrigerator, etc.) located in the utility shed near the spawning channel will be used to store mortalities as it is important for disease considerations to keep steelhead isolated from Chinook at the hatchery. Adult mortalities will be necropsied to determine cause of death, sampled for disease issues, and to evaluate gamete size, egg retention, egg maturation, etc. Carcasses will be treated and returned to local streams. Necropsies will be processed by USFWS lab in Olympia.

Annual water needs

The spawning channel would need to be "watered up" by March 1 of each year of the study. It is anticipated that all fry would be removed from the channel by mid-late autumn of each year of the study, allowing time to clean and prepare the channel for the next brood year. Flows used during the spring chinook study should be sufficient for this experiment as well, though the timing of flow to the channel is different.

Channel cleaning and preparation

Each year, when all fry have exited the channel, the channel would be cleaned, evaluated, and new, appropriate-sized spawning gravels will be distributed in the channel as necessary.

Predation control

At this time, we do not anticipate any special needs or considerations relative to controlling predation in the spawning channel during the annual experimental timeframe.

Disease considerations and contingency plans

Standard bio-containment practices prescribed in Upper Yakima Spring Chinook /

Cle Elum Supplementation and Research Facility (CESRF) Hatchery Genetic Management Plan (2010) will be followed to prevent the spread of fish diseases. If spring Chinook rearing at the Cle Elum facility exhibit evidence of any disease that could be attributed to steelhead in the spawning channel during the course of this experiment, we will immediately work with CESRF staff and USFWS fish health professionals to determine the best course of action.

ESA permits

NOAA has issued a Section 7 Determination of Take for Research Purposes (14-14-CRITFC49) covering these activities. Specifically, the permit allows the collection of up to 250 kelt and 20 maiden steelhead originating upstream of Roza Dam and the collection of 2000 fry from the spawning channel experiment.

PIT-tag retention study

We are still considering an additional objective to evaluate long-term survival of PIT-tagged fish coincident with the kelt reproductive success evaluation in the spawning channel. To test survival of PIT-tagged fish for 3-6 months, we would PIT tag all resident fish prior to placing in the spawning channel. It might also be possible to use the 'elbow' portion of the channel for this evaluation.

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A.2: Publications and Presentations

Publications:

Caldwell, L.K., Pierce A.L., Riley L.G., Duncan C.A. & Nagler J.J. 2014 Plasma nesfatin-1 is not affected by long-term food restriction and does not predict rematuration among iteroparous female rainbow trout (Oncorhynchus mykiss). *PLoS One* **9** e85700.

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Penney, Z. L. and Moffitt, C. M. 2014. Proximate composition and energy density of stream-maturing adult steelhead during upstream migration, sexual maturity, and kelt emigration. Transactions of the American Fisheries Society 143:399-413

Penney, Z.L., and C.M. Moffitt. 2014. Fatty acid profiles of white muscle and liver tissue in stream-maturing steelhead during early migration and kelt emigration. Journal of Fish Biology.

Presentations:

Pierce, A., J. Blodgett, T. Cavileer, J. Boyce, J. Medeiros, L. Caldwell, N. Graham, L. Jenkins, Bosch W., Fast, D., Branstetter, R., Hatch, D., & Nagler, J. Reproductive development in reconditioned female Yakima River steelhead kelts: evidence for consecutive and skip spawning life histories. **Pacific Coast Steelhead Management Meeting, March 18-20 2014, Skamania WA.**

Hatch, D., Stephenson, J., Fast, D.,Bosch, B., Blodgett, J., Branstetter, R., & Pierce, A. Reproductive Success of Reconditioned Kelt Steelhead. **Yakima Basin Science and Management Conference, June 18-20 2014, Ellensburg, WA.**

Pierce, A., Blodgett, J., Bosch W., Cavileer, T., Caldwell, L., Boyce, J., Medeiros, L., Jenkins, L., Branstetter, R., Fast, D., Hatch, D., & Nagler, J. Reproductive development in artificially reconditioned female Yakima River steelhead kelts. **Yakima Basin Science and Management Conference, June 18-20 2014, Ellensburg, WA.**

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Hatch, D., Fast, D., Bosch, W., Branstetter, R., Pierce, A., & Everett. S. Columbia Basin Steelhead Reconditioning Studies. **American Fisheries Society 144th Annual Meeting, Aug 17-21 2014, Quebec City, Canada.**

Jenkins, L., Pierce, A., Everett, S., Graham, N., Cavileer, T., Hatch D., & Nagler J. Reproductive Viability Assessment of Reconditioned Upper-Snake River Tributary B-Run Female Steelhead (Oncorhynchus mykiss) Kelts. **American Fisheries Society 144th Annual Meeting, Aug 17-21 2014, Quebec City, Canada.**

Pierce, A., Blodgett, J., Frederiksen, C., Caldwell, L., Cavileer, T., Medeiros, L., Branstetter, R., Graham, N., Jenkins, L., Bosch, W., Fast, D. Hatch D., & Nagler J. Reproductive Development in Reconditioned Female Yakima River Steelhead Kelts: Evidence for Consecutive and Skip Repeat Spawning Life Histories. **American Fisheries Society 144th Annual Meeting, Aug 17-21 2014, Quebec City, Canada.**

Graham, N., Everett. S., Jenkins, L., Pierce, A., & Hatch D. Collection and Assessment of Emigrating Snake River Steelhead (Oncorhynchus mykiss) Kelts at Lower Granite Dam. **American Fisheries Society 144th Annual Meeting, Aug 17-21 2014, Quebec City, Canada.**

Jenkins, L., Pierce, A., Everett, S., Graham, N., Cavileer, T., Hatch D., & Nagler J. Reproductive Viability Assessment of Reconditioned Upper-Snake River Tributary B-Run Female Steelhead (Oncorhynchus mykiss) Kelts. **1st Annual CRB Trainee Symposium, Oct 28 2014, Pullman WA.**

Pierce, A., J. Blodgett, C. Frederiksen, L. Caldwell, T. Cavileer, J. Boyce, L. Medeiros, N. Graham, L. Jenkins, W. Bosch, D. Fast, R. Branstetter, D. Hatch, & J. Nagler. Reproductive Development in Reconditioned Female Yakima River Steelhead Kelts: Evidence for Consecutive and Skip Repeat Spawning Life Histories. **65th Annual Northwest Fish Culture Conference, Dec 2-4 2014, Pendleton OR.**

Jenkins, L., A. Pierce, S. Everett, N. Graham, T. Cavileer, D. Hatch, & J. Nagler. Reproductive Viability Assessment of Reconditioned Upper-Snake River Tributary B-Run Female Steelhead (Oncorhynchus mykiss) Kelts. **65th Annual Northwest Fish Culture Conference, Dec 2-4 2014, Pendleton OR.**

Graham, N., S. Everett, L. Jenkins, A. Pierce, & D. Hatch. Collection and Assessment of Emigrating Snake River Steelhead (Oncorhynchus mykiss) Kelts at Lower Granite Dam. . **65th Annual Northwest Fish Culture Conference, Dec 2-4 2014, Pendleton OR.**

Caldwell, L.C. Endocrine intersections of growth and reproduction in Oncorhynchus mykiss. Ph.D. Dissertation Defense, May 5 2014, Dept. of Biological Sciences, University of Idaho, Moscow ID.

A.3: List of Metrics and Indicators

Please See Monitoring Methods for further details on protocols. Will include list with monitoring methods linked.

Protocol:

Kelt Reconditioning and Reproductive Success Evaluation:

https://www.monitoringmethods.org/Protocol/Details/2051

Methods

Kelt Collection

Kelt ID: https://www.monitoringmethods.org/Method/Details/5310

Sex ID: https://www.monitoringmethods.org/Method/Details/5334

Coloration Rating: https://www.monitoringmethods.org/Method/Details/5302

Measuring Fork Length: https://www.monitoringmethods.org/Method/Details/4041

Fish Weight: https://www.monitoringmethods.org/Method/Details/1734

Measuring Mid-Orbital Hypural Length:
https://www.monitoringmethods.org/Method/Details/1549

Fish Condition Rating:
https://www.monitoringmethods.org/CustomizedMethod/Details/22915

PIT Tagging: https://www.monitoringmethods.org/Method/Details/1736

Genetic Sampling: https://www.monitoringmethods.org/Method/Details/4087

Blood Sampling: https://www.monitoringmethods.org/Method/Details/4239

Estimating Lipid Content: https://www.monitoringmethods.org/Method/Details/4215

Air-Spawning: https://www.monitoringmethods.org/Method/Details/5343

Genetic Stock Identification (GSI)

Tissue Sampling for PBT: https://www.monitoringmethods.org/Method/Details/1432

SNP Marker Sets: https://www.monitoringmethods.org/Method/Details/1356

SNP Genotyping: https://www.monitoringmethods.org/Method/Details/1332

Genetic Assignment using GeneClass2:
https://www.monitoringmethods.org/Method/Details/487

Predicting Accuracy of GSI:
https://www.monitoringmethods.org/Method/Details/1346

In-River Release

PIT Tagging: https://www.monitoringmethods.org/CustomizedMethod/Details/22818

Downloading Data from PTAGIS:
https://www.monitoringmethods.org/Method/Details/4095

Kelt Reconditioning Physiology Studies

Estradiol Assay: https://www.monitoringmethods.org/Method/Details/5320

Reproductive Success of Artificially Reconditioned Kelt Steelhead

Electrofisher Settings: https://www.monitoringmethods.org/Method/Details/115

Backpack Electrofishing: https://www.monitoringmethods.org/Method/Details/117

Parentage Analysis using Cervus:
https://www.monitoringmethods.org/Method/Details/1430

Radio Tagging:
https://www.monitoringmethods.org/CustomizedMethod/Details/23045

Lotek Receiver Download: https://www.monitoringmethods.org/Method/Details/4244

Metrics

Title	Category	Subcategory	Subcategory Focus 1	Subcategory Focus 2
Kelt abundance"	Fish	Abundance of Fish (ID: 46)	Fish Life Stage: Adult - Outmigrant	Fish Origin: Both
Reconditioned Kelt abundance"			Fish Life Stage: Adult Fish	Fish Origin: Both
"Stock Composition"		Composition: Fish Species Assemblage (ID: 56)	Fish Life Stage: Adult - Outmigrant	Fish Origin:Natural

Maturation Status"		Condition of Life Stage: Fish (ID: 57)	Fish Life Stage: Adult - Returner	NA
"Kelt Condition"			Fish Life Stage: Adult - Outmigrant	NA
Reconditioned Kelt condition"			Fish Life Stage: Adult Fish	NA
Fecundity"		Fecundity: Fish (ID: 68)	NA	NA
Fry Growth"		Growth Rate: Fish (ID: 73)	Fish Life Stage: Juvenile - Fry/Parr	NA
"Fertilization Rate"		Hatchery Practices: Propagation(ID: 87)	Fish Origin: Both	NA
"Kelt length"		Length: Fish Species (ID: 75)	Fish Life Stage: Adult - Outmigrant	NA
"Reconditioned kelt length"			Fish Life Stage: Adult Fish	NA
"Mark Detection"		Mark/Tag Recovery or Detection (ID: 381)	NA	NA
"Parentage Analysis"		Relative Reproductive Success (RRS) (ID: 88)	Fish Origin: Both	NA
"Reproductive success"		Reproductive Success (Nb/N) (ID: 89)	Fish Origin: Natural	NA
"Mark application"		Stock Identity (ID: 95)	Fish Life Stage: Adult - Outmigrant	NA
"Kelt Survival"		Survival Rate: Fish (ID: 99)	Fish Life Stage: Adult - Outmigrant	Fish Origin: Both
"Collection Date"		Timing of Life Stage: Fish (ID: 101)	Fish Life Stage: Adult - Outmigrant	NA
"Release Date"			Fish Life Stage: Adult Fish	NA
"Kelt Weight"		Weight: Fish (ID: 206)	Fish Life Stage: Adult - Outmigrant	Fish Origin: Both

"Reconditioned Kelt weight"			Fish Life Stage: Adult Fish	Fish Origin: Bot