Kelt Reconditioning and Reproductive Success Evaluation Research:

2013 Annual Technical Report

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1. Executive Summary

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. It associates with RPAs 33 and 64 in the Federal Columbia River Power System Biological Opinion. RPA 33 requires the Action Agencies to develop, in cooperation with regional salmon managers, and to then 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 stage steelhead. This project focuses on the reconditioning component. RPA 64 involves resolving artificial propagation critical uncertainties primarily through relative reproductive success studies. This project is working toward evaluating reproductive success of artificially reconditioned kelt steelhead. This research contributed three papers to the published literature in 2013 (Caldwell et al. 2013; Hatch et al. 2013; Penney and Moffitt 2013) and an additional four papers are in review (Buelow et al. in review; Caldwell et al. in press; Penney and Moffitt in review a.; Penney and Moffitt in review b.). Our team presented 14 project presentations in 2013 at basin, regional, national, and international levels.

a. Fish Population Status Monitoring (RM&E)

Reconditioning Processes Strategies

Kelt steelhead reconditioning process evaluations involve fish culturing practices, studying alternative management strategies, and implementing research scale reconditioning programs. Refinements in fish culturing practices this year included a diet experiment that investigated the benefits of top dressing pellets with fish oil or cyclopeeze, and evaluating the effectiveness of ivermectin and emamectin benzoate for parasite control. Effectiveness was measured with survival and rematuration metrics. We used fish in long-term reconditioning for a feeding trial that compared an “orange” diet that was comprised of a mix of customized Bio-oregon brood pellets top coated with a mix of cyclopeeze and Alaskan fish oil versus the standard diet (customized Bio-oregon diet and krill) (Hatch et al 2003a). Both groups received krill at the start of reconditioning for the same duration of time. The diet study just recently concluded in 2013 and data is currently being analyzed. Preliminary results found in the 2012 trial, fish fed the orange diet had higher muscle lipid levels at release than fish fed the standard diet. The orange diet median muscle lipid levels increased by 0.4% in 2013, and 0.6% in 2012. The increase in muscle lipid levels was modest and may be explained due to either increased feed consumption or the higher lipid level in the orange diet. If feasible, the current feeds should have increased lipid content and appetite stimulants added before further diet studies are undertaken. Emamectin benzoate (emamectin) is an alternative to ivermectin that has been developed for control of sea lice (marine copepod ectoparasites) in Atlantic salmon aquaculture, and both drugs have been adapted for use in steelhead kelt reconditioning to reduce *Salmincola* is a genus of parasitic copepod that can inhibit oxygen uptake and gas exchange at the gill lamellae/water surface interface. We

compared the post treatment mortality rates of kelts that were administered ivermectin via gavage as well as a group that was injected. Results demonstrated a dramatic reduction in mortality that was substantial and consistent between years: after 90 days, mortality in emamectin treated fish was 33.2% less than ivermectin treated fish in 2011, and 34.8% in 2012. This experiment did not attempt to quantify levels of copepod infestation in emamectin versus ivermectin treated fish. However, very few fish were observed with significant numbers of copepods at release, suggesting that both ivermectin and emamectin treatment controlled copepods adequately.

Plasma estradiol was extracted and analyzed from long-term reconditioned kelt steelhead to assess rematuration levels. Samples are currently being processed in the laboratory so the final results are not yet available.

Alternative management strategies for kelt steelhead studied thus far include: long-term reconditioning, where fish are collected during out migration, held and feed through the summer and released in the fall; collect and transport kelt steelhead, where fish are collected and immediately transported (unfed) and released below Bonneville Dam, or collected, fed for 4-6 weeks (fed) then transported and released below Bonneville Dam; and direct release, where fish are collected, PIT tagged and released back to river as a control group.

Experimental scale long-term kelt reconditioning survival data was compared from 3 locations including; the Okanogan, Snake, and Yakima rivers, where survival rates in long-term reconditioning was 8.2%, 51.8%, and 48.7% respectively. We also published a manuscript detailing the long-term kelt reconditioning program at Prosser Hatchery (Hatch et al. 2013b).

Specific to Snake River B-run steelhead (addressing RPA 33), our collection locations included kelts at the Lower Granite Dam juvenile bypass separator, air-spawned steelhead being used for the South Fork Clearwater River naturalized broodstock project, and Dworshak National Fish Hatchery returns. Fish collected from Lower Granite Dam were either transported to Dworshak Hatchery for reconditioning, or PIT tagged and released back into the Snake River is represent a control group. Fish collected from the South Fork Clearwater were reconditioned and released, and Dworshak Hatchery fish were used for experiments and not released. Kelt stage steelhead were collected at Lower Granite Dam as they passed through the juvenile bypass system. A total of 110 wild female B-run steelhead were collected at Lower Granite Dam from April to June and transported to Dworshak National Fish Hatchery for reconditioning. Fifty-seven of these fish survived reconditioning and were released downstream of Lower Granite Dam in October 2013. Additionally, 24 B-run kelt steelhead were retained for reconditioning after air spawning at Dworshak Hatchery. These fish were part of project that is developing localized broodstock for the South Fork Clearwater River. Following reconditioning 12 of these fish were released back into the South Fork Clearwater River. In total 69 wild B-run steelhead were reconditioned and released back into the Snake River system aimed at addressing RPA 33.

We did not conduct any transport experiments in 2013 due to their relatively lower benefits compared to long-term reconditioning and the overall lower availability of kelt steelhead. From 2002 through 2011 we collected and transported kelt steelhead from Lower Granite Dam to below Bonneville Dam. These studies collected and transported 2,698 kelt steelhead, of which 29 (0.01%) were detected returning upstream at Bonneville Dam.

We are developing a spreadsheet model of management scenarios to address “what if” simulations. This model will be completed during the first half of 2014.

To establish a control group for management action comparisons, we continue to collect and PIT tag the kelt steelhead run at large to estimate baseline in-river survival. In 2013, we tagged 827 steelhead collected from the Lower Granite Dam juvenile bypass system. None of these fish were detected returning at any FCRPS dam as sequential spawners in 2013. It is possible that some fish from this release will exhibit a skip spawner life history pattern and return in 2014 after spending an additional winter in the ocean. Similar tagging in 2012 resulted in the return of a single fish out of 2,098, resulting in a return rate to Bonneville Dam of 0.05%. Since 2002, we have PIT tagged 9,325 kelt steelhead at Lower Granite Dam and detected 32 (0.34%) of them returning upriver at Bonneville Dam. The 2013 Yakima River control group release included 52 PIT tagged fish. None of these fish were detected returning to Bonneville Dam as sequential spawners. From 2005 through 2013, 669 kelt steelhead were PIT tagged and released in the Yakima River representing the control group and so far 21 (3.4%) fish were detected returning to Bonneville Dam.

The genetic stock structure of Snake River Basin kelt steelhead was examined using 187 SNP markers extracted from emigrating fish collected at Lower Granite Dam. The kelt steelhead population composition at the Lower Granite Dam Juvenile Bypass Separator from 2009-2012 was Upper Salmon (0.39); Grande Ronde/ Lower Snake (Tied) (0.16); Middle Fork Salmon (0.09); and, Imnaha (0.06), reporting groups based on a sample size of 4,138.

Reproductive Success of Artificially Reconditioned Kelt Steelhead

We have investigated reproductive success of artificially reconditioned kelt steelhead at a variety of scales. Experiments have been conducted at the natural stream level, in a hatchery environment, and at the individual fish level.

This evaluation program is designed to investigate the reproductive success of artificially reconditioned kelt steelhead. Since direct examination of reproductive success in the field has proven very difficult, we are concentrating on measuring physiological and endocrinological parameters as an index to rematuration and reproductive success. Additionally, we are evaluating gamete and progeny viability and conducting reproductive success studies in Omak Creek using pedigree analysis. This project is a collaborative effort among four tribes (Nez Perce, Warm Springs, Yakama Nation, and Colville), the University of Idaho, and the Columbia River Inter-Tribal Fish Commission.

At the natural stream level, we have conducted experiments on Omak Creek a tributary to the Okanogan River for several years. We have determined that at least 4 of 11 reconditioned kelt steelhead have successfully produced offspring in Omak Creek. This site has proven challenging for this evaluation primarily due to low kelt steelhead collection numbers and then ultimately few reconditioned fish. Additionally, the reconditioned fish must be released in the Okanogan River to overwinter adding mortality and difficulty to tracking these fish to spawning grounds in Omak Creek. An additional 4 juveniles assigned to reconditioned kelts in the Yakima River. As a result of these challenges, we may transfer future efforts to the Cle Elem Hatchery spawning channel (described below).

In 2013, we radio tagged 70 long-term reconditioned kelt steelhead prior to release in the Yakima River. These fish were known rematuring based on elevated levels of estradiol. We are partnering with the Yakima River Steelhead VSP project to track these fish to spawning beds. Later we will target electrofishing efforts downstream of known reconditioned kelt spawners in an attempt to identify their progeny. In addition, 21

of these radio tagged fish have known detection histories from prior spawnings. These histories are from in-stream PIT tag detection or from previous radio tags. Relocating these fish will provide data on repeat homing fidelity of artificially reconditioned kelt steelhead.

At Parkdale Fish Facility, we conducted experiments to compare performance of maiden and repeat spawnings of artificially reconditioned kelt steelhead using metrics of fecundity, fertilization rates, and early juvenile growth. A total of 21 fish were successfully reconditioned and repeat spawned from a collection of 134 steelhead. Summer-run steelhead had higher fecundity levels in their repeat compared to maiden spawning, and all other metrics showed no significant difference between repeat and maiden spawnings. Winter-run steelhead showed no significant differences in fecundity, fertilization rates, and growth in terms of length and weight, there were significant differences in fry starting length and weight with maiden spawnings resulting in larger fry starting sizes.

To better understand the physiological processes that influence post-spawn steelhead recovery, we published two manuscripts, the first used histological analysis of Snake River steelhead as a model to assess the cellular architecture in the pyloric stomach, ovary, liver, and spleen in sexually mature and kelt steelhead (Penney and Moffitt 2013). The second identified metabolic endocrine factors involved in spawning recovery and rematuration of iteroparous female rainbow trout (Caldwell et al. 2013).

From an adaptive management standpoint in 2015, the experimental spawning channel at Cle Elum Hatchery will be available, so we are currently drafting a proposal and study plan to use the channel in an evaluation of reconditioned steelhead reproductive success. Additional components such as resident rainbow trout contribution and maiden steelhead reproductive success may also be added.

We are also searching for potential stream locations to conduct a relative reproductive success study.

b. Coordination and Data Management (RM&E)

The CRITFC and member Tribes all share a commitment towards collaborative data sharing in the Columbia Basin with many different agencies and interest groups. The CRITFC has a dedicated team of data stewards that are working to establish servers that will serve as data repositories that will enhance collaborations and provide data standardization for the many important projects that are currently in place. The CRITFC also works to provide coordination between the many partners that work on the Kelt Reconditioning project, with planning meetings, education, and services. These commitments by the CRITFC will ensure project success through its concerted efforts at coordination and data management.

The Columbia River Inter-Tribal Fish Commission's Hagerman lab continues to build baseline information on the kelt steelhead stocks that are represented in the juvenile bypass facility at Lower Granite Dam to better direct kelt management in the basin.

The Nez Perce Tribe are making progress on the Master Plan for kelt management in the Snake Basin. Site selection has been narrowed but is still ongoing and we anticipate that the plan will be nearing completion in 2014.

2. Introduction

a. Fish Population Status Monitoring (RM&E)

F&W Program Strategy: Assess the status and trend of natural and hatchery origin abundance of fish populations for various life stages.

Oncorhynchus mykiss are considered to have one of the most diverse life histories in *Salmonidae* (Behnke 1992) with variants that include resident, estuarine, and anadromous ecotypes, widely ranging ages of maturity, timing of juvenile and adult migrations, and various reproductive strategies including precocity, semelparity, and iteroparity. This complex array of life history variation is possibly a compensating or bet hedging device for life in stochastic environments (Taborsky 2001). Overlapping generations provide resources especially for small populations in the event of failure of any brood year due to brief catastrophic events (Seamons and Quinn 2010). While fluctuating populations and overlapping generations may reduce the effective population size (Ne; Waples 2002), retention of genetic diversity and persistence of the species may be favored due to these compensating life histories (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*. Rates of iteroparity are estimated to be as high as 79% for populations in the Utkholok River of Kamchatka, Russia (Savvaitova et al. 1996), and as high as 31% for British Columbia winter-run populations (Withler 1966). Historical rates for the interior Columbia River are not well documented but adult emigrating steelhead averaged 58% of the total upstream runs in the Clackamas River from 1956 to 1964 (Gunsolus and Eicher 1970), 45% of the Snake River upstream run (Jay Hesse personal communication), and 70% of the Yakima River upstream run (Chris Fredrickson personal communication) in recent years. Current iteroparity rates for interior Columbia River Basin steelhead are considerably 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. 1984). Summer steelhead in the South Fork Walla Walla River have expressed 2% to 9% iteroparity rates (J. Gourmand, ODFW, pers. comm.). Hockersmith et al. (1995) reported that repeat spawners composed 4.0% of the Yakima River wild run and recent tagging data shows average return rates to Bonneville Dam of 3.1%.

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

Post-spawn steelhead represent a portion of the population that have successfully survived through an entire life cycle culminating with spawning. Reconditioning these kelts may counter the negative selective forces against iteroparity associated with the hydrosystem (Evans et al. 2008), thereby helping to preserve the evolutionary legacy of the species. Kelt reconditioning starts with the introduction of feed, thereby enabling kelts to survive and rebuild energy reserves required for gonadal development and repeat spawning. Techniques used in kelt reconditioning were initially developed for Atlantic salmon *Salmo salar* and sea-trout *S. trutta*, and a review of these studies and others applicable to steelhead kelts are summarized in Evans et al. (2001). Additional reviews of this subject (Hatch et al. 2002a and 2003b) provide support of the benefits of kelt reconditioning to address population demographic and genetic issues in steelhead recovery. We are estimating survival and return rates of artificially reconditioned kelt steelhead subjected to various management treatments ranging from low to high intensity efforts. Although it is difficult to observe individual fish spawning in the wild and even more difficult to assess the viability and quality of gametes produced in the wild, we are conducting experiments (gamete/progeny viability and reproductive success) that will aid in determining the extent to which reconditioned kelts are contributing to subsequent generations. The overall success of kelt reconditioning, when in full production, can be assessed most accurately based on the number of individuals that successfully spawn in the wild following reconditioning and release.

F&W Program Management Question: What are the status and trend of abundance of natural and hatchery origin fish populations?

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

F&W Program Strategy: Assess the status and trend of diversity of natural and hatchery origin fish populations.

Status and Trends

Natural populations may be suffering from genetic bottlenecks due to low population numbers brought on by habitat destruction and alteration, hydrosystem operation, and overharvest. Climate change may result in negative pressure on listed populations with further unfavorable alteration or destruction of spawning and rearing habitat. Years with bad ocean conditions which may be aggravated by climate change will further reduce the chances of survival for naturally produced steelhead limiting contribution to spawning populations. Historically, the majority of steelhead hatcheries have not been tasked with maintaining the genetic diversity of either the hatchery or their sympatric natural populations.

Uncertainty Research

- How do fecundity, gamete quality, and juvenile quality compare between spawners from the ocean and reconditioned kelts? If problems are identified, can they be rectified by modifications to diet or other aquaculture conditions? Would captive kelts benefit from saltwater rearing?

- What limits the successful spawning of rematuring reconditioned kelts after release? How do the reproductive development, energy reserves, migration patterns, spawn timing, spawning location, and spawning behavior of reconditioned fish compare to those of spawners from the ocean? Would tributary weir collected kelt destined-for-reconditioning produce a higher rate or fitness of successful repeat spawner compared to Lower Granite Dam separator collected kelt?
- Why is the steelhead stock composition at Lower Granite Dam different on the downstream migration compared to the upstream migration?

b. Coordination and Data Management (RM&E)

F&W Program Strategy: Work with regional federal, state and tribal agencies, and non-governmental entities to establish a coordinated, standardized, web-based distributed information network and a regional information management strategy for water, fish, and habitat data. Establish necessary administrative agreements to collaboratively implement and maintain the network and strategy.

F&W Program Management Question: Identification of Management Questions and Strategies

Project Map:

http://qa.cbfish.org/Project.mvc/Map/2007-401-00

Contract Map(s):

http://qa.cbfish.org/Contract.mvc/Map/57554

http://qa.cbfish.org/Contract.mvc/Map/61549

3. Work / Deliverables / Objectives

a. Fish Population Status Monitoring (RM&E)

Reproductive Development

Introduction

Studies applying tools from fish physiology and endocrinology to issues in kelt reconditioning were continued in 2013. These studies aim to achieve a sufficiently detailed understanding of the physiology of reconditioning in kelt steelhead to provide a scientific basis for maximizing the success of reconditioning programs. Major areas of focus include studies in post-spawning rainbow trout as a tractable experimental model for kelt reconditioning, the development of tools for assessing maturation status in kelts during reconditioning, comparison of the energetic and reproductive status of reconditioned kelts versus maiden spawners, development of diets, disease treatments, and other methods to increase survival and rematuration in kelts, and investigation of indicators of subsequent survival and rematuration at the time of intake into reconditioning.

Rainbow Trout Physiology Studies

Very little is known about post-spawning physiology in kelts or in salmonids in general. Lethal sampling and experimental manipulations are difficult with kelts due to the ESA-listed status of fish in most reconditioning programs. The genetic stock and physiological condition of kelts arriving at reconditioning facilities varies widely between individuals, sites, and years, complicating experimental design. Therefore, we have begun studies on post-spawning physiology in rainbow trout. Our initial goal is to construct a profile of growth and reproductive endocrine physiology in post-spawning female rainbow trout. This can then be compared to profiles from kelts, and treatments to stimulate feeding enhance survival, and improve rematuration and reproductive performance can be tested in rainbow trout. Two rainbow trout studies were published by the project during 2013 (Caldwell, et al. 2013; Caldwell, et al. in press). These studies found that restricted ration after spawning prevents female rainbow trout from rematuring, and that restricted ration reduces plasma estradiol level in post-spawning rainbow trout within 10 weeks after spawning. This suggests that the decision to remature occurs within a 10 week time period after spawning. Therefore, feeding level and energy balance in steelhead kelts during the first 10 weeks after intake into reconditioning may determine rematuration rate. Further, liver gene expression levels of the insulin-like growth factor system, IGF2, IGF1, and IGFBP1, were affected by nutritional restriction and recovery from spawning in rainbow trout, suggesting that plasma levels of these factors may predict survival and rematuration in steelhead kelts.

Steelhead Kelt Physiology Studies

Introduction

Columbia basin steelhead have highly complex and plastic life histories at every stage of their life history up through the first spawning (Brannon, et al. 2004). Thus, it is not surprising to find variation in post-reproductive life history. Natural repeat spawning fish may return after a single summer in the ocean (consecutive spawners), or after two or more summers (skip spawners) (Keefer, et al. 2008). Skip spawning is common in seasonally reproducing teleosts, and is driven by energetics (Rideout and Tomkiewicz 2011). Studies from 2009-2012 established that the consecutive and skip spawning life histories are found in reconditioned steelhead kelts. In 2013, we established new laboratory techniques for steroid extraction and assay of plasma estradiol levels in steelhead kelt plasma. These techniques enable us to run large numbers of samples with a rapid turnaround time, enabling us to provide in-season maturation status to project managers. We assayed a large number of plasma samples taken from kelts in 2012 and 2013. Some summary information is available; however, full analysis of these results is ongoing and will be provided in a later report. We conducted the second year of a study evaluating the effect of a supplemented diet on growth, survival, and rematuration in female Prosser kelts. We continued a collaborative project with Yakama Nation Fisheries sampling and radio tagging upriver migrating maiden steelhead at Prosser Dam. This study will allow a direct comparison of the reproductive and energetic status of reconditioned kelts with maiden spawners. We continued a study employing a proteomics approach to search for an indicator of rematuration in plasma samples taken at intake.

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. 2006; 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. in press). In post-spawning fish, energy driven decisions take place in the context of the extreme energy deficit incurred by migration and spawning. Threshold energy levels for rematuration selected before anthropogenic changes occurred in the freshwater and marine environment may no longer be adaptive under current conditions.

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 2013, we established a non-radioactive plate based enzyme immunoassay (EIA) for estradiol that allows rapid turnaround of blood samples. We collected blood from fish in three different reconditioning programs, ran a large number of blood samples from 2012 and 2013, and provided maturation status for Prosser fish to project managers prior to release.

Effects of a supplemented diet on kelt steelhead

Studies conducted from 2009-2011 at the reconditioning project at Prosser showed that muscle lipid levels in the fish at release are strongly related to whether fish show characteristics associated with successful spawning after release (Branstetter, et al. 2010, 2011). Female fish with high muscle lipid levels at release were more likely to be consecutive spawners undergoing active ovarian development at the time of release, whereas females with lower muscle lipid levels at release were more likely to be skip spawners, fish with undeveloped ovaries that would spend an additional year in the ocean prior to maturation in the natural environment (Keefer et al. 2008). Both female and male fish with high muscle lipid levels at release were more likely to be detected migrating upriver after release, and reconditioned kelts that were recaptured during downriver migration the spring after release were fish that had very high muscle lipid levels at release. These findings suggest that treatments which increase muscle lipid levels in the fish at release time will increase the proportion of kelts that migrate and spawn successfully in the river after release.

There is a strong relationship between dietary lipid levels and carcass lipid levels in salmonids (Halver and Hardy 2002). Thus, supplementing our diet with additional fish oil might be effective at increasing muscle lipid levels. The feeding motivation of kelts is low at intake into reconditioning. Cyclopeeze is a microscopic copepod harvested from an Arctic freshwater lake which contains appetite stimulants (Argent 2011). In addition, cyclopeeze contains immune system stimulants, and has been found to improve egg quality in salmonid broodstock. Fish oils also increase palatability of pelleted diets. Preliminary trials suggested that coating feed items with cyclopeeze increased feeding activity in kelt steelhead. To determine whether a fish oil and cyclopeeze supplemented diet has beneficial effects on survival, growth, and rematuration in kelt steelhead, we conducted a diet experiment. The experiment was conducted at Prosser during the 2012 and 2013 reconditioning years. Results from 2012 were reported previously (Hatch, et al. 2013a).

Comparison of reconditioned kelt steelhead and spawning steelhead sampled during upstream migration at Prosser Dam

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

In 2011, a three year radio telemetry study was begun to obtain basic information on steelhead VSP units in the Yakima Basin (Frederiksen, et al. 2012). In this study, upstream migrating steelhead are radio tagged and sampled at the Denil ladder on river right at Prosser Dam. Prosser Dam is less than 1 km upstream from the site where reconditioned kelts are released each fall, and PIT tag detections at Prosser Dam are the principal means by which we monitor whether reconditioned kelts actively migrate toward spawning grounds. During the fall of 2012, we began collaboration with the radio tracking study in which we are obtaining blood samples and Fatmeter readings from upstream migrating female steelhead. Our goals are to compare spawners from the ocean to fish reconditioned at Prosser in terms of: 1) reproductive status using plasma levels of estradiol and vitellogenin; 2) energy reserves using muscle lipid levels measured with the Fatmeter; and 3) migration and spawning success using radio tracking. In addition, measurement of blood hormone levels in samples from spawners from the ocean will contribute information on the stage of reproductive development of these fish for use in the radio telemetry study. Blood hormone levels can be analyzed for relationships to parameters to be estimated in the telemetry study, including pre-spawn mortality, migration patterns, spawn timing, genetic stock, and VSP population assignment. This study was continued and radio tagging and tracking of reconditioned kelts was expanded in the fall of 2013.

It is important to note that reconditioned kelts are not expected to be identical to maiden spawners from the ocean in any of these measures. Our goal is not to show that the performance of reconditioned kelts is identical to that of maiden spawners. Instead, it is to obtain information, which will help us to quantify the benefit of the kelt reconditioning program. In terms of the effect of captive reconditioning on fish performance, the relevant comparison is between reconditioned kelts and natural repeat spawning steelhead. Scales are being taken for the radio tracking study, so it should be possible to identify natural repeat spawners. However, given the number of fish sampled, we would only expect to find 2-4 natural repeat spawners with which to compare reconditioned kelts (Hockersmith, et al. 1995).

Proteomic analysis of female steelhead plasma

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

Rationale: Steelhead 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 is a plasma protein(s) that could fill the role as a biomarker. This might be a protein that is present at higher levels (metabolic indicator) in kelts that will enter a consecutive reproductive cycle, as opposed to fish with much lower or negligible levels of this protein. To discover this protein(s) a plasma proteome approach was employed to identify a biomarker, by comparing the plasma proteomic patterns in kelts that went on to consecutively reproduce with some that did not.

Methods

Protocol Title: Reproductive Development (2007-401-00) v1.0

Protocol Link: *http://qa.monitoringmethods.org/Protocol/Details/185*

Protocol Summary: Hypothesis: The endocrinology of reproductive development is similar between maiden spawners, kelt spawners, and rainbow trout. Site Selection: Sites include the Yakima and Snake River. Site selection was based on fish and facility availability. Sample Size: Depends on the year and site. Duration of Study: Through 2017ish.

Reproductive development in kelt steelhead

Fish Collection and Husbandry

Steelhead kelts were collected and reconditioned at Prosser Hatchery, Washington, Dworshak National Fish Hatchery, Idaho, and Winthrop National Fish Hatchery, Washington as described elsewhere (Abrahamse and Murdoch 2013; Hatch, et al. 2013b).

Blood Sampling

Fish were blood sampled at the indicated dates in 2012 and 2013 (Table 1). During 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).

Estradiol and Vitellogenin Assays

Fish plasma levels of estradiol-17β (E2) and vitellogenin (VG) are indicators 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.

The effect of ether extraction on the EIA was investigated by comparing standard curves that were unextracted, extracted, and extracted with the highest standard serially diluted post-extraction in assay buffer (Fig.1). Extraction shifted the curve downward, indicating that a substance carrying through from the extraction reduced the binding in the assay. Serial dilution of the extracted top standard resulted in a curve that gradually shifted until it was superimposed upon the unextracted standard curve after approximately 100-fold dilution, indicating that the interfering substance diluted out. A serial dilution of extracted fish plasma in extracted EIA buffer was parallel to the extracted standard curve (data not shown). Based on these results, assays were run using an extracted standard curve.

Plasma vitellogenin concentrations were assayed using a rainbow trout vitellogenin enzyme linked immunosorbent assay (ELISA) kit (Biosense, Cayman Chemical, Ann Arbor, MI). Plasma samples were appropriately diluted and duplicate technical replicates assayed in the ELISA according to the manufacturer's instruction manual provided with the kit.

Effects of a supplemented diet on kelt steelhead

Kelt diet pellets were purchased from BioOregon. Pellets were topcoated with Alaskan fish oil and freeze dried cyclopeeze to produce the "orange" diet. Cyclopeeze and fish oil were purchased from Argent Laboratories. The percentage of ingredients and calculated nutritional composition of the diets is listed in Table 1. The percentage of cyclopeeze was reduced from the amount used in preliminary trials due to the cost of this ingredient.

Kelt steelhead arriving at Prosser during the spring of 2013 were processed and stocked into tanks following standard procedures. All fish were scanned for PIT tags at intake, and tagged if no existing tag was found. Fish were stocked into four small tanks (tanks S1-S4, 12' diameter, 20 female fish per tank), and four large tanks (tanks C1-C4, 20' diameter, 99-107 female fish per tank). Odd numbered tanks were fed the orange diet, and even numbered tanks were fed the standard diet. All fish were treated with oxytetracycline and emamectin at intake. Fish were fed *ad libitum*. All fish were fed krill for an initial period of approximately one month before pellets were introduced. Fish were transitioned from krill to pellets by feeding a mixture of the two following standard procedures established at Prosser. Mortality was recorded daily. Only female fish being reconditioned for the first time were included in the analysis (i.e. no males, fish held over the winter, or recaptured fish). Only fish positively identified by PIT tag from intake to exit (mortality or release) were included in the analysis. Muscle lipid levels were measured with the Fatmeter, and specific growth rate in weight was calculated as $\frac{\ell n\,(^{mass_2}/_{mass_1})}{days between measurements} \times 100$. Detections of fish after release were obtained by queries of the PTAGIS database.

Table 1: Composition of experimental diets tested in 2013.

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

Comparison of reconditioned kelt steelhead and spawning steelhead sampled during upstream migration at Prosser Dam

Fish were trapped, sedated, sampled, PIT tagged, and radio tagged at the Prosser Denil as described for the radio tracking study (Frederiksen et al. 2012). Sampling effort and radio tags were distributed throughout the run, with many more fish handled than were radio tagged. Samples were obtained from both tagged and non-tagged fish. In 2012, reconditioned kelts were sampled at the Denil when they were encountered in the trap after release. Reconditioned fish were handled identically to maiden spawners, except that a second blood draw was not taken for those fish handled during the fall migration season. In 2013, reconditioned fish were blood sampled and radio tagged during the release sampling on 10/23/13.

Proteomic analysis of female steelhead plasma

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

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

Protein concentrations were determined using a bicinchonic acid protein assay (Sigma). Samples (100 µg of each) were precipitated overnight in acetone at 4°C followed by resolubilzation 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.

Results

Reproductive development in kelt steelhead

Analysis of plasma hormone levels is ongoing and will be reported in detail in the 2014 annual report to Bonneville Power Administration. Changing our E2 assay technique from a radioimmunoassay to an EIA resulted in a change in the threshold value dividing non-rematuring and rematuring fish from 500 to approximately 1000 pg/ml E2 (Table 1). This change is not unexpected with the change in assays. E2 levels in extracted samples measured with both the EIA and a radioimmunoassay were highly correlated (r2 = 0.88 to 0.92). Thus, we are confident that both assays are precisely measuring plasma E2 levels (Figure 1). For female kelts at Prosser, preliminary rematuration percentages at release were 81% in 2012 and 65% in 2013.

Table 2: Steelhead kelt plasma samples assayed for estradiol and vitellogenin in 2013. DNFH: Dworsak National Fish Hatchery, WNFH: Winthrop National Fish Hatchery, Prosser: Prosser Hatchery, Prosser Denil: Denil trap for upriver fish at Prosser Dam.

Sample date	Location	Fish	Samples	Samples Assayed	Assays
9/25/2012	DNFH	kelts	15	15	E2 and VG
10/3/2012	WNFH	kelts	5	5	E2 and VG
10/10/2012	Prosser	kelts	196	196	E2 and VG
Fall 2012	Prosser Denil	maidens	136	136	E2 and VG
Spring 2013	Prosser Denil	maidens	46	46	E2 only
8/9/2013	DNFH	kelts	144	144	E2 only
8/14/2013	Prosser	kelts	408	408	E2 only
9/26/2013	WNFH	kelts	6	6	E2 only
10/22/2013	Prosser	kelts	98	98	E2 only
Fall 2013	Prosser Denil	maidens	61	47	E2 only
Total			1115	1101	

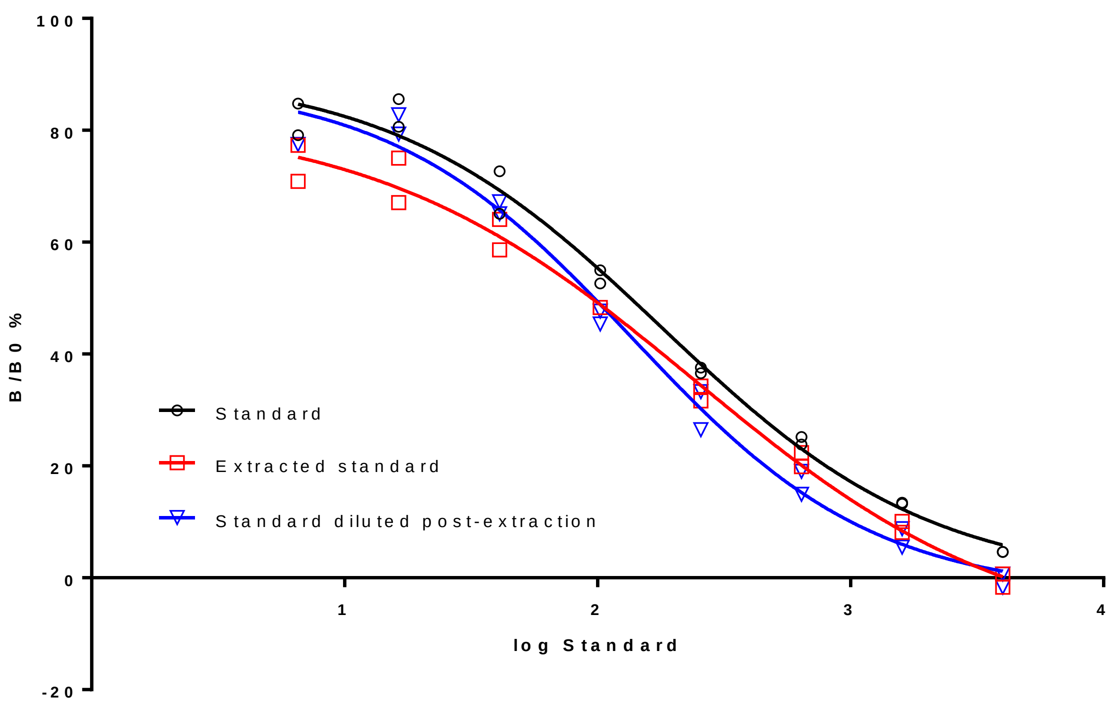


Figure 1: Effect of solvent extraction on the estradiol EIA. Standard: standard curve prepared according to the manufacturer's instructions. Extracted standard: standard curve extracted as described in the Methods section and re-suspended in EIA buffer.

Effects of a supplemented diet on kelt steelhead

This study was completed less than two months ago. There has not yet been sufficient time to complete a full analysis of the results. However, some preliminary results can be reported. As found in the 2012 trial, fish fed the orange diet had higher muscle lipid levels at release than fish fed the standard diet (Fig. 2). Orange diet median muscle lipid levels increased by 0.4% in 2013, versus 0.6% in 2012. The increase in muscle lipid levels may be due to either increased feed consumption or the higher lipid level in the orange diet.

As previously found, there was a significant positive relationship between muscle lipid levels at release and growth rate (Fig. 3). This suggests that Fatmeter readings are informative about the growth status of fish at release time. In salmonids, positive growth rates stimulate smoltification, increase smolt to adult return rate, and stimulate maturation (Beckman 2011; Campbell et al. 2006; Dickhoff, et al. 1997; Picha, et al. 2008). There was a significant difference in the intercept (1.6 in Standard versus 2.1 in Orange diet fish), but not the slope of the linear regression line between Orange and Standard diet fish, suggesting that Orange diet fish had increased muscle lipid levels by approximately 0.5% across a range of growth rates.

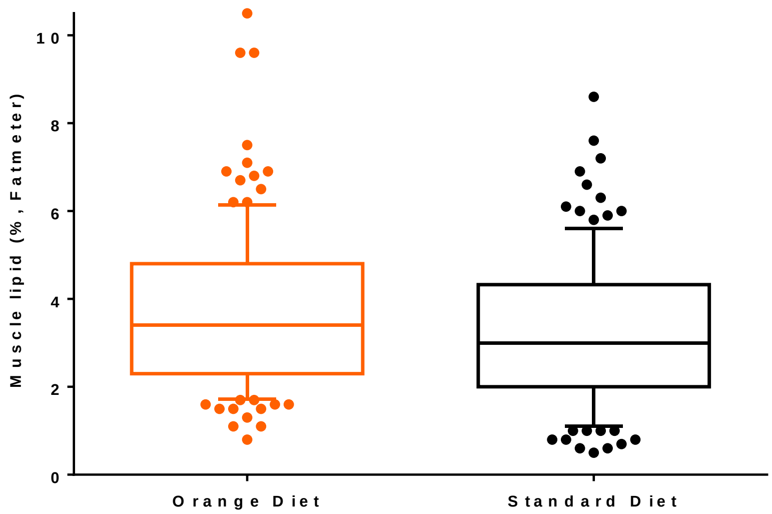


Figure 2: Release muscle lipid levels measured with the Fatmeter in fish fed the Standard and Orange diets at Prosser in 2013. T-test, two tailed, p = 0.0370.

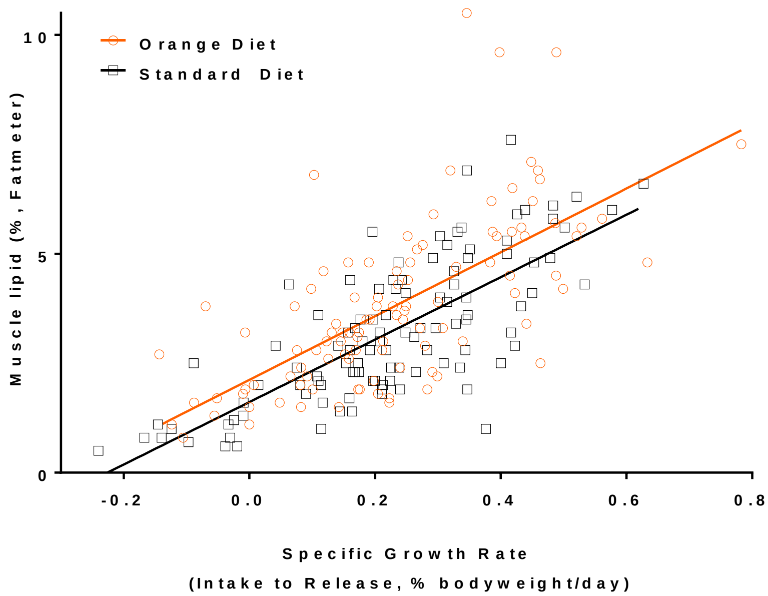


Figure 3: Relationship between release muscle lipid levels measured with the Fatmeter and specific growth rate over the reconditioning period (intake to release) in fish fed the Standard and Orange diets at Prosser in 2013. Linear regression, Standard diet: $p < 0.0001$, r2 = 0.5648, Y=7.131X + 1.613; Orange diet: $p < 0.0001$, r2 = 0.4515, Y=7.284X + 2.120.

Comparison of reconditioned kelt steelhead and spawning steelhead sampled during upstream migration at Prosser Dam

During the 2013 spawn year migration season, blood samples were collected during the period immediately before and during kelt migration through Prosser Dam (Table 3). Surviving long term reconditioned kelts were released on 10/29/12. Additional blood samples were collected during winter and spring migration, which may include additional reconditioned kelts. Blood samples were assayed for estradiol and vitellogenin during 2013. Analysis of these results is ongoing, and will be reported later.

During the 2014 spawn year migration season, blood samples were collected from maiden fish near the time when kelts were released on 10/23/13. Additional samples will be collected during winter and spring migration. Laboratory analysis of blood samples is ongoing and will be reported later.

Preliminary results for the comparison between plasma estradiol levels between maiden spawners and rematuring reconditioned kelts shows that reconditioned fish had higher levels than maiden spawners in 2012, and lower levels in 2013. Preliminary results for plasma vitellogenin show no difference in levels between rematuring reconditioned kelts and maiden spawners in 2012.

Fatmeter readings were taken at the pre-release sampling of kelts on 10/10/12 and 10/23/13, and during sampling of fish in the radio tagging study (Fig. 4). Muscle lipid levels in maiden spawners from the ocean were similar between years (2012 vs. 2013, median 3.5 and 3.7%, $p = 0.22$), ranging from approximately 1.5 to 7.5%, with most values within 1% of the median value.

Table 3: Blood samples collected at Prosser Hatchery and Dam the fall of 2012 to fall of 2013. This does not include all of the fish that were sampled and radio tagged for the radio tagging study. The number of radio tags reported is the number of blood sampled fish that were radio tagged. Additional samples are being collected during Fall 2013-Spring-2014.

Week	Number of blood samples		Number of radio tags	
	maidens	kelts	maidens	kelts
9/23/2012	2			
9/30/2012	5		3	
10/7/2012	13		4	
10/14/2012	20		20	
10/21/2012	24		20	
10/28/2012	41	29	7	10
11/4/2012	26	3	7	
11/11/2012	3		3	
11/18/2012	2		2	
Spring 2013	46		46	
2013 Spawn Year total	**182**	**32**	**112**	**10**
10/6/2013	34		5	
10/13/2013	7		0	
10/20/2013	7	97	4	70
10/27/2013	9		5	
11/3/2013	1		1	
11/10/2013	4		4	
2014 Spawn Year total	**62**	**97**	**19**	**70**

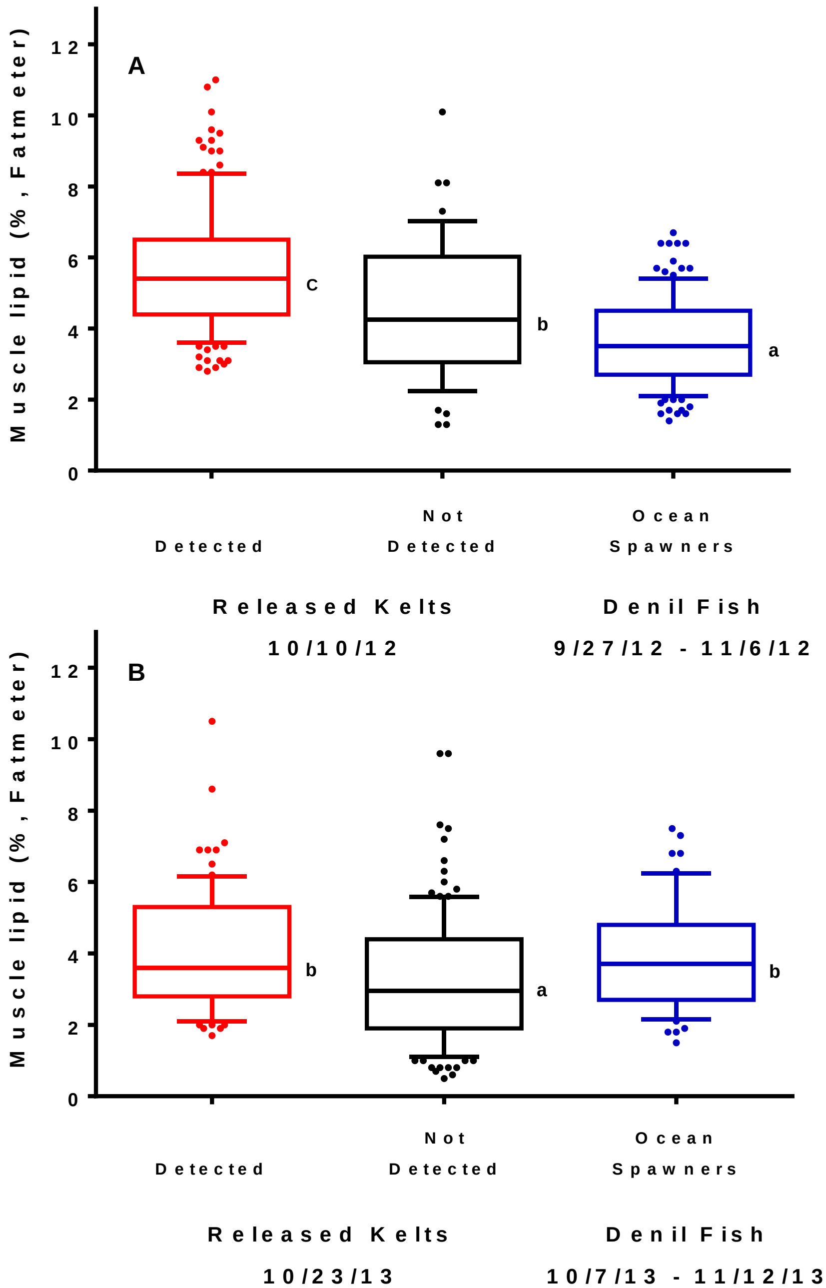


Figure 4: Muscle lipid levels in reconditioned kelts and spawners from the ocean during fall of 2012 (A) and 2013 (B) collected at Prosser Hatchery and Dam. Bars with different letters are significantly different (ANOVA followed by Bonferroni's multiple comparison test, $p < 0.05$). Boxes represent the interquartile range, the median is indicated by a line, and whiskers indicate 10-90% range.

Proteomic analysis of female steelhead plasma

Analysis of the resulting data is ongoing. Approximately 200 proteins have been identified in the plasma samples. Of these, a number of proteins related to iron metabolism (hemoglobins, serotransferrin), histones, and immune response (complement proteins) appear to be differentially expressed between fish which subsequently rematured and those that did not. Identification of additional proteins present in the samples and statistical analysis of differential expression remain to be completed. Results will be presented in the 2014 annual report to Bonneville Power Administration.

Conclusions

Reproductive development in kelt steelhead

Conclusions will be provided in the 2014 annual report to the Bonneville Power Administration.

Effects of a supplemented diet on kelt steelhead

Results are still being analyzed but initial results would suggest that that a diet supplemented with cyclopeeze and fish oil can increase muscle lipid levels in reconditioned fish. Though the increase in muscle lipid level was modest, increased lipid levels are extremely important to survival and rematuration. Therefore, the diet should be reformulated with additional lipid to further increase muscle lipid levels.

Comparison of reconditioned kelt steelhead and spawning steelhead sampled during upstream migration at Prosser Dam

Based on early results, Yakima steelhead store sufficient energy reserves for upstream migration, completion of ovarian development, spawning, and kelt migration. Therefore, the energy reserves of ocean spawners at Prosser Dam are presumably sufficient for the fish to complete these tasks successfully. As previously found, muscle lipid levels in reconditioned kelts were higher in fish actively detected migrating through Prosser Dam after release than in fish that were not detected (Branstetter et al. 2010, 2011; Hatch, et al. 2012). This suggests that measures to increase muscle lipid levels during reconditioning, such as a lipid supplemented diet, are likely to increase the success of released fish. Muscle lipid levels in detected reconditioned kelts were significantly higher than those in spawners from the ocean in 2012, but similar in 2013. These results suggest that reconditioned kelts detected migrating through Prosser Dam have sufficient energy reserves to spawn successfully. The reason for the difference between years in muscle lipid reserves in reconditioned kelts is not known.

Proteomic analysis of female steelhead plasma

Conclusions will be provided in the 2014 annual report to the Bonneville Power Administration.

Kelt Management Scenarios

Protocol Title: Long-term (2007-401-00) v1.0

Protocol Link: *http://qa.monitoringmethods.org/Protocol/Details/180*

Protocol Summary: Hypothesis: Survival rates of steelhead kelt are similar between sites and years. Site Selection: Sites include the Yakima River, Snake River, Hood River, and Omak Creek. Site selection was based on fish and facility availability. Sample Size: Our Goal is to recondition 50% of the post spawn kelts at each location. Total numbers varies between sites and years Duration of Study: Through 2017.

Introduction

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.

Collection

Steelhead kelts are collected from 4 main areas throughout the Columbia River Basin, Prosser, WA, Lower Granite, WA, Omak, WA, and Parkdale, OR. This section details the capture locations, capture methods, and biological information collected from specimens. A small representative portion (9%) of the kelts collected were released back to the river to determine the baseline steelhead kelt iteroparity rate. This information provides a standard with which to compare the experimental approaches. Leaving steelhead kelts in the river represents the least cost option, which is currently the status quo for the majority of the Columbia River Basin.

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. These PIT-tagged kelts provide baseline survival data and an opportunity to compare current repeat spawner rates to other contemporary and historical estimates elsewhere in the Columbia River basin.

Long-term Reconditioning Treatment

We define long-term reconditioning as holding and feeding post-spawn steelhead in a captive environment to increase kelt survival and 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, St. Maries Acclimation Facility, WA, Parkdale Fish Facility, OR, and Dworshak National Fish Hatchery, ID. These fish are typically released in the fall to over-winter and return to the spawning sites volitionally, with the exception of Parkdale and some fish at Dworshak. This treatment represents the highest cost alternative.

Management Scenario Analysis

Management scenarios have consisted of collecting and transporting unfed or fed kelt steelhead downstream and releasing them below Bonneville Dam (Hatch et al 2002b, 2012) and rejuvenating kelts by holding them in large tanks and feeding them until the next season's upstream run occurs when the kelts are released. We present 10 years of data from Prosser Hatchery, 6 years from Lower Granite, and 1 year from John Day Dam (Evans et al. 2008) to evaluate the benefits of transporting kelt steelhead around the hydrosystem. To evaluate success of various management strategies we compared kelt return rates (for transported treatments) and survival rates (for long-term reconditioned kelts) with several "control" groups. Control groups included returns of in-river treatments (fish that were tagged and released back in the river) when available, composition of repeat spawners in the run at large sampled at Bonneville Dam, and values

from the literature (Hockersmith et al. 1995). In 2010, (Branstetter et al. 2011) we compared all treatments and locations across all years, and in 2011 (Hatch et al. 2012) we primarily compare results for just that year and means across years. We have complete return history for transported groups since skip spawners from the 2011 release returned to the Columbia River in the summer of 2012, so we compare all years in this report.

Study Area

Prosser, WA: Yakima River Basin

The Yakima River is approximately 344 km in length and enters the Columbia River at RK 539. The basin is 15,928 km^2 and average discharge is 99 m^3/s. Summer steelhead populations primarily spawn upstream from Prosser Dam in Satus Creek, Toppenish Creek, Naches River, and other tributaries of the Yakima River (TRP 1995) (Figure 5). The Prosser Dam in Prosser, WA is a diversion dam, which collects water for irrigation in the Yakima River valley.

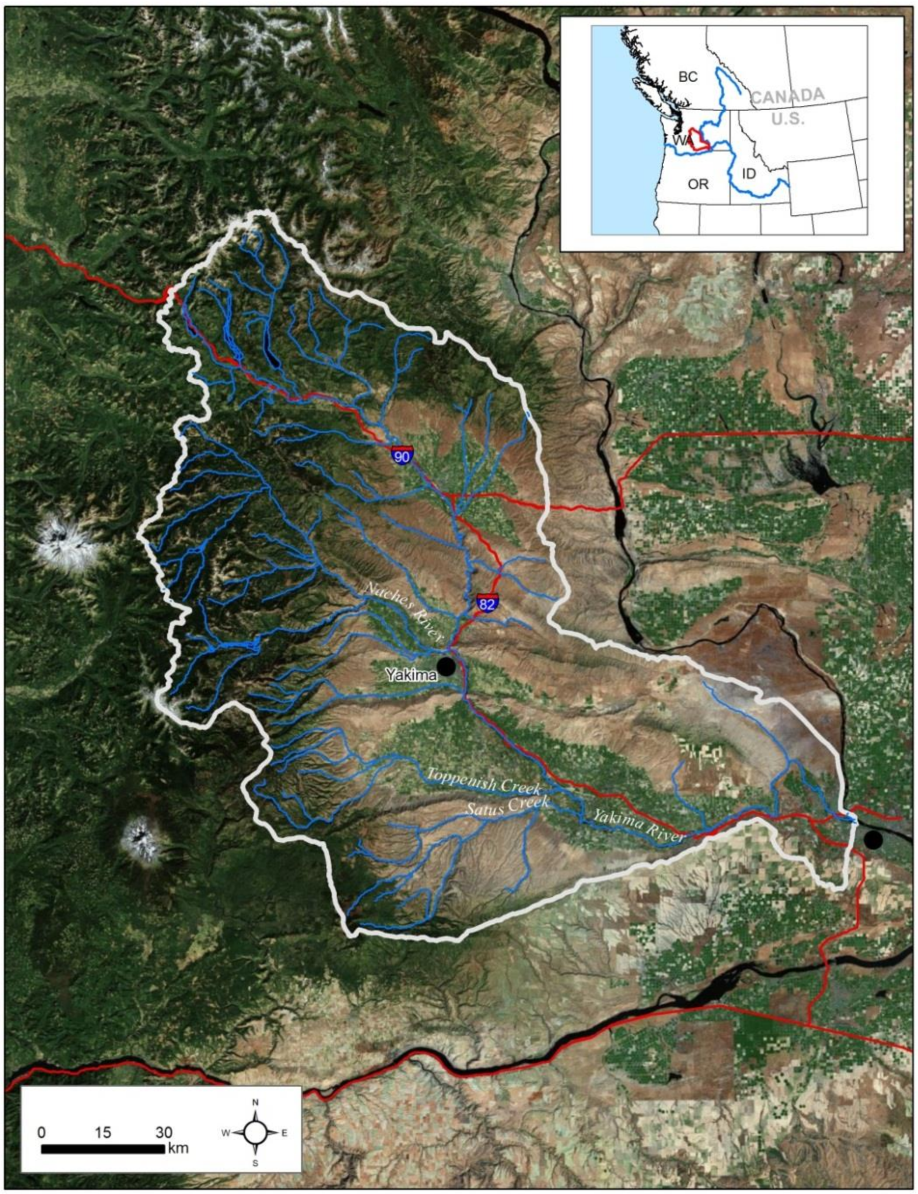


Figure 5: Map of the Yakima River Subbasin.

Lower Granite, WA: Snake River Basin

The Snake River watershed is the tenth largest among North American rivers, and covers almost 280,000 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 6). 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 basin with the vast amount of "b-run" steelhead produced at the Dworshak National Fish Hatchery found on the Clearwater River.

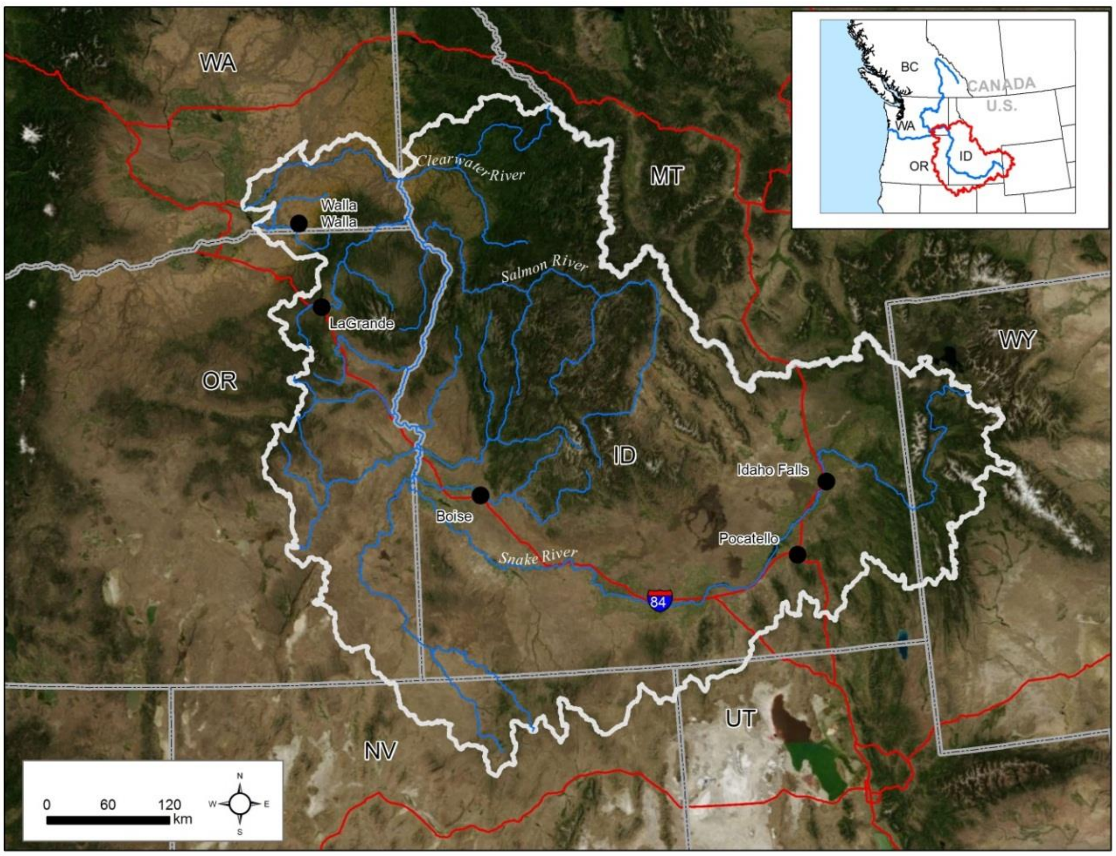


Figure 6: Map of the Snake River Basin.

Omak, WA: Okanogan River Subbasin

The Okanogan River is a tributary of the Columbia River and the confluence is located at RK 858 of the Columbia River. The Okanogan drainage area is 21,238 km^2 with an average discharge rate of 86 m^3/s. Omak Creek, a tributary to the Okanogan River, is located in Okanogan County in North Central Washington, the confluences of Omak Creek is located at RK 52 of the Okanogan River. Omak Creek is approximately 35.4 km in length (Figure 7) running entirely within the Colville Confederated Tribes (CCT) reservation boundaries. Bonaparte Creek's anadromous navigable water runs for 1.6 kilometers and is a tributary to the Okanogan River, which flows through the town of Tonasket. Lower Salmon Creek (6.9 kilometers of Salmon Creek) is a tributary of the Okanogan River that has a diversion dam which prevents upstream fish passage. Steelhead naturally spawn in Omak and Bonaparte Creeks with limited natural spawning in Salmon Creek.

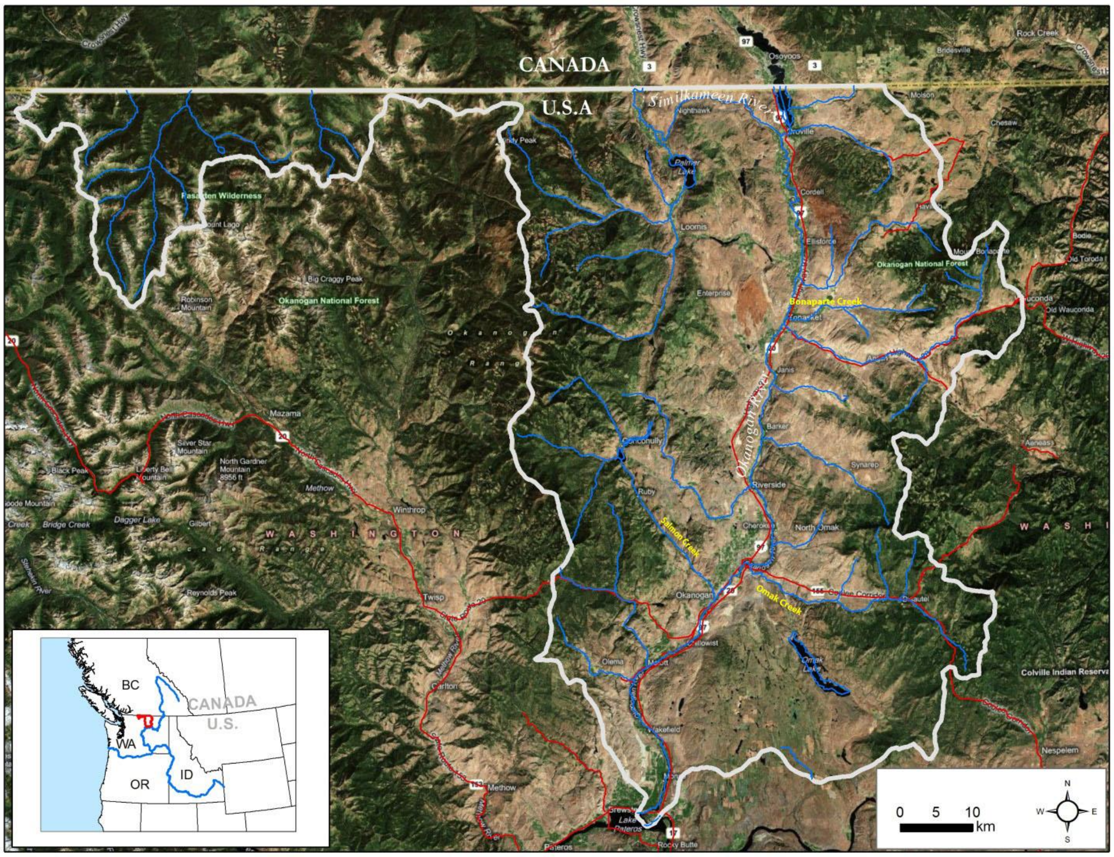


Figure 7: Map of the Okanogan River Subbasin. Omak Creek, Bonaparte Creek, and Salmon Creek in yellow font.

Parkdale, OR: Hood River Subbasin

The Hood River is a tributary of the Columbia River (at RK 272) in northwestern Oregon. Approximately 40 km long from its mouth to its farthest headwaters, the river descends from wilderness areas on Mount Hood and flows through the agricultural Hood River Valley to join the Columbia River in the Columbia River Gorge. The Drainage area is 723[2] km with an average discharge of 28 m^3/s (Figure 8). Currently there is a winter steelhead supplementation program that spawns a small number of hatchery and wild steelhead that are typically reared at the Oak Springs hatchery and acclimated at Parkdale Fish Facility (RK 5.6) and another site on the East Fork Hood River Sand Trap (RK 16)

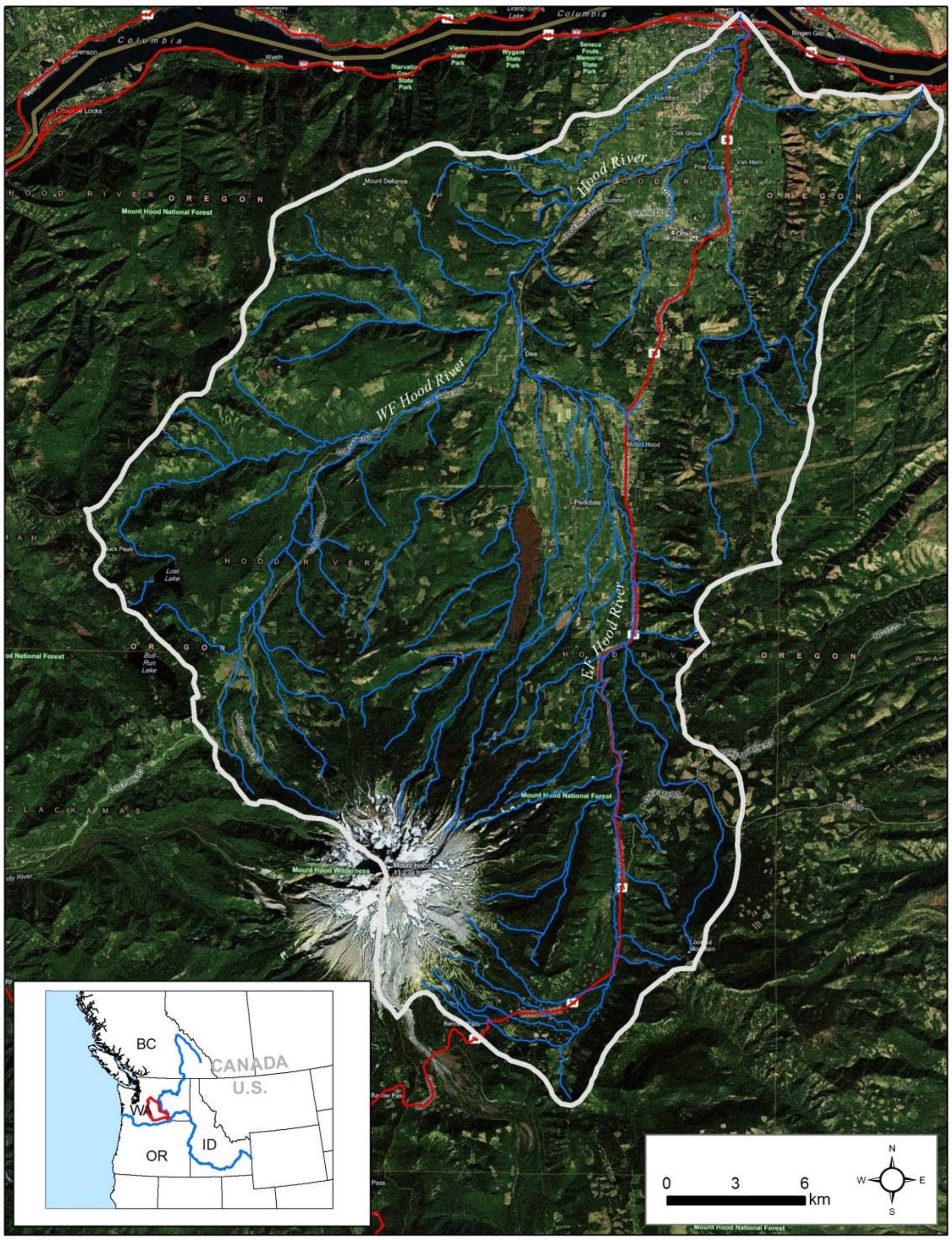


Figure 8: Map of the Hood River Subbasin.

Prosser Hatchery

Prosser Hatchery is located on the Yakima River just downstream of Prosser Dam (RK 75.6). This facility is part of the The Yakima/Klickitat Fisheries Project, a supplementation project designated by the NPPC as the principle means of protecting, mitigating, and enhancing the anadromous fish populations in the Yakima and Klickitat Subbasins. Prosser Hatchery was constructed in 1994 with the primary function of rearing, acclimating, and releasing fall chinook salmon (*O. tshawytscha)*. It is also used for rearing coho salmon (*O. kisutch)* prior to acclimation and release in the upper Yakima River Basin (Figure 9) as well as experimental rearing of white sturgeon *(Acipenser transmontanus)* and Pacific lamprey (*Entosphenus tridentate*).

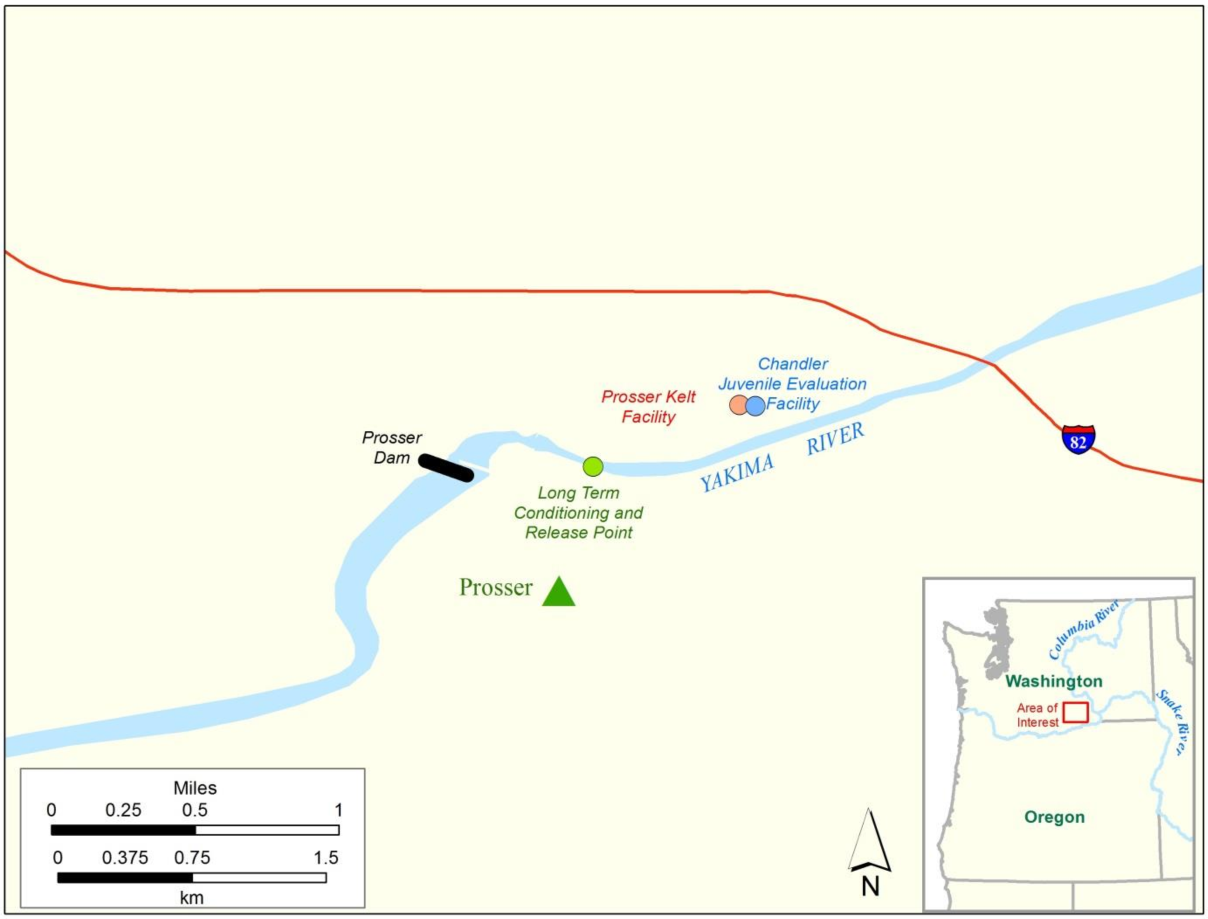


Figure 9: Map showing the location of Prosser Dam and the kelt reconditioning facility at Prosser, WA.

Dworshak National Fish Hatchery

Kelt reconditioning facilities are located at Dworshak National Fish Hatchery (DNFH) in Ahsahka, Idaho (Figure 10). 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).

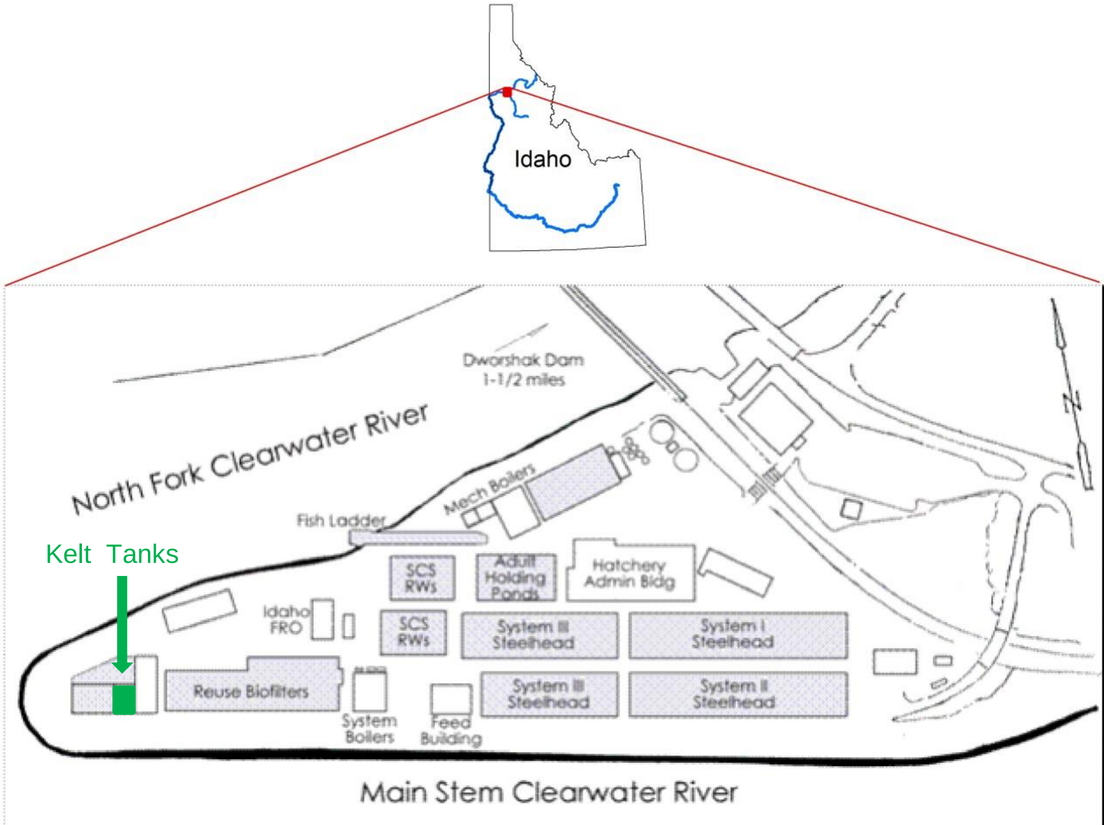


Figure 10: Map showing the location of experimental kelt reconditioning tanks at Dworshak National Fish Hatchery. Figure modified from USFWS 2009.

St. Maries Mission Acclimation Pond

Omak Creek kelt steelhead were reconditioned at the St. Maries acclimation pond located at RK 8.0 of Omak Creek below Mission Falls near the town of Omak (Figure 3). The Colville Confederated Tribes operate the facility which was originally constructed in 2002, as a spring chinook acclimation facility. Spring chinook smolts are acclimated on site from March until release in April.

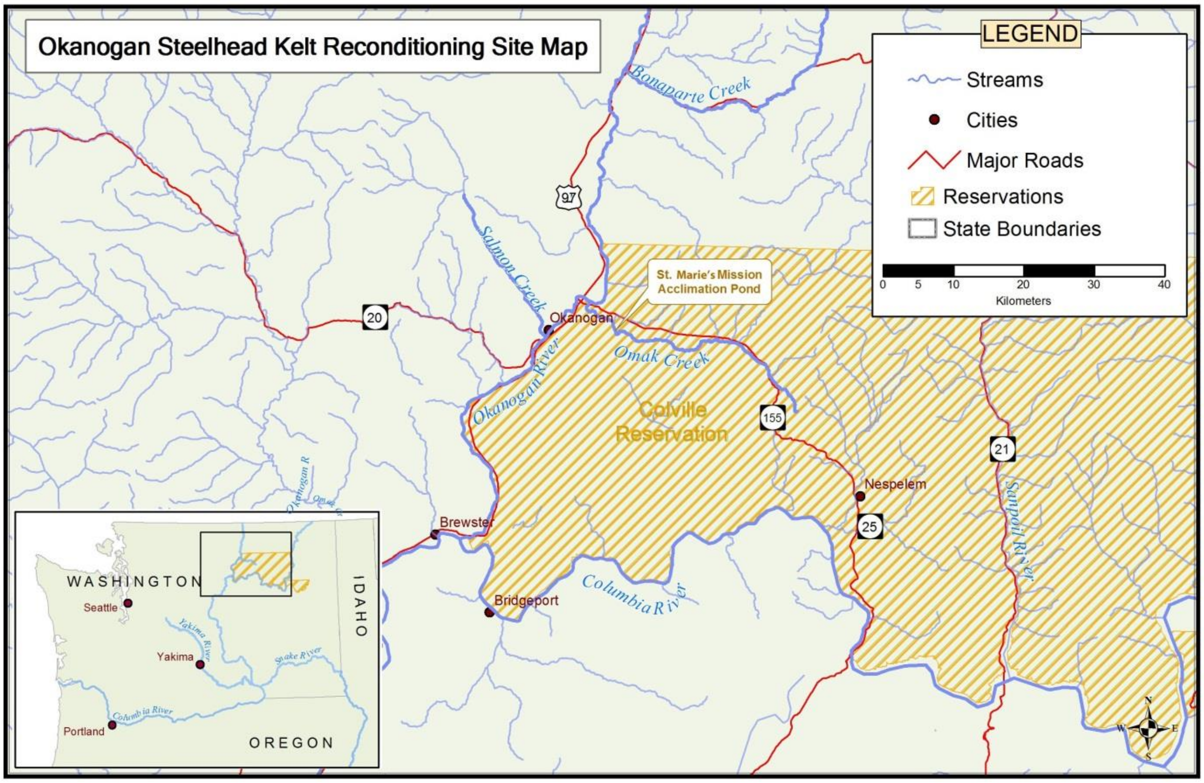


Figure 11: Map showing the locations of Omak Creek, the St. Marie's Mission Acclimation Pond, and the Confederated Tribes of the Colville Reservation.

Parkdale Fish Facility

Steelhead kelt reconditioning for the Hood River was performed at the Parkdale Fish Facility located at RK 5.6 on the Middle Fork of the Hood River (Figure 12). The Bonneville Power Administration originally built the facility in 1998 as an adult holding facility for ESA listed summer/winter steelhead and spring Chinook (reared at Oak Springs). Currently, the facility produces winter steelhead (reared at Oak Springs) and spring Chinook (reared at Parkdale and Oak Springs) for supplementation and terminal fisheries (non-Indian sport, Tribal commercial and Tribal subsistence fisheries). The facility is co-managed by Confederated Tribes of the Warm Springs Reservation and Oregon Department of Fish and Wildlife.

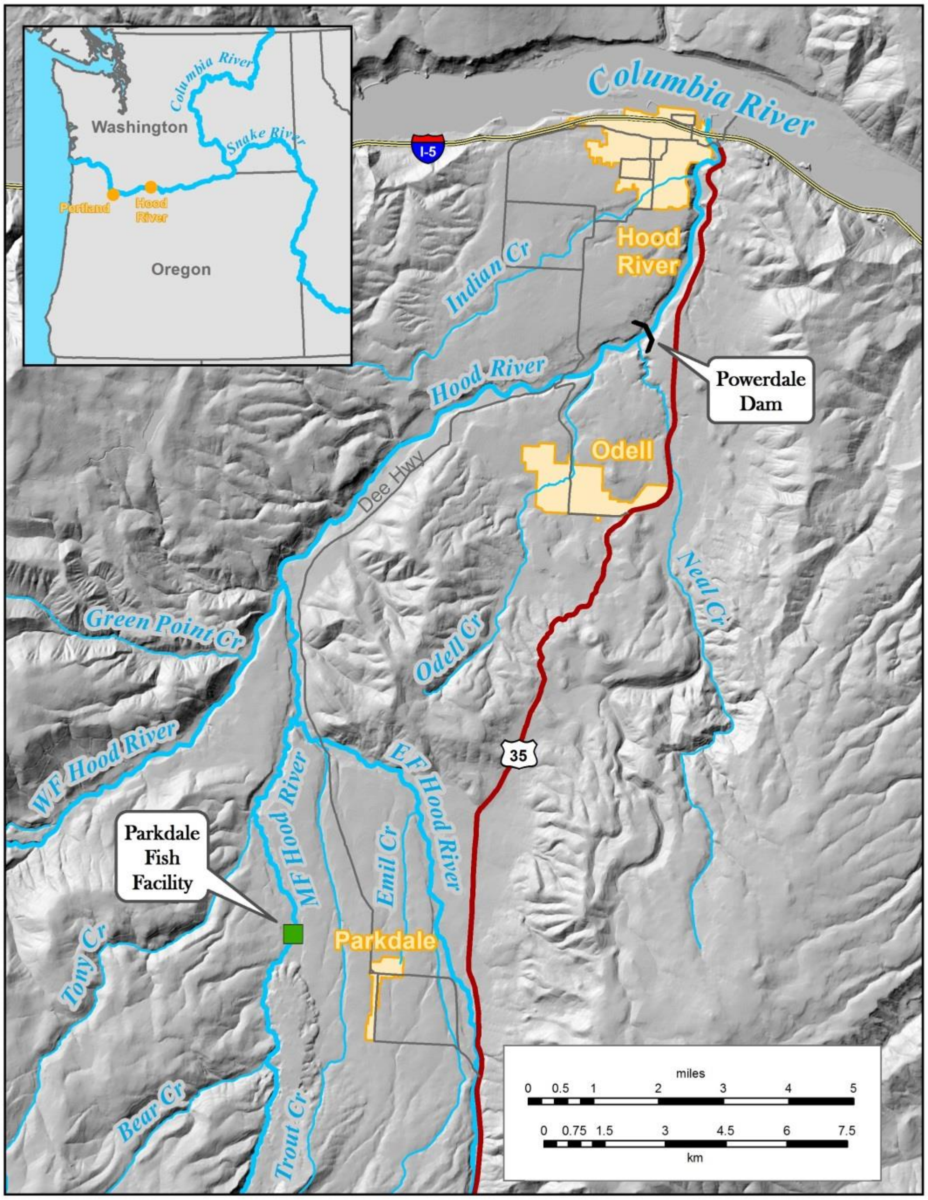


Figure 12: Map showing the location of Parkdale Fish Facility and East Fork Hood River weir.

Methods

Kelt Collection and In-Processing

Chandler Juvenile Monitoring Facility (Yakima River)

Migrating post spawn steelhead are collected at the Chandler Juvenile Monitoring Facility (CJMF) which diverts migratory fishes away from the irrigation canal. Kelts are manually collected from a fish separation device, that smaller juvenile salmonids "fall through" while larger fish can be dipnetted for processing (Figure 13). Yakama Nation staff monitored the Chandler bypass separator during the kelt migration.

Figure 13: 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^{0}C (Figure 14). 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.

The long-term reconditioning continued the feeding trial that compared an "orange" diet that was comprised of a mix of biovita brood pellets top coated with a mix of cyclopeeze and Alaskan fish oil versus the standard diet Bio-brood and krill. Both groups received krill at the start of reconditioning to initiate a feeding response for the same duration of time.

Figure 14: 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) operated by the Army Corps of Engineer (COE) staff. Kelts are collected off the adult fish separator bars and moved to a fish hopper that led into the kelt receiving tank (Figure 15). Both B-run (≥ 70 cm) and A-run (<70 cm) steelhead are selected. In 2013, 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 15: 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 16).

Figure 16: Tanks designed by the UI for holding and sorting kelts at Lower Granite Dam.

Every day, staff from the NPT, UI or CRITFC processed fish. Fish were anesthetized in tricaine methanesulfonate (MS-222) 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.

Omak Creek weirs

The Omak Creek weir (RK 0.8) is utilized to collect broodstock and steelhead kelts for reconditioning (Figure 17). This stock is being used to develop a naturalized steelhead broodstock for the Okanogan River and Omak Creek. All anadromous *O. mykiss,* regardless of up or downstream movement including those

selected for broodstock or reconditioning, were sexed, sampled for length, condition factor, inspected for tags (PIT or other), sampled for DNA and marked with a fin clip. PIT tags were applied if not already present.

Figure 17: Resistance board weir and fish trap located on Omak Creek.

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.

Long term Reconditioning

Facilities

Prosser Hatchery

Kelts were captured at the CJMF 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 to the hatchery (Figure 18). 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 18). 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 18: 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 (approximately 1-2cc). 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 intramuscular 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 is administered approximately five times a week (depending on fungal growth) at 1:6,000 for 1 hour in all reconditioning tanks to treat and prevent fungal outbreaks in cultured kelts.

Dworshak National Fish Hatchery

Transport to Dworshak from Lower Granite Dam

Fish destined for DNFH were dipped netted from the adult holding tank at Lower Granite Dam and placed in a transport truck. Nets were large enough to handle active adult steelhead and consisted of a soft cotton or

natural fiber mesh. The transport truck had a 1.5-kiloliter tank fitted with supplemental regulated, compressed oxygen that was fed via air stones; also a 12-volt powered tank aeration pump was used to circulate oxygenated water. Stress Coat® or PolyAqua® (5ml/38ml of water) was used to replace the natural protective slime coating that may have been compromised by handling. In addition, salt (6.8kg/1.7 kl) 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.

Similar to 2010 and 2012, steelhead were air-spawned at DNFH to augment the number of fish for reconditioning experiments. Fish came from two sources: swim-ins from the adult ladder at DNFH and from the South Fork of the Clearwater River. Steelhead were crowded into collection baskets and anesthetized in tricaine methanesulfonate (MS-222) or Aqui-S® (clove oil). Carbon dioxide was not used because it presents sub-lethal stresses that were likely to be adverse to survival of the kelts. Sorted steelhead were emptied on to a large stainless steel table and assessed by observing several physical factors prior to being selected for air spawning and reconditioning. Fish health was evaluated by: 1) maturation level - only very ripe females and 2) morphological fitness – no physical injuries on the body surface, no obvious fungus present, no fin rot or head burn. Additional fish were air-spawned, PIT tagged, and released into the mainstem Clearwater River after a three-day recovery period.

Selected fish were transferred to an area set aside for the air-spawning procedure. Low-pressure compressed air was injected into the fish using a 20-gauge needle. Eggs were allowed to flow freely with some gentle massage to obtain the remainder (Figure 19). 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 and frozen. A portion of these eggs were donated to Idaho Fish and Game's sturgeon project for use as bait and a portion was used to supplement feed for reconditioning kelts.

Figure 19: Air-spawning steelhead at Dworshak National Fish Hatchery 2013 (Ryan Branstetter to the left, Neil Graham on the right, and not fully pictured Jeremiah Newell).

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 Fatmeter to the outside of the fish. Tagging needles and PIT tags were disinfected before each use by soaking them in 70% ethyl alcohol, and subsequently dried. A 12mm PIT tag was inserted with a sterile 8-gauge trocar midway between the pelvic fins. Length and weight were recorded. Fish received an injection of oxy-tetracycline. After sampling, each fish was placed in a recovery tank for observation prior to transfer to the kelt reconditioning tanks.

Reconditioning Facility and Treatment

Four 4.5m diameter tanks are located at DNFH (Figure 20). 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 20: Experimental kelt reconditioning tanks at DNFH with anti-jump containment curtains and four bucket Koch ring packed columns.

St. Maries Acclimation Pond

Transport to St. Maries

Kelts collected for reconditioning are captured exiting Omak Creek at, or above the trap site and transported to the St. Maries Acclimation Facility. Fish were transported by 1.5 kL tank truck to St. Maries acclimation pond from the Omak Creek weir. Polypond water conditioner was used to protect slime coat and the addition of 3% salt was used to lightly sedate fish.

Reconditioning Facility and Treatment

St. Maries Mission Pond is 22.0m (l) x 3. 7m (w) x 1.2m (h) served by a screened gravity fed water supply from Omak Creek and a well that delivers 11^0C water up to 2.1 kl/m (Figure 21). The kelts were sectioned off in 2013 and initially held in a 3.1m (l) x 3. 7m (w) x1.2m (h) area. Fish were moved after a two-week period

to the other side which was 18.9m (l)x 3. 7m (w) x 1.2m (h). The top of the pond is covered by shade cloth (60% reduction) to reduce stress and control algae growth. Salt was placed at the head of the pond to prevent fungus and algae growth in the pond. An initial injection of liquamyacin was administered based on WDFW recommendations. Every 2 months, fish were checked for copepods, and given emamectin benzoate solution if copepods were present. Feed consisted of squid, prawns, and sand shrimp with a top coating of Vitamin C to provide for immune system enhancement.

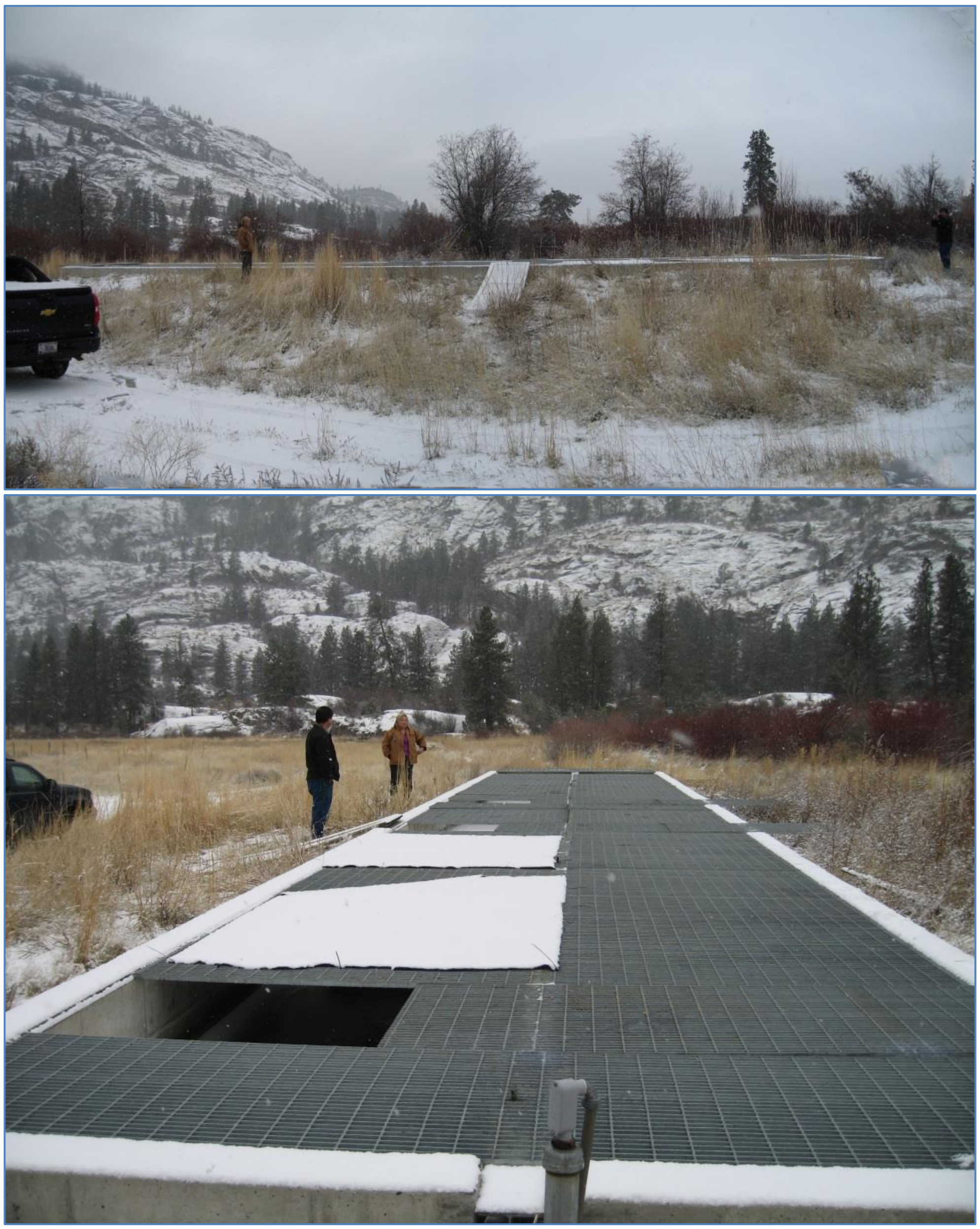


Figure 21: St. Maries Acclimation Pond Omak, WA. Top (side profile), bottom (above) (Doug Hatch to the left and Rhonda Dasher to the right).

Parkdale Fish Facility

Transport to Parkdale Fish Facility

Adult steelhead were collected by Oregon Department of Fish and Wildlife and Parkdale Fish Facility staff at the weir on the East Fork of the Hood River located at RK 20 south of the city of Hood River, Oregon. Fish were transported by truck to the Parkdale Fish Facility.

Reconditioning Facility and Treatment

Maiden steelhead were held, air spawned, and reconditioned at Parkdale Fish Facility. Winter-run and remaining summer-run steelhead kelts are placed into a 12. 2m (l) x 2.4m (w) x 1.2m (h) raceways at 1.5 kpm until ripened and ready for spawning. All incoming fish were inspected for copepods and received a 1-2cc injection of diluted emamectin solution as a parasitic preventative and florfenicol (2cc) injection as a preventative against cold-water disease. Formalin treatments were administered at 1:6000, 3 times weekly for one hour to prevent against fungal infections. After air spawning steelhead were moved to round tanks (1.2m (h) x 3.1 (d)), segregated by run, with water flow at 0.23 kL/m for reconditioning (Figure 22). Post spawn females were administered another dosage of emamectin after completion of air spawning. All kelts were checked in June and again in September for the presence of copepods and administered additional emamectin benzoate treatment if copepods were present. The antibiotics (oxytetracycline and erythromycin) were also administered prophylactically to prevent against cold-water disease in June and reapplied later in the end of August when bacterial related mortality occurred. Administration was Erythromycin 200, (Gallimycin 200) 200 mg/ml, 22 mg/Kg (8 mg/ lb) body weight and Oxytetracyline HCl (Oxytet 100) 100 mg/ml, 10 mg/kg (5 mg/lb) body weight.

Figure 22: Circular tanks at Parkdale Fish Facility used seasonally (late spring into 2013) for reconditioning kelts.

Kelt Mortalities

On discovery of a mortality, fish were collected, scanned for PIT tags, recorded in the database, and examined externally by hatchery personnel to record the suspected time of death, general condition (good, fair, poor), fish color (bright, intermediate, dark), color of the gill arches (red, pink, white), size of the abdomen (fat, thin), presence of any scars or obvious lesions, and any other anomalies. Once the external

exam was completed, an internal examination was conducted to record color of muscle tissue (red, pink, white), type of gonads (ovaries, testes), size of gametes (small, large), and presence of any internal anomalies. PIT tags were removed from mortalities and identification numbers recorded onto computer databases along with growth measurement data. The Lower Columbia Fish Health Center (Prosser), Washington Department of Fish and Wildlife Pathology (Omak), Oregon Department of Fish and Wildlife Pathology (Parkdale) provided disease-monitoring services to insure the health of reconditioned steelhead kelts.

Steelhead Kelt Status and Release

All surviving kelts, prior to release in late fall were scanned for PIT tags, weighed, and measured for fork-length. Reconditioning success was based on the proportion of fish that survived the reconditioning process. Fish were then released to the river to coincide with fall spawning migration and others were retained or terminated. Long-term reconditioned fish located at the Prosser Fish Hatchery were released just below Prosser Dam to utilize PIT-tag detectors in the dam's fish ladders to determine the number of steelhead that are actively migrating to spawning grounds. At Dworshak NFH any unclipped (wild) kelts that survive reconditioning were released in the fall just below Lower Granite Dam or returned to the South Fork Clearwater River if collected there. Fish in the long-term experiments at St. Maries were released approximately at RK 1 of the Okanogan River. Based on previous releases, the majority of long term reconditioned kelts over-winter within the systems they are released to, and volitionally return to the spawning grounds in late winter and spring. PIT tag detectors were monitored for subsequent migration data. The only non-release groups are the Parkdale Fish Facility Steelhead and hatchery fish reconditioned at Dworshak NFH, which were terminated or retained for physiological studies.

Management Scenario Evaluation

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

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

Results

General Population Characteristics of Captured Kelts

Yakima River

A total of 640 live kelts were captured between March 6 and June 25, 2013 at the CJMF. There were 13 steelhead discovered dead upon arrival in the bypass, 42 kelts in poor condition, and 33 prespawn (maiden) steelhead that were released immediately back to the Yakima River on site. A total of 52 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 6, 2013 (Figure 23). The bypass was shutdown from April 6 through April 10 and May 10 through May 19 for the removal of flood transported flotsam, preventing kelt collection. The total number of kelts captured represented 14.3% (686 of 4,787) of the Yakima River spawning migration based on fish ladder counts obtained from Prosser Dam for the period July 1, 2012 through June 30, 2013. This collection only represents the portion of the population that volunteer into the Chandler bypass facility while others migrate over Prosser Dam.

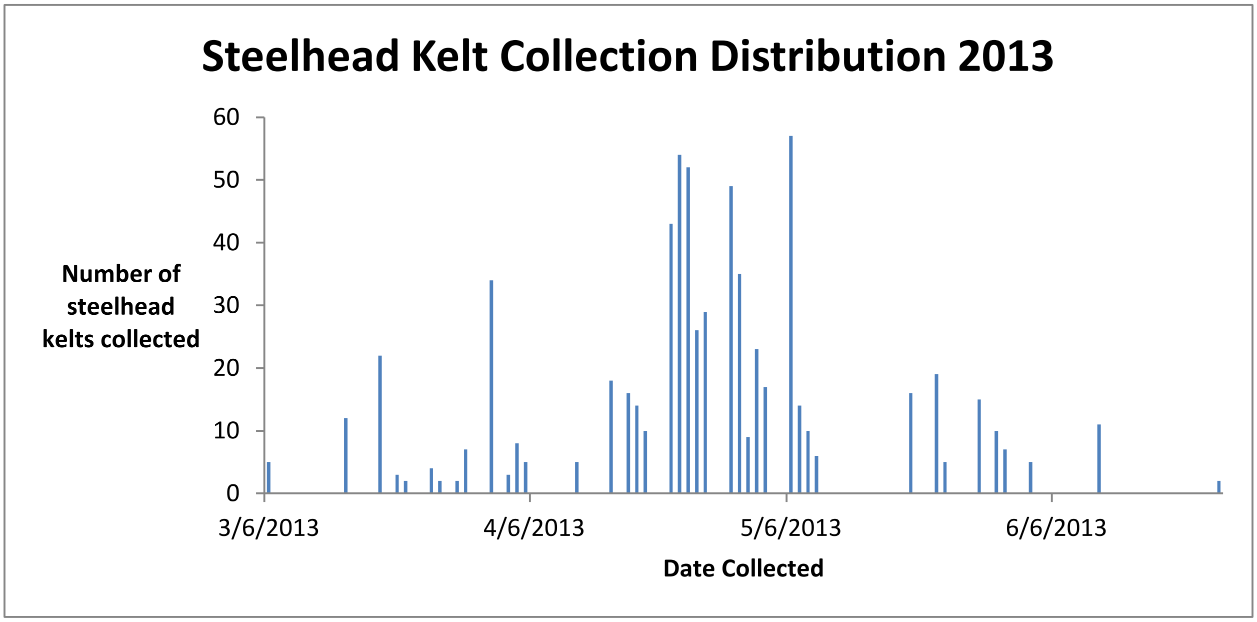


Figure 23: Yakima River kelt steelhead collection at CJMF Prosser, WA in 2013.

Based on visual observations, 581 of 640 (91%) of the kelts were female and 59 (9%) were male which is consistent with previous findings (Hatch et al. 2012). Most kelts were classified as fair (n=383, 60%) condition followed by good condition (n=212, 33%) and finally as poor condition (n=45, 7%). Coloration was predominately intermediate (n=362, 57%) or bright (n=226, 35%) with a small percentage that were dark (n=52, 8%).

Snake River

The LGR JFF between April 7 and July 16, 2013 intercepted a total of 954 kelts. Collection was mostly continuous throughout the season. The separator was shutdown on a few days for a few hours to clear debris. The peak collection (82 fish) occurred on May 12, 2013 (Figure 24). In addition, Pacific Northwest National Laboratory (PNNL) outfitted a subsample of 316 fish with Juvenile Salmon Acoustic Telemetry system (JSATS) tags. These fish were held for one day and released below Lower Granite Dam (Colotelo et al. 2013). Table 4 summarizes the final disposition of kelts collected by the LGR JFF. There were 46 collection/handling mortalities.

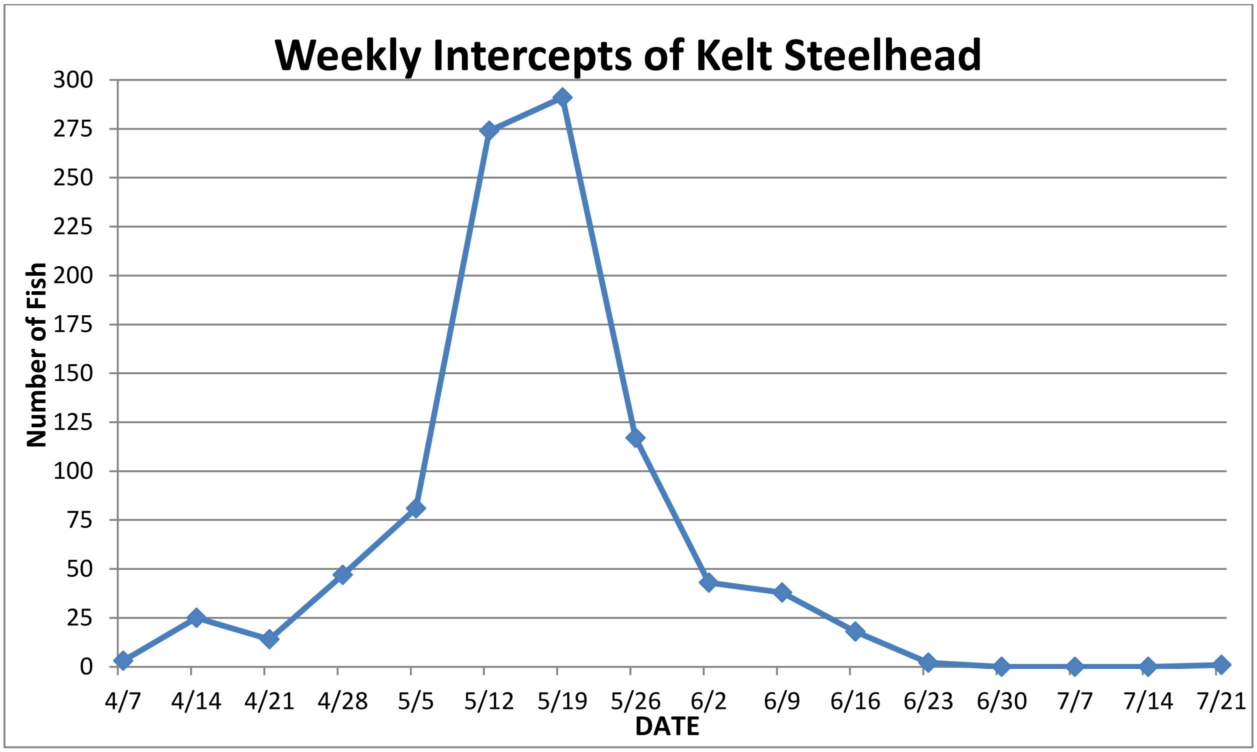


Figure 24: Weekly steelhead kelt collections at LGR JFF in 2013.

Table 4: Summary of final disposition of fish collected at LGR JFF in 2013.

Final Disposition	A-run	B-run	Total
Returned to River	415	92	507
JSAT tagged and released	264	52	316
Transported to DNFH for reconditioning	39	73	112
Mortality	13	6	19
Total	**731**	**223**	**954**

The majority of the fish collected from the Snake River at LGR JFF in 2013 were A-run females in fair condition. Most fish were without any major wounds (scraps, cuts, fungal infections) with the majority of them collected in the month of May (Table 5). Females greater than or equal to 70 cm comprised 22% of the kelts collected at the LGR JFF in 2013 (Table 6). Of these, the proportion rated as being in good condition was 49.5% (Figure 25).

Table 5: Condition of Snake River Kelts collected at the LGR JFF in 2013.

	April	May	June	July	Total
Good	57	340	33	1	431
Fair	41	199	14		533
Poor	29	227	13		269

Table 6: Condition of steelhead kelts by sex and size at the LGR JFF in 2013.

Female	**Good (45.2%)**	**Fair (26.6%)**	**Poor (28.2%)**	**% of collection**
< 70 cm	254	133	146	55.9
≥ 70cm	104	51	55	22.0
			Total	**77.9**
Male				
< 70 cm	68	66	64	20.7
≥ 70cm	5	4	4	1.4
			Total	**22.1**
Total	431	254	269	**954**

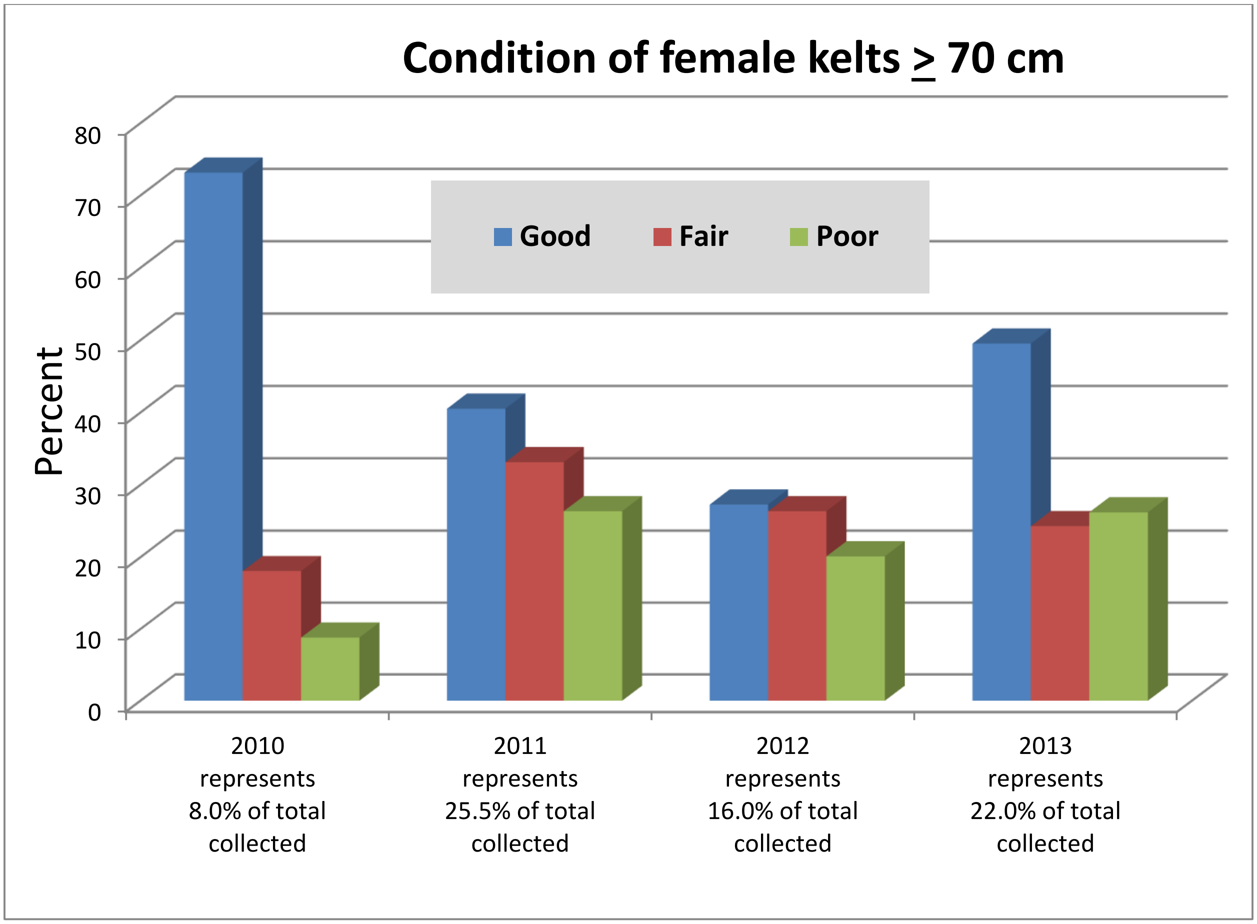


Figure 25: Percent comparison of female kelt steelhead > 70 cm at LGR JFF by condition and collection year.

Similar to 2010-12, fish were observed with recent head injuries (Hatch et al. 2012). These head injuries look very similar in nature (deep tissue wounds), which may indicate something restricting their journey to or through the bypass system (Figure 26). This type of injury is not typically seen in other reconditioning sites and if present, not observed as high a frequency as is found in the Snake River. Overall, the proportion of head injuries was 55.5% (Table 7). The weekly proportion varied between 36.4% (N=4) and 100% (N=1) throughout the collection season; however, no apparent pattern was observed with discharge (Figure 27).

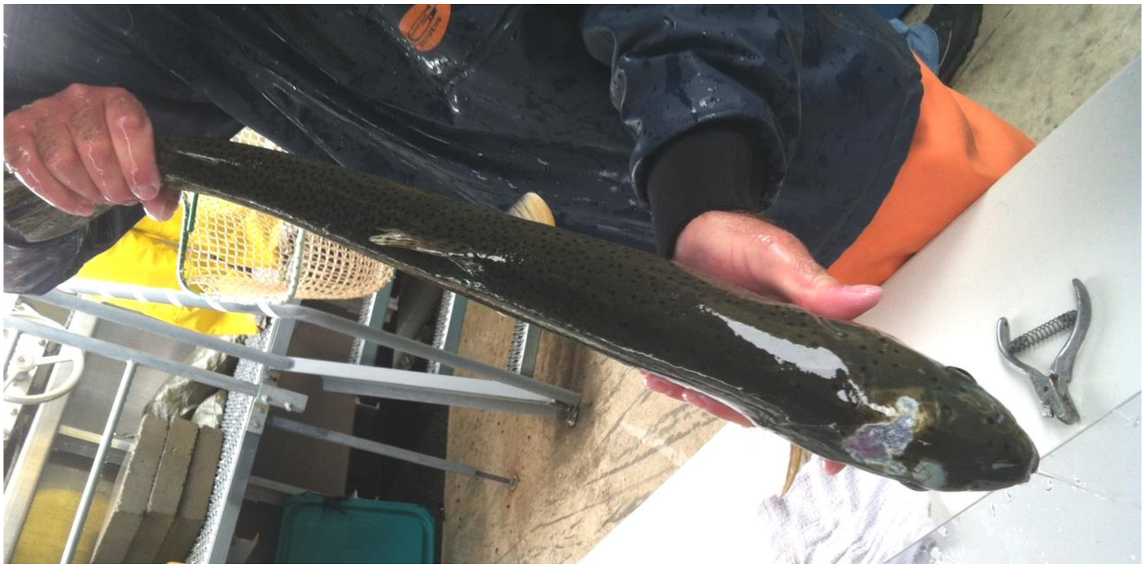


Figure 26: Typical head injury observed on steelheads kelts at LGR JFF.

Table 7: Percent of head injuries on steelhead kelts at the LGR JFF during 2013.

	A-run	B-run	Total
No	34.1	10.4	44.5
	(N=325)	(N=99)	(N=424)
Yes	42.6	12.9	55.5
	(N=406	(N=124)	(N=530)
Total	76.7	23.3	100
	(N=731)	(N=223)	(N=954)

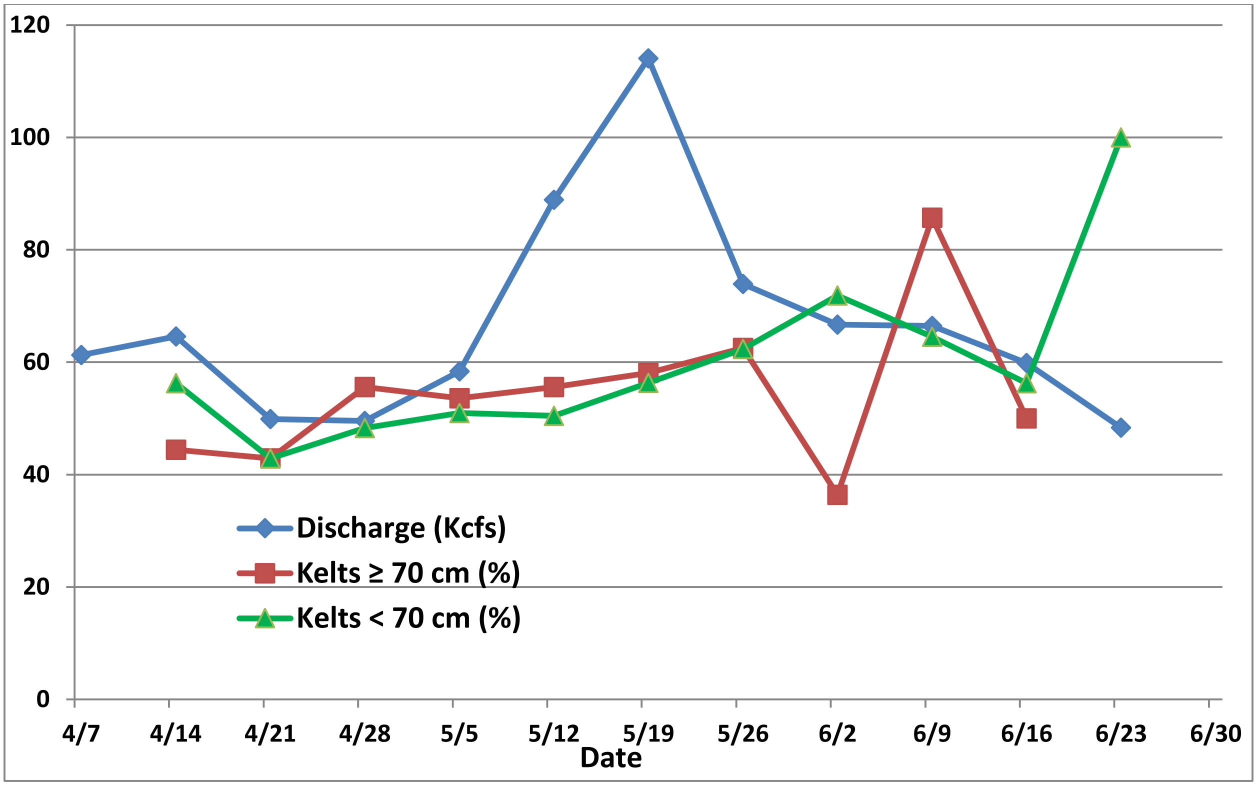


Figure 27: Mean weekly discharge (Kcfs) and percent head injuries observed on steelhead kelts at the LGR JFF in 2013.

Omak Creek

A total of 282 adult summer steelhead were captured and sampled (includes first-time spawners and kelts) between March 5, 2013 and June16, 2013. The panels at the adult weir were compromised (down) from April 3, 2013 through April 19, 2013 allowing an unknown number of steelhead to pass upstream. Prior to the panels going down a total of 94 adults were sampled and passed upstream which consisted of 52 hatchery males, 36 hatchery females, 4 wild males and 2 wild females (Table 8).

After high water subsided panels were repaired, and kelt collections began from April 20-June 16, 2013, a total of 188 kelts were sampled at the trap. A total of 79 (42%) of the sampled upstream migrants were recaptured as kelts. A number of the kelts washed up on the weir as mortalities with 34 hatchery males, 27 hatchery females, 1 wild male, and 3 wild females (Table 8). Sixty three kelts were passed downstream due to poor condition (excessive fungus, wounds). A total of 49 kelts including: 6 hatchery males, 36 hatchery females, 2 wild males, and 5 wild females retained for reconditioning at St. Maries Acclimation Pond. Portions of the collected kelts retained for reconditioning were originally sampled as upstream migrants (3 hatchery males and 3 hatchery females).

Table 8: Percentage and totals of male, female and wild summer steelhead passed above the Omak Creek weir, 2013.

Upstream Sample			Downstream Kelt Sample					
	Total Sample (N)	Non-clipped (N)		Total Sample (N)	Passed Downriver (N)	Dead On Arrival (N)	Retained for Reconditioning (N)	Non-Clipped (N)
Males	56	4	**Males**	87	41	35	11	6
Females	38	2	**Females**	101	27	30	44	10
Total	**94**	**6**	**Total**	**188**	**68**	**65**	**55**	**16**
All Steelhead Sampled Total:			**282**					

Bonaparte Creek

No trapping was conducted on Bonaparte Creek in 2013.

Hood River

No steelhead were collected at the Hood River in 2013.

In-River Release and Return Detection Results

Yakima River

2013 In River Control

A total of 52 kelts were released as in-river control fish in the Yakima River in 2013 with no sequential spawners detected to date. Skip spawning results will be reported in 2014.

2012 In-River Control

Two of the 59 in-river treatment fish released in the Yakima River in 2012 were detected moving upstream at Bonneville Dam in early July through August in 2013 exhibiting a skip spawner life history (Table 9). One fish successfully passed McNary Dam in mid to late September 2013. Neither of these fish was detected passing upriver at Prosser Dam, with no detections passing Priest Rapids Dam or Ice Harbor Dam (Table 9).

Table 9: Yakima in-River releases in 2012 (59 kelts) with return detections by year (2012 sequential spawners and 2013 skip spawners) and dam.

2012 Yakima In- River				
Detection Year	Bonneville Dam	McNary Dam	Prosser Dam	% return of total release to Yakima R.
2012	0	0	0	0%
2013	2	1	0	0%
Total	2 (3.4%)	1 (1.7%)	0	0%

Snake River

2013 Downstream Detection of Control After Release in 2013

As of November 30, 2013, there were a total of 19.4% detected (Table 10) migrating downriver. Data are still being analyzed on site-specific subsequent detections, as well as previous year's releases.

Table 10: Number of kelts PIT tagged and released at Lower Granite Dam in 2013 separated by gender, length and presence (Unclipped) or absence (Clipped) of an adipose fin, as well as the number and percent detected at least once in the Snake or Columbia rivers migrating downriver.

	Tagged and Released			Detected Migrating Downriver			Total
Group and Condition	< 70 cm	≥ 70 cm	Total	< 70 cm	≥ 70 cm	Total	% Detected
Males	**195**	**13**	**208**	**40**	**2**	**42**	**20.2**
Unclipped	128	13	141	25	2	27	19.1
Clipped	67	0	67	15	0	15	22.4
Females	**484**	**133**	**617**	**97**	**21**	**118**	**19.1**
Unclipped	232	80	312	47	13	60	19.2
Clipped	252	53	305	50	8	58	19.0
2013 Total	**679**	**146**	**825**	**137**	**23**	**160**	**19.4**

2013 In-river Control Detections

None of the 2013 in-river release kelts returned to Bonneville Dam as sequential spawners in 2013. Skip spawning for this tag and release year will be determined in the 2014 annual report.

2012 In-river Control Detections

From the 2012 in-river kelt release one sequential spawner returned in 2012 and 5 skip spawners detected returning to Bonneville Dam in 2013 (Table 11). All of these fish were considered a-run fish and were in the 50-60 cm range. They were detected returning in late July/early August of 2013 to Bonneville Dam. Four of these fish were successfully detected migrating passing McNary Dam in late July/early August, Ice Harbor in mid August/early September, and then over Lower Granite Dam in early September of 2013 (Table 11).

Table 11: 2012 Snake In-River release with return year and detection site (2012 and 2013) and the number detected at each dam.

2012 Snake In-River Releases						
Total Tagged and Released: 2,093						
Return Year	Bonneville Dam	McNary Dam	Ice Harbor Dam	Lower Granite Dam	% return of total release to Bonneville Dam	% return of total release to Lower Granite Dam
2012	1	1	1	1	0.05%	0.05%
2013	5	4	4	4	0.24%	0.19%
Total	6	5	5	5	0.29%	0.24%

Long-Term Reconditioning and Survival to Release or Spawning

Prosser Fish Hatchery

A total of 545 kelt steelhead were collected and placed in the long-term reconditioning tanks. Survival to release processing on October 23, 2013 was 266 (49%) (Table 12, Figure 28). A total of 224 long-term reconditioned fish were released to the Yakima River. An additional 42 kelts with low estradiol levels were retained and will be released in the fall of 2014. Steelhead kelts were released, approximately two weeks later than usual to avoid a WDFW sport fishery near the release site. Most migratory movements are expected to occur in late October through November of 2013 but there is typically a small number of kelts that will migrate in February/March of 2014. These fish will be reported in the 2014 annual report. As of early December of 2013, 93 (42%) fish from the long-term release were detected by PIT tag presence migrating past Prosser Dam.

Table 12: Long-term reconditioning results by tank 2013 at Prosser Hatchery.

Long-term Reconditioning									
	Tank								Long-term
	C1	C2	C3	C4	S1	S2	S3	S4	Total
Held for Reconditioning	115	113	122	115	20	20	20	20	545
Surviving fish on 10/23/2013	52	53	49	46	19	15	15	17	266
Survival Rate	45.2%	46.9%	40.2%	40.0%	95.0%	75.0%	75.0%	85.0%	48.8%

Figure 28: Long term reconditioned kelt steelhead from the Yakima River just prior to release in 2012 (Joe Blodgett pictured).

2011 Skip Spawning Long-Term Reconditioned Kelts through 2012

There were a total of 32 non maturing kelts from the 2011 long-term reconditioning program that were retained to determine how well they would recondition through 2012. Nine of these fish survived to release in the fall of 2012. As of May 2013, 6 of these fish were detected migrating upriver over the Prosser Dam indicating successful skip spawner reconditioning.

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

A skip spawning migration pattern was observed in 1 previously long-term reconditioned kelts from the 2011 release that had a PIT-tag detection at Bonneville Dam in late summer (August) of 2012. No further upstream detection was made of this fish.

Dworshak National Fish Hatchery Long-term Reconditioning.

Lower Granite Juvenile Fish Facility Steelhead

A total of 112 fish were transferred from the LGR JFF to DNFH for reconditioning in 2013 (Table 13). Fish survival averaged 98.0 days after transfer to the reconditioning tanks (Figure 29). Fish survived an average 74 more days than the fish transferred in 2012. We experienced consistent but low (<5 per day) mortalities for the first four months. Little mortality was observed after the first week of August (Figure 30). There was a total of 57 (unclipped) fish released to the Snake River during the fall of 2013 to coincide with the returning steelhead run.

Table 13: Snake River steelhead kelts collected at LGR JFF and transferred to DNFH for reconditioning in 2013.

	A-run	B-run	Total
Adipose Clipped	0	1	1
Un-Clipped	39	72	111
Total	**39**	**73**	**112**

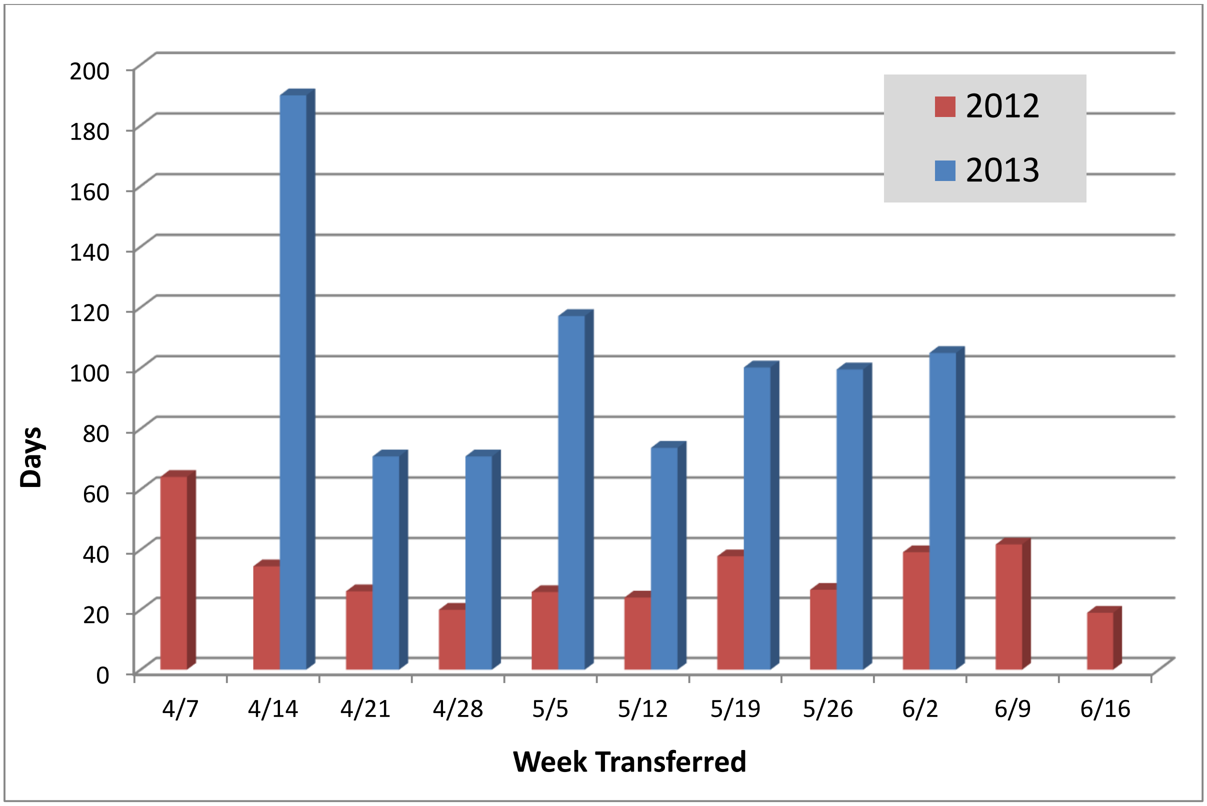


Figure 29: Mean weekly survival (days) of steelhead kelts transferred from LGR JFF to DNFH for reconditioning in 2012 and 2013.

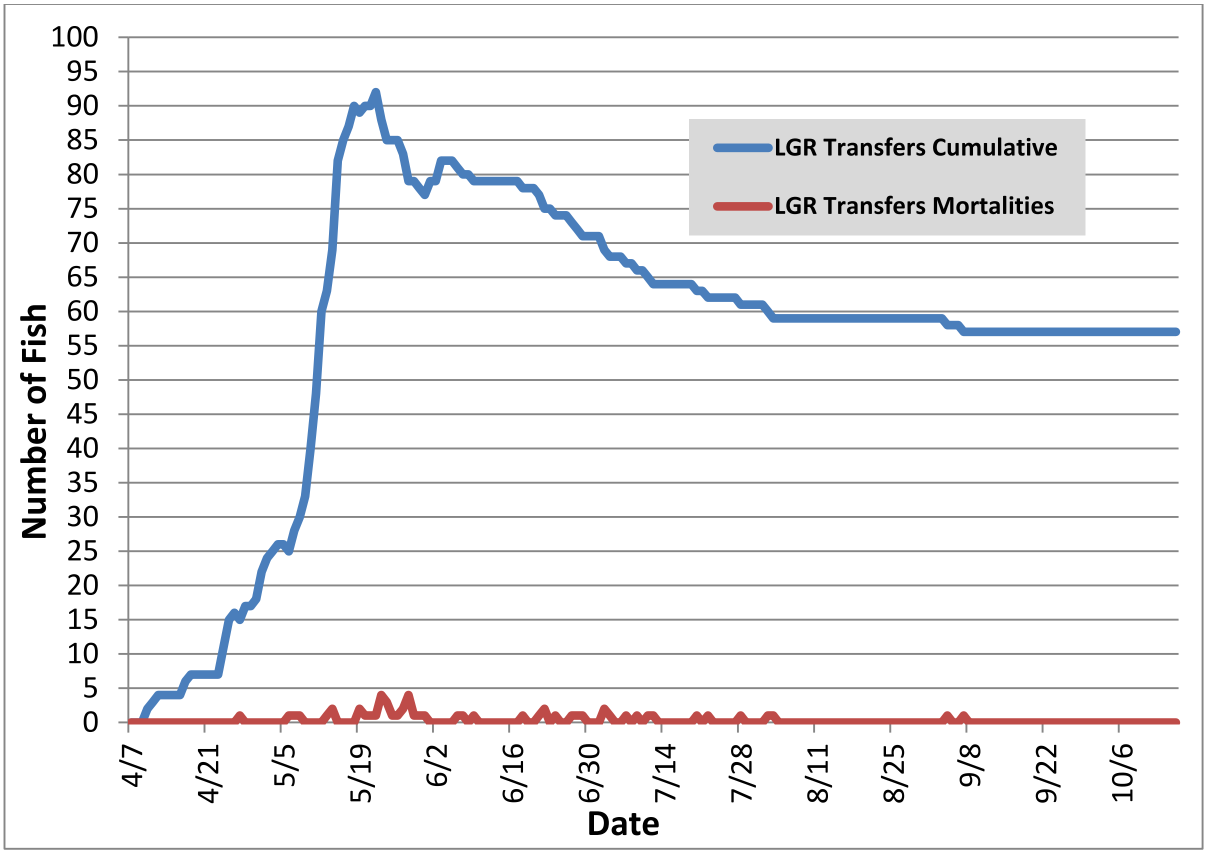


Figure 30: Cumulative on-station holding and daily mortality of steelhead kelts transferred from LGR JFF to DNFH for reconditioning in 2013.

Air-Spawned Steelhead

A total of 524 steelhead were air-spawned on four separate days. Fish were retained for reconditioning only during the first three days (Table 14). Fish from the SF Clearwater survived an average 139 days after transfer to the reconditioning tanks (Figure 31). A total of 12 SF Clearwater River fish were released into the mainstem Clearwater River on October 3, 2013 (Figure 32). Each fish received a radio tag and will be tracked by staff from the NPT and IDFG. As of November 30, 2013, fish from the DNFH ladder survived an average 155 days after transfer to the reconditioning tanks (Figure 32). A total of 52 DNFH ladder fish remain on-station. These fish will continue to be monitored for ripeness. Surviving mature fish will be spawned during the spring of 2014.

Figure 31: 2013 DNFH long term reconditioned kelt steelhead from the SF Clearwater River prior to release (Dr. Andrew Pierce pictured).

Table 14: Snake River steelhead air-spawned and kept for reconditioning in 2013.

	DNFH Ladder		**SF Clearwater River**	
Spawn Date	Air Spawned	Kept for Reconditioning	Air Spawned	Kept for Reconditioning
Feb 26, 2013	162	72	0	0
March 5, 2013	115	68	9	9
March 12, 2013	75	23	30	15
March 17, 2013	135	0	0	0
Total	**487**	**163**	**39**	**24**

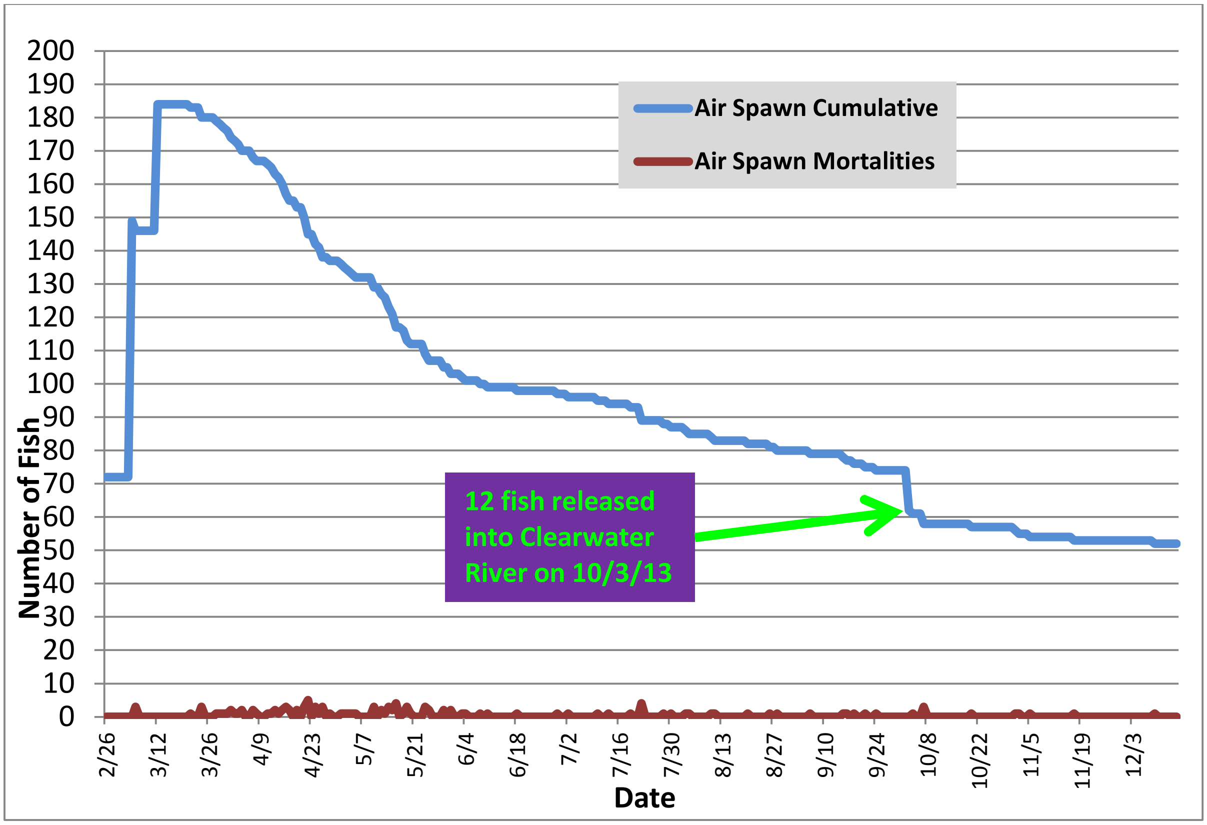


Figure 32: Cumulative on-station holding and daily mortality of steelhead kelts air-spawned at DNFH for reconditioning in 2013.

Reconditioned Kelts Released in 2013

As of November 30, 2013, 11 of the 12 fish released into the Clearwater River have been located by radio receiver. Both NPT and IDFG continue to track these fish in the Clearwater River. As of November 30, 2013, we detected 10 of the 57 reconditioned kelts that were PIT-tagged and released into the Snake River (Table 15). Fish were released 3.5 km below Lower Granite Dam on October 17, 2013. Eight of these kelts were detected ascending the adult ladder at Lower Granite Dam between 2 and 4 days after release. This indicates they may sequentially spawn. The remaining two fish were detected moving downstream. These fish may be expressing a skip spawner or fluvial type life history.

Table 15: 2013 Reconditioned kelts released with detection year and site and the number detected at each dam.

2013 Reconditioned Kelt Releases			
Total Tagged and Released: 57			
Detection Year	Little Goose Dam	Lower Granite Dam	% detected
2013	2 (fall)	8 (fall)	17.5%

St. Maries Fish Acclimation Site

Reconditioning

Of 49 kelts trucked to St. Marie's Acclimation Pond, 4 females survived reconditioning and were released into the mouth of the Okanogan River (4 hatchery) on October 3, 2013. Upon visual inspection all 4 of these fish were in good to excellent condition (Figure 33). PIT-tag arrays will alert staff to the movement of these fish in the basin.

Figure 33: Long-term reconditioned Omak Creek kelt just prior to release in 2013.

Parkdale Hatchery

Summer Run Steelhead Reconditioning

Summer-run kelt collections were discontinued in 2010 with the dismantling of Powerdale Dam (RK 7), and ODFW stopped releasing smolts prior to the dam demolition. The remaining fish in the reconditioning program are remnants of these last captures at Powerdale and are continuing to be reared to get final spawning data. Fish not spawned at the end of June were terminated and gametes were examined to determine ripeness.

2010 Brood

This is the final group of summer-run kelts that were collected at the Powerdale Dam. There was 1 remaining female steelhead by the beginning of 2013. This fish was terminated at the end of June and gametes were examined for ripeness.

2009 Brood

One steelhead kelt successfully reconditioned for the 4th time and was spawned in 2013 and terminated (Table 16). This brood year was the only kelt group which had all skip spawners.

Table 16: Summer steelhead kelt reconditioning success and spawning by year 2006-2013. TBD=To Be Determined. IHN= Infectious hematopoietic necrosis.

Brood Year	2006	2007	2008	2009	2010
Maiden spawn	1	15 (2 culled IHN)	14	12 (3 culled IHN)	22 (7 culled IHN)
1st sequential spawning	1	1	4	0	0
Succ. Recon Rate %	100%	16%	50%	33%	13%
Skip Spawner kelt	0	1	3	2	2
% skip spawner of reconditioned fish	0%	8%	21%	22%	13%
2nd sequential spawning	0	0	2	1	0
3rd sequential spawning	0	0	2	1	0
4th sequential spawning	0	0	2	0	0

Winter Run Steelhead Reconditioning

The winter run steelhead are a locally adapted broodstock that are used for supplementation. These fish were initially captured at the Powerdale trap in 2010. The removal of Powerdale dam changed the capture location starting in 2011 and through 2012 to the East Fork Hood River resistance board weir trap. This collection location likely negatively impacted the survival of winter-run kelts in the reconditioning program.

2012 Brood

There were 14 kelts at the beginning of 2013 (Table 17). We had 4 mortalities in this group between January 1st and spawning time around April. We successfully spawned 3 kelts with the remaining 7 fish were terminated at the end of June. All terminated fish were in good health and were necropsied to determine gamete development (described in: Reproductive Success).

Table 17:Winter-run Kelt Reconditioning at Parkdale Fish Facility 2010-2013. TBD=To Be Determined. IHN= Infectious hematopoietic necrosis.

Brood Year	2010	2011	2012
Maiden spawn	22 (3 culled IHN)	22	22
1st sequential spawning	5	1	3
Succ. Recon Rate %	32%	4%	63%
Skip Spawner	0	0	7*
% kelt skip spawner of reconditioned fish	0	0	?
2nd sequential spawning	1	0	?

*based on necropsies we had 7 possible skip spawners for 2014.

Conclusions

In the following paragraphs we attempt to summarize data from a variety of locations that provides insight into evaluating kelt management scenarios. Comparisons are complicated by data being collected at different locations in different years so in Appendix (B1) we provide a comprehensive table of return rates and survival for all groups.

Comparison groups

Our comparison or control groups consisted of 1. The proportion of repeat spawners in the run at large at Bonneville Dam based on scale pattern analysis; 2. The return rate to Bonneville Dam of fish PIT tagged and released at CJMF and LGR JFF; 3. The return rate to Bonneville Dam of fish PIT tagged and released at John Day Dam; and, 4. the reported proportion of repeat spawners in the run at Prosser Dam based on scale pattern interpretation (Hockersmith et al. 1995) (Table 18). The proportion of repeat spawners in the run at large at Bonneville Dam is based on scale pattern interpretation of 10 years of data collected from 16,095 fish sampled in the adult trap (Miranda et al., 2004, Miranda et al., 2005, Whiteaker et al., 2006, Whiteaker and Fryer 2007, Whiteaker and Fryer 2008, Torbek et al., 2009, Kelsey et al. 2011a, Kelsey et al. 2011b, Nowinski et al. 2013a, Nowinski et al. 2013b, Personal Comm. J. Whiteaker 2013). The weighted mean composition of repeat spawners in the run at large at Bonneville Dam is 0.59%. This indicates that iteroparity is very low in steelhead populations above Bonneville Dam and in 2012 the return rate was 1.1%. The return rate to Bonneville Dam of kelts tagged and released in-river at Prosser Hatchery in 2012 (the last year with complete returns sequential and skip spawners) was 3.39% and the 8 year average of 3.40% is much higher than the run at large at Bonneville Dam suggesting the Yakima River fish may exhibit higher than average iteroparity rates relative to other tributaries. Repeat spawner composition in the Yakima River run based on scale pattern analysis (Hockersmith et al. 1995) was reported at 1.66% (Table 17). This estimate differs from the other control groups in that it is measured at Prosser Hatchery not at Bonneville Dam but further supports the notion that Yakima River steelhead exhibit higher iteroparity rates relative to the run at large measured at Bonneville Dam. The Bonneville Dam return rate of kelt steelhead tagged and released at John Day Dam was 9.76% (Evans and Werthiemer 2008). This is very high relative to other sites and includes only a single year (2002). Kelt returns in 2002 were the highest ever recorded for transported

fish collected at Prosser Hatchery and Lower Granite Dam as well suggesting that the return rate measured at John Day Dam is likely at the high end of the range. It also indicates that when environmental conditions are conducive, high iteroparity rates can be achieved in upriver stocks. The comparison group tagged and released at Lower Granite Dam returned to Bonneville Dam in 2012 at a rate of 0.05%. The 8 year mean return rate to Bonneville Dam for kelts tagged and released at Lower Granite Dam is 0.34%. This is quite low and not statistically different (pooled variance t test; t=-0.7; p=0.496) from the run at large at Bonneville Dam.

Table 18: The return rate in 2013 (last year with complete returns of sequential and skip spawners) and the mean from available years to Bonneville Dam of repeat spawners from various locations used as "controls" or comparison groups. Note that Hockersmith is a return rate to Prosser Hatchery not Bonneville Dam. Starred groups are based on scale pattern analysis; the remaining groups are based on returns of PIT tagged fish.

Return Rate timeframe	Bonneville*	John Day	Prosser	Lower Granite	Hockersmith*
2013	1.1	-	3.39	0.05	-
mean	0.59	9.76	3.40	0.34	1.66

Treatment Groups

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

No kelts were detected returning to Bonneville Dam from fish collected at Lower Granite Dam and transported to Hamilton Island or to Westport, OR. The 6 year mean return rate to Bonneville Dam for fish collected at Lower Granite Dam and transported is 1.12. Four kelts were detected returning to Bonneville Dam from fish collected at Prosser Dam and transported to Hamilton Island (1 fish) and Westport (3 fish). Return rates of Prosser collected fish to Bonneville Dam were 1.00% for the Hamilton Island release and 3.33% for the Westport release. Both of these return rates are lower than the 9 year mean return rate of 4.16%.

The kelts collected at Prosser Hatchery and transported to Hamilton Island had treatment benefits of 0.78, 2.63, and 0.60 relative to the control metrics of in-river, the steelhead run at large at Bonneville Dam, and the Hockersmith (1995) value of 1.66, respectively. The Prosser kelts released at Westport, OR showed higher treatment benefits of 2.58, 8.77, and 2.01 relative to in-river, the steelhead run at large at Bonneville Dam, and the Hockersmith (1995) value of 1.66, respectively. This increase is due to 2 skip spawner returns in 2012. As previously noted, any number greater than 1 is a positive benefit and any number less than 1 is a negative benefit. Nine years of transport data from Prosser origin kelts shows some benefits to transported kelts relative to control groups: 1.35, 8.55, and 2.64 relative to in-river, the steelhead run at large at Bonneville Dam, and the Hockersmith (1995) value of 1.66, respectively (Appendix B1).

Transport benefits for Snake River origin kelts collected at Lower Granite Dam are very low and difficult to measure due to very low returns to Bonneville Dam from these treatment groups. In 2011, no fish returned

to Bonneville Dam from either the Hamilton Island or Westport, OR release of Snake River origin kelt steelhead. No Snake River origin kelt steelhead have returned to Bonneville Dam from 2010 or 2011 releases in the estuary. No Snake River origin kelt steelhead have returned to Bonneville Dam from 2009 through 2011 releases. These very marginal treatment benefits suggest that in most years collecting kelt steelhead then transporting and releasing them below the hydrosystem has very limited benefits. Trucking fish these long distances likely impacts long term survival of transported kelt steelhead.

Survival from release to the ocean was estimated from both collection areas Lower Granite and Prosser Dams and both release sites, in 2011 using sequential detections of acoustic tags. For the kelts collected at Lower Granite Dam, survival to the ocean was 6.5% and 29.8% for the Hamilton Island and Westport, OR release sites, respectively. For the Prosser Dam collected kelts survival to the ocean was 34.0% for both for both the Hamilton Island and Westport, OR release sites. The 7 year mean survival from release at Hamilton Island to the ocean is 44.9%. These low survival rates could be a result of transportation stress on the fish or river environment impacts. For the Snake River origin kelts we found that releasing the fish closer to the ocean resulted in higher survival to the ocean, and increased returns to Bonneville Dam with 2 skip spawners returning in 2012.

In 2013, survival of long-term reconditioned groups was 46.7% for Prosser, 8.16% for Omak, and 51.8% for Lower Granite Dam(Figure 34). Survival for the Prosser and Lower Granite Dam fish was above average, and survival for Omak fish was below average. This data indicates that steelhead kelts can be successfully reconditioned at a variety of locations.

We calculated the benefits of long-term reconditioning in the same manner as we did the transport benefits but instead of return rate to Bonneville we used survival to release for the long-term treated fish. Fish reconditioned at Prosser Hatchery had a 11.4 times survival advantage over the 8 year average return rate to Bonneville Dam for fish left in the river (Figure 35). We used the 8 year mean for comparison since the 2013 in-river group had 0 returns to Bonneville Dam, therefore, the within year comparison is a minimum estimate. Compared to the proportion of repeat spawners in the run at large at Bonneville Dam, long-term reconditioned kelts at Prosser Hatchery had a 63.4 times survival advantage, those from Omak Creek had a 26.5 times advantage, while Snake River steelhead from Lower Granite Dam had a 46.1 times advantage (Figure 35). Long-term reconditioning shows great promise as a tool for restoration based on this data.

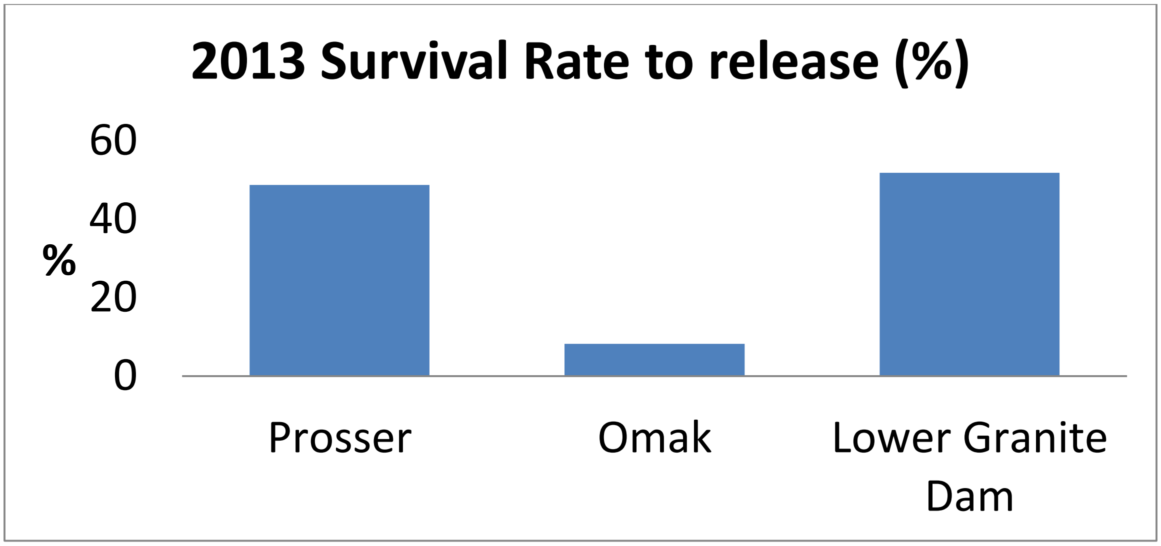


Figure 34: Survival rate (%) of long-term reconditioned kelt steelhead at 3 locations in 2013.

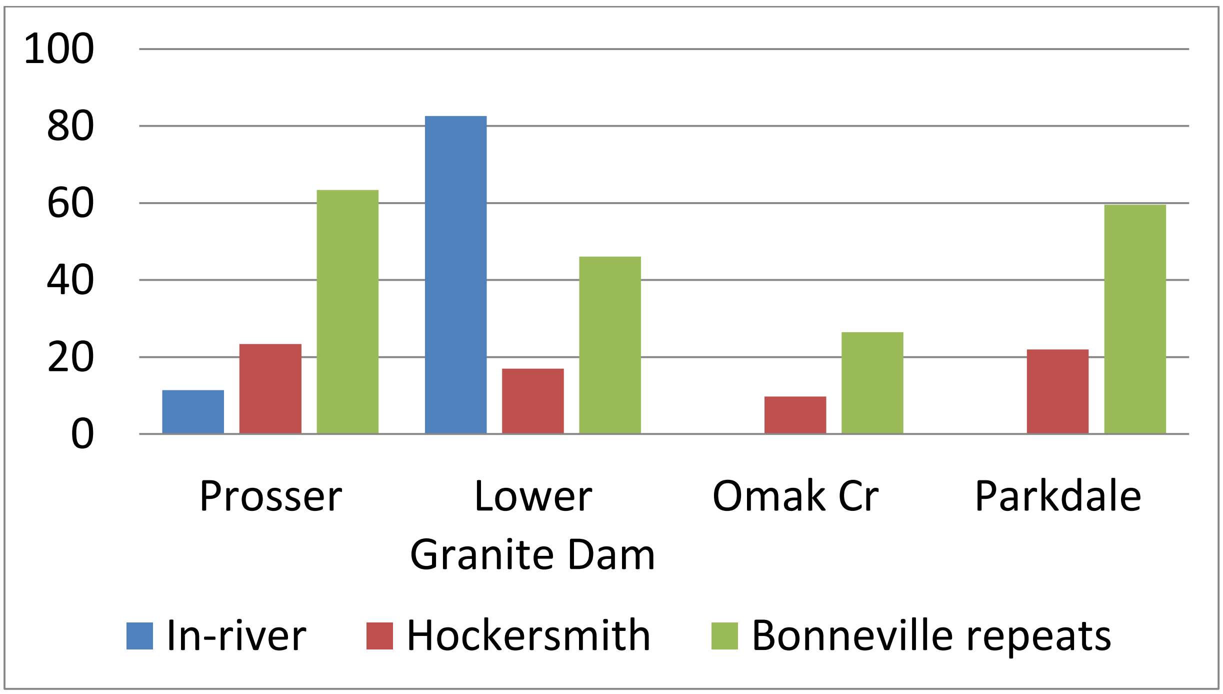


Figure 35: Long-term reconditioning benefits for 2013, calculated by dividing long-term survival rates by control group metrics.

Kelt Management Scenario Model

We are constructing a prototype steelhead model to examine the management implications of kelt reconditioning. The model is 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 will be used to assess the effectiveness of alternate reconditioning strategies, and limitations of the reconditioning program by comparing the rate of population increase

achievable when captured kelts are reconditioned under assumed potential capture rates and assumed survival rates.

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.

$$M_t = a_t S_t e^{-b_t S_t}$$

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, ,$\theta_r, \theta_f, \theta_u, \theta_l$.

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

Introduction

Post-spawn steelhead trout (*Oncorhynchus mykiss*), known as kelt steelhead are found in many watersheds and subbasins throughout the Snake River Basin, but in variable numbers. The spatially distributed differences in incidence of kelts, and potential for iteroparity (repeat spawning migrations), have been shown to be coincident with particular life history attributes including size and age related to A-run and B-run spawning populations (Narum et al. 2008). In this study we used single nucleotide polymorphism (SNP) genotypic data to evaluate genetic structure among Snake River *O. mykiss* stocks, and to conduct an analysis of genetic stock composition among kelt steelhead sampled at Lower Granite Dam (LGD) between 2009 and 2012. The objective of this study was primarily to estimate stock proportions in a mixed stock sample, providing a better understanding of the origins of post-spawn steelhead among the major subbasins (e.g., Clearwater River, Salmon River, Grande Ronde) and major population groups (MPG's) within the Snake River Basin. Results will also relate information about the behavior and population characteristics for genetically assigned kelt stocks.

Methods

The SNP marker panels, laboratory and genotyping methodologies, and descriptive statistics used to evaluate assignment power and to conduct GSI analyses are described in detail in Hess et al. (2012) and Ackerman et al. (2012). A total of 187 SNP loci were used in GSI analyses, and were pared from the original

192 to exclude a sex determining marker, three *O. clarkii* hybrid determining markers, and one of a pair of linked loci. Natural origin kelt steelhead were sampled and successfully genotyped (n=4,138) from across the downstream migration seasons in 2009 through 2012, in coordination with other ongoing monitoring and evaluation efforts at LGD (pers. comm. Scott Everett and Zach Penny of the Nez Perce Tribe). Biological field data for kelts was recorded and provided by Nez Perce Tribe and University of Idaho biologists, including sample date, fork length, gender, disposition and overall condition. Overall condition ratings included "poor", "fair" and "good". Briefly, the rating is based on fish appearance, fungal load, and presence of injuries (e.g., head wounds; pers. comm. Scott Everett, Nez Perce Tribe).

In addition to analysis of natural origin kelts, a total of n=1,434 hatchery kelts were sampled and genotyped. Only hatchery kelts in 2009 (n=41), 2010 (n=51), and 2012 (n=814) were available for GSI and parentage based tagging (PBT) analysis (total n=906). As a quality assurance/ quality control measure, hatchery kelts (excluding 2011 due to missing data) were evaluated using GSI to determine stock proportions for comparison with natural origin kelts. Further, all natural origin kelts were tested against a PBT baseline (Steele et al. 2012) to determine which if any were actually mis-identified hatchery fish. For all natural origin kelts that proved to be hatchery fish based on PBT, a concordance test was conducted from which GSI reporting group assignment would be compared to the region of hatchery-of-origin.

The CRITFC and Idaho Department of Fish and Game (IDFG) SNP baseline for steelhead trout is the frame of reference for determining kelt origins (Hess et al. 2012, Ackerman et al. 2012). It is comprised of 65 discrete collections, providing a genetic characterization of all major subbasins in the Snake River Basin, and typically includes multiple collections (watersheds) per subbasin such that all potential contributing stocks or discrete populations are represented (Table 18; Ackerman 2012). However, for a mixed-stock sample, the assignment of individual fish to specific populations of origin (within a watershed for example) is routinely less accurate than assignment to "reporting groups" that represent larger aggregates of populations (Hess et al. 2012). Therefore, the use of GSI is a regional application for identifying origins, and draws on the scope of demographic influences such as migration, as well as local adaptations to delineate groups of often genetically similar populations.

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

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

the probability of occurrence in the baseline population being evaluated. For the 100% simulations, the mixture sample size parameter was set at 200 and the number of iterations was set at 1000.

In addition to 100% simulations, the baseline has been independently evaluated in detail using several methods including 'leave-one-out' (LOO) individual assignment procedures, performed by Ackerman et al. (2012), and with complementary outcome.

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

Results

Baseline Power Analysis

Based on previously described criteria, the baseline populations were partitioned into 10 reporting groups (RG) for analysis (Figure 36; Table 19). Reporting groups are consistently color coded in figures throughout this document. Baseline assignment accuracy using the 100% simulation method was variable across stream collections and RG's, but was generally at or above an 80% accuracy threshold. Population estimates ranged from 0.36 in the Tucannon River collection (Lower Snake River RG) to 1.00 in upper Big Creek (Middle Salmon River RG). At the population level, there were 37 baseline collections with average population estimates that exceeded 80%, 21 of which were greater than 90%. Estimates were generally lowest in the Lower Salmon (LS) and Lower Snake River (LSN) RG's, and highest in the South Fork Salmon and Upper Clearwater River RG's. At the reporting group level, 61 of 65 collections had average RG estimates greater than 80%, of which 51 exceeded 90%. Again, the LS and LSN reporting groups were generally lowest, but the Middle Fork Salmon River RG was the highest. Mean reporting group accuracy ranged from 81% to 99% (Table 19).

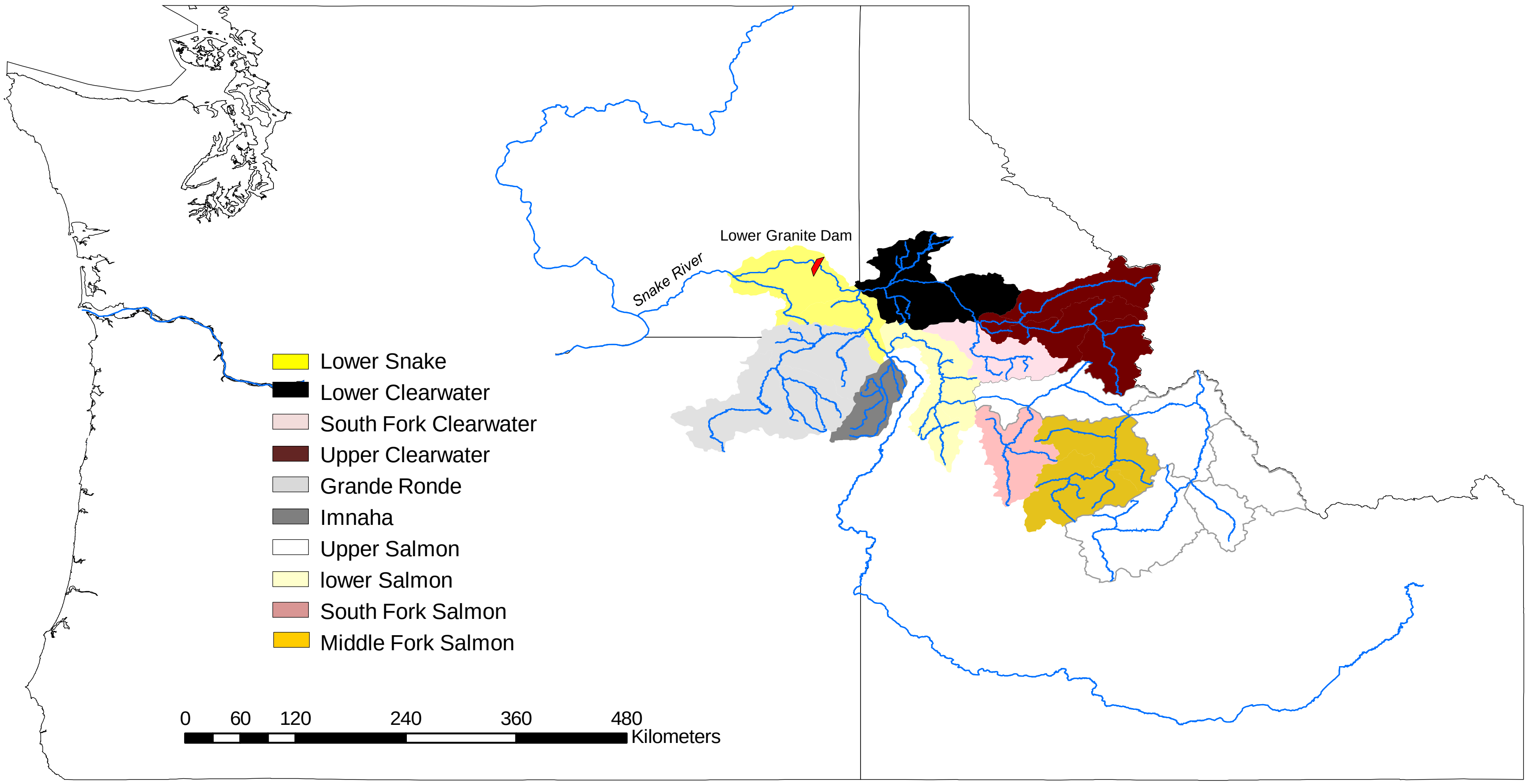


Figure 36: Map of GSI region and reporting groups established on the basis of 65 baseline O. mykiss populations (see Table 19).

Table 19: Populations included in the Snake River O. mykiss SNP baseline. Collections representing baseline populations were partitioned into discrete reporting groups (RG's) for GSI based on assignment probability in leave-one-out bootstrap tests (see Ackerman 2012). The baseline serves as the reference for identifying reporting group of origin for the mixed stock samples of kelt steelhead sampled at Lower Granite Dam. All collections are putative natural-origin fish and all are assumed anadromous. Baseline sample size (n) is shown. Results of the 100% simulation test of ONCOR are: the average population estimate defined as assignment to "self" for each individual stream collection, and average RG estimate defined as assignment to reporting group of origin; mean values within each RG are given. Baseline assignment exceeding 80% accuracy are filled light gray, while those exceeding 90% accuracy are filled dark gray.

Reporting Group (RG)	abbrev.	stream name	abbrev.	(n)	AVG (pop estimate)	AVG (RG estimate)	RG mean
Grande Ronde River	GR	Captain John	CPJN	56	0.91	0.93	---
	GR	Crooked	CROOK	95	0.81	0.89	---
	GR	Elk	ELK	45	0.97	0.99	---
	GR	Joseph	JOSE	45	0.39	0.67	---
	GR	Lostine	LOST	45	0.91	0.96	---
	GR	Little Minam	LTMN	48	0.95	0.97	---
	GR	Menatchee	MENA	68	0.81	0.85	---
	GR	Wenaha	WENA	93	0.79	0.91	0.90
Imnaha River	IM	Big Sheep	BGSP	61	0.87	0.93	---
	IM	Camp	CAMP	23	0.70	0.90	---
	IM	Cow	COW	44	0.74	0.78	---
	IM	Lightning	LTNG	38	0.73	0.90	0.88
lower Clearwater River	LCL	Big Bear	BGBEAR	98	0.68	0.91	---
	LCL	East Fork Potlatch	EFPOT	156	0.80	0.99	---
	LCL	Little Bear	LTBEAR	151	0.89	0.97	---
	LCL	Mission	MISS	49	0.77	0.80	---
	LCL	West Fork Potlatch	WFPOT	85	0.47	0.98	0.93
Lower Salmon River	LS	Boulder	BOUL	47	0.77	0.92	---
	LS	Hazard	HAZA	43	0.39	0.59	---
	LS	Rapid	RAPD	99	0.96	0.97	---
	LS	Slate	SLATE	46	0.63	0.70	---
	LS	Whitebird	WTBR	59	0.84	0.87	0.81
Lower Snake River	LSN	Alpowa	ALPO	98	0.46	0.91	---
	LSN	Asotin	ASOT	147	0.53	0.87	---
	LSN	George	GEO	95	0.39	0.87	---
	LSN	Tucannon	TUCA	105	0.36	0.92	0.89
Middle Fork Salmon	MFS	Bargamin	BARG	46	0.83	0.88	---

River							
	MFS	Camas	CAMA	56	0.86	1.00	---
	MFS	Chamberlain	CHAM	46	0.86	0.91	---
	MFS	Lower Big	LOBIG	46	0.89	1.00	---
	MFS	Loon	LOON	84	0.96	1.00	---
	MFS	Marsh	MARS	59	0.99	1.00	---
	MFS	Pistol	PIST	23	0.72	1.00	---
	MFS	Rapid	RAPD	31	0.93	1.00	---
	MFS	Sulphur	SULP	42	0.89	1.00	---
	MFS	Upper Big	UPBIG	45	1.00	1.00	0.98
South Fork Clearwater River	SFC	Tenmile	10MI	46	0.99	1.00	---
	SFC	Clear	CLEAR	45	0.92	0.97	---
	SFC	Crooked	CROOK	104	0.99	0.99	---
	SFC	Johns	JONH	36	0.75	0.87	0.96
South Fork Salmon River	SFS	East Fork South Fork Salmon	EFSFS	45	0.97	1.00	---
	SFS	Lick	LICK	39	0.96	0.99	---
	SFS	Secesh	SECH	45	0.98	0.99	---
	SFS	Stolle Meadows	STOL	45	0.96	0.99	0.99
Upper Clearwater River	UPC	Three Links/ Selway	3LIN	47	0.94	1.00	---
	UPC	Bear/ Selway	BEAR	35	0.67	1.00	---
	UPC	Canyon/ Lochsa	CANY	46	0.87	0.98	---
	UPC	Colt/ Lochsa	COLT	38	0.65	1.00	---
	UPC	Crooked Fork/ Lochsa	CRFK	44	0.77	0.99	---
	UPC	Fish/ Lochsa	FISH	99	0.87	1.00	---
	UPC	Gedney/ Selway	GEDN	45	0.54	0.99	---
	UPC	Lake/ Lochsa	LAKE	47	0.71	1.00	---
	UPC	Little Clearwater/ Selway	LTCL	59	0.93	1.00	---
	UPC	N. F. Moose/ Selway	MFMO	92	0.96	1.00	---
	UPC	OHara/ Selway	OHAR	47	0.73	0.92	---
	UPC	Mainstem Selway	SELW	76	0.97	1.00	---
	UPC	Storm/ Lochsa	STORM	38	0.78	1.00	---
	UPC	Whitecap/ Selway	WTCP	76	0.97	1.00	0.99
Upper Salmon River	UPS	Hayden	HAYD	84	0.80	0.96	---
	UPS	Morgan	MORG	37	0.68	0.97	---
	UPS	North Fork Salmon	NFSA	99	0.69	0.84	---
	UPS	Pahsimeroi Weir	PAHS	96	0.79	0.96	---
	UPS	Sawtooth Weir	SAW	105	0.82	0.97	---
	UPS	Valley	VALL	44	0.49	0.93	---
	UPS	West Fork Yankee Fork	WFYF	117	0.85	0.97	0.94

Kelt Mixture Assignments

Of the 4,138 kelt samples evaluated, 2,108 (51%) assigned with at least 80% probability (robust assignments; Table 20). Ordering of the 10 reporting groups by average probability of assignment was generally consistent from year to year. Regions believed to be dominated by A-run steelhead (Grande Ronde - GR, Imnaha - IM, lower Salmon - LS, and lower Snake River) ranked lowest as did the lower Clearwater River RG, while regions believed to be dominated by B-run fish were highest, particularly South Fork Salmon River - SFS, South Fork Clearwater River - SFC and upper Clearwater River - UCL reporting groups. Mean assignment probabilities ranged from 62% in LS to 92% in UPC. Note that the upper Salmon River RG typically scored better than the other A-run counterparts (Table 20). For RG's that ranked lowest in proportion of individuals that assigned with at least 80% probability (GR, IM, LS, or LCL), second ranked assignments were dominated by LSN and UPS reporting group assignments. Middle Fork Salmon River (MFS) and SFS reporting groups had second ranked assignments dominated by alternate Salmon River groups, as did SFC and UPC respective of Clearwater River alternate RG's (Table 21).

Table 20: Reporting group (RG) assignment probability summary for kelts (origin unknown) sampled at Lower Granite Dam. The total number sampled was 4,138. Only 2,108 met an individual assignment probability threshold of 80%; (%n) are sample proportions by RG that did not meet the threshold. The average assignment probability for reporting group of origin among baseline populations is shown for comparison (100% sim.).

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

Table 21. Individual kelts were assigned to a reporting group of origin based on probability rankings (best estimate = highest probability regardless of a threshold criteria). For all "best" assignments the alternative or next more likely reporting groups of origin are identified by frequency of occurrence (gray fill indicates alternate probability > 20%). For example, among Grande Ronde (GR) best assignments, the next most likely reporting group of origin was Lower Snake River (LSN) in 56% of observations.

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

Stock proportions estimated using the entire sample (n=4,138) were similar but slightly skewed from stock proportions based on an 80% threshold (n=2,108; Figure 37). The largest differences occurred in UPS and MFC (greater proportion at strict criteria) and LSN and GR (both with lower proportion at strict criteria). Greater proportional assignment to UPS presumably made up much of the differences in GR and LSN proportions (Figure 37; Table 22). Stock proportions of kelts determined by GSI analysis were compared to the stock proportions determined by GSI of returning steelhead sampled at LGD during spawn years 2009-2011 (extrapolated to estimate total escapement to LGD). Both analyses included only natural origin adult steelhead, defined as having no detectable marks or tags. Stock proportions for estimated escapement were evaluated, and data contributed by Mike Ackerman (IDFG); based on an 80% probability threshold only. Note that results for kelts varied widely from estimates from total escapement in the same years, where stock proportions among the returns were far more uniform across reporting groups (Figure 37; Table 22). The difference was particularly poignant in regions with relatively few kelts (UPC, SFC, SFS).

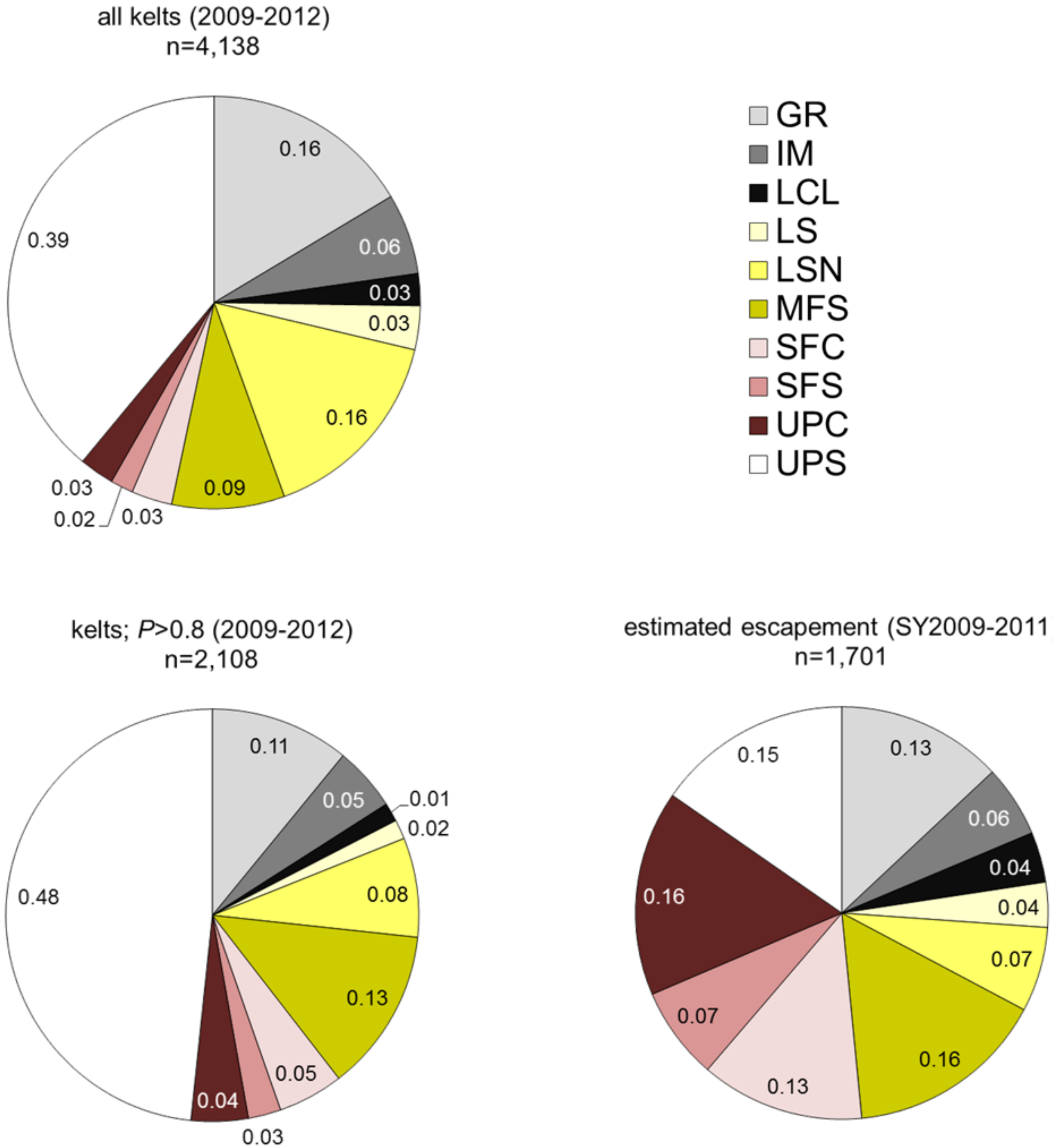


Figure 37: . Stock proportions by reporting group of origin (GSI assignment) for all kelts, and for estimated escapement of steelhead sampled at LGD for spawn year 2009-2011; spawn year data provided by Mike Ackerman, IDFG.

Table 22: Stock proportions for estimated escapement vs. Lower Granite Dam kelt samples. Individuals were sampled from across the migration/outmigration in each year (2012 data for escapement estimate not available). Results summarize individual assignments to reporting group for all sampled kelts, and kelt assignments at or above an 80% probability threshold. Stock proportions that exceed 10% absolute difference (abs.) between escapement and kelts are denoted (*), and reporting groups with kelt proportions exceeding 20% appear in bold gray fill.

		LGD kelt (*all*)		LGD kelt (*P*>0.8)		escapement (*P*>0.8)		
year	reporting group	(n)	proportion	(n)	proportion	(n)	proportion	abs.
2009	GR	38	0.14	10	0.08	60	0.11	0.04
	IM	19	0.07	7	0.05	31	0.06	0.01
	LCL	6	0.02	0	0.00	28	0.05	0.05
	LS	9	0.03	4	0.03	29	0.05	0.02
	LSN	32	0.12	5	0.04	28	0.05	0.02
	MFS	40	0.15	31	***0.23***	74	0.14	0.09
	SFC	6	0.02	4	0.03	75	0.14	***0.11**
	SFS	6	0.02	5	0.04	35	0.07	0.03
	UPC	7	0.03	6	0.05	108	0.20	0.16
	UPS	102	***0.38***	61	***0.46***	61	0.12	***0.34**
	Total	265		133		529		
2010	GR	186	0.14	75	0.10	129	0.14	0.04
	IM	104	0.08	43	0.06	56	0.06	0.01
	LCL	21	0.02	2	0.00	24	0.03	0.02
	LS	43	0.03	10	0.01	24	0.03	0.01
	LSN	178	0.13	52	0.07	78	0.09	0.02
	MFS	124	0.09	98	0.13	174	0.19	0.06
	SFC	24	0.02	22	0.03	97	0.11	0.08
	SFS	25	0.02	21	0.03	60	0.07	0.04
	UPC	34	0.02	30	0.04	112	0.12	0.08
	UPS	624	***0.46***	425	***0.55***	163	0.18	***0.37**
	Total	1363		778		917		
2011	GR	199	0.17	72	0.13	144	0.13	0.00
	IM	79	0.07	26	0.05	57	0.05	0.00
	LCL	29	0.02	13	0.02	49	0.04	0.02
	LS	41	0.03	6	0.01	37	0.03	0.02
	LSN	201	0.17	43	0.08	64	0.06	0.02
	MFS	115	0.10	78	0.14	153	0.14	0.00
	SFC	31	0.03	22	0.04	156	0.14	0.10
	SFS	25	0.02	17	0.03	91	0.08	0.05
	UPC	41	0.03	33	0.06	192	0.17	***0.11**
	UPS	419	***0.36***	241	***0.44***	168	0.15	***0.29**
	Total	1180		551		1111		

2012	GR	256	0.19	73	0.11	na	---	---
	IM	60	0.05	30	0.05	na	---	---
	LCL	48	0.04	16	0.02	na	---	---
	LS	49	0.04	12	0.02	na	---	---
	LSN	242	0.18	64	0.10	na	---	---
	MFS	87	0.07	63	0.10	na	---	---
	SFC	71	0.05	60	0.09	na	---	---
	SFS	18	0.01	11	0.02	na	---	---
	UPC	31	0.02	25	0.04	na	---	---
	UPS	468	***0.35***	292	***0.45***	na	---	---
	Total	1330		646		na		

Demographic Correlations

For these exploratory analyses, all assignments were considered regardless of probability. Sex ratios were largely consistent across RG's that had sufficient numbers of assigned fish from each gender for comparison. The RG mean proportion of female kelts ranged from 74.2% in SFC to 85.5% in IM (Figure 38; Appendix B2). Across RG's, the mean proportion of kelts identified as female ranged from 71% in 2010 to 87% in 2011 (Figure 38). The mean across RG's was 77.0% female, and results were generally consistent with Keefer et al. (2008).

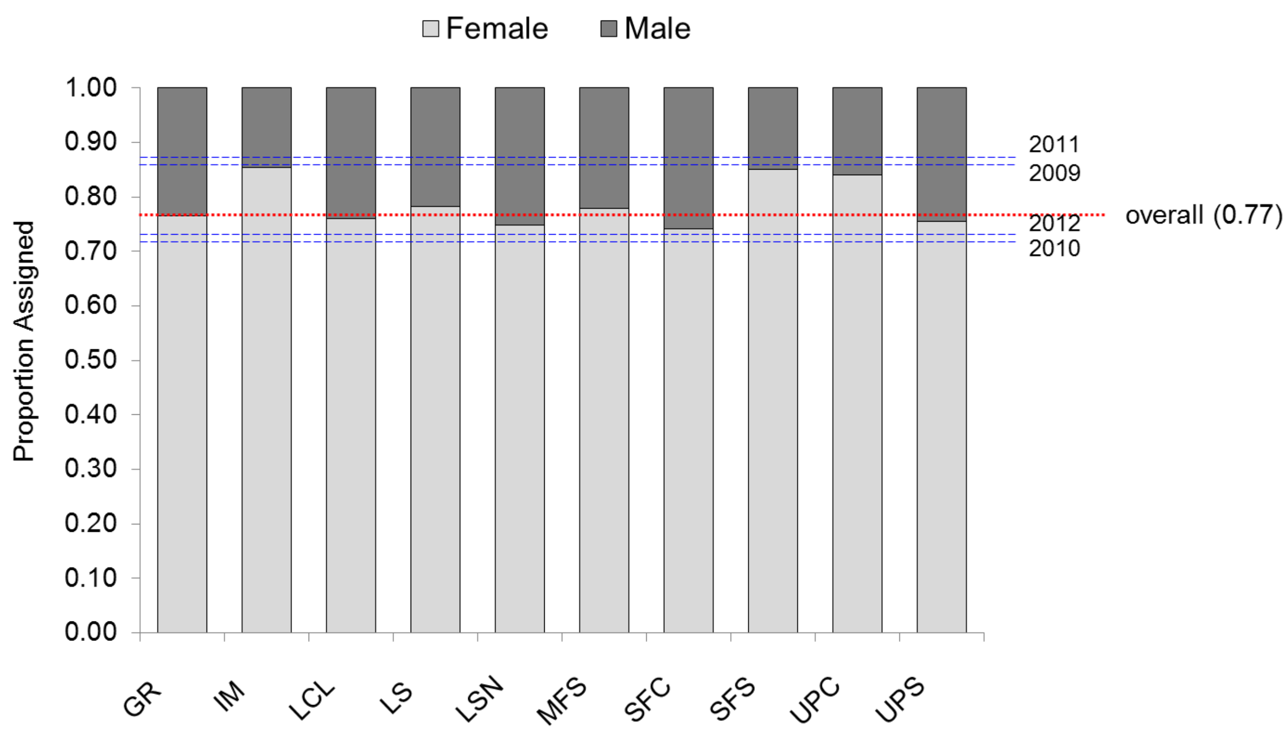
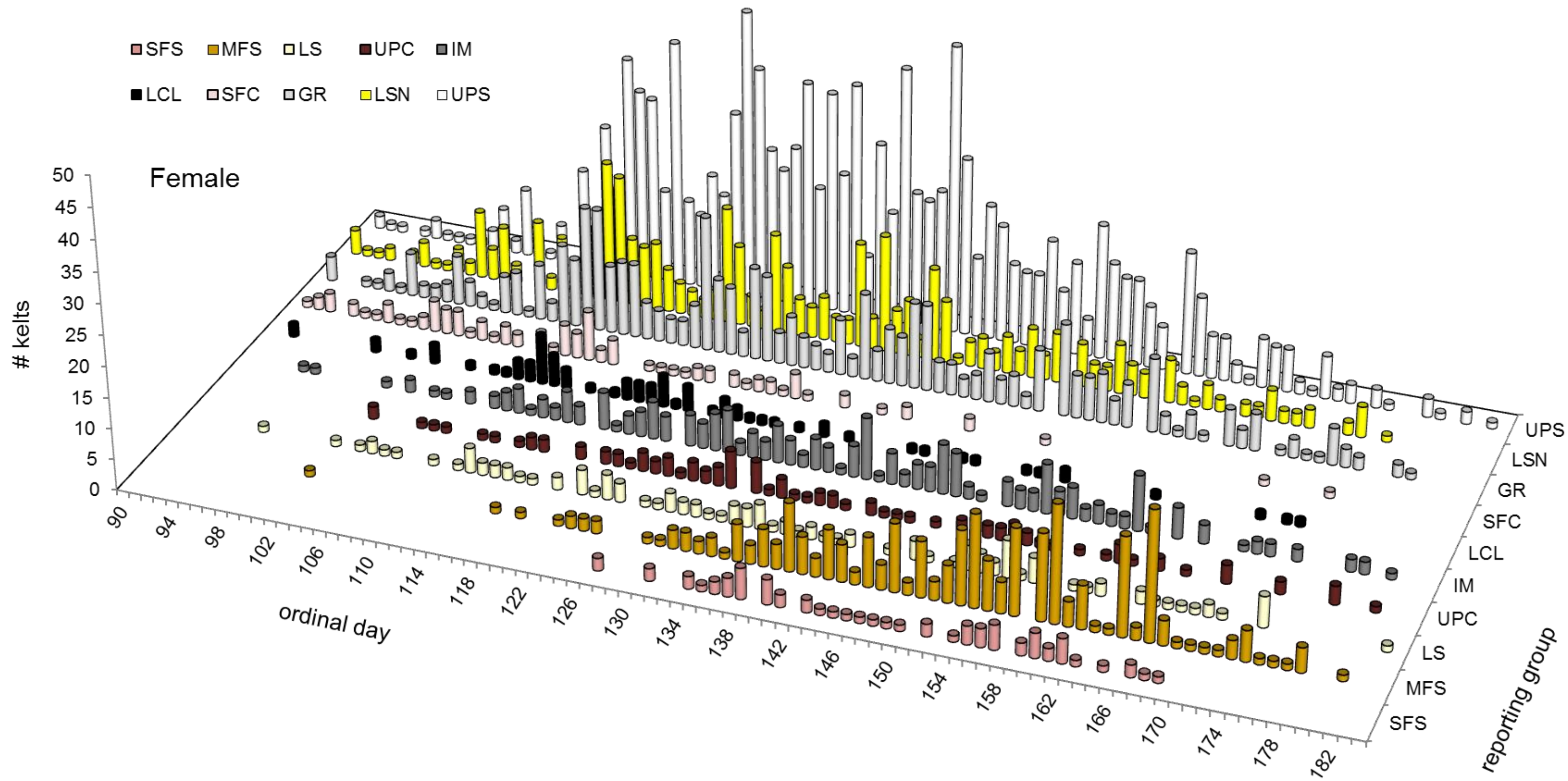


Figure 38: Proportion of male and female kelts by reporting group. The histogram depicts mean proportion across all years (2009-2012). The dashed blue lines identifiy the mean female proportion across reporting groups in each sample year; overall is the global mean female proportion across years and reporting groups.

Capture date at Lower Granite Dam served as a proxy for outmigration date, which was enumerated as ordinal day (January 1st = day 1). Generally, male kelts migrated downstream later than females, and the trend was consistent across years and RG's; exceptions occurred when too few males were assigned to an RG in a given year. The mean outmigration days for females and males were day 134 and day 141 respectively (Figure 39; Appendix B2). Among RG's the overall average outmigration day ranged from 115 in SFC to 153 in MFS for females, and from 129 in SFC to 165 in SFS for males. The Lower Clearwater River (LCL) and South Fork Clearwater River (SFC) groups were consistently the earliest outmigrants, and often the difference in mean day of outmigration between males and females was greatest in these two RG's. Interestingly and in direct contrast to kelt outmigration timing, the South Fork Clearwater River was the latest returning (LCL was also among the latest) and the Middle Fork Salmon and South Fork Salmon rivers were the earliest returning based on GSI for steelhead returns at LGD from 2009 through 2011 (pers. comm. Mike Ackerman, IDFG).



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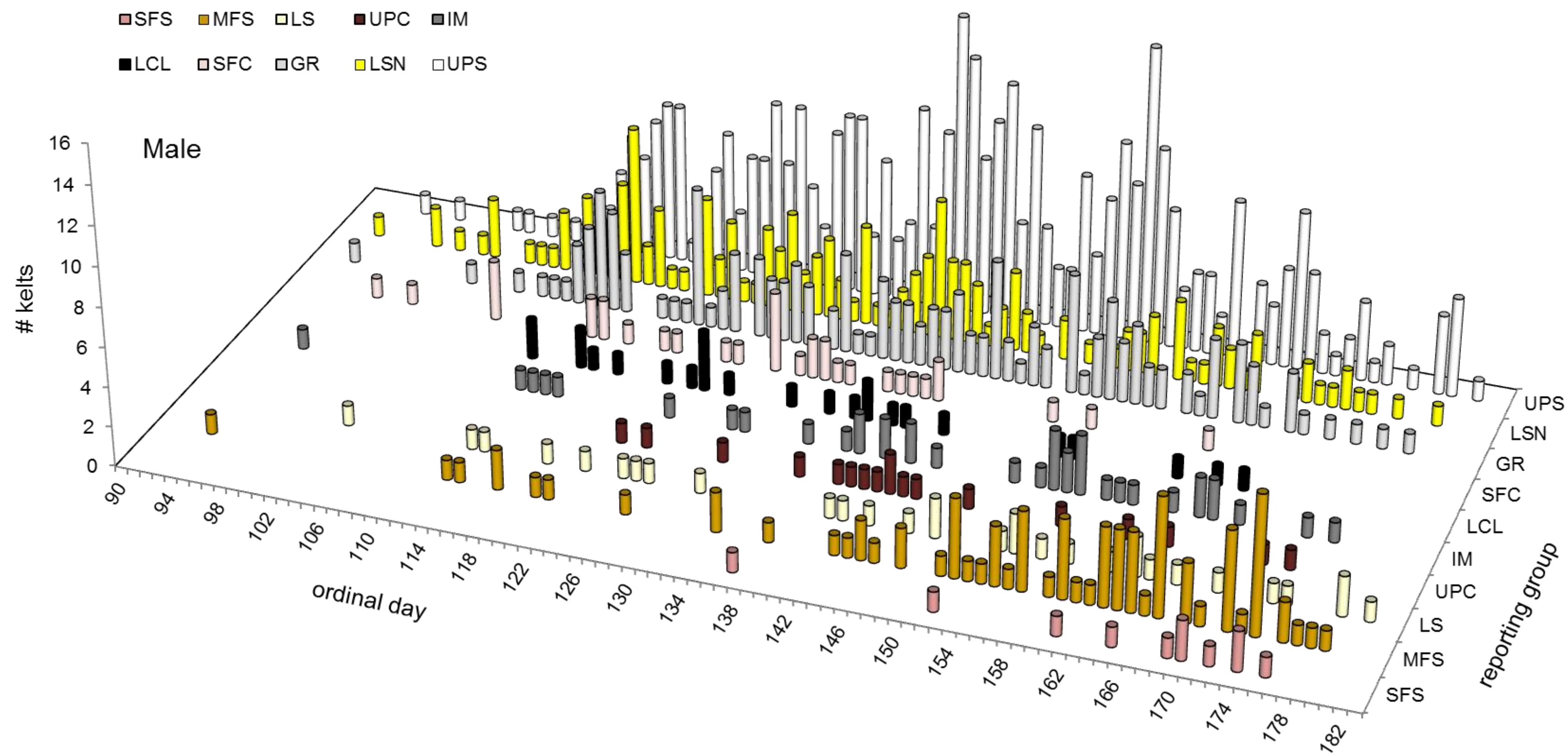


b)

Figure 39: Sample-date distribution for kelts captured at LGD during downstream out-migration (2009-2012). Kelts are differentiated by reporting group assignment, and day is the ordinal day (January 1st = day 1).

Kelt size (fork length) significantly trended toward larger fish in regions generally considered to support predominantly B-run steelhead populations (e. g., South Fork Salmon River and Clearwater River), and the trend was apparent for both sexes (Figure 40). In each year, kelts assigning to the SFS, SFC and UPC reporting groups were the largest observed. Among RG's the mean female kelt length ranged from 65.1cm in 2010 to 69.1cm in 2011 (Figure 40, Appendix B2). Within each year (2009-2012) and on average, male kelts were smaller in length than females (62.1cm and 67.6cm respectively). These results are in agreement with previously published studies that describe kelt distribution and characterization based on GSI (Narum et al. 2008).

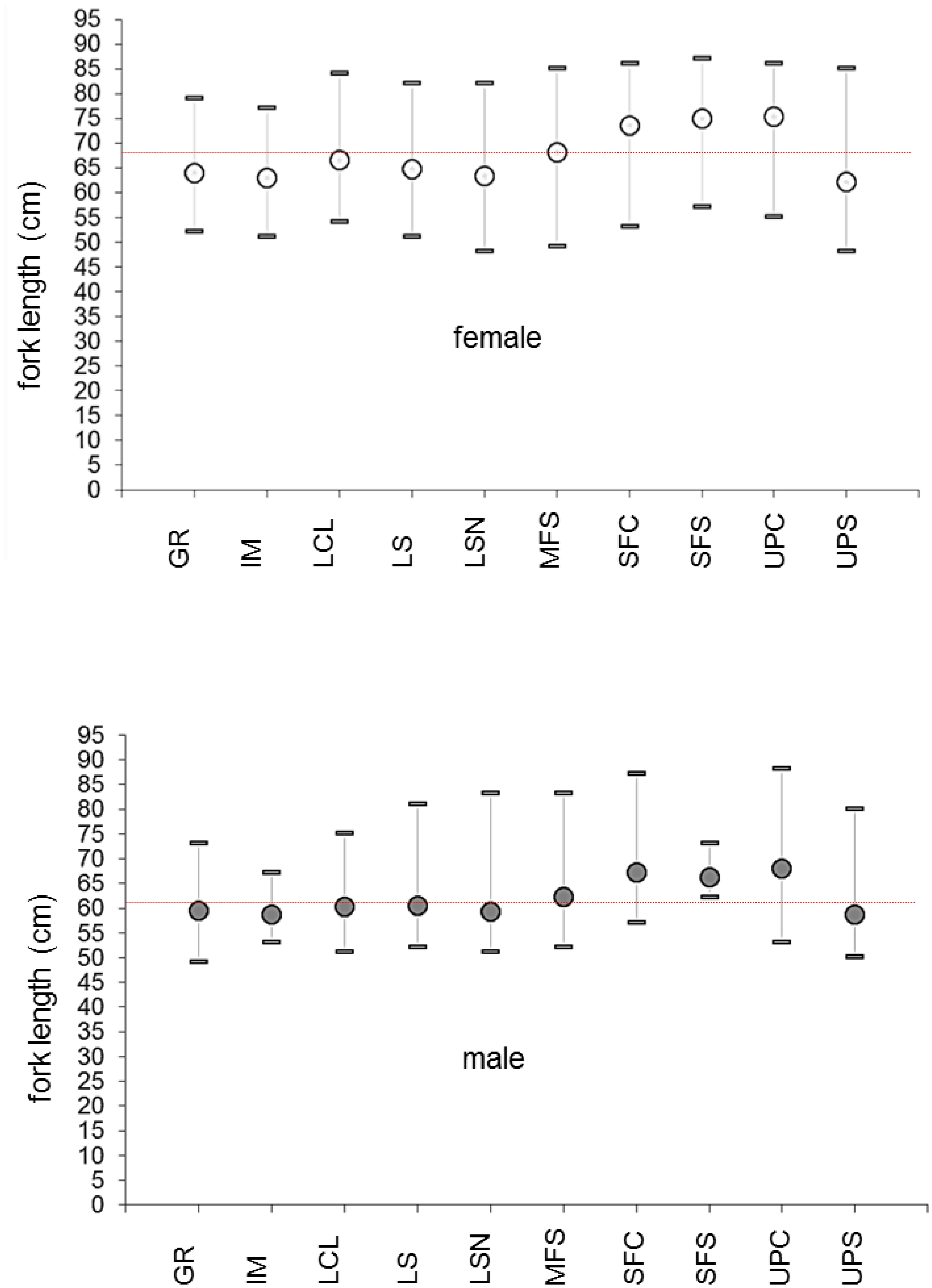


Figure 40: Mean length of kelts assigned to each reporting group. The vertical dashed lines with caps represent the range of observed lengths. The horizontal red lines are mean length across RG's (Appendix B2).

The condition rating of female kelts was generally better than male kelts, but there was no clear correlation or relationship between reporting group and overall condition (e.g. % good) for either gender (Figure 41). To further explore possible correlations between kelt condition and demographic variables, condition was plotted against fork length and against outmigration time using the entire kelt sample (n=4,138), across gender and reporting groups. There was no relationship observed between kelt length and condition. However, the relationship between outmigration date and overall condition rating of individuals (Figure 42) was significant, where earlier outmigration showed a smaller proportion of kelts in “good” condition.

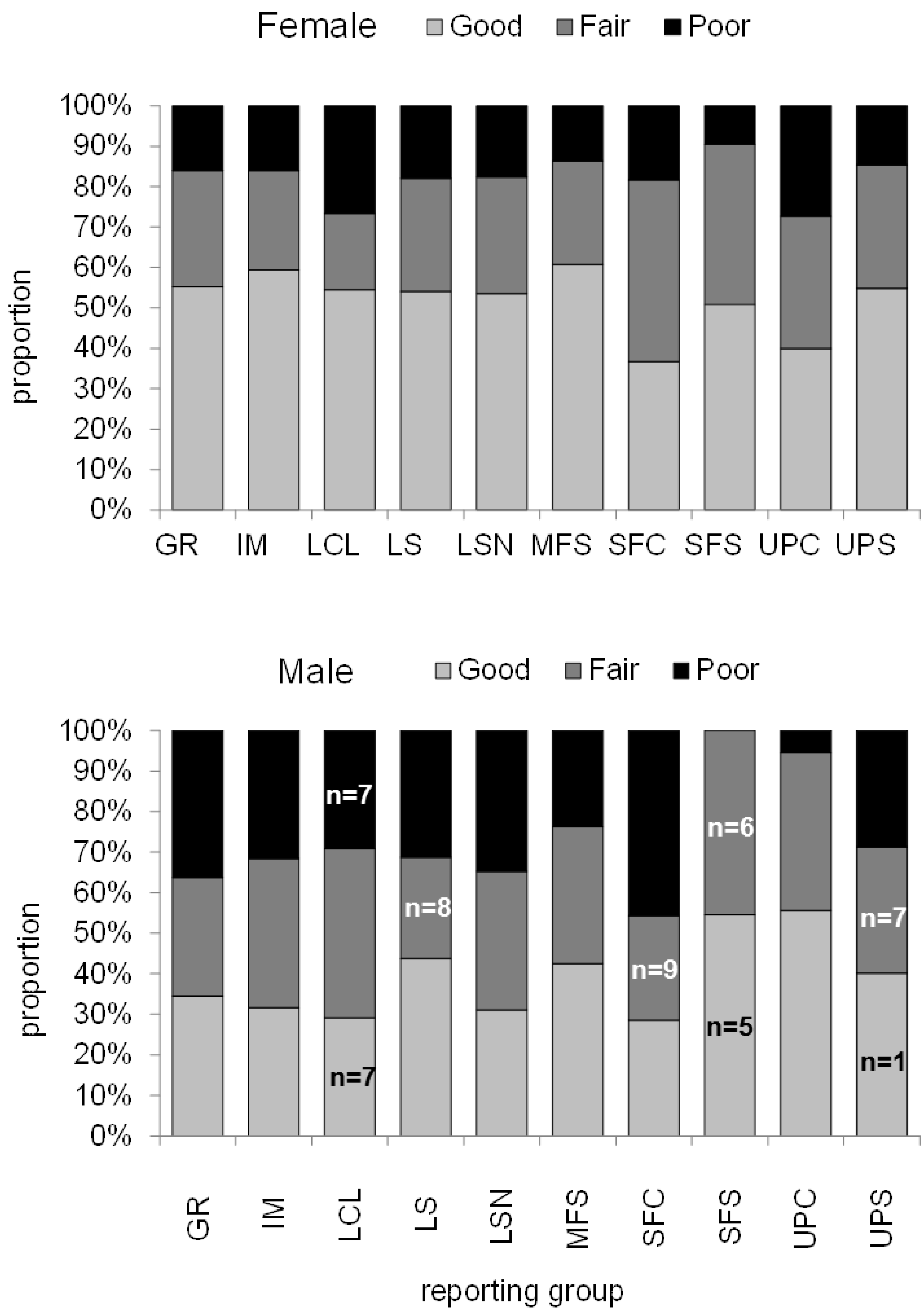
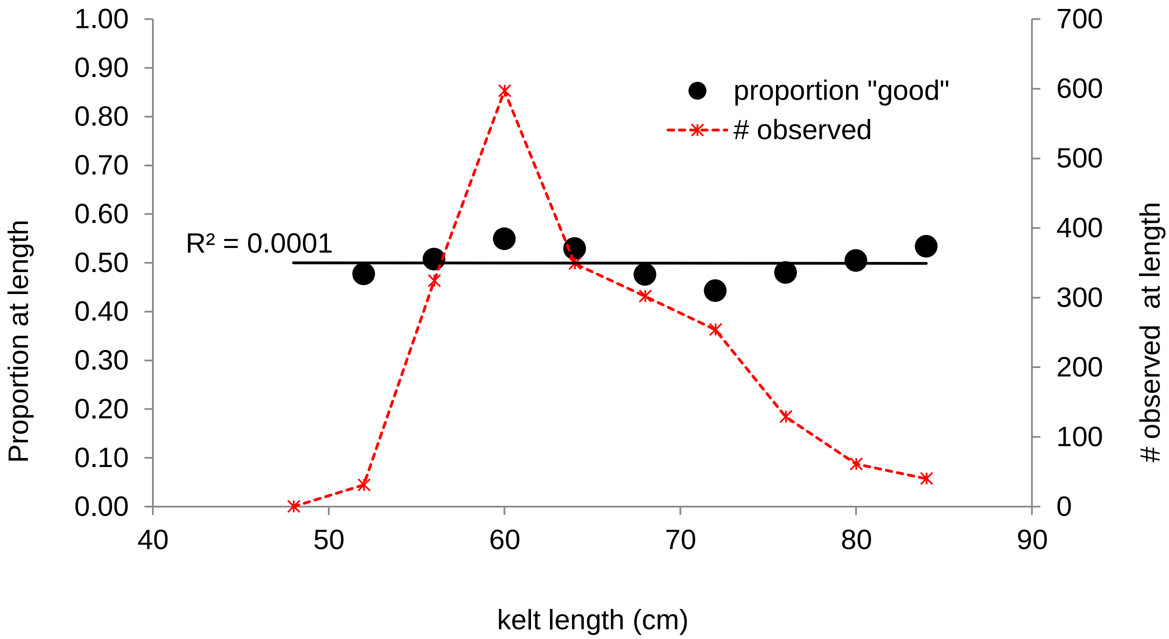


Figure 41: Overall condition of kelts sampled at LGD (2009-2012). Results are the proportion of fish in good, fair, and poor condition, partitioned by gender and reporting group assignment. Condition is contingent on presence and degree of injuries, fungus and other physiological factors. Proportions based on sample sizes less than n=10 are shown in the histograms.



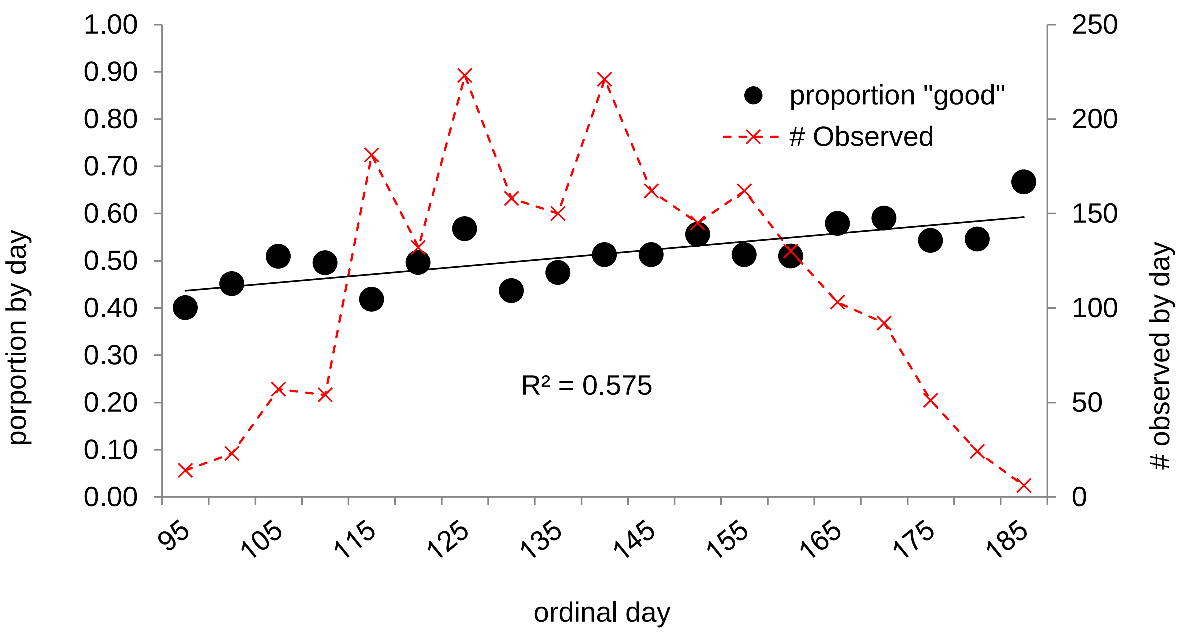


Figure 42: Correlation between life history variables. Condition of kelts ("good") is shown in association with (a) length or size of kelts, and (b) in association with sample date which is treated as a proxy for outmigration time. All sampled kelts (n=2,900) are included in the analysis, but because number of males was limited, results are not partitioned by gender.

Hatchery origin fish would presumably not assign to reporting groups as accurately as natural origin fish using a GSI baseline comprised exclusively of putative natural origin collections. Stock transfers and outplanting, and phases of the rearing cycles that occur in non-local facilities are factors also likely to further confound the ability to confidently assign hatchery fish to regions of origin based on GSI reporting group assignment. The GSI stock proportions for hatchery kelts varied greatly from those observed for natural origin kelts. For example, UPS reporting group made up as much as 66% of all sampled hatchery kelts. Results shifted using an 80% criteria for assignment probability in which proportions from GR and LSN shrank and conversely grew in UPS and SFC (Figure 43). There were n=331 natural origin kelts sampled between 2009 and 2012 (8% of the total) that were assigned as hatchery progeny using the PBT method. Note that this procedure followed primary GSI analyses, and therefore these fish were included in previously described analysis and results for natural origin kelts. Nevertheless, there was a high degree of concordance between GSI reporting group assignment and the RG's from which hatchery PBT assigned kelts originated (e.g. Sawtooth Hatchery located in the UPS reporting group). For example, of n=71 presumed natural origin kelts that were PBT assigned to Sawtooth Hatchery broodstock, 93% also GSI assigned to the upper Salmon River (UPS) reporting group, and all four Tucannon River hatchery fish assigned to the lower Snake River reporting group (Table 23).

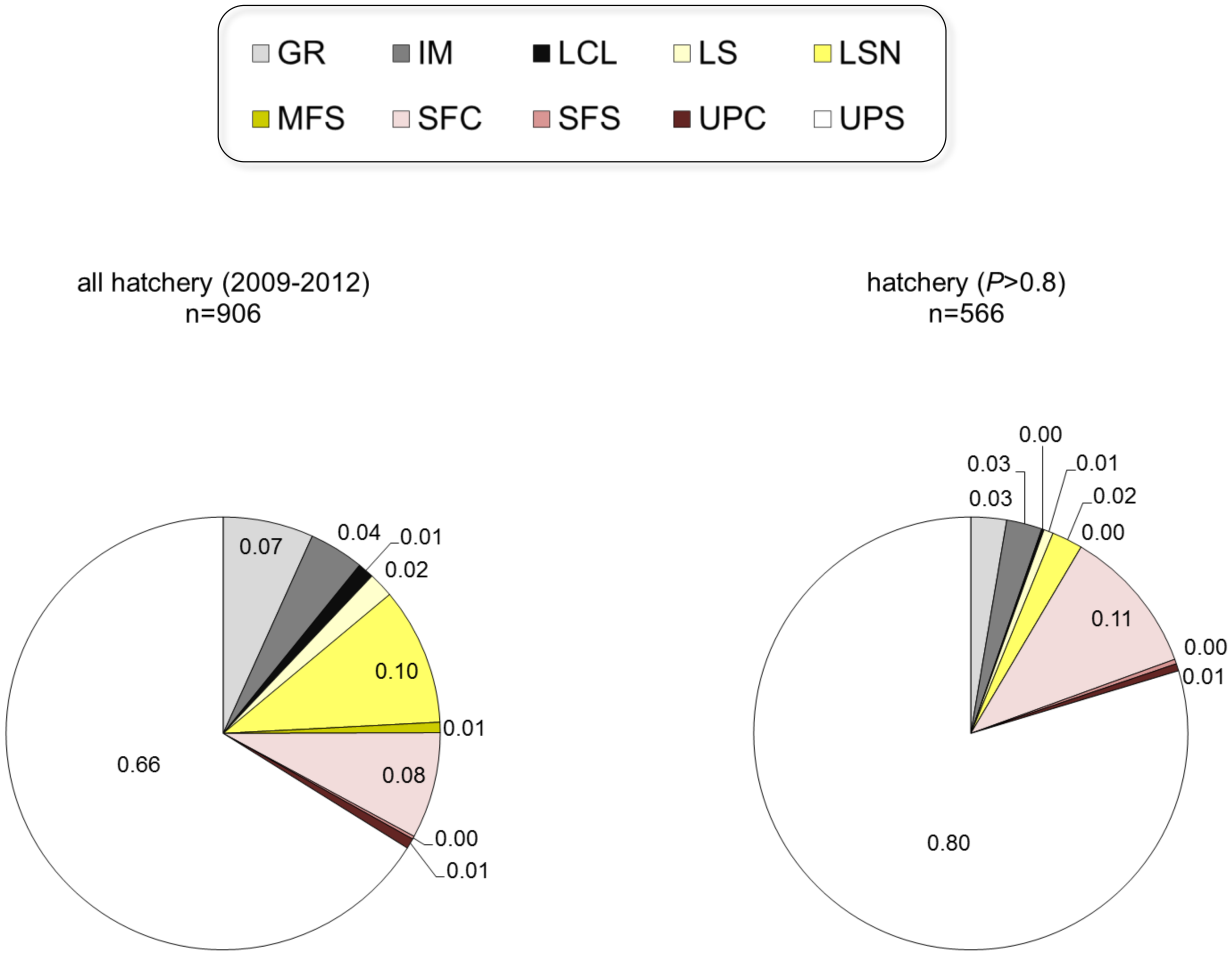


Figure 43: Hatchery GSI

Table 23: Concordance between PBT assignment and GSI assignment for 331 kelts identified as putative natural-origin based on the absence of marks (i.e. adipose clip) or tags. For fish with an identified ("known") hatchery-of-origin, the reporting group assignment that occurred with greatest frequency is shaded and bordered. Hatchery locations are: 1) Clearwater River, 2) Tucannon River – lower Snake River, 3) Snake River – Hells Canyon, 4) upper Salmon River, 5) upper Salmon River, 6) acclimation/release site, 7) acclimation/release site, 8) Tucannon River – lower Snake River, 9) Grande Ronde River.

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

Discussion

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

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

Among the kelts representing in this data set, there was no significant correlation observed between fish size and the proportion of fish rated “good” in overall condition (contrasting previously reported results (Hatch et al. 2012). There is some concern that size selectivity at the Lower Granite Dam bypass may be a contributing factor to condition of kelts. The bypass entrance orifices are undersized (12") and these small orifices are thought to result in a high incidence of head wounds on large fish (affecting the condition rating). Moreover, entrances may be selecting for smaller fish and excluding larger ones. In a related study, 90% of B-run steelhead that were acoustically tagged at a weir in Fish Creek (Middle Clearwater River) were detected in the Lower Granite Dam forebay, but none of the fish were collected in the bypass (pers. comm. Christine Moffitt, University of Idaho and Doug Hatch, CRITFC)**.** To date, the results of kelt GSI regarding physical condition vs. size of fish are inconclusive. In contrast to size, a later date of downstream migration was significantly associated with overall condition of the kelts (higher proportion “good” condition). However, the biological significance of this finding is difficult to discern on the basis of fresh water residence time. For example, some of the earliest steelhead to return to LGD during spawn years were often some of the last to be detected at LGD post-spawn (e.g. Middle Fork Salmon River). The opposite is true for some Clearwater River reporting groups, where steelhead were among the last to return to LGD during the spawn year, but GSI assigned kelts were among the first to outmigrate. Observing the proportion of good condition larger sized kelt steelhead is similar to smaller fish is encouraging for the kelt reconditioning program targeting B-run steelhead since fish in better condition tend to survive reconditioning at higher rates (Hatch et al. 2013b).

These results describing the distribution of kelts to their regions of origin, and related group distinctions (characteristics) are biologically intuitive based on prior information (Busby et al. 1996, Keefer et al. 2008, Narum et al. 2008). The GSI assignment proportions of kelts reported here verify or substantiate some information about the life history of the species *O. mykiss*. Specifically, female rates of iteroparity are higher than for males regardless of region or ecotype, and that the A-run ecotype (generally younger

age) are more prone to repeat migrations across their distribution range. Those regions dominated by the A-run ecotype are somewhat less accurately differentiated than B-run using GSI, yet these results provide a reasonable level of confidence in evaluating which regions produce greater proportions of potentially iteroparous individuals. This is an important attribute contributing to population productivity, and monitoring of the relative abundances of both A-run and B-run forms will inform specific management of each with important implications for conservation. As expected, note that the assignment proportions were markedly different for the estimated total escapement across RG's compared to the reporting group of origin for the kelt sample (Figure 2). With refined methods and more data it may be possible to confidently estimate relative rates of iteroparity among RG's based on escapement using such comparisons. Hatchery GSI results suggest this method may perform reasonably well for identifying kelt proportions from specific hatchery programs. We will continue to update the baseline to strive for the highest level of population and reporting group resolution possible. Additional kelt samples in following years will be valuable in evaluating the consistency (or temporal variability) of the observations presented here.

2013 GSI Analysis

Genetic samples collected in 2013 at Lower Granite Dam are currently being analyzed and will be reported in the 2014 annual report to Bonneville Power Administration.

Reproductive Success

Introduction

Protocol Title: Parentage analysis (2007-401-00) v1.0

Protocol Link: *http://qa.monitoringmethods.org/Protocol/Details/183*

Protocol Summary: Hypothesis: Offspring production per spawning (maiden and kelt) are similar. Site Selection: Sites include the Parkdale Fish Facility, Yakima River, and Omak Creek. Site selection was based on fish and facility availability. Sample Size: Depends on the year and site.

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?

Steelhead Kelt Gamete and Progeny Viability at the Parkdale Fish Facility

Reproductive success is difficult to document under natural conditions. Steelhead, in particular, are problematic as migration and spawn timing are associated with high flow events in the late winter and spring. This limits the operation of weirs and traps and makes direct observation of spawning difficult. In addition to the problems associated with sampling migratory anadromous adults, resident *O.mykiss* can represent a substantial portion spawning population (Araki et al. 2007), and are often unsampled. The design of this study is to collect hatchery-origin prespawn adults and transport them to the hatchery to evaluate them in a more controlled environment which will help to understand if repeat spawners produce viable offspring and how that compares with first time spawner success. We initially began this experiment in 2006 utilizing summer-run steelhead. In 2010, we started collected locally adapted winter steelhead for comparative purposes while phasing out the summer-run portion of the experiment. This was the final year

that kelts were reconditioned at Parkdale. Remaining kelts were terminated and gametes were examined for maturation.

This experiment utilizes a replicated, repeated measures experimental design to assess and compare egg and progeny viability of maiden versus reconditioned spawners. We compared the performance of artificially reconditioned repeat spawning steelhead with their previous maiden performance using measures of fecundity, fertilization rates, fry length change, and fry weight change over ten weeks. The same individual performance metrics were used for the group comparison.

The steelhead kelts exhibit at least two strategies for repeat spawning, sequential and skip spawning (Burgner et al. 1992). In the sequential strategy a kelt stage steelhead emigrates to the ocean in spring, spends the late spring and early summer in the ocean, returns to fresh water in the late summer or fall (August through November) for repeat spawning the successive spring. In the skip spawner strategy, the fish spend an additional year or more in the ocean and return in the late summer or fall in a subsequent spawning migration. Both sequential and skip spawner strategies were exhibited by individual kelt steelhead in our study. These repeat spawning strategies provide a backdrop for understanding the reproductive strategies of individual fish. Individuals were collected and spawned as maidens, then held, reconditioned and spawned as a repeat spawner the following spring or as a skip spawner two years later. Some of these kelts would spawn consecutively up to 3 or 4 times.

Yakima and Omak Parentage Analysis

In this study we utilize DNA markers and pedigree analysis to address these questions for kelt steelhead in tributaries of the Yakima River basin and Omak Creek. We are using genetic information collected from steelhead kelts that are artificially reconditioned as described in the Kelt Management Scenarios section from Prosser Fish Facility on the Yakima River and from the St. Maries acclimation pond near Omak Creek. This experiment then collects juvenile fish by primarily electrofishing and rarely by screw trap and attempts to determine if kelt progeny are present. This experiment is difficult in both places due to the large resident population and physical size of the Yakima basin and the small number of kelts successfully reconditioned in Omak Creek.

Study Location

Parkdale Fish Facility

Reconditioning, spawning, and rearing was performed at the Parkdale Fish Facility located at RK 5.6 on the Middle Fork of the Hood River (Figure 44). This facility is co-managed by The Confederated Tribes of Warm Springs and the Oregon Department of Fish and Wildlife. The hatchery receives surface water from the Middle Fork of the Hood River and spring water from Rogers Creek. This facility currently operates as a supplementation hatchery for winter steelhead and spring Chinook but has been used in the past for supplementing summer steelhead and spring Chinook.

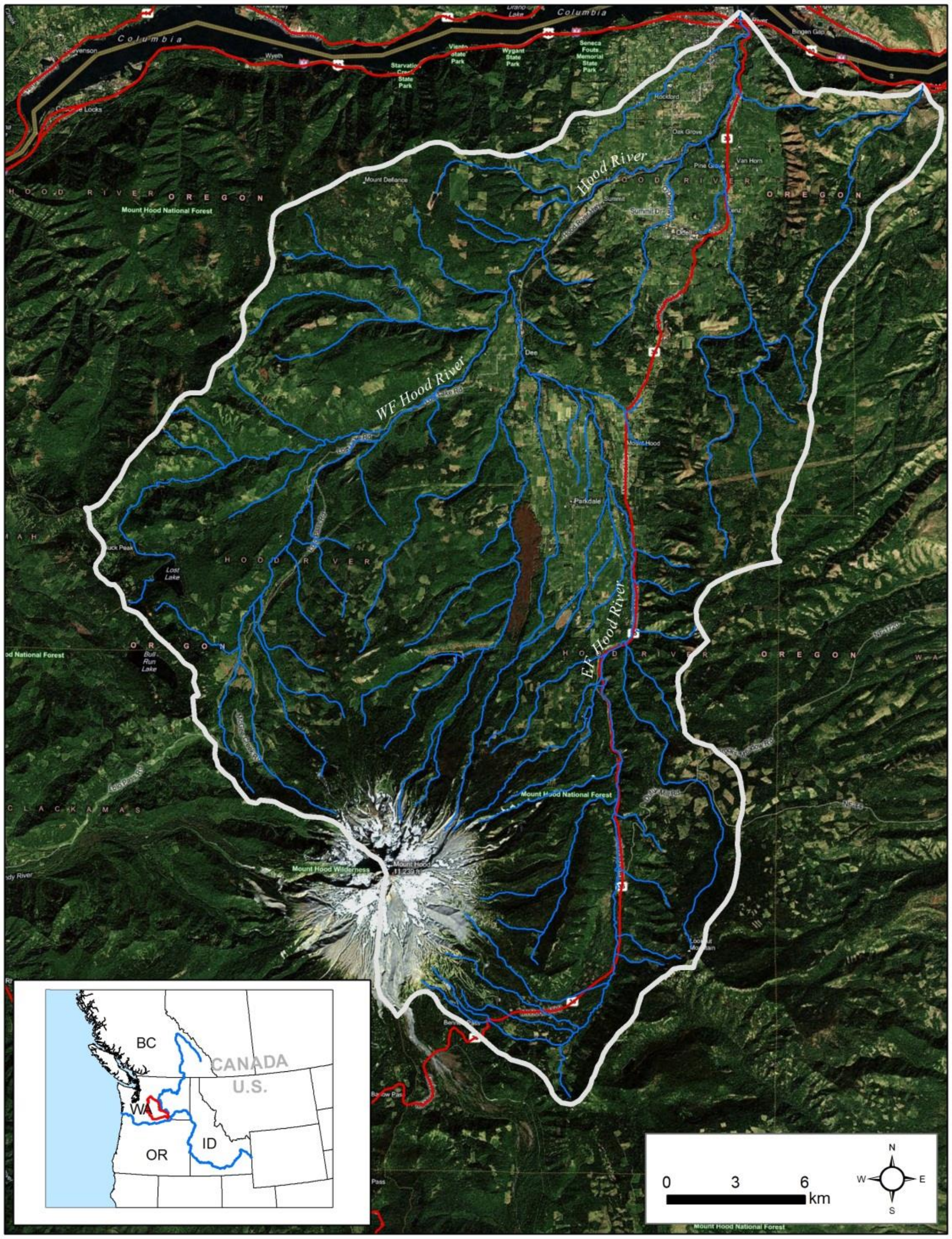


Figure 44: Hood River basin: Fish were collected at the east fork hood river weir trap and reconditioned at Parkdale Fish Facility.

Yakima River and Omak Creek

Work for genetic collections occurred on the mainstem and tributaries of the Yakima River (Figure 45). Steelhead kelt genetic collection occurred as fish were in-processed from the Chandler Juvenile Evaluation Facility bypass to the Prosser Hatchery and at the Omak Creek weir to the St. Maries Acclimation Pond. The primary areas for the collection of steelhead progeny occurred at the tributaries of the systems we were working in. These tributary locations in the order of collection size in the Yakima River Basin include Satus, Toppenish, Naches, and Ahtanum Creeks while collections occurred solely at Omak Creek, a tributary of the Okanogan River (Figure 46).

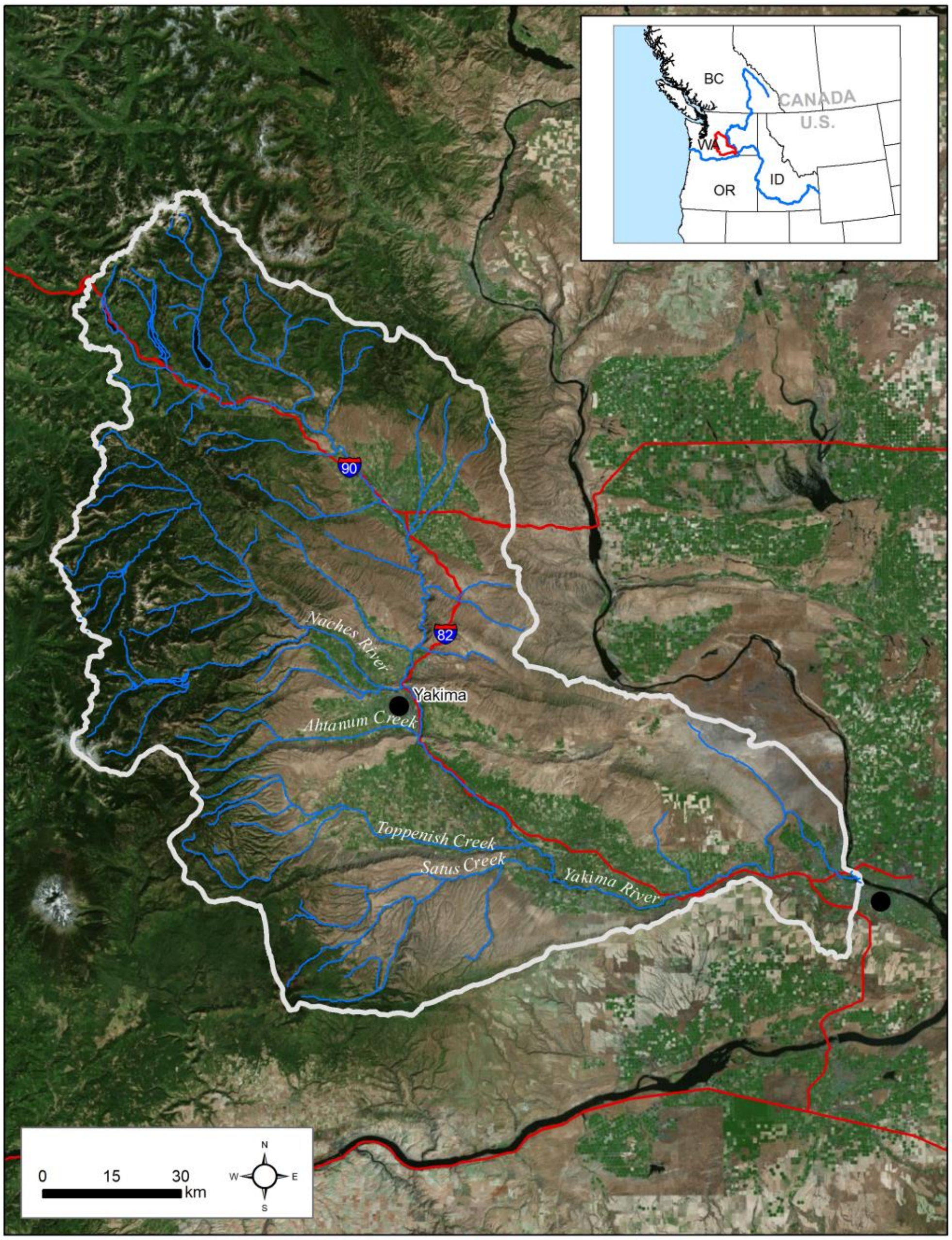


Figure 45: Yakima River basin with sampled tributaries. Juvenile samples were collected using electroshocking and rotary screw trap while adults were processed at Prosser Hatchery.

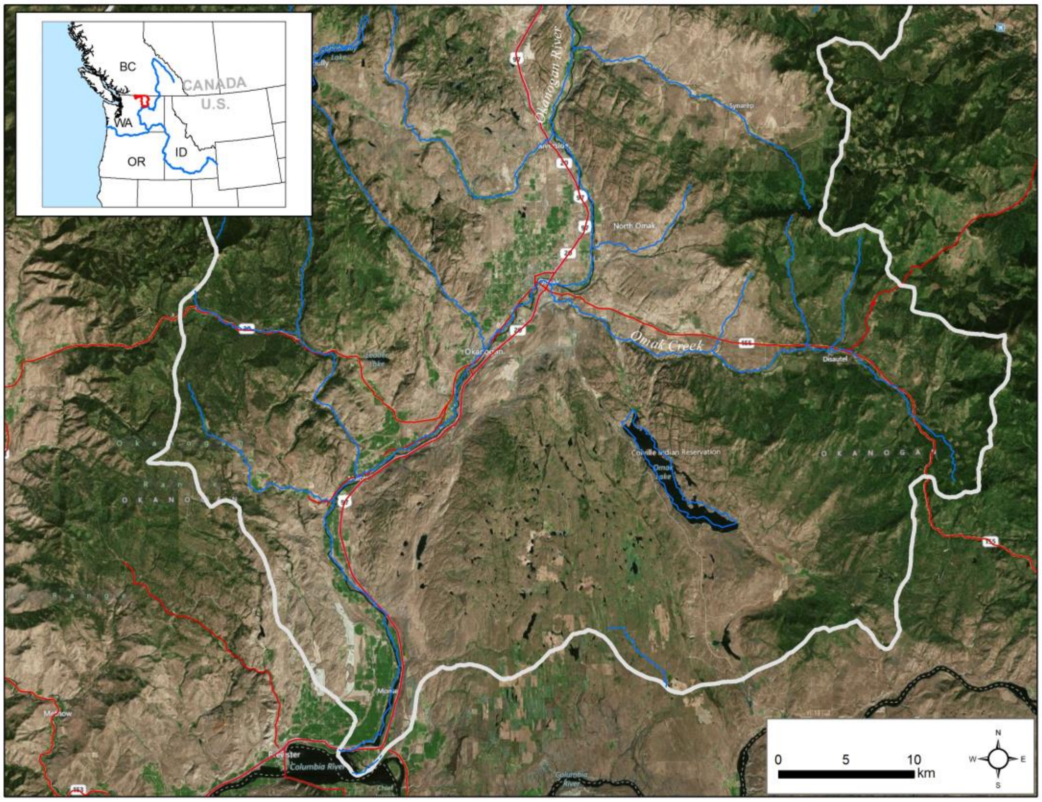


Figure 46: Omak Creek basin. All genetic samples were collected from various stretches from just above the mouth to below the barrier falls (8.6 RK).

Methods

Steelhead Kelt Gamete and Progeny Viability at the Parkdale Fish Facility

Parkdale Fish Facility cryopreservation and spawning

For collection and reconditioning methods see Kelt Management Scenarios section. Fish were identified primarily using PIT-tags throughout the reconditioning and evaluation period, and for simplified reporting only the last 4 digits (ID:xxxx) are used to identify individuals. Staff sorted fish biweekly from February through June checking for ripeness. Male gametes were collected manually and cryogenically stored (Cloud and Osborne 1997) prior to egg fertilization. This allowed us to use the same males for both maiden and reconditioned spawning events, controlling any male effect. Female gametes were collected by air spawning (Leitritz and Lewis 1980) as seen in Figure (47).

Figure 47: Air spawning female steelhead at Parkdale Fish Facility. Pictured left to right is Ryan Branstetter, Jim Gidley, and Albert Santos.

Organ tissue and gamete samples were collected from post spawn males. Ovarian fluid samples were collected during air spawning and submitted to the ODFW pathology lab to screen for infectious diseases including Infectious Hematopoietic Necrosis virus (IHNV) and Bacterial Kidney Disease (BKD). Disease screens can take upwards of 4-6 weeks to be fully processed. A positive screen from either parent prompted the disposal of eggs and all possibly infected juveniles and mothers were euthanized. After air spawning, the total number of eggs was estimated utilizing the Von Bayer method (Wedemeyer 2002). Approximately 1500 eggs from each female were spawned and subdivided into three groups. Each egg group was held in an isolation basket and mixed with thawed cryopreserved milt (ODFW 2008) from two different males (Figure 48). This minimized the loss of sample units as a result of positive disease tests. Surviving females were reconditioned at the Parkdale Fish Facility and spawned a second time with cryopreserved milt from the same male combinations.

Figure 48: Utilizing cryopreserved milt to fertilize steelhead eggs

Water hardened eggs were disinfected with a diluted solution of iodaphor povadine (1:100 ppm) (Argentyne) prior to placement into vertical stack incubators. Eggs were incubated at 5.5^{0}C water and treated with formalin 3 times weekly at 1:600 for 15 minutes. Eggs were subsampled (N=20) on day 15 (120 temperature units), which developmentally, is at the epiboly stage. The collected eggs are fixed in Stockard's solution to estimate initial fertilization by counting the number of keels present. The proportion of eggs that were successfully fertilized post cold shock (Pennel and Barton 1996) and alevin that died post hatch was also recorded.

The fry subgroups were transferred to a fiberglass picking troughs (4.3 m (l) x 42.0 cm (w) x 11.4 cm (d) troughs which were subdivided (88.4cm x 41.9cm or 141.0 cm x 41.9 cm depending on stocking density) for isolation purposes (Figure 49). Single pass water is fed via downspout flowing at 56.8 liters/min with a constant temperature 5.5^{0}C. Fry were fed Biovita starter feed #0 to satiation every hour during daylight hours for the first 4 weeks then gradually moved to Biovita #1 and #2 at a rate of 4 times daily to satiation for the remaining 6-10 weeks. Fry were sampled by collecting two random quick-netted subsamples of juveniles every week for 10 weeks. All juvenile fish were anesthetized with MS-222 to reduce stress and simplify sampling. Wet weights and lengths were measured on 20 individuals from the collection. At the end of the 8-10- week period, all juvenile fry were euthanized with the administration of a fatal dosage of MS-222. All mortalities and intentional terminations were landfilled.

Prior to the Parkdale Fish Facility experiment the University of Idaho Aquculture Reasearch Institute (ARI) conducted experiments in 2006 and 2007. The ARI had higher water temperatures and different rearing

containers that could not be replicated at Parkdale (Branstetter et al 2007 and Branstetter et al. 2008) so these juvenile growth values could not be compared against kelts raised at Parkdale.

Figure 49: CRITFC-intern (Hardo Lopez) sampling juvenile fish from picking trough to collect weight and length measures.

Data Analysis

We evaluated performance of artificially reconditioned kelt steelhead at the Parkdale Fish Facility by collecting pre-spawn fish, air spawning, holding, artificially reconditioning and repeat spawning those fish. Winter- and summer-run repeat spawner performance was evaluated using fecundity, fertilization rate, juvenile starting size and growth rate from maiden spawning compared to repeat spawning events in a repeat measures design using paired t tests. We then compared first spawn performance of successful repeat spawners with the collection at large to determine if successful repeat spawners differed from the overall collection using two sample t tests.

Yakima and Omak Parentage Analysis

Genetic Sample Collection

Yakima River

Anadromous adult steelhead were collected as downstream kelt migrants at the Chandler Juvenile Monitoring Facility after presumably spawning in the spring. Age-0 juveniles (juveniles collected in the same calendar year as the spawning event) were targeted using electrofishing techniques (NMFS 2000 Electrofishing Guidelines) during the fall in natal tributaries. Sampling was targeted near areas where steelhead spawning was observed. Sample numbers for each collection at the tributary level are reported in Table (28). Fork length was recorded and graphed by each collection site as dates varied within the Satus and Toppenish drainages. Age-0 length range was determined by choosing the first break in slope seen on a scatter plot based on fish lengths.

Omak Creek

Adult steelhead were collected as upstream or downstream (kelts) migrants via an adult trap at a semi-permanent weir on Omak Creek. A PIT tag antennae array was also operated upstream of the Omak Creek confluence with the Okanogan River. Adults were collected at the weir in 2012 (n=137), and two individuals that were collected in 2011 were resampled or detected by PIT array in 2012.

Age-0 juveniles (juveniles collected in the same calendar year as the spawning event) were collected in the fall using electrofishing techniques. Age-0 status was determined by length (fish <100mm) during fall collections. In 2012, electrofishing started at the mouth of Omak Creek, and went as high as the barrier falls. Six of the sections are described in Miller (2013), and the other eight sections are all located between the mouth of Omak Creek and the upper end of Moomah Road.

Reconditioning efforts and subsequent detections of returning adults are quantified in Table (31). Juvenile sampling and genotyping was designed to preferentially sample fish of appropriate age to the post-reconditioning spawning event. In 2012 the PIT tag antennae detected two of the fish reconditioned in 2011. One fish was not detected again, while the second was captured at the adult weir following spawning.

Genetic Analysis

Fin tissue samples were collected and stored dry on whatman paper, or paper slips in coin envelopes for preservation of DNA. Genetic analysis was conducted at the Hagerman Fish Culture Experiment Station in Hagerman, ID. DNA was extracted from tissue samples using standard manufacturer's protocols from Qiagen® DNeasy™ extraction kit. Current genotyping efforts utilize the 192 Single Nucleotide Polymorphism (SNP) markers and methods described in Hess et al. (2012). Statistical analysis used 188 of the 192 markers. Of the four not used, three are diagnostic for cutthroat, and one marker (OmyY1_2SEX) is a sex-determining marker that was used only to determine fish gender for the study. Significant linkage disequilibrium was previously observed in one pair of loci: Omy_GHSR-121 and Omy_mapK3-103 by Hess et al. (2012). Because linked loci are still informative for parentage analysis, both linked markers were included in this study.

Prior to statistical analysis, confirmed duplicate samples, samples with incomplete genotypes, and non-target species samples were omitted and are not included in the results. In order to evaluate genetic diversity, expected and observed heterozygosity were calculated using Excel Microsatellite Toolkit (Park 2001). Deviation from Hardy-Weinberg equilibrium was evaluated using exact tests (Haldane 1954, Weir 1990, Guo and Thompson 1992) implemented in GENEPOP v3.4 (Raymond and Rousset 1995). Corrections to the significant value were made using the Bonferroni method (Rice, 1989).

Parentage analysis was performed using CERVUS v 3.0 (Marshall et al. 1998, Kalinowski et al. 2007). Information on fish gender was not included in the analysis. To minimize incorrect assignments, simulations were performed to determine a 99.0% confidence LOD value. Assignments were excluded unless they met the critical LOD value, had a minimum of 180 loci comparisons, and zero mismatches.

Yakima

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

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

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

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

Probability of At least one parent $= (S_{cr} * S_{cr})+(2*((S_{cr}) – (S_{cr} * S_{cr})))$

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

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

Results

Parkdale Fish Facility egg production, fertilization, and juvenile development

A total of 66 winter-run steelhead were collected and 68 summer-run fish were collected over a 7 year period from 2006 to 2013. A total of 12 summer- and 9 winter-run fish survived reconditioning and were repeat spawned.

Cumulative Winter-run Repeat Spawners 2010-2013.

In this section we compare maiden and repeat spawners using fecundity, fertilization rates, fry weight and length gain. In 2011, kelts were captured at a resistance board weir versus collection at the Powerdale Dam which may confound results.

Individual Repeat Spawning Versus Individual Maiden Spawner Reproductive Variables

For the 9 individuals that survived and repeat spawned, we compared their performance metrics between the two spawnings using paired t tests. Overall, performance metrics were not significantly different between the two spawnings. Analysis of individual metrics revealed no significant differences among egg production (p=0.746); fertilization rates (p=0.359); fry growth in terms of length change (p=0.862) and weight change (p=0.764) through 4 weeks of growth. The starting length and weight of fry were significantly larger in the maiden spawning than the repeat spawning (p=0.013 and p<0.000), respectively (Table 24).

Kelt Maiden Versus Grouped Maiden Reproductive Variables

To determine if repeat spawners represented a unique segment of the overall collection, we compared first spawning performance between the 9 fish that survived and repeated spawned with the total collection of 68 winter-run steelhead using two sample t-tests. We found no significant differences in any of the metrics analyzed including: egg production (p=0.335); fertilization rates (p=0.15); fry starting weight (p=0.318); fry starting length (p=0.454); fry weight growth (p=0.539); and, fry length growth (p=0.793) (Table 25).

Table 24: Parkdale winter steelhead progeny and gamete comparisons of maiden spawning with repeat spawning.

	Egg Production		Fertilization		Starting weight (g)		Weight Change (g)		Starting Length (cm)		Length Change (cm)	
Kelt ID	Maiden	Repeat	Maiden	Repeat	Maiden	Repeat	Maiden	Repeat	Maiden	Repeat	Maiden	Repeat
02768	4008	5256	6	55.7	0.30	0.15	0.26	0.13	3.3	2.8	0.8	0.4
02785	2640	2320	87.2	2.5	0.22	0.18	0.08	0.15	3.2	2.9	0.4	0.4
02808	4212	3810	5.5	70.5	0.23	0.13	0.09	0.15	3.2	2.8	0.5	0.3
02820	3542	5144	82.8	50.2	0.22	0.17	0.04	0.16	3.2	2.9	0.3	0.4
02821	4432	3952	8	25.9	0.29	0.22	0.10	0.16	3.5	3.0	0.3	0.5
9565	4752	4563	39	3.1	0.24	0.19	0.14	0.04	3.2	2.8	0.3	0.4
7831	6371	5904	54.7	41	0.25	0.21	0.10	0.05	3.2	2.9	0.3	0.3
2987	2672	3899	70.6	1	0.18	0.11	0.11	.	2.8	3.2	0.5	.
5439	6090	4864	68.3	22	0.24	0.19	0.12	0.17	3.1	2.8	0.3	0.4
mean	4302.11	4412.44	46.90	30.21	0.24	0.17	0.11	0.13	3.18	2.90	0.42	0.39
n	9		9		9		8		9		8	
Paired t	-0.336		0.972		5.763		-0.312		3.194		0.18	
df	8		8		8		7		8		7	
P	0.746		0.359		0.000		0.764		0.013		0.862	

Table 25: Parkdale winter steelhead progeny and gamete comparisons from fish that survived artificial reconditioning and repeat spawned versus all the 1st spawning of the collected population.

	Egg Production		Fertilization		Starting weight (g)		Weight Change (g)		Starting Length (cm)		Length Change (cm)	
	Repeat	All Maiden	Repeat	All Maiden	Repeat	All Maiden	Repeat	All Maiden	Repeat	All Maiden	Repeat	All Maiden
mean	4302	4819	47	65	0.24	0.23	0.11	0.13	3.18	3.14	0.42	0.39
n	9	68	9	636	9.00	62	9	58	9	64	9	63
2 sample t	-1.013		-1.573		1.051		-0.639		0.78		0.27	
df	10.171		9.088		9.965		9.177		9.56		9.427	
P	0.335		0.15		0.318		0.539		0.454		0.793	

Cumulative Summer-run Repeat Spawners 2006-2013.

In this section we compare summer run maiden and repeat spawners using fecundity, fertilization rates, fry starting length and weight and weight and length gain.

Individual Repeat Spawning Versus Individual Maiden Spawner Reproductive Variables

For the 12 individuals that survived and repeat spawned, we compared their performance metrics between the two spawnings using paired t tests. Overall, performance metrics were not significantly different between the two spawnings. Analysis of individual metrics revealed no significant differences among egg production (p=0.087); fertilization rates (p=0.995); fry starting weight (p=0.107); fry starting length (p=0.740); fry growth in terms of length change (p=0.361) and weight change (p=0.269) (Table 26).

Kelt Maiden Versus Grouped Maiden Reproductive Variables

To determine if repeat spawners represented a unique segment of the overall collection, we compared first spawning performance between the 12 fish that survived and repeated spawned with the total collection of 66 summer-run steelhead using two sample t-tests. We found that successful repeat spawners had higher egg production at their first spawning compared to the collection at large (p=0.005). We found no other significant differences in any of the other metrics analyzed including: fertilization rates (p=0.712); fry starting weight (p=0.120); fry starting length (p=0.188); fry weight growth (p=0.332); and, fry length growth (p=0.549) (Table 27).

Table 26: Parkdale summer steelhead progeny and gamete comparisons of maiden spawning with repeat spawning.

	Egg Production		Fertilization		Starting weight (g)		Weight Change (g)		Starting Length (cm)		Length Change (cm)	
Kelt ID	Maiden	Repeat	Maiden	Repeat	Maiden	Repeat	Maiden	Repeat	Maiden	Repeat	Maiden	Repeat
2006Kelt	4618	3208	75	17			.		.	.	.	.
2007kelt	4864	6604	84	49		0.31	.	0.72	.	3.2	.	1.6
2008KS1	3810	4320	45	48	0.17	0.31	0.67	1.03	2.9	3.1	1.7	1.8
2008KS2	6318	6642	42	88	0.21	0.15	0.66	0.57	3.1	2.5	1.6	1.7
2008KS3	3696	4320	52	78	0.23	0.65	0.75	0.62	3.2	2.9	1.4	2
2008KS4	5280	7202	15	78	0.21	0.23	0.6	1.06	3.2	3.2	1.3	2.1
2008KS5	4191	4320	50	70	0.28	0.29	0.92	1.08	3	2.9	1.7	2.5
2008KS6	4080	5184	46	2	0.24	0.4	.	.	3	3.3	.	.
2009KS1	3340	4104	79	79	0.18	0.29	0.81	0.77	3	3.3	1.7	1.5
2009KS2	4864	3952	78	64	0.25	.	.	.	3	.	.	.
2010KS1	4048	6578	28	75	0.25	0.25	0.52	0.75	3.2	3.2	1.8	1.5
2010KS2	5525	5540	68	15	0.21	0.2	0.78	0.63	3.1	3	1.8	1.3
mean	4552.83	5164.50	55.17	55.25	0.22	0.31	0.71	0.80	3.07	3.06	1.63	1.78
n	12		12		9		8		9		8	
Paired t	-1.876		-0.007		-1.814		-1.2		0.343		-0.977	
df	11		11		8		7		8		7	
P	0.087		0.995		0.107		0.269		0.74		0.361	

Table 27: Parkdale summer steelhead progeny and gamete comparisons from fish that survived artificial reconditioning and repeat spawned versus all the 1st spawning of the collected population.

	Egg Production		Fertilization		Starting weight (g)		Weight Change (g)		Starting Length (cm)		Length Change (cm)	
	Repeat	All Maiden	Repeat	All Maiden	Repeat	All Maiden	Repeat	All Maiden	Repeat	All Maiden	Repeat	All Maiden
mean	4553	3743	55	58	0.22	0.24	0.71	0.77	3.07	3.12	1.63	1.67
n	12	66	12	57	10	39	8	37	10	39	8	38
Paired t	3.136		-0.375		-1.634		-1.006		-1.366		-0.613	
df	21.087		17.916		17.737		13.929		18.643		15.049	
P	0.005		0.712		0.12		0.332		0.188		0.549	

Yakima Basin

To determine reproductive success of artificially reconditioned repeat spawners in the Yakima Basin, a total of 1163 samples were successfully genotyped (Table 28). Juvenile plotted lengths (Appendix B3) for each collection show likely break points representing different age groups. Although sometimes ambiguous, the first break in the slope of lengths varied between 50 and 90 mm in fork length.

Numbers for each collection, by location and year, can be seen in Table (28). Departures from Hardy-Weinberg equilibrium (critical level =0.05 /188 loci = 0.000266) were seen in three collections. Parentage analysis proceeded as normal as it does not require Hardy-Weinberg or Linkage equilibrium.

Table 28: Population Statistics. Each collection is reported in terms of sample size (n), expected heterozygosity (HE), observed heterozygosity (H_O), number of loci out of Hardy-Weinberg equilibrium (HW), and number of pairwise loci comparisons showing significant linkage disequilibrium (LD).

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

Across all juvenile collections, 13 offspring were successfully assigned to at least 1 repeat spawning adult (Table 29). Assigned offspring include 5 to Satus Cr., 4 to Toppenish Cr., 2 to Nile Cr., and 1 to Little Rattlesnake Cr. Twelve of the assignments were between 48 and 70 mm in fork length, while 1 was seen at 88mm in length. Table (30) shows the expected, observed and percent of expected parentage assignments for each of the juvenile classes. Assuming all 611 potential offspring were of age-0, 85.9 would be expected to assign back to at least one parent, while 13 assignments were found.

Table 29: Juvenile offspring assigned to at least one parent.

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

Table 30: Assignment probabilities. Each class of assignment is listed for the probability of assignment by individual juvenile progeny, and the expected and observed numbers for all individuals.

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

Omak

To determine reproductive success of artificially reconditioned repeat spawners in Omak Creek, a total of 352 samples were successfully genotyped. Numbers by life stage can be seen in Table (31). Of the 137 adult samples genotyped in 2012, 135 were putative first time spawners. Two fish were from the 2011 capture, reconditioning and release efforts. There were 100 genotypic males, 35 genotypic females, and 2 undetermined individuals. Departures from Hardy-Weinberg equilibrium (critical level =0.05 /191 loci = 0.000266) were seen in both adults and juveniles. This may be the result of kinship or the Wahlund effect resulting from population admixture of distinct resident and anadromous populations (Branstetter et al. 2011). Parentage analysis proceeded as normal as it does not require Hardy-Weinberg equilibrium to be informative unless the cause is allelic dropout.

Table 31: Population Statistics. Each collection is reported in terms of sample size (n), expected heterozygosity (H_E) and observed heterozygosity (H_O).

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

A total of 103 juveniles were successfully assigned back to at least one adult parent. Of the 103, 17 were assigned to a parent pair. Table (32) shows the number of age 0 offspring genotyped and successfully assigned by each site. Sites are listed starting at the mouth (Section 1) moving upstream to the barrier falls

(Section 14) (Table 32). Overall, 48.4% of the juveniles genotyped were assigned to at least one parent. Section 1 had a single one of the 16 genotyped juveniles successfully assigned back to at least one parent.

Table 32: Parentage Assignments. Each section is reported in numbers of genotyped fish (n), and the number and percentage of successful assignments.

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

A single female accounted for 11 of the 103 progeny that were assigned to at least one parent, while the majority of adults had no progeny assigned to them (Table 33 and Figure 50). The lack of reproductive success detection was more pronounced in males (73%) than females (48%). A small subset of these fish (5 males, 3 females) were likely removed prior to spawning when they were collected as hatchery broodstock.

Table 33: Progeny Per Adult. Percent of adults having between 0 and 11 offspring assigned to them

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

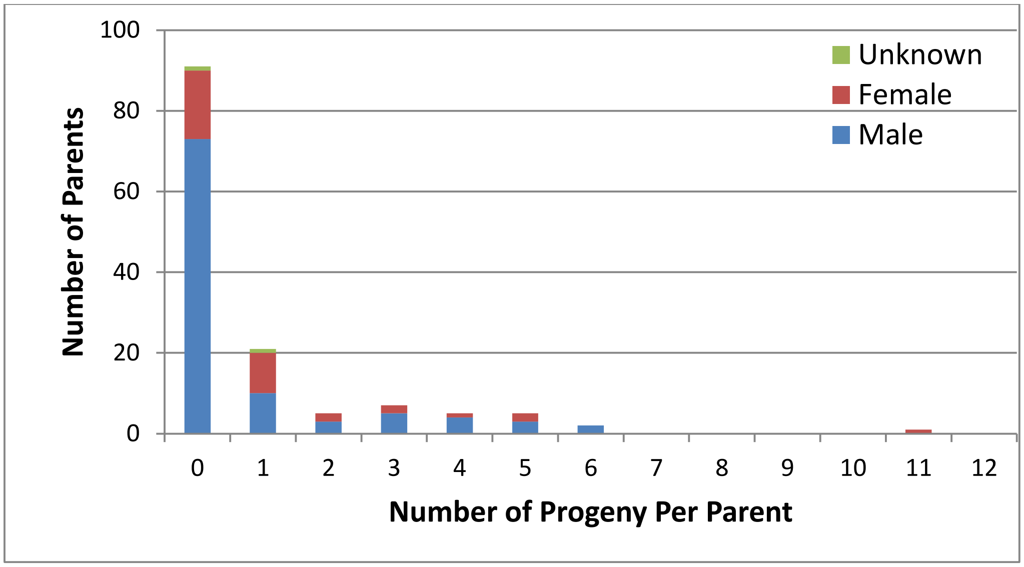


Figure 50 Progeny per adults. Number of adults having between 0 and 11 offspring assigned to them.

Table (34) shows a summary of reproductive success attributed to fish that went through the reconditioning process and returned to spawn again. Successful reproduction has been confirmed for four of the eleven reconditioned kelts that were detected returning to Omak Creek. Reconditioned kelt progeny were not detected in 2012.

Table 34: Summary of reproductive success. Each detection of reproductive success reported below

Progeny	Stage	Length	Sample year	Brood Year	Kelt ID	Kelt Gender
OMRST-216	Smolt	103	2007	2006	OCKELT-2	Male
OMRST-171	Smolt	97	2007	2006	OCKELT-2	Male
OMRST-575	Smolt	135	2007	2006	OCKELT-2	Male
OMRST263	Smolt	152	2008	2006	OCKELT-2	Male
OMRST109	Smolt	193	2008	2006	OCKELT-2	Male
OMRST75	Smolt	182	2008	2006	OCKELT-1	Female
Redd-A1	Fry		2008	2008	OMCT5	Female
OMRST-45	Smolt	163	2009	2008	OMCT5	Female
OmyOCS2011j-0175	Age-0	77	2011	2011	Omy-AE27	Female
OmyOCS2011j-1012	Age-0	78	2011	2011	Omy-AE27	Female

Conclusions

Parkdale Fish Facility

This study was initiated to evaluate repeat spawning performance of steelhead in captivity. Winter- and summer-run steelhead were independently evaluated as maiden spawners, artificially reconditioned and evaluated as repeat spawners. Reproductive success performance metrics were all not significantly different between maiden and repeat spawnings for winter- and summer-run fish except for the starting fry size, both and length at weight for winter-run steelhead suggesting that artificial reconditioning imparted no negative impact on the fish (Tables 24,26). The difference in fry size was likely attributed to changing

collection locations. Additionally, the fish that survived to repeat spawn were not significantly different from the collections at large (Tables 25,27). A total of 21 fish from the two runs were successfully reconditioned, some multiple times.

Limited holding space at the Parkdale Fish Facility, a change in collection location, stress, and an accidental mixing of progeny in one year, restricted sample sizes. Adult holding space was limited to about 40 adults, including both males and females as well as maiden and repeat spawners. Juvenile rearing space was also a limitation, with total rearing capacity of about 50 broods. Prior to 2011, maiden fish were collected at Powerdale Dam (RK 7) located low on the mainstem Hood River. After the removal of Powerdale Dam, maiden fish collections were relocated to a resistance board weir on the East Fork of the Hood River. The majority of the 2011 maiden fish died shortly after spawning, leaving only 3 kelts to recondition. Fish quality declined with the new trap placement following the decommissioning of the Powerdale trap in 2010. The fish collected at the weir suffered numerous fungal infections and stress related diseases not observed in prior collections. The hatchery manager also noted that many of the other brood fish (both chinook and steelhead) have also been in poorer shape (higher mortality and lower fertilization rates) than in his prior years (>20) of experience at the facility (pers. conv. Gidley, J., 2013). Fish collected at Powerdale Dam required less active disease treatment than fish collected at the weir. Consequently, we were slower to treat fish collected at the weir in the first year (2011); this likely resulted to additional mortality. In 2012, we took an aggressive response to mortalities in the hatchery and treated all kelts with oxytetracycline and erythromycin if mortalities would cluster around specific dates. Parkdale staff also assisted with collection of kelts and checked the trap boxes at a higher frequency in 2012 than in 2011, which likely improved the quality of kelts, which were present at the facility. These aggressive approaches likely improved reconditioning survival to one of the highest on record at Parkdale (60%). The 2010 winter kelt progeny cumulative weight and length change measures were compared at week 4 due to sampling errors in 2010 and cleaning error in 2011 which resulted in some groups becoming mixed at week 5. This left a gap for week 5-10 with which to effectively compare change in weight and length for those brood years.

The remaining kelts were terminated in June 2013 following the end of this portion of the project. All of these kelts showed early stages of gamete development and likely would have produced additional eggs in the spring of 2014 (Figure 57).

Figure 57: Necropsied kelt reconditioned at the Parkdale Fish Facility demonstrating developing gametes.

We expected winter-run fish to artificially recondition better than the summer-run fish as a result of less time spent fasting and improved water quality during freshwater residence, similar to results at Snow Creek (Seamons and Quinn 2010). The initial winter-run collection was made at Powerdale Dam and results were quite positive with no significant difference detected in performance metrics from 5 repeat spawners. The change in collection location resulted in more variable results.

We observed that repeat spawners may be foregoing active growth and instead directing the majority of energy into egg production, similar to findings in Quinn et al. (2011). Some fish repeat spawned multiple times, there were 9 winter-run repeat spawnings (10 repeat events) and 12 summer-run repeat spawners (17 spawning events). Since the study was terminated, we were not able to follow the 2012 winter-run through the entirety of their potential spawning; skip-spawning in 2014 may well have provided additional repeat spawners and yielded important data. However, we did determine that winter-run kelts are capable of being artificially reconditioned without negative effect on them.

Performance metrics were not statistically different between maiden and repeat spawnings in summer steelhead. However, successful repeat spawners did produce larger egg numbers ($p=0.005$) in their maiden spawning than the maiden spawnings from the overall collection.

To increase the knowledge base of repeat spawner production, the geographic replication of this experiment with the addition of following progeny through an extended rearing and eventual release, so that smolt to adult ratios could be obtained, which would provide great insight into the reproductive capabilities of steelhead kelts and their progeny. Based on our data a summer-run kelt program could benefit the Hood River, since the distinct summer-run population (Matala 2009) is depressed. Unfortunately

several logistical hurdles would need to be overcome including finding a facility with holding space and a means to collect large numbers of steelhead kelts.

Yakima River

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

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

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

The collection of Age-0 juveniles should continue in future years as the primary method of offspring collections. However, it may be necessary to collect additional length data from all size classes to demonstrate separation of age classes. Up to 30% of the juveniles genotyped in 2012 are likely attributed to age-1 or greater fish, which explains a portion of the difference between expected and observed juvenile parentage assignments.

While reproductive success has been confirmed for four reconditioned kelts spawning in the Yakima drainage in 2011, we are currently unable to calculate relative reproductive success estimates. The small number of samples that are being successfully assigned limits statistical power to compare reproductive success among other groups such as first time spawners. Increasing the proportion of adult spawners and number of juveniles sampled will help with this issue. A second issue is the lack of unbiased data for first time spawners. Samples collected at the Chandler facility as post-spawn kelts are putative first time spawners for the year they are collected. However, they are not random samples. Post-spawn kelts have survived the full life cycle and are assumed to have successfully spawned. Alternatively, kelts that are released following reconditioning are still exposed to over-wintering and pre-spawn mortality.

Reconditioned kelts detected at Prosser Dam and first time spawner adults sampled at Chandler Dam in the fall are likely a good comparison, but sample sizes for these two groups are low relative to overall adult

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

Omak Creek

Reproductive success has been confirmed for four individuals following reconditioning efforts. Two progeny were seen from a female kelt reconditioned in 2010 and detected at the PIT tag array in 2011. A female observed digging below the weir in 2008 had progeny detected as an age-0 emergent fry in 2008 and age-1 in 2009. One of the females returning in 2006 was also shown to reproduce with the detection of an age-2 progeny in 2008. The male reconditioned kelt that passed above the Omak Creek picket weir in 2006 successfully spawned with progeny detected as both age-1 in 2007 and age-2 in 2008.

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

Determination of kelt reproductive success is dependent upon separation of first and second time spawning events. While some years (2007 and 2008) have shown discrete age-1 and age-2 histograms in spring collections at the screwtrap, variable growth rates preclude reliable age assignment by length. Potential alternatives include sampling at age-0, full parental sampling, and scale analysis. Full parental sampling has been unattainable so far, and scale analysis has not been shown as accurate at aging juvenile steelhead. The successful sampling of age-0 fish in fall 2011 and 2012 demonstrated the ability to sample juveniles at a length that is identifiable, and will be repeated in future years.

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

2013 Parentage Analysis

We currently are in the process of analyzing collection data from 2013 work conducted in the Yakima River, which is collected in the fall. These results will be published in the 2014 annual report. We are working on trying to narrow fish spawning locations with radio tags and utilizing in-stream PIT-tag arrays. This attempt at finding spawning locations should help us to get a more accurate determination of reconditioned kelt

spawning success in the wild. No fish were detected in Omak Creek so no collection of juvenile fish was made in Omak Creek in 2013 for parentage analysis. The 4 kelts that were released in the fall will be monitored for detection into Omak Creek and possible subsequent juvenile collection. We are exploring the option of another site in Idaho that would utilize artificially reconditioned b-run kelts for parentage analysis as our kelt numbers from this location were 3 times that of Omak Creek with potential for much higher than that in 2014.

b. Coordination and Data Management (RM&E)

The CRITFC and its member/partner tribes work with a number of agencies throughout the Columbia River Basin. Coordination and data sharing are very important components of the CRTFC's mission to enhance fish throughout the basin. The CRITFC provides Annual Reports which are available on CRITFC website: CRITFC Science Reports. The CRITFC routinely collaborates and shares data with other entities. The CRITFC is also integral in helping to develop policy and management throughout the basin such as the *kelt management plan* and kelt master plan (still in development) for the Snake River Basin.

Presentations:

Our team presented findings at the following professional meetings in 2013:

- American Fisheries Society Annual Meeting – 3 presentations
- Yakima Basin Science and Management Conference – 3 presentations
- Western Division American Fisheries Society – 3 presentations
- World Aquaculture Society – 1 presentation
- Center for Reproductive Biology – 2 presentations

Caldwell LC, Riley LG, Duncan CA, Pierce AL, and Nagler JJ. Metabolic and reproductive endocrine signals of repeat spawning in post-spawned Oncorhynchus mykiss. Oral presentation, World Aquaculture Society Triennial Meeting, Feb 21-25 2013, Nashville, Tennessee.

Branstetter R, Hatch D, Whiteaker J, Pierce A, Bosch B, Blodgett J, Moffitt C, Everett S. Survival and migration characteristics in the Lower Columbia River of post-spawn steelhead (Oncorhynchus mykiss) kelts transported from the mid-Columbia and Snake rivers and released below Bonneville Dam. Poster presentation American Fisheries Society Annual Meeting, April 15-18, 2013, Boise, Idaho.

Hatch D, Fast D, Bosch W, Blodgett J. Survival and traits of Reconditioned Kelt Steelhead Oncorhynchus mykiss in the Yakima River, Washington. Oral presentation American Fisheries Society Annual Meeting, April 15-18, 2013, Boise, Idaho.

Penney Z, Moffitt C. Histological assessment of selected tissues in maturing and post spawning Snake River steelhead. Oral presentation American Fisheries Society Annual Meeting, April 15-18, 2013, Boise, Idaho.

Pierce AL, Cavileer TD, Boyce J, Medeiros LR, Caldwell LC, Blodgett JW, Bosch WJ, Fast DE, Branstetter R, Hatch DR, and Nagler JJ. Columbia River basin steelhead kelt reconditioning: reproductive development, energy stores, and post-release migration of long-term reconditioned female kelts in the Yakima River. Poster presentation, 17th Annual Center for Reproductive Biology Retreat, May 14 2013, Pullman, Washington.

Caldwell LK, Pierce AL, and Nagler JJ. Reproductive steroids involved in rematuration of iteroparous female rainbow trout. Poster presentation, 17th Annual Center for Reproductive Biology Retreat, May 14 2013, Pullman, Washington.

Hatch D, Fast D, Bosch W, Blodgett J, Whiteaker J, Branstetter R, Pierce A. Survival and Traits of Reconditioned Kelt Steelhead *Oncorhynchus mykiss* in the Yakima River, Washington. Oral presentation, Yakima Basin Science and Management Conference, June 12-13 2013, Ellensburg, Washington.

Pierce AL, Blodgett JW, Fiander M, Bosch WJ, Turner K, Everett S.R., Branstetter R, Fast DE, and Hatch DR. Control of parasitic copepods in steelhead kelts. Oral presentation, Yakima Basin Science and Management Conference, June 12-13 2013, Ellensburg, Washington.

Trammel J. An Evaluation of the Yakama Fisheries Kelt Reconditioning. Oral presentation, Yakima Basin Science and Management Conference, June 12-13 2013, Ellensburg, Washington.

Hatch D, Fast D, Bosch W, Blodgett J, Whiteaker J, Branstetter R, Pierce A. Survival and traits of reconditioned kelt steelhead *Oncorhynchus mykiss* in the Yakima River. Oral presentation American Fisheries Society Annual Meeting September 8-12, 2013, Little Rock, Arkansas.

Penney Z. Finding Death: the relation between energy and iteroparity in steelhead trout. Oral presentation American Fisheries Society Annual Meeting September 8-12, 2013, Little Rock, Arkansas.

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Publications:

Three Publications have been produced with another 4 in review.

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Appendix A: Use of Data & Products

Appendix B: Detailed Results

B1. Steelhead Kelt Reconditioning Treatments

Strategy	Year	Location	# Collected	# released	S @ release (%)	# @ ocean	S @ ocean (%)	# @ Bonneville	Return Rate to Bonneville (%)	Transportation (or treatment) Benefit relative to in-river	Treatment benefit relative to Hockersmith 1.66	Transportation (or treatment) Benefit relative to Bonneville natural
In-river	2005	Prosser	67	67				3	4.48	1.32	2.70	25.61
In-river	2006	Prosser	52	52				1	1.92	0.57	1.16	3.10
In-river	2007	Prosser	53	53				3	5.66	1.66	3.41	9.28
In-river	2008	Prosser	88	88				4	4.55	1.34	2.74	6.64
In-river	2009	Prosser	58	58				3	5.17	1.52	3.12	11.54
In-river	2010	Prosser	155	155				2	1.29	0.38	0.78	3.74
In-river	2011	Prosser	85	85				3	3.53	1.04	2.13	6.92
In-river	2012	Prosser	59	59				2	3.39	1.00	2.04	6.65
In-river	2013	Prosser	52	52				0	0.00	0.00	0.00	0.00
Total and weighted mean			***669***	***669***				***2.63***	***3.40***	***1.00***	***2.05***	***5.56***
In-river	2002	Lower Granite*	1209	1209				8	0.66	1.94	0.40	
In-river	2003	Lower Granite*	865	865				3	0.35	1.02	0.21	
In-river	2004	Lower Granite*	1138	1138				10	0.88	2.57	0.53	1.49
In-river	2009	Lower Granite	178	176				2	1.12	3.29	0.68	1.51
In-river	2010	Lower Granite	1411	1399				5	0.35	1.04	0.21	0.62
In-river	2011	Lower Granite	1633	1613				3	0.18	0.54	0.11	0.22
In-river	2012	Lower Granite	2098	2098				6	0.29	0.72	0.17	0.34
In-river	2013	Lower Granite	840	827				0	0.00	0.00	0.00	0.00
Total and weighted mean			***9372***	***9325***				***5.69***	***0.39***	***1.00***	***0.26***	***0.65***
In-river	2002	John Day*	***287***	***287***				***28***	***9.76***	***1.00***	***5.88***	***15.94***
Total and weighted mean												
Transported (Hamilton Island)	2002	Lower Granite*	750	750				19	2.53	3.83	1.53	
Transported (Hamilton Island)	2003	Lower Granite*	376	376				3	0.80	2.30	0.48	
Transported (Hamilton Island)	2004	Lower Granite*	982	982				7	0.71	0.81	0.43	2.00
Transported (Hamilton Island)	2009	Lower Granite	71	68				0	0.00	0.00	0.00	0.00
Transported (Hamilton Island)	2010	Lower Granite	301	301		13/108	12.04	0	0.00	0.00	0.00	0.00
Transported (Hamilton Island)	2011	Lower Granite	109	109		3/47	6.38	0	0.00	0.00	0.00	0.00
Total and weighted mean			***2589***	***2586***			***9.21***	***8.59***	***1.12***	***1.74***	***0.67***	***1.83***
Transported (estuary release)	2010	Lower Granite	23	22		4/10	40.00	0	0.00	0.00	0.00	0.00
Transported (estuary release)	2011	Lower Granite	91	90		14/46	30.43	0	0.00	0.00	0.00	0.00
Total and weighted mean			***114***	***112***			***35.22***	***0.00***	***0.00***	***0.00***	***0.00***	***0.00***
Transported	2002	John Day*	***271***	***271***				***34***	***12.55***	***1.29***	***7.56***	***20.50***
Total and weighted mean												

Transported (unfed Hamilton Island)	2004	Prosser	75	63		15/28	53.57	5	6.67		4.02	18.75
Transported (unfed Hamilton Island)	2005	Prosser	98	96		14/57	24.56	1	1.02	0.23	0.61	5.84
Transported (unfed Hamilton Island)	2006	Prosser	55	49		31/49	63.27	2	3.64	1.89	2.19	5.87
Transported (unfed Hamilton Island)	2007	Prosser	43	38		14/35	40.00	0	0.00	0.00	0.00	0.00
Transported (unfed Hamilton Island)	2008	Prosser	100	100		26/49	53.06	3	3.00	0.66	1.81	4.38
Transported (unfed Hamilton Island)	2010	Prosser	124	123		27/59	45.76	1	0.81	0.16	0.49	2.34
Transported (unfed Hamilton Island)	2011	Prosser	100	100		16/47	34.04	1	1.00	0.78	0.60	1.96
Total and weighted mean			***595***	***569***			***44.89***	***1.86***	***2.18***	***0.64***	***1.32***	***3.57***
Transported (unfed estuary release)	2010	Prosser	113	113		13/60	21.67	1	0.88	0.69	0.53	2.57
Transported (unfed estuary release)	2011	Prosser	90	89		16/47	34.04	3	3.33	2.58	2.01	6.54
Total and weighted mean			***203***	***202***			***27.85***	***1.00***	***1.97***	***1.63***	***1.19***	***3.22***
Transported (fed Hamilton Island)	2002	Prosser	479	334				43	8.98		5.41	
Transported (fed Hamilton Island)	2003	Prosser	208	187				8	3.85		2.32	
Transported (fed Hamilton Island)	2004	Prosser	105	83		11/26	42.31	5	4.76		2.87	13.39
Transported (fed Hamilton Island)	2005	Prosser	106	96		6/56	10.71	0	0.00	0.00	0.00	0.00
Transported (fed Hamilton Island)	2006	Prosser	56	50		32/50	64.00	0	0.00	0.00	0.00	0.00
Transported (fed Hamilton Island)	2007	Prosser	40	38		19/27	70.37	1	2.50	0.44	1.51	4.10
Transported (fed Hamilton Island)	2008	Prosser	108	100		28/50	56.00	7	6.48	1.43	3.90	9.47
Total and weighted mean			***1102***	***888***			***48.68***	***21.40***	***5.81***	***1.71***	***3.50***	***9.49***
Transported (pooled groups)	2002	Prosser	479	334				43	8.98		5.41	
Transported (pooled groups)	2003	Prosser	*208*	187				8	3.85		2.32	
Transported (pooled groups)	2004	Prosser	203	179		26/54	48.15	10	4.93		2.97	13.85
Transported (pooled groups)	2005	Prosser	161	145		20/113	17.70	1	0.62	0.14	0.37	3.55
Transported (pooled groups)	2006	Prosser	99	88		63/99	63.64	2	2.02	1.05	1.22	3.26
Transported (pooled groups)	2007	Prosser	140	138		33/62	53.23	1	0.71	0.13	0.43	1.17
Transported (pooled groups)	2008	Prosser	232	223		54/99	54.55	10	4.31	0.95	2.60	6.30
Transported (pooled groups)	2010	Prosser	237	236		40/119	33.61	2	0.84	0.16	0.51	2.45
Transported (pooled groups)	2011	Prosser	190	189		32/94	34.04	4	2.11	1.63	1.27	4.13
Total and weighted mean			***1759***	***1530***			***45.14***	***15.68***	***4.16***	***1.22***	***2.64***	***6.79***
Long-term	2000	Prosser	512	91	17.77						10.71	
Long-term	2001	Prosser	551	197	35.75						21.54	
Long-term	2002	Prosser	420	140	33.33						20.08	
Long-term	2003	Prosser	482	298	61.83						37.24	
Long-term	2004	Prosser	662	253	38.22						23.02	107.49
Long-term	2005	Prosser	386	86	22.28					4.98	13.42	127.44
Long-term	2006	Prosser	279	85	30.47					15.84	18.35	49.15
Long-term	2007	Prosser	422	221	52.37					9.25	31.55	85.84

Long-term	2008	Prosser	472	269	56.99	12.54	34.33	83.27
Long-term	2009	Prosser	510	140	27.45	5.31	16.54	61.24
Long-term	2010	Prosser	1157	404	34.92	27.06	21.03	101.26
Long-term	2011	Prosser	680	223	32.79	9.29	19.76	64.30
Long-term	2012	Prosser	550	340	61.82	17.52	37.24	56.20
Long-term	2013	Prosser	546	266	48.72	14.37	29.35	44.29
Total and weighted mean			***7629***	***3013***	***38.78***	***11.39***	***23.36***	***63.37***
Long-term	2005	Shitike Cr	9	1	11.11		6.69	63.56
Long-term	2006	Shitike Cr	4	0	0.00		0.00	0.00
Long-term	2007	Shitike Cr	14	1	7.14		4.30	11.71
Long-term	2008	Shitike Cr	11	0	0.00		0.00	0.00
Total and weighted mean			***38***	***2***	***5.26***		***3.17***	***8.60***
Long-term	2005	Omak Cr	17	3	17.65		10.63	100.94
Long-term	2006	Omak Cr	27	2	7.41		4.46	11.95
Long-term	2007	Omak Cr	43	8	18.60		11.21	30.50
Long-term	2008	Omak Cr	32	9	28.13		16.94	41.09
Long-term	2009	Omak Cr	17	2	11.76		7.09	26.25
Long-term	2010	Omak Cr	13	6	46.15		27.80	133.85
Long-term	2011	Omak Cr	20	4	20.00		12.05	39.22
Long-term	2012	Omak Cr	65	4	6.15		3.71	5.59
Long-term	2013	Omak Cr	49	4	8.16		4.92	
Total and weighted mean			***283***	***42***	***16.24***		***9.78***	***26.54***
Long-term	2006	Parkdale	1	1.0	100.00		60.24	161.33
Long-term	2007	Parkdale	13	1.0	7.69		4.63	12.61
Long-term	2008	Parkdale	14	7	50.00		30.12	73.06
Long-term	2009	Parkdale	9	4	44.44		26.77	99.15
Long-term	2010	Parkdale	15	4	26.67		16.06	77.33
Long-term	2011	Parkdale	23	5	21.74		13.10	42.63
Long-term	2012	Parkdale	21	13	61.90		37.29	56.28
Total and weighted mean			***96***	***35.0***	***36.46***		***21.96***	***59.57***
Long-term	2012	DNFH	143	5.0	3.50		2.11	3.18
Long-term	2013	DNFH	163	55.0	33.74		20.33	30.67
Total and weighted mean			***306***	***60***	***19.61***		***7.84***	***32.04***
Long-term	2012	Lower Granite	124	9.0	7.26	152.27	4.37	6.60
Long-term	2013	Lower Granite	110	57.0	51.82	151.76	31.22	47.11
Total and weighted mean			***234***	***66***	***28.21***	***82.61***	***16.99***	***46.09***
Long-term	2013	S.F. Clearwater	24	12.0	50.00	146.44	30.12	45.45
Total and weighted mean			***24***	***12***				

Natural repeat	2004	Bonneville Dam	1125	4	0.36
Natural repeat	2005	Bonneville Dam	572	1	0.17
Natural repeat	2006	Bonneville Dam	1452	9	0.62
Natural repeat	2007	Bonneville Dam	1967	12	0.61
Natural repeat	2008	Bonneville Dam	2630	18	0.68
Natural repeat	2009	Bonneville Dam	2454	11	0.45
Natural repeat	2010	Bonneville Dam	1740	6	0.34
Natural repeat	2011	Bonneville Dam	1391	7	0.51
Natural repeat	2012	Bonneville Dam	1486	16	1.1
Natural repeat	2013	Bonneville Dam	1278	14	1.1
			16095		***0.61***

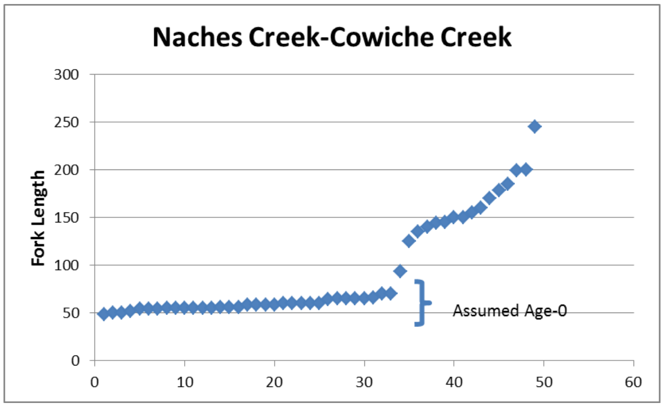
* Lower Granite and John Day data from Evans, A.F., R.H. Wertheimer, M.L. Keefer, C.T. Boggs, C.A. Peery, and K. Collis. 2008. Transportation of steelhead kelts to increase iteroparity in the Columbia and Snake Rivers.

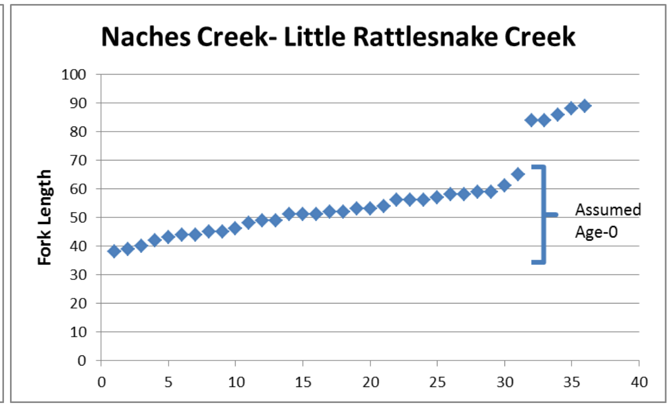
B2. Summary demographic statistics by reporting group and gender, for kelts sampled at Lower Granite Dam (2009-2012). "M-F" for outmigration date (i.e. ordinal day) is the difference in mean day between genders. Under condition, G=good, F=fair, and P=poor.

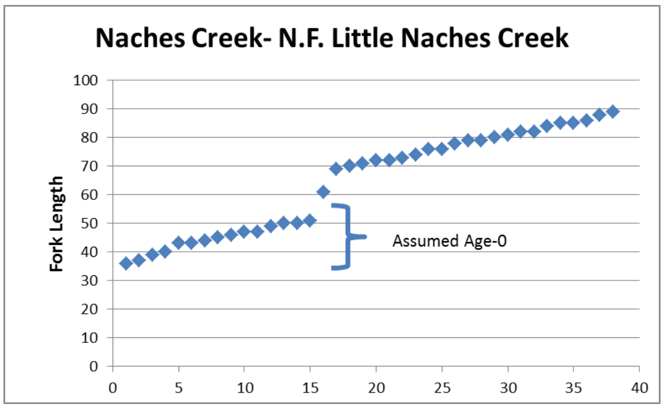
		Gender					ordinal day (od)			condition (F)			condition (M)			mean FL (cm)	
Yea r	reporting group	F	M	tot al	% F	% M	F	M	M- F	G	F	P	G	F	P	F	M
200	GR	31	7	38	0.	0.	152	163	11	0.	0.	0.	0.	0.	0.	61.	59.
	IM	19	0	19	1.	0.	153	--	--	0.	0.	0.	--	--	--	63.	---
	LCL	5	1	6	0.	0.	133	166	33	1.	0.	0.	0.	0.	0.	64.	63.
	LS	8	1	9	0.	0.	159	175	16	0.	0.	0.	1.	0.	0.	69.	59.
	LSN	26	6	32	0.	0.	153	159	6	0.	0.	0.	0.	0.	0.	61.	58.
	MFS	39	1	40	0.	0.	159	177	18	0.	0.	0.	0.	0.	0.	65.	65.
	SFC	5	1	6	0.	0.	116	155	39	0.	0.	0.	1.	0.	0.	80.	61.
	SFS	6	0	6	1.	0.	160	--	--	0.	0.	0.	--	--	--	77.	---
	UPC	7	0	7	1.	0.	155	--	--	0.	0.	0.	--	--	--	73.	---
	UPS	81	2	10	0.	0.	150	157	7	0.	0.	0.	0.	0.	0.	60.	59.
	total/mea	**22**	**3**	**26**	**0.**	**0.**	**149**	**164**	**15**	**0.**	**0.**	**0.**	**0.**	**0.**	**0.**	**67.**	**60.**
201	GR	13	5	18	0.	0.	130	147	17	0.	0.	0.	0.	0.	0.	62.	60.
	IM	78	2	10	0.	0.	140	149	9	0.	0.	0.	0.	0.	0.	60.	59.
	LCL	13	8	21	0.	0.	125	142	17	0.	0.	0.	0.	0.	0.	64.	59.
	LS	27	1	43	0.	0.	141	156	15	0.	0.	0.	0.	0.	0.	62.	62.
	LSN	13	4	17	0.	0.	130	141	11	0.	0.	0.	0.	0.	0.	61.	59.
	MFS	74	5	12	0.	0.	153	162	9	0.	0.	0.	0.	0.	0.	65.	61.
	SFC	15	9	24	0.	0.	123	132	9	0.	0.	0.	0.	0.	0.	69.	68.
	SFS	15	1	25	0.	0.	152	168	16	0.	0.	0.	0.	0.	0.	71.	66.
	UPC	24	1	34	0.	0.	140	150	10	0.	0.	0.	0.	0.	0.	74.	65.
	UPS	45	1	62	0.	0.	132	144	12	0.	0.	0.	0.	0.	0.	59.	58.
	total/mea	**96**	**3**	**13**	**0.**	**0.**	**137**	**149**	**12**	**0.**	**0.**	**0.**	**0.**	**0.**	**0.**	**65.**	**62.**
201	GR	17	2	19	0.	0.	136	141	5	0.	0.	0.	0.	0.	0.	65.	59.
	IM	74	5	79	0.	0.	140	155	15	0.	0.	0.	0.	0.	0.	64.	56.
	LCL	27	2	29	0.	0.	125	142	17	0.	0.	0.	0.	0.	0.	67.	60.
	LS	36	5	41	0.	0.	141	146	5	0.	0.	0.	0.	0.	0.	64.	58.
	LSN	16	3	20	0.	0.	131	140	9	0.	0.	0.	0.	0.	0.	64.	59.
	MFS	10	1	11	0.	0.	155	149	-6	0.	0.	0.	0.	0.	0.	71.	61.
	SFC	28	3	31	0.	0.	122	115	-7	0.	0.	0.	0.	0.	1.	73.	66.
	SFS	25	0	25	1.	0.	148	--	--	0.	0.	0.	--	--	--	77.	---
	UPC	40	1	41	0.	0.	139	165	26	0.	0.	0.	0.	0.	0.	76.	88.
	UPS	35	6	41	0.	0.	134	145	11	0.	0.	0.	0.	0.	0.	64.	58.
	total/mea	**10**	**1**	**11**	**0.**	**0.**	**137**	**144**	**7.**	**0.**	**0.**	**0.**	**0.**	**0.**	**0.**	**69.**	**63.**
201	GR	18	6	25	0.	0.	124	129	5	0.	0.	0.	0.	0.	0.	64.	58.
	IM	53	7	60	0.	0.	134	136	2	0.	0.	0.	0.	0.	0.	64.	59.
	LCL	34	1	48	0.	0.	120	127	7	0.	0.	0.	0.	0.	0.	66.	60.
	LS	40	9	49	0.	0.	132	134	2	0.	0.	0.	0.	0.	0.	65.	58.
	LSN	16	7	24	0.	0.	122	126	4	0.	0.	0.	0.	0.	0.	63.	59.
	MFS	68	1	87	0.	0.	147	148	1	0.	0.	0.	0.	0.	0.	68.	63.
	SFC	50	2	71	0.	0.	109	128	19	0.	0.	0.	0.	0.	0.	74.	67.
	SFS	17	1	18	0.	0.	145	137	-8	0.	0.	0.	0.	0.	0.	73.	64.
	UPC	24	7	31	0.	0.	135	139	4	0.	0.	0.	0.	0.	0.	74.	69.
	UPS	32	1	46	0.	0.	128	135	7	0.	0.	0.	0.	0.	0.	63.	58.
	total/mea	**95**	**3**	**13**	**0.**	**0.**	**130**	**133**	**4.**	**0.**	**0.**	**0.**	**0.**	**0.**	**0.**	**67.**	**62.**

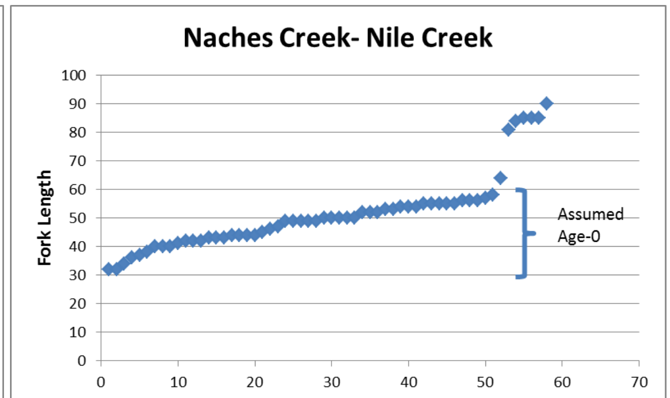
ove	GR	51	1	67	0.	0.	131	139	8	0.	0.	0.	0.	0.	0.	64.	59.
	IM	22	3	26	0.	0.	140	147	7	0.	0.	0.	0.	0.	0.	63.	58.
	LCL	79	2	10	0.	0.	123	135	12	0.	0.	0.	0.	0.	0.	66.	60.
	LS	11	3	14	0.	0.	139	149	10	0.	0.	0.	0.	0.	0.	64.	60.
	LSN	48	1	65	0.	0.	129	134	5	0.	0.	0.	0.	0.	0.	63.	59.
	MFS	28	8	36	0.	0.	153	157	4	0.	0.	0.	0.	0.	0.	68.	62.
	SFC	98	3	13	0.	0.	115	129	14	0.	0.	0.	0.	0.	0.	73.	67.
	SFS	63	1	74	0.	0.	149	165	16	0.	0.	0.	0.	0.	0.	74.	66.
	UPC	95	1	11	0.	0.	139	147	8	0.	0.	0.	0.	0.	0.	75.	68.
	UPS	12	3	16	0.	0.	133	141	8	0.	0.	0.	0.	0.	0.	62.	58.
	total/mea	**31**	**9**	**41**	**0.**	**0.**	**134**	**141**	**7.**	**0.**	**0.**	**0.**	**0.**	**0.**	**0.**	**67.**	**62.**

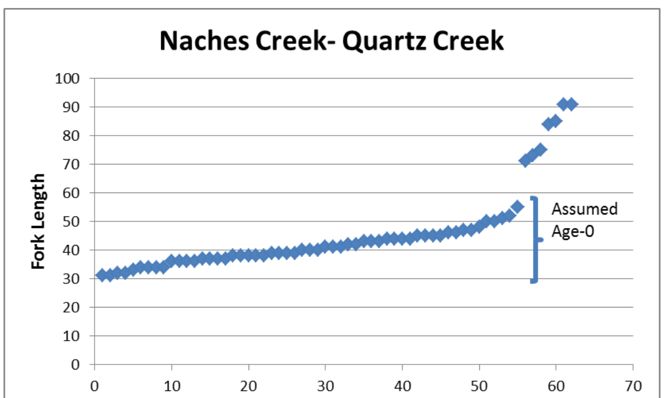
Appendix B.3: Plotted lengths of genotyped samples. Graphs are divided by sections within each major tributary.

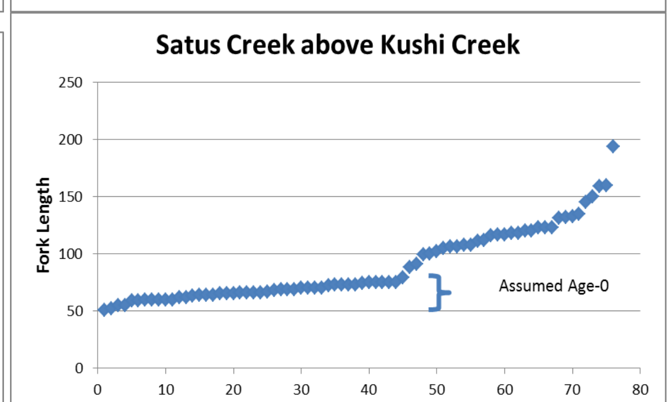


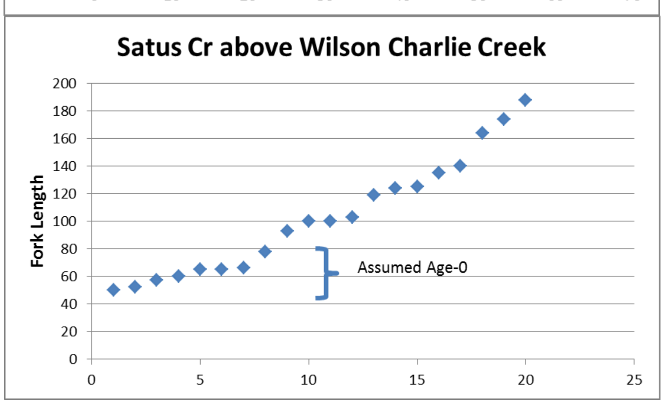


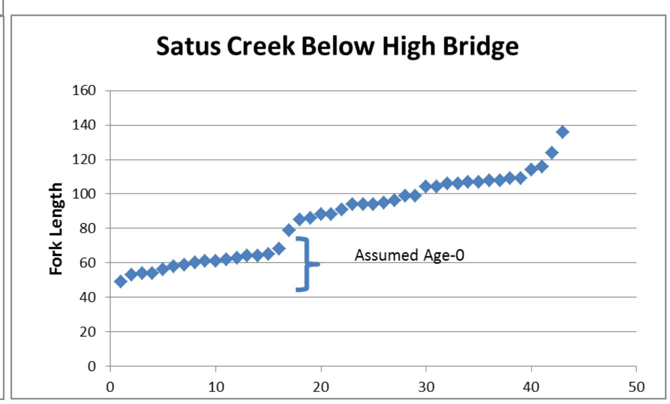


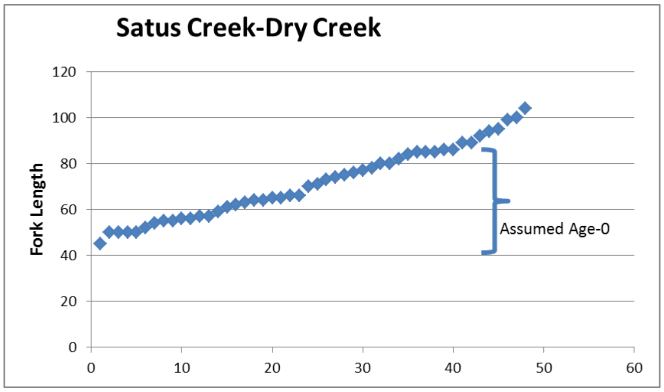


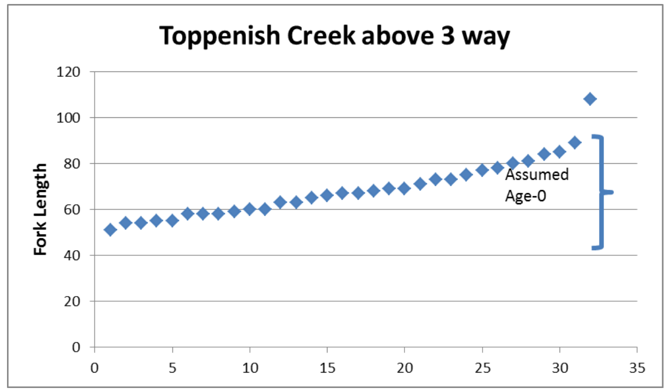


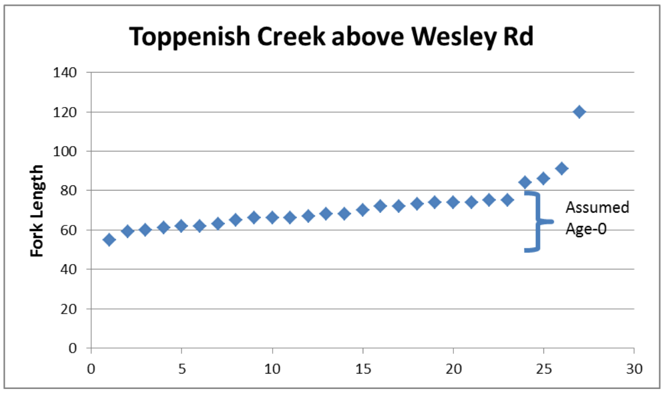


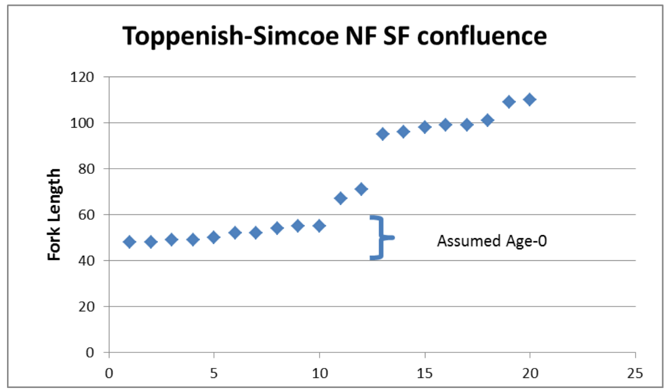


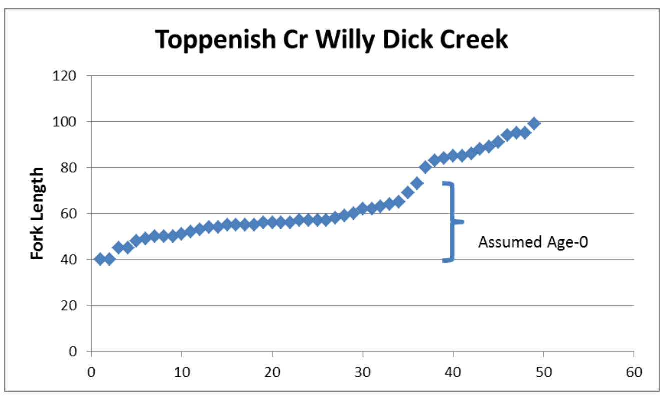


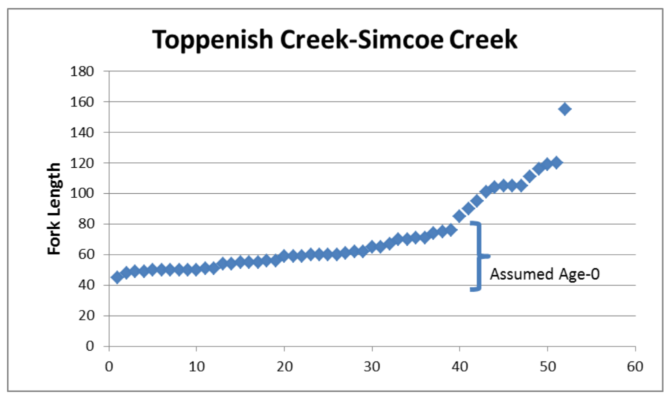

Satus Creek-Dry Creek
Fork Length
0
20
40
60
80
100
120
0
10
20
30
40
50
60
Assumed Age-0


Toppenish Creek above 3 way
Fork Length
0
20
40
60
80
100
120
0
5
10
15
20
25
30
35
Assumed Age-0


Toppenish Creek above Wesley Rd
Fork Length
0
20
40
60
80
100
120
140
0
5
10
15
20
25
30
Assumed Age-0


Toppenish-Simcoe NF SF confluence
Fork Length
0
20
40
60
80
100
120
0
5
10
15
20
25
Assumed Age-0


Toppenish Cr Willy Dick Creek
Fork Length
0
20
40
60
80
100
120
0
10
20
30
40
50
60
Assumed Age-0


Toppenish Creek-Simcoe Creek
Fork Length
0
20
40
60
80
100
120
140
160
180
0
10
20
30
40
50
60
Assumed Age-0

Appendix C: List of Metrics and Indicators

Category	Subcategory	Subcategory Focus 1	Subcategory Focus 2	Specific Metric Title
Fish	Relative Reproductive Success (RRS)	Fish Origin: Both		
Fish	Survival Rate: Fish	Fish Life Stage: RANGE: Adult to Adult	Fish Origin: Both	
Fish	Relative Reproductive Success (RRS)	Fish Origin: Both		
Fish	Survival Rate: Fish	Fish Life Stage: RANGE: Adult to Adult	Fish Origin: Both	
Fish	Relative Reproductive Success (RRS)	Fish Origin: Both		
Fish	Survival Rate: Fish	Fish Life Stage: RANGE: Adult to Adult	Fish Origin: Both	
Fish	Relative Reproductive Success (RRS)	Fish Origin: Both		
Fish	Survival Rate: Fish	Fish Life Stage: RANGE: Adult to Adult	Fish Origin: Both	
Fish	Relative Reproductive Success (RRS)	Fish Origin: Both		
Fish	Survival Rate: Fish	Fish Life Stage: RANGE: Adult to Adult	Fish Origin: Both	
Fish	Relative Reproductive Success (RRS)	Fish Origin: Both		
Fish	Survival Rate: Fish	Fish Life Stage: RANGE: Adult to Adult	Fish Origin: Both	
Fish	Relative Reproductive Success (RRS)	Fish Origin: Both		
Fish	Survival Rate: Fish	Fish Life Stage: RANGE: Adult to Adult	Fish Origin: Both	

Fish	**Relative Reproductive Success (RRS)**	**Fish Origin: Both**		
Fish	**Survival Rate: Fish**	**Fish Life Stage: RANGE: Adult to Adult**	**Fish Origin: Both**	
Fish	**Relative Reproductive Success (RRS)**	**Fish Origin: Both**		
Fish	**Survival Rate: Fish**	**Fish Life Stage: RANGE: Adult to Adult**	**Fish Origin: Both**	
Fish	**Abundance of Fish**	**Fish Life Stage: Adult - Outmigrant**	**Fish Origin: Both**	**Kelt abundance**
Fish	**Abundance of Fish**	**Fish Life Stage: Adult Fish**	**Fish Origin: Both**	**Reconditioned Kelt abundance**
Fish	**Condition of Life Stage: Fish**	**Fish Life Stage: Adult - Outmigrant**		**Kelt Condition**
Fish	**Condition of Life Stage: Fish**	**Fish Life Stage: Adult Fish**		**Reconditioned Kelt condition**
Fish	**Length: Fish Species**	**Fish Life Stage: Adult - Outmigrant**		**Kelt length**
Fish	**Length: Fish Species**	**Fish Life Stage: Adult Fish**		**Reconditioned kelt length**
Fish	**Mark/Tag Recovery**			**Mark Detection**
Fish	**Stock Identity**	**Fish Life Stage: Adult - Outmigrant**		**Mark application**
Fish	**Survival Rate: Fish**	**Fish Life Stage: Adult - Outmigrant**	**Fish Origin: Both**	**Kelt Survival**
Fish	**Timing of Life Stage: Fish**	**Fish Life Stage: Adult - Outmigrant**		**Collection Date**
Fish	**Timing of Life Stage: Fish**	**Fish Life Stage: Adult Fish**		**Release Date**
Fish	**Weight: Fish**	**Fish Life Stage: Adult - Outmigrant**	**Fish Origin: Both**	**Kelt Weight**
Fish	**Weight: Fish**	**Fish Life Stage: Adult Fish**	**Fish Origin: Both**	**Reconditioned Kelt weight**

Fish	**Relative Reproductive Success (RRS)**	**Fish Origin: Both**		
Fish	**Survival Rate: Fish**	**Fish Life Stage: RANGE: Adult to Adult**	**Fish Origin: Both**	
Fish	**Relative Reproductive Success (RRS)**	**Fish Origin: Both**		
Fish	**Survival Rate: Fish**	**Fish Life Stage: RANGE: Adult to Adult**	**Fish Origin: Both**	
Fish	**Relative Reproductive Success (RRS)**	**Fish Origin: Both**		
Fish	**Survival Rate: Fish**	**Fish Life Stage: RANGE: Adult to Adult**	**Fish Origin: Both**	
Fish	**Relative Reproductive Success (RRS)**	**Fish Origin: Both**		
Fish	**Survival Rate: Fish**	**Fish Life Stage: RANGE: Adult to Adult**	**Fish Origin: Both**	
Fish	**Relative Reproductive Success (RRS)**	**Fish Origin: Both**		
Fish	**Survival Rate: Fish**	**Fish Life Stage: RANGE: Adult to Adult**	**Fish Origin: Both**	
Fish	**Relative Reproductive Success (RRS)**	**Fish Origin: Both**		
Fish	**Survival Rate: Fish**	**Fish Life Stage: RANGE: Adult to Adult**	**Fish Origin: Both**	
Fish	**Relative Reproductive Success (RRS)**	**Fish Origin: Both**		
Fish	**Survival Rate: Fish**	**Fish Life Stage: RANGE: Adult to Adult**	**Fish Origin: Both**	